The Molecular Genetics of Bipolar Affective Disorder: South African Populations, Endophenotypes, and Environmental Influence.

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Declaration.

This study was performed from 2002-2006 under the supervision of Professor Raj Ramesar and Professor Mark Solms.

I hereby certify that this is my own unaided work and has not been presented for a degree at another university.

Signed by Candidate

Signature Removed

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May, 2006.

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List of Abbreviations used in the Thesis.

ANOVA: Analysis of Variance

ANPS: Affective Neuroscience Personality Scale

ApoE: Apolipoprotein E

BDNF: Brain Derived Neurotrophic Factor

BPD I: Bipolar Disorder Type I

BPD II: Bipolar Disorder Type II

BPD NOS: Bipolar Disorder Not Otherwise Specified

CDCV: Common Disease Common Variant hypothesis

cM: centimorgan (a distance of 1 centimorgan corresponds to a 1% probability of a recombination event taking place between two loci)

COMT: Catechol-O-Methyltransferase

CT: Cyclothymic Temperament Scale of TEMPS-A.

CTQ: Child Trauma Questionnaire

CVLT: California Verbal Learning Test

DA: Dopamine

DAT: Dopamine Transporter

DES: Dissociative Experiences Scale

DLPFC: Dorsolateral Prefrontal Cortex

DRD2: Dopamine 2 Receptor

DRD4: Dopamine 4 Receptor

DSM-IV: Diagnostic and Statistical Manual of the American Psychiatric Association, Version IV

DT: Dysthymic Temperament Scale of TEMPS-A

EPQ: Eysenck Personality Questionnaire.

fMRI: functional Magnetic Resonance Imaging

HA: Harm Avoidance Scale of TCI

HLOD: Heterogeneity LOD.

HPS: Hypomanic Personality Scale

HT: Hyperthymic Temperament Scale of TEMPS-A

IT: Irritable Temperament Scale of TEMPS-A

Kb: Kilo-bases (one thousand base pairs)

LD: Linkage Disequilibrium.

LOD: Logarithm of the Odds

MAO-A: Monoamine Oxidase A

Mb: Mega-bases (one million base pairs).

MDE-R: Major Unipolar Depression – Two or More Episodes

MDE-S: Major Unipolar Depression – One Episode

MDI: Manic Depressive Illness

Mega2: Manipulation Engine for Genetic Analysis, Version 2

Merlin: Multi-point Engine for Rapid Likelihood Inference

MLOD: Modified LOD

NPL: Non-Parametric Linkage

NS: Novelty Seeking Scale of TCI

OR: Odds Ratio

PAG: Periaqueductal Gray Matter

PCA: Principle Components Analysis

PCR: Polymerase Chain Reaction

PFC: Prefrontal Cortex

PTSD: Post-Traumatic Stress Disorder

Q-TDT: Quantitative Transmission Disequilibrium Test

RAVLT: Rey Auditory Verbal Learning Test

RCF: Rey Complex Figure

REM: Rapid Eye Movement

SERT: Serotonin Transporter

SNP: Single Nucleotide Polymorphism

SPS: Schizotypal Personality Scale

STA: Schizotypal Personlaity Scale

STB: Borderline Personality Scale

TCI: Temperament and Character Inventory

TDT: Transmission Disequilibrium Test

TEMPS-A: Temperament Inventory of Memphis, Pisa, and San Diego

UTR: Untranslated Region

VNTR: Variable Number Tandem Repeat

WAIS: Wechsler Adult Intelligence Scale

WCST: Wisconsin Card Sorting Test

Abstract.

The identification of the genetic variants underpinning bipolar disorder (BPD) has been impeded by a complex pattern of inheritance that may include by genetic heterogeneity, genetic epistasis, gene-environment interactions, incomplete penetrance and variable expressivity. In this thesis three strategies were employed to ameliorate these confounding factors.

The first strategy was to focus on a theoretically genetically-homogenous population with BPD. A unique South African sample including 190 individuals of the relatively reproductively-isolated Afrikaner population yielded promising evidence of linkage to chromosome 1q31-32 and weaker peaks at 10q23 and 13q32, regions previously implicated in the disorder. A family-based analysis suggested that the 3' variable number tandem repeat (VNTR) variant of the dopamine transporter gene (*DAT*) is associated with bipolar-spectrum illness in the 132-strong sample of British ancestry.

The second strategy was to carry out genetic linkage and association analyses using quantitative traits (endophenotypes) that were closely associated with BPD. As part of this process a variety of personality traits were evaluated in the cohort, and anxiety-related, novelty-seeking, hyperthymic, and cyclothymic personality traits were found to aggregate in participants with BPD and to a lesser extent repeated unipolar illness (MDE-R). These traits were therefore used as quantitative markers or endophenotypes of BPD.

The quantitative linkage analysis indicated that a variant in the region of 13q32 may influence the development of novelty-seeking-related traits in the largest Afrikaner pedigree, while the personality trait, "Stability", was weakly linked to 4p16 in the total sample. The catechol-o-methytransferase (*COMT*) Val158Met and the Brain Derived Neurotrophic Factor (*BDNF*) Val66Met polymorphisms were associated with mood-labile-cyclothymic and hyperthymic-novelty-seeking traits, respectively. The *DAT* VNTR and the *Notch4* exonic repeat variants were associated with a broad range of "pathological" personality traits in the samples of British and Afrikaner origin, respectively.

The sample was also evaluated with a battery of neuropsychological tasks and the BPD I and MDE-R groups displayed both verbal and visual memory recall deficits while the BPD I sample also suffered from recognition memory deficits. The neurocognitive trait, "Memory" was therefore used as a second endophenotype generating potential linkage signals on 10q23 and 22q11. The exonic 48bp VNTR polymorphism in the dopamine 4 receptor (*DRD4*) gene was associated with "Memory" performance.

As a third strategy, a potentially important aetiological factor, childhood trauma, was measured, and used to test for gene-environment interactions between the various candidate genes and bipolar-illness or BPD-related endophenotypes in the cohort. BPD and MDE-R individuals displayed significantly higher levels of emotional and physical abuse, and the former variable was also associated with the development of anxiety-related and unstable personality traits. A functional variant of the *COMT* gene was found to interact with abuse to predispose to anxiety-related, unstable-cyclothymic and novelty-seeking-related personality traits. The combination of childhood abuse and possession of low-activity *MAO-A* gene variants was also associated the development of more anxious and unstable personality traits. An interaction between sexual abuse and the *BDNF* gene modulated performance on verbal and visual memory tasks. A similar interaction between the *ApoE* gene and sexual abuse was observed.

Although a number of theoretical obstacles remain to be resolved, the analyses of isolated populations coupled with the use of endophenotypes and the testing of gene-environment interactions, holds out great promise for the eventual elucidation of the genetic basis of bipolar affective disorder.

Chapter 1.

"Once the attack of mania is over the sick persons become slowed down, docile, taciturn and sad, and when they recall the illness they have been through they feel anguish at their wretchedness". Aretaeus of Cappadocia, quoted by Koukopoulos et al. (2001, p 316).

1.1. Introduction.

Bipolar disorder (BPD) is a severe, chronic and potentially malignant psychiatric illness that is associated with significant morbidity and mortality. In 1990 the World Health Organisation listed BPD as the sixth leading cause of disability in the world among people in the 15-44 year-old category (Pini et al. 2005); a greater degree of disability than chronic diseases such as osteoarthritis, diabetes and asthma (Sajatovic 2005). The illness imposes a significant load on health-care systems around the world with the total economic burden in the United States totalling approximately 45 billion dollars (Hirschfeld and Vornik 2005). About 60% of (even university-educated) BPD individuals are unemployed, and of those employed almost 50 annual workdays are lost per person per year (Sajatovic 2005). More seriously, up to 30% of patients attempt suicide, with an odds ratio for attempts of about six compared to three for unipolar depression (Bauer and Pfennig, 2005).

BPD runs in families, and has a strong genetic basis (Craddock and Jones 1999). Given the human and capital costs associated with the illness, a world-wide effort is being made to isolate putative disease loci: an undertaking that promises to help elucidate the pathophysiology of BPD and catalyse the development of more efficacious therapeutic strategies. Despite millions of dollars worth of research, no genetic variant has unequivocally been shown to render individuals susceptible to the

disorder, and at best the findings are contradictory. The causal role of multiple loci of small effect size, gene-gene interactions, the contribution of unknown or unquantified environmental factors and the possible pathophysiologically and genetically heterogeneous nature of the disorder has blunted the edge of foregoing molecular investigations (Gould and Gottesman 2006; Hasler et al. 2006).

In this thesis three different strategies are adopted in order to try and counter some of these problems. Each strategy forms the basis of a separate chapter or chapters of the thesis. These chapters are self-contained with each consisting of:

(1) A review of the relevant literature.
(2). A summary of the rationale behind the strategy employed.
(3). An outline of the methodological details of the study.
(4). A section in which the results of the analysis are presented.
(5). A discussion of these results.

Information that is believed to be important but cannot be included in the main body of the thesis without making it unwieldy is presented in the various appendices at the end of the thesis. In Appendix A general background information on linkage and association analyses is offered as it is relevant to the interpretation of the results and the evaluation of the discussion. Appendix B contains a detailed tabular summary of the results of published linkage analyses. Appendix C addresses methodological details such as primer sequences, and Appendix D is a pedigree drawing of the largest family. Appendices E and F contain psychometric information about the personality and neuropsychological tests used in the thesis.

Before moving on to the central aspects of the thesis, a general background discussion of bipolar illness is presented here, including its symptomatology, diagnosis and epidemiology. A final chapter (Chapter 6) sums up the thesis in its entirety.

1.2. BPD: Symptomatology and Diagnosis.

I knew hatred
Hatred for
The blood
and marrow
that courses
through
my cursed being

Hatred
for the other
So distant and discreet
Pissing in dark alleys
Screaming and intent
I seek revenge
To free this
wreckage of a body
The fragments of a life interred
With the stench of body fluids
Less innocent than mine

Knowledge and belief
know no template
Buried in this bone
Borne to bear a faulty gene
This spirit incarnate
seeks the unseekable
Meets the unspeakable
and, I juxtapose, quite formally,
Eyes spilling blood, and your stately
gaze.

Molly Message, (2001), a participant in this study.

The light emanates
From glass unshattered
the wind blows
through trees unblighted
with infant ease
and
I implode
and feel the beauty.

The light splits
through crags enclimbed
The bush exudes
native tenderness
and the ease and freshness
touches unframed bodies
to pass into
the beauty of a
world in need of nothing.

Molly Message, (2001).

Manic-depressive illness is a pathological disturbance of affective regulation, which is characterised, in its prototypical form, by a seemingly capricious oscillation between extreme elation or mania and its polar opposite, devastating depression - hence the formal name, "bipolar disorder" (APA, 1994).

A description of mania:

There is a peculiar kind of pain, elation, loneliness and terror involved in this kind of madness. When you're high it's tremendous. The ideas and feelings are fast and frequent like shooting stars, and you follow them until you find better and brighter ones. Shyness goes, the right words and gestures are suddenly there, the power to captivate others a felt certainty. ... Feelings of ease, intensity, power, well-being, financial omnipotence, and euphoria invade one's marrow. But somewhere this changes. The fast ideas are too fast... overwhelming confusion replaces clarity. ... Everything previously moving with the grain is now against you - you are irritable, angry, frightened, uncontrollable, and enmeshed totally in the blackest caves of the mind.

Kay Redfield Jamison, (1995, p 67).

A manic episode is a profound disturbance of emotion that is characterised by an abnormally elevated, expansive or irritable mood (APA 1994). Elation and euphoria, expressed in the guise of laughter, punning and joking are typically observed, and manic individuals are usually grandiose, loquacious, hedonistic, and impulsive (Akiskal 1995). The enormous increase in mental and physical energy associated with the episode may be expressed in the form of racing thoughts, pressured speech, sexual promiscuity, distractibility, psychomotor agitation and decreased need for sleep (APA 1994).

The mood state is not always stable and it may be punctuated by tearfulness or ferocious irritability and hostility, particularly when the patient does not get his/her own way (Akiskal 1995). In these cases, the patient may be diagnosed with a mixed manic episode: the simultaneous presentation of depressive and manic symptoms. Mania is not always pleasant. Sometimes the high can become so intense that the mood actually becomes dysphoric in nature (Akiskal 1995). Manic individuals often leave a trail of destruction in their wake - in particular, damaged relationships and financial debt - which only serves to exacerbate the period of deep depression which invariably follows the unnatural emotional high (Jamison 1995).

A description of depression:

"It is like falling into a deep black pit; or being drawn down into a dark vortex... With it goes all feeling. There is no despair for there is no meaning; all is as white as the absence of colour, as black as all colour. It is a state of non-being... the closest I can come /to giving it a description/ *is that of a void; of being condemned to life. And as the ability to live recedes, the most terrifying part of it all is that it leaves a certain serenity. At that point only the idea of death itself gives hope".*

Claire Dubois quoted by Peter Whybrow (1997, p 23).

The term depression *"... has slithered through language like a slug, leaving little trace of its intrinsic malevolence and prevented by its very insipidity, a general awareness of the horrible intensity of the disease when out of control".*

William Styron (1991).

Depression is not only associated with subjective feelings of sadness and emptiness, but typically causes a variety of physical disturbances such as insomnia or hypersomnia, weight gain or loss, psychomotor agitation or retardation, fatigue and lassitude (APA 1994). Common psychological sequelae of the disorder include anhedonia, exaggerated feelings of worthlessness, inappropriate guilt, and the inability to think clearly or concentrate (APA 1994). In more severe cases, the patient often presents with psychotic features which are prototypically characterised by themes of worthlessness, sinfulness and persecution (Akiskal 1995).

At first glance the intensity and cyclical nature of the illness appears to lend itself to ease of diagnosis. The reality is somewhat different. A long battle between divergent psychiatric traditions has raged over the nosological status of bipolar depression and related conditions.

The initial insight that depression and mania occur as part of the same illness is attributed to Aretaeus of Cappadocia of first century Alexandria (Angst 2000;

Marneros and Angst 2000). Modern conceptions of the disorder have their origin in the work of Jean-Pierre Falret, who in 1851 described a discrete category of illness, *folie circulaire*, and Baillarger who described *folie a double forme* three years later (Angst 2000; Marneros and Angst 2000). Up until the early 20th century psychosis was seen as a unitary phenomenon. Emil Kraepelin then distinguished between two main forms of psychosis, *dementia praecox* (schizophrenia) and *manic depressive insanity*, a nosology that has had an unparalleled influence on the classification of psychiatric disorders. Kraepelin (1913) posited that manic depressives have a good clinical prognosis free from long-term cognitive deterioration, but as the name suggests, "precocious dementia" is characterised by chronic psychosis and a deterioration in intellectual functioning. Kraepelin's observations have proved to be fairly robust and the DSM-IV currently considers schizophrenia and BPD to be discrete entities.

In addition to their long-term cognitive sequelae, the two disorders are differentiated by the character of the psychosis. Although, manic-depressives may present with religious and paranoid hallucinations, incoherent speech, loose associations and other hallmarks of schizophrenia, the leitmotif and duration of the psychosis is inextricably linked with mood (Blacker and Tsuang 1992). In the case of schizophrenia, there is no underlying mood disorder, although an intermediate category, schizoaffective disorder, which is characterised by mood-incongruent psychotic features, does exist (Blacker and Tsuang 1992).

Kraepelin did not however make a distinction between unipolar and bipolar forms of the illness, and manic depressive illness was an inclusive label for a wide range of affective conditions (Marneros and Angst 2000). This started to change in the 1960's when research began to suggest that a nosological differentiation between bipolar and unipolar forms of depression should be made. Unipolar depression was found to differ from BPD in terms of symptomatology, course of the illness, genetics, gender ratios, and premorbid personality (Marneros and Angst 2000).

The movement towards the differentiation of bipolar and unipolar forms of illness, culminated in the DSM IV of the American Psychiatric Association (APA 1994) which makes use of a very narrow definition of BPD (Akiskal and Pinto 2000). The

tide appears to be turning however, and a return towards a more unitary position is gaining momentum. The renaissance of the Kraepelinian diagnostic schema has been driven largely by Akiskal and colleagues who have noted that patients with DSM-IV unipolar depression often display subtle features of bipolarity which are overlooked by the clinician (Akiskal and Pinto 2000; Koukopoulos et al. 2000).

A percentage of the bipolar population experience a less potent form of mania known as hypomania which is a period of mildly elevated mood, characterised by sharpened thinking, increased energy and activity levels, several days to weeks in duration (APA 1994). Hypomania does not result in psychosis and the associated disruption of social or occupational functioning that typically leads to hospitalisation (Akiskal 1995; Akiskal 1986), and this form of the illness, which is also punctuated by severe depressive episodes, is known as bipolar disorder type 2 (BPD II).

The DSM-IV specifies that the hypomanic period must be of at least four days duration in order for the patient to meet the criteria for BPD II (APA 1994). This leaves a significant portion of the clinical population who suffer from brief (less than four days) hypomanic episodes in a nosological no-man's land, and they are currently subsumed under the unipolar banner (Akiskal and Pinto 2000). Other "unipolar" patients suffer from short-lived hypomanic episodes as a result of pharmacotherapy (Akiskal et al. 2003b). These individuals who do not fall neatly into the BPD I or II categories but present with so-called "soft" bipolar signs may account for up to a third of bipolar cases (Akiskal and Mallya 1987). The distinction is not only academic in nature, but has treatment implications because "soft" bipolars may show an adverse response to treatment with anti-depressants, especially the tricyclic and selective serotonin reuptake inhibitor (SSRI) classes of drugs (Akiskal and Mallya 1987).

In summary then, an entire spectrum of bipolar illness from dramatic schizophrenia-like psychoses to chronic dysthymia exists (see Tables 1.1 and 1.2, below). These disorders may share a common or at least partly overlapping aetiology which is problematic for geneticists who have traditionally relied on DSM-IV diagnoses to label study participants as affected or unaffected.

Table 1.1: The Pisa/San-Diego Nosology of BPD. (Adapted from Akiskal, 1986; Akiskal and Mallya, 1987; Akiskal, 2001; Akiskal and Pinto, 2000; Angst et al. 2002; *Perugi and Akiskal, 2002; Perugi et al. 2003*; Akiskal et al. 2003).

Nomenclature.	**Typical DSM-IV Diagnosis.**	**Symptomatology/Signs.**
BPD Type 0.5.	*Bipolar Type I or Schizoaffective Disorder.*	Illness dominated by mania rather than depression.
BPD Type I.	*Bipolar Type I.*	Classical alternating periods of depression and mania.
BPD Type 1.5.	*BPD Type I or II.*	Protracted hypomania with episodes of depression.
BPD Type II.	*Bipolar Type II.*	Major depression with hypomania of at least 4 days duration. Individuals have a "sunny" disposition.
BPD Type 2.5.	*Cyclothymia, Borderline PD or Atypical Depression.*	Present with depression and hypomanic episodes of 1-3 days duration or less. Rapid swings in mood, cognition and behaviour. Irritable, mood-labile, risk-taking, rejection avoidance, interpersonal sensitivity and impulsivity.
BPD Type III.	*Major unipolar depression; recurrent minor depression, dysthymia or double depression.*	Present with chronic mild depression or major depression. Hypomania and/or rapid cycling upon TCA or MAO challenge. Bipolar family history, early onset and high frequency of episodes.
BPD Type 3.5.	*Major Unipolar Depression with Substance Abuse.*	Depressive and hypomanic swings associated with stimulants and other substances of abuse.
BPD Type IV.	*Major Unipolar Depression*	Present with depression - often mixed depressive states. Agitated depression which occurs later in life: often males in 50's, dysphoria, anxiety, increased sexual drive. Hyperactivity in youth.

Table 1.2. The Diagnostic Schema of Angst. (Angst et al. 2003).

Diagnosis.	**Description.**
Bipolar I	Hospitalised Mania + Major Depression.
Bipolar II	Major Depressive Episodes with any hypomanic symptoms.
Minor BPD	Dysthymia, minor depression or recurrent brief depression with any hypomanic symptoms.
Pure hypomania	Hypomanic symptomatology without depression.

1.3. Epidemiology.

According to the DSM-IV, the lifetime prevalence of BPD is 0.4-1.6% for BPD I and 0.5% for BPD II (APA 1994). These figures fall broadly in line with more recent epidemiological surveys. Pini et al. (2005) summarised the results of epidemiological studies in ten European countries, with most studies suggesting a 12-month prevalence of BPD I of approximately 1%. The prevalence rate for BPD II varied more between studies with some surveys reporting BPD II to be more common than BPD I, and others indicating the opposite pattern (Pini et al. 2005). The same trend is observable in an earlier review by Angst (1998) who suggested lifetime prevalence rates of 0.2-3% for BPD II. The lifetime prevalence rate for the "softer" spectrum of bipolar illness is significantly higher with estimates ranging from 2.8 to 6.5% (Bauer and Pfennig 2005). Angst et al. (2003) reported prevalence rates of 0.5% for BPD I, 1.7% for BPD II and 21.3% for major depressive disorder when traditional DSM-IV criteria were used. Employment of their alternative classification (described in Table 1.2) however, resulted in new prevalence rates of 11% for the soft bipolar spectrum and 11.4% for major depressive disorder (Angst et al. 2003). In other words, up to half of patients currently diagnosed with unipolar depression could have a bipolar diathesis!

Women are about twice as likely as men to develop major depressive disorder (MDD), but this skewed sex ratio is not observed in BPD (Blazer 1995). BPD can develop at any time between childhood and middle age, but the vast majority of individuals fall ill during their adolescence or early twenties (Blazer 1995). The mean age of onset of a large sample (2 839) of patients was recorded by the Stanley Foundation BPD registry to be 19.8 years (Kupfer et al. 2002).

Co-morbidity is common - especially drug or alcohol abuse. Regier et al. (1990) noted that approximately 25% of their sample of patients met DSM criteria for substance abuse. The European epidemiological studies reviewed by Pini et al. (2005) put this figure even higher at close to 70%. Anxiety disorders, including generalised anxiety disorders, panic disorder, post-traumatic stress disorder and specific phobias are also salient (Pini et al. 2005). In fact, Angst (1998) found that patients with BPD II were 10 times more likely to suffer from panic attacks than non-bipolar controls. Somatic conditions such as cardiovascular disease, cancer, and cerebrovascular disease are also a problem (Bauer and Pfennig 2005).

Genetic factors play a significant role in the aetiology of BPD. Family based research is predicated upon the notion that if the disease of interest is genetic or at least partially genetic, then the risk of developing that disorder will be greatest among family members who are closely related to the proband or affected individual. The results of these studies are expressed in terms of relative risk. That is, the ratio of risk in the first degree relatives of the bipolar probands to the risk in the first degree relatives of controls. In their review Cradock and Jones (1999) report that all studies have shown an increased risk of BPD amongst the first degree relatives of bipolar probands, with the relative risk ranging from 2 to 20 (mean 7) depending on the study quoted. A more recent review by Merinkangas and Low (2004) examined "well-controlled" studies of mood disorders amongst the relatives of bipolar probands, and found that the relative risk ranged between 3.7 and 17.7, with a weighted average of 9.2.

These data are illustrative of the fact that BPD tends to aggregate in families. The relative risk of developing unipolar depression, BPD and schizoaffective disorder is also enhanced in the relatives of BPD probands (Craddock and Jones 1999).

Family based studies are however open to the counter criticism that BPD aggregates in families due to environmental risk factors: the notion that disturbed parents will bring-up pathological offspring. Twin studies seek to eliminate this possible objection by comparing the concordance rates of monozygotic and dizygotic twins raised in identical environments. The outcome of this type of study is expressed as a heritability value - the proportion of the statistical variance of the trait (diagnosis of BPD) that can be attributed to genetic variation in a particular population.

As a basic principle, heritability values can be estimated as double the difference between monozygotic and dizogotic correlations in liability (since monozygotic twins share 50% more DNA than dizygotic twins) to give the full genetic effect size. In practice, however, many different types of statistical corrections are made to increase the accuracy of the results.

A summary of the largest twin studies that the author is aware of is displayed in Table 1.3. Heritability values for narrowly defined BPD range from 59%-93%, with four out of the five studies indicating heritability scores of upwards of 80%. The moderately high monozygotic twin concordance rates of about 40% illustrate the importance of non-genetic factors in the precipitation of the disorder, and these data are discussed in Chapter 5.

Table 1.3. Twin Studies of BPD

Study.	**MZ Pairs**	**Concordance**	**DZ Pairs**	**Concordance**	**Heritability**
Bertelsen et al. (1977).	34	62%	37	8%	59%
Kendler et al. (1995).	13	38.5%	22	4.5%	79%
Cardno et al. (1999).	25	44%	33	9.1%	87%
McGuffin et al. (2003)	30	40%	37	5.4%	85%
Kieseppa et al. (2004)	7	43%	18	6%	93%

According to Craddock and Jones (1999), only two adoption studies have made use of current conceptualisations of BPD. Mendlewicz and Rainer (1977) found that the biological parents of bipolar adoptees had about an 18% chance of developing a broadly defined affective disorder (BPD, schizoaffective disorder or unipolar depression), while the adoptive parents of these selfsame individuals had about a 7% risk of developing affective problems. Wender et al. (1986), used a much smaller sample (10 subjects), and also found that the biological relatives of BPD adoptees were at a greater risk of developing the disorder than the adoptive family members, although the results were not statistically significant.

1.4. Aim of the Study.

In this thesis a trilateral approach to the elucidation of the genetic origins of bipolar affective illness is presented. The three pillars of this strategy are described in Chapters 2, 3, 4, and 5.

(1). Traditional linkage and association studies were conducted on the cohort of South African bipolar families with special emphasis on individuals of Afrikaner origin (Chapter 2).

(2). Quantitative linkage and association analyses using personality traits and neuropsychological data as endophenotypes for BPD were performed as described in Chapters 3 and 4.

(3). Association analyses and linkage analyses were enhanced by controlling for one potential environmental risk factor: childhood abuse or neglect (Chapter 5).

Chapter 2.

The Molecular Genetics of Bipolar Affective Disorder: South African Populations.

The last of the clan was Phyllis. She was about nine years older than Edgar and nursed at St Bartholomew's hospital. She was always feeling that she was being persecuted and once when she had one of her mental breakdowns ... I was sent to fetch her ... I had a black MG open sports car and I put Aunt Phyllis in the front with me... and throughout the journey she'd be shouting and screaming wherever we stopped and whatever we did, and I being quite a young man – I was a medical student – found it most embarrassing that she would shout and scream and talk to practically everyone, particularly if we stopped for lunch or tea or a meal.

Extract from an autobiography of the late Edgar Hacking, a participant in this study, describing the manic behaviour of his aunt in the UK before immigrating to South Africa.

2.1. Linkage Analysis.

In the following sections the application of linkage methodologies to BPD is examined, and the most promising chromosomal regions that have been linked to the illness reviewed. For each chromosome that is discussed, a figure summarising the linkage results is presented, and detailed information such as sample size, statistical techniques, markers used and affection status is presented in a separate table in Appendix B.

A vast number of linkage analyses have been carried out on BPD over the last 15 years and a number of promising loci have been identified. In the following section

these chromosomal regions are highlighted. All chromosomal distances are quoted in megabases which were obtained from the August 2005 build of the Ensembl Human Genome Database (www.ensembl.org).

Chromosome 1.

Turecki et al. (1995) provided the initial impetus for investigations of chromosome 1, when they reported an association between a fragile site on chromosome 1q32 and BPD. Detera-Wadleigh et al. (1999) replicated this finding in a sample of 22 multiplex pedigrees. A LOD score of 2.67 at 205.3 Mb and a multipoint affected sib-pair NPL score of 1.78 between markers at 206.1 and 211.1 Mb (1q32-1q41) were obtained. A sample of 65 North American families, implicated a slightly more distal region with the marker D1S549 at 216 Mb returning a NPL score of 2.27 (McInnes et al. 2003). A recent study of families with bipolar, schizoaffective and schizophrenia provided evidence of linkage to a marker at 231 Mb with a LOD score of 3.54 (Hamshere et al. 2005). Similar results were obtained by MacGregor et al. (2004) in their investigation of 22 Scottish families, with a two-point parametric LOD score of 2.63 at 227.2 Mb (1q42.2) under a recessive inheritance model.

Various other studies have been weakly suggestive of linkage to this region. A genome-wide scan carried out on a large pedigree from Quebec, Canada produced a NPL score of 1.99 with marker D1S229 at 213.5 Mb, although parametric tests failed to confirm these data (Morissette et al. 1999). An affected sib-pair analysis conducted on an isolated population from Finland produced a NPL score of 1.8 for the marker D1S1660 at 195.3 Mb (Ekholm et al. 2003). The follow-up study of the French Canadian sample again produced tacit evidence of linkage to 1q32 with a parametric LOD score of 1.23 at 195.4 Mb (Shink et al. 2004).

The region around chromosome 1q42 has also been linked to schizophrenia (Hovatta et al. 1999; Ekelund et al. 2001; Hwu et al. 2003). More evidence implicating the region comes from a pedigree in which a chromosomal translocation (1; 11) (q42; q14.3) was found to be cosegregating with a schizophrenia spectrum, bipolar or depressive phenotype (Blackwood et al. 2001).

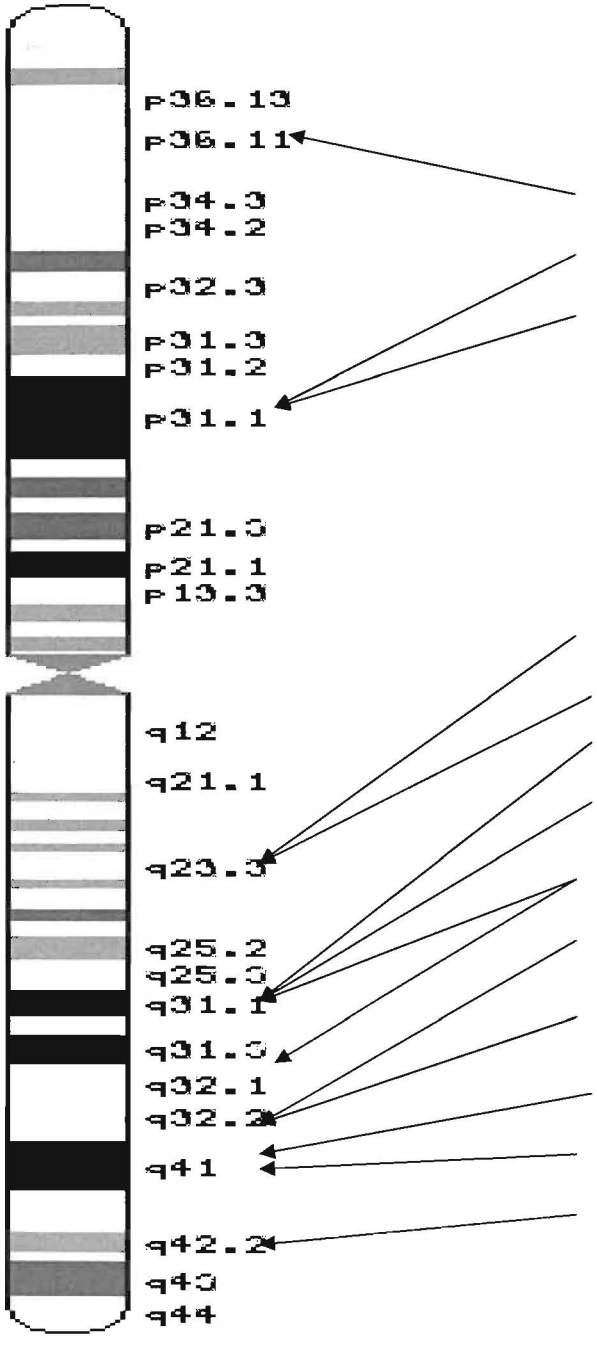
The first wave of the NIMH collaborative sample of 97 families, with 540 genotyped individuals, of whom 232 were diagnosed with BPD I, and 72 with BPD II, highlighted a different region, 1p31.1. Rice et al. (1997) reported a highest MOD (Modified Logarithm of the Odds) score of 1.94 at D1S1648 (73.1 Mb). A peak between markers D1S224 and D1S1648 in the same region was also observed in their multipoint analysis (Rice et al. 1997). A genome-wide scan carried out by Ewald et al. (2002) in two large Danish families supported these data. An affected-only parametric two-point LOD score of 2.75 at 77.4 Mb, and three point LOD score of 2.98 at 68 Mb were reported.

Another locus on chromosome 1 that has been linked to BPD in at least two studies is 1q23. Fallin et al. (2004) detected an NPL score of 3.047 at 1q23.3 (157.6 Mb) in Ashkenazi Jewish families and weaker evidence of linkage to this region was also obtained by Shink et al. (2004) in their French-Canadian sample. A linkage disequilibrium study of an Arab population with schizophrenia spectrum illness produced a statistically significant score for a marker at 148 Mb (Kohn et al. 2004).

Of particular interest to this thesis is a study by Abecasis et al. (2004) who carried out a genome-wide scan in 34 South African schizophrenia families of Afrikaner descent. Schizophrenia and BPD have been hypothesised to share susceptibility variants (Berrettini 2004; Craddock et al. 2005). No evidence of linkage to 1q32-42 was detected. A novel signal on the short arm of the chromosome was however reported by the authors.

In summary then, three regions on chromosome 1 have been linked to BPD in at least two studies: 1p31, 1q23, and a broad region, 1q32-1q42 (see Appendix B for details). While none of these loci are particularly convincing candidates, the author has chosen to investigate the area around 1q32 further on the basis of the fact that the region has been linked to BPD in four independent samples.

Figure 2.1. Linkage Data for Chromosome 1.



Study	Region	LOD	NPL
Kohn et al. (2004)	148 Mb	2.4	
Ewald et al. (2002)	68-77 Mb	2.75	11.91
Rice et al. (1997).	73.1 Mb		1.94
Fallin et al. (2004)	157.6 Mb	1.73	2.46
Shink et al. (2004)	157.6 Mb	1.14	
Shink et al. (2004)	195.4 Mb	1.23	
Ekholm et al. (2003)	195.3 Mb		1.8
This Thesis	***194-200 Mb***		
Detera-Wadleigh et al. (2004)	205.3 Mb and 206-211 Mb	2.37	1.78 and 2.67
Morissette et al. (1999)	213.5 Mb	NS	1.99
McInnes et al. (2003)	216 Mb		2.27
MacGregor et al. (2004)	227.1 Mb	2.77	

Chromosome 2.

The most exciting findings on chromosome 2 have been related to unipolar rather than bipolar depression. Zubenko et al. (2002) examined 81 families characterised by early-onset recurrent major depression with 6 markers around the cAMP response element binding protein (CREB1) on 2q33.3. LOD scores of 6.331 and 6.866 in female affected relative pairs only were reported for the markers, D2S2321 and D2S2208 which lie 451 Kb apart at 208.1 and 208.5 Mb, respectively. Gender and linkage to 2q were entered into the model as covariates. A follow-up genome-wide scan of the 81 families this time produced a non-parametric LOD score of 8.19 at D2S2321, after co-varying for gender and linkage to 2q (Zubenko et al. 2003). Another secondary linkage peak with a score of 6.72 was obtained for the marker D2S427 at 232 Mb (Zubenko et al. 2003).

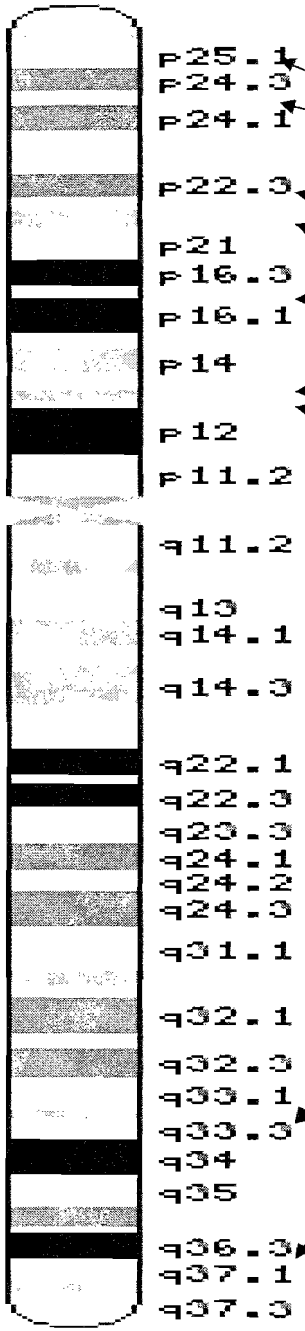
Although these results were obtained in families with a unipolar depressive phenotype, there is a substantial overlap between BPD and UPD. A review of the literature concluded that on average, relatives of BPD probands are 3-fold more likely to develop UPD than an individual from the background population (Smoller and Finn 2003). Similarly, the relatives of UPD probands are at a significantly increased risk of developing BPD (Smoller and Finn 2003).

The covariate, "linkage to 2q" was specified as a dichotomous variable by fractionating the sample into those families with LOD scores above and below zero. This methodological approach may however have artificially inflated the reported LOD scores. Nevertheless an independent group have reported an association between the 124bp allele of D2S2944 a tetranucleotide marker on 2q35 and recurrent major depression in females (Philibert et al. 2003).

Venken et al. (2005) have reported linkage to the same region in BPD pedigrees. Nine families from a geographically isolated part of northern Sweden returned a multipoint parametric LOD score of 2.16 between 198.1 and 233 Mb. A genome-screen of 154 narrowly defined and 258 broadly defined affected sibling pairs from the UK implicated a more distal region, with a maximum NPL score of 1.70 at marker D2S125 on 2q37.3 (240.9 Mb) (Bennett et al. 2002).

Other areas of chromosome 2 that have produced interesting findings include 2p13 (McInnes et al. 2003; Park et al. 2004), and 2p22 (McInnes et al. 2003; Middleton et al. 2004). Maziade et al. (2004) detected linkage to 2q14.1 and 2q22.3 with a maximum parametric LOD score of 2.76. The latter region was also implicated by Middleton et al. (2004) in a genome-wide scan using over 11 000 SNPs.

Figure 2.2. Linkage Data for Chromosome 2.



Study	Region	LOD	NPL
Detera-Wadleigh et al. (1999).	2pter and 11.6-17.4 Mb		2.0 and 1.11
McInnes et al. (2003).	36.2 Mb		1.75
Middleton et al. (2004).	38 Mb and 57 Mb		3.83 and 2.98
McInnes et al. (2003).	72.2 Mb		2.54
Park et al. (2004).	73 Mb		1.34
Maziade et al. (2004).	114.3 and 148.2 Mb	2.76	
Middleton et al. (2004).	145 Mb		3.09
Zubenko et al. (2002).	208 Mb		4.8
This Thesis	**208 Mb**		
Zubenko et al. (2003).	208 and 232 Mb		8.19 and
Venken et al. (2005).	217-230 Mb	2.35	2.93
Bennett et al. (2002).	241 Mb		1.70

Chromosome 4.

While vast areas of chromosomes 1 and 2 have been implicated in BPD, a somewhat greater consensus seems to have been reached with respect to chromosome 4. Three distinct regions on the chromosome have been suggested to harbour bipolar susceptibility genes: 4p16, 4p15-14 and 4q32-35 (see Table 3). Writing in *Nature Genetics*, Blackwood et al. (1996) reported a two-point parametric LOD score of 4.1 at D4S394 (7 Mb) in a single 120 individual strong Scottish family. A three-point analysis with surrounding markers further increased the LOD score to 4.8. Eleven other smaller families were then typed, and a two-point heterogeneity LOD score of 4.1 was again obtained at D4S394 on 4p16.1 (Blackwood et al. 1996).

Ewald et al. (1998) replicated the findings of Blackwood et al. (1996) in two Danish pedigrees with a two-point parametric LOD score of 2 at the same marker, D4S394 under a recessive model of inheritance. No statistically significant results were forthcoming however, when a dominant inheritance model was used. The same group followed up this report by typing additional markers and produced an almost identical LOD score of 1.97, again under a recessive inheritance model (Ewald et al. 2002).

More recently, Als et al. (2004) identified 11 patients with schizophrenia and 17 patients with BPD from a relatively isolated population on the Faroe Islands. The authors carried out a linkage disequilibrium analysis, based on the premise that affected Faroese individuals share a common ancestry and therefore disease genes. Various haplotypes of markers on 4p16.1 (8.3-10.3 Mb) were associated with both the schizophrenia and BPD samples, although interestingly the strongest evidence for association was provided by the combined data set (Als et al. 2004).

Visscher et al. (2005) used the samples of Blackwood et al. (1996), Ewald et al. (1998) and Ginns et al. (1998) to produce a consensus map of the region at 7-10 Mb although resolution of the region was not significantly improved, a result partially attributed to genetic or locus heterogeneity.

Asherson et al. (1998) examined 191 individuals in 24, mostly UK families with a history of both schizophrenia and bipolar spectrum conditions, and found weak

evidence for linkage to the marker D4S403 on 4p15.3 (13.4 Mb), with a two-point parametric LOD score of 1.12 under both dominant and recessive models of inheritance. One particular family however, appeared to contribute predominantly to this positive LOD score, providing evidence for genetic heterogeneity (Asherson et al. 1998).

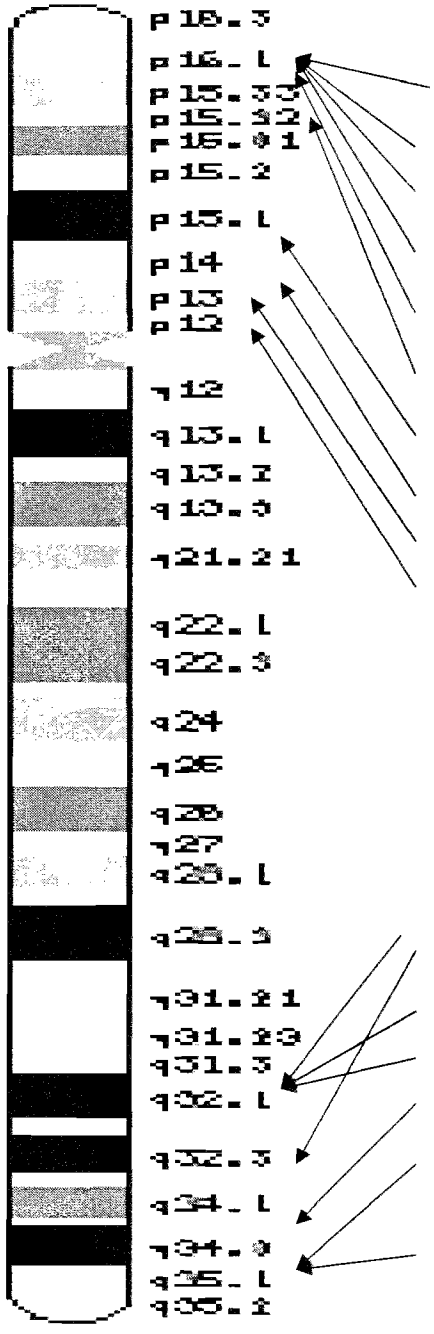
Other researchers have implicated a region on chromosome 4p that lies closer to the centromere. A multi-point sib-pair analysis returned a LOD score of 1.77 for Detera-Wadleigh et al's, (1999) collection of 22 BPD families, but more significantly, one of the largest pedigrees returned a parametric LOD score of 3.24 at 4p15.1 (35.5 Mb) under a dominant model of inheritance with 50% penetrance. Bennett et al. (2002) collected a sample of 154 narrowly defined and 258 broadly defined BPD sibling pairs from the UK and Ireland and reported a weakly positive maximum multipoint LOD score of 0.79 at 4p14 (40.2 Mb). Using the NIMH pedigree set, Willour et al. (2003) detected a linkage peak between 39.1 and 42.5 MB with the affected only analysis returning a NPL score of 2.16.

An area of chromosome 4 that has more recently attracted interest is 4q32-35. Adams et al. (1998) carried out a genome-wide scan on a large Australian pedigree, detecting a maximum two-point parametric LOD score of 2.20 at D4S1652 (190.4 Mb) and a three point LOD score of 3.19 between markers on 4q35.2. The follow-up analysis by Badenhop et al (2003), this time with a sample of 55 families, produced a two-point parametric LOD score of 3.2 at D4S1652 under a dominant model of inheritance with several other markers in the region producing LOD scores greater than 1.5. Friddle et al. (2000) reported an HLOD score of 2.11 in a region only 1-5 Mb away in a sample of 50 American families, a result replicated by McInnes et al. (2003) who obtained a non-parametric LOD score of 2.80 at 188.4 Mb.

A region 10 Mb towards the centromere at 4q34.3 (179 Mb) produced a LOD score of 1.46 under a recessive model of inheritance in an isolated population group from northern Sweden (Venken et al. 2005). Ekholm et al. (2003), McInnes et al. (2003) and Willour et al. (2003) implicated an even more centromeric region with the same marker (D4S1629) on 4q32.1 (158.7 Mb), achieving LOD scores of 2.6, 2.8, and 2.49, respectively. The latter two authors made use of an overlapping sample of the NIMH

dataset. Ekholm et al.'s (2003) finding was augmented by a three-point analysis which returned a maximum LOD score of 3.6.

In summary, an area between 7 and 13 Mb on the short arm of chromosome 4 has been linked with BPD in at least four independent samples with a maximum LOD score of 4.8 in Blackwood et al's (1996) study. The region between 35 and 42 Mb has been highlighted in three independent samples although the LOD scores are weaker, peaking at 2.16. Finally, a vast region between 158 and 190 Mb on the long arm of the chromosome has generated interest with statistically significant results obtained in four independent samples, with a maximum LOD score of 3.6. Given the size of this area, and the weakness of the linkage peaks detected between 35 and 42 Mb, the region around 4p16.1 will be concentrated on in this thesis (see section 2.5).



Study	Region	LOD	NPL
Blackwood et al. (1996).	7 Mb	4.1	
Ewald et al. (1998).	7.1 Mb	2.0	
Ewald et al. (2003).	7.1 Mb	1.97	
This Thesis	**7-10 Mb**		
Als et al. (2004).	8.3-10.3 Mb	NA	NA
Asherson et al. (1998).	13.4 Mb	1.12	
Detera-Wadleigh et al. (1997).	26.9-27.3 Mb		Not given
Detera-Wadleigh et al. (1999).	31-35.5 Mb	3.24 (one pedigree)	1.77
Bennett et al. (2002).	40.2 Mb		0.79
Willour et al. (2003).	39.1-42.5 Mb		2.16
McInnes et al. (2003).	158.7 and 188.4 Mb		2.80 and 2.43
Ekholm et al. (2003)	158.7 Mb	2.6	
Willour et al. (2003).	158.7 Mb		2.49
Venken et al. (2005)	179 Mb	1.46	
Friddle et al. (2000).	185.5-189.4 Mb	2.11	
Badenhop et al. (2003)	190.4 Mb	3.19	
Adams et al. (1998).	190.4 Mb	2.20	

Chromosome 10.

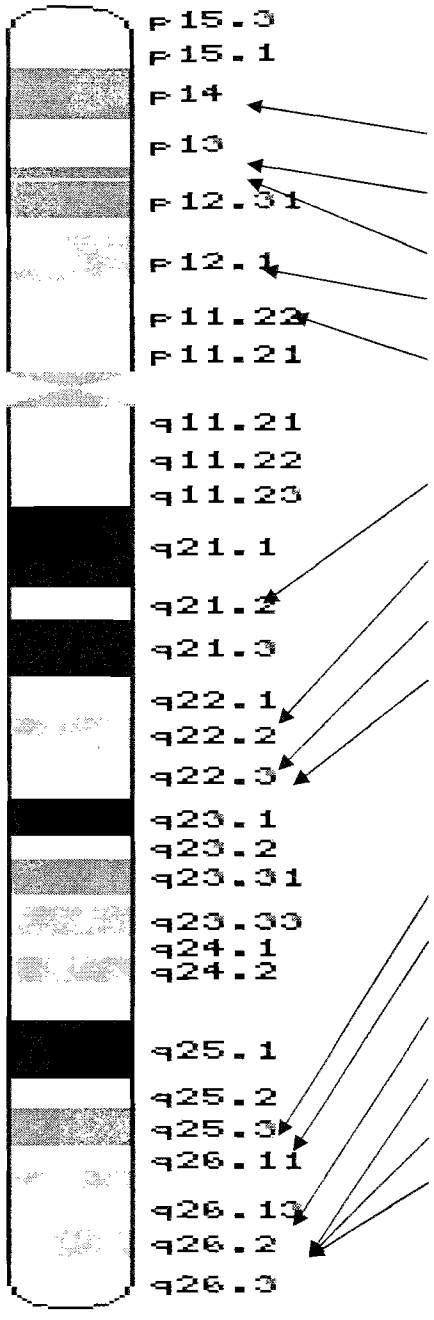
Two major regions of chromosome 10 have been implicated by linkage studies; 10p12-13 and 10q26, with the latter chromosomal band appearing to be the stronger candidate. Detera-Wadleigh et al. (1999) first reported weak evidence of linkage to 10q25.3 in a sample of 22 families, with a pair-wise parametric LOD score of 1.69 under a recessive model of inheritance. A slightly more distal region on 10q26 was more clearly implicated in an independent sample of predominantly German families (Cichon et al. 2001). A two-point LOD score of 2.86 at marker D10S217 (129.4 Mb) under a dominant model of inheritance, and an NPL score of 3.12 at markers D10S587, D10S1723 and D10S216 implicated a 15cM candidate region flanked by markers D10S1483 and D10S217 (Cichon et al. 2001).

A genome-wide survey of 20 US families produced a two-point parametric LOD score of 2.27 under a recessive model of inheritance at 129.1 Mb (Kelsoe et al. 2001), while the same approach in two large Danish pedigrees further supported this result with an NPL score of 2.60 at an almost identical region on 10q26.2 (129.4 Mb) (Ewald et al. 2002). McInnes et al. (2003) reported a genome-wide non-parametric LOD score of 2.12, peaking at the same marker (D10S1223) highlighted by Kelsoe et al. (2001). Park et al. (2004) carried out a genome-wide scan with a mixture of North American and Israeli pedigrees, detecting weak linkage to 10q26.3 (132.4 Mb) as evinced by multipoint affected sib-pair analysis NPL of 1.18. No significant data were forthcoming however when a two-point parametric analysis was run on the data. The genetic variant or variants located in this region may also be a risk factor for unipolar depression. Zubenko et al. (2003) obtained a maximum multipoint LOD score of 5.39 at marker D10S1656 (126.1 Mb) in a sample of patients with major depression.

A study of 97 NIMH families produced non-parametric LOD scores of 2.5 and 1.5 for markers at 19.5 and 30.5 Mb (10p12-11), respectively (Foroud et al. 2000). More recently, Bennet et al. (2002) carried out a genome-wide screen in 151 UK nuclear families, producing weak multipoint scores of 0.96 and 0.98 at 10p14-12. The same group then genotyped additional markers of interest in the same region, this time at a density of 5cM, and obtained a two-point LOD score of 3.18 at 26.6 Mb (Lambert et al. 2005). Maziada et al. (2004) recruited 21 multigenerational families from Quebec

and also implicated this area of chromosome 10, with a MOD score of 2.66 at 10p13-12 (16.6-23.6 Mb).

Figure 2.4: Linkage Data for Chromosome 10.



Study	Position	LOD	NPL
Zubenko et al. (2003).	9.3 Mb		4.70
Bennett et al. (2002).	10.6-18.8 Mb		0.98
Maziade et al. (2004).	16.6-23.6 Mb	2.66	
Lambert et al. (2005).	26.6 Mb		3.18
Foroud et al. (2000).	19.5 and 30.5 Mb		2.5 and 1.5
Shink et al. (2004).	61.1 Mb	1.45	
Rice et al. (1997).	75 Mb	3.47	
Park et al. (2004).	80.4 Mb	1.60	1.51
This Thesis	**92.5 Mb**		
Detera-Wadleigh et al. (1999).	118.6 Mb	1.69	
Cichon et al. (2001).	125.2 – 129.4 Mb	2.86 and 1.48	Z=3.12
Zubenko et al. (2003).	126.1 Mb		5.3
Kelsoe et al. (2001).	129.1 Mb	2.27	
McInnes et al. (2003).	129.1 Mb		2.12
Ewald et al. (2003).	129.4 Mb	2.17	

Chromosome 12.

Arguably the best replicated finding in the BPD genetics literature is the possible linkage to 12q23-24. Craddock et al. (1994) originally reported a pedigree in which five individuals presented with both affective disorders and Darier's disease, a dermatological condition. Although the Darier's disease gene (*ATP2A2*) could theoretically predispose to affective illness, the most likely explanation is that the genetic variant involved in affective illness is in linkage disequilibrium with the Darier's disease locus (Craddock et al. 1994). Jacobsen et al. (2001) performed mutational screening of the *ATP2A2* coding, promoter and 3' untranslated regions, in a large sample of bipolar patients but were unable to detect any pertinent changes, lending credence to their hypothesis. Craddock et al.'s (1994) pedigree was consistent with an autosomal dominant form of transmission of affective illness, suggestive of a gene of major effect. A follow-up study by Jones et al. (2002) confirmed linkage to *ATP2A2*, the Darier's disease locus, and in a sample of 45 families, the same group reported a heterogeneity LOD score of 3.52 at *PLA2*, a marker gene in the region.

This chromosomal area has since been intensively investigated. In fact, at least 18 studies have linked markers in a 40-50 Mb region on 12q21-24 to the presence of the bipolar phenotype (see Table 5, Appendix B). The most proximal of these findings were weak effects detected by Bennett et al. (2002) and Venken et al. (2005) with the marker D12S326 on 12q21.2 (76.4 Mb). In their initial genome scan of the NIMH cohort, Rice et al. (1997) produced a MOD score of 1.89 at 12q21.3 (83.4 Mb) using a broad affection status model. The majority of linkage analyses, however, have emphasised a narrower region on 12q23-24.

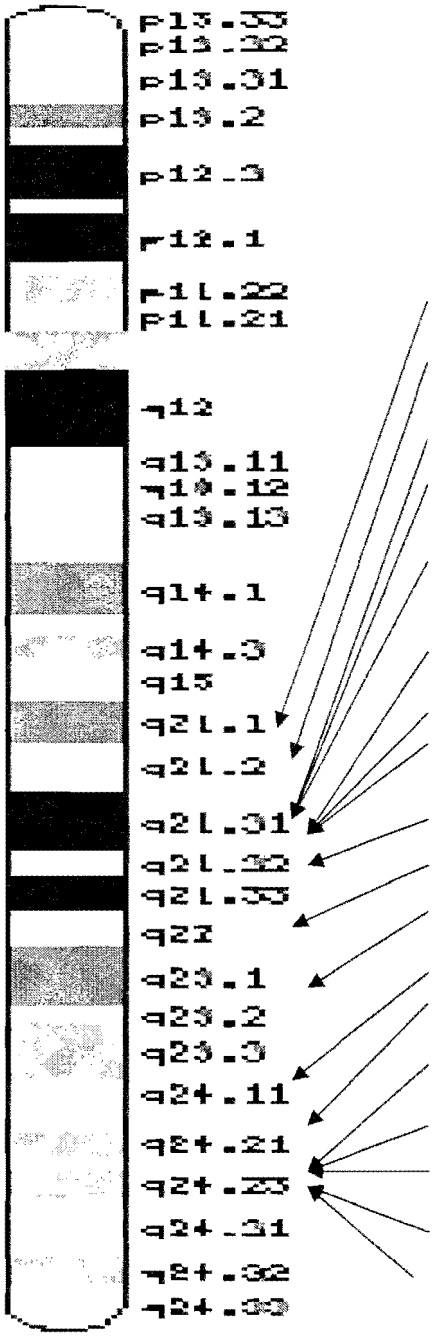
Abkevich et al. (2003) carried out a genome-wide scan in 110 Mormon families with a history of major depression. A multipoint parametric analysis under a dominant inheritance model returned an HLOD score of 4.6 at 12q23.1 (97 Mb). Turecki et al. (2001) reported a much weaker LOD score (1.27) at the same marker in a cohort of 31 multiplex families in which the probands were efficaciously treated with lithium.

McInnes et al. (2003) detected a linkage peak only 2 Mb distal from the Abkevich et al. (2003) and Turecki et al. (2001) results in their sample of 65 American BPD pedigrees. A NPL score of 2.08 was obtained under a broad affection status model. This report was echoed by the more recent finding of Maziade et al. (2004) who reported a MOD score of 3.53 at 101.4 Mb under a recessive model of inheritance. An almost identical region was implicated by Venken et al. (2005) at 102.7 Mb in a sample of Swedish origin. Finally, Green et al. (2005) examined their two families with Darier's disease using 45 markers across the 12q23-24 chromosomal region in an attempt to further delineate the region of interest. Multipoint parametric analysis pinpointed an area between 106.7 Mb (12q23.3) and 113.3 Mb (12q24.2) with a LOD score of 4.47.

Complementary evidence supporting the role of this region in affective disorders is derived from the report of Fullerton et al. (2003) who identified a putative quantitative trait locus (QTL) influencing variation in the personality trait, neuroticism at 12q23.1 (98 Mb). Kendler et al. (1993) argued that approximately half of the genetic liability to depression is related to levels of neuroticism. This area of the chromosome has also been implicated in schizophrenia with Brzustowicz et al. (2000) and Wilcox et al. (2002) reporting LOD scores of 2.6 and 3.0, respectively, at 12q23.1 (approximately 100 Mb).

Nevertheless, a more telomeric region has also been suggested to harbour BPD susceptibility genes. Shink et al. (2004) obtained a two-point parametric LOD score of 3.35 and an ASP derived NPL score of 5.05 for marker D12S378 at 123.2 Mb in their French Canadian sample. Bailer et al. (2002) used a multipoint analysis to identify a linkage peak on 12q24.3 (124.3-128.9 Mb) with a maximum NPL score of 2.43. A third independent sample also implicated this region. Ewald et al's (2003) study of two large Danish pedigrees returned a two-point parametric LOD score of 3.42, and an NPL score of 2.29 at 124.3 Mb. This region was further highlighted by Degn et al. (2001) who reported increased haplotype sharing between 124.4 and 126.7 Mb on 12q24.3 among bipolar patients from the isolated Faroese population.

Figure 2.5. Linkage Data for Chromosome 12.



Study	Position	LOD	NPL
Bennett et al. (2002).	76.4 Mb		0.77
Rice et al. (1997).	83.4 Mb	1.89	
Turecki et al. (2001).	97 Mb	1.27	
Abkevich et al. (2003).	97 Mb	4.6	
Craddock et al. (1994).	95-125 Mb	2.11	
Dawson et al. (1995).	95-125 Mb	3.52	
Morissette et al. (1999).	95-125 Mb	1.61	3.92
Venken et al. (2005).	98 – 102.7 Mb	1.65	
McInnes et al. (2003).	99 Mb		2.08
Ekholm et al. (2003).	100 Mb		2.0
Maziade et al. (2004).	99.4-101.4 Mb	3.53	
Green et al. (2005).	106.7-113.3 Mb	4.47	
Detera-Wadleigh et al. (1999).	114.5 Mb		1.24
This Thesis	**117-123 Mb**		
Shink et al. (2004).	123.2 Mb	3.35	5.05
Ewald et al. (2003).	124.3 Mb	3.42	
Curtis et al. (2003).	124.4 Mb	2.9	
Ewald et al. (1998).	125 Mb	3.37	
Bailer et al. (2002).	124.3 - 128.9 Mb	1.24	Z=2.43

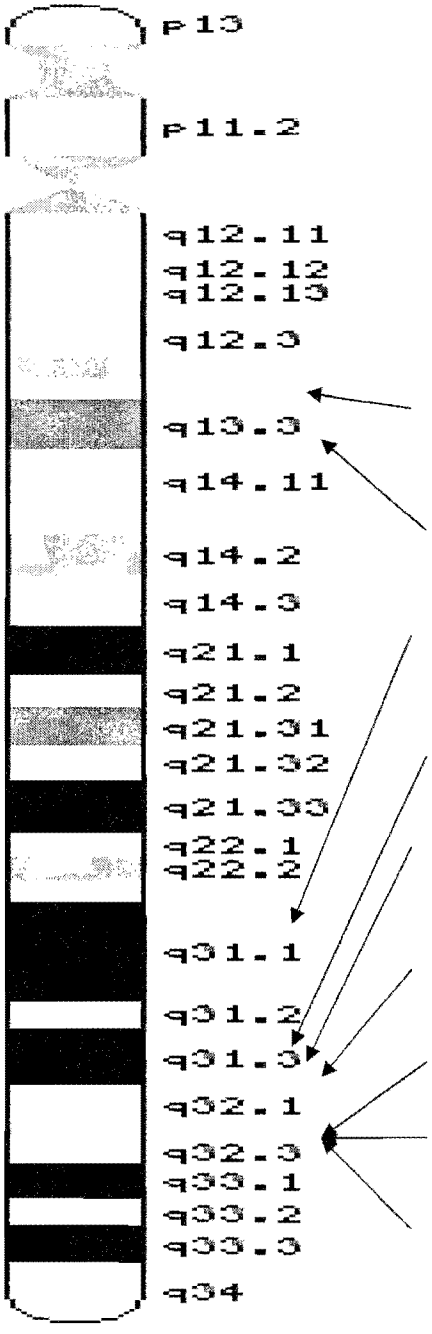
Chromosome 13.

Detera-Wadleigh et al. (1999) first implicated the region around 13q32 in a genome-wide scan of 22 pedigrees. A parametric LOD score of 2.06 under a recessive model of inheritance, and a non-parametric score of 3.4 were detected around the marker D13S779 at 100.3 Mb. A fine mapping follow-up study by the same group supported the original finding, with a multipoint non-parametric analysis returning a LOD score of 3.25 in the identical region of the chromosome.

Kelsoe et al. (2001) replicated these results in another North American cohort. The authors reported two-point LOD scores of 2.4 and 2.34 for the markers D13S154 and D13S796 at 95 and 106.7 Mb, respectively. Other salient results have been published by Shaw et al. (2003) and Park et al. (2004) with significant parametric and non-parametric scores obtained for markers between 95.1 and 100.3 Mb. Using the transmission disequilibrium test, Ferraren et al. (2005) returned results of nominal significance between 100.3 and 102.3 Mb. The other important finding in a more centromeric region was a NPL score of 3.56 at 13q31.1 (81 Mb) by Potash et al. (2003).

Another area of chromosome 13 that has been highlighted by two separate groups is 13q13. McInnes et al. (2003) obtained a non-parametric multipoint LOD score of 2.53 at 32.9 Mb, while Maziade et al. (2004) reported a multipoint parametric score of 2.27 at 37.5 Mb. These findings do not however appear to be as promising as the 13q32-33 region which has also been linked to schizophrenia. Perhaps the most impressive results are those of Blouin et al. (1998) who detected a NPL score of 4.18 at 13q32 in a sample of 54 families with schizophrenia. Abecasis et al. (2004) reported a multipoint non-parametric LOD score of 2.23 slightly distal to the Blouin et al. (1998) finding in a sample of 34 Afrikaner families.

Figure 2.6. Linkage Data for Chromosome 13.



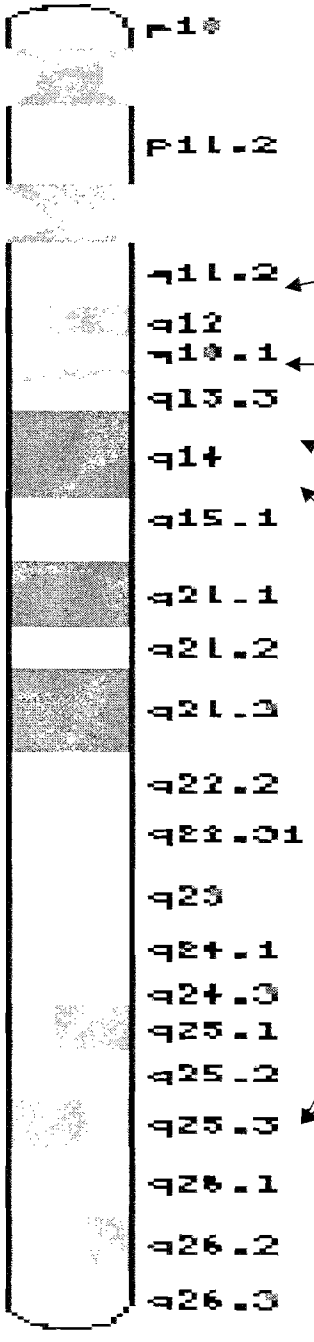
Study	Position	LOD	NPL
McInnes et al. (2003).	32.9 Mb		2.53
Maziade et al. (2004)	37.5 - 42.1 Mb	2.27	
Potash et al. (2003).	81.6 Mb		3.56
Shaw et al. (2003).	95.1 – 103 Mb	2.29 and 3.39	
Kelsoe et al. (2001).	95 – 106.7 Mb	2.4 and 2.34	
This Thesis	**97.8 Mb**		
Park et al. (2004).	100.3 Mb	1.96	1.55
Detera-Wadleigh et al. (1999).	100.3 Mb	2.06	3.4
Liu et al. (2001)	100.3 Mb		3.25

Chromosome 15.

Turecki et al. (2001) first reported significant linkage to 15q14 (32.8 Mb) in BPD families where the probands demonstrated a good response to lithium. A parametric LOD score of 3.43 was obtained under a recessive model of inheritance with a broadly defined phenotype. Zubenko et al. (2003), in their study of recurrent unipolar depression detected weak evidence of linkage 4 Mb away from the Turecki et al. (2001) finding (36.8 Mb), with a maximum multipoint NPL score of 1.96 at marker D15S1012. The data of Maziade et al. (2004), derived from a sample of pedigrees from eastern Quebec, pointed to a region closer to the centromere with a parametric multipoint LOD score of 4.59 at 15q12 (23.2 Mb). Of interest is the fact that this region has also been implicated in schizophrenia with Kauffman et al. (1998) and Stober et al. (2000) describing LOD scores of 1.96 (NPL) at 15q11 and 2.75 at 15q15, respectively. The study of Freedman et al. (1997) deserves a special mention as a LOD score of 5.3 was obtained at D15S128 in schizophrenic families by using a P50 gating deficit as a neurophysiological marker for the disorder. Faraone et al. (2004) identified a region at 50 Mb on 15q that putatively influences the age of onset of mania. Whether this genetic factor also contributes to the risk of developing the disorder is a matter of debate.

Another region highlighted by linkage studies is 15q26. In a sample of 21 Quebec pedigrees, Maziade et al. (2004) detected a linkage signal at 95.8 Mb (Z=4.55) with a diagnostic model including both schizophrenia and BPD. A mixed sample of US and Israeli families with a history of BPD characterised by psychosis yielded a two-point LOD score of 1.96 and a multipoint ASP non-parametric score of 2.62 at 90.3 Mb (Park et al. 2004).

Figure 2.7. Linkage Data for Chromosome 15.



Study	Region	LOD	NPL
Maziade et al. (2004).	23.2 Mb	4.59	
This Thesis	**23.2-27.8 Mb**		
Turecki et al. (2001).	32.8 Mb	3.43	
Zubenko et al. (2003).	36.8 Mb		1.96
Park et al. (2004).	90.3 Mb	1.96	2.62
Maziade et al. (2004).	95.8 Mb	Z=4.55	

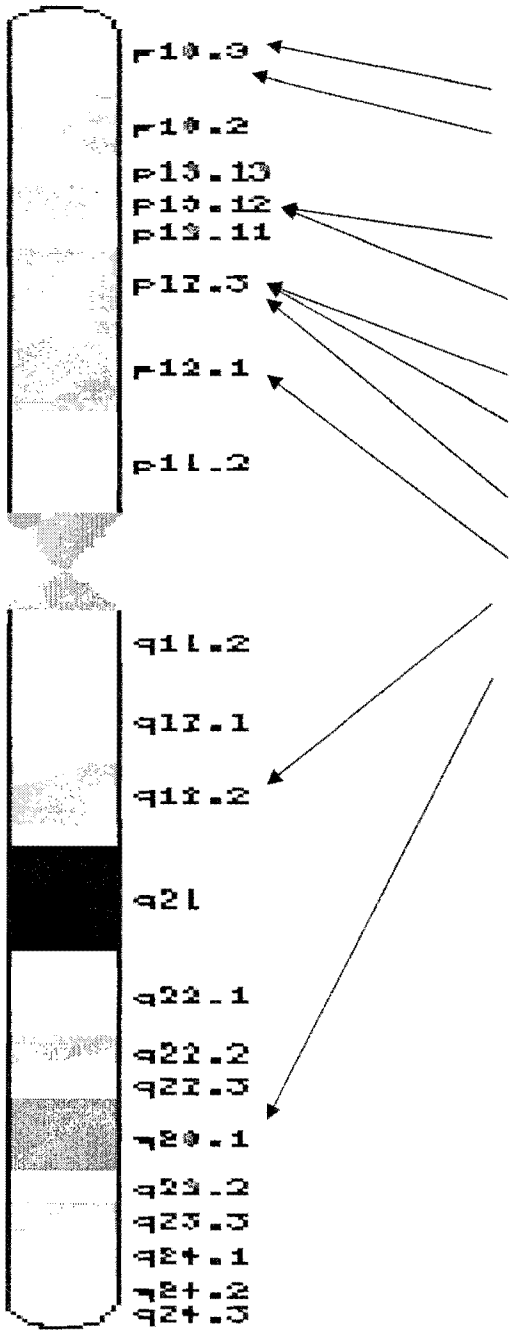
Chromosome 16.

Ewald et al. (1995) obtained a LOD score of 2.5 at D16S510 under a recessive model of inheritance in two large Danish pedigrees. The follow-up study using the same families again produced a significant parametric LOD score of 2.23 at 16p13.3 (5.70 Mb) under a recessive model of inheritance. Non-parametric testing was however, non-significant (Ewald et al. 2002).

In a Costa Rican family, McInnes et al. (1996) suggested linkage to D16S521 - approximately 10cM distal to the results of Ewald and colleagues, and as part of a genome-wide linkage analysis, Edenberg et al. (1997) found evidence of linkage to a marker on 16p13.1 (13.6 Mb) using a non-parametric sib pair analysis in the NIMH cohort. The second wave investigation by the same group confirmed the weak effect, with a maximum non-parametric LOD score of 1.7 at D16S749 on 16p12 under a broad diagnostic model (Foroud et al. 2000). A replication analysis in another sample of 56 NIMH families again provided evidence for linkage to 16p13.1 (13.6 Mb) in the guise of affected relative pair and affected sib pair analyses with LOD scores of 2.1 and 2.3, respectively (Dick et al. 2002).

More positive results were once again obtained from the French-Canadian sample of Maziade et al. (2004) with a multipoint analysis generating a modified LOD score of 4.05 between the markers D16S410 and D16S403 on 16p12.3 (19-19.3 Mb). Middleton et al. (2004) used a high density single-nucleotide polymorphism (SNP) assay in 25 Portuguese families and detected a NPL score of 2.89 in the same region (20 Mb). In a genome-wide scan conducted by Ekholm et al. (2003), a linkage peak with a parametric LOD score of 2.5 was detected even closer to the centromere at 16p12.1 (26 Mb).

Maziade et al. (2004) detected a linkage signal on chromosome 16q12.2 with a parametric LOD score of 2.19 under a dominant model of inheritance. Bailer et al. (2002) also implicated the long arm of the chromosome in a linkage analysis of 5 schizophrenia and 3 BPD Austrian families. Their findings were however, decidedly mixed with a parametric LOD score of -0.41 but a marginally significant NPL score of 1.76 at D16S289.



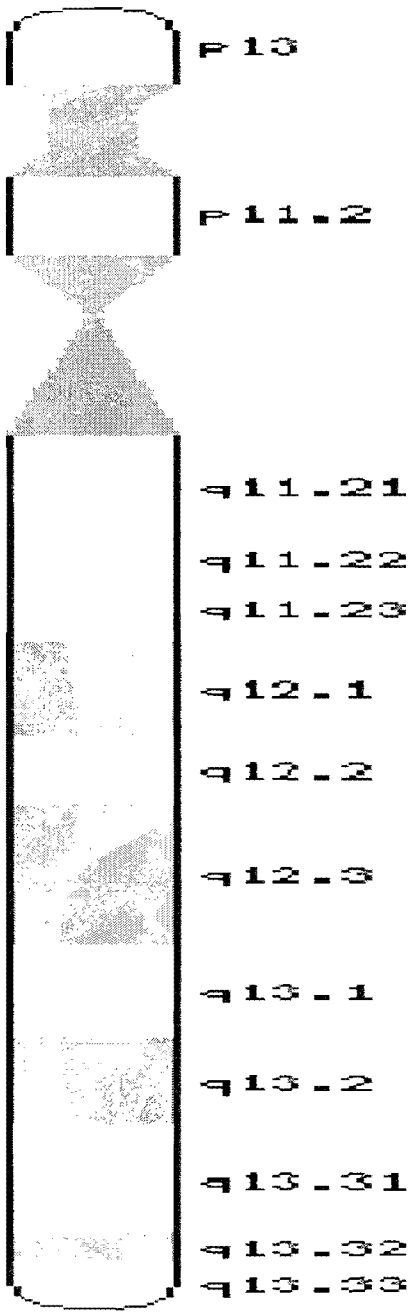
Study	Region	LOD	NPL
This Thesis	**1-7 Mb**		
Ewald et al. (2002).	5.70 Mb	2.23	
Edenburg et al. (1997).	13.6 Mb		1.9
Dick et al. (2002).	13.6 Mb		2.13
Foroud et al. (2000)	19.7 Mb		1.7
Maziade et al. (2004).	19-19.3 Mb	4.05	
Middleton et al. (2004).	20 Mb		2.89
Ekholm et al. (2001).	26 Mb	2.5	
Maziade et al. (2004).	53.3 Mb	2.19	
Bailer et al. (2002)	76.2 Mb	- 0.41	1.76

Chromosome 22.

A first pass analysis of 97 NIMH families yielded a LOD score of 2.46 at 24.1 Mb (22q11.2) as evinced by multipoint sib-pair methods (Edenburg et al. 1997). Since then, the region around chromosome 22q12 has been linked to BPD in a number of independent samples (Detera-Wadleigh et al. 1999; Kelsoe et al. 2001; Potash et al. 2003, and Shink et al. 2004). The most promising findings were those of Kelsoe et al. (2001), who carried out a genome scan of 20 families and reported a parametric LOD score of 3.8 at 34.7 Mb (22q12.3), and Potash et al. (2003) who achieved a non-parametric LOD score of 3.06 at 34.6 Mb in 10 families with a history of psychotic BPD. Hamshere et al. (2005) reported evidence for linkage to marker D22S240 at 16 Mb (22q11.1) with a LOD score of 1.96 in a sample of families with schizoaffective disorder. Shink et al. (2004) obtained weaker parametric and non-parametric LOD scores of 1.21 and 1.63, respectively at 43.6 Mb (22q13.3).

A number of studies have also demonstrated that adults with 22q11 deletions have a higher than normal risk of developing schizophrenia (Bassett et al. 1998), with Murphy et al. (1999) estimating the risk to be approximately 24%.

Figure 2.9. Linkage Data for Chromosome 22.



Study	Position	LOD	NPL
Hamshere et al. (2005)	16 Mb		1.96
Turecki et al. (2001).	16.2 Mb	1.91	NS
Edenberg et al. (1997).	24.1 Mb		2.46
This Thesis	**24.3-24.5 Mb**		
Detera-Wadleigh et al. (1999).	27.1 Mb		2.1
Potash et al. (2003).	34.6 Mb		3.32 and 3.06
Kelsoe et al. (2001).	34.7-34.8 Mb	3.84 and 2.89	
Shink et al. (2004).	43.6 Mb	1.21	1.63

Three meta-analyses of published linkage data have provided modest evidence supporting linkage between some of the regions discussed above and BPD. Badner and Gershon (2002) pooled data from all available genome-wide scans and reported highest peaks on 13q and 22q. No region achieved genome-wide significance in the analysis of Segurado et al. (2003) with strongest peaks at 9p22-21, 10q11-22, and 14q24-32. Most recently, the 6q and 8q regions were most strongly significant under a narrow and broad model, respectively (McQueen et al. 2005). Nevertheless, given potential difficulties with genetic heterogeneity (see below), it is unclear how accurate the results of meta-analyses will turn out to be.

While some researchers remain adamant that linkage analysis is the best strategy for identifying bipolar susceptibility genes, others favour an association study based approach. In the following section some of the genes that are candidates for playing a role in increasing susceptibility to BPD are examined.

2.2. Association Analysis.

"What is rare is not that someone should be in despair; no, what is rare, the great rarity, is that one should truly not be in despair". Soren Kierkegaard.

By April, 2004, more than 1000 original reports on the genetics of BPD had been published (DePaulo 2004) and it was thus decided that a reasonable point of departure was to examine the conclusions of review articles published in the last five years that have addressed this topic. These data are shown in Table 2.1, below.

Table 2.1: Reviews of Genetic Association Studies of BPD.

Study.	**Most Promising Candidate Genes.**
Potash and DePaulo (2000).	*SERT; MAO-A; COMT*
Stoltenberg and Burmeister (2000).	*SERT; COMT*
Craddock and Jones (2001).	*SERT; COMT*
Craddock et al. (2001).	*SERT; COMT; MAO-A*
Johansson et al. (2001).	*SERT; COMT*
Jones and Craddock (2001).	*SERT; 5-HT2A; TH; COMT; MAO-A.*
Kato et al. (2001).	*SERT; MAO-A; COMT*
Baron (2002).	*None reported.*
Inoue and Lupski (2003).	*COMT*
Kennedy et al. (2003).	*G72/G30; BDNF*
Schulze and McMahon (2003).	*SERT; COMT*
Tsuang et al. (2003).	*SERT; DRD2; DRD4; 5-HT2A*
Craddock et al. (2005).	*BDNF; G72/G30; DISC1; NRG1; COMT.*
Maier et al. (2005).	*G72/G30; COMT; BDNF; PIPK2A.*
Payne et al. (2005).	*G72/G30; BDNF; SERT; MAO-A; COMT*

5-HT2A=Serotonin Receptor 2A; *BDNF*=Brain-Derived Neurotrophic Factor; *COMT*=Catechol-O-Methyl-Transferase; DISC1=Disrupted in Schizophrenia 1; *DRD2*=Dopamine 2 Receptor; *DRD4*=Dopamine 4 Receptor; *MAO-A*=Monoamine Oxidase A; *NRG1*=Neuregulin 1; *SERT*=Serotonin Transporter; *TH*=Tyrosine Hydroxylase.

In the following sections some of the studies that have implicated the above candidate genes in the aetiology of BPD are discussed.

The Serotonin Transporter (SERT).

SERT encodes a protein that is responsible for the reuptake of serotonin in the synaptic clefts of the neurones. It is coded for by a gene on chromosome 17q11.1-q12 that is 31kb in length and is made up of 14 exons (Lesch and Mossner 1998). Two *SERT* polymorphisms have been used in association analyses. The variant that has captured most of the attention is a 44bp insertion/deletion polymorphism (5-HTTLPR) in the promoter region of the gene, which is about 1kb upstream from transcription initiation site (Lesch and Mossner 1998). The 5-HTTLPR polymorphism gives rise to two common alleles – the *long* (16 repeat insertion) and the *short* (14 repeat deletion) - which display functional differences (Lesch et al. 1996). The *short* or deletion allele possesses lower transcriptional activity leading to a relative reduction in mRNA levels, serotonin binding, and reuptake (Lesch et al. 1996; Heils et al. 1997; Little et al. 1998).

The Weinberger group have demonstrated that individuals with one or two copies of the *short* allele show greater amygdaloid activity as evinced by functional magnetic resonance imaging (fMRI) when exposed to fearful stimuli than individuals who are homozygous for the *long* allele (Hariri et al. 2002). A follow-up study indicated that the 5-HTTLPR variant impacts the behaviour of an amygdala-cingulate negative feedback circuit that extinguishes negative affect (Pezawas et al. 2005). The activity of the aforementioned feedback system was relatively uncoupled to the processing of fearful stimuli in the *short* allele carriers (Pezawas et al. 2005). Given the putative role of the *SERT* in modulating normal emotional activity, genetic variation in the activity of this enzyme may constitute a susceptibility mechanism for affective disorders.

In fact, because of the effect of antidepressants on serotonin activity, genetic studies of *SERT* variants and their relationship to affective disorders stretch back almost a decade. Collier et al. (1996) were the first group to report a significant association between the *short* allele of the 5-HTTLPR and BPD in a sample of 304 bipolar patients and 281 Caucasian controls. Rotondo et al. (2002) and Hauser et al. (2003) replicated this finding in Italian and Polish samples, respectively. In a family-based analysis, Mynett-Johnson et al. (2000) reported over-transmission of the *short* allele to

affected individuals in bipolar pedigrees. Nevertheless, the majority of studies have returned negative findings (Rees et al. 1997; Kunugi et al. 1997; Bellivier et al. 1998; Furlong et al. 1998; Piccardi et al. 2002).

Meta-analytic work carried out by Anguelova et al. (2003) returned a significant odds ratio (OR) of 1.14 but sensitivity analysis suggested that the association with the *short* allele could be attributed to the effect of one large study: that of Collier et al. (1996). Lotrich and Pollock, (2004) performed a meta-analysis using random-effects modelling to control for inter-study variation. Among unipolar depressives, the *short/short* genotype was found to be a risk factor, with an OR of 1.16. A similar trend was observed among bipolar individuals although the results fell short of statistical significance (Lotrich and Pollock, 2004). The most recent meta-analysis supports the hypothesis that the presence of the *short* allele or *short/short* genotype of the 5-HTTLPR variant is a small risk factor for BPD with a recorded OR of 1.12 (Cho et al. 2005).

The 5-HTTLPR *short* allele has also been associated with a number of phenotypes that are indirectly related to mood disorders. It has been suggested that the *short* allele is a risk factor for suicide (Bondy et al. 2000; Courtet et al. 2001; Baca-Garcia et al. 2002; Joiner et al. 2002; Bayle et al. 2003) although again the majority of studies return negative results (for example Geijer et al. 2000; Ho et al. 2000; Courtet et al. 2003; Pooley et al. 2003).

The other notable polymorphism is a variable number tandem repeat (VNTR) in the second intron of the *SERT* gene which gives rise to three alleles denoted STin2.9 (9 repeats), STin2.10 (10 repeats) and STin2.12 (12 repeats). This VNTR appears to be in moderate linkage disequilibrium with the 5-HTTLPR variant: D' is estimated to be approximately 0.5 (Fan and Sklar, 2005). Evidence exists that this VNTR acts as a transcriptional regulator with the 12 repeat allele transcribed more efficiently than the shorter variants (MacKenzie and Quinn 1999; Fiskerstrand et al. 1999).

Ogilvie et al. (1996) first reported an association between the STin2.9 allele and major depression in a small sample of 39 unipolar patients and 193 controls. A slew of publications have since followed in bipolar populations. Most of these studies have

not achieved statistical significance (Battersby et al. 1996; Bellivier et al. 1998; Liu et al. 1999) although there have been some notable exceptions. Collier et al. (1996) found an association between the 12 repeat allele and bipolar illness in a substantial sample of 380 cases and 374 controls. This finding was replicated in smaller samples of participants (Rees et al. 1997; Heiden et al. 2000). In the Japanese population, Kunugi et al. (1997) reported an excess of the STin2.12 allele in a sample of 284 bipolar patients compared to a group of 424 controls.

An early meta-analysis using data from studies of Caucasian subjects failed to reveal any statistically significant association between the VNTR polymorphism and affective disorders (Furlong et al. 1998) but this is contradicted by more recent data. Anguelova et al. (2003) carried out a meta-analysis on different types of affective disorders, reporting a non-significant odds ratio (OR) of 1.03 in unipolar depression, but a significant association between the 12 repeat allele and BPD, with an OR of 1.18. Cho et al. (2005) meta-analysed 16 studies comprising 1764 cases and 2703 controls and again concluded that the STin2.12 allele increases the risk of developing BPD (OR = 1.12). Of additional interest is a meta-analytical study of schizophrenia pooling 2177 cases and 2369 controls, and finding a significant relationship between the STin2.12 allele and the disorder, with an OR of 1.24 (Fan and Sklar 2005).

One of the possible factors leading to the contradictory pattern of findings in the literature is the mediating role of environmental adversity. Recent studies have demonstrated that the 5-HTTLPR variant interacts with life stress (Caspi et al. 2003) and childhood maltreatment (Kaufman et al. 2004) to influence susceptibility to depressive symptomatology later in life. These findings will be discussed in more detail in Chapter 5.

Catechol-O-Methyl-Transferase (COMT).

An evolutionary recent functional single nucleotide (SNP) polymorphism of the *COMT* gene (Val158Met) located on chromosome 22q11 that results in the substitution of valine with methionine at codon 158 of the protein sequence has been described. The methionine (*met*) allele produces an enzyme that is unstable at body

temperature and has only a quarter the activity of the valine (*val*) containing polypeptide (Egan et al. 2001).

The *COMT* gene is a strong candidate for involvement in schizophrenia. At least four studies have demonstrated an association between schizophrenia and SNP haplotypes in and around the gene (Shifman et al. 2002; Chen et al. 2004; Saunders et al. 2004; Handoko et al. 2004) although Craddock et al. (2005) are sceptical that the *COMT* gene itself exerts an effect because of a weaker association between the functional Val158Met variant and the disorder.

Association studies of the Val158Met polymorphism and BPD have generally been disappointing with negative results predominating (Gutierrez et al. 1997; Kunugi et al. 1997; Lachman et al. 1997; Cusin 2002). Li et al. (1997) reported an association between the low activity *met* allele/genotype and BPD in a small sample of 93 Chinese patients and 98 controls and the same allele was implicated in unipolar depression in a Japanese cohort (Ohara et al. 1998). Most studies that have returned significant results, however, have made use of specific bipolar or unipolar phenotypes.

Kirov et al. (1998) reported that the *met* allele was associated with the rapid-cycling, but not the classical form of BPD and this was confirmed by Papolos et al. (1998) who also suggested an association with ultradian rapid cycling. On the basis of a 62-strong sample of patients, Rotondo et al. (2002) argued that the *met* allele is a risk factor for BPD without panic disorder. Finally, a recent multi-centre collaboration suggested an association between the high activity *val* allele and early-onset unipolar depression although the same relationship could not be detected in their bipolar cohort (Massat et al. 2005).

Shifman et al. (2004) compared allele and haplotype frequencies in 217 bipolar patients and approximately 4000 healthy controls from the Ashkenazi Jewish population. Although the Val158Met variant was not associated with BPD, again both a two-SNP and a three-SNP haplotype was over-represented in the bipolar population (OR=1.3). The implication here is that another functional variant within or near the *COMT* gene is contributing to the risk for psychiatric illness.

Brain-Derived Neurotrophic Factor.

The *BDNF* gene is located on chromosome 11p13 and is composed of 5 or more exons, each with its own promoter region allowing for differential mRNA splicing (Jiang et al. 2005). A frequent, non-conservative single nucleotide polymorphism in the gene, producing a valine (*val*) to methionine (*met*) amino-acid substitution at codon 66 (val66met) of the pro-*BDNF* sequence, was shown by Egan et al. (2003) to affect the activity-dependent secretion of *BDNF*. Depolarisation-dependent secretion of *BDNF* is impaired by the *met* allele. The *met*-BDNF may not be correctly transferred from the Golgi apparatus to its appropriate secretory granules despite the fact that mature protein function is unaltered by the polymorphism (Egan et al. 2003).

Sklar et al. (2002) and Neves-Pereira et al. (2002) reported excess transmission of the *val* allele to patients in family based analyses of BPD. In the latter study, a total of 283 triads consisting of affected probands and their parents yielded a highly significant result with the use of the transmission disequilibrium test (TDT). A smaller sample of 53 trios in which the probands were child and adolescent bipolar cases supported the notion of preferential transmission of the *val* allele (Geller et al. 2004).

Neves-Perriera et al. (2005) conducted a case-control association study with 321 schizophrenics, 263 patients with BPD and 350 controls. The *val* allele was over-represented in the schizophrenia cohort and there was a non-significant trend towards over-representation of this allele in the bipolar cohort (Neves-Pereira et al. 2005). An even larger case-control study has lent credence to these findings. Lohoff et al. (2005) genotyped 621 patients with BPD I and 998 controls. The *val* allele was once more found to be significantly increased in bipolar patients with an OR of 1.22 (Lohoff et al. 2005).

Again the picture is clouded by contradictory results. A multi-centre study in a large Japanese population of 519 patients with BPD and 588 controls failed to detect any relationship between the Val66Met variant and BPD (Kunugi et al. 2004). Negative results have also been obtained by other groups (Hong et al. 2003; Nakata et al. 2003; Oswald et al. 2004; Skibinska et al. 2004), although Nakata et al. (2003) and Oswald et al. (2004) did not type the Val66Met polymorphism. Furthermore Tsai et al. (2003)

failed to detect a significant relationship between the Val66Met polymorphism and unipolar depression or response to fluoxetine treatment in these patients.

The Dopamine 4 Receptor (DRD4).

The dopamine four receptor gene (*DRD4*), one of the most variable yet studied, is situated on chromosome 11p15.5 (Ding et al. 2002). The D4 receptor is a G-protein coupled receptor belonging to the D2 family of receptors, which exert an inhibitory effect on the adenylate cyclase-mediated secondary messenger pathway (Kandel 2000).

A 48bp VNTR polymorphism in the third exon of the gene has been the main focus of attention. Between 2 and 11 repeated elements have been reported in the literature, although the two predominant alleles in Caucasians consist of four (4R) and seven repeats (7R), respectively (Ding et al. 2002). The polymorphic repeated segment codes for amino-acids in the third intracellular loop of the receptor, a region that couples to G proteins and therefore mediates intracellular signalling (Asghari et al. 1995). The strategic position of the polymorphism suggests that the *DRD4* receptor variants differ in function. Asghari et al. (1995) found differences in cyclic adenosine monophosphate (cAMP) inhibition between the 4R and 7R alleles, as well as between a 2R and 4R combined group, and the 7R allele. This is congruent with the data of Ding et al. (2002) who argue that the evolution of the 7R polymorphism required six separate steps, and therefore originated relatively recently as a rare mutational event, and increased in frequency because of positive selection. Another group reported that the 2R allele displays suboptimal binding to (and therefore inhibition of) adenylyl cyclase thereby influencing secondary messenger mediated signal transduction (Watts et al. 1999).

Studies testing for an association between alleles of the 48bp VNTR polymorphism and BPD are largely negative (Lim et al. 1994; Perez de Castro et al. 1994). Three family-based analyses of the *DRD4* VNTR and BPD have also been published. Bocchetta et al. (1999) reported non-significant results. While Serretti et al (2002) found evidence for the preferential transmission of the 2R allele, Muglia et al. (2002) observed a parent of origin effect in their sample of 145 nuclear families. The 4R

allele of maternal but not paternal origin was preferentially transmitted to bipolar individuals, while the 2R allele was under-transmitted to affected offspring. The authors speculate that the *DRD4* gene may be imprinted in humans because of its location close to a cluster of imprinted genes on the telomeric region of the chromosome.

Lopez-Leon et al. (2005) performed a meta-analysis of association (but not family-based) studies investigating the relationship between *DRD4* VNTR alleles and unipolar and BPD. The authors pooled data from 917 patients and 1164 controls and detected a significant association between the 2R allele and unipolar depression (OR=1.73) and the combined unipolar and bipolar groups (OR=1.41). The bipolar data analysed separately did not produce a statistically significant result (OR=1.26) (Lopez-Leon et al. 2005). These data contradict Muglia et al.'s, (2002) assertion that the 2R allele appears to exert a protective effect.

Some of the contradictory findings in the literature may be explained by the presence of another functional variant in the *DRD4* gene. A 120 bp tandem duplication first identified by Seaman et al. (1999) and located 1.2 Kb upstream from the transcription initiation codon of the gene, was found by D'Souza et al. (2004) to exert a functional effect on gene expression. The tandem duplication polymorphism contains consensus sequence binding sites for several transcription factors, perhaps explaining why the longer allele is under-expressed relative to the shorter allele (D' Souza et al. 2004). The long allele has been associated with schizophrenia (Xing et al. 2003) and a linkage study implicated the tandem duplication in Attention Deficit Hyperactivity Disorder (ADHD) (McCracken et al. 2000).

Monoamine Oxidase A (MAO-A).

The *MAO-A* gene, located on Xp11 codes for a mitochondrial enzyme that degrades noradrenaline, serotonin and dopamine. Sabol et al. (1998) identified a functional 30 bp VNTR in the promoter region of the *MAO-A* gene which is present in 2, 3, 3.5, 4 or 5 copies. The longer 3.5 and 4 repeat alleles appear to display greater enzymatic activity than the shorter 2 and 3 repeat alleles (Sabol et al. 1998). However, while

Sabol et al. (1998) suggested that the 5 repeat allele also exhibits low transcriptional activity, Deckert et al. (1999) reported that the 5 repeat allele is a high activity variant.

Lim et al. (1995) detected an association between three different *MAO-A* variants, including the promoter VNTR, and BPD. There appears to have been a gender-specific relationship with the effect stronger in females than males. The follow-up batch of replication studies showed the predictable pattern of inconsistency. Kawada et al. (1995) and Rubinsztein et al. (1996) replicated the results of Lim et al. (1995) for one of these variants; a CA repeat polymorphism, but Craddock et al. (1995) and Nothen et al. (1995) returned negative results, the latter using a family-based design.

Recently published studies have been equally inconclusive. Furlong et al. (1999) typed 106 bipolar patients and 250 controls for a variety of *MAO-A* variants. Neither the aforementioned CA-repeat polymorphism nor the promoter VNTR was associated with BPD, but when data were pooled for a meta-analysis it was found that the CA-repeat was associated with BPD in both Japanese and Caucasian populations (Furlong et al. 1999). No meta-analysis was undertaken for the VNTR polymorphism. Another study that made use of 272 bipolar patients and 122 controls detected a relationship between the CA-repeat variant and BPD although the replication was only partial because a different allele to previous studies was associated with psychiatric illness (Preisig et al. 2000). No association was reported for the promoter VNTR (Preisig et al. 2000).

Schulze et al. (2000) found an excess of the long (high activity) VNTR alleles in female patients with recurrent major depression, a gender-specific association that is congruent with Deckert et al.'s (1999) report that long alleles are over-represented in female panic disorder patients. On the other hand, male but not female patients with schizophrenia may be more likely to carry the shorter, less efficiently transcribed alleles (Jonsson et al. 2003).

G72/G30.

G72 and *G30* are two genes that overlap on the complementary strands of chromosome 13q32-33. In a family-based analysis, Hattori et al. (2003) found that a

haplotype consisting of 5-6 SNPs was over-transmitted to bipolar patients in two independent pedigrees. A haplotype of seven SNPs was also found by Schumacher et al. (2004) to be significantly associated with BPD in a sample of 299 patients and 300 controls. A smaller case-control sample of 139 cases and 113 controls again suggested a relationship between *G72/G30* and BPD although different SNPs were associated with the illness than in the Hattori et al. (2003) study.

The Dopamine 2 Receptor (DRD2).

The *DRD2* gene is about 270kb long and contains 8 exons. A commonly studied SNP, TaqIA, is located in the 3' untranslated region of the gene (Noble et al. 2000). The functional status of the TaqI A variant is not entirely clear but some preliminary evidence indicated that A1 allele was associated with reduced density of *DRD2* receptors in the striatum (Pohjalainen et al. 1998). More recently, Duan et al. (2003) found that a synonymous SNP (C957T) affects mRNA stability and therefore receptor expression, and this was confirmed *in vivo* by Hirvonen et al. (2004). The C957T variant was found to be in linkage disequilibrium with the TaqI A variant in a Caucasian, but not an African-American sample (Duan et al. 2003).

An early study with a small sample of subjects of German origin failed to detect any relationship between the TaqI A variant and BPD (Nothen et al. 1992). Similar negative results were published by Savoye et al. (1998) using the TaqI A variant and other groups who genotyped a variety of polymorphisms in the *DRD2* gene (Manki et al. 1996; Stober et al. 1998; Heiden et al. 2000). Li et al. (1999) observed a significant increase in the A1 allele of the TaqI A variant in a Chinese sample of bipolar patients; however the result could not be replicated in a separate group of Caucasians, indicating that the former result was a false positive or that genetic heterogeneity is present.

The one exception to this pattern was a multi-centre association study making use of 358 individuals with BPD and 358 controls which reported an association between an allele of a microsatellite marker in the second intron of the gene and the presence of affective illness (Massat et al. 2002).

The Dopamine Transporter (DAT).

The dopamine transporter gene, located on chromosome 5p15.3 mediates the reuptake of dopamine into neurones. A 40bp VNTR in the 3' untranslated region (UTR) of the gene has differential enhancer activity with the 10 repeat allele resulting in greater gene expression than the 9 repeat allele (Mill et al. 2002). Variants of the *DAT* have generally been associated with susceptibility to psychiatric conditions like post-traumatic stress disorder (PTSD) (Segman et al. 2002) and ADHD (Cornish et al. 2005) rather than BPD (Gomez-Casero et al. 1996; Bocchetta et al. 1999; Georgieva et al. 2002). Nevertheless some exceptions do exist.

Kelsoe et al. (1996) reported linkage to a locus in the region of the *DAT* gene on chromosome 5p15. The same group carried out a linkage disequilibrium analysis with a haplotype comprised of five SNPs spanning the gene and found a significant association between the haplotype and BPD (Greenwood et al. 2001). More recently, Horschitz et al. (2005) discovered a rare missense mutation in the *DAT* gene in two patients with BPD that was not found in any of their control sample. The mutation in question prevents the DAT protein from being transported to the cell surface despite the fact that it is transcribed and translated (Horschitz et al. 2005).

Notch4.

The *Notch4* gene, located on 6p21.31 is made up of 30 exons distributed over a length of 28 kb. The gene is involved in neuronal development including stem-cell differentiation, the timing of apoptosis, the outgrowth of neurons and dendrites, and the maintenance of their synaptic connections (Wassink et al. 2003). Investigation of the *Notch4* gene began auspiciously with a publication in *Nature Genetics* reporting an association with schizophrenia. Wei and Hemmings (2000) observed excess transmission of particular *Notch4* alleles from heterozygous parents to schizophrenic offspring. Replication attempts have been particularly disappointing with at least three well controlled studies failing to confirm the original findings (Sklar et al. 2001; McGinnis et al. 2001; Ujike et al. 2001). The author's interest in the gene was piqued however, by the report of Wassink et al. (2003) who found an association between the 6R allele of an exonic CTG polymorphism and performance on the Wisconsin Card

Sorting Test (WCST) as well as frontal grey-matter volume in a sample of schizophrenic patients and controls.

ApoE.

The Apolipoprotein E (*ApoE*), gene which is found on chromosome 19q13.2 and codes for a glycoprotein that is involved in the transport of cholesterol and lipids, is the major risk factor for the later-onset, sporadic form of the Alzheimer's disease (AD) (Saunders et al. 1993). Two single nucleotide polymorphisms in close proximity to each other result in three different alleles: ε2 (cysteine at both positions), ε3 (cysteine at position 112 and arginine at position 158), and ε4 (arginine at both positions) (Price et al. 1998). Individuals homozygous for the ε4 allele are 14 times more likely to develop AD, and ε4 heterozygotes have a 3-fold increased risk over non-carriers of being diagnosed with the disorder (Farrer et al.1997).

Krishnan et al. (1996) reported an increased presence of the *ApoE* ε4 allele in late-onset compared to early-onset patients with major depression while Zubenko et al. (1996) postulated an association between the presence of the ε4 allele and the development of psychotic features in elderly patients with unipolar depression. Both these results may however, in the author's opinion reflect the effects of incipient dementia.

Nevertheless, based on their finding of higher APOE levels in the caudate and putamen of post-mortem BPD subjects, Dean et al. (2005) argue that the *APOE* gene plays an aetiological role in the pathophysiology of BPD. In addition, an intriguing relationship between dementia and unipolar and bipolar depression has been detailed (Kessing and Nilsson 2003). The authors examined a cohort of close to 14 000 people hospitalised with a mood disorder, 81 380 patients with osteoarthritis and 69 149 patients with diabetes. The risk of receiving a diagnosis of dementia on subsequent readmission was elevated in the affective disorder group compared to the two control groups (Kessing and Nilsson 2003).

2.3. Isolated Populations.

How can one ameliorate the contradictory pattern of findings that pervades the bipolar genetics literature? One way of addressing the problem of genetic heterogeneity is to conduct genetic studies on isolated population groups, a methodological approach pioneered by Leena Peltonen in the Finnish population. Finns are descended from a small founder population which immigrated to the area about 2000 years ago before increasing in size to today's estimate of more than five million (Peltonen et al. 1999). The limited genetic variation associated with this founding population coupled with their vulnerability to genetic drift resulted in extensive linkage disequilibrium (LD) and unusually high locus and allelic homogeneity, facilitating the identification of 32 different disease genes (Peltonen 1999).

The Afrikaner population of South Africa is also an attractive target for molecular genetic work. A small founder population of 1000-2000 Dutch settlers arrived at the Cape of Good Hope in 1652 to set up a refuelling station. The population expanded largely through natural growth although a trickle of immigrants, mostly from the Netherlands and Germany arrived over the first few decades (Giliomee 2004). There was also a degree of inter-breeding with the indigenous population. In fact, according to some estimates seven percent of Afrikaner families have a non-European progenitress (Giliomee 2004).

When the British occupied the Cape in 1806 a proportion of the population refused to acquiesce to their rule and trekked into the interior of country forming *Boer* republics in the Transvaal and Orange Free State (Brink 1988). The discovery of gold and diamonds in these areas precipitated continued conflict with the British, culminating in the Anglo-Boer war at the turn of the 20th Century (Brink 1988). The duel British and black African threat to the independence of the Afrikaner, together with a strong cultural identity forged by instruments such as the Dutch Reformed Church, has lent itself to 13-15 generations of largely natural population growth with minimal external contributions to the gene pool beyond the first few generations (Brink 1988; Abecasis et al. 2004).

Like other population isolates, a founder effect in the Afrikaner population coupled with genetic drift has resulted in high frequencies of rare mendelian disorders such as variegate porphyria, pseudoxanthoma elasticum, hypercholesterolaemia, and Huntington's chorea (Botha and Beighton 1983; Jenkins 1990; Torrington and Viljoen 1991), with the extent of conserved haplotypes surrounding these disease genes estimated to be between 8 and 11 cM (Groenewald et al. 1998). Gordon et al. (2000) investigated the extent of background LD in the Afrikaner population and found LD between markers up to 5.5 cM apart. A more detailed follow-up study by the same group replicated the finding of significant LD in the 3-6 cM range and suggested that the Afrikaner population displays a greater degree of LD than both outbred and other founder populations such as the Finns (Hall et al. 2002).

The extent of the conserved haplotypes around disease loci is important for both linkage and association studies which benefit from higher levels of LD. The greater the extent of LD in a population, the greater the probability that an allele of a randomly selected marker will be associated with the hypothetical disease locus. The same reasoning can be applied to linkage analysis: the larger the conserved chromosomal block surrounding a disease locus, the greater the probability that a randomly selected marker will be part of that block and therefore inherited IBD from the common affected ancestor (Hall et al. 2002).

Perhaps the most advantageous aspect of isolated populations is that they have reduced genetic diversity because of past population bottle-necks. This suggests that disease susceptibility loci have originated from a small number of individuals reducing the genetic complexity and heterogeneity that tends to undermine genetic investigations (Peltonen et al. 1999). In other words, any two affected individuals selected at random are reasonably likely to have received the same disease-predisposing allele identical by descent from a common ancestor.

Another often overlooked advantage of studying population isolates is the potentially greater homogeneity in environmental background which may result in increased phenotypic homogeneity (Arcos-Burgos and Muenke 2002). The effects of environment are difficult to quantify but it is highly unlikely that genetic variants that predispose to complex disorders operate independently of environmental influence.

2.4 Rationale.

Given its small founding population and relative reproductive isolation, the Afrikaner population is an attractive target for genetic research. The circumscribed gene pool associated with the small founding population should theoretically reduce the problem of genetic heterogeneity. In other words, different Afrikaner families are possibly more likely to share genetic variants that induce susceptibility to BPD than families of a typical outbred population. The greater extent of LD in the Afrikaner population also facilitates genetic work by increasing the power of linkage and association tests. The analysis of Afrikaner ancestry families therefore constitutes one pillar of this thesis. As will be discussed below, a linkage analysis using markers in regions previously implicated in BPD, and an association analysis using polymorphisms of various candidate genes was conducted.

2.5. Methodology.

2.5.1 Subjects.

The UCT Division of Human Genetics neuropsychiatric genetics research project commenced in 1997, with the recruitment of South African families with BPD. Over a period of four years a number of clinically-trained psychiatric nurses undertook several visits to disparate regions of the country to interview prospective participants. Families who met the criteria for inclusion in the study consisted of a BPD I index proband and usually at least one first-degree relative with a BPD I or BPD II diagnosis. The mean number of BPD patients per family was 2.0. Both Caucasian and Mixed-Ancestry individuals were recruited. The former group included families of mostly Afrikaner and British origin with a small number of Ashkenazi Jewish pedigrees. The South African Mixed-Ancestry population is believed to have San, Khoi-Khoi, Madagascan, Javan and Western European ancestry (Cupido et al. 2005).

Probands and their relatives were interviewed with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First et al. 1998). Following the interview, a

psychiatrist made a diagnosis for each subject on the basis of the SCID interview as well as all available family and medical records. Individuals who were acutely depressed or manic were interviewed once they were stable. No children under the age of 16 were interviewed unless they had already been diagnosed with BPD by a psychiatrist. All subjects signed the UCT Research Ethics Committee-approved (081/96 and 195/2000) consent forms. In addition, 20 ml of blood was obtained from each participant for DNA extraction and storage in the molecular genetics laboratory of the Division of Human Genetics.

For reasons that will be discussed in Chapters 4, only Caucasian families were included in both the linkage and association analyses. The Caucasian sample was divided approximately evenly between individuals of British and Afrikaner origin. A detailed breakdown of the ethnic origins and demographic characteristics of the sample is provided in Tables 2.2 and 2.3, below.

Table 2.2. Ethnic Origin of Families.

Origin.	**Number of Pedigrees**	**Total Sample Size**	**Minimum Pedigree Size.**	**Maximum Pedigree Size.**	**Average Pedigree Size.**
Afrikaner	17	190	1	77	11.18
British	22	132	1	25	6
Other Caucasian	8	28	1	8	3.62
Total.	47	350	1	77	7.21

Table 2.3. Demographic Characteristics of the UCT Sample.

Origin.	**Average Age.**	**Average Education Level (Years).**	**Gender (% Male).**
Afrikaner	47.39	15.12	44.7
British	49.05	14.1	47.7
Other Caucasian	51.14	13.6	47.8
Total.	50.48	14.66	45.8

The most common psychiatric diagnosis in the sample was major depression. As is the trend internationally a distinction was made between individuals who met DSM-IV criteria for a major depressive episode on one occasion (MDE S), and participants who had a history of two or more episodes of depression (MDE-R). Approximately equal numbers of individuals were diagnosed with BPD I and MDE-R (see Table 2.4), and about 30% of the sample were unaffected. Other less common diagnoses included BPD not otherwise specified (BP NOS), ADHD, alcoholism, borderline personality disorder, delusional disorder, schizophrenia, and obsessive compulsive disorder.

Table 2.4. Diagnostic Characteristics of Sample.

Ethnic Origin.	**Total Sample Size**	**BPD I**	**BPD II**	**MDE R**	**MDE S**	**Unaffected**	**Other Diagnosis.**
Afrikaner	190	25 (13.2%)	17 (8.9%)	42 (22.1%)	27 (14.2%)	60 (31.6%)	19 (10%)
British	132	29 (22%)	11 (8.3%)	19 (14.4%)	21 (15.9%)	38 (28.8%)	14 (10.6%)
Other Caucasian	28	9 (32.1%)	0	6 (21.4%)	4 (14.3%)	6 (21.4%)	3 (10.7%)
Total.	350	64 (18.3%)	28 (8%)	67 (19.1%)	52 (14.9%)	103 (29.4%)	36 (10.3%)

2.5.2 Genotyping.

Blood (5-20ml) was collected from participants in EDTA-containing plastic tubes labelled with the patients' particulars. The blood samples and the consent form for the study was stored in the molecular genetics laboratory of the Division of Human Genetics at UCT, the former at -20°C. Relevant information was uploaded onto a Microsoft Access database. The Genomix extraction kit (*Talent*, Italy) was used to extract DNA from the lymphocyte cells, and working dilutions of 200ng/ul were made from the stock solutions.

For the linkage analysis, dinucleotide and tetranucleotide repeat microsatellite markers were selected from the literature and the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Marker heterozygosity ranged from 0.60 to 0.96 with a mean of 0.85. The average inter-distance on each chromosome was 3cM. Primers sequences were obtained from the literature or designed online at http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/Default.aspx

Standard polymerase chain reaction (PCR) methods were used for multiplex DNA amplification: 200ng DNA; 10pmol of each primer (purchased from the Synthetic DNA Laboratory at UCT) labelled with HEX or FAM dye; reaction buffer containing 10mM Tris-HCl (pH=8.3) and 50mM KCl; 1.5mM $MgCl_2$; 200uM dNTP (*Invitrogen,* UK), and 0.25-0.5 units of taq DNA polymerase (Biotaq: *Bioline,* UK). A list of the primer sequences used for each marker can be found in Appendix C (Table C.1). Amplifications were carried out on a DNA Thermal Cycler (*Perkin Elmer*, USA). A 30 cycle touch-down program was used with a denaturation step of 94°C for 15 seconds, an annealing temperature ranging from 58-53°C for 15 seconds, and a 30 second-long amplification step at 72°C.

Samples were scored on the Applied Biosystems (ABI) 3100 sequencer (Perkin Elmer) using either the GeneScan or the GeneMapper software programs. The accuracy of the genotyping was checked using the SimWalk2 (2.91) (Sobel and Lange 1996) and PedCheck packages (O'Connell and Weeks 1998) which identify non-mendelian inheritance patterns in pedigrees. Problematic samples were checked and if necessary re-genotyped, and where mendelian inconsistencies could not be resolved, excluded from the analysis.

Regarding the association analysis, candidate genes were selected on the basis of published studies. Given time and resource constraints, where possible known functional polymorphisms were selected in order to circumvent the need for genotyping large numbers of SNP's in each of these genes. Primer sequences were largely obtained from the literature although where PCR problems were encountered primers were redesigned using the Integrated DNA Technologies primer program: http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/Default.aspx

Again, standard PCR methods were used. Primer sequences and annealing temperatures for the different PCR reactions are listed in Appendix C (Table C.2). Amplifications were carried out on a DNA Thermal Cycler (*Perkin Elmer*, USA). A 30 cycle program was used with a denaturation step of 94°C for 30 seconds, an annealing temperature ranging from 67-54.5°C for 30 seconds depending on the variant being genotyped, and a 30 second-long amplification step at 72°C.

VNTR and insertion/deletion polymorphisms were scored on the Applied Biosystems (ABI PRISM) 3100 sequencer (*Hitachi*, Japan) using the Applied Biosystem's GeneScan computer program. SNPs were typed using restriction enzyme digests and resolved on either 2% agarose or 6% polyacrylamide gels. More details are available in Appendix C (Table C.3).

2.5.3. Procedure and Data Analysis.

In order to test for linkage to BPD in the South African cohort, 25 highly polymorphic markers found in regions previously associated with BPD were typed by the author (see Figures 2.1-2.9, above, and Table C.1 in Appendix C). The original plan was to type 27 markers (3 per region of interest on 9 chromosomes), but two of the markers did not genotype well, and were left out of the final analysis.

A decision was made to concentrate on non-parametric linkage methods as these model-free approaches do not require the specification of parameters such as mode of inheritance, gene frequency and penetrance which are unknown in complex disorders such as manic depression. When these parameters are incorrectly specified the accuracy of the analysis may be adversely affected and thus non-parametric methods are likely to be more robust than parametric approaches (Thomas 2004).

Nonetheless, the large size of some of the pedigrees rendered them refractory to certain types of non-parametric analyses. This led to adoption of two strategies: firstly to break down the large pedigrees into smaller families, and secondly to leave the larger pedigrees intact and make use of different linkage algorithms.

The Multipoint Engine for Rapid Likelihood Inference (Merlin) (Abecasis et al. 2002), which implements a statistical method derived from the Lander-Green algorithm, was run on the sample after splitting up the large pedigrees into smaller units. The Lander-Green algorithm computes all possible patterns of gene-flow (inheritance vectors) within a pedigree and calculates linkage statistics for the pedigree conditional on each alternative gene-flow pattern (Abecasis et al. 2002). While computational resources only increase linearly with the number of markers, the algorithm becomes problematic in large families because the complexity of the calculation increases exponentially with the number of inheritance vectors (Abecasis et al. 2002). Abecasis et al. (2002) reasoned that since many of the inheritance vectors contain redundant information, they can be combined together producing a sparse gene-flow tree which allows for more efficient analysis of larger pedigree sets.

Merlin uses these sparse gene flow trees to test for allele sharing among affected pedigree members, producing NPL All and NPL Pair scores. The latter score reflects the degree to which all the distinct affected relative pairs in the pedigree share alleles IBD. When considering larger groups of affected individuals, the NPL All score places more weight on those relatives who share alleles IBD. Despite these improvements to the original incarnation of the Lander-Green algorithm, large pedigrees (approximately 20 or more individuals) still pose problems for the Merlin calculation and hence the need for Monte Carlo chain modelling in the guise of SimWalk2.

SimWalk2 (version 2.91) (Sobel and Lange 1996; Sobel et al. 2001) is able to cope with the analysis of very large pedigrees (>200 individuals) and numbers of markers (>30) because computational time scales in a linear fashion with both pedigree size and markers typed. In addition, missing genotyping data has only a mildly detrimental effect on the computational resources required to provide a solution (Sobel and Lange 1996). The program measures the degree to which marker alleles descended from founders (family members whose parents have not been genotyped) aggregate in affected relatives. The program relies on the Markov chain Monte Carlo (MCMC) and simulated annealing algorithms to provide estimated NPL Pair and NPL All scores. The NPL Pair statistic corresponds to the sum of conditional kinship coefficients for

all affected relative pairs whereas the NPL All statistic is a measure of whether certain founder alleles are over-represented in affected individuals (Sobel et al. 2001).

The second strategy was to therefore carry out a non-parametric linkage analysis using a combination of the Merlin and SimWalk2 packages. This option (# 24) is available on the Mega2 (Manipulation Engine for Genetic Analysis) (Mukhopadhyay et al. 1999) conversion program which produces a script allowing for the dual analysis of pedigrees with both the Merlin and SimWalk2 algorithms. The Merlin algorithm is run on the smaller pedigrees producing exact NPL score calculations and the SimWalk2 algorithm is used on the large families producing NPL score estimations. These computations are then summed together to produce an overall NPL score.

For both the Merlin and Merlin-SimWalk2 analyses a relatively broad affection status model was used. Individuals with a diagnosis of schizophrenia, BPD I, BPD II, cyclothymia and MDE-R were considered to be affected. Participants with all other diagnoses were labelled as unknown. As mentioned above family studies show a significant overlap between UPD and BPD illness. Similarly, the risk of developing BPD is doubled in schizophrenic probands compared to the general population (Kendler and Gardner 1997). Moerover, Cardno et al. (2002) found a significant correlation (r=0.68) in genetic liability between individuals with manic and schizophrenic syndromes.

The author also genotyped the cohort for 11 different variants of specific genes implicated in BPD (see Table C.2, Appendix C) and conducted an association analysis with these genes using the QTDT (Quantitative Transmission Disequilibrium Tests) program (Abecasis et al. 2000). The original transmission disequilibrium test (TDT) (Spielman et al. 1993) is based on the assumption that a parent heterozygous for a disease susceptibility allele should pass on the susceptibility allele to the affected child more often than one would expect by chance (50%). Here the allele that is not transmitted acts as the "control" that enables the detection of the distorted transfer of alleles in triads. Curtis (1997) then extended this method to discordant sibling pairs allowing for the extraction of information from cases whose parents were not available.

The weakness of these original TDT tests is that information from parents and siblings is alone used to define allelic transmission causing a great deal of information that is present in extended pedigrees to be lost, with a concomitant reduction in statistical power (Abecasis et al. 2000a). Abecasis et al. (2000a) and Abecasis et al. (2000b) generalised these TDT tests to include all available ancestors in the analysis by using each typed family member to construct an expected genotype for each non-founder and calculate the degree of deviation from this genotype (Abecasis et al. 2000b). The QTDT program thus not only provides a fast, efficient way of detecting association in extended pedigrees, but is immune to the confounding influence of population stratification and admixture.

Once again a broad model which defined people with schizophrenia, BPD I, BPD II, cyclothymia, and MDE-R as affected was used. Ethnicity, gender, and age were entered into the model as covariates.

2.6. Results.

2.6.1. Linkage Analysis.

The NPL scores and p-values obtained in the various statistical analyses are presented in Tables 2.5-2.11, below. NPL scores of 1.5 and Z scores of 2 or above are shown in boldface. Probability values of less than 0.05 are indicated by * and p-values of less than 0.01 are indicated by **.

The combined Merlin-SimWalk2 analysis returned significant ($p<0.05$) NPL scores of 1.82, 1.50 and 1.34 for the three chromosome 1q32 markers. Statistically significant findings were also obtained for the markers D16S3024 (NPL=1.47, $p<0.05$), D16S3027 (NPL=1.84, p=0.0339), and D16S3088 (NPL=1.37; p=0.0430). No other markers showed any evidence for linkage to BPD (see Table 2.5).

Table 2.5. Results of Combined Merlin-Simwalk Analyses - All Pedigrees.

Marker	**NPL-Pair**	**p-value**
D1S1660	**1.82**	**0.0151***
D1S2655	**1.50**	**0.0319***
D1S1678	*1.34*	*0.0454**
D2S2321	0.45	0.3534
D2S2208	0.25	0.5648
D4S394	0.35	0.4412
D4S983	0.12	0.7665
D4S1582	0.19	0.6442
D10S1753	0.33	0.4754
D10S564	0.30	0.5044
D10S1171	0.58	0.2619
D12S86	0.50	0.3135
D12S1612	0.57	0.2682
D13S1298	0.29	0.5151
D13S1271	1.20	0.0630
D13S1284	0.51	0.3079
D15S122	0.52	0.3012
D15S1002	0.53	0.2755
D15S1048	0.13	0.7468
D16S3024	*1.47*	*0.0339**
D16S3027	**1.84**	**0.0144***
D16S3088	*1.37*	*0.0430**
D22S421	0.04	0.9106
D22S315	0.10	0.8029
D22S1164	0.04	0.9124

The large pedigrees were then fractionated into independent units and a non-parametric affected pedigree member analysis run on the sample with the program Merlin. Again significant evidence for linkage to chromosome 1 was present with NPL All scores of 2.52 ($p<0.01$), 0.69 ($p<0.05$) and 1.52 ($p<0.01$) obtained for the

chromosome 1 markers. In contrast to the Merlin-SimWalk2 analysis which did not detect any indication for linkage to chromosome 10, 2 out of the 3 typed markers on 10q23 yielded weak evidence for linkage to the disease phenotype. NPL scores of 1.15 and 0.77 ($p<0.05$) were obtained for the markers D10S54 and D10S1171. At D12S86 an NPL score of 1.18 ($p<0.01$) was present but D12S1612 was not linked to the disorder. A highly significant NPL All score of 2.17 ($P<0.01$) at the marker D13S1271 was not echoed by the neighbouring markers D13S1284 and D13S1298 which returned non-significant NPL scores. Only one marker on chromosome 16, D16S3024 was weakly significant with an NPL All score of 0.60 ($p<0.05$). Detailed results can be found in Table 2.6.

Table 2.6. Results of Single-Point Merlin Analysis – All Families.

Marker	ZMean Pair	p-value	LOD Pair	p-value	ZMean All	p-value	LOD All	p-value
D1S1660	**2.99**	**0.0014****	**2.14**	**0.0008****	**3.16**	**0.0008****	**2.52**	**0.0003****
D1S2655	*1.53*	*0.06*	*0.57*	*0.05**	*1.40*	*0.08*	*0.69*	*0.04**
D1S1678	**2.03**	**0.02***	**1.72**	**0.002****	**1.56**	**0.06**	**1.52**	**0.004****
D2S2321	0.76	0.20	0.17	0.20	0.46	0.30	0.09	0.30
D2S2208	1.17	0.12	0.41	0.08	1.18	0.12	0.44	0.08
D4S394	0.32	0.40	0.08	0.30	0.10	0.50	0.01	0.40
D4S2983	0.35	0.40	0.04	0.30	0.48	0.30	0.07	0.30
D4S1582	0.30	0.40	0.03	0.40	0.84	0.20	0.24	0.15
D10S1753	0.32	0.40	0.04	0.30	0.06	0.50	0	0.50
D10S564	1.59	0.06	1.13	0.011*	1.37	0.09	1.15	0.011*
D10S1171	1.78	0.04*	1.06	0.014*	1.38	0.08	0.77	0.03*
D12S86	**2.02**	**0.02***	**1.19**	**0.01****	**1.80**	**0.04***	**1.18**	**0.01****
D12S1612	0.88	0.20	0.25	0.14	0.50	0.30	0.10	0.20
D13S1298	1.39	0.08	0.45	0.08	1.21	0.11	0.39	0.09
D13S1271	**2.97**	**0.0015****	**1.93**	**0.0014****	**2.93**	**0.002****	**2.17**	**0.0008****
D13S1284	0.26	0.40	0.02	0.40	0.15	0.40	0.01	0.40
D15S122	0.72	0.20	0.30	0.12	0.43	0.30	0.14	0.02
D15S1002	0.21	0.40	0.02	0.40	-0.26	0.60	-0.01	0.60
D15S1048	-0.60	0.70	-0.12	0.80	-0.93	0.80	-0.06	0.70
D16S3024	1.85	0.03*	0.76	0.03*	1.47	0.07	0.60	0.05*
D16S3027	1.43	0.08	0.54	0.06	1.10	0.14	0.43	0.08
D16S3088	0.02	0.50	0	0.50	-0.34	0.60	-0.02	0.60
D22S421	0.95	0.20	0.32	0.11	0.68	0.20	0.22	0.20
D22S315	0.48	0.30	0.14	0.20	0.07	0.50	0	0.40
D22S1164	0.18	0.40	0.01	0.40	0.13	0.40	0.01	0.40

When the pedigrees of Afrikaner origin were analysed separately the significant evidence for linkage to chromosome 1q32 and 10q23 seen in the entire sample was no longer present. Two out of 3 markers on chromosome 16p13 yielded statistically

significant NPL scores and a trend in the same direction for marker D16S3088 (p=0.077) was apparent. See Table 2.7 for more details.

Table 2.7. Results of Combined Merlin-Simwalk Analyses – Afrikaner Pedigrees.

Marker.	**NPL-Pair.**	**p-value**
D1S1660	0.61	0.2433
D1S2655	0.89	0.1295
D1S1678	0.75	0.1770
D2S2321	0.75	0.1793
D2S2208	0.24	0.57
D4S394	0.73	0.1880
D4S2983	0.37	0.4243
D4S1582	0.23	0.5935
D10S1753	0.08	0.8355
D10S564	0.08	0.8270
D10S1171	0.07	0.8533
D12S86	0.24	0.5647
D12S1612	0.21	0.6161
D13S1298	0.44	0.3593
D13S1271	0.82	0.1522
D13S1284	0.35	0.4449
D15S122	0.84	0.1432
D15S1002	0.64	0.2312
D15S1048	0.32	0.4775
D16S3024	1.39	0.0407*
D16S3027	1.50	0.0315*
D16S3088	1.11	0.0777
D22S421	0.03	0.9353
D22S315	0.12	0.7641
D22S1164	0.06	0.8619

The combined Merlin-SimWalk2 analysis of family "30", the 77-strong pedigree of Afrikaner origin (see Appendix D) was disappointing. The only significant NPL score

of 1.58 (p=0.0265) was obtained for the marker D16S3024 at 16p13 with a weak trend towards significance for another nearby marker, D16S3027.

Table 2.8. Results of Combined Merlin-Simwalk Analyses: Family 30.

Marker	**NPL-Pair**	**p-value**
D1S1660	0.71	0.1956
D1S2655	0.60	0.2535
D1S1678	0.45	0.3560
D2S2321	0.23	0.5957
D2S2208	0.16	0.6864
D4S394	0.65	0.2237
D4S2983	0.39	0.4035
D4S1582	0.27	0.5415
D10S1753	0.03	0.9233
D10S564	0.01	0.9745
D10S1171	0.10	0.7983
D12S86	0.37	0.4274
D12S1612	0.44	0.3621
D13S1298	0.19	0.6521
D13S1271	0.56	0.2761
D13S1284	0.18	0.6543
D15S122	0.89	0.1283
D15S1002	0.83	0.1477
D15S1048	0.43	0.3742
D16S3024	**1.58**	**0.0265***
D16S3027	0.94	0.1156
D16S3088	0.71	0.1965
D22S421	0.12	0.7625
D22S315	0.46	0.3433
D22S1164	0.12	0.7625

The Merlin analysis of the Afrikaner ancestry pedigrees again produced relatively different results from the Merlin-SimWalk2 calculation. Statistically significant but relatively modest NPL scores were obtained for 2 out of 3 of the markers on chromosome 1. Tacit evidence of linkage to chromosome 2q33 was also apparent with NPL All scores of 0.77 ($p<0.05$) and 0.58 ($p=0.05$) for the markers D2S2321 and D2S2208. Markers D12S86 and D13S1271 yielded more evidence for linkage as did two of the chromosome 16 variants (see Table 2.9).

Table 2.9. Results of Single-Point Merlin Analysis: Afrikaner Ancestry Pedigrees.

Marker	**ZMean Pair**	**p-value**	**LOD Pair**	**p-value**	**ZMean All**	**p-value**	**LOD All**	**p-value**
D1S1660	*1.47*	*0.07*	*0.61*	*0.05**	*1.64*	*0.05**	*0.72*	*0.03**
D1S2655	0.92	0.20	0.22	0.20	0.70	0.20	0.22	0.20
D1S1678	*1.71*	*0.04**	*1.02*	*0.02**	*1.42*	*0.08*	*0.96*	*0.02**
D2S2321	*1.57*	*0.06*	*0.79*	*0.03**	*1.18*	*0.14*	*0.77*	*0.03**
D2S2208	*1.16*	*0.12*	*0.49*	*0.07*	*1.10*	*0.14*	*0.58*	*0.05**
D4S394	0.40	0.30	0.12	0.20	0.49	0.30	0.17	0.20
D4S983	*1.61*	*0.05**	*0.85*	*0.02**	*1.29*	*0.10*	*0.54*	*0.06*
D4S1582	-0.06	0.50	0	0.50	0.57	0.30	0.09	0.30
D10S1753	-0.55	0.70	-0.09	0.70	-0.47	0.70	-0.02	0.60
D10S564	1.00	0.20	0.53	0.06	0.92	0.20	0.61	0.05*
D10S1171	0.59	0.30	0.12	0.20	0.23	0.40	0.03	0.40
D12S86	1.58	0.06	0.86	0.02*	1.29	0.10	0.86	0.02*
D12S1612	-0.23	0.60	-0.01	0.60	-0.68	0.8	-0.03	0.7
D13S1298	0.47	0.30	0.07	0.30	0.16	0.40	0.01	0.40
D13S1271	**2.42**	**0.008****	**1.00**	**0.02***	**2.44**	**0.007****	**1.16**	**0.01***
D13S1284	-0.17	0.60	-0.01	0.60	-0.32	0.60	-0.02	0.60
D15S122	1.03	0.20	0.60	0.05*	0.71	0.20	0.46	0.07
D15S1002	0.95	0.20	0.38	0.09	0.85	0.20	0.37	0.10
D15S1048	-0.37	0.60	-0.05	0.07	-0.51	0.70	-0.02	0.60
D16S3024	*1.68*	*0.05**	*0.61*	*0.05**	*1.19*	*0.12*	*0.46*	*0.07*
D16S3027	**2.07**	**0.02***	**1.01**	**0.02***	**1.71**	**0.04***	**1.14**	**0.011***
D16S3088	1.06	0.15	0.52	0.06	0.23	0.40	0.06	0.30
D22S421	0.45	0.30	0.08	0.30	0.29	0.40	0.07	0.30
D22S315	0.70	0.20	0.21	0.20	0.20	0.40	0.03	0.40
D22S1164	-0.30	0.60	-0.02	0.60	-0.28	0.60	-0.01	0.60

The combined Merlin-SimWalk2 analysis of the families of British origin also implicated chromosome 1q32 with a maximum NPL All of 1.56 ($p<0.05$) for the marker D1S1660. In addition, NPL Pair scores for the three chromosome 10 markers were significant with a maximum score of 2.01 ($p<0.01$) at D10S1171.

Table 2.10. Results of Combined Merlin-Simwalk Analysis: British Ancestry Pedigrees.

Marker	**NPL-Pair**	**p-value**	**NPL-All**	**p-value**
D1S1660	**1.64**	**0.0227***	**1.56**	**0.0277***
D1S2655	*1.31*	*0.0491**	*1.11*	*0.0782*
D1S1678	*1.34*	*0.0458**	*1.12*	*0.0751*
D2S2321	0.09	0.8100	0.18	0.6547
D2S2208	0.15	0.7002	0.26	0.5536
D4S394	0.10	0.7936	0.20	0.6259
D4S983	0.05	0.8895	0.07	0.8538
D4S1582	0.25	0.55630	0.28	0.5308
D10S1753	**1.56**	**0.0278***	**0.94**	**0.1149**
D10S564	**1.56**	**0.0277***	**0.94**	**0.1147**
D10S1171	**2.01**	**0.0097****	**1.51**	**0.0313***
D12S86	0.59	0.22569	0.63	0.2319
D12S1612	0.78	0.1657	0.73	0.1836
D13S1298	0.57	0.2703	0.72	0.1927
D13S1271	0.86	0.1383	1.19	0.0642
D13S1284	0.61	0.2448	0.87	0.1336
D15S122	0.21	0.6237	0.15	0.7056
D15S1002	0.05	0.8824	0.02	0.9522
D15S1048	0.05	0.8992	0.02	0.9554
D16S3024	0.58	0.2629	0.31	0.4924
D16S3027	0.53	0.29	0.32	0.4791
D16S3088	0.58	0.26	0.48	0.33
D22S421	0.13	0.7373	0.14	0.7165
D22S315	0.16	0.6864	0.15	0.71
D22S1164	0.10	0.7921	0.11	0.7780

The Merlin only analysis provided support to the hypothesis that the three chromosome 1 markers are linked to BPD with a maximum NPL All score of 1.57 ($p<0.01$) at D1S1660. The region around chromosome 10q23 also yielded significant NPL scores, peaking at 1.56 ($p<0.01$) for the marker D10S1171. The markers D12S86 and D13S1171 were also linked to BPD in the British ancestry sample. See Table 2.11, below.

Table 2.11. Results of Merlin Analysis – British Ancestry Pedigrees.

Marker	ZMean Pair	p-value	LOD Pair	p-value	ZMean All	p-value	LOD All	p-value
D1S1660	**2.33**	**0.01****	**1.56**	**0.004**	**2.41**	**0.008****	**1.57**	**0.004****
D1S2655	1.25	0.11	0.34	0.11	1.40	0.08	0.51	0.06
D1S1678	*1.19*	*0.12*	*0.93*	*0.02**	*0.80*	*0.20*	*0.62*	*0.05**
D2S2321	-0.78	0.80	-0.19	0.80	-0.69	0.08	-0.16	0.80
D2S2208	0.44	0.30	0.05	0.30	0.61	0.30	0.09	0.30
D4S394	-0.21	0.60	-0.04	0.70	-0.32	0.60	-0.08	0.70
D4S983	-1.10	0.90	-0.38	0.90	-1.17	0.90	-0.38	0.90
D4S1582	0.55	0.30	0.13	0.20	0.71	0.20	0.23	0.20
D10S1753	*1.39*	*0.08**	*1.00*	*0.02**	*0.86*	*0.20*	*0.50*	*0.07*
D10S564	*1.60*	*0.05**	*1.14*	*0.011**	*1.30*	*0.10*	*0.93*	*0.02**
D10S1171	**2.13**	**0.02***	**1.56**	**0.004****	**1.92**	**0.03***	**1.34**	**0.006****
D12S86	1.27	0.10	0.35	0.10	1.36	0.09	0.42	0.08
D12S1612	*1.32*	*0.09*	*0.87*	*0.02**	*1.18*	*0.12*	*0.78*	*0.03**
D13S1298	1.58	0.06	0.44	0.08	1.66	0.05*	0.51	0.06
D13S1271	*1.74*	*0.04**	*1.09*	*0.012**	*1.72*	*0.04**	*1.13*	*0.011**
D13S1284	0.56	0.30	0.10	0.20	0.58	0.30	0.11	0.20
D15S122	-0.05	0.50	0	0.50	-0.16	0.60	-0.02	0.60
D15S1002	-0.75	0.80	-0.23	0.08	-1.33	0.90	-0.45	0.90
D15S1048	-0.62	0.70	-0.11	0.80	-0.91	0.80	-0.24	0.80
D16S3024	0.91	0.20	0.17	0.20	0.92	0.20	0.17	0.20
D16S3027	-0.48	0.70	-0.08	0.70	-0.53	0.70	-0.10	0.80
D16S3088	-1.06	0.90	-0.37	0.90	-0.70	0.80	-0.21	0.80
D22S421	0.74	0.20	0.17	0.20	0.55	0.30	0.09	0.30
D22S315	-0.09	0.50	-0.01	0.60	-0.08	0.50	-0.02	0.60
D22S1164	0.55	0.30	0.13	0.20	0.56	0.30	0.14	0.20

2.6.2. Association Analysis.

The QTDT association analysis on the sample as a whole did not produce any statistically significant associations between variants of the candidate genes and bipolar spectrum illness. All individuals diagnosed with schizophrenia, BPD I, BPD II, cyclothymia and MDE-R were labelled as "affected". Three covariates were also included in the variants components model: age, gender and ethnicity. Details of the analysis can be found in Table 2.12, below.

Table 2.12. QTDT Association Analysis: All Pedigrees.

Variant	Sample Size	Chi Square	p-value	Risk Allele
COMT	187	1.98	0.1595	NA
5-HTT LPR	187	0.11	0.7388	NA
SERT VNTR	185	0.18	0.9140	NA
DRD4	186	6.06	0.1944	NA
D4 120	182	0.09	0.7676	NA
DRD2	180	0.00	0.9790	NA
DAT	186	0.94	0.6242	NA
BDNF	187	0.05	0.8292	NA
ApoE	186	3.02	0.2211	NA
Notch	181	5.58	0.2328	NA
Prion	184	0.20	0.6586	NA

As was the case with the linkage analysis, the author was interested in examining if the pedigrees of Afrikaner and British origin differed from each other in any way. In the Afrikaner ancestry sample, no significant association at an α level of 0.05 was apparent. There was however, a weak trend towards significance for two of the candidate polymorphisms, the *DRD4* 48bp VNTR (χ^2=8.52, p=0.0742), and the Prion Met129Val SNP (χ^2=3.13, p=0.0820). Full data are listed in Table 2.13, below.

Table 2.13. QTDT Association Analysis Afrikaner Ancestry Sample.

Variant	Sample Size	Chi Square	p-value	Risk Allele
COMT	106	0.03	0.8604	NA
5-HTT LPR	106	0.19	0.6664	NA
SERT VNTR	105	0.57	0.7536	NA
DRD4	*105*	*8.52*	*0.0742*	*4R*
D4 120	104	1.49	0.2216	NA
DRD2	103	0.22	0.6414	NA
DAT	106	0.81	0.3673	NA
BDNF	106	1.40	0.2364	NA
ApoE	106	0.20	0.9036	NA
Notch	103	6.13	0.1897	NA
Prion	*106*	*3.13*	*0.0820*	*Met*

In contrast the British ancestry sample yielded χ^2 scores of borderline significance for the *COMT* Val158Met variant ($p=0.0585$) with the *Val* allele found more frequently than expected by chance in the bipolar group. The *DAT* VNTR was statistically significant ($p<0.01$) with the 10 repeat allele over-represented in the bipolar group and the 9R allele under-represented in the control group (see Table 2.14, below). There was no statistically significant relationship between any of the other genetic variants and the disease phenotype.

Table 2.14. QTDT Association Analysis: British Ancestry Families.

Variant	**Sample Size**	**Chi Square**	**p-value**	**Risk Allele**
COMT	*70*	*3.58*	*0.0585*	*Val*
5-HTT LPR	71	0	0.9703	NA
SERT VNTR	70	0.48	0.7847	NA
DRD4	71	2.37	0.6684	NA
D4 120	68	2.41	0.1207	NA
DRD2	67	0.10	0.7504	NA
DAT	**70**	**6.99**	**0.0082****	**10R**
BDNF	71	0.52	0.4697	NA
ApoE	70	2.38	0.3039	NA
Notch	67	2.56	0.6343	NA
Prion	68	0.64	0.4240	NA

2.6. Discussion.

2.6.1 Linkage Analysis.

A visual summary of the linkage results is presented in Table 2.15, below.

Table 2.15. Summary of BPD Linkage Data.

Marker	Total Sample	Afrikaner	British	Family 30 (No Merlin)
D1S1660	√√	X√	√√	X
D1S2655	√√	XX	√X	X
D1S1678	√√	X√	X√	X
D2S2321	XX	X√	XX	X
D2S2208	XX	X√	XX	X
D4S394	XX	XX	XX	X
D4S983	XX	XX	XX	X
D4S1582	XX	XX	XX	X
D10S1753	XX	XX	√√	X
D10S564	X√	X√	√√	X
D10S1171	X√	XX	√√	X
D12S86	√√	√√	XX	X
D12S1612	XX	XX	X√	X
D13S1298	XX	XX	X√	X
D13S1271	X√	X√	X√	X
D13S1284	XX	XX	XX	X
D15S122	XX	XX	XX	X
D15S1002	XX	XX	XX	X
D15S1048	XX	XX	XX	X
D16S3024	√√	√√	XX	√
D16S3027	√X	√√	XX	X
D16S3088	√X	XX	XX	X
D22S421	XX	XX	XX	X
D22S315	XX	XX	XX	X
D22S1164	XX	XX	XX	X

√√ = Statistically significant result in combined analysis and Merlin analysis.
√X= Statistically significant result for combined analysis but not Merlin analysis.
X√= Statistically significant result for Merlin but not combined analysis.
XX= No statistically significant findings.

Table 2.15 is based on nominally significant p-values. As discussed in Appendix A, the suggested threshold for significance in genome-wide scans is stringent: 3.3. In this case, only 25 markers on 9 different chromosomal regions were typed. Furthermore, *a priori* evidence for linkage to these regions exists, and it is therefore unclear what constitutes appropriate cut-off scores.

The most promising finding was the Merlin derived single-point NPL (pair) score of 2.14 ($p<0.001$) and NPL (All) score of 2.52 ($p<0.001$) obtained at the marker D1S1660 on 1q31.1 (194.8 Mb). This finding appears to be robust as the two nearby markers, D1S2655, and D1S1678 both showed statistically significant evidence for linkage, particularly D1S1678 which returned a NPL (pair) score of 1.72 ($p=0.002$). A multipoint analysis implicated the entire 6 Mb region (194-200 Mb) with peak NPL scores of 1.78 at 194 and 1.58 at 200 Mb, respectively. In addition, the combined Merlin-SimWalk2 analysis produced similar findings, with NPL (pair) scores of 1.82 ($p=0.0151$), 1.50 ($p=0.0319$), and 1.34 ($p=0.0454$) for the markers D1S1660, D1S2655, and D1S1678, respectively.

Caucasians of both British and Afrikaner origin showed significant evidence of linkage to this region although the effect seemed to be weaker in the Afrikaner ancestry population with a maximum NPL (All) score of 0.96 ($p=0.02$) at D1S1678. The smaller British ancestry sample returned a maximum NPL score of 1.57 ($p=0.004$) at D1S1660.

Two other groups have described linkage to markers in an almost identical region of the chromosome. Ekholm et al. (2003) reported weak evidence for linkage to BPD with an affected sibling pair derived Z score of 1.8 at the same marker (D1S1660) in a

Finnish population, while Shink et al. (2004) recorded a modest parametric LOD score of 1.23 at the marker D1S413 (which is located only 1 Mb away from D1S1660) under a recessive model of inheritance in their French Canadian sample.

A search of the PubMed database suggests that the maximum NPL score of 2.52 obtained in this study is one of the highest ever reported in the region (see Figure 2.1). Morissette et al. (1999) conducted an affected-relative pair analysis of one large Quebec pedigree and found evidence for linkage to a marker only a few Mb distal from Detera-Wadleigh et al.'s (2004) peak, with an NPL score of 1.99 at 213.5 Mb. The analysis of the Scottish sample of MacGregor et al. (2004) implicated an even more distal region with a multipoint parametric LOD score of 2.77 at 227.1 Mb.

Given the fact that a linkage signal for a complex disease may occur up to 30cM away from the original finding (Park et al. 2004) and that true LOD score peaks are usually broader than false peaks (*Terwilliger et al. 1997*), it is likely that the peak at 195 Mb represents the same disease locus detected by researchers in the region around 1q41. There have however been two reports of linkage to a 1q23.3 about 40 Mb proximal to the UCT finding (see Figure 2.1), and it is thus theoretically possible that linkage to this hypothetical disease locus has been detected instead. The area around the 3 significant markers needs further investigation and future typing of the UCT cohort should include a variety of markers in the broad region between 157 and 230 Mb.

The Merlin analysis produced NPL Pair and All scores of 1.93 ($p<0.001$) and 2.17 ($p<0.001$) at the marker D13S1271. The two adjacent markers however, rather disconcertingly produced weakly positive NPL (pair and all) scores of 0.11-0.45 and 0.02-0.40, respectively. What can account for the difference in Merlin-derived NPL scores across the three 13q32 markers? Given the small distance between these markers (1 cM) inter-marker recombination can be effectively ruled out as a possible explanation for the divergent results. The degree of marker informativity can also be ruled out as a contributing factor. See Figure 2.10, below. Eleven different alleles of D13S1271 were observed in the sample while D13S1298 and D13S1284 had 15 and 9 alleles, respectively.

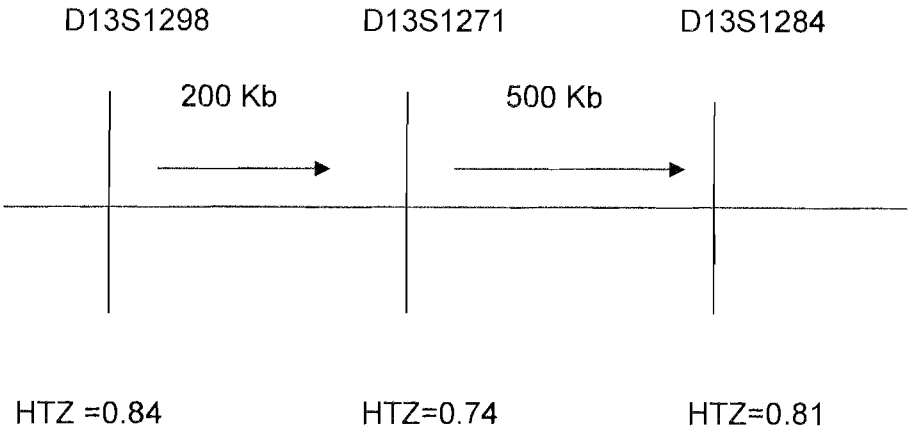


Figure 2.10 Location and Heterozygosity of Chromosome 13 Markers.

Genotyping error is a possible explanation. Given the fact that two out of the three markers produced non-significant Merlin results, it could be argued *ceteris paribus* that the significant NPL score at D13S1271 is artefactual, especially given the non-significant Merlin-SimWalk findings. On the other hand, a re-examination of the author's linkage files indicated that D13S1271 genotyped better than the other two negative markers. It is clearly going to be necessary to re-evaluate the region around 13q32 in this sample especially given the *a priori* evidence for linkage to this region.

Park et al. (2004), Detera-Wadleigh et al. (1999) and Liu et al. (2001) all detected significant linkage peaks at 100 Mb, approximately 2 Mb distal to the potentially significant markers in this study. Furthermore, Kelsoe et al. (2001) reported a parametric LOD score of 2.4 at 95 Mb, 3 Mb proximal to the equivocal results obtained in this thesis. The same region has also been implicated a number of times in schizophrenia. Lin and Bale (1997) reported a LOD score of 2.58 at the markers D13S122 and D13S128, and this was replicated by Blouin et al. (1998) who detected a maximum LOD score of 3.6 in a genome-wide scan of a large number of schizophrenia pedigrees. Brzustowicz et al. (1999) found an even higher LOD score of 3.92 in an independent sample of schizophrenia families.

The linkage results from chromosomes 10 and 16 provide preliminary support to the strategy of discriminating between subjects of British and Afrikaner origin. No statistically significant scores for the markers D10S1753, D10S564, and D10S1171

were observed in the pedigrees of Afrikaner origin with Merlin-derived NPL (pair) scores of -0.09; 0.53, and 0.20, respectively. The smaller British ancestry sample in contrast, yielded significant NPL (pair) scores of 1.00 (p=0.02), 1.14 (p=0.011), and 1.56 (p=0.004). The combined Merlin-SimWalk2 analysis discriminated even more sharply between the two groups with NPL (pair) scores of 0.08, 0.08, and 0.06 in the Afrikaner ancestry sample and 1.56, 1.56, and 2.01 in the British ancestry Caucasian group (see Tables 2.7 and 2.10). The reverse pattern obtains on chromosome 16 with NPL (pair) scores of 0.61, 1.01, and 0.52 as evinced by Merlin in the Afrikaner ancestry subgroup and NPL scores of 0.17, -0.08, and -0.37 in the British ancestry sample (see tables 2.9 and 2.11).

Although there is a dearth of epidemiological studies of BPD in African populations, the author is not aware of evidence to suggest that the disorder is any more or less common in these populations. On the balance of probability, the author suggests that the genetic changes that predispose to manic depressive illness are likely to have arisen before the large scale migration of modern *Homo sapiens* from Africa to Europe, Asia and the New World approximately 100 000 years ago (Stringer and McKie 1996). Extant Caucasian populations presumably carry a proportion of these variants in their gene pool, and founding populations such as the Afrikaner, an even smaller percentage of these polymorphisms or mutations. It is therefore plausible for British Caucasians to possess a disease susceptibility locus on chromosome 10q23.31 that is absent in the Afrikaner population The reverse situation in which the Afrikaner but not British ancestry pedigrees showed evidence of linkage to chromosome 16 markers is most probably a false positive result given the relatively small NPL scores obtained. Nevertheless, it is likely that the UCT British ancestry sample is not representative of all the susceptibility variants found in the British BPD population.

The modest evidence for linkage to 10q23.31 (92.5 Mb) in the British ancestry Caucasian sample echoes most directly the study of Park et al. (2004). These authors carried out a genome-wide scan with 343 markers in 40 Caucasian pedigrees from the United States and Israel, recording a multipoint affected sib-pair NPL score of 1.51 and a single-point parametric LOD score of 1.60 at 80.4 Mb (10q22.2). The interesting discrepancy between the two studies is that Park et al. (2004) defined affected individuals on the basis of psychotic symptomatology whereas the majority

of affected individuals in the UCT sample have not shown evidence of psychosis, especially those patients diagnosed with MDE-R. The issue of psychosis is pertinent because Fallin et al. (2003) produced some of the strongest evidence yet that this region is involved in susceptibility to schizophrenia and schizoaffective disorder. These authors conducted a genome-wide scan in Ashkenazi Jewish families and recorded a maximum NPL (all) score of 3.35 and heterogeneity (HLOD) score of 3.14 under a dominant model of inheritance at 10q22.3. Fine mapping with additional markers in the region increased the NPL score to 4.27 (Fallin et al. 2003). Yet when the same group investigated their sample of Ashkenazi Jewish families for linkage to BPD, only weak evidence for linkage in this region could be obtained (Fallin et al. 2004).

Rice et al. (1997) reported the highest parametric LOD score (3.47) in the region at 75 Mb (see Figure 2.4) under an affection status model that included schizoaffective disorder, BPD I, and BPD II. When the model was broadened to include MDE-R individuals, the score dropped to 2.35.

A host of other studies have implicated a more distal region ranging between 118.6 and 129.4 Mb on the long arm of chromosome 10 (see Figure 2.4). The most promising of these results were those of Cichon et al. (2001) who obtained a maximum parametric LOD score of 2.86 under a broad affection status and dominant model of inheritance, and a maximum Z score of 3.12 at 125 Mb. It seems unlikely that results of this thesis (if genuine) and those of Rice et al. (1997), Park et al. (2004), and Fallin et al. (2003) are reflecting linkage to the same susceptibility locus implicated by Cichon et al. (2001), Kelsoe et al. (2001), McInnes et al. (2003) and other groups. There may thus be two susceptibility loci on 10q, one in the region of 10q22-23 and another at 10q25-26. Further research is necessary to test this hypothesis and possible candidate genes are suggested in the concluding chapter of the thesis.

As mentioned above, weakly significant NPL scores were obtained for the chromosome 16 markers (1-7 Mb) in the Afrikaner ancestry sample (see Tables 2.7 and 2.9). Ewald et al. (2002) reported evidence for linkage to markers in the same region (5.70 Mb) in their Danish pedigrees with a parametric LOD score of 2.23 (see

Figure 2.8) under a recessive model of inheritance. Edenburg et al. (1997) and Dick et al. (2002) reported significant NPL scores of 1.9 and 2.13, respectively at markers approximately 10 Mb away from the UCT peak score (13.6 Mb) in the NIMH series of bipolar pedigrees. The strongest linkage peak in the region was detected at 20 Mb (see Figure 2.8) about 15 Mb distal from the author's weakly significant NPL scores. Maziade et al. (2004) examined 21 pedigrees from Quebec. Seven of these families were predominantly afflicted with schizophrenia, six with BPD, and 8 were characterised by mixed schizophrenia and BPD diagnoses. The BPD pedigrees yielded a modified LOD (MOD) score of 4.05 under a recessive inheritance model. A broad affection status model that included both schizophrenia and BPD yielded a multipoint MOD score of 3.66 (Maziade et al. 2004).

Chromosome 12 yielded suggestive but somewhat contradictory evidence for linkage. The sample as a whole produced Merlin-derived NPL (pair) scores of 1.19 ($p=0.01$) at D12S86 (117 Mb), and 0.25 at D12S1612 (123 Mb). The SimWalk-Merlin statistic was more congruent with NPL (pair) scores of 0.50 and 0.57 at D12S86 and D12S1612. Splitting the sample up into the different ethnic groups helped to elucidate the situation. The group of Afrikaner origin returned Merlin NPL (pair) scores of 0.86 ($p=0.02$) and -0.01 at D12S86 and D12S1612, respectively, while NPL scores of 0.35 and 0.87 ($p=0.02$) were achieved for the British ancestry group at the same markers. The fact that the latter sample returned a statistically significant NPL score for D12S1612, while the Afrikaner ancestry group yielded a considerably higher NPL for D12S86 suggests that the most parsimonious explanation for the discrepant results is undetected genotyping errors at D12S1612 in the Afrikaner ancestry sub-sample which artificially deflated the linkage statistic. In a follow-up study, it is planned to genotype additional markers in this region to clarify the situation especially given the amount of interest that 12q23-24 has generated in the literature (see Figure 2.5). Three different samples have provided strong evidence for linkage to the region around 12q24 (125 Mb) with LOD or NPL scores above 2.5 (Curtis et al. 2003; Ewald et al. 2003; Shink et al. 2004). For details please see section 2.1, above.

A perusal of Table 2.9, above indicates that weakly significantly NPL scores of 0.77 ($p<0.05$) and 0.58 ($p<0.05$) were obtained at the markers D2S2208 and D2S2321 on chromosome 2q33 (208 Mb). Zubenko et al. (2003) carried out a genome-wide scan in

81 families with recurrent major unipolar depression and detected evidence of linkage to an identical region (208 Mb) in the vicinity of the CREB gene. The results obtained at these markers in this thesis may well be a false positive as they are very weak. Nevertheless it is interesting that the suggestion of linkage occurred in the Afrikaner ancestry subgroup. This is because the largest Afrikaner ancestry pedigree in the sample with 77 members has a higher incidence of recurrent unipolar depression than BPD with 20 MDE-R individuals and 5 BPD I members. Perhaps family "30" is genetically closer to the unipolar spectrum illness than BPD *per se*, and this might also account for the disappointing absence of linkage to the other markers typed in the study. It might be useful in follow-up work to type this family for markers that have been specifically linked to unipolar depression.

There are a number of possible reasons for the failure to replicate reports of linkage to regions of chromosome 2, 4, 15, and 22. False negative results may be a consequence of under-powered studies. An important factor determining power is the effect size of the gene or locus under study and because these data are unknown, power cannot be accurately determined. A variant that leads to a small increase in the probability of developing psychopathology may be ubiquitous in the general population and thus difficult to detect using linkage techniques.

The UCT study sample size of 350, although relatively modest, compares favourably with a number of studies that have reported linkage to BPD on chromosomes 2, 4, 15, and 22. Middleton et al. (2004) obtained a Z score of 3.09 on 2q22 with a sample of 233 individuals, and Venken et al. (2005) reported a maximum NPL score of 2.93 between 2q31-35 in a sample of 171 individuals (see Appendix B). On chromosome 4p15-16 significant LOD or NPL scores were obtained by Asherson et al. (1998), Detera-Wadleigh et al. (1999) and Willour et al. (2003) with samples of 171, 396 and 354 individuals, respectively. Significant linkage to chromosome 22q11-12 was reported with samples of 396 and 164 individuals (Detera-Wadleigh et al. 1999 and Kelsoe et al. 2001).

Power is not only a function of sample size but pedigree structure and the density of affected individuals within families. Generally deeper (the number of generations) and larger pedigrees contain more informative meioses and present the researcher with a

better chance of detecting linkage to a locus within the family. In fact, Kwok et al. (1999) argue that most of the significant LOD scores reported in BPD have been obtained with single large pedigrees indicative of an oligogenic effect. A case in point is the study of Blackwood et al. (1996) who obtained a LOD score of 4.1 at 4p16 in a single 120 member-strong family. In this sense the UCT cohort compares favourably with previously published work with a 77-strong family of Afrikaner origin as the *piece de resistance.*

Most of the UCT study families are characterised by a relatively high density of affective illness (see Table 2.4). Approximately 45% of individuals met DSM-IV criteria for BPD I, BPD II or MDE-R, increasing the power of non-parametric analysis. A perusal of Appendix B indicates the density of affected individuals in the UCT sample is equal to or higher than most other published reports in the literature.

At first glance one would assume that power increases with the number of pedigrees included in the study and that the largest published studies are therefore by necessity the most statistically powerful. This is only true however if the different families included in the study share common genetic risk factors. In the presence of genetic heterogeneity - the phenomenon where different genetic variants cause the same disorder in different individuals, families or population groups – analyses of multiple small families may prove fruitless (Terwilliger and Goring 2000). Genetic heterogeneity is an umbrella term for two phenomena: non-allelic heterogeneity where the loci that predispose to illness differ across families and allelic heterogeneity where more than one allele of the same gene predisposes to illness.

The problem of genetic heterogeneity should be ameliorated in the UCT sample, at least within the Afrikaner ancestry subgroup and this further increases the probability of detecting linkage to susceptibility loci if they are present in the population. In sum then, it is unlikely that the failure to replicate previous linkage reports in a number of chromosomal regions is due to a lack of power. The genetically heterogeneous nature of the disorder makes it far more likely that the relevant disease variants are simply not present in this particular South African sample at significant levels.

A possible weakness of this study is the fact that only 2-3 polymorphic markers were typed per region of interest. Assuming that the markers are informative enough (the average heterozygosity of the markers was 0.85) this should not be a problem as long as the true susceptibility locus lies close to the pair or trio of dinucleotide repeats. On the other hand, a susceptibility locus that lies 15-30 Mb from the typed markers may easily be missed because of recombination events in the intervening DNA sequence.

Can this lack of marker coverage account for some of the negative findings in this study? It is certainly plausible but if the reader examines Figures 2.3, 2.7 and 2.9, above, it is clear that the typed dinucleotide repeats lie within 10 Mb of the vast majority of markers that have been linked to BPD on these chromosomes. The typing of additional markers might well have increased the statistical significance of the NPL scores, but whether they would have uncovered latent linkage to BPD on chromosomes 4, 15 and 22 is in the author's opinion doubtful. Negative findings may also have resulted from false positive signals in previous studies.

2.6.2. Association Analysis.

Analysis of the sample as a whole yielded no statistically significant results. However, when the largest Afrikaner ancestry pedigree (family 30) was removed from the analysis significant results were obtained for *COMT* (χ^2=5.97; p=0.0020) and *DAT* (χ^2=7.32; p=0.0410), with the *val* and 10R variants, respectively, as risk factors. The data are not shown in tabular format.

The *COMT* gene is considered to be an excellent candidate for involvement in schizophrenia both because of its monoamine catabolic activity and its position on 22q11, a chromosomal region deleted in velocardiofacial syndrome which is characterised by a high incidence of psychosis (Harrison and Owen 2003). Two case-control association studies detected an association between the low activity *met* allele and schizophrenia although sample sizes were relatively modest. Kotler et al. (1999) typed 92 schizophrenics and 415 controls of various ethnic backgrounds while Ohmori et al.'s, (1998) study included 150 Japanese cases and controls.

On the other hand, Wonodi et al. (2003) reported an association between the high activity *val* allele and schizophrenia and Shifman et al. (2002) in the largest study to date with 720 patients and 4000 controls reported a haplotype which included the *val* allele of the V158M variant to be significantly associated with schizophrenia.

Nevertheless, some of the largest case-control studies have failed to detect a significant effect. Arinami et al. (2001) and Norton et al. (2002) genotyped over 300 cases and 300 controls but there was no evidence that one or other of the *COMT* V158M alleles was over-represented in cases or controls. More recently, Fan et al. (2005) typed 862 schizophrenics and 928 controls in the Han Chinese population and once again failed to detect a statistically significant association between the V158M variant and the disorder although there was a trend towards overrepresentation of the *val* allele in the schizophrenic patients.

The more robust family-based association studies support the recently published case-control studies which suggest that the *val* allele is a risk factor for the schizophrenia. Li et al. (2000) adopted a linkage disequilibrium mapping approach and reported that haplotypes containing the *val* variant of the Val158Met polymorphism were over-transmitted to affected offspring. The *val* variant was similarly implicated by Egan et al. (2001) who also found it to be associated with cognitive deficits characteristic of schizophrenia, and the small sample of Kunugi et al. (1997) was indicative of a trend in the same direction. A recent study provided further support for the notion that the *val* allele is a risk factor for schizophrenia (Ohnishi et al. 2005). The authors demonstrated that schizophrenics with the *val/val* genotype showed significant volume reductions of the left anterior cingulate cortex and right middle temporal gyrus relative to *met* carriers.

The disjunction in outcome between the various published association studies was attributed by Glatt et al. (2003) to genetic heterogeneity across ethnic groups. Glatt et al. (2003) argued that the *val* allele is a small risk factor for the development of schizophrenia in Caucasian populations. The authors also pointed out that the Val158Met variant – though functional – may be in linkage disequilibrium with the true disease susceptibility variant confounding previous association studies. A number of recent studies reporting an association between schizophrenia and SNP haplotypes

in and around the *COMT* gene but not between the V158M polymorphism and the disorder have strengthened this hypothesis (Shifman et al. 2002; Chen et al. 2004; Saunders et al. 2004; Handoko et al. 2005). Like Glatt et al. (2003), Handoko et al. (2005) argue on the basis of their haplotype analysis that the V158M polymorphism is not disease-causing but is in linkage disequilibrium with the true unidentified disease-causing variant approximately 20 kb away.

As discussed in section 2.2, the *met* allele of the *COMT* V158M variant has been implicated in the rapid-cycling form of BPD (Kirov et al. 1998; Papolos et al. 1998). Two undersized studies have also argued that the *met* allele is a risk factor for the development of the more common phenotype (Li et al. 1997; Ohara et al. 1998). These data are at odds with the schizophrenia literature which on balance has implicated the *val* allele in the disorder. Nevertheless, Massat et al. (2005) typed 378 patients with major depression, 506 bipolar individuals and 628 Caucasian controls, and reported an association between the *val* allele and early onset depression.

Harris et al. (2005) reported an association between the V158M genotype and the personality trait intellect/imagination. This time however, the heterozygote group achieved higher scores than either of the homozygote groups, and it is therefore suspected that this is a false positive finding. Nevertheless, a genuine association between *COMT* variants and imagination would provide support to the hypothetical role of this gene in BPD. There is a well known association between creative accomplishment in the arts and affective illness (Andreasen et al. 1987, Jamison 1993), and a similar relationship has been reported in science and politics (Ludwig 1992; Sulloway 1994). Goodwin and Jamison (1990) and Gilvarry et al. (2000) have extended this observation to general intelligence as measured by IQ scores.

A strong association between the 10R allele of the 40-bp VNTR in the 3' untranslated region of the *DAT* gene and BPD was detected (χ^2=6.99; p=0.0082 in the British ancestry sample). Four other studies, three of them published by the Kelsoe group, have implicated this gene in BPD. Kelsoe et al. (1996) first reported that a region close to the *DAT* gene on chromosome 5p15.3 was linked to BPD in their sample. Subsequently, a series of SNPs in the gene were typed and a haplotype comprising 5 SNPs in the 3' region of gene was found to be associated with BPD with a TDT

family-based association analysis of 50 parent-proband trios returning a p-value of 0.001 (Greenwood et al. 2001). Nevertheless, the 40bp VNTR was not itself significantly associated with the disease phenotype. Greenwood et al. (2005) recruited a completely independent sample of 70 trios, genotyping 22 SNPs and the 3' 40-bp VNTR. Again haplotype blocks in the 3' region of the gene were found to be significantly associated with BPD but the VNTR polymorphism was not associated with the disorder *per se*, replicating their original finding.

Greenwood et al. (2005) argue that either the VNTR variant fails to play a causal role in BPD, or if it does exert some kind of effect, then it must act in conjunction with other *DAT* variants. The finding of this thesis that the 10R *DAT* variant is associated with affective illness can be interpreted as contradicting Greenwood et al's (2005) hypothesis. Alternatively, the *DAT* VNTR may be in LD with other causal *DAT* variants in the UCT sample but not in the US populations of Greenwood et al. (2001) and Greenwood et al. (2005).

Horshitz et al. (2005) may have identified one of these potential causal variants. The authors discovered a rare missense mutation which was inherited by a BPD patient from her affected father. The mutation, which is believed to inhibit cell surface expression of the transporter protein, was not detected in any control samples (Horshitz et al. 2005).

Is it reasonable to view the associations between *COMT*, *DAT* and affective illness as genuine given the fact that family 30 was excluded from the association analysis? From the vantage point of the statistician this kind of analysis is tantamount to data manipulation and the results thereof should therefore be treated with scepticism. However, as will be argued throughout this thesis, psychiatric geneticists need to adopt a different mindset to that employed in the identification of monogenic disorders. Genetic heterogeneity in bipolar patients is likely to be significant because of the complexity of neural networks and the molecular pathways upon which these circuits are built. Clearly the arbitrary fractionation of samples will lead to false positive results but if cogent rationale for the subdivision of samples exists, the strategy should not be dismissed. In this case, elimination of one large family that is likely to share particular genetic risk factors seems reasonable, especially since the

family does appear to differ phenotypically from most of the the other bipolar pedigrees. A perusal of Table 2.4 indicates that the majority of families display approximately equivalent frequencies of unipolar and bipolar illness. In family 30, however, 40% of the sample has had at least one episode of unipolar depression, while 8% of the sample has been diagnosed with bipolar illness (data not shown).

Given the argument about genetic heterogeneity, the sample was then divided into families of British and Afrikaner origin. The Afrikaner ancestry sample yielded some weak evidence of an association between the *DRD4* VNTR and the Met129Val *Prion* SNP and BPD. Papassotiropolous et al. (2005) reported that individuals with the *Prion Met* allele recalled more information on a memory task than their *Val/Val* counterparts. There is evidence to suggest that neurocognitive functions like memory are disturbed in BPD (see Chapter 4), but give the fact that the *Met* rather than the *Val* allele was associated with BPD in this sample, and the association was not statistically significant ($p= 0.0820$) this result is unlikely to be meaningful.

The 4R allele of the *DRD4* 48bp VNTR polymorphism was found to be marginally over-represented in affected individuals ($\chi^2=8.52$, $p=0.0742$). There are very little data implicating the *DRD4* gene in the aetiology of BPD and the data that are available tend to suggest that the 2R allele, rather than the 4R allele is the susceptibility variant. As mentioned in section 2.2, Lopez-Leon et al. (2005), in their meta-analysis, detected a significant association between the 2R allele and unipolar depression (OR=1.73) as well as the 2R allele and their combined unipolar and bipolar sample (OR=1.41). However, when the BPD data were analysed separately the trend towards over-representation of the 2R variant was no longer statistically significant (OR=1.26) (Lopez-Leon et al. 2005). Nevertheless, the finding of the UCT study is supported by the family-based association study of Muglia et al. (2002) who argue for an imprinting effect with the 4R allele of maternal but not paternal origin preferentially transmitted to affected offspring.

More interesting results were found in the British ancestry sample. The functional V158M *COMT* polymorphism returned a χ^2 score that was of borderline significance ($p=0.0585$) with the *val* allele over-represented in affected individuals. The *DAT* 10R allele was also found to be over-represented in the affectively ill ($\chi^2=6.99$, $p=0.0082$).

These results support the strategy of excluding family 30 from the analysis as both the British group and the sample as a whole *sans* family 30 showed an association with the *COMT* and *DAT* variants.

While all research has methodological limitations, it is important to emphasize the strength of one's findings. One of the most discussed methodological issues in genetic association analyses of complex traits is that of population stratification. It is often stated in the literature that if two or more different ethnic groups are sampled in a study and if the frequencies of the alleles under consideration differ between these groups, then any observed genotype-phenotype correlations may in fact result from the use of an ethnically stratified sample (although see Cardon and Palmer 2003). The use of the family-based QTDT analysis has circumvented this problem. The significant association between the *COMT* V158M variant and the *DAT* 40bp VNTR cannot be attributed to hidden population stratification. The fact that the association was present in both the British and Afrikaner ancestry sub-samples (without family 30), and that some of the p-values were highly significant ($p < 0.01$) also adds credence to the results.

Given the fact that 11 different genetic variants were tested for association with BPD, it could be argued that the significant *COMT* and *DAT* results are false positives. Theoretically, for each independent statistical test the probability of a false positive result increases by 5% because of the commonly accepted type 1 error rate, and therefore in this case α should be adjusted to 0.005. As will be discussed in more detail in Chapter 3, the Bonferroni correction for multiple testing is overly conservative. Nevertheless, even after correction for multiple testing, the p value of 0.002 obtained for *COMT* remains statistically significant and the p-value of 0.0082 for DAT is very close to significance.

The failure to detect a significant association between BPD and variants of some of the most promising genes such as *BDNF* was disappointing but not that surprising given the contradictory picture of the literature discussed in section 2.2. One potential explanation is lack of statistical power. Genetic association analyses of complex traits are predicted upon the CDCV hypothesis (see Appendix A) and therefore genetic effect sizes are by definition modest with negative implications for statistical power.

If the UCT sample does indeed suffer from a lack of statistical power than the effects exerted by *DAT* and *COMT* on the disease phenotype may be considerably greater than the other variants that were typed.

It could be argued that the investigation of the eleven candidate genes was not thorough enough because only one or two polymorphisms per locus were genotyped. This may be a problem if an unknown variant within the relevant candidate gene is the true risk allele and therefore multiple markers need to be typed to ensure that at least one of them is in LD with the causal variant. However, with the exception of the *DRD2* Taq SNP (which is probably in LD with the functional SNP) and the *Notch4* repeat polymorphism, all of the variants that the author genotyped have been shown to exert some kind of functional effect. If these candidate genes are involved in the aetiology of BPD then it is reasonable to presuppose that that an association with the functional variant should be present. This supposition is supported by the literature reviewed in section 2.2 which has largely implicated these same functional variants in the aetiology of BPD.

In the author's opinion, the most likely explanation for the failure to replicate previously published findings is the existence of multiple subtypes of BPD which are superficially identical but vary genetically. Genetic heterogeneity has been observed in many disorders including some supposedly "simple" mendelian conditions such as Retinitis Pigmentosa (RP).

RP is a mendelian, monogenic high penetrance disorder of the retina which leads to visual deficits and blindness. It is however, genetically heterogeneous. Terwilliger and Goring (2000) note that mutations in 11 different genes cause an autosomal dominant form of RP, 7 different loci result in an autosomal recessive form of the illness and 4 genes on the X chromosome produce a sex-linked form of RP. Terwilliger and Goring, (2000) calculated that because of this heterogeneity a random sample of up to 20 000 affected sibling pairs would be required to obtain a LOD score of 3 for one of these causal mutations! If gene-gene interactions and environmental risk factors are taken into account, the situation is even bleaker.

If this is the level of complexity observed in a phenotypically "simple" and highly penetrant condition affecting the retina then an incompletely penetrant psychiatric disorder associated with functional changes to convoluted neural circuits is likely to be many orders of magnitude more intricate. The implications for genetic heterogeneity are clear and discouraging for gene identification efforts (Terwilliger and Goring, 2000). How can this conundrum be solved?

As stated above, the results of genetic association (and linkage) studies should not be so readily dismissed if there is an inconsistent pattern of replication in the literature. Nevertheless, this does not answer the question of how one should go about remedying the situation. One potential method is to study isolated population groups such as the Afrikaner (Terwilliger and Goring 2000). Promising evidence of linkage to chromosome 1q32 has been detected here but the association analysis was more disappointing. Nevertheless, even within the Afrikaner population genetic heterogeneity may be present. Ascertaining large pedigrees with multiple affected individuals such as family "30" increases the chance that the affected individuals are carrying the same genetic risk factors (Terwilliger and Goring 2000) but in this case the largest pedigree did not provide any highly significant results.

Another strategy is to improve the phenotyping of the sample so that different phenotypic subtypes of BPD presumably underpinned by distinct genetic factors can be identified. These bipolar variants can then be analysed separately improving the chances of detecting linkage or association to genetic variants. This is a good plan in theory but there is no guarantee that a linear relationship between phenotype and genotype is present. The same genetic variant may produce dissimilar bipolar phenotypes in the presence of alternative genetic or environmental backgrounds (Kelsoe 2003).

A subtly different strategy is to search for simpler quantitative traits that are intimately associated with the overall phenotype - the classic reductionistic approach to science. Understanding the genetic basis of these component endophenotypes will theoretically help to unravel the genetic basis of the larger more complex disease phenotype. In Chapter 3 the use of one potential endophenotype, personality is evaluated.

Chapter 3.

Personality as an Endophenotype.

"There are men who, though not quite indifferent or dull, are not markedly affected by joy or sorrow. Others will shout with joy or dissolve in tears at the slightest provocation, and others again are moved by a few things only, but these the more deeply and lastingly. All this indicates that there is something that decides the moods of the soul: this is the degree of vitality of temperament. Heinroth, (1818), quoted in Koukopolous et al. (2001, p329).

List of Abbreviations of Genes Referred to in this Chapter.

BDNF	Brain Derived Neurotrophic Factor
BDNF Val66Met	Functional variant giving rise to 2 alleles: *val* (high activity) and *met*
COMT	Catechol-O-Methyltransferase
COMT Val158Met	Functional variant giving rise to 2 alleles: *val* (high activity) and *met*
DAT	Dopamine Transporter
DAT VNTR	Functional variant with 2 predominant alleles: 9R and 10R
Notch4	CTG Exonic Repeat with alleles: 6R, 9R, 10R, 11R, 12R.
SERT	Serotonin Transporter
SERT 5-HTTLPR	Functional Ins/Del promoter variant producing 2 alleles: *short* (low activity) and *long*.
SERT VNTR	Functional intronic variant producing 3 alleles: 9R, 10R & 12R.

In order to facilitate matters a list of the abbreviations used for the various personality scales is also presented, below. Detailed information is also to be found in Appendix E. The different questionnaires are highlighted throughout the chapter in distinct type-face.

List of Abbreviations of Personality Scales.

Questionnaire	**Abbreviation**
TEMPS-A Cyclothymic Temperament Scale	**CT**
TEMPS-A Dysthymic Temperament Scale	**DT**
TEMPS-A Hyperthymic Temperament Scale	**HT**
TEMPS-A Irritable Temperament Scale	**IT**
TEMPS-A Anxious Temperament Scale	**AT**
TCI Novelty Seeking	NS
TCI Harm Avoidance	HA
TCI Reward Dependence	RD
TCI Persistence	P
TCI Self-Directedness	SD
TCI Cooperativeness	C
TCI Self-Transcendence	ST
Hypomanic Personality Scale	HPS
Affective Neuroscience Personality Scale Seek	*Seek*
ANPS Fear	*Fear*
ANPS Care	*Care*
ANPS Anger	*Anger*
ANPS Play	*Play*
ANPS Sadness	*Sadness*
ANPS Spirituality	*Spirit*

Aspects of this chapter of the thesis are adapted from Savitz & Ramesar (2006). Personality: Is it a viable endophenotype for genetic studies of bipolar affective disorder? *Bipolar Disorders.* In Press.

3.1. The Concept of the Endophenotype.

As discussed previously, the promise of psychiatric genetics remains unfulfilled as researchers grapple with the difficulties of dealing with phenotypically and genetically heterogeneous conditions, controlling for gene-environment interactions and uncovering the contributions of multiple loci of small effect size. BPD is no exception to this pattern. Part of the difficulty may be that the DSM-IV criteria traditionally used in genetic studies are not reflective of "natural types" of psychopathology, that is, they do not correspond with the underlying neurobiology of psychiatric disorders (Gottesman and Gould 2003; Craddock et al. 2004). Geneticists have thus searched for alternative methods to phenotype study participants, culminating in the current popularity of the endophenotypic approach. In fact, a National Institute of Mental Health (NIMH) workgroup has recently recommended endophenotypes be used as an adjunct to genetic investigations of affective disorders (Merikangas et al. 2002).

The concept of the endophenotype, borrowed from evolutionary genetics and applied to psychiatry by Gottesman and Shields (1973) initially referred to the biochemically based identification of hidden internal phenotypes. The notion has increased in sophistication in recent years as the use of endophenotypes becomes more common. For a detailed review of the concept see Gottesman and Gould (2003).

An endophenotype is a trait that lies somewhere on the developmental pathway from genes to phenotype (Gottesman and Gould 2003). In other words, if the psychiatric illness of interest is the DSM-IV defined phenotype – the final product of different genetic and environmental factors, then the endophenotype is a more elementary trait that is correlated with the phenotype. The assumption here is that the reduced phenotypic complexity of an endophenotype is reflective of a more basic aetiological process and should therefore be less refractory to genetic investigation.

Gottesman and Gould (2003) list five conditions that need to be satisfied in order for a trait or marker to be classified as an endophenotype:
(1). The trait must be associated with the illness in the relevant population.

(2). The trait must be largely state independent, manifesting in the individual during both periods of health and illness.
(3). The trait must be heritable.
(4). Within families, the trait and illness should co-segregate.
(5). The trait found in affected individuals should be found in non-affected family members at a higher rate than in the general population.

In the following section these five criteria are used to evaluate the feasibility of using personality as an endophenotype for genetic studies of BPD. Before evaluating these data the issue of temperament and personality needs to be addressed. Some researchers make a hard distinction between temperament, which is usually described as the innate proclivity of the nervous system to respond to stimuli in a consistent manner, and personality, which is reflective of the adaptation of the individual to environmental contingencies (Cloninger et al. 1993). In reality, like the notion of "nature versus nurture", the distinction between temperament and personality is heuristic: genetically mediated tendencies modify the effects of environmental exposures (Plomin and Daniels 1987; Plomin and Bergeman 1991). While this thesis reviews studies addressing both "temperament" and "personality" in BPD, for the purposes of evaluating the endophenotypic approach, these two terms are used synonymously.

3.2. Personality as an Endophenotype.

3.2.1. Are Specific Personality Traits Associated with BPD?

The recognised association between affective illness and temperament can be traced back to ancient Greece and Rome (Angst 2000).

"Those prone to the disease [BPD] *are such as are naturally passionate, irritable, of active habits, of an easy disposition, joyous, puerile: likewise those whose disposition inclines to the opposite condition, namely, such as are sluggish, sorrowful, slow to learn, but patient in labour, and who when they learn anything soon forget it; those*

likewise are more prone to melancholy who have formerly been in a mad condition". Aretaeus of Cappadocia (AD 30-90) cited in (Angst 2000), p178.

The interplay between pathology and temperament found resonance in the writings of nineteenth century European psychiatrists such as Esquirol, Falret, and Kahlbaum, but was epitomised by Kraepelin's seminal work on manic-depressive insanity (MDI) (Angst 2000). Kraepelin viewed major depression and mania as manifestations of an identical underlying pathology which is genetic in aetiology and expressed in the form of multiple phenotypes or clinical subtypes (Akiskal and Pinto 2000). While these variants of MDI are not always fully penetrant, the inchoate expression of morbidity is discernible in its temperamental guise (Akiskal and Pinto 2000).

"There are certain temperaments which may be regarded as rudiments of manic-depressive insanity. They may throughout the whole of life exist as peculiar forms of psychic personality without further development; but they may also become the point of departure for a morbid process which develops under peculiar conditions and runs its course in isolated attacks". (Kraepelin 1921) p 118.

Four dispositions or temperaments: depressive (**DT**), hyperthymic (**HT**), cyclothymic (**CT**), and irritable (**IT**), were asserted by Kraepelin to predispose to MDI. See Appendix E for a description of these temperament types. Kraepelin found that the **DT** was more common in a largely depressive form of MDI, while predominantly manic patients tend to be hyperthymic or irritable in character. The **CT** appears to be predominantly associated with a predilection for the classical form of the illness (Kraepelin 1913 cited in Angst 2000). The last two decades have witnessed a plethora of studies examining the association between these temperament types and affective illness. It should be noted that at present the terms dysthymia and cyclothymia refer to both DSM-IV defined sub-affective disorders as well as particular temperament types, and this is reflected in the data below.

(a). The Dysthymic Temperament (DT).

The person with a **DT** is described by Akiskal and Mallya (1987) as gloomy, pessimistic and incapable of fun; quiet, passive and indecisive; broody and given to

worry. In Cassano et al.'s (1992) sample, approximately 40% of the BPD I and 20% of the BPD II group presented with **DT**. A high prevalence of **DT** and double depression was also characteristic of a sample of depressed patients who exhibited anti-depressant induced hypomania (Akiskal et al. 2003b). In Haykel and Akiskal's (1999) sample, dramatic hyperthymic switches occurred in 12% of the dysthymic subjects. In fact, Klein et al. (1988) and Rihmer (1990) cited in Akiskal (2001) suggest that brief hypomanic switches occur in up to 30% of dysthymic patients. Iatrogenic hypomania may be indicative of a BPD diathesis (Akiskal et al. 2003b; Benazzi 1997; Altshuler et al. 1995). More recently, Evans et al. (2005) reported higher scores on the **DT** subscale of the TEMPS-A in their sample of 155 BPD I and II patients and 63 unrelated controls. Similar results were obtained by Mendlowicz et al. (2005) in their sample of 23 remitted BPD subjects and 102 unrelated controls.

(b). The Cyclothymic Temperament (CT).

Individuals with a **CT** vacillate between being grandiose, talkative and creative to suffering from pessimism, low self-esteem and mental confusion (Akiskal & Mallya, 1987). A **CT** is most commonly associated with BPD II, with 44% of Hantouche et al.'s, (1998) 100 strong sample displaying cyclothymic characteristics, and 88% of cyclothymes diagnosed with BPD II. Benazzi and Akiskal (2005) replicated the results of Hantouche et al. (1998) and postulate that trait mood lability is a pathognomonic sign of BPD type II. Akiskal et al. (2003a) argue for the existence of a variant of BPD II, dubbed BPD 2.5 underpinned by a **CT**, and characterised by a "darker", unstable, irritable risk-taking profile that is confused with an erratic personality disorder. Again Evans et al. (2005) and Mendlowicz et al. (2005) demonstrated in controlled studies that BPD patients scored significantly higher on the **CT** scale than either unaffected relatives or controls.

(c). The Hyperthymic Temperament (HT).

HT individuals are usually irritable, cheerful, extraverted, bombastic, loquacious and insubordinate (Akiska and Mallya 1987). Cassano et al. (1992) found comparable rates of **HT** (25%) in both BPD I and BPD II groups. Hantouche et al. (1998) reported that 15% of their sample of BPD II individuals displayed a **HT**, and similarly, of 28

patients classified by Perugi et al. (1998) as BPD II, 5 (18%) had a **HT**. While melancholic and anxious personality traits (DT) are more common in unipolar depression, manic personality traits (**HT**) were reported by Hecht et al. (1998) to aggregate in BPD II and particularly BPD I groups. In a similar vein, Henry et al. (1999) argued that the presence of a **HT** predisposes patients with BPD to suffer from a greater number of manic episodes.

Methodologically superior studies employing a control group have nevertheless produced mixed findings. In their comparison of 100 BPD patients and 100 controls, Kesebir et al. (2005), found an excess of **HT** in the patient group. Three large controlled studies have however, shown hyperthymic traits to be elevated in controls compared to BPD patients (Evans et al. 2005; Mendlewicz et al. 2005; Matsumoto et al. 2004).

(d). The Irritable Temperament (IT).

Kraepelin characterised the irritable disposition as the co-occurrence of depressive and hyperthymic temperaments (Akiskal 1981). The **IT** as defined by the Temperament Evaluation of Memphis, Pisa, Paris and San Diego (TEMPS-A) or its forerunners appears to be rare with a non-gaussian distribution so that only about 2% of the population score above the second standard deviation (Placidi et al. 1998). Individuals with this temperament are often labelled as borderline personalities and they usually suffer from major depressions interspersed with hypomania and mixed episodic features (Akiskal 1995). Akiskal (1981) and Akiskal et al. (1985b) suggest that the DSM category of borderline personality disorder is not representative of a specific psychopathological syndrome, and that most cases can be subsumed under a BPD nosology. Both Evans et al. (2005) and Mendlowicz et al. (2005) found higher levels of **IT** in their samples of remitted patients with BPD relative to healthy relatives and unrelated controls.

While Akiskal and colleagues have pursued the traditional European model of affective temperaments, an alternative approach has been the administration of self-report questionnaires based cognitive and bio-genetic paradigms, which putatively measure the underlying factors that capture most of the variation in personality. Three

models in particular have achieved pre-eminence: Eysenck's tridimensional model (neuroticism, extroversion, and psychoticism), the Five Factor Model (FFM) (Costa and McCrae 1989) measuring extroversion, neuroticism, conscientiousness, agreeableness and openness to experience, and the Biopsychosocial Model (Cloninger et al. 1993) which is measured by the Temperament and Character Inventory (TCI).

Cloninger et al. (1998) assessed 804 adults from the general population in an attempt to describe **DT, CT, HT** and **IT** with the TCI. Those individuals who scored low on all three character dimensions of the TCI (SD, C, ST) are described in Cloninger et al. (1998) as depressive personalities. Individuals with **HT** in contrast, score highly on all three character dimensions. The **CT** type is theoretically low in SD but high in C and ST, while the **IT** is low in SD and ST but high in C (Cloninger et al. 1998).

Unfortunately, there are no studies which have specifically set out to examine the prevalence of these higher order character traits in BPD samples. Nevertheless, Engstrom et al. (2004) in a comparison of 100 BPD I cases and 100 controls reported that the former group were significantly higher in HA, but lower in RD, SD and C. Nowakowska et al. (2005) found higher ST but lower SD in their BPD sample while Evans et al. (2005) also detected higher levels of ST and lower SD and C among their BPD group.

Most studies have highlighted the temperament dimension of negative affectivity. Negative affectivity is described by the Eysenck Personality Inventory (EPQ) and the FFM as neuroticism: a higher order trait that measures a predisposition to experience anger, anxiety, depression, guilt and other negative emotions or cognitions. The TCI comprises a similar scale called HA which is purported to measure the tendency to avoid punishment, non-reward or novelty.

The association between high levels of neuroticism or anxiety-related traits and BPD has been replicated by many investigators (Evans et al. 2005; Engstrom et al. 2004; Osher et al. 1996; Solomon et al. 1996; Strakowski et al. 1992; Young et al. 1995; Matussek and Feil 1983). In one of the more interesting studies, Nowakowska et al. (2005) compared 49 BPD patients to 32 students in creative disciplines and 47 healthy

controls. The BPD and creative groups showed higher levels of neuroticism and conscientiousness as defined by the FFM and increased HA as evinced by the TCI. In a well controlled study, Evans et al. (2005) examined about 150 patients with BPD and 63 controls detecting higher levels of HA among the BPD subjects.

The next strongest finding in the literature concerns traits such as disinhibition, novelty seeking and extroversion. Cronin et al. (1992) reported that their sample of 14 hospitalised patients scored higher than a control group of 17 hospital staff on the Sensation Seeking Scale. Young et al. (1995) obtained similar results with the TCI in a larger sample of patients and controls. Nowakowski et al. (2005) reported that both their BPD and creative groups displayed higher levels of NS than background controls, and Evans et al. (2005) also found evidence for elevated NS in their sample. Janowsky et al. (1999) found higher levels of NS in their BPD group relative to unipolar subjects but the absence of a control group limits the conclusions that can be drawn from this study. Osher et al. (1999) reported *lower* NS in their sample of euthymic patients but population norms were used instead of a control group.

Studies of paediatric BPD patients although limited, broadly support the data from adult research. High levels of emotional reactivity - defined as the tendency to have intense emotional reactions that are difficult to soothe – a trait that incorporates aspects of neuroticism or HA are commonly reported (Hirshfeld-Becker et al. 2003). NS, operationalised in young children as the tendency to approach new situations, rigidity and dysphoric mood states were also found to be elevated in the offspring of BPD patients (Chang et al. 2003a).

3.2.2. Are the BPD Associated Personality Traits State Independent?

It is a well established fact that scores on self-report questionnaires are sensitive to the effects of mood, especially depression (Coppen et al. 1965; Knowles et al. 1965; Kendell et al. 1968; Kerr et al. 1970; Akiskal et al. 1983; Brown et al. 1992; Joffe et al. 1993) and thus in order to qualify as an endophenotype, mood-independent personality trait-BPD associations will have to be demonstrated. This can be demonstrated in one of two ways: (a). An assessment of euthymic patients with BPD,

and (b) an analysis of the premorbid personality features of individuals who go on to develop the disorder. The literature covering the former will be discussed first.

(a). Personality Traits in Euthymic Patients.

Despite the fact that the acute effects of depression result in inflated scores on scales measuring traits like neuroticism and HA, individuals with BPD still appear to be more emotionally unstable or anxious than their unaffected counterparts even when tested in a euthymic state (Evans et al. 2005; Engstrom et al. 2004; Nowakowska et al. 2005; Young et al. 1995; Osher et al. 1996; Solomon et al. 1996).

The weight of evidence also indicates that euthymic BPD individuals tend to be more extraverted and inclined towards NS than controls or unipolar depressives (Nowakowska et al. 2005; Henry et al. 2001; Bagby et al. 1996; Young et al. 1995). Bagby et al. (1996) and Nowakowska et al. (2005) reported higher "openness to experience" to be associated with BPD although these studies are limited by the absence of a normal comparison group. High "openness to experience" individuals tend to be imaginative, aesthetically sensitive, curious and attentive to inner feelings, (Bagby et al. 1996).

(b). Longitudinal Research Assessing Premorbid Personality in BPD.

The hypothesis that particular personality types predispose to affective illness is best assessed by rigorous prospective research (Akiskal et al. 1983). Very few studies have examined risk factors for BPD *per se*. In their longitudinal analysis of 6315 army conscripts, Angst et al. (1986) found that the 16 individuals who later developed BPD, scored in the normal range on all scales of the Freiburg Personality Inventory. In their later follow-up study of 591 individuals between 20 and 35 years of age, emotional lability was reported to be a risk factor for BPD (Angst et al. 2003). Akiskal et al. (1995) carried out an 11-year prospective study which examined the characteristics of patients with unipolar depression who later developed BPD II. Those individuals with high TCI C (dependent on others) and ST (imaginative) scores were more likely to develop hypomanic symptoms over time.

Longitudinal research has been dominated by temperament-orientated research which indicates that the association of **DT, CT, HT** and **IT** with BPD predates the onset of illness and is therefore unlikely to be an artefact of mood.

Kraepelin and Akiskal point out that in a minority of cases dysthymia may constitute the premorbid temperament of manic depressive illness (Akiskal et al. 1978). Kovacs et al. (1994) monitored a cohort of dysthymic children and reported that 76% subsequently developed a major depressive episode, and 13% were diagnosed with BPD after a 3-12 year interval.

Akiskal et al. (1977) followed 46 individuals with cyclothymia over a 2-3 year period and reported that 44% of the group experienced anti-depressant associated hypomania, and 35% developed full-blown major depression, hypomania or mania. In a later study, Akiskal et al. (1979), longitudinally assessed a clinical sample of diagnosed cyclothymic patients and found that 25% later suffered from major depressive breakdowns, and 6% developed manic episodes. These data are congruent with Depue et al.'s, (1981) prospective report which concluded that approximately one third of patients with cyclothymia develop major depression and hypomanic episodes over time.

More recent research has differentiated between BPD I and II subtypes. In a prospective study of 559 patients labelled as unipolar at entry, high trait mood lability was found to be predictive of the conversion of diagnosis to BPD II over the 11 year period (Akiskal et al. 1995). In a prospective study of 80 depressed children and adolescents, a **CT** at baseline was also shown to predict a switch to BPD by the end of the follow-up period (Kochman et al. 2004).

Kwapil et al. (2000) compared groups of high and average scorers on the Hypomanic Personality Scale (HPS) (which is nominally equivalent to the Akiskalian **HT**) after a thirteen year hiatus, and reported that 25% of the high scorers, and 0% of the average scorers qualified for a BPD diagnosis. In a similar study, Meyer and Hautzinger (2003) found that high scorers on the HPS suffered more manic or hypomanic episodes, but not more depressive episodes. Thus the rate

of bipolar disorders in the risk group was 20.8% compared to 1.3% in the control group, but the two groups did not differ in their incidence of unipolar depression (Meyer and Hautzinger 2003). A more recent prospective study by the same group showed that individuals who scored on the upper decile of the HPS were more prone to develop hypomanic or manic symptoms two years later (Blechert and Meyer 2005).

The author is aware of only one study that has prospectively analysed individuals with an **IT**. In a sample (N=100) of borderlines followed by Akiskal et al. (1985b) for up to 6 years, 66 met criteria for recurrent depressive, dysthymic, cyclothymic or BPD II disorders.

3.2.3. Is Personality Heritable?

Heritability is usually defined as the extent to which phenotypic variation is accounted for by genetic variation. Twin studies have suggested heritability estimates for the FFM (Costa and McCrae 1989) personality traits of between 40 and 60% (Loehlin 1992; Jang et al. 1996; Riemann et al. 1997). Almost identical results have been reported for the TCI's four dimensions of temperament (Cloninger et al. 1994). Adoption studies yield lower estimates – in the region of 30% - possibly because of non-additive genetic variance (Bouchard and Loehlin 2001).

The behavioural genetics data are congruent with molecular genetic work that has allowed for the identification of genetic variants that appear to influence specific personality traits. Lesch et al. (1996) and Ebstein et al. (1996) first reported that anxiety-related and novelty-seeking related traits were associated with polymorphisms of the serotonin transporter (*SERT*) and dopamine four receptor (*DRD4*) genes, respectively. In the interim, a plethora of positive and negative findings have been reported although the original findings appear to be genuine (Savitz and Ramesar 2004). Clearly personality traits are at least partly influenced by genetic factors but the role of environmental factors in the development of personality cannot be dismissed.

3.2.4. Does Personality and BPD Illness Co-segregate in Families?

No single premorbid personality profile is pathognomonic of BPD. Temperament orientated research has demonstrated that four different temperaments – **DT, HT, CT,** and **IT** – can all give rise to the full spectrum of affective illness. Anxiety-related traits, tendencies towards NS, extroversion and ST as well as reduced SD and C have also been associated with BPD but are unlikely to all be elevated or reduced in a single individual. Nevertheless, different forms of affective illness may coalesce around each of these temperaments or personality traits.

3.2.5. Are Particular Personality Traits Found in Unaffected Family Members More Frequently Than the General Population?

Rosenthal et al. (1981) reported that dysthymics with stable personality traits and a truncated rapid eye movement (REM) sleep latency had a family history of affective illness. About 30% of the sample had relatives with unipolar depression, and 10% had kin with a BPD history. Akiskal et al. (1985a) examined the juvenile offspring or siblings of BPD probands, and discovered that 12 out of 68 subjects met their criteria for dysthymia. Evans et al. (2005) found that unaffected relatives scored midway between BPD individuals and unrelated controls on the **DT** scale.

Akiskal et al. (1977) found that the prevalence of bipolar disorders in the biological relatives of patients with BPD and cyclothymia was almost identical. Klein et al. (1986) evaluated this putative familial relationship and found that nine out of 37 BPD offspring, but no control group offspring met criteria for cyclothymia. In a similar family-based study, Maier et al. (1995) showed that a **CT** as well as a higher rate of compulsive personality disorder and rigidity was found in excess among healthy relatives of BPD probands relative to a control group. In another controlled study, Chiaroni et al. (2005) assessed the temperaments of 100 normal individuals with a negative family history of affective disorders, 37 symptom-free individuals with a family history of unipolar depression and 40 symptom-free volunteers with a BPD family history. **CT** traits were highest in the group with a BPD family history, intermediate in the unipolar group and lowest in controls (Chiaroni et al. 2005). Mendlowicz et al. (2005) compared 23 remitted patients with BPD to 52 unaffected

BPD relatives and 102 normal controls. Once again **CT** scores were highest in the BPD probands followed by their relatives and the control group. Identical results were obtained by Evans et al. (2005) in their study of 85 BPD families and 63 controls.

Hoffmann (1921) and Leonhard et al. (1962), in early explorations of Kraepelin's theory, both suggested an excess of **HT** in the first-degree relatives of their BPD probands. A number of decades later, Weissman et al. (1984) similarly concluded that **CT** and **HT** aggregate in the relatives of BPD I probands.

In a more recent controlled study, Kesebir et al. (2005) compared 100 BPD probands and their 219 unaffected first degree relatives to a control group without any family-history of BPD, and reported an excess of **HT** in BPD subjects and their relatives. Mendlowicz et al. (2005) and Evans et al. (2005) however, reported significantly higher **HT** scores in their normal control group compared to BPD patients and their unaffected relatives.

In a sample (N=100) of borderlines followed by Akiskal et al. (1985b) for up to 6 years, 66 met criteria for recurrent depressive, dysthymic, cyclothymic or BPD II disorders. In addition, many of these patients had a positive family history of BPD and presented with pharmacologically induced hypomania (Akiskal et al. 1985b). Evans et al. (2005) found that unaffected relatives obtained higher scores on the **IT** scale than background controls.

3.3. Genetic Factors Involved in Personality.

As part of their analysis of the literature, Savitz and Ramesar (2004) reviewed 36 studies that examined the relationship between the *SERT* 5-HTTLPR polymorphism and anxiety-related personality traits. Of these analyses, 18 reported positive results, although in 6 instances the observed association was in the "wrong" direction, leaving a total of 12 studies (33%) that suggested a relationship between the 5-HTTLPR and anxiety-related personality traits. It was argued that although the surfeit of small sample-size studies reporting positive results is a cause for concern, gene-environment interactions, genetic heterogeneity and variation in genetic background,

may introduce noise into the analysis of data, concealing a true association (Savitz and Ramesar 2004).

A recent negative report, however, deserves mention. Willis-Owen et al. (2005) tested two independent samples with the EPQ: 564 extremely concordant and discordant sibling pairs, and 1001 individuals with extreme scores (below the 5th and above the 95th percentiles) on the questionnaire. The latter group was derived from a quite extraordinary sample of 88 142 individuals. No significant association between the 5-HTTLPR variant and neuroticism was observed despite calculations indicating 100% power to detect an effect size of 0.5% of the phenotypic variance (Willis-Owen et al. 2005). As will be argued throughout this thesis, however, the use of very large sample sizes does not necessarily guarantee success because of the confounding influence of genetic heterogeneity.

The *SERT* intron 2 VNTR has also been investigated in personality research. Out of nine studies that examined the relationship between the *SERT* VNTR polymorphism and anxiety-related traits, only three reported statistically significant results (Savitz and Ramesar 2004).

Savitz and Ramesar (2004) also found that 18 out of 37 studies addressing the role of the dopamine 4 receptor gene (*DRD4*) VNTR polymorphism in personality report statistically significant results. Seventeen out of these 18 studies found an association between either the 7R allele or "long alleles" and NS related personality traits, producing an overall "positive" rate of 46%. Again it was concluded that gene-gene interactions, genetic heterogeneity, gene-environment interactions and lack of statistical power may account for the negative findings reported in about half the published studies. The accuracy of phenotypic measurement is also a perennial cause for concern. Of greater relevance to this thesis is a study by Rogers et al. (2004) who reported an association between the short allele (or genotype) and elevated NS in a 267 strong sample of BPD patients and their relatives.

The Catechol-O-Methyltransferase (*COMT*) Val158Met variant may also moderate the development of personality traits. Benjamin et al. (2000) reported a three-way

interaction between *COMT*, the *DRD4* and *SERT* and this was replicated by Strobel et al. (2003). These researchers found that a variant of *DRD4* predisposes to higher levels of novelty seeking traits in the presence of the *long/long SERT* genotype and the *val/val COMT* genotype.

Monoamine Oxidase A (*MAO-A*) has also been implicated in aggressive and impulsive behaviours, including suicidal behaviour (Du et al. 2002). Manuck et al. (2000) argued that the low activity 3R and 5R alleles which result in greater concentrations of catecholamines, were associated with low aggressivity as evinced by a self-report questionnaire. On the other hand, Caspi et al. (2002), in a longitudinal study, demonstrated that maltreated children with the high activity *MAO-A* VNTR alleles, were less likely to exhibit violent behaviour. Newman et al. (2005) also demonstrated evidence of a gene-environment interaction in Rhesus monkeys: monkeys reared by their mothers were most aggressive if they carried a low activity *MAO-A* allele, while peer-reared monkeys with low MAO activity demonstrated the least degree of competitive aggression.

The brain derived neurotrophic factor (*BDNF*) Val66Met polymorphism has also been implicated in personality. Sen et al. (2003) reported that the *met* allele and *met/met* genotype was associated with lower FFM neuroticism scores and hence may be a protective factor against depression. These data were replicated by Lang et al. (2005) and extended by the finding that lower *BDNF* concentrations in the serum samples of healthy subjects predict higher levels of neuroticism (Lang et al. 2004). It is unclear however, why the low activity *met* variant is associated with lower neuroticism scores when low serum concentrations of *BDNF* predict higher levels of neuroticism. A study in the Japanese population failed to reproduce the aforementioned correlation with anxiety-related traits but the *met/met* group achieved higher scores on the extroversion scale of the FFM (Itoh et al. 2004).

The dopamine two receptor gene (*DRD2*) has attracted more interest in domains other than personality. It is hypothesised to act as a reinforcement or reward gene, and it has been implicated in a plethora of conditions: from alcoholism and drug abuse through to obesity and pathological gambling (Noble 2000). The role of *DRD2* in personality is equivocal. Farde et al. (1997) found a significant correlation between D2 receptor

density in the putamen and scores on the detachment scale of the Karolinska Scales of Personality; a result replicated by Breier et al. (1998). Since then a number of researchers have examined the relationship between *DRD2* polymorphisms and personality traits, with mixed results. Noble and colleagues (Noble et al. 1998; Berman et al. 2002) have suggested that the TaqA1 allele may predispose individuals to substance abuse through its effects on the development of novelty seeking and harm avoidance personality traits.

In summary then, the genetic variants most favoured by the literature for playing a role in the development of personality traits are listed below.

SERT 5-HTTLPR: *Short* Allele ----------> Anxiety-related traits.
DRD4 VNTR: 7R Allele ----------> Novelty Seeking-related traits.
MAO-A VNTR: Low Activity Variants ------> Aggressive traits.
BDNF Val66Met SNP: *Val* Allele ----------> Anxiety-related traits

3.4. Rationale.

The above review of the literature indicates that personality is at least partly heritable, that particular temperaments or personality traits aggregate in individuals with BPD, and that these traits are found in unaffected relatives at a greater frequency than in the background population. These temperamental peculiarities may constitute a more penetrant expression of the emotional dysregulation that characterises affective illness (Savitz and Ramesar 2006). Given this assumption, it is hypothesised that various measures of temperament could be used as an alternative to DSM-IV diagnoses in molecular genetic analyses.

The use of DSM-IV categories in genetic analyses are potentially problematic because given the psychiatric community's limited aetiological understanding of psychiatric illness, patients are grouped together on the basis of signs and symptoms which may have very different causes. As Kelsoe (2003) points out, BPD is most probably a syndrome – a collection of superficially similar conditions with distinct aetiologies. In the genetic parlance, BPD is a genetically heterogeneous condition. Grouping large

samples of DSM-IV defined patients together will therefore diminish the potential for detecting genetic association or linkage as the effects of different risk alleles neutralise trends in the pattern of data.

Theoretically, an endophenotype or intermediate trait should be less genetically heterogeneous than the psychiatric disorder so that grouping patients together on the basis of the endophenotype will increase the statistical power needed to detect underlying genetic effects (Gould and Gottesman 2006). In this chapter of the thesis this hypothesis is tested by using various personality traits as quantitative markers of different types of BPD illness.

3.5. Methodology.

3.5.1 Subjects.

The personality assessment component of the research was approved by the UCT research ethics committee (ref 269/2002) and all participants signed the relevant consent forms. The same group of probands and their family members on which the traditional binary linkage and association analyses were conducted (described in Chapter 2), participated in the endophenotype study. The original cohort of BPD pedigrees were recruited between 1997 and 2000, and in the intervening years natural attrition of the sample took place through death, illness and emigration. Some members of the UCT cohort also refused to participate in the follow-up study and thus personality data were not available for all 350 individuals. At least some personality data were obtained from 266 members of the cohort (76%). The exact figure varied from one questionnaire to another because of incomplete or incorrect entries. The reasons for the missing data were as follows: refusal to participate 40 (11.4%); deaths 22 (6.3%), emigration 18 (5.1%), and physical illness 4 (1.1%).

3.5.2 Genotyping.

The same genetic variants and therefore genotyping methods described in Chapter 2 apply to this section of the thesis.

3.5.3. Psychometric Measures.

Seven different personality questionnaires comprising a total of 589 items were administered by the author. For details concerning the type of traits measured by each questionnaire as well as the relevant psychometric data, the reader is referred to Appendix E.

The personality questionnaires were translated into Afrikaans and then back-translated into English by a professional translator. However, because English is the *lingua franca* of South Africa and most of the Afrikaner cohort is fully fluent in English, many of these individuals in any case elected to complete the original versions of the questionnaires. The scoring of the TCI is automated, while the other questionnaires were scored by hand by the author. The following questionnaires were used in the study.

(1). The Temperament and Character Inventory (Cloninger et al. 1994).
(2). The Hypomanic Personality Questionnaire (Eckblad and Chapman 1986).
(3). The Affective Neuroscience Personality Scale (Davis et al. 2003).
(4). *The Temperament Assessment of Memphis, Pisa, Paris, and San Diego Auto-questionnaire (TEMPS-A) (Perugi and Akiskal 2002).*
(5). *The Schizotypal Traits Questionnaire* (Claridge and Brocks 1984).
(6). *The Beck Depression Inventory (BDI)* (Beck and Steer 1993).
(7). *The Altman Self-Rating Mania Scale (ASRM)* (Altman et al. 1997).

3.5.4 Procedure.

All affected individuals were tested in a euthymic or at least relatively euthymic state. Very few participants had recently been hospitalised and in fact numerous individuals had been stable for many years. The majority of individuals were tested individually at their homes. A small minority completed the questionnaires in a counselling room in the Division of Human Genetics at UCT. The battery of questionnaires took approximately 1-2 hours to fill in, depending on the level of functioning of the research participants. Some patients became too tired to complete the questionnaires

in one sitting, and were allowed to take the forms home to finish in their own time. These were then posted or couriered back to the research centre. In addition, a number of individuals who lived in remote areas of the country were contacted telephonically and asked to participate in this section of the BPD study. Questionnaires were sent to these individuals by post and the return of completed questionnaires was facilitated by a courier service.

3.5.5. Data Analysis.

A mixed-model Analysis of Variance (ANOVA) was used to compare personality scores across the various diagnostic groups. The following covariates were entered into the model: age, gender and self-rated depression and mania scores. Family of origin was used as a random factor. In order to calculate whether a number of underlying factors could account for the majority of variation in personality scores across the seven different questionnaires, a principle components analysis (PCA) was conducted on the data. Means and standard deviations were rescaled for each personality instrument.

A Spearman's rank correlation was used to examine the correlations between the various personality endophenotypes. This statistic was used because a small number of the variables did not have a completely symmetrical distribution.

The QTDT (Abecasis et al. 2000) is designed to test for association or linkage between a quantitative trait and a particular genetic marker. Association is calculated by testing if the mean trait value differs between alleles of the marker of interest while taking the degree of relatedness between individuals in the family and other covariates into account. Linkage on the other hand is calculated by examining the variance of the trait at each allele. The smaller the spread of the trait values at each allele, the greater the evidence for linkage (Abecasis et al. 2000).

The QTDT also provides a measure of the heritability of a quantitative trait by comparing a null model which assumes that none of the statistical variance in the trait is due to genetic factors to the alternative model that the trait variance has both an

environmental and a genetic (polygenic) component. Here the polygenic component is the degree of covariance of the trait inside families. High heritability scores are reflective of a situation in which trait values correlate strongly with the degree of genetic relatedness inside families. In other words, a low heritability score indicates that distantly related individuals in a pedigree are just as likely as closely related individuals to achieve similar scores on the relevant trait.

The *MAO-A* gene is located on the X chromosome and cannot therefore be analysed using the QTDT algorithm. It was therefore decided, in consultation with Goncalo Abecasis, to use a mixed-model ANOVA with family of origin entered into the model as a random factor in order to control for the fact that individuals in the sample were related to each other. Age, gender, ethnicity, and self-reported depression and mania scores were used as covariates.

3.6. Results.

A mixed-model ANOVA was run on the data in order to examine personality differences across the various diagnostic groups. The BPD I and MDE-R groups scored higher than unaffected family members (controls) on the **CT** subscale of the TEMPS-A ($F=5.34$, $p<0.0001$) and the *Fear* subscale of the ANPS ($F=3.0$, $p=0.0241$). The same diagnostic groups also scored significantly lower on the SD subscale of the TCI ($F=2.0$, $p=0.0394$)

The BPD I, BPD II and MDE-R groups achieved higher scores than the control group on the **IT** scale of the TEMPS-A ($F=5.52$, $p<0.0001$) and the borderline personality scale (STB) ($F=4.29$, $p=0.0005$). The BPD but not depressive groups scored higher than the control sample on the HPS ($F=6.0$, $p<0.0001$). While the MDE-R group was more anxious than their counterparts as evinced by the **AT** scale of the TEMPS-A ($F=2.35$, $p=0.0381$), all the BPD and depression samples achieved higher scores than controls on the *Sadness* scale of the ANPS ($F=4.0$, $p=0.0009$). A good number of these results remain statistically significant after correction for multiple testing. For a clearer picture of the results see Table 3.1, below. Statistically significant scores are highlighted.

Table 3.1. Results of the Mixed Model ANOVA Comparing Personality Scores Across the Diagnostic Groups.

Instrument.	Sample Size.	F-Value.	p-Value.	Significant after Bonferroni?	Nature of Relationship.
TEMPS-A DT	193	1.2	0.33	NA	NA
TEMPS-A CT	**217**	**5.34**	**<0.0001****	**Yes**	**BPD I >MDE-R >C**
TEMPS-A HT	**193**	**2.39**	**0.0313***	**No**	**Misc group < C**
TEMPS-A IT	**193**	**5.52**	**<0.0001****	**Yes**	**BPD I + BPD II > MDE-R > C**
TEMPS-A AT	**122**	**2.35**	**0.0381***	**No**	**MDE-R > controls**
HPS	**234**	**6.0**	**<0.0001****	**Yes**	**BPD I + BPD II > C**
STA	220	1.6	0.1584	NA	NA
STB	**221**	**4.29**	**0.0005****	**Yes**	**BPD I + BPD II > MDE-R > C**
ANPS Seek	219	0.01	0.9099	NA	NA
ANPS Fear	**220**	**3.0**	**0.0241***	**No**	**BPD I + MDE-R > C**
ANPS Care	220	1.0	0.5353	NA	NA
ANPS Anger	220	2.0	0.1560	NA	NA
ANPS Play	220	1.0	0.2217	NA	NA
ANPS Sadness	**220**	**4.0**	**0.0009****	**Yes**	**BPD I, BPD II, MDE-R + MDE-S > C**
ANPS Spirit	219	0.8	0.5675	NA	NA
TCI HA	**222**	**2.4**	**0.0297***	**No**	**BPD I > controls.**
TCI NS	222	1.5	0.1730	NA	NA
TCI RD	222	1.0	0.1842	NA	NA
TCI Per	222	2.0	0.1764	NA	NA
TCI SD	**222**	**2.0**	**0.0394***	**No**	**BPD I < MDE-R < C**
TCI COOP	222	2.0	0.0975	NA	NA
TCI ST	222	1.6	0.1628	NA	NA

* $p<0.05$

** $p<0.01$

C = Controls

The 22 different personality scales (or sub-scales) listed above constitute a thorough examination of the construct of personality but with so many scales, the possibility of false positive results due to multiple testing becomes a concern. Reducing these data into a few key variables would also facilitate the genetic analyses. A decision was therefore made to carry out a PCA on the data (see Table 3.2). Some questionnaires that have not been discussed at this point are listed in Table 3.2 and these will be covered in greater detail in Chapter 5. Significant values are highlighted in red.

Table 3.2. PCA: Personality Data.

Loadings:	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11	Comp.12	Comp.13	Comp.14	Comp.15
DES	-0.244				-0.280	0.154	-0.208	0.200	0.195	-0.202		0.145			-0.228
TEMPS-A DT	-0.222			-0.214	-0.194	0.146	-0.108		**-0.360**	0.209			-0.345		0.135
TEMPS-A CT	-0.283				-0.149			-0.126	-0.175	-0.122		-0.141		0.33	
TEMPS-A HT		-0.368		0.251	-0.126		-0.213	0.225	-0.297		-0.217	0.107	0.347		
TEMPS-A IT	-0.257			0.107					**-0.305**	0.220	0.193	0.2	0.123	0.278	-0.148
TEMPS-A AT	-0.282		-0.104						-0.219	-0.222	0.102		-0.168		-0.225
HPS	-0.223	-0.136		0.290			-0.186					-0.321		-0.282	0.202
STA	-0.272					0.130	0.164			-0.326			0.141		
STB	-0.293					0.117				-0.122	-0.172	-0.128	0.207	0.109	
CTQ emotional abuse	-0.195	-0.268		-0.252	0.113		0.258			0.107	0.126	-0.153			0.123
CTQ physical abuse	-0.108	-0.354		-0.171	0.117		0.267			0.116	-0.128	-0.399	0.163	0.29	
CTQ sexual abuse	-0.173			-0.272	-0.131		-0.361		0.398		0.428	0.157	0.225		0.293
CTQ emotional neglect	-0.150	-0.307		-0.168		-0.105		-0.135	0.145	0.221	-0.130	0.405	-0.197	-0.149	
CTQ physical neglect	-0.125	-0.321	0.116	-0.219		0.315			0.169		-0.244		-0.193	-0.177	-0.263
CTQ denial		0.306				0.496		0.307	0.157	0.318		-0.341	-0.155		
Seek		-0.130		0.413	0.156	0.347	0.305	-0.132	0.253	-0.176	0.232	0.141	-0.187		0.261
Fear	-0.243	0.168	-0.113		0.198			-0.147			-0.234			-0.347	-0.212
Care			-0.350	-0.165	0.200	0.217	-0.166	-0.432	-0.127	0.222	0.138	-0.201	0.2	-0.246	-0.286
Anger	-0.221			0.245	0.351				0.193				0.222	0.102	-0.145
Play	0.175	-0.145	-0.125	0.308	-0.111	0.209		-0.360	-0.12	0.137			-0.147	0.252	
Sadness	-0.192	0.125	-0.265	0.103	0.196	-0.176			0.197	0.349	-0.275			0.203	
Spirit		-0.116	-0.471			-0.110	0.126	0.325			0.238	0.102	-0.227	0.224	-0.254
TCI HA	-0.187	0.324		-0.134		-0.174	0.178	-0.148			0.119	-0.13		-0.118	0.161
TCI NS		-0.146		0.251	-0.373	-0.411	-0.101	-0.244	0.299	0.121	0.108	-0.366	-0.264		-0.244
TCI RD			-0.485		-0.136	0.118	-0.224	-0.132	0.184		-0.269		0.157		0.128

TCI.Persistence		-0.166			0.555		-0.508			-0.175		-0.151	-0.384	0.172	0.172
TCI SD	0.261				0.111					-0.299	0.302				-0.392
TCI Cooperativeness	0.185		-0.247	-0.275			0.119	-0.201		-0.338	-0.296			0.225	0.18
TCI ST		-0.230	-0.409			-0.225	0.157	0.332	-0.103		0.124			-0.334	0.145
Importance of components:															
	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11	Comp.12	Comp.13	Comp.14	Comp.15
SD	3.055	1.875	1.645	1.560	1.238	1.095	0.976	0.940	0.882	0.856	0.800	0.783	0.684	0.662	0.601
% of variance	32%	12%	9%	8%	5%	4%	3%	3%	3%	3%	2%	2%	2%	2%	1%
Cumulative	32%	44%	54%	62%	67%	71%	75%	78%	80%	83%	85%	87%	89%	90%	92%

Unfortunately the PCA did not provide clear components that were usable and made theoretical sense, and a decision was therefore taken to construct independent super-variables based largely on theory, but also with some input from the PCA. These super-variables are listed in Table 3.3, below.

Table 3.3. Personality Super-Variables.

Super-Variable.	**Sub-Components.**
Anxiety	TEMPS-A DT, TEMPS-A AT, ANPS-Fear, ANPS-Anger, ANPS-Sadness, TCI-HA
Stability	TEMPS-A CT, TEMPS-A IT, ANPS-Anger, STB
Hyperthymia	TEMPS-A HT, HPS, ANPS-Seek, ANPS-Play, TCI-NS.
Spirituality	ANPS-Care, ANPS-Spirituality, TCI-RD, TCI-ST, TCI-Cooperativeness.

For each individual, a standardised super-variable was calculated by re-scaling the sub-variables so that they all had the same mean and standard deviation, and then averaging them. A mixed model ANOVA testing the relationship between scores on these variables and psychiatric diagnosis was carried out, once again employing family of origin as a random factor and age, gender, level of depression and level of hypomanic symptomatology as covariates.

The BPD I and MDE-R groups scored significantly higher than controls on the Anxiety construct ($F=3.46$, $p=0.0029$) while the BPD I, BPD II and MDE-R groups achieved higher scores (indicating lower stability) on the Stability super-variable ($F=5.89$, $p<0.001$). Both of these results remained significant after multiple testing. There were no significant differences between the diagnostic groups on the Hyperthymia and Spirituality super-variables. See Table 3.4 for details.

Table 3.4. Mixed Model ANOVA Results for Personality Super-Variables.

Super-Variable	Sample Size	F-value	p-value	Significant after Correction?	Nature of Relationship
Anxiety	**236**	**3.46**	**0.0029****	**Yes**	**BPD I + MDE-R > C (more anxious)**
Stability	**236**	**5.89**	**<0.001****	**Yes**	**BPD1 BPD2 and MDE-R > C**
Hyperthymia	237	1.863	0.0895	NA	NA
Spirituality	232	1.294	0.2621	NA	NA

Based on these results and the above review of the literature a decision was taken to use five personality traits as endophenotypes for the genetic analysis: The Anxiety and Stability super-variables, the HPS, the **CT** scale of the TEMPS-A, and the NS scale of the TCI. Theoretically, endophenotypes should be significantly heritable and the QTDT program was therefore used to calculate familiality scores for the relevant variables.

Both the Anxiety and Stability super-variables displayed a significant degree of familiality. See Table 3.5. There was a trend towards significance for the NS scale (χ^2=3.26, p=0.0710) but the HPS and **CT** do not appear to be highly heritable in this sample.

Table 3.5. Familiality of Personality Traits as Measured by QTDT.

Trait	Chi Square	p-value
Anxiety	**7.16**	**0.0075****
Stability	**7.98**	**0.0047****
HPS	0.33	0.5653
TEMPS-A CT	0.52	0.4720
TCI NS	3.26	0.0710

It was decided to also examine the correlations between these putative endophenotypes using the Spearman's statistic. The data are listed in Table 3.6, below.

Table 3.6. Correlations Between the Personality Endophenotypes.

Anxiety				
r =0.7513	**Stability**			
r =0.3784	r =0.6386	**HPS**		
r =0.6399	r =0.8621	r =0.6154	**CT**	
r = -0.008	r =0.2144	r =0.4172	r = 0.1629	**TCI NS**

A quantitative linkage analysis using these five personality traits was then carried out with the QTDT program. Since the vast majority of results did not reach statistical significance, only significant data will be reported in tabular form.

For the Stability variable, weak evidence of linkage in the total sample to chromosome 4p16.1 was obtained with the markers D4S394 (χ^2=1.09, p=0.2961), D4S2983 (χ^2=5.78, p=0.0162), and D4S1582 (χ^2=2.99, p=0.0836). The **CT** trait was very weakly linked to 2 out of the 3 chromosome 16 markers typed, with χ^2 scores of 3.29 (p=0.0699) and 2.87 (p=0.0902) at the markers D16S3027 and D16S3088, respectively. Marker D16S3024 was not associated with CT scores (χ^2=0.08, p=0.7839). See Table 3.7, below.

Table 3.7. Linkage Data in Full Sample.

Marker	**Stability**	**CT**
D4S394	χ^2= 1.09 (p=0.2961)	NS
D4S2983	**χ^2=5.78 (p=0.0168)**	NS
D4S1582	*χ^2=2.99 (p=0.0836)*	NS
D16S3024	NS	χ^2=0.08 (p=0.7839)
D16S3027	NS	*χ^2=3.29 (p=0.0629)*
D16S3088	NS	*χ^2=2.87 (p=0.0902)*

There was no statistically significant linkage between the personality endophenotypes and the 25 polymorphic markers in either the British or the Afrikaner ancestry samples. Nevertheless, in family 30, there was a strong association between NS and all three markers on chromosome 13q32. The data are listed in Table 3.8, below. A weak association between one of the chromosome 22 markers and Anxiety was also observed.

Table 3.8. Linkage Between Family 30 and Personality Endophenotypes.

Marker	**Anxiety**	**TCI NS**
D13S1298	NS	**χ^2=7.63 (p=0.0058)**
D13S1271	NS	**χ^2=7.83 (p=0.0051)**
D13S1284	NS	**χ^2=5.52 (p=0.0188)**
D22S421	χ^2=1.93 (p=0.1650)	NS
D22S315	χ^2=2.27 (p=0.1323)	NS
D22S1164	*χ^2=3.83 (p=0.0503)*	NS

The QTDT association analysis produced more positive results. The 4R allele of the *DRD4* VNTR polymorphism and the 6R allele of the *Notch4* CTG exon 1 microsatellite were associated with higher Anxiety scores in the cohort of Afrikaner origin. No other associations reached statistical significance although there were some trends towards significance in the various samples. Details are displayed in Tables 3.9-3.11, below. Significant results are shown in bold-type and trends towards significance ($p<0.1$) are displayed in italics.

Table 3.9. Results of QTDT Genetic Association Analysis All Pedigrees: Anxiety.

Variant	N	Chi Square	p-value	Risk Allele
COMT	229	1.65	0.1990	NA
5-HTT LPR	*230*	*3.48*	*0.0622*	*Short*
SERT VNTR	228	3.03	0.2202	NA
DRD4 VNTR	*229*	*8.31*	*0.0808*	*4R*
D4 120	225	0.02	0.8920	NA
DRD2	221	0.81	0.3668	NA
DAT	*229*	*5.14*	*0.0766*	*10R*
BDNF	231	0.50	0.4778	NA
ApoE	227	2.56	0.2780	NA
Notch4	223	4.87	0.4322	NA
Prion	*226*	*2.80*	*0.0945*	*Met*

Table 3.10. Results of QTDT Association Analysis. Afrikaner Pedigrees. Anxiety.

Variant	N	Chi Square	p-value	Risk Allele
COMT	125	0.22	0.6359	NA
5-HTT LPR	125	2.01	0.1564	NA
SERT VNTR	124	1.59	0.4514	NA
DRD4 VNTR	**124**	**12.19**	**0.0160***	**4R**
D4 120	123	0.98	0.3229	NA
DRD2	121	1.86	0.1722	NA
DAT	125	2.19	0.1385	NA
BDNF	126	1.15	0.2829	NA
ApoE	124	2.78	0.2493	NA
Notch4	**122**	**13.83**	**0.0079****	**6R**
Prion	125	2.53	0.1118	NA

Table 3.11. QTDT Association Analysis British Ancestry Pedigrees. Anxiety.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	*86*	*3.00*	*0.0832*	*Val*
5-HTT LPR	88	1.17	0.2800	NA
SERT VNTR	87	0.38	0.8251	NA
DRD4 VNTR	88	0.87	0.4090	NA
D4 120	85	0.87	0.3500	NA
DRD2	83	0.01	0.9129	NA
DAT	87	2.36	0.1248	NA
BDNF	88	0.00	1.0000	NA
ApoE	86	0.14	0.9322	NA
Notch4	84	4.02	0.4036	NA
Prion	84	0.11	0.7347	NA

The *val* allele of the *COMT* Val158Met SNP was associated with higher Stability scores (a more unstable personality) in both the overall (χ^2=8.47, p=0.0045) and the British ancestry (χ^2=7.43, p=0.0064) samples. In the Afrikaner ancestry cohort both *BDNF* (χ^2=4.34, p=0.0373) and *Notch4* (χ^2=20.60, p=0.0004) were associated with Stability, while in the cohort of British origin the *DAT* VNTR returned significant results (χ^2=10.54, p=0.0012). Details are listed in Tables 3.12-3.14.

Table 3.12. QTDT Association Analysis. All Pedigrees: Stability.

Variant	N	Chi Square	p-value	Risk Allele
COMT	**229**	**8.07**	**0.0045****	**Val**
5-HTT LPR	230	0.68	0.4109	NA
SERT VNTR	228	0.19	0.9095	NA
DRD4 VNTR	229	2.65	0.6172	NA
D4 120	225	0.19	0.6641	NA
DRD2	221	0.44	0.5056	NA
DAT	*229*	*5.76*	*0.0561*	*10R*
BDNF	231	2.48	0.1152	NA
ApoE	227	0.38	0.8288	NA
Notch4	223	6.62	0.2504	NA
Prion	226	0.87	0.3499	NA

Table 3.13. QTDT Association Analysis. Afrikaner Sample: Stability.

Variant	N	Chi Square	p-value	Risk Allele
COMT	126	2.67	0.1021	NA
5-HTT LPR	126	1.66	0.1982	NA
SERT VNTR	125	0.11	0.9455	NA
DRD4 VNTR	125	3.57	0.4668	NA
D4 120	124	0.10	0.7577	NA
DRD2	122	0.50	0.4811	NA
DAT	126	0.53	0.4653	NA
BDNF	**127**	**4.34**	**0.0373***	***val***
ApoE	227	2.56	0.2780	NA
Notch4	**123**	**20.60**	**0.0004****	**6R**
Prion	126	0.16	0.6931	NA

Table 3.14. QTDT Association Analysis. British Ancestry Sample: Stability.

Variant	N	Chi Square	p-value	Risk Allele
COMT	**86**	**7.43**	**0.0064****	**Val**
5-HTT LPR	88	0.10	0.7565	NA
SERT VNTR	87	3.10	0.2119	NA
DRD4 VNTR	88	0.32	0.9885	NA
D4 120	85	0.86	0.3527	NA
DRD2	83	0.05	0.8273	NA
DAT	**87**	**10.54**	**0.0012****	**10R**
BDNF	88	0.07	0.7948	NA
ApoE	86	1.12	0.5701	NA
Notch4	84	2.64	0.6206	NA
Prion	84	0.93	0.3343	NA

The *val* allele of the Val66Met *BDNF* polymorphism was significantly associated with higher HPS scores in both the overall (χ^2=5.93, p=0.0149) and Afrikaner ancestry (χ^2=9.87, p=0.0017) sample. The *Notch4* and the *DAT* variants were significantly associated with hypomanic personality traits in the Afrikaner (χ^2=13.83, p=0.0079) and British ancestry cohorts (14.40, p=0.001), respectively. Details are available in Tables 3.15-3.17.

Table 3.15. QTDT Association Analysis. All Pedigrees: HPS.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	225	1.30	0.2551	NA
5-HTT LPR	226	1.80	0.1792	NA
SERT VNTR	224	0.45	0.7985	NA
DRD4 VNTR	225	1.40	0.8437	NA
D4 120	221	1.59	0.2069	NA
DRD2	217	1.28	0.2576	NA
DAT	225	0.72	0.6965	NA
BDNF	**227**	**5.93**	**0.0149***	**Val**
ApoE	223	2.68	0.2623	NA
Notch4	219	4.56	0.4725	NA
Prion	222	0.62	0.4317	NA

Table 3.16. QTDT Association Analysis. Afrikaner Sample: HPS

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	125	0.08	0.7779	NA
5-HTT LPR	125	1.31	0.2525	NA
SERT VNTR	124	2.64	0.2668	NA
DRD4 VNTR	124	1.80	0.7718	NA
D4 120	123	1.20	0.2743	NA
DRD2	121	0.81	0.3668	NA
DAT	125	1.60	0.2053	NA
BDNF	**126**	**9.87**	**0.0017****	**Val**
ApoE	124	1.77	0.4132	NA
Notch4	**122**	**13.83**	**0.0079****	**6R and 10R**
Prion	125	0.38	0.5362	NA

Table 3.17. QTDT Association Analysis. British Ancestry Pedigrees: HPS.

Variant	N	Chi Square	p-value	Risk Allele
COMT	84	0.54	0.4606	NA
5-HTT LPR	86	1.75	0.1854	NA
SERT VNTR	85	1.66	0.4357	NA
DRD4 VNTR	86	4.38	0.3572	NA
D4 120	83	2.27	0.1322	NA
DRD2	81	1.68	0.1943	NA
DAT	**85**	**14.40**	**0.0001****	**10R**
BDNF	86	0.35	0.5543	NA
ApoE	84	1.22	0.5445	NA
Notch4	82	0.42	0.9811	NA
Prion	82	0.03	0.8721	NA

As far as the **CT** association analysis was concerned, only the *DAT* VNTR showed any significant association with cyclothymic personality traits, and this was in the British ancestry sample (χ^2=4.15, p=0.0417). The results are listed in Tables 3.16-3.18.

Table 3.18. QTDT Association Analysis. All Pedigrees: TEMPSA-CT.

Variant	N	Chi Square	p-value	Risk Allele
COMT	*213*	*3.42*	*0.0643*	*Val*
5-HTT LPR	215	0.34	0.5622	NA
SERT VNTR	213	0.59	0.7441	NA
D4 VNTR	214	2.10	0.7110	NA
D4 120	210	0.07	0.7845	NA
D2	208	0.03	0.8672	NA
DAT	*214*	*4.70*	*0.0956*	*10R*
BDNF	205	0.01	0.9086	NA
ApoE	212	0.49	0.7846	NA
Notch4	209	3.44	0.6332	NA
Prion	210	0.68	0.4106	NA

Table 3.19. QTDT Association Analysis. Afrikaner Sample: TEMPS-A CT.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	119	1.64	0.2007	NA
5-HTT LPR	120	0.02	0.8988	NA
SERT VNTR	119	0.05	0.9746	NA
D4 VNTR	119	3.63	0.4591	NA
D4 120	118	0.52	0.4722	NA
D2	117	0.21	0.6447	NA
DAT	120	0.21	0.6460	NA
BDNF	120	2.17	0.1406	NA
ApoE	119	1.02	0.6012	NA
Notch4	*118*	*9.25*	*0.0553*	*6R*
Prion	119	0.48	0.4886	NA

Table 3.20. QTDT Association Analysis British Ancestry Sample: TEMPS-A CT.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	77	1.04	0.3074	NA
5-HTT LPR	79	0.30	0.5845	NA
SERT VNTR	78	2.80	0.2468	NA
D4 VNTR	79	1.29	0.8631	NA
D4 120	79	0.00	0.9733	NA
D2	75	0.31	0.5784	NA
DAT	**78**	**4.15**	**0.0417***	**10R**
BDNF	*79*	*3.39*	*0.0657*	*NA*
ApoE	77	1.85	0.3966	NA
Notch4	75	3.39	0.4942	NA
Prion	75	0.43	0.5128	NA

The *val* allele of the *BDNF* variant was associated with a tendency towards novelty-seeking personality traits in both the overall (χ^2=5.08, p=0.0242) and British ancestry samples (χ^2=4.40, p=0.039). None of the other variants reached statistical significance. Scores are listed in Tables 3.21-3.23, below.

Table 3.21. QTDT Association Analysis. All Pedigrees: Novelty Seeking.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	213	0.93	0.3339	NA
5-HTT LPR	215	0.36	0.5492	NA
SERT VNTR	213	0.22	0.8976	NA
D4 VNTR	214	3.52	0.4753	NA
D4 120	210	0.31	0.5755	NA
D2	206	0.72	0.3948	NA
DAT	214	0.29	0.8632	NA
BDNF	**215**	**5.08**	**0.0242***	**val**
ApoE	212	0.51	0.7765	NA
Notch	208	5.34	0.3762	NA
Prion	210	1.91	0.1671	NA

Table 3.22. QTDT Association Analysis. Afrikaner Sample: TCI NS.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	116	0.05	0.8241	NA
5-HTT LPR	117	0.00	0.9853	NA
SERT VNTR	116	1.36	0.5064	NA
D4 VNTR	116	3.93	0.4158	NA
D4 120	115	0.14	0.7115	NA
D2	*113*	*3.37*	*0.0665*	*A2*
DAT	*117*	*3.05*	*0.0806*	*9R*
BDNF	117	1.21	0.2710	NA
ApoE	117	3.16	0.2055	NA
Notch4	114	4.20	0.3802	NA
Prion	116	1.01	0.3142	NA

Table 3.23. QTDT Association Analysis. British Ancestry Pedigrees: TCI NS.

Variant	N	Chi Square	p-value	Risk Allele
COMT	*81*	*3.01*	*0.0826*	*Val*
5-HTT LPR	83	1.19	0.2759	NA
SERT VNTR	82	0.41	0.8136	NA
D4 VNTR	83	3.17	0.5298	NA
D4 120	80	0.20	0.6572	NA
D2	78	1.06	0.3029	NA
DAT	82	2.11	0.1467	NA
BDNF	**82**	**4.40**	**0.0359***	**val**
ApoE	81	4.88	0.0871	NA
Notch4	79	7.72	0.1025	NA
Prion	79	0.04	0.8336	NA

In order to examine whether the *MAO-A* promoter VNTR exerted any significant effect on the development of the personality endophenotypes, a mixed-model ANOVA controlling for age, gender, ethnicity, self-reported symptoms of depression and mania was carried out on the data. Family of origin was used as a random factor to control for the relatedness of individuals as advised by Goncalo Abecasis (Abecasis 2005, personal communication). The only result that reached statistical significance was an association between the *MAO-A* VNTR polymorphism and HPS score in the cohort of Afrikaner origin. The low activity alleles (2R, 3R + 5R) were associated with lower HPS scores (t=-2.613, p=0.0104) but this relationship did not reach statistical significance in either the full (t=-1.300, p=0.1953) or British ancestry (t=0.436, p=0.6641) cohorts.

3.7. Discussion.

3.7.1. Personality Traits in BPD Affective Disorder.

A mixed model ANOVA controlling for a number of covariates was used to compare personality traits across the various diagnostic groups (see Table 3.1). As far as the TEMPS-A is concerned highly significant differences were observed between the groups with the BPD I and MDE-R samples scoring higher than controls on the **CT** ($F=5.34$, $p<0.0001$) and the BPD I, BPD II, and MDE-R groups achieving higher scores than controls on the **IT** scale ($F=5.52$, $p<0.0001$). The MDE-R but not the BPD samples scored higher than controls on the **AT** inventory ($F=2.35$, $p<0.05$). Here controls refer to unaffected relatives as an unrelated control group was not sampled.

The results of Evans et al. (2005), Mendlowicz et al. (2005), and Nowakowska et al. (2005) who found that BPD patients scored higher than their unaffected relatives on the **CT** and **IT** subscales of the TEMPS-A have been replicated here. Evans et al. (2005) and Nowakowsak et al. (2005) also reported that their unipolar depression group scored higher than unaffected relatives on these subscales echoing the results of this study. Nevertheless, these authors reported significant differences between BPD and control subjects on the **DT** and **AT** subscales, which were not observed in the UCT sample. One of the possible reasons for this is the failure of Evans et al. (2005), Nowakowska et al. (2005) and Mendlowicz et al. (2005) to control for mood state at the time of testing. As discussed in section 3.2 many personality measures have been shown to be sensitive to depressive symptomatology and the **DT** scale appears to conform to this rule. The **AT** is probably also sensitive to the effects of mood but this does not explain why the BPD group of Evans et al. (2005) achieved significantly higher scores on this scale than their unipolar group while the reverse pattern was obtained in the UCT sample.

The most glaring divergence from reports in the literature is the failure of the **CT** scale to differentiate between the BPD II group and unaffected relatives in this study. Kochman et al. (2005) studied 109 consecutive admissions to a child and adolescent psychiatry unit and showed that a cyclothymic temperament as evinced by the **CT**

predicted a later diagnosis of BPD II. Another French study compared 250 patients with major depression and argued that a **CT** was a significant marker of a latent BPD II disorder (Hantouche et al. 1998). Both of these studies are longitudinal in nature and unfortunately neither Evans et al. (2005), Mendlowicz et al. (2005) nor Nowakowska et al. (2005) differentiated between BPD I and BPD II disorder in their analyses so that no comparable cross-sectional study is available with which the results of this thesis can be judged. Besides the differences in methodology, there are a number of other possible explanations for the non-significant finding in the UCT BPD II group.

BPD II is a difficult diagnosis to make because of the subjective nature of hypomanic symptomatology and is therefore probably one of the least reliable diagnoses in psychiatry. The Paris and San Diego groups may have been inclined to include a "softer" profile of patients in their BPD II groups than permitted by a strict interpretation of the DSM-IV. Since the MDE-R group which presumably includes a good percentage of these "soft" BPD patients scored significantly higher than healthy relatives on the **CT,** this may account for the discrepancy in results. Another possible basis for the negative result is a lack of statistical power to detect a genuine effect because of the small size of the BPD II sample (N=21).

The most statistically significant finding was the very strong association between scores on the HPS and both BPD I and BPD II individuals ($F=6.0$, $p<0.0001$). These data confirm the results of Kwapil et al. (1998) who followed up 40 individuals with scores above 1.82 standard deviations (SD) from the mean and 40 controls with scores below 0.5 SD from the mean out of a total sample of 2 500 students who had completed the HPS in 1982. Twenty-five percent of the high scoring group and 0% of the control group were diagnosed with BPD in the intervening years. Meyer and Hautzinger (2003) tested an independent sample of 224 students and replicated these results with approximately 20% of the high scoring individuals and 1% of the low scoring group receiving a diagnosis of BPD.

The UCT BPD and MDE-R groups displayed elevated levels of anxiety-related traits even after controlling for levels of depression at the time of testing (see Table 3.3).

The BPD I and MDE-R groups scored higher than controls on the *Fear* subscale of the ANPS ($F=3.0$, $p<0.05$) while the BPD I group scored higher than controls on the TCI HA scale ($F=2.4$, $p<0.05$). All the patient groups (except for the miscellaneous category) received significantly higher scores than the controls on the ANPS *Sadness* scale ($F=4$, $p<0.01$).

No study has attempted to characterise BPD and unipolar individuals with the ANPS, nevertheless these results are to be expected given the strong correlations between *Fear, Anger* and *Sadness*, and neuroticism as defined by the FFM (see section 3.5.3). The association between HA and BPD has been replicated many times in the literature (Evans et al. 2005; Engstrom et al. 2004; Nowakowska et al. 2005) but it was surprising that the MDE-R group did not differ significantly from the control group on this measure. Both Evans et al. (2005) and Nowakowska et al. (2005) reported that their unipolar depression groups showed elevated HA scores compared to unaffected relatives and the UCT unipolar depressives were characterised by elevated scores on the *Fear* and *Sadness* ANPS subscales.

Another unexpected finding was the non-significant group differences on scales which measure appetitive drive such as *Seek* and NS. Young et al. (1995) tested 45 consecutive BPD individuals at an out-patient unit with the Tridimensional Personality Questionnaire (TPQ) (a forerunner of the TCI) and reported elevated NS scores among the patients in comparison to healthy controls. Both Nowakowska et al. (2005) and Evans et al. (2005) found elevated NS scores to be characteristic of their patient groups but the effect was much smaller than observed in the case of HA. These authors, however included a healthy control group as well as a group of unaffected relatives and in the case of the Nowakowska et al. (2005) study, the significant difference in NS scores was between the BPD group and unrelated controls rather than unaffected relatives. Unlike previously published research, hypomanic symptomatology which may lead to elevated scores on scales that measure novelty-seeking traits has been controlled for. This could also account for the differences in results. Alternatively, the TPQ measure of NS may differ psychometrically from the more modern TCI used in this study.

The other TCI measure that differed between the groups was SD with the BPD I and MDE-R groups scoring lower than controls ($F=2.0$, $p<0.05$). This finding is congruent with the data of Nowakowska et al. (2005) and Evans et al. (2005) who also reported reduced SD scores in their BPD and unipolar samples. In addition, Engstrom et al. (2004) tested 100 euthymic BPD patients and controls and detected lower SD and C scores in the patient sample. There was a trend towards reduced C in this sample ($F=2.0$, $p=0.0975$). Cloninger et al. (1998) has proposed that individuals low in SD and cooperativeness tend to have dependent or depressive personalities characterised by immature, submissive and emotionally reactive behaviours.

The BPD I, BPD II, and MDE-R groups scored significantly higher than their unaffected relatives on the STB ($F=4.29$, $p<0.01$). The STB cannot provide a clinical diagnosis of borderline personality disorder but it does tap traits associated with the condition. A PCA yielded two factors: hopelessness (dejection, thoughts associated with suicide and self-harm) and impulsiveness (self-destructive behaviour such as physical aggression towards others and alcohol abuse) (Rawlings et al. 2001).

People with borderline personality disorder are affectively unstable and present with dysphoric episodes of anger, irritability, suicidal and other impulsive behaviour, as well as depression and transient psychotic symptoms (APA 1994). Between 4 and 20% of borderline individuals are co-morbid for BPD and not surprisingly some researchers have argued that there is a strong link between borderline personality disorder and mood disorders (Widiger and Trull 1991). Personality disorders are common in BPD samples with the incidence ranging from 3-80% depending on the study quoted (Brieger, 2000; Vieta et al. 1997; Peselow et al. 1995).

A perusal of Table 3.1 indicates that the other interesting aspect of the results of this thesis is the divergent characteristics of the MDE-R and MDE-S group. The former sample resembled the BPD cohort, particularly the BPD I group on many facets of personality while the MDE-S group did not differ significantly from controls. These results are not a function of sample size (67 MDE-R versus 52 MDE-S) and strongly suggest that relatives of BPD probands who suffer from multiple episodes of depression, share genetic risk factors with their BPD relatives while in those

individuals with only one life-time episode of major depression environmental risk factors are likely to be more salient. These data support the author's strategy of labelling MDE-R but not MDE-S participants as affected in the binary linkage and association analyses described in Chapter 2.

The one weakness of this section of the study was the absence of a control group consisting of healthy unrelated individuals. The presence of an unrelated control group would have allowed more detailed comparisons with the data of Evans et al. (2005) and Nowakowska et al. (2005) to be made. It would have also allowed the author to test whether the sample of unaffected relatives scored midway between affected patients and background controls as is predicted of an endophenotype. Unfortunately, because this was a genetic study the emphasis lay in the recruitment of bipolar probands and their family members.

Nevertheless, the observed inter-group differences in scores on the comprehensive battery of personality measures employed in this study have reinforced the author's hypothesis (which was based on a review of the literature) that certain personality traits aggregate in people with BPD spectrum illness and may therefore be used as endophenotypes for genetic investigations. Unfortunately testing for genetic linkage and association with each one of these personality traits is not only impractical but may lead to false-positive results through multiple testing.

In order to ameliorate this problem, personality super-variables were developed based on the hypothetical constructs measured by the various scales (see Table 3.3). The Anxiety construct which included scales like the TEMPS-A **AT**, the ANPS *Fear*, and the TCI HA, was significantly associated with affective illness ($F=3.46$, $p<0.01$). Since significant differences were apparent between the groups on most of the subscales comprising Anxiety, it was no surprise that both the BPD I and MDE-R groups were more "anxious" than their unaffected relatives ($F=3.46$, $p<0.01$). It is unclear why the BPD II group did not show the same effect and as hypothesised previously it may be an issue of sample size.

The super-variable labelled "Stability" which is comprised of cyclothymic, borderline and irritable personality traits was also very strongly associated with affective illness.

The BPD I, BPD II, and MDE-R groups all displayed less "stability" than the unaffected relatives ($F=5.89$, $p<0.001$).

There was a trend towards significance on the Hyperthymia super-variable ($F=1.863$, $p=0.0895$). A perusal of Table 3.3 indicates that while some of the variables that make up the Hyperthymia trait are highly significantly associated with BPD (for example the HPS), most of the others like *Seek* show no trend towards statistical significance whatsoever. Clearly, the HPS is measuring a very different trait from the rest of the Hyperthymia variables. The personality dimension Spirituality, showed no statistical evidence of association with any of the patient groups ($F=1.294$, $p=0.2621$) and was not considered for the genetic analyses.

Based on the ANOVA results for the personality variables and the above review of the literature, five personality traits were selected for the genetic analyses: Anxiety, Stability, TEMPS-A **CT**, HPS, and TCI NS. The Anxiety and Stability variables are good general summaries of anxiety-related and impulsive-irritable-mood labile traits measured by the battery of tests. Nevertheless, the author was reluctant to rely solely on these somewhat diffuse measures of personality and thus included three sub-scales from the original battery.

Although the **CT** scale is represented in the Stability variable, a host of studies have reported associations between scores on the **CT** scale and BPD spectrum illness (see section 3.2), and in fact both Perugi and Akiskal (2002) and Chiaroni et al. (2005) have suggested that the scale be used as an endophenotype for genetic studies of BPD. The **CT** was also very strongly associated with BPD and major depression in the UCT sample and therefore included as an endophenotype.

The HPS was selected because of its robust association with BPD and major depression in the current study as well as the literature. As mentioned in section 3.2, the scale has also been shown to predict which individuals go on to develop BPD related illnesses.

Finally the NS sub-scale of the TCI was selected as an endophenotype based on data indicating increased levels of extraversion and tendencies towards novelty-seeking behaviour in people with BPD (see section 3.2).

A perusal of Table 3.6 indicates that the majority of variables were significantly correlated with each other and this is one potential weakness of the study. Nevertheless, the square of the rho values which estimates the degree to which the variance explained by different questionnaires overlap, indicates that even highly correlated personality endophenotypes differ substantially from each other. For example the correlation between CT and Anxiety is 0.64 but this means that there is only 40% overlap in the variance explained by these two traits. A decision was therefore made to use all five personality variables in the genetic analyses.

As was discussed in section 3.1, Gottesman and Gould (2003) note that a trait must have a genetic basis in order for it to be a useful endophenotype. On a purely philosophical level, an *a priori* argument based on evolutionary reasoning can be made that all personality traits are at least partly genetic (Buss 1991; Turkheimer 1998). Nevertheless, it is the degree of genetic influence that is important for the evaluation of prospective endophenotypes. This cannot be measured directly but rather inferred with heritability estimates. Heritability refers to the extent to which genetic variation accounts for the phenotypic variation in a trait. Here variation is nominally equivalent to statistical variance so that in mathematical terms:

$$H^2 = V(g)/V(p) = V(g)/[V(g)+V(e)+V(i)]$$

where V(g)=genetic variance, V(p)= phenotypic variance, V(e)=environmental variance, and V(i)=variance due to gene-environment interactions.

In the absence of gold-standard twin and adoption studies, the best way to estimate heritability is to ask whether the degree of similarity between family members for a particular trait is explained by the extent of their genetic relatedness. In other words, when personality trait values correlate more strongly among closely related individuals than distantly related individuals this may be due to shared genetic factors. Technically however, this measure is not a true heritability score because closely

related people are more likely than distantly related individuals to be exposed to the same environmental influences (McCrae et al. 2001). Nevertheless, examination of the intra-familial covariance of the five selected personality variables, Anxiety, Stability, **CT**, HPS, and NS, provides a useful estimate of their promise as endophenotypes.

Both the Anxiety ($F=7.16$, $p<0.001$) and Stability ($F=7.98$, $p<0.001$) super-variables showed high levels of familiality, and a trend in the same direction was apparent for novelty-seeking behaviour ($F=3.26$, $p=0.0710$). On the other hand, scores on the personality scales HPS and **CT** were not more correlated among closely related individuals within pedigrees and this suggests that these traits may be strongly influenced by non-shared environmental factors. Nevertheless because these data are not reflective of a true heritability analysis (twin or adoption study) the findings should be interpreted with caution.

3.7.2. Linkage Analysis.

The evidence of linkage to the Stability trait that was obtained at the markers D4S394 ($\chi^2=1.09$, $p=0.2961$), D4S2983 ($\chi^2=5.78$, $p=0.0162$), and D4S1582 ($\chi^2=2.99$, $p=0.0836$), suggests the possible presence of a locus in this region that mediates the risk of developing unstable personality traits. Nevertheless, the weakness of the linkage statistic is most likely indicative of a false peak at D4S2983. If these data are accurate however it underlines the difficulty of detecting linkage to variants that are likely to be common and therefore exert a small phenotypic effect.

In the full sample, the **CT** trait was very weakly linked to 2 out of the 3 chromosome 16 markers typed, with χ^2 scores of 3.29 ($p=0.0699$) and 2.87 ($p=0.0902$) at the markers D16S3027 and D16S3088, respectively. Again given the weakness of the linkage statistics this is most likely a false positive finding but it would be useful to type additional markers in the region at a future date.

The best evidence for linkage was at 13q32 where the personality endophenotype NS was significantly associated with all three markers typed in this location in family 30.

These data are interesting because there was no evidence of linkage between NS and 13q32 in the total Afrikaner and British ancestry samples. If this result is valid, then it indicates that there is a variant in the 13q32 region that exerts a large effect (hence detectable by linkage analysis) on the development of novelty-seeking related traits in this family only. In other words, it suggests that personality traits may also be genetically heterogeneous. This issue is elaborated upon in Chapter 6. In addition, no significant evidence of linkage was detected in pedigree 30 in the binary linkage analysis (see Chapter 2) but this may be due to the fact that the subtype of BPD found in this family is not underpinned by strong hypomanic or novelty seeking-related tendencies. It would be easy to dismiss the 13q32-NS linkage as a false positive finding but the fact that all three markers were highly significant suggests to the author the possible presence of a genuine personality QTL.

As mentioned in Chapter 2, the region around 13q32 has been linked to BPD on a number of occasions. Park et al. (2004), Detera-Wadleigh et al. (1999) and Liu et al. (2001) all detected significant linkage peaks at 100 Mb, approximately 2 Mb distal to the potentially significant markers in this study, while Kelsoe et al. (2001) reported a parametric LOD score of 2.4 at 95 Mb, 3 Mb proximal to these linkage findings. The same region has also been implicated a number of times in schizophrenia. Lin and Bale (1997) reported a LOD score of 2.58 while Blouin et al. (1998) detected a maximum LOD score of 3.6 in a genome-wide scan of a large number of schizophrenia pedigrees.

The potentially genuine association between 13q32 and NS not withstanding, how can the general absence of linkage to the five personality endophenotypes be explained in light of the positive results obtained in the binary linkage (see Chapter 2) and quantitative association analyses? At first glance one would expect the statistically powerful quantitative linkage analysis to produce even stronger findings than the traditional approach but the data need to be interpreted in terms of the underlying biology.

Linkage analyses in general are designed to detect genetic changes that have a large effect size while all types of association analyses including the QTDT are able to detect much smaller genetic effects (Abecasis 2005, personal communication).

Personality is most likely a normally distributed trait reflecting the actions of many genetic variants of relatively small effect, hence the positive results obtained with the QTDT association analysis but the absence of detectable linkage. Following this reasoning, replicated reports of linkage to BPD in the literature (and indeed the binary analysis) must reflect the actions of relatively rare variants that exert a large effect on the risk of developing BPD. In other words, if one accepts the validity of large swathes of the genetic association and linkage literature, one should concede the point that both rare variants and common polymorphisms contribute to the development of BPD. This issue will be followed up in Chapter 6.

3.7.3. Association Analysis.

The results of the family-based association analyses were more promising. A summary of the findings from the large numbers of tables presented in section 3.6 can be found in Table 3.24. The data in Table 3.24 include the analysis of the sample without family 30 but for the sake of brevity, these data are not displayed in the results section. Each genetic variant will be covered separately in the discussion which follows below.

Table 3.24. Summary of Association Findings.

	COMT	*5-HTT*	*SERT VNTR*	*D4 VNTR*	*D4-120*	*DRD2*	*DAT*	*BDNF*	*ApoE*	*Notch4*	*Prion*	*MAO-A*
Anxiety All	X	T	X	T	X	X	X	X	X	X	T	X
Anxiety Afr	X	X	X	√	X	X	T	X	X	√	X	X
Anxiety Brit	T	X	X	X	X	X	X	X	X	X	X	X
Anxiety -30	√	√	X	X	X	X	T	X	X	X	X	No test
Stability All	√	X	X	X	X	X	T	X	X	X	X	X
Stability Afr	X	X	X	X	X	X	X	√	X	√	X	X
Stability Brit	√	X	X	X	X	X	√	X	X	X	X	X
Stability -30	√	X	X	X	X	X	√	X	X	X	X	No test
HPS All	X	X	X	X	X	X	X	√	X	X	X	X
HPS Afr	X	X	X	X	X	X	X	√	X	√	X	√
HPS Brit	X	X	X	X	X	X	√	X	X	X	X	X
HPS -30	X	X	X	X	X	X	X	√	X	X	X	No test
CT All	T	X	X	X	X	X	T	X	X	X	X	X
CT Afr	X	X	X	X	X	X	X	X	X	T	X	X
CT Brit	X	X	X	X	X	X	√	T	X	X	X	X
CT -30	√	X	X	X	X	X	X	X	X	X	X	No test
NS All	X	X	X	X	X	X	X	√	X	X	X	X
NS Afr	X	X	X	X	X	T	T	X	X	X	X	X
NS Brit	T	X	X	X	X	X	X	√	X	X	X	X
NS -30	X	X	X	X	X	X	X	√	X	X	X	No test

X = No evidence for association.

√ = Statistically significant ($p<0.05$).

T = Trend towards significance ($p<0.1$)

COMT.

The *COMT* V158M variant was significantly associated with anxiety in the sample *sans* family 30 (χ^2=4.82, p=0.0281), and a trend in the same direction was observed in the British ancestry group (χ^2=3.00, p=0.08) with the *val* allele associated with higher anxiety scores. These data are congruent with the results of McGrath et al. (2004) who reported an association between the *val/val* genotype and phobic anxiety (OR=1.99) as evinced by the Crown-Crisp phobic anxiety scale. Eley et al. (2003) detected a trend towards higher peer-rated neuroticism scores among *val* allele carriers, but among male study participants only. On the other hand, Enoch et al. (2003) found that 75 female Caucasian *met/met* homozygotes displayed higher HA scores than their counterparts, and a small study comparing 51 Korean panic disorder patients and 45 controls reported an excess of the *met* allele in the patient group (Woo et al. 2002). Further support for a role for *COMT* in anxiety-related traits is derived from Hamilton et al's (2002) study where linkage to markers around the *COMT* locus was reported in a sample of panic disorder patients.

The *COMT* V158M variant was also very strongly associated with the impulsive-irritable-mood labile trait, Stability in the overall sample both with and without family 30 (χ^2=8.07, p=0.0045; χ^2=10.80, p=0.0010), and the British ancestry subgroup (χ^2=7.43, p=0.0064). The **CT** scale which forms part of the Stability variable was itself associated with *COMT* (χ^2=7.42, p=0.0065) when family 30 was removed from the analysis.

The *COMT* variant has been implicated in aggressive, self-destructive and suicidal behaviour (see Savitz et al. 2006c). Most of these studies have however, reported an association between the low activity *met* variant and violence. Nolan et al. (2000) found that *met* allele was over-represented in schizophrenics with a history of violent suicide attempts and at least three studies have found this allele to be associated with violent and antisocial behaviour among this population (Strous et al. 1997; Lachman et al. 1998; Kotler et al. 1999). In contrast, Jones et al. (2001) found that *val/val* homozygotes with schizophrenia showed higher levels of self-reported aggression than their heterozygote counterparts. Can the results of this thesis together with those

of Jones et al. (2001) be reconciled with the strong reported association between violent suicide and the low activity *met* allele?

Rujescu et al. (2003) may have provided a solution. The authors found that their sample of *val/val* carriers scored higher on a self-reported anger questionnaire, but that the *met* allele was more common among violent suicide attempters. Rujescu et al. (2003) reasoned that the low activity homozygotes express their anger more outwardly, while the *val/val* homozygotes internalise their anger and hence report more antagonistic feelings when questioned. In other words, irritable-impulsive-mood labile individuals do not necessarily engage in violent and anti-social behaviour.

There was a very weak association between *COMT* and NS in the British ancestry subgroup (χ^2=3.01, p=0.0826) with the presence of *val* allele inflating novelty seeking tendencies. This may well be a false positive result although a more robust association may be disguised by gene-gene interactions: Strobel et al. (2003) showed that NS scores are higher in individuals with the 7R allele of the *DRD4* VNTR, but only in the presence of the *long* 5-HTTLPR allele and the *val/val COMT* genotype.

5-HTTLPR.

An association between the *SERT* 5-HTTLPR polymorphism and anxiety-related personality traits (χ^2=6.78, p<0.01) was detected in the overall sample when family 30 was excluded from the analysis. The *short* allele was associated with higher anxiety scores. A trend in the same direction was observed when family 30 was included in the analysis (χ^2=3.48, p=0.0622).

Lesch et al. (1996) first reported an association between neuroticism as measured by the FFM and the *short* 5-HTTLPR allele and this finding has since been replicated many times (see Savitz and Ramesar 2004). The focus has now moved to identifying the neurobiological pathways that mediate this trait. Hariri et al. (2002) found that relative to individuals homozygous for the *long* 5-HTTLPR allele, carriers of the *short* allele exhibit increased activity in the right amygdala in response to fearful stimuli. The differential processing of affective stimuli among carriers of the *short*

allele was replicated by Battaglia et al. (2005) who found that children with this variant showed a pattern of reduced cortical but increased subcortical activity during the processing of hostile facial cues. The increased subcortical or amygdaloid activity appears to be due to a "short-circuit" of an amygdala-cingulate negative feedback loop that normally acts to extinguish anxiety (Pezawas et al. 2005).

The 5-HTTLPR-anxiety association was expected given the extensive literature on the topic, and in fact it was surprising that the relationship was not more robust. The author suspects that the conservative approach of controlling for depressive and anxious symptomatology adopted here attenuated the relationship between the two variables. Theoretically an endophenotype should be associated with the disorder of interest in the absence of any psychiatric symptomatology and hence the extensive use of covariate modelling. The situation is more complex, however when one is using self-report measures of symptomatology because individuals with high trait anxiety (*short* allele carriers) are also likely to report higher levels of state anxiety and thus controlling for state effects is probably overly conservative.

BDNF.

A significant association between *BDNF* and hypomanic personality traits as evinced by the HPS was apparent in the overall sample (χ^2=5.93, p=0.0149), the total sample *sans* family 30 (χ^2=5.99, p<0.0144), and the Afrikaner ancestry sample (χ^2=9.87, p=0.0017). NS which correlated with HPS was also significantly associated with the *BDNF* Val66Met variant in the total sample (χ^2=5.08, p=0.0242), the total sample without pedigree 30 (χ^2=6.79, p=0.0091), and the British ancestry sample (χ^2=4.40, p=0.0359). The *val* allele was associated with increased scores on the HPS and NS scales.

To the best of the author's knowledge only one published study has touched on this relationship. Itoh et al. (2004) found that healthy females but not males with the *met/met* genotype scores higher on the FFM extroversion scale (which has similarities to the TCI NS scale) than their counterparts. It is suspected that this is a false positive result because of the modest sample size, the gender specific relationship, and the

implication of the *met* allele in NS traits. The finding that the *val* allele is a risk factor for elevated HPS and NS scores is supported by the literature on BPD and schizophrenia which confirms that the high activity *val* allele is a risk factor for psychopathology (see Chapter 2.2). The *val* allele has however also been associated with elevated levels of anxiety-related traits (Lang et al. 2005) which are often conceptualised as the converse of NS and hypomanic traits. These data may indicate that high levels of NS and hypomanic traits do not preclude the experience of anxiety.

The *BDNF* val66met variant also showed some degree of association with the personality trait Stability with a χ^2 value of 4.34 in the Afrikaner sample which translated into a p-value of 0.0373. Again higher scores were associated with the *val* allele. None of the other groups displayed a significant relationship with the functional *BDNF* variant. Since there are no published studies which have examined the correlation between mood-labile-irritable personality traits and genetic polymorphisms it is difficult to evaluate this result. There is however some evidence to suggest that *BDNF* is associated with aggression. Mice with one copy of the *BDNF* gene knocked out develop enhanced aggressiveness which can be ameliorated with fluoxetine, suggesting that the *BDNF* protein plays a critical role in the normal development of the serotonin system (Lyons et al. 1999). Dwivedi et al. (2003) reported that mRNA levels of *BDNF* and its receptor trkB were significantly reduced in the both the prefrontal cortex and hippocampus of patients who had committed suicide.

DAT.

There was a trend towards a statistically significant association between the *DAT* VNTR and anxiety in the overall sample, both including (χ^2=5.14, p=0.07) and excluding (χ^2=5.0; p=0.07) family 30, with the 10R allele associated with higher scores. Rowe et al. (1998) provided support to the author's hypothesis that the 10R allele increases the risk for anxiety in a study of childhood internalising disorders. The severity of generalised anxiety disorder, social phobia and obsessive compulsive disorder was found to increase with the number of 10R alleles possessed by the child (Rowe et al. 1998).

The *DAT* VNTR polymorphism was also associated with the Stability super-variable in the total sample minus pedigree 30 (χ^2=5.1, p=0.02) and as well as the British ancestry sample (χ^2=10.54, p=0.0012). In addition, there was a trend towards significance in the overall sample (χ^2=5.76, p=0.0561). A similar but less convincing pattern of results obtained for the **CT** trait with a trend towards significance in the overall sample (χ^2=4.70, p=0.0956) and the British ancestry sample yielding a χ^2 score of 4.15 (p=0.0417). Here the 10R allele was associated with higher mood-labile-irritability scores.

In contrast to the UCT data, Gerra et al. (2005) found that heroin addicts with the 9R/9R genotype scored higher than heterozygotes or 10R/10R homozygotes on the Buss-Durkee Hostility Inventory (BDHI). The discrepancy in outcome between the studies may be attributed to differences in sample (drug addicts versus BPD patients), or the psychometric (BDHI versus CT/Stability) characteristics of the relevant instruments. Other human and animal studies have suggested that abnormalities of dopamine transmission – particularly increased dopaminergic function is associated with aggressive behaviour. For example, Kuikka et al. (1998) showed that impulsive, aggressive alcoholics showed a greater heterogeneity of striatal *DAT* density than controls. As was pointed out earlier in the chapter, however, it is questionable to what degree the Stability construct overlaps with a proclivity for aggressive behaviour.

Individuals of British but not Afrikaner ancestry possessing one or more copies of the 10R allele also tended to score higher than their counterparts on the HPS (χ^2=14.40, p=0.0001). The author is unaware of any similar study in the literature that can provide a basis for comparison although Cornish et al. (2005) demonstrated that the 10R/10R genotype was associated with greater ADHD symptomatology and cognitive disinhibition.

It is interesting that such a highly significant result was obtained in the pedigrees of British origin but not the in other sample combinations. The association between Stability-CT and the *DAT* polymorphism was also much stronger in the smaller group of British origin. Whether the VNTR polymorphism (or another variant) of the *DAT* gene is a risk factor for BPD-related pathology, or more specifically unstable or hypomanic personality traits, in this particular sample or all populations of British

origin is a moot point. The other studies that have indicated that the *DAT* may be a risk factor for BPD made use of Caucasian samples of North European origin, and an Old Order Amish pedigree (Greenwood et al. 2005). The Old Order Amish originated in 16th century Germany (Patton 2005).

Notch4.

Another variant for which there appears to be an ethnic specific distribution of risk is the exonic CTG repeat in the *Notch4* gene. The 6R allele was associated with increased anxiety (χ^2=13.83, p=0.0079), instability (χ^2=20.60, p=0.0004), HPS (χ^2=13.83, p=0.0079), and **CT** (χ^2=9.25, p=0.05) in the Afrikaner ancestry sample only. Wei and Hemmings (2000) tested 80 British parent-offspring trios and reported that the 10R CTG allele was transmitted more often than expected by chance to individuals with schizophrenia. Although the immediate attempts to replicate Wei and Hemmings' (2000) original finding was disappointing (Sklar et al. 2001; McGinnis et al. 2001), recent reports have been more encouraging.

Skol et al. (2003) typed 392 schizophrenic individuals in a racially mixed sample of 166 families and reported excess transmission of two alleles of another microsatellite marker in the *Notch4* gene to affected individuals. Luo et al. (2004) found a two-SNP haplotype to be associated with schizophrenia in an African American but not a Caucasian sample of patients. A follow-up study by Wei and Hemmings (2004) in 122 UK trios demonstrated an association between schizophrenia and a number of SNPs close to the *Notch4* gene. Two of these SNPs were located in the nearby TNXB gene, another possible candidate gene (Wei and Hemmings 2004).

Wassink et al. (2003) found that variation in the *Notch4* gene produces divergent neurocognitive effects in schizophrenics and controls with the 6R schizophrenia group performing better on neuropsychological testing but the 6R control carriers performing worse than their counterparts. Kaneko et al. (2004) examined 78 Japanese trios and found the 6R CTG microsatellite allele to be over-represented in cases compared to controls (p=0.012). Remarkably, the authors then dismiss their results as irrelevant because they tested for 5 different variants and thus their statistic just missed out on statistical significance after a Bonferroni correction!

Glatt et al. (2005) conducted a meta-analysis with 1953 cases and 2180 controls and found no overall evidence that variation in the *Notch4* gene induces susceptibility to schizophrenia. The authors do however point out that a remarkable variation in effect size estimates exists across studies indicating that some cryptic factor is leading the *Notch4* variants to be associated with disease in a percentage of the samples studied. This is another way of stating that genetic heterogeneity may be present in the sample. Another possibility for the inconsistency across samples is the presence of a gene-gene interaction. Antilla et al. (2004) studied the effect of various polymorphisms on neuroleptic response in schizophrenic patients and found that patients with the *met/met COMT* genotype and particular *Notch4* SNP genotype were 10-times more likely than other individuals to be refractory to treatment.

A search of the PubMed database (www.pubmed.com) suggests that only one study has evaluated the role of the *Notch4* gene in BPD. Prathikanti et al. (2004) typed 153 parent-offspring trios but found no evidence of an association between variants in the gene and BPD illness. Carmine et al. (2003) examined a variety of phenotypes including personality in their sample of 74 Swedish schizophrenics and 135 control participants. An association between a SNP and the irritability scale of the Karolinska Scales of Personality, and the CTG variant and the extraversion scale of the FFM inventory was reported (Carmine et al. 2003). Again the authors concluded that the *Notch4* gene does not induce susceptibility to schizophrenia or related traits on the basis that controlling for multiple testing rendered their results non-significant.

There are 5 common CTG microsatellite alleles, 6, 9, 10, 11 and 12 repeats and since the polymorphism is exonic it is plausible that the number of leucine amino-acids in the protein may exert some kind of functional effect (Glatt et al. 2005). An encouraging aspect to finding of this thesis is the fact that the same 6R allele that was demonstrated by Wassink et al. (2003) and Kaneko et al. (2004) to be implicated in schizophrenia was associated with more pathological personality traits in the UCT sample. In addition, the 10R allele which was over-represented in Wei and Hemmings' (2000) patient group, was associated with higher HPS scores in this sample (see Table 3.13).

The *Notch4* gene is also a good theoretical candidate for involvement in psychopathology and neurocognition. The gene codes for the human homolog of the *Drosophila melanogaster Notch4* protein that plays a role in inter-cellular signalling and cell fate determination (Glatt et al. 2005). The Notch signalling pathway exerts a diverse influence on neuronal development including stem-cell differentiation, the timing of apoptosis, the outgrowth of neurons and dendrites, and the maintenance of their synaptic connections (reviewed in Wassink et al. 2003). The 6p21-24 region has also been implicated numerous times in linkage analyses of schizophrenia (see Owen et al. 2004) and to a lesser extent BPD (Rice et al. 1997; Schulze et al. 2004). Of interest is the study of Straub et al. (1995) who reported a heterogeneity LOD score of 3.51 in 265 Irish pedigrees with schizophrenia. Straub et al. (1995) argued for the existence of substantial genetic heterogeneity since the locus only influenced susceptibility to schizophrenia in 15-30% of their families.

MAO-A.

In the cohort of Afrikaner origin, scores on the HPS scale tended to decrease with the number of low activity variants possessed by the individual ($t=-2.613$, $p=0.0104$). The same effect was not however apparent in the full sample or the cohort of British ancestry. It is unclear therefore if this is a false positive result or another demonstration of genetic heterogeneity. The *MAO-A* has not been implicated in hypomanic personality traits but as mentioned above, the low activity variants of the VNTR polymorphism have been associated with low aggressivity (Manuck et al. 2000) although the direction of association appears to moderated by environmental exposures (Caspi et al. 2000; Newman et al. 2005). Nevertheless, it is unlikely that these data can be extrapolated to hyperthymic personality traits although individuals who achieve high scores on the HPS tend to be rude and insubordinate (Meyer and Keller 2000).

A possible weakness of this section of the thesis is the absence of significant evidence for heritability of the **CT** and HPS. With the possible exception of the *DAT*, CT scores were not particularly strongly associated with any of the genetic variants. On the other hand, the HPS was strongly associated with genes such as *BDNF* and *Notch4* and at

face-value is a good candidate endophenotype. It may well be worthwhile testing the heritability of these traits in other cohorts as heritability scores are known to be sample specific.

In summary then, the use of quantitative markers of BPD has yielded promising results with associations between five genes and various personality traits. The *Notch4* gene was associated with anxiety-related, mood-labile-irritable-cyclothymic and hypomanic traits in the Afrikaner ancestry subgroup. The *DAT* VNTR polymorphism, in contrast, was strongly associated with unstable and hyperthymic personality features in the British ancestry sample. The *BDNF* val66met polymorphism was predominantly associated with hypomanic and novelty seeking traits in both populations while the *COMT* Val158Met variant was related to mood-labile traits when family 30 was removed from the equation. As expected, the 5-HTTLPR variant modulated levels of anxiety although the effect was attenuated by controlling for state effects.

Despite these interesting findings it could be argued that with 12 polymorphisms and 5 personality traits tested across 4 different combinations of samples, the author's results are false positives induced by multiple testing. There are a number of reasons why it is believed that this is not the case.

(1). The different personality questionnaires are correlated with each other and thus the statistical tests carried out on the data are not strictly independent.
(2). The alleles of these 5 genes that were associated with "pathological" personality scores are the same alleles implicated in BPD or schizophrenia. If these results were due to multiple testing one would expect only half of the associations to be in the "correct" direction.
(3). There is also a non-random pattern of results across the two ethnic groups with families of Afrikaner origin showing an association with *Notch4* across various personality traits and families of British origin more strongly associated with the DAT.
(4). There is significant *a priori* evidence linking these genetic variants and personality traits to each other and to BPD. The stringent use of Bonferroni

corrections should not apply to cases where evidence of a relationship already exists (Perneger, 1998).

(5). Many of the positive results obtained are not of borderline significance but rather statistically significant at an α value of less than 1%, further decreasing the probability that they are simply random type I errors.

The potentially exciting results yielded by the association analysis of various quantitative personality traits underlines the promise of the endophenotypic approach. In Chapter 4 the author plans to build on these nascent successes by investigating another class of endophenotype, neurocognition.

Chapter 4.

Neurocognitive Function as an Endophenotype.

The night
was late and soggy: It was
New York in July.
I was in my room, hiding,
hating the need to swallow.

Elizabeth Prince quoted by Andrew Solomon, (2001).

List of Abbreviations Commonly Used in this Chapter.

BDNF	Brain Derived Neurotrophic Factor
BDNF Val66Met	Functional variant giving rise to 2 alleles: *val* (high activity) and *met*
COMT	Catechol-O-Methyltransferase
COMT Val158Met	Functional variant giving rise to 2 alleles: *val* (high activity) and *met*
DRD4	Dopamine 4 Receptor
DRD4 VNTR	Exonic polymorphism producing 7 different alleles (2R-8R)
RAVLT	Rey Auditory Verbal Learning Test
RCF	Rey Complex Figure
QTDT	Quantitative Transmission Disequilibrium Tests
SERT	Serotonin Transporter
SERT 5-HTTLPR	Functional Ins/Del promoter variant producing 2 alleles: *short* and *long*.
SERT VNTR	Functional intronic variant producing 3 alleles: 9R, 10R & 12R.
WCST	Wisconsin Card Sorting Test.
WMS	Wechsler Memory Scales

4.1 Neurocognition as an Endophenotype of BPD.

In Chapter 3 positive associations between various functional genetic polymorphisms and BPD-associated personality traits were demonstrated. Given these data and the potential promise of the endophenotypic approach for ameliorating the challenges posed by genetic heterogeneity and arbitrary diagnostic criteria, the feasibility of using another category of endophenotype, neurocognitive function, is evaluated here. Aspects of this chapter have recently been reported in the following papers:

Savitz, J., Solms, M., Ramesar, R. (2006a). The Molecular Genetics of Cognition: Dopamine, *COMT*, and *BDNF*. *Genes, Brain and Behavior*. In Press.

Savitz et al. (2005a). Neurocognitive function as an endophenotype for genetic studies of bipolar affective disorder. *NeuroMolecular Medicine*. 7, 275-286.

Savitz et al. (2005b). Neuropsychological Deficits in Bipolar Affective Disorder: A Critical Opinion. *Bipolar Disorders*. 7, 216-235.

4.1.1. Are Specific Neurocognitive Deficits Associated with BPD?

Neuropsychological deficits have been widely reported in acutely depressed patients, with memory and executive function emphasised by most authors (Henry et al. 1973; Miller et al. 1975; Sternberg and Jarvik 1976; Cohen et al. 1982; Cole et al. 1984; Elliot et al. 1996; Elliot et al. 1997).

More specifically, depressed individuals with BPD exhibit deficits in sustained attention (Rund et al. 1992; van den Bosch et al. 1996; Hart et al. 1998); working memory (Ali et al. 2000; Martinez-Aran et al. 2004); verbal fluency (Ali et al. 2000; Borkowska and Rybakowski 2001; Martinez-Aran et al. 2004) inhibition of prepotent responses (Borkowska and Rybakowski 2001; Dixon et al. 2004; Martinez-Aran et al. 2004); cognitive flexibility (Borkowska and Rybakowski 2001; Martinez-Aran et al. 2004) visual recognition memory (Sweeney et al. 2000), recall of verbal material (Wolfe et al. 1987; Massman et al. 1992; Fossati et al. 2004), and decision making and planning (Murphy et al. 2001; Dixon et al. 2004).

The cause of this cognitive dysfunction is unclear but has been attributed to reduced motivation (Cohen et al. 1982; Hockey 1986), impaired attention (Cole et al. 1984) or concentration (Keitner et al. 1996); intrusive thoughts (Zielinsky et al. 1991), and slowness of mentation and movement (Naismith et al. 2003). Anxiety or stress has been shown to adversely affect cognition in both humans and animal models by decreasing working memory function (McEwen 2002). The physiological basis of this effect is unclear although a number of studies have suggested that the effect may be mediated by hypercortisolaemia (Bohnen et al. 1990; Bohnen et al. 1992; Altshuler 1993).

Mania is understudied in comparison with depression, but executive dysfunction appears to be the salient cognitive feature of this mood state. Manic patients have been demonstrated to suffer from difficulties with sustained attention (Sax et al. 1999; Clark et al. 2001; Seidman et al. 2002; Swann et al. 2003), working memory (Sweeney et al. 2000), verbal fluency (Lebowitz et al. 2001; Dixon et al. 2004) impaired inhibitory control, decision making and planning (Murphy et al. 1999; Murphy et al. 2001; Swann et al. 2003; Dixon et al. 2004); spatial recognition memory (Sweeney et al. 2000) and recall and recognition of verbal information (Henry et al. 1971; Taylor and Abrams 1986; Sweeney et al. 2000; Clark et al. 2001; Basso et al. 2002; Fleck et al. 2003).

Clearly, acute episodes of depression and mania may have an adverse effect on the cognitive performance of people with BPD. The wide range of cognitive domains implicated in neuropsychological investigations of BPD; however, make it difficult to demonstrate that *specific* neurocognitive impairments are associated with the illness.

4.1.2. Are the Bipolar Associated Neuropsychological Deficits State Independent?

The data suggest that neurocognitive deficits are also present in the euthymic phase of the illness. In fact, in a recent review by Savitz et al. (2005b) it was found that 37 out of 40 studies reported some degree of cognitive impairment in nominally euthymic bipolar patients. More specifically, deficits of sustained attention (Wilder-Willis et al. 2001; Liu et al. 2002; Clarke et al. 2002; Clarke et al. 2005), cognitive inhibition

(Zubieta et al. 2001; Dixon et al. 2004; Martinez-Aran et al. 2004; Zalla et al. 2004); planning (El-Badri et al. 2001); executive control of working memory (Ferrier et al. 1999); verbal memory (Sapin et al. 1987; van Gorp et al. 1999; Zubieta et al. 2001; Cavanagh et al. 2002; Deckersbach et al. 2002; Donaldson et al. 2003; Fleck et al. 2003; Martinez-Aran et al. 2004), visual memory (Rubinsztein et al. 2000; El-Badri et al. 2001; Deckersbach et al. 2004) and psychomotor speed (Rubinsztein et al. 2000; Wilder-Willis et al. 2001; Dixon et al. 2004) have all been hypothesised to be state independent characteristics of bipolar illness.

Ferrier et al. (1999), Rubinsztein et al. (2000) and Clark et al. (2002) point out, however, that study participants who are labelled as euthymic, may in fact display residual affective symptoms or may not exhibit enough symptoms to meet the DSM-IV criteria for depression or mania. Scott et al. (2000) remarked on the fact that 30% of their sample had Beck Depression Inventory (BDI) scores greater than 10 (indicative of depression) despite being rated as euthymic by clinicians.

Nevertheless, a number of well designed investigations have recently been published that mitigate these concerns. Zubieta et al. (2001) and Clark et al. (2002) controlled for low levels of affective symptomatology using the Hamilton Rating Scale for Depression and the Young Mania Rating Scale. The same is true of Rubinsztein et al.'s (2000) sample although these authors also controlled for subjective ratings of depression using the BDI. Thompson et al. (2005) went one step further and confirmed that their patient group was euthymic during, and for at least a month prior to testing, by making use of clinical and self-rating scales. Saliva samples were also collected to ensure that the results were not confounded by hypercortisolaemia. The group with BPD performed significantly worse than controls on a wide variety of cognitive tasks measuring attention, aspects of executive function, spatial working memory, and verbal and visual declarative memory (Thompson et al. 2005).

Another method of demonstrating that bipolar-associated neurocognitive deficits are state independent is to examine the premorbid cognitive profile of individuals who go on to develop BPD. Retrospective case control studies suggest that affective disorders may have neurodevelopmental antecedents. Crow et al. (1994) found that adults

presenting with affective psychosis displayed an excess of premorbid motor and intellectual deficits compared to controls from the same birth cohort, while van Os et al. (1997) observed that children with affective disorders were delayed in reaching motor-development milestones, and suffered from twice as many speech abnormalities as their peers. More recently, Sigurdsson et al. (1999) report an increased prevalence of retarded language, social and motor development in a group of adolescents with BPD or depressive psychosis.

In a prospective study, Hellgren et al. (1994) followed 56 Swedish adolescents who evinced developmental deficits at the age of 6 years, and observed an increased rate of psychiatric disorder (especially major depression) relative to controls from the same cohort. Seven percent of the developmental delay group went on to develop BPD, compared to 0% of the control group (Hellgren et al. 1994). Meyer et al. (2004) followed a group of adolescents who were at risk of developing BPD and found that 67% of the individuals who went on to develop BPD in later years, displayed executive deficits as measured by the Wisconsin Card Sorting Test (WCST) in adolescence.

On the other hand, some studies have failed to demonstrate cognitive deficits in premorbid cases of BPD. Quakenbush et al. (1996) analysed the premorbid academic functioning of a cohort of adolescent bipolars and argued that 85% of the sample demonstrated "good to excellent" premorbid academic achievement. In similar vein, Kutcher et al. (1998) conducted a retrospective evaluation of the premorbid functioning of 28 adolescents with BPD by reviewing their school records, and reported that approximately 67% of the sample evinced good to excellent academic achievement prior to their illness. These data are supported by more recent studies (Reichenberg et al. 2002; Guerra et al. 2002) finding that a premorbid group of non-psychotic bipolar patients did not differ from unaffected controls on any measure of intellectual functioning; and that neither mania nor depression was associated with childhood dysfunction, respectively. A cohort of 60 bipolar offspring was reported to demonstrate a good level of functioning as evinced by the Global Assessment of Functioning (76±12), and the Wide Range Achievement Test, a measure of academic performance (Chang and Steiner 2003).

In sum, the data from prospective and retrospective analyses of premorbid cognition appears to be mixed although general intellectual functioning in particular seems to be largely unaffected in premorbid individuals. The discrepant results might be due to the fact that only a sub-population of individuals who go on to develop bipolar spectrum illness suffers from neurocognitive deficits. While Quakenbush et al. (1996) and Kutcher et al. (1998) demonstrated that their cohorts were generally cognitively intact before becoming ill, one can reinterpret the data to suggest that up to 15% and 33% of study participants, respectively, were performing at average to below average levels before illness onset.

Another variable which confounds the interpretation of these studies is the nature of the prodromal period. Even if study participants do not meet the criteria for BPD, they may suffer from prodromal symptoms or present with other psychopathology that causes neurocognitive dysfunction. Psychotic illnesses have been shown to have a lengthy prodromal period (Nopolous et al. 1995), with symptoms of depression, hyperactivity, and hypomania (Lish et al. 1994).

4.1.3. Is Neuropsychological Function Heritable?

While the role that genetic factors play in cognition has long been a topic of considerable interest, the field has been dominated by polarised debates over the heritability of general intelligence to the exclusion of research into the heritability of module-specific cognitive processes. Nevertheless, these data are valuable given the assertion that intelligence quotient (IQ) scores predict concurrent neuropsychological performance across the entire spectrum of intelligence in neurologically *normal* individuals (Bell and Roper 1998; Dodrill 1999; Jung et al. 2000; Diaz-Asper et al. 2004).

Heritability estimates of standardised IQ tests converge on the 50-80% range (Bouchard Jr 1998; Wright et al. 2001; Bartels et al. 2002), and these data are supported by neuroanatomical data. In a magnetic resonance imaging (MRI) study of monozygotic and dizygotic twins, Thompson et al. (2001) found that large areas of the brain, including Broca's and Wernicke's areas and frontal gray matter volume, are

under significant genetic control and that frontal gray matter volumes correlated with a general intelligence.

More recently, researchers have begun to focus on the genetic contribution to discrete (as opposed to global) cognitive processes such as attention and working memory. A version of the continuous performance test (CPT), a measure of sustained attention was reported to have a heritability value of 0.49 (Cornblatt et al. 1988). Similarly, Myles-Worsley and Coon (1997) calculated the heritability of a selective attention task to be 41% and Groot et al. (2004) also demonstrated some degree of genetic influence on the attentional performance of preschool children. Anokhin et al. (2003) suggested that performance on the WCST, a measure of cognitive flexibility has a heritability value in the region of 37-46%. Working memory is also significantly influenced by genetic factors with heritability values ranging from 30-60% (Finkel and McGue 1993; Finkel et al. 1995; Ando et al. 2001; Luciano et al. 2001).

These data are congruent with molecular genetic work which has implicated particular polymorphisms in neurocognitive function. For instance, Egan et al. (2001) reported that the high activity allele of a variant in the catechol-O-methyltransferase (*COMT*) gene was associated with poorer performance on the WCST and reduced efficiency of physiological response in the dorsolateral prefrontal cortex during a working memory task. The same group demonstrated that a functional variant of the brain-derived neurotrophic factor (*BDNF*) gene was associated with poorer episodic memory and a disruption of the normal hippocampal functional Magnetic Resonance Imaging (fMRI) disengagement pattern during a working memory task (Egan et al. 2003).

4.1.4. Do Patterns of Neuropsychological Dysfunction and Bipolar Illness Co-segregate in Families?

Although the literature indicates that on average groups of euthymic persons with BPD perform more poorly on neuropsychological tasks than healthy controls, a great deal of intra-group variation is present. For example, Altshuler et al. (2004) reported executive and verbal memory deficits in their bipolar group but the neurocognitive performance was bimodally distributed suggesting the presence of two subgroups, one with impairment and one with relatively normal neurocognitive functioning.

Thompson et al. (2005) detected significant performance decrements on a wide range of neurocognitive tasks in a sample of euthymic bipolar patients. The number of bipolar individuals who fell below the fifth percentile, however, varied from approximately 15-25% depending on the neuropsychological task involved (Thompson et al. 2005). In other words, a minority of patients appeared to account for the widespread patient-control group performance differences detected by the authors.

Thus, the statistical association between cognitive performance and bipolar illness does not necessarily mean that neuropsychological dysfunction co-segregates with the disorder in families. Perhaps neurocognitive dysfunction is only present in a particular subtype of bipolar illness but in the absence of genetic knowledge how does one demonstrate that subtypes of BPD run true in families?

4.1.5. Is There Any Evidence for Neuropsychological Dysfunction in the Unaffected Family Members of Bipolar Probands?

The earliest neurocognitive investigations of biological relatives of bipolar patients made use of general intelligence tests. Kestenbaum (1979) and Decina et al. (1983) examined the high-risk offspring of bipolar parents and asserted that a significantly higher verbal than performance IQ may be a signature of genetic susceptibility. More recently, McDonough-Ryan et al. (2002) reported that 8-12 year-old children who had at least one parent with BPD performed worse on executive and non-verbal intelligence test-associated tasks than a matched group of children with healthy parents. The finding still held when the children with sub-syndromal mood symptoms were excluded from the statistical analysis. Zalla et al. (2004) produced data showing that bipolar patients and their unaffected relatives performed poorly on the Stroop test, a measure of disinhibition and susceptibility to interference.

Gourovitch et al. (1999) compared the neuropsychological performance of monozygotic twins discordant for BPD and found memory deficits - as evinced by the Wechsler Memory Scale (WMS) and the California Verbal Learning Test (CVLT) - in both the non-affected and affected members of the twin pair. The affected twins did however display a more pervasive pattern of memory deficits than their unaffected

siblings: they showed recognition as well as recall difficulties while their healthy counterparts displayed only recall problems. This result was replicated by Keri et al. (2001) who showed that the unaffected relatives of their bipolar group displayed a greater degree of verbal recall difficulties than a group of unrelated controls.

Visual memory impairments may also be characteristic of a bipolar diathesis. Ferrier et al. (2004) compared the performance of 17 unaffected first-degree relatives and 17 unrelated controls on a battery of neuropsychological tests which included measures of verbal learning and memory, inhibition, verbal fluency, working memory, sustained attention and visual-spatial memory. Only the latter domain distinguished the two groups with the controls outperforming the relatives of individuals with BPD (Ferrier et al. 2004).

Sobczak et al. (2002) found that an experimentally induced acute tryptophan depletion and therefore central serotonergic activity reduction resulted in impaired planning, working memory and verbal recall, but not recognition performance in the first degree relatives of bipolar patients compared to unrelated controls. These data were interpreted to indicate that an inherited serotonergic-mediated dysfunction in frontal lobe function may constitute a vulnerability marker for BPD (Soczak et al. 2002).

Nevertheless, not all studies of "at-risk" relatives have reported the presence of cognitive deficits. Kremen et al. (1998) and McNeil and Schubert (2003) report a wide range of cognitive deficits in the relatives of schizophrenics, but not BPD. Similarly, Gilvarry et al. (2000) were unable to detect significant neuropsychological deficits in the biological relatives of manic depressive individuals. MacQueen et al. (2004) showed that the offspring of bipolar individuals with no lifetime history of illness performed at the same levels as unrelated controls on a visual backward masking task. On the other hand, euthymic offspring with a past history of affective illness were more error prone on the test. Clark et al. (2005) detected deficits of sustained attention in euthymic patients with BPD but their first degree relatives did not perform significantly worse than a matched group of unrelated controls.

A weakness inherent in studies of at risk relatives is the higher rate of psychopathology and exposure to significant environmental stressors in the at-risk

groups (DelBello and Geller 2001; Chang et al. 2003). Studies evaluating the cognitive performance of the children of bipolar parents run into the same methodological difficulties as longitudinal studies: prodromal symptoms and the presence of other psychopathology with neurocognitive sequelae need to be controlled for. In the Ferrier et al. (2004) study for example, 3 of the "unaffected" relatives had a history of major depression and were on treatment with antidepressants at the time of the assessment.

In sum, the literature suggests that executive dysfunction is characteristic of people with BPD in both the acute and chronic stages of the illness, that neurocognitive function is influenced by genetic factors, and that neuropsychological deficits have been reported in the non-affected relatives of bipolar probands. It is unclear however, whether neuropsychological dysfunction co-segregates with affectively ill individuals. Neuropsychological performance may nevertheless be a marker of a bipolar diathesis, and therefore a useful endophenotype for molecular genetic investigations. Genes that have been implicated in cognition would therefore be useful candidates with which to evaluate the endophenotypic potential of bipolar-associated cognitive dysfunction.

4.2. Genes Implicated in Cognition.

One of the most promising of these candidates is *COMT*. On the basis of an extensive review of the literature, Savitz et al. (2006a) suggested that the effect of *COMT* genotype on prefrontal cortex-mediated cognitive functions may be as follows: The *val* (high activity) allele of the val158met polymorphism theoretically results in lowered stability of prefrontal cortex (PFC) neural networks which may manifest in decreased ability to maintain information in working memory (Bilder et al. 2004; Winterer and Weinberger 2004), but enhanced ability to update the contents of working memory with new information or switch cognitive sets (Cools and Robbins 2004). The *met* allele, which is associated with an elevated PFC dopamine (DA) level and increased D1 receptor binding, is predicted to enhance the stability of PFC networks and thus the performance of cognitive tasks involving maintenance of information, but lead to decreased cognitive flexibility (Bilder et al. 2004; Winterer

and Weinberger 2004). More information on the neurobiological mechanisms underlying this process is available in Savitz et al. (2006a).

Savitz et al. (2006a) reviewed 26 studies that examined the association between the *COMT* val158met polymorphism and cognitive function. The results are impressive even if one acknowledges the possible effects of publication bias. Twenty out of 26 studies reported an association between the *COMT* val158met polymorphism and aspects of cognitive function, and all of them bar two suggested that the low activity *met* allele allows for better performance on cognitive tasks that have a working memory component.

Savitz et al. (2006a) concluded that neurocognitive tasks that require the individual to hold information "on-line" (i.e. working memory) and perhaps inhibit responses to prepotent or distracting stimuli, appear to be most sensitive to differential *COMT* activity. Some preliminary evidence suggests that the effect of *COMT* variants may extend to other cognitive domains such as memory (Bates et al. 2003; de Frias et al. 2004), but these results may also be indicative of poor executive functioning (Savitz et al. 2005a).

BDNF is another strong candidate for involvement in cognition. In a sample of schizophrenic and healthy subjects, the *met* allele of the functional val66met variant was associated with poorer episodic memory as evinced by immediate and delayed recall of WMS stories, a disruption of the normal hippocampal fMRI disengagement pattern during a working memory task, and lower hippocampal n-acetyl aspartate (NAA), an intra-cellular marker of neuronal function (Egan et al. 2003). Similarly, Hariri et al. (2003) demonstrated that *met-BDNF* carriers displayed reduced hippocampal engagement during both the encoding and retrieval of a spatial task, and made significantly more recognition errors on this task than *val/val* homozygotes. These results were partially replicated by Dempster et al. (2005). Another study of declarative memory in affectively ill individuals however, failed to confirm these original findings (Strauss et al. 2004). In MRI investigations, Pezawas et al. (2004) and Szeszko et al. (2005) extended the findings of Egan et al. (2003) and Hariri et al. (2003) by demonstrating that *val/met* heterozygotes displayed lower hippocampal volumes than their *val/val* counterparts.

Savitz et al. (2006a) concluded that: (1). Animal studies suggest that *BDNF* protects neurons from the effects of damage, plays a role in modifying synaptic connections, and modulates hippocampal long term potentiation (LTP). (2). Human studies have demonstrated that the *met* allele of the functional val66met polymorphism is associated with lower hippocampal volume or functional activity. (3). Genetic studies with the functional variant have indicated that the *met* allele is associated with weaker performance on tests of memory and "executive" function. Given these parallel sources of evidence it is likely that the val66met *BDNF* variant exerts an effect on memory performance and perhaps executive function, although more evidence is required for the latter.

The prion protein (PrP) coded for by the *PRNP* gene on the short arm of chromosome 20 (20p13), is infamous for its role in the pathogenesis of scrapie, bovine spongiform encephalopathy (BSE) and various neurodegenerative disorders in humans such as Creutzfeld-Jakob disease (CJD). The function of PrP remained a mystery for many years but has recently been implicated in the formation of long-term memory (Shorter and Lindquist 2005). These revelations coupled with the salient dementing process that characterises the various incarnations of CJD (Berr et al. 1998), have attracted the attention of researchers in the dementia field.

Berr et al. (1998) assessed the cognitive functioning of a large community-based sample of individuals between the ages of 59 and 71 with the Mini Mental Status Examination (MMSE), a commonly used, if rather crude estimate of cognitive impairment. An association between the *val/val* genotype of a common SNP at codon 129 (val129met) of the gene and susceptibility to cognitive impairment was reported (Berr et al. 1998).

Papassotiropoulos et al. (2005) demonstrated that the presence of the *met* allele of the val129met polymorphism was associated with better long-term memory in the form of word list learning in a sample of 354 healthy subjects. On the other hand, Rujescu et al. (2003) found that healthy individuals with the *val/val* genotype performed significantly better than their heterozygous and *met/met* homozygous counterparts on a general intelligence test.

The *DRD4* VNTR polymorphism has also been implicated in cognition. In studies of Attention Deficit Hyperactivity Disorder (ADHD), Swanson et al. (2000), Fossella et al. (2002) and Manor et al. (2002) found that children with a 4R allele of the *DRD4* VNTR polymorphism or other shorter (2-5R) alleles performed more poorly on tests of attention and executive functioning. Langley et al. (2004), however report increased impulsivity and therefore worse performance on a test of behavioural inhibition in carriers of the 7R allele.

Savitz et al. (2006a) reviewed five studies examining the effect of the *DRD2* TaqIA polymorphism and cognition. Tsai et al. (2002) and Petrill et al. (1997) made use of standardised IQ tests. The former reported that A1 homozygotes outperformed their counterparts on the performance subscale of the Wechsler Adult Intelligence Scale - revised version (WAIS-R), while the latter found no significant association between genotype and IQ. Using the Rey Auditory Verbal Learning Test (RAVLT), a measure of verbal memory and learning, Bartes-Faz et al. (2002) reported a result in the same direction as Tsai et al. (2002): reduced performance in A2 homozygotes. However, in a sample of 10-14-year-old boys, Noble et al. (1994) and Berman and Noble (1995) reported prolonged P300 latency and reduced visuospatial performance in A1 allele carriers. These contradictory findings together with the modest sample sizes of the relevant studies suggest the possibility of false positive results.

4.3. Rationale.

A diversity of psychiatric conditions tend to aggregate in bipolar pedigrees and the South African cohort is no exception to this pattern. Diagnoses ranged from psychotic illnesses such as schizophrenia and delusional disorder to mild conditions such as dysthymia and adjustment disorder. Conditions that are commonly co-morbid with BPD such as alcoholism and anxiety disorders were also regularly observed in the relatives of the UCT study probands. Given the high heritability values for bipolar illness, it is likely that genetic factors underpin the condition. It is unclear however, if the range of psychopathology seen in bipolar cohorts has a common genetic aetiology. That is, do individuals with BPD share susceptibility variants with schizophrenics, dysthymics, alcoholics and other psychiatric patients?

Some categories of illness are most probably at least partly genetically related to each other and this is the dilemma for the geneticist. Traditional linkage and association techniques require the researcher to dichotomously label individuals as affected or unaffected. An oft adopted conservative approach is to limit the label of "affected" to bipolar patients but this results in a loss of valuable information and greater sample sizes are hence required to compensate for the loss of statistical power. Greater sample size is however a double-edged sword because the more families included in the study, the greater the problem of genetic heterogeneity.

As discussed in Chapter 3, genetic heterogeneity is an even more serious obstacle than the vagaries of psychiatric diagnosis to elucidating the aetiology of bipolar affective disorder. Progress in identifying the genetic variants underpinning BPD has been retarded by the presence of distinct risk alleles in different bipolar individuals and their families leading to the non-replication of results (Gould and Gottesman 2006).

One approach to mitigating these difficulties is the use of endophenotypes. As discussed in Savitz et al. (2005b) the genetically-driven variation in the dynamic neural networks underpinning emotion most probably impacts cognition. Therefore people who carry bipolar susceptibility alleles may display subtle neuropsychological deficits even in the absence of any overt psychological symptomatology. The use of neuropsychological endophenotyping may thus eliminate the need to "guess" on the basis of symptomatology which subjects carry disease predisposing variants. Since particular aspects of neurocognition theoretically have a simpler genetic basis than BPD, the potential for genetic heterogeneity is also reduced. Understanding the genetic basis of bipolar-related neurocognitive changes should lead to an enhanced understanding of the molecular basis of the illness itself.

In this chapter it has been argued that "executive" type deficits may be an endophenotype for BPD. Glahn et al. (2004) came to a similar conclusion following their review of the literature: "neurocognitive measures of executive functioning and declarative memory are clear candidate endophenotypes worthy of investigation in BPD [bipolar disorder]" (Glahn et al. 2004, p 178). In the following sections the use of these endophenotypes in association and linkage analyses of BPD is described.

4.4. Methodology.

4.4.1. Subjects.

The neuropsychological assessment protocol was approved by the UCT research committee (ref 269/2002). The same sample of families who were referred to in Chapters 2 and 3 participated in the neuropsychological endophenotyping aspect of the study. The cohort of individuals who completed the battery of neuropsychological tasks did not overlap completely with the sample that completed the personality questionnaires because certain individuals on the UCT database live in remote regions of the country and could not be assessed by the author. These individuals were however sent the personality questionnaires by post. At least partial neuropsychological data were available for 225 individuals or 64.3% of the sample. Reasons for an absence of data are as follows: remote location 36 (10.3%); refusal to participate 42 (12%); deaths 22 (6.3%), emigration 18 (5.1%), and physical illness or confounding neurological condition (e.g. history of stroke) 7 (2%). Out of the 225 strong sample, 48 individuals (21.3%) were BPD I, 16 (7.1%) BPD II, 44 (19.5%) MDE-R, 34 (15.1%) MDE-S, 65 (28.9%) unaffected and 18 (8%) were labelled with another diagnosis.

For historical reasons the mixed ancestry community has on average received far less education than Caucasian populations. There is also a greater disparity in education levels between individuals in this community. This makes it difficult to obtain an adequate measure of neurocognitive function because many neuropsychological tests, especially tests of so-called "executive" function are sensitive to level of education. In order to enhance the accuracy of the data, a decision was therefore taken to focus on the Caucasian population who have on average had equal access to educational opportunities.

4.4.2. Genotyping.

Please refer to section 2.5.2 and Appendix C for the relevant genotyping information. In brief, a total of 25 polymorphic markers on 9 different chromosomes were typed

for the linkage analysis, while 12 different genetic polymorphisms in 10 candidate genes were genotyped for the association analysis aspect of the study.

4.4.3. Neuropsychological Tasks.

Well known and respected paper and pencil neuropsychological tests developed many decades ago, were used to assess the UCT cohort. Tests that measure various aspects of "executive" function as well as verbal and visual memory were the focus of this study since the literature suggests that these cognitive domains may be impaired in bipolar spectrum illness. Table 4.1 below describes the neurocognitive domains (and their theoretical neural correlates) tapped by each of these tasks. It should be noted at the outset, however that neuropsychological testing – at least in the traditional sense, cannot provide a "clean" measure of the functioning of one particular neural circuit in isolation. For psychometric characteristics of the tests and further details please see Appendix F.

Table 4.1. Characteristics of the Neuropsychological Assessment Battery.

Test	**Neurocognitive Domain**	**Theoretical Anatomical Correlates**
General knowledge subtest of the South African Wechsler Adult Intelligence Scale (SA-WAIS).	IQ estimator	NA
Digit Span (F & R).	Working Memory	Dorsolateral prefrontal cortex (DLPFC)
Controlled Oral Word Association Test (COWAT).	Verbal fluency or generativity	Deep ventromesial frontal white matter, supplementary motor area.
Rey Complex Figure Test (RCF).	Visual-spatial function, planning, visual memory	Right hemisphere, DLPFC, medial temporal lobe
Stroop Colour Word Test	Divided attention or inhibition of prepotent cognitive stimuli.	DLPFC, orbital-basal prefrontal cortex
Rey Auditory-Verbal Learning Test (RAVLT)	Self-monitoring, working memory, verbal memory	DLPFC, medial temporal lobes
Wisconsin Card Sorting Test (WCST)	Cognitive-flexibility, hypothesis testing, problem solving.	DLPFC

4.4.4. Procedure.

The large majority of participants were assessed by the author over a period of four years. Approximately 50 subjects were tested by an experienced psychiatric nurse who was trained to administer the neuropsychological battery. As was the case with the collection of personality data, the vast majority of participants were tested in their own homes and were euthymic at the time of assessment. A small number of individuals were assessed at the Division of Human Genetics at UCT.

The neuropsychological assessment which took approximately one hour per person to complete, was always administered before the personality component of the assessment was commenced. The tasks were administered in the following order: SA-WAIS General Knowledge, Digits (F+R), COWAT, RCF, Stroop, RAVLT, WCST. The delayed recognition component of the RAVLT was administered after the completion of the WCST.

Since the majority of participants in the Afrikaner group are fluent in English, the instructions of the tasks are easy to understand, and the author can speak some Afrikaans, the results of the assessment were not confounded by language. A group of predominantly Afrikaans-speaking people who live in the central part of the country were however assessed by the aforementioned psychiatric nurse whose home language is Afrikaans.

4.4.5. Data Analysis.

A mixed-model ANOVA was used to compare neuropsychological task scores across the various diagnostic groups. The following covariates were entered into the model: age, gender and self-rated depression and mania scores. Family of origin was used as a random factor. Level of education was not used as a covariate because if individuals become ill at an early age their educational progress tends to be curtailed and thus covarying for this variable may constitute a case of "over-control".

In order to calculate whether a number of underlying factors could account for the majority of variation in neurocognitive function across the various cognitive tasks, a principle components analysis (PCA) was conducted on the data. Means and standard deviations were rescaled for each instrument.

The QTDT (Abecasis et al. 2000) was used to test for linkage and association between the quantitative cognitive scores and the various genetic markers. The program was also used to provide a heritability estimate of the relevant cognitive traits. For more information on the QTDT, please see Chapter 3.

As mentioned in Chapter 3, because the *MAO-A* gene is located on the X chromosome, a mixed-model ANOVA was used instead of the QTDT program to test for association between the *MAO-A* VNTR polymorphism and cognition.

4.5. Results.

The BPD I and MDE-R groups performed significantly worse than unaffected relatives (controls) on the recall, but not copy component of the RCF ($F=2.7$, $p=0.0157$). They also performed worse than controls when the quality of the copy performance, which can influence recall was taken into account (Snow's correction) ($F=2.7$, $p=0.0163$). Secondly, the BPD I group performed worse than controls on the RAVLT learning rate ($F=2.9$, $p=0.0105$) which provides a measure of the extent of learning that has taken place over the 5 recall trials (see Appendix F). The same group also performed worse than their unaffected relatives on the delayed recognition component of the RAVLT ($F=2.9$, $p=0.0104$). There were no statistically significant differences between the groups on any of the other neuropsychological tasks, including the general knowledge subscale of the SA-WAIS which was used as an estimate of premorbid intelligence. For more details please see Table 4.2, below.

Table 4.2. Mixed Model ANOVA Results: Comparisons between Diagnostic Subgroups on Neuropsychological Measures.

Instrument.	**Sample Size.**	**F-Value.**	**p-Value.**	**Significant after Bonferroni?**	**Nature of Relationship.**
Digits Forward.	215	0.8	0.5915	NA	NA
Digits Reverse.	215	0.9	0.4874	NA	NA
COWAT.	215	1.2	0.3265	NA	NA
RCF copy.	204	1.0	0.5477	NA	NA
RCF recall.	**202**	**2.7**	**0.0157***	**No**	**C > MDE-R > BP I**
RCF snow.	**199**	**2.7**	**0.0163***	**No**	**C > MDE-R > BP I**
Stroop # words.	201	1.0	0.3716	NA	NA
Stroop errors.	*201*	*1.82*	*0.0996*	*NA*	*NA*
RAVLT (immediate memory)	212	0.6	0.7641	NA	NA
RAVLT (learning rate).	**212**	**2.9**	**0.0105***	**No**	**BPD I < C**
RAVLT (total learning).	212	2.0	0.1006	NA	NA
RAVLT (delayed recognition).	**207**	**2.9**	**0.0104***	**No**	**BPD I < C**
WCST (# categories).	211	0.4	0.8725	NA	NA
WCST (trials to first categories).	211	0.33	0.9193	NA	NA
WCST (failure to maintain cognitive set).	211	0.62	0.7150	NA	NA
WCST (perseverative errors).	210	0.41	0.8704	NA	NA
IQ Estimate.	211	1.0	0.7719	NA	NA

Since the use of so many neuropsychological tasks as endophenotypes for genetic analysis is both impractical and problematic from a multiple testing point of view, a PCA was carried out on the data in order to draw out the key components measured by the test battery. The first category which explained 24% of the statistical variance in neuropsychological scores consisted of the following tests: RCF recall, RAVLT total learning, RAVLT recognition, and somewhat surprisingly WCST number of categories (see Table 4.3). The second, component consisted of the Digits (F+R), the COWAT, and the general knowledge sub-test of the SA-WAIS. The remaining components were less easy to subsume under existing neuropsychological theory. The details are listed in Table 4.3, below.

Table 4.3. Principle Components Analysis of Neuropsychological Data.

Loadings:											
	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11
Digits(F)		**-0.490**		-0.103				**-0.443**	**-0.380**		**0.304**
Digits(R)	-0.157	**-0.497**	-0.167	-0.102		-0.144		-0.235	-0.103	-0.257	-0.200
COWAT	-0.145	**-0.305**	**-0.314**	0.146	**-0.370**		0.164	0.293	**0.380**		0.272
Bryden				**0.409**	**-0.566**		**-0.388**	**-0.410**	0.199		
RCFcopy	-0.240		0.231	-0.202	-0.235		-0.263	**0.366**	-0.284	**-0.581**	-0.185
RCFrecall	**-0.326**			-0.249		-0.149	-0.168	0.126	0.128	-0.127	**0.571**
Stroop Colour-Words	-0.284						**0.535**	-0.184	**0.420**	-0.223	**-0.372**
Stroop Errors		0.218	-0.148		**-0.423**	**-0.340**	**0.534**		**-0.488**	0.116	0.163
RAVLT Immediate memory	-0.296		**0.301**	**0.489**	0.166	-0.263			-0.124	0.109	
RAVLT Learning Rate	-0.226			**-0.494**	**-0.341**	**0.409**				**0.348**	
RAVLT Total Learning	**-0.419**		0.264	0.178						0.263	
RAVLT Recognition	**-0.393**		0.247		-0.167	0.150				0.265	
WCST categories	**-0.305**	0.274	**-0.329**		0.197		-0.116	-0.119		-0.138	
WCST trials to 1st category	0.239	-0.295	**0.399**				0.115	0.147	0.246		0.266
WCST Failure to maintain set			0.135	**-0.336**		**-0.689**	-0.286	-0.155	0.110	0.279	-0.237
WCST perseverative errors	0.249	-0.236	**0.372**	0.145	-0.263	0.271			-0.178	-0.158	-0.174

IQ		**-0.371**	**-0.355**	0.166			-0.174	**0.491**		**0.323**	-0.308

Importance of components:

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11
SD	2.034	1.373	1.247	1.165	1.077	1.050	0.969	0.940	0.884	0.835	0.746
% of variance	24%	11%	9%	8%	7%	6%	6%	5%	5%	4%	3%
Cumulative	24%	35%	45%	53%	59%	66%	71%	77%	81%	85%	89%

In order to try and avoid the problem of multiple testing the PCA results were used in conjunction with basic neuropsychological theory to reduce the 19 variables listed in Table 4.2 into four super categories. These have been loosely labelled as Memory, Verbal Intelligence, Cognitive Flexibility, and Cognitive Control. The first two components of the PCA were left largely intact except that number of categories obtained on the WCST was removed from the first PCA component. The third component seemed to load mostly on WCST variables except for the theoretically incongruous immediate memory component of the RAVLT which was excluded from the super-variable. The two measures of Stroop performance were added together to obtain the Cognitive Control component. For each individual, a standardised super-variable was calculated by re-scaling the sub-variables so that they all had the same mean and standard deviation, and then averaging them. The neurocognitive tests making up each super-variable are listed in Table 4.4, below.

Table 4.4. Neurocognitive Supervariables.

Super-Variable	**Sub-Components**
Memory	RCF (recall); RAVLT (total learning and recognition).
Verbal Intelligence	Digit Span (F+R); COWAT; SA-WAIS (general knowledge).
Cognitive Flexibility	WCST (number of categories + perseverative errors).
Cognitive Control	Stroop (number of colour-words + errors)

A mixed model ANOVA testing the relationship between scores on these variables and psychiatric diagnosis was carried out, once again employing family of origin as a random factor and age, gender, level of depression and level of hypomanic symptomatology as covariates. The results are listed in Table 4.5, below.

The BPD I group performed significantly worse than controls on the Memory component ($F=3.38$; $p<0.01$) of the cognitive battery. No significant differences

between the diagnostic groups could be detected on the other three components, Verbal Intelligence, Cognitive Flexibility and Cognitive Control.

Table 4.5. Mixed Model ANOVA with Neuropsychological Super-Variables.

Super-variable	**Sample Size**	**F-value**	**p-value**	**Significant After Correction?**	**Nature of Relationship**
Memory	**212**	**3.38**	**0.0037****	**Yes**	**BPD 1 < C**
Verbal Intelligence	216	0.754	0.6075	NA	NA
Cognitive Flexibility	215	0.368	0.8983	NA	NA
Cognitive Control	201	0.882	0.5096	No	NA

Based on the results of the above mixed-model ANOVA, a decision was taken to make use of the Memory endophenotype for the genetic analysis. The QTDT program was run on the data in order to obtain an estimate of the familiality of this construct. A weak trend towards significance was observed for the Memory super-variable ($F=2.80$, $p=0.0945$).

The QTDT algorithm was then used to detect evidence for linkage between the 25 polymorphic markers on nine chromosomes and the Memory endophenotype. The analysis was characterised by a preponderance of negative results and thus the data are not displayed in tabular format. In the total cohort the most promising suggestion of linkage to the Memory trait was found for the marker D10S564 ($\chi^2=6.59$, $p=0.0102$) on 10q23.3, but the two adjacent markers which lie less than 1 Mb away were not statistically significant. D10S1753 and D10S1171 returned χ^2 scores of 0.94 ($p=0.3319$) and 2.59 ($p=0.1072$), respectively. A score of borderline significance was also obtained for the marker D22S421 ($\chi^2=3.65$, $p=0.0562$) at 22q11.2, but again the adjacent markers did not produce statistically significant results (D22S315: $\chi^2=0.55$, $p=0.4579$; D22S1164: $\chi^2=2.16$, $p=0.1414$). In addition, no significant results were forthcoming when the Afrikaner and British ancestry groups were analysed

independently, however in pedigree 30, 2 out of the 3 markers on chromosome 22q11 were highly significant. See Table 4.6, below.

Table 4.6. Results of Quantitative Linkage Analysis using Neurocognitive Markers: Family 30.

Marker	**Memory**
D22S421	**χ^2=33.38 (p<0.0001)**
D22S315	**χ^2=7.41 (p=0.0065)**
D22S1164	χ^2=0 (p=1)

Regarding the association analysis, a significant relationship between *DRD4* and the memory construct was detected in the full sample, (χ^2=12.79, p=0.0123), and the Afrikaner ancestry cohort (χ^2=14.21, p=0.0067). A trend towards significance was also observable for the subset of individuals of self-reported British origin (χ^2=8.49, p=0.0741). The 4R allele was associated with lower "memory" scores. In addition, the *DRD4* 120bp ins/del polymorphism was of borderline significance in the Afrikaner ancestry sample (χ^2=3.82, p=0.0506) with the *short* (deletion) allele associated with poorer performance. No other statistically significant associations were obtained with the QTDT analysis. The details are available in Tables 4.7-4.9, below.

Table 4.7. QTDT Association Analysis. All Subjects: Memory.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	203	0.97	0.3244	NA
5-HTT LPR	204	0.13	0.7188	NA
SERT VNTR	202	0.13	0.9375	NA
D4 VNTR	**204**	**12.79**	**0.0123***	**4R**
D4 120	199	2.02	0.1553	NA
D2	195	2.62	0.1053	NA
DAT	203	1.41	0.4935	NA
BDNF	205	0.02	0.8845	NA
ApoE	201	3.48	0.1758	NA
Notch4	197	3.08	0.6882	NA
Prion	200	0.94	0.3329	NA

Table 4.8. QTDT Association Analysis. Afrikaner Ancestry Sample: Memory.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	113	0.05	0.8292	NA
5-HTT LPR	113	0.00	0.9474	NA
SERT VNTR	112	0.91	0.6350	NA
D4 VNTR	**113**	**14.21**	**0.0067****	**4R**
D4 120	*111*	*3.82*	*0.0506*	*Short*
D2	109	2.62	0.1099	NA
DAT	109	0.13	0.7148	NA
BDNF	114	0.30	0.5869	NA
ApoE	112	2.84	0.2422	NA
Notch4	110	2.79	0.5943	NA
Prion	113	0.10	0.7502	NA

Table 4.9. QTDT Association Analysis. British Ancestry Pedigrees. Memory.

Variant	**N**	**Chi Square**	**p-value**	**Risk Allele**
COMT	72	0.55	0.4592	NA
5-HTT LPR	74	0.13	0.7188	NA
SERT VNTR	73	0.70	0.7050	NA
D4 VNTR	*74*	*8.49*	*0.0751*	*4R*
D4 120	71	0.23	0.6296	NA
D2	69	1.28	0.2582	NA
DAT	73	0.52	0.4692	NA
BDNF	74	0.06	0.8037	NA
ApoE	72	1.89	0.3894	NA
Notch4	70	7.40	0.1163	NA
Prion	70	0.65	0.4215	NA

A mixed-model ANOVA was used to test for an association between the *MAO-A* variant and the Memory super-variable. In the full sample, possession of the low-activity variants (2R, 3R, 5R) of the *MAO-A* promoter polymorphism appeared to result in lowered performance on the visual and verbal memory tasks (t=-2.011, p=0.0461). The same association between Memory and *MAO-A* held for the sample of Afrikaner ancestry (t=-2.101, p=0.0394) but no statistically significant relationship was observed in the British ancestry cohort (t=-1.020, p=0.3120).

4.6. Discussion.

4.6.1. Neuropsychological Differences Between the Diagnostic Groups.

The RCF is a test of visual-spatial function, strategy and visual memory. The diagnostic groups did not differ from each other on the copy component of the task indicating that visual-spatial deficits of the type that typically result from structural lesions to the right cerebral cortex are not characteristic of the affectively ill sample. Nevertheless, both the BPD I and MDE-R groups performed worse than their unaffected relatives on the recall component of the RCF (χ^2=2.7, p=0.0157). This result may be indicative of memory deficits, in other words, the inability to convert information stored in short-term memory to long-term memory. Alternatively it may reflect poor strategy on the part of the affectively ill group when copying the Rey diagram. Since a measure of copying strategy was not used, these two possibilities cannot be differentiated and this is a methodological weakness. Nevertheless, an examination of the results of the verbal memory task, the RAVLT, suggests that the deficit in performance may be the result of memory difficulties rather than strategic thinking, planning or cognitive organisation.

The BPD I group performed worse than controls on the learning rate component of the RAVLT (F=2.9, p=0.0105) which is the difference in score between the fifth and first attempt at recalling the list of words read to the individual. This score is indicative of the verbal learning that has taken place over the trials. The BPD I group also showed performance decrements on the delayed recognition component of the task (F=2.9, p=0.0104) in which the examinee is given a sheet of words and asked to

pick out the words that were read to him or her earlier. If a true memory problem exists, that is, the individual is unable to encode the information read to him or her, then being presented with a forced-choice list of word prompts will not aid recall to a significant degree. On the other hand, if the information is encoded in long-term memory but simply cannot be accessed by the individual, then reducing the executive demands of the task – in this case presenting the patient with a written list of words containing the previously dictated words, will greatly benefit performance (Squire 1987). The fact that the BPD I group also performed significantly more poorly than controls on the recognition component of the RAVLT indicates that these individuals may suffer from a true encoding or storage problem.

If poor scores on the RCF and RAVLT are not an artefact of poor strategic thinking or planning, then the next step is to ask whether the encoding problem is the result of (structural or functional) damage to the hippocampal structures of the medial temporal lobe, or whether the memory deficits result from poor concentration or attention. A number of researchers have argued that euthymic bipolar patients suffer from attentional problems, in particular difficulties with sustained attention (Clark et al. 2002; Clark et al. 2005) and it is therefore plausible that this is the cause of the "memory" deficits observed in the UCT sample. A test of sustained attention *per se* has not been included in the battery but if an attention deficit is to blame for the deficient RAVLT recognition performance than the effects of this deficit should be seen on other neuropsychological tasks. More specifically, one would expect equally poor performance on tests such as the Digit Span, the failure to maintain cognitive set component of the WCST, or even the first recall trial of the RAVLT. A perusal of Table 4.2 indicates that no statistically significant differences in performance between the various diagnostic groups were found on these tasks.

The results of this study are concordant with a number of neuropsychological investigations of euthymic bipolar patients. Cavanagh et al. (2002) tested 20 matched cases and unrelated controls and found that their bipolar group performed significantly worse than the controls on the recall component of the CVLT a similar task to the RAVLT. There was also a trend towards significance on the recognition component of the task ($p=0.09$) and given the modest sample size this result may be clinically if not statistically significant. A more recent study by Thompson et al.

(2005) that controlled very strictly for state effects found strongly significant differences between bipolar and control subjects on both the recall and recognition components of the RAVLT.

Regarding visual memory, Altshuler et al. (2004) found that their bipolar group performed significantly worse than unrelated controls on the recall component of RCF while van Gorp et al. (1998) compared 25 bipolar patients and 22 unrelated controls on the same test and reported a score of borderline significance for the difference in recall performance between the two groups ($p=0.07$). Since strategic planning of the Rey diagram is not usually measured by researchers, like in this study, it is unclear whether these deficits in visual recall are caused by an executive or a true memory dysfunction. Nevertheless, there is some evidence for the latter phenomenon with Rubinsztein et al. (2000) detecting BPD-specific visual memory recognition deficits on the Cambridge Neuropsychological Test Automated Battery (CAN-TAB). On the other hand, Deckersbach et al. (2004) did make use of a quantitative measure of the organisational strategy of the RCF copy test and found that differences between bipolar and control individuals in the quality of the figure recall were no longer significant after the effects of organisation were partialled out of the statistical analysis.

Other findings stand in contradistinction to the study results. Ferrier et al. (1999) detected significant differences between their bipolar and control groups on the RAVLT and recall component of the RCF, but these differences disappeared after controlling for depressive symptomatology. Clark et al. (2002), Martinez-Aran et al. (2004) and Altshuler et al. (2004) reported verbal recall but not recognition deficits as evinced by the CVLT. Jones et al. (1994) found no difference between their bipolar group and controls on the RCF while Zubieta et al. (2001) were unable to detect visual memory performance differences between their cases and controls on the WMS. Given their small sample of 15 bipolar patients and healthy controls, however this may be a function of statistical power.

As the above discussion indicates, it is unclear from the literature whether the "memory" dysfunction characteristic of euthymic bipolar patients is a consequence of poor mental organisation and strategic thinking, attentional deficits, or damage to the

memory machinery of the medial temporal lobes. Savitz et al (2005c) concluded that the neuropsychological deficits associated with BPD are the result of genetically driven variation in highly dynamical and interdependent networks supporting various aspects of cognition, especially executive-type processes. The reader is referred to this paper for a more detailed argument. As discussed in Savitz et al (2005a) and Savitz et al. (2005b) studies of unaffected relatives of bipolar patients support the notion that neuropsychological dysfunction in BPD is underpinned by genetic variation. These data may help to elucidate the underlying cause of the memory deficits characteristic of BPD.

Gourovitch et al. (1999) compared the neuropsychological performance of monozygotic twins discordant for BPD and found memory deficits - as evinced by the WMS and the CVLT - in both the non-affected and affected members of the twin pair. The affected twins showed recognition as well as recall difficulties while their healthy counterparts displayed only recall problems. Another study of unaffected relatives confirmed that verbal recall difficulties seem to be a neuropsychological marker of a genetic liability to BPD (Keri et al. 2001). McIntosh et al. (2005) also reported that the unaffected relatives of their bipolar cohort showed memory deficits as evinced by the Rivermead Behavioural Memory Test, but the authors do not report whether recall and recognition memory or just recall performance was worse in the relative group. Visual memory impairments may be equally characteristic of a bipolar diathesis. Ferrier et al. (2004) reported that their control group outperformed the relatives of individuals with BPD on a spatial recognition memory task but no statistical difference between the groups was observed on the RAVLT (Ferrier et al. 2004).

Unfortunately, these data have not completely resolved the issue. Two out of the three neuropsychological analyses of memory performance in at-risk relatives indicate that poor verbal recall but not recognition memory may be an endophenotype of affective illness, while one study suggests that visual recognition memory is a marker for a bipolar diathesis. On the other hand, the neuroimaging literature (reviewed in Savitz et al. 2005b) tends to suggest that executive rather than memory (hippocampal) dysfunction *per se* is a potential marker of a genetic susceptibility to bipolar illness although the salient weakness of these studies is a dearth of information from euthymic bipolar cohorts.

Functional imaging studies of depression have consistently reported hypofrontality, with the dorsolateral prefrontal cortex (Soares and Mann 1997b); the subgenual cortex (Drevets et al. 1997; Pizzagalli et al. 2004); the anterior cingulate (Mayberg et al. 1994; Mayberg et al. 2000), and the medial prefrontal cortex (Dolan et al. 1994; Kruger et al. 2003) all implicated. Blumberg et al. (2003) contend, however, that the weight of evidence points to decreased dorsal prefrontal cortex and anterior cingulate gyrus activation, but increased activity of the ventral prefrontal cortex during depression. Mania is associated with the opposite pattern with decreased ventral and increased dorsal activity of the prefrontal cortex (Blumberg et al. 1999; Blumberg et al. 2000).

Concerning the structural imaging data, volume reductions of the anterior cingulate cortex have been described on a number of occasions, especially the left subgenual of the anterior cingulate (Drevets et al. 1997; Hirayasu et al. 1999). Volume reduction of the anterior cingulate cortex has also been found in subjects at high familial risk for BPD and has been suggested by Hasler et al. (2006) to be a potential endophenotype for the disorder.

White matter hyperintensities (WMH) as evinced by T2 weighted MRI are known to increase with age and are associated with conditions such as hypertension and arteriosclerosis, indicating an ischemic aetiology (Bearden et al. 2001). One of the most replicated findings in the literature is the presence of WMH of the subcortical white matter of the frontal lobes and basal ganglia (Videbach 1997; Lyoo et al. 2002). Figiel et al. (1991) announced that they had found WMH in two bipolar patients in their mid-twenties. Botteron et al. (1992) discuss a case of 14-year-old patient with BPD who displayed pronounced WMH on imaging. In a sample of 8 bipolar children, the same group reported WMH in two of the subjects (Botteron et al. 1995). Strakowski et al.'s (1993) 18-strong first episode sample of patients with mania exhibited 1.7 times as many WMH as controls, but the difference was not statistically significant. Aylward et al. (1994), and Dupont et al. (1995), however, both uncovered significantly higher percentages of WMH in their middle age bipolar samples relative to matched control groups, and Lyoo et al. (2002) report a WMH prevalence of 2.4% and 17.9% in their 42 and 56 strong schizophrenia and bipolar samples, respectively. Pillai et al. (2002) uncovered an even stronger effect: Ten out of 15 of their sample of

adolescent bipolar patients showed evidence of WMH, approximately double the prevalence of schizophrenic or control subjects. Videbech (1997), Soares and Mann (1997a), and Sheline (2003) postulate that the location of the WMH is suggestive of a defective frontal-basal ganglia circuit, evoking parallels with the frontostriatal circuit implicated in the functional studies of affective illness described above.

In sum, it has been demonstrated that bipolar patients show visual recall and verbal recall and recognition problems. The results of this study mirror precisely the conclusion reached by Glatt et al. (2004) in their review of the literature that executive dysfunction and declarative memory deficits are characteristic of BPD. Based on another review of the neuropsychological and neuroimaging literature it was concluded by Savitz et al. (2005b) that the most likely explanation for the available data is a genetically-driven disturbance of the fronto-striatal neural networks, impairing emotional regulation and executive function. This conclusion appears to contradict the finding of this study that bipolar patients show deficits on both recall and recognition memory. How can these conclusions be reconciled?

One potentially confounding variable that has not been discussed is medication. The medical regimen of the individuals who participated in the neuropsychological testing aspect of the study is detailed in Table 4.10, below. The numbers of individuals on each type of treatment is displayed separately for the various diagnostic groups. Many of these patients are medicated with more than one type of drug and for practical reasons these combinatorial data are not shown. The miscellaneous group consists *inter alia* of patients with schizophrenia and hence the presence of antipsychotic medication in this column of the table.

The BPD I group was much more heavily medicated with anti-psychotics and mood stabilizing agents, in particular lithium, than the other diagnostic groups. Similar levels of anti-depressant and benzodiazepine consumption were observed across the BPD I, BPD II, and MDE-R groups. The MDE-S and MDE-R groups also showed similar treatment profiles although less of the former group were receiving treatment. Clearly anti-depressant medication is not responsible for the cognitive deficits seen in the BPD I and MDE-R groups but it is much more difficult to rule out the effect of the other classes of drugs.

Donaldson et al. (2003) reported that treatment of their bipolar cohort with antipsychotic medication at the time of testing was a statistically significant predictor of cognitive, including memory performance. This result is congruent with some of the literature that has examined the cognitive effects of psychotropic medication.

Pachet and Wisniewski (2003) reviewed all studies of the cognitive effects of lithium published between 1968 and 2000, and concluded that lithium adversely affects psychomotor speed and verbal memory, but not visual-spatial function or attention. In line with this finding, a study of the effects of lithium on the cognitive performance of healthy individuals was carried out by Stip et al. (2000) who reported that the placebo group recalled significantly more words than the lithium group on a test of "long-term memory". Valproate and carbemazepine, on the other hand have been reported to cause attentional difficulties (Thompson and Trimble 1982). In a more recent study, Prevey et al. (1996) found no decline in neurocognitive performance pre and post-treatment with either carbemazepine or valproate. Prevey et al. (1996) do state however, that their treatment group did not show the practice effects observed in the control sample suggesting the presence of "subtle" medication-related cognitive deficits. Neuroleptics have been associated with sustained attention and visuomotor speed deficits (King 1994), and benzodiazepines are known to interfere with memory (Stein and Strickland 1998). Zubieta et al. (2001) found that performance on the WCST was negatively correlated with years of exposure to antipsychotic drugs.

In a longitudinal study, however, Engelsmann et al. (1988) failed to detect evidence of cognitive decline over a six year period in a sample of BPD patients treated with lithium. Comparison of long-term and short-term lithium treatment groups also failed to yield significant memory score differentials (Engelsmann et al. 1988). More recent work (Drevets 2000; Manji et al. 2000) indicates that lithium and sodium valproate, rather than impacting negatively on cognition, actually exert a neuroprotective effect on neuronal tissue. As noted by Murphy and Sahakian (2001) and Bearden et al. (2001) however, patients with BPD are often treated with complex combinations of mood stabilisers, anti-depressants, antipsychotics and anxiolytics, and the combinatorial effects of these drugs on cognition is a matter of speculation.

Table 4.10. Pharmacotherapy of the Sample Assessed with Neurocognitive Tests.

Medication	**BPD I**	**BPD II**	**MDE-R**	**MDE-S**	**Other**
SSRI/SNRI	11	8	12	3	3
AD Other	9	3	7	2	1
Lithium	23	0	2	0	0
Valproate	12	2	1	0	0
Carbamazepine	6	0	0	0	0
Lamotrigine	8	3	1	0	0
Topiramate	3	2	0	0	0
Phenytoin	1	0	0	0	0
Clozapine	5	0	0	0	1
Zuclopenthixol	2	0	0	0	0
Risperidone	1	0	0	0	0
Thioridazine	4	0	1	0	0
Trifluoperazine	0	0	0	0	2
Orphenadrine	0	0	0	0	1
Clopixal	3	0	0	0	0
Trihexyphenidyl	1	0	0	0	0
Benzodiazepine	4	3	3	1	0
Nil	6	6	23	29	15

In conclusion then, a genetically-driven dysfunction of fronto-striatal networks underpinning various aspects of neurocognition is most likely pathognomonic of BPD and accounts for the deficits in recall memory performance seen in this study and other published work. The finding of impaired recognition memory, a phenomenon which has also been inconsistently reported in the literature, could be the result of either temporal lobe dysfunction or iatrogenic treatment effects. The latter hypothesis is favoured by the author but the genetically heterogeneous nature of BPD renders the situation more complicated, because of the plausible presence of distinct subtypes of the illness with and without a dysfunctional medial temporal cortex.

This neuropsychological study of BPD differs from already published studies in a number of important respects. To the best of the author's knowledge this is one of the largest, or even the largest sample of euthymic patients with BPD spectrum illness ever tested. It is also one of the few studies to examine a BPD II cohort separately from a BPD I group, and to differentiate between individuals with MDE-R and MDE-S. In addition, although a trilateral comparison of the performance of unipolar, BPD and control subjects has been reported many times in the literature, these unipolar groups were not recruited from BPD families. Thus this study afforded a novel opportunity to compare patients with BPD spectrum illness to BPD I individuals and unaffected relatives. However because the primary purpose of this thesis was a genetic study of BPD families, an unrelated group of controls has unfortunately not been assessed, and this is the major weakness of this component of the study.

The MDE-R but not the MDE-S group resembled the BPD I group in performing worse than controls on the recall component of the RCF. No differences between the MDE-R group and the unaffected relatives were however apparent on the verbal memory task (see Table 4.2). Basso and Bornstein, (1999) compared patients with a first episode of major depression and individuals with a history of multiple episodes of depression and concluded that the MDE-R group but not the first episode cohort exhibited verbal recall but not recognition deficits on the CVLT. Similar results were obtained by Fossatti et al. (2004) with a slightly different memory test. These results are nominally equivalent to the findings of this study with the exception that verbal as opposed to visual memory was found to be impaired. Nevertheless, acutely ill unipolar patients were recruited by these authors without specifying whether they had

a family history of bipolar illness and thus it is doubtful whether the results of this thesis should be considered to be a replication of these findings.

While Basso and Bornstein (1999) and Fossatti et al. (2004) interpret their results in light of the putative brain-damaging effects of multiple episodes of depression, it is hypothesised here that the MDE-R and BPD I groups share some degree of genetic liability to affective illness and hence the observed deficits in visual recall. The MDE-S group on the other hand, probably has no genetic predisposition to BPD and resembles the average cohort with sporadic, mild depression. This conclusion is supported by the finding (see Chapter 3) that the MDE-S group also differed from the MDE-R group in terms of personality characteristics.

Although there has been some suggestion that the experience of chronic affective illness changes personality over time (Hirschfeld et al. 1989) other studies have disputed this conclusion. Katz and McGuffin (1987) concluded that past depressive episodes only explained 1% of the variance in neuroticism scores in their sample. Shea et al. (1996) assessed their sample before and after an episode of clinical depression and found no evidence that a single depressive episode results in personality change, although the effect of chronic depression on personality structure remains hypothetical.

As in the case of the personality analysis the BPD II group differed from both the BPD I and MDE-R groups and did not perform significantly differently from unaffected relatives. Whether this is an issue of sample size and by implication statistical power or whether the UCT BPD II cohort represents a distinct nosological entity is unclear. Coryell et al. (1984) found similar rates of BPD I and BPD II in BPD I probands. In the first-degree relatives of BPD II probands however, 3% were diagnosed with BPD I but 30% suffered from BPD II (Coryell et al. 1984). This may indicate that BPD II is not just a less severe form of the bipolar phenotype caused by the presence of less risk alleles than in the case of BPD I (see Chapter 6), but is at least a partially distinct genetic condition.

In order to ameliorate the problem of multiple testing, a PCA was conducted on the data in order to identify a smaller number of underlying components which could be

used as potential endophenotypes. The PCA results were used in conjunction with neuropsychological theory to identify four potential endophenotypes: Memory, Verbal Intelligence, Cognitive Flexibility and Cognitive Control. Performance on the Memory ($F=3.38$, $p=0.0037$) but not the Verbal Intelligence, Cognitive Flexibility and Cognitive Control super-variables differed between the BPD I and MDE-R group and their unaffected relatives. The Memory variable was therefore selected as an endophenotype for the QTDT linkage and association analyses.

There was a weak trend towards significance for the Memory construct heritability calculation ($\chi^2 = 2.80$, $p=0.0945$). The selection of the Memory super-variable as an endophenotypes for molecular genetic analyses of bipolar disorder is not only supported by this heritability calculation, but by the literature as a whole. As discussed in detail above, performance on memory tasks has been shown by numerous researchers to distinguish bipolar patients and to a lesser extent their healthy relatives, from unrelated control populations.

4.6.2. Linkage Analysis.

The analysis of pedigree 30 led to suggestive evidence that a variant influencing Memory performance is located on chromosome 22q11, with one marker returning a χ^2 score of 33.38 ($p<0.0001$), and the adjacent marker also statistically significant ($\chi^2=7.41$, $p=0.0065$). On the other hand, the third typed marker was not linked to Memory performance ($\chi^2=0$, $p=1$). If genuine linkage between the quantitative Memory trait and 22q11 exists it is not clear why the binary linkage analysis in family 30 returned negative results (see Chapter 2). Perhaps the quantitative linkage analysis is more powerful. Alternatively, the low density of BPD individuals in family 30 may mean that the pedigree differs genetically and neuropsychologically from other bipolar families. It is also surprising that two of the markers were strongly linked with Memory but the third marker returned a χ^2 score of 0. Approximately equal numbers of alleles were observed for both sets of these markers in the UCT cohort and thus differences in marker informativity are unlikely to account for the above results. See Figure 4.1, below. It could be argued *ceteris paribus* that there are likely to be

genotyping errors in marker D22S1164 which led to the non-significant results. This region around chromosome 22q11 will have to be investigated further in family 30.

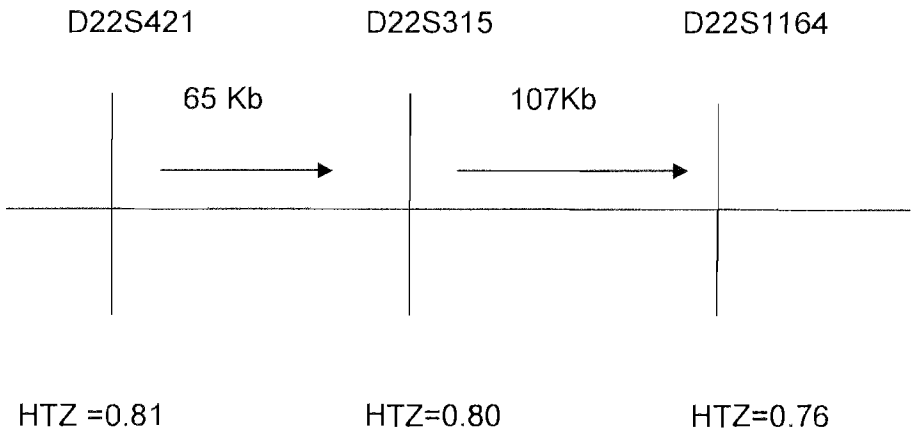


Figure 4.1. Location and Heterozygosity of Chromosome 22 Markers.

Perhaps owing to the possible linkage detected in family 30, weak evidence of a prospective memory QTL on chromosome 22q11.2 was also obtained in full sample with a maximum χ^2 score of 3.65 (p=0.0562) for the marker D22S421. Again there was a very slight trend towards significance in one of the adjacent markers (D22S1164: χ^2=2.16, p=0.1414), with the third marker returning a non-significant score (D22S315: χ^2=0.55, p=0.4579).

It is tempting to speculate that the linkage between the 22q11 markers and Memory performance is due to the actions of the *COMT* gene Val158Met polymorphism which is located in this region of the genome. It does however, seem unlikely that the putatively small functional effect of this variant could be detected in a linkage analysis. Perhaps another *COMT* variant or a polymorphism in the proximity of the gene exerts a much more significant effect on neurocognition.

Tacit evidence of a quantitative trait locus (QTL) for verbal and visual memory on chromosome 10q23.3 was also observed, with the marker D10S564 returning a statistically significant χ^2 score of 6.59 (p=0.0102) in the full sample. A weak trend towards significance was also observed for the neighbouring marker, D10S1171 (χ^2=2.59, p=0.1072). The third marker in the region, D10S1753 (χ^2=0.94, p=0.3319)

was not statistically significant. Marker heterozygosity as provided by the *Human Genome Database* (www.gdb.org) was approximately equivalent across the markers and this was confirmed in the UCT sample with 14-16 alleles detected. Thus marker informativity is unlikely to vary significantly between the triad of markers. See Figure 4.2, below.

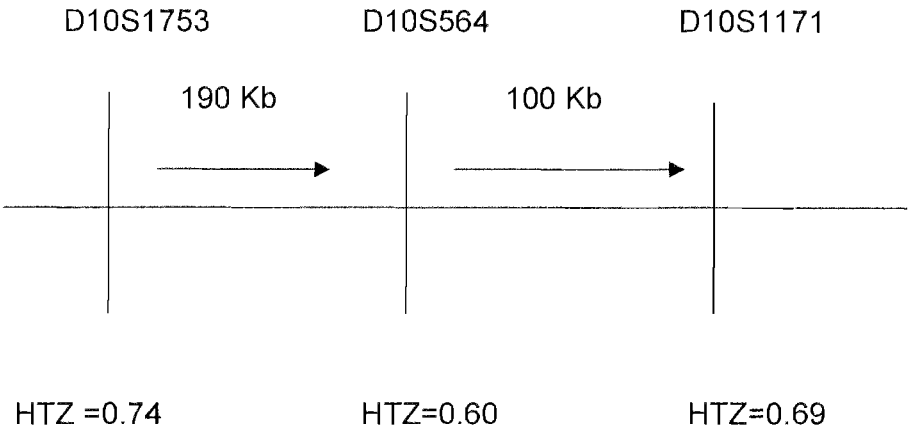


Figure 4.2. Location and Heterozygosity Scores of the Chromosome 10 Markers.

As described in Chapter 2, combined Merlin-SimWalk2 NPL scores of 1.56, 1.56, and 2.01 were obtained at the markers D10S1753, D10S564, and D10S1171, respectively, in the Caucasian group of self-reported British origin under the broad affection status model. Assuming the validity of the neurocognitive linkage statistics, it could be argued that the same genetic variants that disrupt the integrity of neural circuits involved in emotional regulation, lead to functional alterations of neural pathways that mediate cognitive processes like executive processing or memory, and that one or more of these variants are to be found in the region around 10q23.

The relative novelty of the author's endophenotyping strategy means that no direct comparisons can be made with previous published work in the bipolar literature. A few recent studies have, however attempted to identify neurocognitive endophenotypes for other forms of psychopathology. Paunio et al. (2004) conducted a genome-wide analysis in a large Finnish schizophrenia sample comprising 598 individuals (179 with schizophrenia) from 168 nuclear families. These researchers

made use of the variance-components program, SOLAR which has similarities to QTDT, and detected potential QTL for verbal learning and memory on 4q21 (Z=3.01), and visual working memory on 2q36 (Z=2.80). The interesting aspect of the Paunio et al. (2004) study was that the authors used a similar battery of tasks to that of the UCT study, but elected to make use of 11 different endophenotypes, obviating the need for PCA analyses but increasing the risk of Type I errors.

In a similar type of study, a sample of 217 pedigrees with a history of alcoholism showed significant linkage between performance on the Digit Symbol Substitution Test (a measure of attention and executive function), and markers on chromosome 14q11.2 (Buyske et al. 2006). Evidence for linkage between performance on the Digit Span test and markers on in the region of chromosome 11q25 was also reported (Buyske et al. 2006). Finally, Posthuma et al. (2005) carried out a genome-wide scan with 634 healthy sibling pairs and reported linkage between performance and verbal IQ scores and markers in the region of 2q24-31 and 6p25-22, with LOD scores of 4.40 and 2.33, respectively.

Given these positive results, how can the largely negative results reported in this study be explained? Firstly, none of the above-mentioned studies detected QTL for cognition in the chromosomal regions that were typed in this study, and therefore it is plausible that genetic factors influencing cognition or more specifically memory do not exist in these areas of the genome. Nonetheless, given the hypothesised role of neurocognitive factors in BPD there may be other reasons for the absence of positive results

A sample size of 225, while large for a neuropsychological study, is modest by the standards of genetic analyses. It is therefore plausible that this sample suffers from a lack of power to detect true effects although this does not explain the host of statistically significant findings described in Chapter 2.

A more appealing explanation is that neuropsychological analysis is a rather blunt tool for detecting functional perturbations in discrete neural circuits. As pointed out above, there is no linear relationship between performance on a neuropsychological task and damage to or dysfunction of a specific neural network. Another weakness of

neuropsychological analyses is there are many reasons beyond neurological deficits for poor performance on a particular task. These factors include mental alertness (tiredness), motivation, anxiety, hunger, thirst, emotional state, level of education, vocational experience, and general intelligence. An attempt was made to control for some of these factors (anxiety and mood) and a conscious decision was taken not to control for education level (see section 4.4.5), but nevertheless, a small genetic effect can easily be lost in the noise of the overall assessment. This is one of the reasons why there has been a recent trend towards the use of neuroimaging modalities for the detection of possible neurocognitive endophenotypes. At this point in time, however, the financial and temporal cost of imaging hundreds of individuals is prohibitive.

The fourth plausible reason for the general absence of statistically significant evidence for linkage is the underlying genetic architecture of cognition. Whereas pathology, psychiatric or otherwise, can be intuitively conceptualised as the product of rare genetic variants with large phenotypic effects, subtle bipolar-associated neurocognitive abnormalities are perhaps more likely to result from common genetic variants which are not amenable to linkage analysis. On the other hand, if this hypothesis is true then the putative QTL uncovered by Paunio et al. (2004) and Posthuma et al. (2005) and the equivocal evidence for a "memory" QTL on 22q11 in this study could be construed as Type I errors since linkage analyses do not have the power to identify weak susceptibility variants. This issue is discussed in greater detail in Chapter 6.

4.6.3. The Association Analysis.

The 48-bp VNTR repeat in the *DRD4* gene was strongly associated with both visual and verbal memory (the Memory super-variable) in the total sample (χ^2=12.79, p=0.0123) and the Afrikaner ancestry group (χ^2=14.21, p=0.0067). A trend in the same direction was observable in the British ancestry subgroup (χ^2=8.49, p=0.0751). In all cases, the 4R allele was associated with poorer memory performance. In addition, the *DRD4* 120bp tandem-duplication promoter polymorphism was weakly associated with Memory in the Afrikaner ancestry cohort (χ^2=3.82, p=0.0506) with

the *short* allele a potential risk factor for lowered Memory function. The *short* allele is over-expressed relative to the *long* variant (D' Souza et al. 2004).

The author is not aware of published data implicating the *DRD4* gene in memory. Four papers have however described an association between alleles of the *DRD4* VNTR polymorphism and attentional performance. Three out of the four studies were carried out on individuals with ADHD, possibly because the *DRD4* gene is a candidate gene for this disorder. While Swanson et al. (2000), Fossella et al. (2002) and Manor et al. (2002) found that children with a 4R allele or other shorter (2-5R) alleles performed more poorly on tests of attention and executive functioning, Langley et al. (2004) report increased impulsivity and therefore worse performance on a test of behavioural inhibition in carriers of the 7R allele. The results of this thesis could therefore be broadly interpreted as being consistent with the notion that shorter alleles of the *DRD4* VNTR polymorphism reduce the efficacy of cognitive function. There are however two caveats associated with this interpretation.

Firstly, the neuropsychological tests used by Swanson et al. (2000), Fossella et al. (2002) and Manor et al. (2002) such as the Attention Network Test (ANT) and Test of Variables of Attention (TOVA) are not tests of memory. Almost all neuropsychological tests, however, including the RCF and RAVLT have an executive component to them – in this case, planning and strategising performance - and are thus sensitive to deficits of the prefrontal cortex. It is therefore not inconceivable to imagine that the same genetic variants influence performance across a range of neuropsychological tasks.

Secondly, the interpretation of the results from the ADHD literature is complicated by Swanson et al.'s, (2000) hypothesis that there may be two basic etiological subtypes of ADHD. One sector of the ADHD population displays a profile suggestive of significant cognitive impairment. The balance of the population is hypothesised to carry the 7R allele of the *DRD4* VNTR polymorphism implicated in novelty seeking behaviour (Savitz and Ramesar 2004). This genetic subtype may represent an extreme of temperament, manifesting the behavioural but not the neurocognitive problems associated with ADHD (Swanson et al. 2000). If this postulate is correct then those individuals with a "genetic subtype" of ADHD (i.e. carriers of the 7R allele) may

outperform their counterparts who have sustained some exogenous form of damage to the brain during development. This latter ADHD group will probably not display an excess of any particular *DRD4* VNTR allele. Thus in ADHD samples, although the 7R allele may be associated with better cognitive performance, it may play no role in cognition and may simply be a proxy for ADHD aetiology.

If the 4R allele does indeed modulate cognitive performance then what might be the mechanism by which it exerts its putative effects? The 4R allele has been reported to be more responsive to pharmacological dopamine agonists (Fossella et al. 2002). If this is the case then one would expect more efficient signal transduction in 4R carriers and a subsensitive response to dopamine in 7R carriers. The effect of the 4R allele may be amplified by the presence of the *short DRD4* 120bp tandem repeat allele which leads to relative over-expression of the gene. Theoretically the 4R allele could therefore be associated with over-activity of the mesocorticolimbic DA pathway (Swanson et al. 2000) although to the author's knowledge this remains to be conclusively demonstrated. This hypothesis does not however explain the hypothesised association between improved cognition and the *COMT met* allele (which elevates the PFC DA level, see section 4.2).

The low activity variants of the *MAO-A* VNTR promoter polymorphism were associated with poorer performance on the verbal and visual memory tasks (Memory) in both the total (t=-2.011, p=0.0461) and Afrikaner ancestry (t=-2.101, p=0.0394) cohorts. A search of the *PubMed* database (www.pubmed.com) suggests that only one study has examined the effects of the *MAO-A* promoter polymorphism on cognition. Passamonti et al. (2005) obtained fMRI data from 24 healthy males while they completed a Go/No-Go task which measures response inhibition. The right ventrolateral prefrontal cortex and the right superior parietal lobe were preferentially activated in high-activity and low activity allele carriers, respectively. Passamonti et al. (2005) argue that this demonstrates that response inhibition is dominated by frontal modulation in the former group while parietal modulation of caudate outflow is characteristic of low-activity allele carriers. It is unlikely that this result can be generalised to performance on tests of verbal and visual memory which do not measure response inhibition, but since the MAO-A enzyme catabolises a variety of neurotransmitters such as dopamine which are involved in cognition (Savitz et al.

2005a), the *MAO-A*-Memory association should not be prematurely dismissed as a false positive result.

The absence of a significant relationship between the *BDNF* gene and to a lesser extent the *COMT* gene and "Memory" performance was something of a surprise given the relatively robust association between these two variables in the literature (Savitz et al. 2006a). It has been argued previously that genetic heterogeneity is a confounding variable in genetic analyses of BPD but theoretically endophenotypes should have a simpler genetic architecture and therefore display greater homogeneity. Whether the absence of a *BDNF*-memory association in the UCT sample is due to genetic heterogeneity is unclear. Another possible cause is lack of statistical power. Although a sample size of 225 individuals is very large from the point of view of a neuropsychological study, from a genetic perspective it is modest. The lack of quantitative neurocognitive data for 125 (350-225) members of the total UCT sample would have also reduced the statistical power necessary to detect a small effect. Nevertheless, this study is larger than 6 out of the 8 samples used in previously published studies on the topic (see Savitz et al. 2006a).

In summary then, the personality and neuropsychological endophenotypes have shown some degree of potential for facilitating the identification of disease genes but even these simpler phenotypes are most likely influenced by environmental factors and this is reflected in some of the heritability calculations described above. The nature of the environmental factors that meditate the development of BPD and associated phenotypes is unknown. Nevertheless, there is a degree of consensus that one factor, exposure to childhood abuse and trauma predisposes to various types of psychopathology.

It is intuitively plausible that particular genetic variants act as risk factors for BPD when coupled with early exposure to abusive experiences. It also seems reasonable to hypothesise that particular personality endophenotypes are underpinned by a pattern of gene-environment interactions. The case for early abuse as a mediator of neurocognitive function is less clear-cut. Nevertheless, if perturbations in the neural networks underpinning emotion disrupt neurocognitive function then the role of early environmental experiences cannot be discounted. Childhood neglect may also directly

affect the development of neurological pathways sub-serving aspects of cognitive functioning. Secondly, as will be described in Chapter 5, there is some evidence that stressful experiences arising from emotional trauma can lead to a degree of neuropsychological dysfunction. Thus, in following chapter the role of childhood trauma as a modulator of the genetic risk for BPD, "pathological" personality traits and neurocognitive dysfunction is explored.

Chapter 5

The Influence of the Environment.

"I did not sleep much that night, and could not get up the following day.... I lay very still and thought about speaking, trying to figure out how to do it. I moved my tongue but there were no sounds. I had forgotten how to talk. Then I began to cry, but there were no tears only a heaving incoherence. I was on my back. I wanted to turn over but I couldn't remember how to do that either. I tried to think about it but the task seemed colossal. I thought perhaps I'd had a stroke, and then I cried again for a while". Andrew Solomon, (2001, p 49).

5.1. Childhood Abuse and Neglect.

Mueser et al. (1998) point out that most surveys indicate that 30-50% of patients with severe mental illness report past exposure to sexual or physical abuse, and many of these patients suffer from post-traumatic stress disorder (PTSD). The prevalence of abuse in the background population is difficult to gauge accurately but a recent World Health Organisation (WHO) report suggests that approximately 20% of women and 5-10% of men are victims of sexual abuse (WHO 2002).

Trauma in childhood appears to be associated with a range of different psychiatric disorders. Almost all of this research is conducted in a retrospective fashion because of the practical and ethical difficulties of longitudinal research. A number of studies have found that exposure to sexual or physical abuse in childhood is a risk factor for major depression, anxiety disorders and substance abuse in adulthood (reviewed in Browne and Finkelhor 1986; Hill et al. 2001). In one well-controlled example, Bifulco et al. (1991) studied 286 "working-class" mothers and estimated the prevalence of sexual abuse to be 9%. Of these abused individuals 64% presented with

an episode of major depression within three years of the research interview. The incidence of depression in the control group of non-abused women was 26% (Bifulco et al. 1991). Another important study was that of McCauley et al. (1997) who interviewed a community-based cohort of more than 2000 women. Subjects with a history of childhood physical or sexual abuse (but not abuse during their adult years) presented with more depression and anxiety, and attempted suicide more often than their abuse negative controls (McCauley et al. 1997).

The prevalence of childhood abuse is also significantly raised in patients with psychotic disorders. Greenfield et al. (1994) examined 38 patients admitted for a first episode of psychosis (including individuals with BPD) and found that 20 of these individuals reported being abused in childhood. Out of 62 chronic inpatients with psychosis studied by Goff et al. (1991), 43% reported childhood abuse. The abused patients also presented with higher levels of dissociative symptoms than their non-abused counterparts. Zimmerman and Mattia (1999) compared psychotic and non-psychotic unipolar depressives and found that the incidence of PTSD was four times higher in the former.

There is a surprising dearth of research examining the effects of childhood trauma on BPD *per se*. Both Levitan et al. (1998) and Hyun et al. (2000) reported a significantly higher incidence of childhood abuse in their bipolar cohorts compared to their unipolar cohorts. In the latter study, 60% of bipolar males and 44% of bipolar females reported some kind of abuse compared to 32% of males and 35% of females in the unipolar cohort (Hyun et al. 2000). Levitan et al. (1998) assessed 653 individuals with major depression and found a "strong" relationship between the presence of mania and childhood physical abuse.

Childhood abuse also has an adverse effect on the clinical course of BPD. Leverich et al. (2002) studied 631 consecutive bipolar admissions finding that 49% of the female patients reported physical or sexual abuse in childhood or adolescence, while 36% of the male cohort appeared to have been abused in some way in their formative years. The abused group had an earlier age of onset, cycled faster, abused more alcohol and drugs and suffered from a greater degree of co-morbidity than their non-abused counterparts (Leverich et al. 2002). Garno et al. (2005) assessed 100 consecutive BPD

patients (predominantly BPD I) and identified the presence of childhood abuse with the Childhood Trauma Questionnaire (CTQ). The authors found that severe childhood abuse occurred in approximately half of their sample, with one third of the cohort experiencing multiple types of abuse (Garno et al. 2005). Like Leverich et al. (2002), Garno et al. (2005) detected a relationship between an abuse history and rapid cycling.

Of the 96 bipolar patients studied by Hammersley et al. (1993), 16% had a history of childhood sexual abuse and 20% reported being physically assaulted. The rate of hallucinations in the abused group was 2-3 times greater than the group without a history of sexual abuse. The putative association between a history of abuse and rapid cycling or hallucinations has implications for genetic studies of BPD as both of these traits have been used as genetic subtypes of the disorder in linkage and association studies.

Childhood neglect is rarely studied as a separate phenomenon from abuse but may be just as serious because of its greater chronicity (see Hildyard and Wolfe 2002 for a review). Factors such as chronic poverty, care-giving deficits, homelessness and poor medical care do not only have physical sequelae but have been shown increase the risk of psychopathology (Brooks-Gunn and Duncan 1997; McCall and Groark 2000). Physically neglected toddlers become angry more easily and are more impulsive than control children when confronted with tasks that they cannot solve (Egeland et al. 1983). They also tend to suffer from low self-esteem and generally become socially isolated and withdrawn as they get older because of avoidant behaviour (Camras and Rappaport 1993). Similar symptoms beset emotionally neglected children including poor coping skills and disturbances of emotional regulation during stressful situations (Hildyard and Wolfe 2002).

In one of the few longitudinal studies conducted, physically and emotionally neglected children were reported to be at a greater risk for delinquent and violent criminal behaviour during adolescence and adulthood (Maxfield and Widon 1996). Johnson et al. (1999) found that neglected individuals had a four-fold increased risk of being diagnosed with various personality disorders, and a follow-up study by the

same group again emphasised the risk for personality disorders as well as symptoms of anxiety and depression (Johnson et al. 2000).

The risk posed by childhood trauma for the later development of psychopathology may be mediated in part through the personality trait of neuroticism (N). Ernst et al. (1993) and Lysaker et al. (2001) both reported that exposure to childhood sexual abuse was positively correlated with adult N levels. Roy (2002) tested 532 individuals with the CTQ and the Eysenck Personality Questionnaire (EPQ), and found a significant relationship between all types of abuse and neglect - emotional, physical and sexual - and N as measured by the CTQ.

The physiological mechanisms by which early trauma impacts later psychopathology has become a burgeoning field of research. Heim and Nemeroff (2001), in a detailed review, argue that early exposure to trauma results in long term modification of the neural circuits that mediate response to stress. The most important of these systems is the hypothalamic-pituitary-adrenal axis. During a stressful event, cortisol-releasing factor (CRF) is released from the hypothalamus, driving the release of adreno-corticotrophic releasing hormone (ACTH) and other peptides from the pituitary gland, which in turn results in the release of glucocorticoids from the adrenal cortex (Heim and Nemeroff 2000). Various studies reviewed in Heim and Nemeroff (2001) are consistent with the hypothesis that childhood trauma disturbs the functioning of HPA system, resulting in hyper or hypo-responsiveness to stress. This same circuit is often impaired in cases of major depression and other affective illness (Heim and Nemeroff 2001).

Research into the environmental factors such as childhood trauma that contribute to the aetiology of psychiatric disorders has been largely overshadowed by the excitement of genetic work. Nevertheless these environmental influences may interact with genetic factors to induce susceptibility to psychiatric illnesses, and should ideally be controlled for in genetic research (Farmer et al. 2005). In the next section, some of the early studies that have begun to elucidate these gene-environment interactions are discussed.

5.2. Gene-Environment Interactions.

The potentially important role of environmental factors in mediating psychopathology coupled with the meagre successes of traditional genetic methodologies has catalysed attempts to elucidate the nature of gene-environment interactions. Research has thus far concentrated on a functional insertion/deletion variant in the promoter of the *SERT* gene (5-HTTLPR) and a functional 30bp VNTR in the promoter region of the *MAO-A* gene. The latter is covered first.

Caspi et al. (2002) carried out a longitudinal study of a cohort of over 1000 children and followed them into adulthood. Children who were maltreated and carried the low activity variants of *MAO-A* VNTR were more likely to develop antisocial personality traits. In contrast, the high activity *MAO-A* variants conferred a protective effect on maltreated children making them less likely to develop antisocial problems (Caspi et al. 2002). These results were replicated by Foley et al. (2004) who observed that low MAO-A activity increased risk for conduct disorder in the presence of an adverse childhood environment. Huang et al. (2004) tested 663 individuals with various psychiatric disorders. No association between the functional *MAO-A* variant and mood disorders was detected. The lower expression variant was however, associated with a history of childhood abuse and higher impulsivity in males (Huang et al. 2004). The authors interpret these results to indicate that the low activity variant sensitises the individual to the effects of abuse causing impulsive behaviour in adulthood (Huang et al. 2004).

The New Zealand birth-cohort followed by Caspi and colleagues yielded more interesting data when it was found that the influence of life stress is modulated by the 5-HTTLPR variant. Individuals with one or two copies of the *short* allele (lower transcriptional efficacy) presented with more depressive symptomatology, DSM-IV diagnosable depression, and suicidality after exposure to stress than their counterparts who were homozygous for the *long* allele (Caspi et al. 2003). A similar effect was detected by Grabe et al. (2005) in their assessment of the mental and physical distress of 1005 people from the general population. Genotype did not independently predict mental distress but females who were unemployed and carried a *short* allele displayed

higher levels of psychological and physical distress than their unemployed counterparts homozygous for the *long* allele (Grabe et al. 2005).

Kaufman et al. (2004) investigated a three-way interaction between 5-HTTLPR genotype, childhood abuse and level of social support, partially replicating the findings of Caspi et al. (2003). Maltreated children with the *short/short* genotype and poor social support had the highest levels of depression - double that of non-maltreated children with the same genotype, indicating that positive social support can ameliorate the risk posed by the *short/short* genotype and childhood abuse (Kaufman et al. 2004). On the other hand, Gillespie et al. (2004) set out to replicate the results of Caspi et al. (2003) in a sample of 1091 twin pairs and found no evidence for a main effect of 5-HTTLPR genotype on vulnerability to depression or any data supporting an interaction between genotype and stressful life events.

Two recent animal studies have provided more evidence for the interactive effect of environment and 5-HTTLPR genotype. Barr et al. (2004) investigated the role of serotonin transporter gene polymorphisms and early adversity (maternal separation) among rhesus macaques. Animals carrying the *short* allele had higher levels of ACTH after acute stress than *long/long* individuals. Among females, ACTH hormone levels were higher in maternally separated heterozygotes than *long/long* animals separated at birth (Barr et al. 2004). Another study found that both nursery-reared and mother-reared rhesus monkeys heterozygous for the 5-HTTLPR variant were more distressed and displayed greater affective reactivity than their *long/long* counterparts (Champoux et al. 2002). Nursery-reared, but not mother-reared heterozygotes displayed lower orientation scores than monkeys who did not carry the *short* allele.

Kendler et al. (2003) modelled the effect of family dysfunction (the general emotional tone of the home) on the heritability of N as defined by the EPQ. Kendler et al. (2003) predicted that the genetic effects on N would increase in importance together with the level of familial dysfunction and the presence of the *short* 5-HTTLPR allele. The heritability of N did not however vary with levels of family dysfunction suggesting that this variable does not moderate the genetic factors which influence the development of anxiety-related traits. Novelty-seeking (NS) traits on the other hand, may be affected by family environment. Keltinkangas-Jarvinen et al. (2003) followed

92 children over 14 years into adulthood. The authors found that children with two or five repeat alleles of the *DRD4* gene VNTR polymorphism reared in a hostile environment showed elevated NS scores on the TCI, but in the absence of this environment, the *DRD4* genotype had no effect on personality scores (Keltinkangas-Jarvinen et al. 2003).

5.3. Rationale.

Various types of childhood trauma appear to increase the risk of developing adult psychopathology. The most heavily investigated category of psychological trauma, sexual or physical abuse, has been demonstrated to be significantly increased in patients with major depressive illness, BPD and psychotic disorders. It is plausible to hypothesise that these adverse early experiences interact with genetic variants to bias neurodevelopmental processes in a manner that predisposes to later psychopathology, including BPD. Some support for this type of gene-environmental interaction already exists in the form of data indicating that a functional polymorphism of the SERT gene modulates the effect of life stressors (Caspi et al. 2003) and influences the risk of developing depressive disorders (Kaufman et al. 2004).

This approach has not yet been applied to genetic studies of BPD. The aim of this section of the thesis is therefore to search for gene-environment interactions that induce susceptibility to BPD or endophenotypes of BPD. The hypothesis here is that certain genetic variants may only exert an effect in combination with particular environmental contingencies, or may potentiate the effect of childhood trauma partly accounting for the inconsistencies that characterise the literature (Moffitt et al. 2005). In order to explore this hypothesis the author carried out an association analysis with the various candidate genes described in Chapter 2 using mixed-model ANOVAS. Methodological details, results and a discussion of these findings follow below.

5.4. Methodology.

5.4.1. Subjects and Genotyping.

Please refer to the methodology sections of Chapters 2, 3 and 4 for the relevant information about the UCT cohort and the genotyping thereof.

5.4.2. Assessment of Childhood Trauma.

A well-respected and widely used self-report instrument, the *Child Trauma Questionnaire* (Short Version – Bernstein et al. 2003) was used to measure levels of childhood abuse among the UCT cohort (see Appendix G). A decision was also made to measure dissociative tendencies in the cohort with the *Dissociative Experiences Scale* (Bernstein and Putnam 1986; Carlson and Putnam 1993). Abused individuals tend to display elevated scores on this scale although high *DES* scores are not necessarily indicative of childhood abuse. A positive correlation between the two variables exists because individuals exposed to chronic abuse may dissociate as a defence mechanism to protect themselves from the traumatic experience. Dissociation is defined by Bernstein and Putnam (1986) as an inability to properly integrate thoughts, feelings, and experiences into consciousness and memory. More information about the DES and CTQ is available in Appendix G.

5.4.3. Procedure and Data Analysis.

The CTQ and DES II instruments were administered as part of the battery of personality tests described in Chapter 3. For details please see Chapter 3.5.4. Mixed-model ANOVAS were used to calculate the relationship between diagnosis or the relevant quantitative trait and CTQ performance after controlling for age, gender, ethnicity and self-reported depression and mania. Similarly, mixed-model ANOVAS were constructed for each gene-phenotype association of interest. Fixed effects included age, ethnicity, gender, depression and mania scores. The effect of the genetic variant and abuse on the quantitative traits constituted the main effects of the model.

An interaction effect between the specific polymorphism and abuse was then calculated. Family of origin was once again used as a random factor. For a detailed explanation of the interaction effect, please see Appendix H.

5.5. Results.

5.5.1. The Relationship Between Childhood Trauma, DSM-IV Diagnosis and BPD Endophenotypes.

In order to test whether there was any relationship between diagnostic status and self-reported levels of abuse, a mixed model ANOVA was run on the data with family as a random factor, and gender, age, level of depression and hypomania entered into the model as covariates.

The BPD I, BPD II, and MDE-R groups scored significantly higher than controls (unaffected relatives) on the emotional abuse subscale of the CTQ ($p<0.05$), and the latter two groups also appeared to be exposed to significantly more physical abuse ($p<0.05$). There were no statistically significant differences between the diagnostic groups on any of the other sub-scales of the CTQ. The BPD I and MDE-R groups also displayed elevated dissociation scores ($p=0.05$) as measured by the DES II.

Table 5.1. Results of Mixed Model ANOVA Comparing Levels of Abuse Across Diagnostic Groups.

Instrument.	**Sample Size.**	**F-Value.**	**p-Value.**	**Significant after Correction?**	**Nature of Relationship.**
DES	**175**	**2.15**	**0.05***	**No**	**BPD I + MDE-R > C**
CTQ: Emotional Abuse	**234**	**2.7**	**0.0149***	**No**	**BPD I, BPD II + MDE-R > C**
Physical Abuse	**234**	**2.59**	**0.0198***	**No**	**BPD II + MDE-R > C**
Sexual Abuse	234	1.4	0.2219	NA	NA
Emotional Neglect	233	1.5	0.1771	NA	NA
Physical Neglect	234	0.7	0.6267	NA	NA
CTQ: Denial	233	0.69	0.6574	NA	NA

CTQ scores were also significantly associated with the endophenotypes Anxiety, Stability and Memory after controlling for age, gender, ethnicity, and level of depression and mania. See Table 5.2, below. The variables emotional and physical neglect were combined into one composite variable, neglect. Exposure to emotional abuse tended to increase Anxiety (t=2.386, p=0.0181) and Stability scores (t=2.280, p=0.0232) but childhood physical abuse was associated with lower levels of Anxiety (t=-3.608, p=0.0004). Self-reported exposure to sexual abuse was associated with poorer performance on the Memory super-variable (t=-1.972, p=0.050).

Table 5.2. The Relationship Between Childhood Trauma and BPD Endophenotypes: Full Sample.

Variable	**Emotional Abuse [t-value (p)]**	**Physical Abuse**	**Sexual Abuse**	**Neglect**
Anxiety	**2.386 (0.0181)***	**-3.608 (0.0004)****	1.663 (0.0981)	-1.056 (0.2926)
Stability	**2.280 (0.0232)***	-1.227 (0.2215)	1.430 (0.1545)	-0.684 (0.4946)
HPS	1.651 (0.1006)	0.208 (0.8357)	0.523 (0.6017)	-0.700 (0.4849)
CT	1.663 (0.0982)	-0.968 (0.3344)	1.1187 (0.2371)	-0.876 (0.3821)
NS	0.770 (0.4424)	0.106 (0.9157)	-0.899 (0.3698)	1.459 (0.1464)
Memory	1.078 (0.2828)	0.184 (0.8543)	**-1.972 (0.050)***	-1.787 (0.0759)

5.5.2. Testing for Gene-Environment Interactions: Strategy 1.

The one option for testing gene-environment interactions in this study was to search for possible interactions between all the candidate genes and all the binary and quantitative variables. This would entail re-analysing all the association analyses from Chapters 2, 3, and 4. If one were to examine the full, Afrikaner and British ancestry

sub-samples separately, each test would be carried out three times. Secondly, since there are five CTQ subscales, the number of tests carried out would be further increased by a factor of five.

In order to minimise the number of statistical tests carried out on the data only certain potential gene-phenotype interactions were tested. The first criterion was to examine those endophenotypes that were significantly associated with a specific type of abuse or neglect. A perusal of Table 5.2 indicates that the personality traits Anxiety and Stability were significantly associated with emotional and physical abuse as measured by the CTQ. It was therefore decided to test for gene-environment associations between these 4 variables (Anxiety + Stability and emotional + physical abuse) and the 11 candidate genes described previously. Similarly, the neurocognitive endophenotype, Memory was significantly associated with sexual abuse and thus the effect on Memory of the various gene-sexual abuse interactions was measured. A summary of Strategy 1 is shown in Figure 5.1, below.

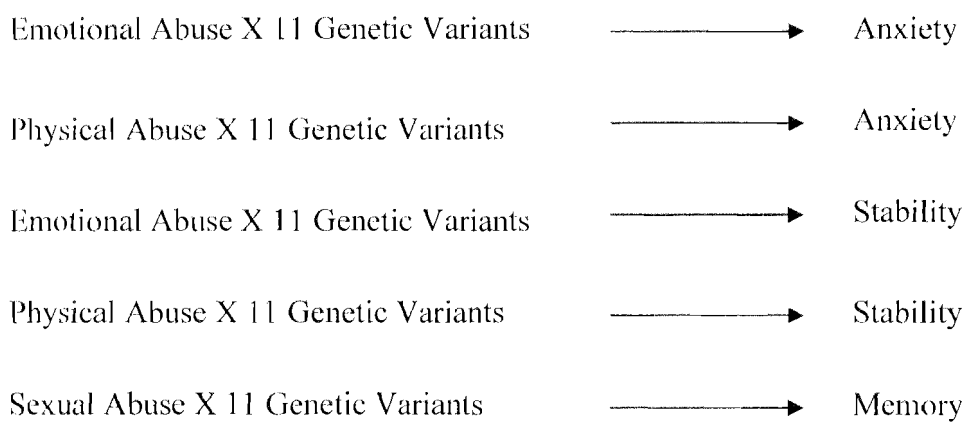


Figure 5.1. Diagram of Strategy 1.

These strategy 1 gene-environment interaction data are displayed in Tables 5.3-5.5 below. For the sake of brevity, interaction terms only are shown. In cases where one of the polymorphisms returned significant results, the data for all alleles were displayed.

The A1 allele of the TaqIA *DRD2* polymorphism interacted with abuse to influence scores on the Anxiety and Stability endophenotypes. However, while emotional abuse

was associated with higher levels of anxiety-related and mood-labile personality traits, the A1 allele interacted with physical abuse to reduce Anxiety and Stability scores. The main effects of genotype and emotional and physical abuse were not significant. The 11R repeat of the *Notch4* exonic microsatellite polymorphism reduced Stability scores when considered in isolation (t=-2.024, p=0.0445) but appeared to elevate Stability scores in the presence of emotional abuse (t=2.385, p=0.0182).

As far as Memory performance was concerned, both the 9R *SERT* VNTR variant (t=-2.044, p=0.0426) and sexual abuse (t=-2.890, p=0.0044) were associated with reduced memory scores. However, the 9R allele of *SERT* interacted with sexual abuse to produce higher Memory scores (t=2.052, p=0.0419). Neither sexual abuse (t=-0.667, p=0.5056) nor the *BDNF* val66met polymorphism (t=1.789, p=0.0824) exerted a statistically significant effect on Memory when considered separately. Nonetheless, the *met* allele of the functional *BDNF* variant interacted with sexual abuse to result in poorer performance on the verbal and visual memory tasks (t=-2.287, p=0.0236). A similar interaction was apparent for the *Apolipoprotein E* gene. Exposure to sexual abuse (t=-2.030, p=0.0442) reduced memory scores, but the ε2 allele of *ApoE* had no significant effect on Memory scores when considered in isolation (t=0.581, p=0.5622). The ε2 allele however, interacted with sexual abuse to reduce Memory performance (t=-1.913, p=0.0577). The ε4 allele considered in isolation was associated with improved performance on the Memory variable (t=2.490, p=0.0139) but the presence of the allele in conjunction with exposure to sexual abuse exerted a negative effect on Memory scores (t=-2.616, p=0.0098). Concerning the ε3 allele, no significant ANOVA main effect was observed for genotype (t=-1.626, p=0.1062) although sexual abuse by itself was detrimental to Memory performance (t=-3.224, p=0.0016). The ε3 allele however, appeared to exert a protective effect in the presence of sexual abuse (t=2.647, p=0.0090). See Table 5.5, below.

Table 5.3. Gene-CTQ Interactions: Anxiety.

Interaction	t-value	p-value	Nature of Interaction
*COMT*EA*	0.324	0.7464	NA
*COMT*PA*	0.640	0.5230	NA
*MAO*EA*	1.059	0.2911	NA
*MAO*PA*	-0.323	0.7471	NA
*5HTTLPR*EA*	-0.555	0.5796	NA
*5HTTLPR*PA*	0.197	0.8444	NA
*SERT VNTR*EA*	0.465	0.6424	NA
*SERT VNTR*PA*	-0.398	0.6908	NA
*D4-120*EA*	0.909	0.3648	NA
*D4-120*PA*	0.431	0.6671	NA
DRD2*EA	**2.042**	**0.0427***	**A1 allele and EA = higher Anxiety**
DRD2*PA	**-2.025**	**0.0444***	**A1 allele and PA = lower Anxiety**
*DAT*EA*	-0.556	0.5789	NA
*DAT*PA*	0.912	0.3630	NA
*BDNF*EA*	1.367	0.1734	NA
*BDNF*PA*	-0.343	0.7321	NA
*APOE*EA*	0.721	0.4719	NA
*APOE*PA*	1.108	0.2695	NA
*NOTCH6*EA*	-1.057	0.2921	NA
*NOTCH6*PA*	0.691	0.4906	NA
*NOTCH9*EA*	1.652	0.1005	NA
*NOTCH9*PA*	-1.810	0.0720	NA
*NOTCH10*EA*	-1.538	0.1260	NA
*NOTCH10*PA*	1.675	0.0958	NA
*NOTCH11*EA*	1.423	0.1565	NA
*NOTCH11*PA*	-0.684	0.4949	NA
*NOTCH12*EA*	-0.605	0.5462	NA
*NOTCH12*PA*	-0.249	0.8040	NA
*Prion*EA*	-0.201	0.8409	NA
*Prion*PA*	0.966	0.3356	NA

Table 5.4. Gene-CTQ Interactions: Stability.

Interaction	t-value	p-value	Nature of Interaction
*COMT*EA*	1.174	0.2420	NA
*COMT*PA*	0.565	0.5727	NA
*MAO*EA*	0.572	0.5680	NA
*MAO*PA*	1.288	0.1996	NA
*5HTTLPR*EA*	-1.178	0.2404	NA
*5HTTLPR*PA*	0.923	0.8444	NA
*SERT VNTR*EA*	0.465	0.6424	NA
*SERT VNTR*PA*	-0.398	0.6908	NA
*D4-120*EA*	-0.561	0.5756	NA
*D4-120*PA*	0.691	0.4904	NA
DRD2*EA	**2.550**	**0.0117***	**A1 allele + EA = higher Stability**
DRD2*PA	**-2.238**	**0.0211***	**A1 allele + PA = lower Stability**
*DAT*EA*	0.137	0.8912	NA
*DAT*PA*	-0.428	0.6689	NA
*BDNF*EA*	0.113	0.9100	NA
*BDNF*PA*	-0.176	0.8606	NA
*APOE*EA*	0.721	0.4719	NA
*APOE*PA*	1.108	0.2695	NA
*NOTCH6*EA*	-1.510	0.1330	NA
*NOTCH6*PA*	1.579	0.1163	NA
*NOTCH9*EA*	1.139	0.2564	NA
*NOTCH9*PA*	-1.683	0.0942	NA
*NOTCH10*EA*	-1.339	0.1824	NA
*NOTCH10*PA*	1.604	0.1106	NA
NOTCH11*EA	**2.385**	**0.0182***	**Combination of EA and 11R results in higher Stability scores**
*NOTCH11*PA*	-1.238	0.2174	NA
*NOTCH12*EA*	-1.160	0.2475	NA
*NOTCH12*PA*	-0.482	0.6382	NA
*Prion*EA*	-0.970	0.3333	NA
*Prion*PA*	1.530	0.1278	NA

Table 5.5. Gene-CTQ Interactions: Memory.

Interaction	t-value	p-value	Nature of Interaction
*COMT*SA*	-0.673	0.5019	NA
*5HTTLPR*SA*	1.085	0.2795	NA
SERT 9R*SA	**2.052**	**0.0419***	**9R allele is protective in presence of SA**
*SERT 10R*SA*	-0.731	0.4660	NA
*SERT 12R*SA*	-0.100	0.9208	NA
*D4-120*SA*	-0.969	0.3344	NA
*DRD2*SA*	0.179	0.8580	NA
*DAT*SA*	1.459	0.1467	NA
BDNF*SA	**-2.287**	**0.0236***	**Met allele interacts with SA to reduce Memory performance.**
*APOE2*SA*	*-1.913*	*0.0577*	*NA*
APOE3*SA	**2.647**	**0.0090****	**E3 allele exerts a protective effect on Memory in presence of SA**
APOE4*SA	**-2.616**	**0.0098****	**E4 allele interacts with SA to reduce Memory scores**
*NOTCH*SA*	-1.126	0.2620	NA
*Prion*SA*	-1.189	0.2362	NA

Table 5.5.3. Testing for Gene-Environment Interactions: Strategy 2.

In strategy 1, analyses were conducted in cases where the endophenotypes were associated with CTQ scores. In contrast, in strategy 2 potential gene-environment interactions were examined in cases where the one or more of the candidate genes were significantly associated with the binary disease phenotype or one of the personality or neurocognitive endophenotypes (see Chapters 2-4). In other words, every significant result from the association analyses using the binary notion of affective illness (Chapter 2), personality (Chapter 3) and neurocognitive function (Chapter 4) was tested for a gene-abuse interaction here.

Because of the large number of positive associations detected in the previous three chapters another super-variable, dubbed "Abuse" was created to circumvent testing for interactions with each one of the CTQ variables. Given the positive correlation between borderline and dissociative symptoms and a history of childhood abuse (Putnam et al. 1986; Carlson and Putnam 1993; Gunderson and Sabo 1993), the means and standard deviations of the CTQ, the DES and the STB were re-standardised and the scores averaged across these scales to produce the Abuse super-variable. Once again a mixed model ANOVA was used to compare the various diagnostic groups on this variable. Both BPD groups as well as the MDE-R sample scored significantly higher than controls on the super-variable, Abuse.

Table 5.6. Mixed Model ANOVA Comparing the Diagnostic Groups on the Standardised Super-Variable.

Super-Variable	**Sample Size**	**F-value**	**p-value**	**Nature of the Relationship**
Abuse	**236**	**4.40**	**0.0004****	**BPD I + BPD II + MDE-R > C**

A mixed-model ANOVA was then carried out for each gene-endophenotype association containing the fixed-effects predictors age, gender, ethnicity, depression and hypomania scores, the main effects of the gene and Abuse super-variable, and the interaction term gene*abuse. Family of origin was entered into the model as a random factor. The particular tests that were carried out are displayed in Table 5.7, below. Due to the large amount of data collected, detailed information is only listed for the statistically significant results that were obtained. These are highlighted in bold in Table 5.7, and are displayed in Table 5.8, below. Again only the interaction term is shown.

Table 5.7. List of the ANOVA Analyses Carried Out Under Strategy 2.

Phenotype	**Cohort**	**Gene Variant**	**Significant Interaction?**
Affective Illness	Afrikaner	*DRD4* VNTR	No
Affective Illness	British	*COMT*	No
Affective Illness	British	*DAT*	No
Anxiety	Total	*DRD4* VNTR	No
Anxiety	Total	*DAT*	No
Anxiety	Afrikaner	*DRD4* VNTR	No
Anxiety	Afrikaner	*Notch4*	No
Anxiety	**British**	***COMT***	**Yes**
Stability	**Total**	***COMT***	**Yes**
Stability	Total	*DAT*	No
Stability	Afrikaner	*BDNF*	No
Stability	Afrikaner	*Notch4*	No
Stability	**British**	***COMT***	**Yes**
Stability	British	*DAT*	No
HPS	Total	*BDNF*	No
HPS	Afrikaner	*Notch4*	No
HPS	British	*DAT*	No
CT	Total	*DAT*	No
CT	**Total**	***COMT***	**Yes**
CT	Afrikaner	*BDNF*	No
CT	Afrikaner	*Notch4*	No
CT	British	*DAT*	No
NS	Total	*BDNF*	No
NS	Afrikaner	*DAT*	No
NS	Afrikaner	*BDNF*	No
NS	**British**	***COMT***	**Yes**
Memory	**Total**	***DRD4* VNTR**	**Yes**
Memory	**Afrikaner**	***DRD4* VNTR**	**Yes**
Memory	British	*DRD4* VNTR	No

In the total cohort, Abuse and the 3R allele of the *DRD4* gene interacted with each other to reduce Memory performance (t=-3.845, p=0.0002), and a similar effect was apparent in the Afrikaner ancestry cohort (t=-3.716, p=0.004). Neither Abuse nor the 3R variant exerted significant effects on Memory when considered in isolation. In the Afrikaner ancestry group, the 4R allele considered in isolation was not significant (t=1.086, p=0.2805) but the variant exerted a protective effect on Memory performance when there was a history of sexual abuse (t=2.317, p=0.0228).

In the full sample the *val* allele of the Val158Met *COMT* polymorphism interacted with childhood trauma to increase levels of unstable-mood-labile traits as evinced by the Stability construct (t=2.375, p=0.0186) and the TEMPS-A CT scale (t=2.012, p=0.0458). There were no statistically significant main ANOVA effects. There was also a weak trend towards statistical significance for the Anxiety super-variable with the *val* variant once again the risk allele in the presence of Abuse (t=1.661, p=0.0985).

The same interaction effect was observed in the British ancestry cohort for Anxiety (t=2.248, p=0.0278), Stability (t=2.029, p=0.0465), CT (t=2.157, p=0.0352) and NS (t=1.986, p=0.0515). Once again there were no statistically significant main ANOVA effects. In the British ancestry sample, visual and verbal memory performance was also found to decrease in individuals with the *val* allele who were also exposed to abuse (t=-2.820, p=0.0066). There were no significant main ANOVA effects.

In the Afrikaner ancestry sample, both the main ANOVA effects, Abuse (t=-3.622, p=0.0005) and genotype (t=2.740, p=0.0074) exerted a significance effect. The *val* allele was associated with better Memory performance. The *val* allele-abuse interaction was also associated with better Memory performance (t=2.400, p=0.0185). Interestingly, the *COMT*-abuse interaction effect for the personality traits did not reach statistical significance in the cohort of Afrikaner ancestry. See Table 5.8, below.

Table 5.8.Gene-Abuse Interactions: Significant Results-Strategy 2.

Interaction	t-score	p-value	Nature of relationship
Anxiety (British ancestry): *COMTAbuse**	**2.248**	**0.0278***	**Val allele + Abuse = higher Anxiety**
Stability (total sample): *COMTAbuse**	**2.375**	**0.0186***	**Val allele + Abuse = higher Stability**
Stability (British ancestry): *COMTAbuse**	**2.029**	**0.0465***	**Val allele + Abuse = higher Stability**
CT (total sample) *COMTAbuse**	**2.062**	**0.0408***	**Val allele + Abuse = higher NS**
*NS (British ancestry): COMT*Abuse*	*1.986*	*0.0515*	*Val allele + Abuse = higher NS.*
Memory (total sample): *DRD4* VNTR 3R*Abuse	**-3.845**	**0.0002****	**3R allele + Abuse = reduced Memory**
Memory (Afrikaner ancestry): *DRD4* VNTR 3R*Abuse	**-3.716**	**0.0004****	**3R allele + Abuse = reduced Memory**
Memory (Afrikaner ancestry): *DRD4* VNTR 4R*Abuse	**2.317**	**0.0228***	**4R allele + Abuse = higher Memory scores**

As can be seen from a perusal of Table 5.8, the *COMT* val158met polymorphism appeared to interact with abuse to modify a large number of BPD endophenotypes. It was therefore decided to explore the effect of this gene more thoroughly. In the cohort of British ancestry, Anxiety levels were demonstrated to increase with the number of *COMT* gene *val* alleles carried by the individual when there was a history of self-reported abuse (t=2.248, p=0.0278). A similar trend was apparent in the full sample (t=1.661, p=0.0985), but not the Afrikaner ancestry sample (t=-0.542, p=0.5893). In the total cohort (t=2.375, p=0.0186) and sample of British ancestry (t=2.029, p=0.0465), the *COMT val* allele also appeared to interact with abuse to elevate Stability scores but again statistically significant results were not obtained in the

Afrikaner ancestry cohort (t=1.062, p=0.2906). The same kind of *val*-abuse interaction was observed in the full (t=2.012, p=0.0458; t=2.215, p=0.0281) and British ancestry (t=2.257, p=0.0352; t=1.986, p=0.05) cohorts for the CT and NS traits, respectively. The details are displayed in Table 5.9, below.

An interesting pattern of results was obtained for the Memory endophenotype. In the Afrikaner ancestry cohort, Memory performance improved with the number of *val* alleles when there was a history of abuse (t=2.400, p=0.0185). The *val* allele was also associated with improved Memory performance when Abuse was not taken into account (t=2.740, p=0.0074). On the other hand, in the cohort of British origin, Memory scores declined with the number of *val* alleles carried by the individual when there was a history of abuse (t=-2.820, p=0.0066). *COMT* genotype was not statistically significant when considered in isolation. See Table 5.9.

Table 5.9. Mixed Model ANOVA Results: *COMT**Abuse Interactions.

Phenotype	**t-score**	**p-value**	**Nature of Interaction**
Affective Illness (total)	-0.838	0.4032	NA
Affective Illness (Afr)	0.077	0.9390	NA
Affective Illness (Brit)	-1.314	0.1949	NA
Anxiety (total)	*1.661*	*0.0985*	Val + Abuse = higher Anxiety
Anxiety (Afr)	-0.542	0.5893	NA
Anxiety (Brit)	**2.248**	**0.0278***	**Val + Abuse = higher Anxiety**
Stability (total)	**2.375**	**0.0186***	**Val + Abuse = higher Stability**
Stability (Afr)	1.062	0.2906	NA
Stability (Brit)	**2.029**	**0.0465***	**Val + Abuse = higher Stability**
HPS (total)	0.794	0.4281	NA
HPS (Afr)	0.844	0.4007	NA
HPS (Brit)	1.439	0.1550	NA
CT (total)	**2.012**	**0.0458***	**Val + Abuse = higher CT**
CT (Afr)	0.386	0.7002	NA
CT (Brit)	**2.157**	**0.0352***	**Val + Abuse = higher CT**
NS (total)	**2.215**	**0.0281***	**Val + Abuse = higher NS**
NS (Afr)	0.127	0.8994	NA
NS (Brit)	*1.986*	*0.0515*	*Val + Abuse = higher NS*
Memory (total)	-1.151	0.2516	NA
Memory (Afr)	**2.400**	**0.0185***	**Val + Abuse = higher Memory**
Memory (Brit)	**-2.820**	**0.0066****	**Val + Abuse = lower Memory**

Table 5.5.4. Testing for Gene-Environment Interactions: Strategy 3.

The third strategy was to thoroughly investigate those genetic variants which have previously been demonstrated to be moderated by environmental contingencies. As discussed in section 5.2 the ins/del promoter variant of the *SERT* gene (5-HTTLPR) and the VNTR polymorphism in the promoter of the *MAO-A* gene have been reported to interact with environmental factors to increase the risk of developing various types of psychopathology. Once again, a mixed-model ANOVA was used to test for interactions between these polymorphisms and the Abuse super-variable. There were no significant interactions between the 5-HTTLPR *SERT* variant and Abuse (see Table 5.10).

Table 5.10. Interaction Between 5-HTTLPR and Childhood Abuse.

Phenotype	**t-score**	**p-value**	**Nature of Interaction**
Affective Illness (total)	-0.580	0.5627	NA
Affective Illness (Afr)	-0.181	0.1311	NA
Affective Illness (Brit)	-0.294	0.7700	NA
Anxiety (total)	-0.414	0.6792	NA
Anxiety (Afr)	-1.522	0.1311	NA
Anxiety (Brit)	0.448	0.6555	NA
Stability (total)	-0.302	0.7633	NA
Stability (Afr)	-0.589	0.5568	NA
Stability (Brit)	-0.070	0.9445	NA
HPS (total)	0.394	0.6942	NA
HPS (Afr)	-0.031	0.9750	NA
HPS (Brit)	0.616	0.5402	NA
CT (total)	-0.317	0.7518	NA
CT (Afr)	-0.431	0.6677	NA
CT (Brit)	-0.267	0.7905	NA
NS (total)	-0.003	0.9977	NA
NS (Afr)	1.517	0.1326	NA
NS (Brit)	-1.522	0.1329	NA
Memory (total)	0.603	0.5472	NA
Memory (Afr)	-0.171	0.8649	NA
Memory (Brit)	0.866	0.3902	NA

In order to test for an interaction between the *MAO*-A VNTR polymorphism and childhood trauma, the various alleles were grouped together on the basis of their functional activity. More specifically, the 2R, 3R and 5R alleles have been shown to result in reduced expression of the MAO-A enzyme and were labelled as low activity variants, while the 3.5R and 4R alleles result in higher levels of enzymatic activity.

In the total sample, the VNTR in the promoter region of the *MAO-A* gene showed a weak statistical trend towards an interaction with abuse with the low activity variants producing higher Anxiety scores in individuals exposed to childhood Abuse (t=1.842, p=0.0672). The genotype and Abuse main-effects were not statistically significant. An almost identical effect was observed for the Stability endophenotype with the low activity *MAO-A* gene variants interacting with Abuse to increase mood-labile-unstable-cyclothymic personality traits (t=1.826, p=0.0697). Abuse scores considered on their own also raised Stability scores (t=2.228, p=0.0272). The *MAO-A**Abuse interaction reached statistical significance in the sample of British ancestry (t=2.210, p=0.0306). The data are displayed in Table 5.11.

Table 5.11. Interaction Between MAO-A VNTR and Childhood Abuse.

Phenotype	**t-score**	**p-value**	**Nature of Interaction**
Anxiety (total)	*1.842*	*0.0672*	*Low activity*Abuse = higher Anxiety*
Anxiety (Afr)	1.532	0.1288	NA
Anxiety (Brit)	1.214	0.2290	NA
Stability (total)	*1.826*	*0.0697*	*Low activity*Abuse = higher Stability*
Stability (Afr)	0.761	0.4487	NA
Stability (Brit)	**2.210**	**0.0306***	**Low activity*Abuse = higher Stability**
HPS (total)	0.748	0.4557	NA
HPS (Afr)	-0.737	0.4626	NA
HPS (Brit)	1.645	0.1048	NA
CT (total)	1.058	0.2917	NA
CT (Afr)	0.644	0.5212	NA
CT (Brit)	1.163	0.2497	NA
NS (total)	0.284	0.7767	NA
NS (Afr)	-1.601	0.1121	NA
NS (Brit)	1.796	0.0774	*Low activity*Abuse = higher NS*
Memory (total)	-0.112	0.9102	NA
Memory (Afr)	-0.406	0.6861	NA
Memory (Brit)	0.170	0.8659	NA

5.6. Discussion.

5.6.1. The Relationship Between Childhood Trauma, DSM-IV Diagnosis and BPD Endophenotypes.

The BPD I, BPD II and MDE-R groups scored significantly higher than their unaffected relatives on the emotional abuse sub-scale of the CTQ ($F=2.7$, $p=0.0149$). The latter two groups also displayed significantly higher scores on the physical abuse scale of the CTQ ($F=2.59$, $p=0.0198$), but no inter-group differences were apparent for sexual abuse or childhood neglect. As discussed in section 5.1, childhood sexual and physical abuse has been shown to be a risk factor for the later development of depression (Bifulco et al. 1991; McCauley et al. 1997), psychotic illness (Goff et al. 1991; Greenfield et al. 1994) and rapid-cycling forms of BPD in particular (Leverich et al. 2002; Garno et al. 2005). With the exception of Garno et al. (2005) who found that emotional and physical, but not sexual abuse was associated with a rapid-cycling phenotype, the other studies mentioned above have not specifically measured emotional abuse. Emotional abuse might therefore be an important but neglected aetiological factor in the development of affective illness, including BPD, and further research is required.

An association between emotional abuse and the BPD endophenotypes, Anxiety ($t=2.386$, $p=0.0181$) and Stability ($t=2.280$, $p=0.0232$) was also observed. While the correlation between abuse and psychopathology is the subject of widespread research, very few studies have examined the effect of childhood abuse on the development of personality traits *per se*. Nevertheless, the relationship between Stability and emotional abuse is congruent with data from the literature which have repeatedly demonstrated that borderline personality disorder is associated with childhood abuse (Gunderson and Sabo 1993; Lysaker et al. 2004). Borderline personality disorder is a condition characterised by marked mood instability (APA 1994). The association between emotional abuse and Anxiety, reported here, can be considered to be a replication of the result of Roy (2002) who found a significant relationship between all types of abuse and neglect including emotional abuse and N, a trait which overlaps with the Anxiety construct.

The finding that higher levels of childhood physical abuse were associated with lower levels of Anxiety (t=-3.608, p=0.0004) is very difficult to explain. Perhaps scores on the physical abuse subscale of the CTQ reflect cultural characteristics rather than abuse *per se*. For example one of the questions from this scale is: "When I was growing up I was punished with a belt, a board, a cord, or some other hard object". This may be an anomalous item because the author notes that a significant number of individuals received a relatively high score on this question but low scores on the other 4 items of the scale. Since the total physical abuse scores were generally low across the sample then responses to this item may have biased the data, resulting in counter-intuitive findings. The mean physical abuse score for the total sample was 7.79 on a scale where scores range from 5-25, with higher scores indicating more severe trauma (see Appendix G).

Self-reported exposure to childhood sexual abuse was also associated with decreased visual and verbal memory performance (t=-1.972, p=0.05). There is extensive evidence from both animal and to a lesser extent human studies that exposure to stress, particularly chronic stress, may result in damage to or dysfunction of specific neuro-anatomical pathways. The preponderance of research has investigated the hippocampal response to stress as the CA1-CA4 fields of the hippocampus are among the most vulnerable areas of the brain to toxic insults (O'Brien 1997). As discussed in section 5.1, the hypothalamic-pituitary-adrenal (HPA) axis is the primary physiological pathway through which the body regulates its response to stress, culminating in the release of glucocorticoids from the adrenal cortex (Heim and Nemeroff 2000). Sapolsky et al. (1986) reported an association between high levels of corticosterone in rats, hippocampal degeneration and impairments in learning and memory, while young rats administered glucocorticoids display hippocampal changes and memory deficits reminiscent of those seen in older animals (Levy et al. 1994). Similar findings hold for non-human primates (Uno et al. 1989; Brooke et al. 1994).

Bremner (1999) cites a large number of human studies demonstrating that:
(a) Administration of normal therapeutic doses of glucocorticoids to healthy subjects impairs verbal memory performance.
(b) Stress-induced elevations in cortisol are associated with memory deficits which improve with declining cortisol levels. For example, Bremner et al. (1993) and

Yehuda et al. (1995) demonstrated found that Vietnam combat veterans suffering from PTSD displayed deficits in verbal declarative memory.
(c) Patients with Cushing's disease, characterised by elevated cortisol levels, show deficits in verbal declarative memory which are correlated with hippocampal volume reductions on MRI.

There are less data on the role that childhood abuse plays in memory dysfunction. Nevertheless, deficits in verbal memory as evinced by the Wechsler Memory Scales (WMS) have been reported to be characteristic of adults exposed to childhood physical and sexual abuse (Bremner et al. 1993). These data are supported by structural imaging studies which have highlighted the effects of abuse on the left hippocampus. Bremner et al. (1997) examined hippocampal volume in a series of patients with a history of severe childhood sexual and physical abuse and detected a 12% reduction in the volume of the left hippocampus in abused subjects relative to a matched control group, a result replicated by Stein et al. (1997) in a sample of sexually abused women. More recent data indicate that childhood abuse *per se* does not cause hippocampal dysfunction but rather PTSD which may or may not be secondary to sexual abuse in childhood (Bremner et al. 2004).

Based on the above data it seems reasonable to hypothesise that damage to or dysfunction of the hippocampus is responsible for the association between sexual abuse and Memory performance in this study. In evaluating this result it should however be noted that previous reports of hippocampal dysfunction and impaired memory performance have been obtained in samples exposed to significant degrees of childhood trauma or stress, hence the development of PTSD in later life. In the UCT cohort, however, the incidence of PTSD was low and the mean score on the sexual abuse scale of the CTQ was 7.31. The maximum possible score is 25. If the effect of sexual abuse on Memory performance is genuine then it suggests that even low levels of trauma can have a detrimental effect on later cognitive functioning. On the other hand, a significant proportion of the UCT cohort may have already had a genetically-based neurocognitive vulnerability given the family-history of BPD-spectrum illness. It could be hypothesised that the sub-group of BPD patients who displayed verbal memory recognition deficits had a history of past sexual abuse which interacted with

a genetically-mediated weakness to result in both executive and hippocampal dysfunction.

Nevertheless, the situation is rendered even more complex by the fact that the effect of stress on the brain may extend beyond the hippocampus. Acute administration of a benzodiazepine inverse agonist has been demonstrated to impair the prefrontal cortex-mediated cognitive function of rats through excess catecholamine release (Arnsten 1998; Shansky et al. 2003). Similarly, primates exposed to noise stress were found to perform more poorly on a spatial working memory task dependent on the function of the pre-frontal cortex (Arnsten and Goldman-Rakic 1998).

5.6.2 Testing for Gene-Abuse Interactions: Strategy 1.

The A1 allele of the *DRD2* Taq1A polymorphism interacted with emotional abuse to result in higher levels of anxiety-related ($t=2.042$, $p=0.0427$) and mood-labile personality traits ($t=2.550$, $p=0.0117$). The same A1 allele, however, interacted with physical abuse to reduce Anxiety ($t=-2.025$, $p=0.0444$) and Stability ($t=-2.238$, $p=0.0211$) scores.

As discussed in section, 5.6.1, the data obtained from the physical abuse subscale of the CTQ may not be as accurate as the other subscales, and therefore if the *DRD2* TaqIA polymorphism is exerting a genuine effect on the Anxiety and Stability endophenotypes in the presence of abuse, the risk allele is likely to be the A1 variant.

There are no previously published reports of an interaction between abuse (or another environmental variable) and the *DRD2* gene and thus it is difficult to evaluate this result. There are however data to suggest that over-activation of dopaminergic neurotransmission and DRD2 receptors may be an evolutionary adaptive response to stress that takes the prefrontal cortex "off-line" and allows for more instinctive, subcortically-mediated responses (Arnsten and Goldman-Rakic 1998). Arnsten and Goldman-Rakic (1998) found that they were able to block stress-induced working memory deficits in primates by antagonising DRD2 receptors prior to application of the stressor. However, since the Taq1A variant is not functional but simply in

purported linkage disequilibrium with another functional SNP, it is not possible to tell whether the A1 allele is associated with up or down-regulation of the *DRD2* gene.

In the author's opinion the interaction between the 11R variant of the *Notch4* gene and abuse leading to higher Stability scores (t=2.385, p=0.0182) is a false positive result. Not only is the 11R allele relatively rare, but none of the other *Notch4* alleles interacted with abuse to exert a significant effect on Stability. Likewise it is hypothesised that the interaction between the 9R allele of the *SERT* VNTR polymorphism and abuse should not be taken seriously as the 9R allele was very uncommon in the UCT sample. The potential interactions between the *BDNF* and *APOE* genes, sexual abuse and Memory are however, very interesting.

In section 4.6.3, it was noted that the absence of an association between Memory performance and the *BDNF* val66met polymorphism was surprising given the fact that this is one of the most promising candidates for a role in mediating cognitive function (see also Savitz et al. 2006a). As discussed in section 4.2, the low activity *met* allele of the *BDNF* gene has been associated with decreased executive and memory function. Here it was found that in individuals exposed to sexual abuse, Memory performance decreased with increasing numbers of *met* alleles (t=-2.287, p=0.0236), a result congruent with the literature.

What might be the physiological mechanism behind this putative interaction? BDNF is a neurotrophin, a class of molecule that exerts long-term effects on neuronal survival, migration, dendritic and axonal growth (Pang and Lu 2004). BDNF has been shown to prevent the spontaneous death of dopaminergic rat neurons (Hyman et al. 1994) and exert a protective effect in the presence of neurotoxins (Hung and Lee 1996) or ischemia (Yamashita et al. 1997) when injected into the brain. This protective effect is particularly salient in the hippocampus where the *BDNF* gene is most strongly expressed (Yan et al. 1997).

Chronic stress is hypothesised to result in excessive release of glucocorticoids from the adrenal gland, directly and indirectly causing atrophy and death of vulnerable neurons (particularly in the hippocampus) through the actions of cortisol and the inhibition of BDNF synthesis, respectively (Duman et al. 1997). The high activity *val*

allele of the *BDNF* gene might partially counteract the stress-induced inhibition of BDNF synthesis, perhaps explaining why the *met* allele is a risk factor for poor Memory performance when sexual abuse is taken into account.

Concerning Apolipoprotein E, a weakly significant interaction between the ε2 allele of the *ApoE* gene and sexual abuse was observed ($t = -1.913$, $p=0.0577$) leading to decreasing Memory scores. Similarly, higher sexual abuse scores with increasing numbers of the ε4 allele exerted a detrimental effect on Memory performance ($t = -2.616$, $p=0.0098$). On the other hand, the interaction between the ε3 allele and childhood abuse was protective, leading to improved visual and verbal memory ($t=2.657$, $p=0.0090$).

The ε4 allele is known to be a risk factor for the development of Alzheimer's disease (AD). Individuals homozygous for the ε4 allele are 14 times more likely to develop AD, and ε4 heterozygotes have a 3-fold increased risk over non-carriers of being diagnosed with the disorder (Farrer et al. 1997). The pathophysiological mechanism behind the action of the ε4 isoform is poorly understood but four main hypotheses exist. The ε4 isoform has been postulated to bind more strongly to the beta amyloid peptide forming a stable complex which may facilitate plaque formation (Strittmatter et al. 1993). The ε2 and ε3 isoforms of *ApoE* have also been demonstrated to bind more efficiently to microtubule-associated protein tau than the ε4 isoform which renders tau vulnerable to phosphorylation and therefore neurofibrillary tangle (NFT) formation (Strittmatter et al. 1994; Polvikoski et al. 1995). More recently, Harris et al. (2003) have demonstrated that the ε4 protein is particularly vulnerable to cleavage by a serine protease, generating a biologically active truncated product that produced NFT-like intra-neuronal inclusion bodies and elicited neurodegenerative and behavioural deficits in transgenic mice.

The fourth and most relevant suggestion in terms of this thesis, is that *ApoE* stimulates neurite outgrowth in response to cellular injury and degeneration (Fagan et al. 1996), but that the ε4 allele is not only deficient at facilitating neuronal repair, but actually interferes with the normal protective function of the ε3 isoform (Buttini et al. 2000). The retardation of dendritic growth may lead to the loss of synaptic connections and in turn, a progressive decline in the efficacy of neural network

function with associated cognitive impairment (Turic et al. 2001). The findings of a number of studies have lent credence to this proposal. Jordan et al. (1997) assessed 30 professional boxers with the Chronic Traumatic Brain Injury scale (CTBI) and found that a combination of high exposure (many bouts) and the presence of the ε4 allele was associated with greater levels of cognitive impairment. Kutner et al. (2000) showed that older ex American football players with an ε4 allele exhibited poorer attention, processing speed, and general cognitive function than their colleagues without this allele, or young players with the ε4 allele. Teasdale et al. (1997) and Crawford et al, (2002) demonstrated that memory performance was worse in ε4 carriers than non-carriers in a group of patients who had suffered traumatic brain injury.

A recent and somewhat controversial debate over the role of the ε4 isoform in the memory and cognitive functioning of non-demented individuals has evolved in the literature. Savitz et al. (2006b) carried out a detailed review of the literature and concluded that: (a). Elderly and to a lesser extent middle-aged non-demented individuals with a copy of the ε4 allele perform worse on neuropsychological tasks than non-carriers of the ε4 allele. (b). Non-demented adults with at least one copy of the ε4 variant present with structural (reduced medial temporal volumes) and metabolic (increased or decreased prefrontal, parietal and temporal activation) imaging abnormalities. (c). These neuropsychological and neuroradiological changes mirror those seen in AD and are thus unlikely to be reflective of normal ageing alone. (d). There is no evidence to suggest that the *ApoE* genotype has neurodevelopmental implications for IQ or cognition in a broader sense. On the basis of these data it is hypothesised that the reported *ApoE* genotype-cognition associations in the literature are due to the sampling of individuals with incipient AD.

How are these data relevant to the finding that the ε4 allele and to a lesser extent the ε2 allele interact with sexual abuse to reduce Memory function while the ε3 allele has a protective effect? As discussed above there is some data, albeit inconclusive, from animal and human studies that exposure to chronic stress (possibly in the form of sexual abuse) may cause damage to or at least dysfunction of the medial temporal lobe. It is therefore plausible to hypothesise that because the ε4 allele is deficient at facilitating neuronal repair and may indeed interfere with the normal repair processes

of the ε3 allele, the combination of sexual abuse and one or more ε4 alleles leads to long-term dysfunction of the neural networks underpinning verbal and visual memory.

A potential weakness with this hypothesis however, is the finding that the ε2 allele which putatively lowers the risk of developing AD (Nagy et al. 1995) also interacts with sexual abuse to reduce memory scores. Nevertheless, the potentially protective role of the ε2 isoform remains inconclusive. For example, den Heijer et al. (2002) assessed the impact of the different *ApoE* ε isoforms on hippocampal and global brain atrophy and found that ε4 carriers displayed greater atrophy but the effect of the ε2 allele did not differ from that of the ε3 allele. Lung et al. (2005) hypothesise that the ε2 allele only decreases the risk of AD when paired with an ε4 allele. The ε2 allele has also been suggested to exert a pathogenic effect leading to autistic spectrum pathology (Persico et al. 2004). As always a false positive result cannot be ruled out but the *a priori* evidence linking the *ApoE* gene to cognition and in particular, memory, suggests that this association may be worth trying to replicate in an independent sample.

5.6.3. Testing for Gene-Abuse Interactions: Strategy 2.

The finding that the 3R allele of the *DRD4* VNTR polymorphism appeared to interact with exposure to childhood abuse to reduce performance on the various memory scales in both the full (t=-3.845, p=0.0002) and Afrikaner ancestry (t=-3.716, p=0.0004) samples while the 4R allele exerted a possibly protective effect in the presence of abuse in the latter cohort (t=2.317, p=0.0228) is likely to be a false positive result. The 3R allele of the exonic VNTR polymorphism was relatively rare in the UCT sample, and thus a chance result owing to a small sample is probable. It is also unclear how the 4R allele, which was associated with poorer performance on the "Memory" construct (see Chapter 4), would exert a protective effect in the face of childhood trauma.

On the other hand, exposure to childhood trauma is postulated by the author to modulate the effects of the *COMT* Val158Met variant on anxiety-related, novelty-

seeking-related, and mood-labile-cyclothymic personality traits, with the *val* allele a risk factor for "pathological" traits. There are three reasons why this potential interaction may be genuine:

(a) The effect was observed in the total as well as the smaller British ancestry sample.

(b) The effect was apparent for a variety of phenotypes – Anxiety, Stability, CT and another relatively uncorrelated trait, NS.

(c) As discussed in Chapter 3, the *COMT* Val158Met polymorphism was significantly associated with a mood-labile-cyclothymic phenotype. Individuals with borderline personality disorder are characterised by affective instability, unstable and intense personal relationships, a capricious self-image, and severe dissociative symptoms (APA 1994). People with borderline personality disorder are also impulsive, a trait that is positively correlated with NS scores (NS contains a subscale called "impulsiveness"). The important point *apropos* borderline personality disorder is that early trauma in the form of sexual and physical abuse is postulated to be a key aetiological factor in the development of the illness (Gunderson and Sabo 1993; APA 1994). It therefore makes theoretical sense for exposure to childhood abuse to potentiate the effects of a genetic variant (possibly *COMT* Val158Met) which influences the development of mood-labile-cyclothymic personality traits. It is hypothesised that the *val* allele constitutes a risk factor for the development of both mood-labile-cyclothymic traits and borderline personality disorder, and one way to check the validity of the *COMT*-abuse interaction finding reported here, is to carry out a genetic association study in a sample of individuals with borderline personality disorder.

(d) The potential interaction between *COMT* and abuse is also biologically plausible. The endogenous opioid system is activated in response to both physical and psychological stressors particularly those of a chronic and unpredictable nature where the opioids act to attenuate the distressing affective component of the stimuli (see Ribeiro et al. 2005 for a review). Zubieta et al. (2003) found that the *COMT* val158met polymorphism modulates the stress-induced activation of the μ-opioid neurotransmitter system and thus "influences the human experience of pain and may

underlie individual differences in the adaptation and responses to pain and other stressful stimuli". Zubieta et al. (2003) observed that *met/met* homozygotes had a lower capacity to activate μ-opioid neurotransmission than their heterozygous and *val/val* homozygous counterparts and that this effect was associated with a compensatory up-regulation of μ-opioid receptors. Berthele et al. (2005), in a post-mortem study, showed that the presence of the *COMT met* allele is indeed associated with a higher expression of μ-opioid receptors in diverse areas of the brain including the caudate and thalamus. Based on these data, one would intuitively hypothesise that the *met* allele rather than the *val* allele should be the risk factor for an adverse response to stressful experiences like abuse. Nevertheless the scientific community's understanding of the complex molecular interactions underpinning these pathways is too embryonic to draw firm conclusions.

The potential effect of the interaction between the *COMT* Val158Met polymorphism and abuse on Memory performance is more equivocal. In the sample of British origin the *val* allele interacted with abuse to significantly reduce scores on the Memory supervariable (t=-2.820, p=0.0066). On the other hand, in the cohort of Afrikaner origin the interaction between the *val* variant and abuse yielded better memory scores (t=2.400, p=0.0185). It is unclear why the same allele would exert two very different effects on visual and verbal memory in two different samples exposed to the same environmental factor. One possibility is that the actions of a given variant are modified by genetic background. As discussed in Chapter 4, a potential QTL for Memory was observed in the region of the *COMT* gene on chromosome 22q11 but only in family 30, the largest of the Afrikaner ancestry pedigrees. The more parsimonious explanation however, is that these contradictory data are indicative of a false positive result. Nevertheless, the fact that there is prior evidence implicating the *COMT* gene in memory and executive function (Savitz et al. 2005a) indicates that these findings are worthy of further investigation.

5.6.3. Testing for Gene-Abuse Interactions: Strategy 3.

The absence of any statistically significant interactions between the 5-HTTLPR variant of the *SERT* gene and abuse was something of a surprise as the *short* 5-

HTTLPR allele has been demonstrated to interact with adverse environmental events to predispose to mental distress and depression (see section 5.2.). On the other hand, the results from this thesis are congruent with the findings of Gillespie et al. (2004) who reported that the 5-HTTLPR genotype did not interact with life stress to predispose to depression.

There are two possible reasons for the absence of significant results in this study. Self-reported childhood trauma as an adverse environmental agent cannot necessarily be equated with life-stress or levels of social support which have been previously demonstrated to modulate 5-HTTLPR genotype effects. Secondly, the positive reports in the literature have focused on unipolar depression and mental distress as outcome variables. In this study, affective illness in BPD pedigrees and various hypothesised endophenotypes for BPD were the dependent variables. It is plausible that the 5-HTTLPR genotype-life-stress interaction is limited to unipolar illness.

A weak interaction between the *MAO-A* VNTR polymorphism and the Abuse super-variable was observed with low activity variants producing higher Anxiety scores in individuals with higher childhood Abuse scores ($t=1.842$, $p=0.0672$). An almost identical effect was observed for the Stability endophenotype ($t=1.826$, $p=0.0697$). The latter interaction reached statistical significance in the sample of British ancestry ($t=2.210$, $p=0.0306$).

These data are at least nominally consistent with the studies of Caspi et al. (2002) and Foley et al. (2004) who found that maltreated children with the low activity allele of *MAO-A* gene VNTR polymorphism were more likely to develop antisocial and conduct disorders than carriers of the protective high-activity variant. On the other hand, the possible effect of the interaction between the *MAO-A* gene and Abuse on the development of cyclothymic-mood-labile traits is congruent with the data of Huang et al. (2004) who found that the lower expression variant was associated with a history of childhood abuse and higher impulsivity.

5.6.4. Weaknesses.

A potentially significant difficulty with attempting to control for the effects of environmental variables in genetic studies deserves mention. It is in practice very difficult to obtain a measure of environmental influence that is not contaminated by genetic effects (Plomin and Bergeman 1991). Rowe (1981) first reported this counter-intuitive notion by showing that adolescent twins' reports of their parents' levels of accepting and rejecting behaviour were under genetic influence. This type of finding has been extended to retrospective measures of family warmth and parental control (Plomin et al. 1988) as well as family cohesion and encouragement of individual growth (Bouchard and McGue 1990).

Krueger et al. (2003) demonstrated that the heritability of recalled family environment is partly explained by the heritability of personality. The authors studied 180 pairs of twins who were separated at an early age and reared apart with multiple measures of personality and recalled childhood family environment. The same genotypes that lead to individual differences in personality appear to influence the way individuals remember their family environment (Krueger et al. 2003).

Following this line of reasoning it may be unwise to assume that correlations between childhood trauma and psychopathology are indicative of the causal primacy of abuse or neglect. The situation is rendered even more complicated when one takes into account the possibility that genetic factors influence the probability of exposure to environmental events. The impulsive risk-taking child or the inhibited socially withdrawn individual may both be vulnerable to the nefarious influence of social predators (Hill, 2003).

Yet another possibility is that a history of abuse simply supervenes on the family history of BPD. In other words, BPD may be largely genetic in aetiology. The familial pattern of abusive relationships may be a consequence of genetically-driven mood instability rather than a cause of psychiatric illness (Garno et al. 2005).

Another weakness of the gene-environment interactions described in this thesis is the ambiguous nature of some of the findings. The results of genetic analyses of complex disorders are difficult to interpret given the phenomena of incomplete penetrance, variable expressivity, genetic epistasis and genetic heterogeneity as well as the ubiquitous possibility of type 1 errors. When environmental variables and their interactions with candidate genes are taken into account the complexity of the situation increases exponentially and this together with science's embryonic understanding of the neurobiology of psychopathology renders the legitimacy of novel findings open to debate.

In addition, only one self-reported measure of childhood abuse, the *Childhood Trauma Questionnaire* was used as a basis for the analysis of the gene-abuse interactions. More objective, perhaps clinician-based ratings would have facilitated the evaluation of the results of this section of the thesis. It may also be worthwhile to reanalyse the data with another "abuse" super-variable this time excluding dissociative and borderline symptomatology.

Finally, the modest sample size which may have reduced the power needed to detect small effects is noted. Replication studies are necessary in order to test the validity of the results.

Chapter 6.

Conclusion: An Evaluation of Findings and Future Strategies.

Joy and woe are woven fine,
A clothing for the soul divine;
Under every grief and pine
Runs a joy with silken twine.
It is right it should be so;
Man was made for joy and woe;
And, when this we rightly know,
Safely through the world we go.

William Blake
Auguries of Innocence (1863).

6.1. Novel Findings.

The overarching theme of this thesis has been the emphasis on the neurobiological and genetic complexity of bipolar affective disorder and the difficulties this poses for the phenotypic characterisation of the condition and consequently for gene identification. Three novel strategies were adopted as a response to these challenges:

(1). Affected probands and their family members from a relatively reproductively-isolated population, the Afrikaner, were recruited to participate in the current research project. Theoretically, populations that have expanded in size from a small number of founding members should display reduced genetic heterogeneity and increased linkage disequilibrium compared to out-bred populations.

There is no published work detailing molecular genetic investigations of BPD in this ethnic group or indeed any South African population. Abecasis et al, (2004) however, produced evidence for a linkage signal on the short arm of chromosome 1 as well as chromosome 13q32 in a sample of Afrikaner families with schizophrenia.

The original findings from this component of the thesis include significant evidence for linkage in the region of 1q31-32 with a maximum Merlin NPL (All) score of 2.52, and a statistically significant association between the 10R allele of the DAT gene and affective illness detected in the sample of British origin.

(2). The main thrust of the thesis was the novel use of personality traits and neurocognitive function as endophenotypes for genetic analyses of BPD. Understanding the genetic basis of these theoretically simpler phenotypic traits could constitute the first step in the ascent towards the aetiological understanding of the more complex psychiatric phenotype.

The BPD I group and to a lesser extent the MDE-R sample displayed higher scores on personality scales that measure anxiety-related, mood-labile-irritable, and hypomanic personality traits. The potentially distinct personality profiles of the BPD II and MDE-S groups is interesting and worthy of follow-up. The anxiety-related and mood-labile traits showed significant evidence of heritability, confirming their potential as phenotypic markers of affective illness.

Original findings include:
(a) Strong evidence for linkage between the 13q32 region and NS in the largest Afrikaner pedigree.
(b) Weak evidence for linkage between the Stability trait and markers on chromosome 4p16.
(c) An association between the COMTVal158Met polymorphism and mood-labile-cyclothymic traits.
(d) An association between the BDNF Val66Met variant and hypomanic or novelty-seeking related personality traits.
(e) An association between the 3' VNTR variant of the DAT gene and anxiety-related, mood-labile-cyclothymic, and hypomanic personality traits in the subgroup of British origin.
(f) An association between the Notch4 exonic CTG repeat and anxiety-related, mood-labile-cyclothymic, and hypomanic personality traits in the Afrikaner population.
Another quantitative trait, neurocognition, was used as a second experimental endophenoype. The BPD I and MDE-R groups performed significantly worse than

unaffected relatives on both verbal and visual memory recall tasks, but only the BPD I group showed deficient recognition memory performance. The Memory super-variable, the composite score of RCF (recall) and RAVLT (total learning and recognition), showed some degree of heritability supporting its choice as an endophenotypic marker.

Novel findings from this aspect of the thesis include:

(a) The fact that the MDE-R group as well as the BPD I group performed more poorly than unaffected relatives on visual and verbal memory recall tasks suggesting at least some overlap in the risk alleles carried by bipolar probands and their unipolar relatives.

(b) Potential linkage signals for visual and verbal memory on 10q23 and 22q11.

(c) An association between the exonic DRD4 gene 48bp VNTR polymorphism and memory performance.

(3). The third original aspect to this thesis was the attempt to control for an environmental factor, childhood abuse, that may interact with genetic susceptibility variants to precipitate affective and psychotic illness. The BPD I, BPD II, and MDE-R groups all reported more emotional abuse than controls, while the latter two groups also had a greater history of physical abuse than their unaffected relatives.

Novel results include:

(a) Whereas sexual abuse is highlighted as a risk factor for psychopathology in the psychological literature, a higher prevalence of emotional and to a lesser extent physical abuse, but not sexual abuse was characteristic of the BPD and MDE-R groups in this study.

(b) Emotional abuse was also found to elevate Anxiety and Stability (unstable traits) scores.

(c) A potential interaction between early childhood trauma, the presence of the *val* allele of the *COMT* Val158Met polymorphism and elevated Anxiety, Stability and NS traits.

(d) A potential interaction between exposure to sexual abuse, the low activity *met* variant of *BDNF* and impaired performance on verbal and visual memory tasks.

(e) A possible interaction between sexual abuse and the ε4 allele of the *ApoE* gene, resulting in reduced visual and verbal memory task performance.
(f) A possible interaction between abuse and the low activity variants of the *MAO-A* gene leading to increased anxiety-related and mood-labile-cyclothymic traits.

6.2. Methodological Weaknesses of this Study.

The binary linkage and association analyses were carried out on a modest sample of 350 individuals and thus the study was potentially under-powered. Personality and neuropsychological data were then collected on a proportion of this sample further increasing the possibility of Type II errors.

A large number of personality questionnaires and neuropsychological tasks were administered to the cohort. A select few of these psychological tests then had to be selected as endophenotypes to ease the potential for multiple testing-related errors. Unless a PCA gives a very clear indication of the underlying components of the battery, the selection of tests can become an exercise in subjectivity. The other alternative is for the researcher to make an *a priori* decision to use particular tests as phenotypic markers of BPD. When this thesis commenced in 2002 however, there was less clarity in the literature about which temperaments and neurocognitive deficits could be used as endophenotypes of BPD, circumventing this option.

Thirdly, even with the limited number of endophenotypes - five in the case of personality and only one in the case of neurocognitive function – used in this study, it may be argued that the results are confounded by the large number of independent tests carried out on the data which exacerbates the risk of false positive associations.

The issue of correction for multiple testing is controversial and statistically complex. Bonferroni corrections are usually deemed to be overly conservative in cases where *a priori* evidence of an association is present or when the outcome measures are correlated with each other (Perneger 1998). Some statisticians however, dispute the theoretical validity of adjusting for multiple testing. Perneger (1998 p1236) writes that

"Bonferroni adjustments are, at best, unnecessary and at worst deleterious to sound statistical inference".

Researchers are interested in testing the validity of each association between independent and dependent variables but Perneger (1998) argues that Bonferroni adjustments do not provide this information but rather lead to the acceptance or the rejection of an irrelevant null hypothesis that the groups are identical on all variables under consideration.

Secondly, Perneger (1998) notes that inference based on the results of Bonferroni corrections defy common sense because a given comparison will be interpreted differently according to how many other tests were performed. The example of a patient who is sent by a doctor for diagnostic tests is illustrative of the point. Assuming that each test has a baseline risk of returning a false positive result, the patient's diagnosis could be determined by how many laboratory tests were ordered!

Given time and resource constraints, not all regions of interest and promising candidate genes were typed by the author. Examples include chromosome 6p22, 8p22, 10q25-26, 12q21, *G72/G30*, Disrupted in Schizophrenia (*DISC1*), Dysbindin, Neuregulin (*NRG*), G-protein-coupled receptor kinase (*GRK3*) and Proline dehydrogenase (*PRODH2*). See section 6.4, below.

Both the psychological testing and the genotyping were performed by the author who was consequently not blind to psychiatric diagnosis during the collection of these data.

The majority of diagnostic interviews were performed during the initial recruitment phase of the project between 1998 and 2000, and therefore DSM-IV diagnoses may have shifted over time in a percentage of the sample. Nevertheless, since the genetic analyses with the quantitative endophenotypes did not rely on formal psychiatric diagnoses, these data should be immune to any potentially confounding diagnostic changes.

The analysis of potential gene-childhood trauma interactions was based on only one measure of childhood abuse, *The Childhood Trauma Questionnaire*. In particular, (possibly) more objective clinician-based ratings of childhood abuse could have led to different results.

6.3. Theoretical Problems with the Endophenotypic and Gene-Environment Interaction Approach.

While the use of genetically simpler endophenotypes may help to ameliorate the difficulties posed by the genetically heterogeneous nature of BPD, there is no guarantee that a particular endophenotype will not itself display a certain level of genetic heterogeneity. This is particularly true of more complex endophenotypes such Kraepelinian or Akiskalian temperaments. Another potential example in this thesis is the strong evidence for linkage between NS and 13q32 markers in pedigree 30 only. As illustrated in Chapter 5, many of the endophenotypes used in this study were significantly influenced by an environmental factor, level of childhood trauma. Thus these personality phenotypes may not necessarily be easier to elucidate genetically than psychiatric symptomatology. The selection of theoretically simpler phenotypes may reduce this problem but these traits are less likely to show specificity for the illness.

A possible example of an endophenotype that shows poor illness specificity is executive dysfunction. Executive dysfunction has been widely reported to be present in people with unipolar depression, schizophrenia and their unaffected relatives (Davidson et al. 2002; Hoff and Kremen 2003) which is to be expected given that these illnesses are hypothesised to share nosological features and genetic risk factors with BPD (Akiskal and Pinto 2000; Bramon and Sham 2001; Berrettini 2004). A number of disorders which bear a more tenuous nosological relationship to BPD are however also characterised by executive dysfunction. The following list is by no means comprehensive but includes ADHD (Barkley 1997), autism (Hill 2004) eating disorders (Lena et al. 2004), obsessive compulsive disorder (Shin et al. 2004), antisocial personality disorder (Hiatt et al. 2004) and borderline personality disorder (Monarch et al. 2004). In fact, the possibility that signs of executive dysfunction are a necessary feature of all psychiatric illness is raised. Unless the processes involved in

executive functioning can be fractionated and each disorder correlated with a unique disturbance, the generic term "executive dysfunction" will likely signify a susceptibility to psychiatric illness rather than BPD *per se*. Anxiety-related personality is another potential example of an over-inclusive endophenotype. Anxiety is associated with a myriad of nosologically distinct psychiatric conditions. Understanding the genetic basis of psychopathology-associated executive function and harm avoidance-inhibitory personality styles will be invaluable but will not necessarily elucidate the susceptibility variants that make bipolar affective disorder unique from other forms of psychiatric illness.

The counter argument to this potential difficulty with the use of endophenotypes is that the author is guilty of the reification of DSM-IV psychiatric categories. The *raison d'etre* of endophenotyping is the avoidance of DSM categorisation. If supposedly distinct categories of psychiatric illness share genetic risk factors then this may be indicative of the failure of current diagnostic systems rather than the inferiority of the relevant endophenotype.

As was mentioned in Chapter 5, self-report measures of environmental influence such as exposure to childhood abuse are not objective measures of the exposure to trauma and are in fact strongly heritable.

On a philosophical note, one cannot in the author's opinion, demonstrate *a priori* that a particular trait is a genuine endophenotype for BPD (or any other psychiatric illness) because unless the variants that induce susceptibility to BPD are known it is impossible to prove that the marker is co-segregating with BPD in a family. Firstly, it is theoretically possible that certain individuals with diagnoses of BPD do not carry susceptibility genes. Thus the absence of a phenotypic marker in an affected individual may mean that the marker is not associated with the disorder, that it is not associated with that putative subtype of bipolar illness, or that the individual in question is a phenocopy. Secondly, it is not always clear what phenotype should be equated with the presence of the illness. An individual with a bipolar diathesis may present with a variety of profiles such as schizoaffective disorder, unipolar depression, alcoholism, and panic disorder. Equally, individuals with these conditions may or may not be genetically predisposed to BPD and thus the presence or absence

of the putative endophenotypic marker in these persons cannot prove or disprove a co-segregation of marker and trait.

This point can be extended to the field of psychiatric genetics in general. The key issue is the burden of proof required to convince the research community that a particular gene-disorder association is genuine. If a rare variant is responsible for a psychiatric illness then a demonstration that this variant tracks with the illness in a particular family is probably sufficient to demonstrate the aetiological importance of the variant in question. When common polymorphisms are the focus of research efforts how can the consumer of scientific publications differentiate between false positive results, inconsistent replications due to genetic heterogeneity, and true associations? In Popperian terms, genetic association studies are currently unfalsifiable, a label traditionally reserved for "pseudoscientific" disciplines such as psychoanalysis. In the author's opinion the only long-term solution is for hypothesised gene-disorder associations to be proven by higher level human and animal experiments conducted by the neuroscientific community.

6.4. Future Directions.

The pilot analysis of personality and neurocognitive endophenotypes described in this thesis needs to be broadened and deepened. The typing of variants in at least some of the candidate genes described in Table 6.1, below, would be a useful addendum to the project. These tabulated data have been derived from various sources including papers by Ogden et al. (2004), Sullivan (2005) and Hattori (2005), as well as Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM). Tissue specific gene expression data were obtained from the Novartis Foundation expression atlas (http://symatlas.gnf.org).

Most of the genes listed in Table 6.1 have been either independently associated with schizophrenia or BPD, or are located in regions linked to these disorders, especially the candidate regions listed in Chapter 2. Given the evidence for linkage between chromosome 1q32 markers and affective disorder in this study, the region between 1q23 and 1q42 was emphasised. Other genes have been chosen because of their

proximity to strong candidates based on data from this study such as DAT (5p15) and NOTCH4 (6p21).

Table 6.1. Hypothetical Disease Susceptibility Candidates.

Gene	**Name**	**Location**	**Expression in PFC π**	**Expression Cingulate Cortex π**	**Expression Amygdala π**	**Linkage to Region (Chapter 2.1)**	**Association Evidence**
RGS4	Regulator of G-protein signalling 4	1q23.3	+++	+++	+++	Yes (SCZ BPD)	Yes (SCZ)
CAPON	C-terminal PDZ domain ligand of neuronal nitric oxide synthase	1q23.3	+	-	-	Yes (SCZ BPD)	Yes (SCZ BPD)
GLUL	Glutamate-ammonia ligase (glutamine synthase)	1q25.3	+++	+	+	Yes (SCZ BPD)	No
ADORA1	Adenosine A1 receptor	1q32.1	++	++	++	Yes (SCZ BPD)	No
PIGR	Polymeric immunoglobulin receptor	1q32.1	+	+	+	Yes (SCZ BPD)	No
PIK3C2B	Phosphoinositide-3-kinase, class 2, beta polypeptide	1q32.1	+	+	++	Yes (SCZ BPD	No

PPP2R5A	Protein phosphatase 2. regulatory subunit B (B56), alpha isoform	1q32.3	+	-	+	Yes (SCZ BPD)	No
DISC1	Disrupted in Schizophrenia	1q42.2	+	-	+	Yes (SCZ BPD)	Yes (SCZ BPD Cognition)
RGS7	Regulator of G-protein signalling 7	1q43	+++	+++	+++	Yes (SCZ BPD)	No
GPR78	G protein-coupled receptor 78	4p16.1	-	+	-	Yes (BPD)	Yes (BPD)
ADCY2	Adenylate Cyclase 2	5p15.31	-	+	+	Yes (BPD)	No
BASP1	Brain abundant, membrane attached signal protein 1	5p15.1-p14	+++	+++	+++	Yes (BPD)	No
GRM4	Metabotropic glutamate receptor 4	6p21.31	+	+	++	Yes (SCZ BPD)	No
GABBR1	GABA B receptor 1	6p22.1	-	-	-	Yes (SCZ BPD)	Yes (SCZ)
CAP2	Adenylate cyclase-associated protein, 2	6p22.3	+++	+++	+++	Yes (SCZ BPD)	No
DTNBP1	Dystrobrevin binding protein 1	6p22.3	+	+	+	Yes (SCZ BPD)	Yes (SCZ & BPD)

NRG1	Neuregulin 1	8p12	+	-	-	Yes (SCZ)	Yes (SCZ BPD)
PSAP	Prosaposin	10q22.1	+	-	+	Yes (BPD)	No
CAMK2G	Calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	10q22.2	+	+	-	Yes (BPD)	No
SGPL1	Sphingosine-1-phosphate lyase 1	10q22.1	+	-	+	Yes (BPD)	No
TACR2	Tachykinins receptor 2	10q22.1	+	+	-	Yes (BPD)	No
CIT	Citron	12q24	-	+	-	Yes (BPD)	Yes (BPD)
DAO	D-amino-acid oxidase	12q24	+	+	-	Yes (SCZ BPD)	No
NOS 1	nitric oxide synthase 1 (neuronal)	12q24	+	-	-	Yes (SCZ BPD)	Yes (SCZ BPD)
G30/G72	LG30 & G72	13q33.2	Unknown	Unknown	Unknown	Yes (BPD & SCZ)	Yes (BPD & SCZ)
ABAT	4-Aminobutyrate aminotransferase precursor	16p13.2	+	+	+	Yes (BPD & SCZ)	Yes (Autism)
ADCY9	Adenylate cyclase 9	16p13.3	++	+	+	Yes (BPD SCZ)	No
PRODH	Proline dehydrogenase 1	22q11.2	+++	+	++	Yes (BPD	No

						SCZ)	
PITPNB	Phosphatidylinositol transfer protein. beta	22q12.1	+	+	+	Yes (SCZ BPD)	No
RBM9	RNA binding motif protein 9	22q12.3	+++	++	+	Yes (SCZ BPD)	No
NPTXR	neuronal pentraxin receptor	22q13.1	+++	+++	+++	Yes (SCZ BPD)	No

PFC=Prefrontal Cortex

SCZ=Schizophrenia

π Novartis Research Foundation (http://symatlas.gnf.org) + expression above median ++ 3* median expression +++ 10* median expression – below median expression

A number of the genes listed in Table 6.1, were suggested as candidates for BPD and schizophrenia by Ogden et al. (2004) based on an approach dubbed "Convergent Functional Genomics". Ogden et al. (2004) selected a set of genes that showed altered levels of expression in response to methamphetamine and valproate administration in an animal model. A subset of these genes that are expressed in key neuronal regions were then derived from the master list and cross-referenced against regions of the genome known to be linked to schizophrenia and BPD, as well as post-mortem data from these patients indicating neuronal gene expression changes (Ogden et al. 2004). The exploitation of data from multiple disciplines and their sieving through bioinformatics–based methodologies will probably become the principal future strategy for identifying genes worthy of exploration. A similar scheme could be used to identify candidate genes involved in neurocognitive function. A baseline list of candidate genes could be derived from microarray-based studies of medication-induced cognitive deficits in either animals or humans and then cross-referenced in a similar manner to that of Ogden et al. (2004). Up or down-regulation of genes involved in approach-avoidance behaviour may be construed as candidates for temperament. The caveat is that the biological basis of animal behaviour may be just as complex as the mechanisms underlying human behaviour and psychiatric disorders.

While *a priori* identification of candidate genes is important, a systematic evaluation of reported associations is an even greater imperative. As was alluded to in the above discussion, psychiatric geneticists need to work together with molecular biologists and neuroscientists in order to demonstrate that the genetic variants associated with psychiatric illness exert an effect that leads to dysfunction of neural circuits involved in emotion or cognition. A nascent movement in this direction is already detectable. For instance Egan et al. (2001), Hariri et al. (2002) and Hariri et al. (2003) demonstrated that particular polymorphisms associated with schizophrenia were also associated with functional imaging and neurocognitive changes. Stefansson et al. (2002) demonstrated that Neuregulin 1 (associated with schizophrenia) knockout mice showed reduced expression of N-methyl-D-aspartate receptors suggesting that glutamatergic abnormalities may be characteristic of the disorder. Brandon et al. (2004) and Schurov et al. (2004) have explored the neurodevelopmental role played by DISC 1 in an animal model.

The complexity of intra-cellular signalling pathways suggests that changes to more than one gene involved in a molecular cascade may be necessary to induce susceptibility to psychiatric illness. On a genetic level this is theoretically reflected in the phenomenon of gene-gene interactions. Ogden et al. (2004) and Hattori et al (2005) have thus advocated a systematic approach in which all the genes involved in a particular molecular pathway are considered to be potential candidates for psychopathology. It may therefore be germane to investigate the circuits driven by *COMT, BDNF, DAT, Notch4* and other genes that have been associated with BPD.

This approach is however predicated upon the assumption that the statistical techniques and power necessary to compute these potentially complex gene-gene interactions are available. Assuming these technical difficulties can be overcome testing data for genetic epistasis may prove to be very fruitful. In one example, Schulze et al. (2004) observed a positive correlation between bipolar-family-specific linkage scores on chromosome 6q and 6p22.2 (which lie approximately 70 cM apart) and argued that this correlation was reflective of an interaction between the two loci. Linkage analysis calculations for each locus conditioned on evidence of linkage to the other locus increased the LOD scores from 2.26 to 5.42 on 6q and from 0.35 to 2.26 on 6p22.2 (Schulze et al. 2004).

As far as the endophenotypic approach is concerned it may be instructive to identify simpler endophenotypes – endophenotypes of personality and neuropsychological testing-defined neurocognitive function – that will introduce less noise into genetic analyses. Concerning personality, one emerging possibility is the extensive body of work describing asymmetrical prefrontal cortical activation as evinced by electroencephalograph (EEG) and its relationship to positive (appetitive drive or behavioural activation) and negative (inhibitory) affective style (Davidson 1998; Demaree et al 2005). Other neuroimaging modalities such as functional magnetic resonance imaging (fMRI) may provide a more sensitive and domain-specific measure of neurocognitive dysfunction. A hypothetical schematic of the phenotypic and perhaps genetic complexity of various endophenotypes for bipolar disorder is shown in Figure 6.1, below.

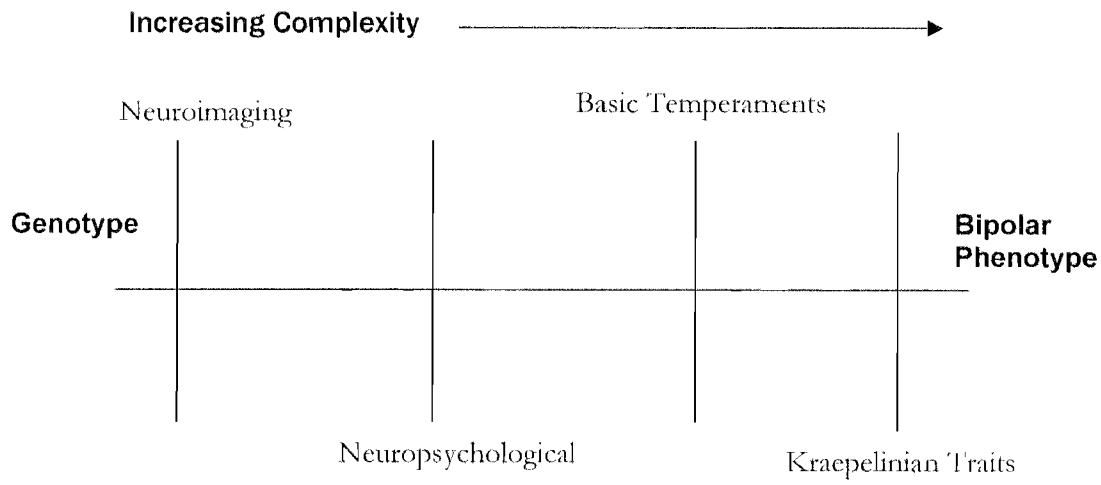


Figure 6.1. Complexity of Candidate Endophenotypes.

One additional potential endophenotype which has been suggested in the literature is relative hand-skill (RHS) which serves as an indirect measure of cerebral lateralisation (Leask and Crow 2001). A more direct, imaging derived measure of the degree of hemispheric lateralisation may therefore be a useful endophenotype for both BPD and schizophrenia populations.

In keeping with the leitmotif of this thesis, that of genetic heterogeneity, linkage study-derived data could be analysed differently. Instead of adding the LOD scores together from all pedigrees in a sample, the sub-group of families which are positively linked to the region in question could be compared to the negative-linkage group on various phenotypic measures. If the two groups differ from each other phenotypically, an independent sample with the specific phenotype of interest could be recruited and assessed for linkage. In other words, one could work backwards and search for endophenotypes in an *a posteriori* manner instead of trying to determine the identity of endophenotypes *a priori*. The difficulty with this approach, however, is the possible absence of a simple, linear correlation between genotype and phenotype. In other words, the identical phenotype may be underpinned by different genotypes or the same genotype may give rise to different phenotypes.

In future analyses of gene-environment interactions it would be helpful to try and obtain a more objective (perhaps clinician-derived) measure of the environmental

variable under study. One possibility is early loss or separation from parents although this variable may relate more to the risk of developing unipolar depression than BPD.

6.5. A Hypothetical Genetic Model of BPD.

BPD and other complex disorders are often conceptualised in the literature as continuous, normally distributed traits underpinned by the actions of numerous genes, each with a small effect. When enough susceptibility alleles aggregate in an individual, a threshold is breached, resulting in disease. This polygenic model (PGM), or a slightly more sophisticated version of it involving genetic epistasis, is favoured by proponents of the CDCV hypothesis discussed in Appendix A. On the other end of the spectrum, a monogenic or major locus model (MLM) in which one genetic variant contributes to all or most of the risk for a disease has less commonly been proposed.

Kelsoe (2003) evaluated the strength of these models *apropos* BPD. The PGM receives support from epidemiological surveys which indicate a lower prevalence of the more severe phenotypes like BPD I and schizoaffective disorder, an intermediate prevalence of the BPD II phenotype, and a higher prevalence of the milder bipolar spectrum conditions (Kelsoe 2003). This model is also consistent with data from this thesis indicating that the personality traits and neurocognitive profile of the MDE-R group was intermediate between the BPD I and unaffected relative samples. Nevertheless, the BPD II group did not conform to this pattern. The PGM is also supported by the literature reviewed in Chapter 2 which indicates that common polymorphisms of genes like *COMT, SERT, BDNF* and *DAT* have been repeatedly associated with BPD.

As pointed out by Kelsoe (2003) however, the MLM can also account for the prevalence data of the various categories of BPD illness by invoking the phenomenon of incomplete penetrance. That is, relatives of the BPD proband may inherit the same disease susceptibility variant but display milder forms of illness because of the absence of precipitating environmental factors (Kelsoe 2003). The importance of gene-environmental interactions is highlighted by the results of this study which indicate that a combination of exposure to childhood abuse and inheritance of (a) *val*

allele(s) of the *COMT* Val158Met polymorphism may predispose to the development of affective illness as well as anxiety-related, mood-labile, and novelty-seeking-related personality traits. The MLM is also supported by the replicated reports of linkage to particular regions of the genome which is suggestive of the action of genetic variants of large effect. One such region, 1q31-32 was also found to be linked to affective disorder in this study.

How can these divergent models underpinned by association and linkage data, respectively, be reconciled? Kelsoe (2003) has postulated a so-called mixed-model in which susceptibility to BPD is driven by a small number of loci of large effect acting on a polygenic background. This basic schema is then complicated by genetic heterogeneity and epistasis producing a pattern of overlapping and distinct phenotypes underpinned by distinct or at least partially distinct genetic factors (Kelsoe 2003).

Data from this thesis is applied to the schema of Kelsoe (2003) to produce a concrete but admittedly highly speculative example of a mixed model in action. Regarding the PGM component, common variants of the *DAT* and *Notch4* gene are hypothesised to exert a non-specific phenotypic effect and to therefore constitute risk factors for the development of a broad range of bipolar phenotypes. On the other hand, the *short* allele of the *SERT* 5-HTTLPR is postulated to be a general risk factor for anxiety and thus associated with subgroups of highly anxious bipolar patients only. The *BDNF* Val66Met variant is hypothesised to predispose to BPD through its effect on hyperthymic or hypomanic traits. On the other hand, BPD individuals with an irritable-mood-labile temperament will be most likely to display an excess of the *COMT val* allele, and this association may be modulated by early traumatic experiences. Finally the 4R allele of the *DRD4* gene may predispose to a type of BPD characterised by impaired executive or memory function. Against this polygenic background, rare variants in the region of 1q32 and 10q23 contribute substantially to the risk of developing BPD spectrum illness, while rare variants in the region of 13q32 and 22q11 are risk factors for the development of NS-related personality traits and neurocognitive dysfunction, respectively. This model, which is summarised in Table 6.2, below could well turn out to be incorrect but its real value may lie in its heuristic conceptualisation of BPD as a heterogeneous collection of phenotypic syndromes underpinned by divergent genetic and perhaps environmental risk factors.

Table 6.2. Hypothesised Subtypes of BPD and Associated Genetic Variants.

Phenotype	**Akiskal's Nomenclature (see Table 1.1)**	**Associated Gene**	**Risk Allele**	**Modulating Factor**	**Linked Region**
Broad	?	*DAT and Notch4*	10R and 6R	?	1q32 and 10q23
Hyperthymic subtype	Type 1.5/II	*BDNF*	Val66Met: *val*	?	13q32
Mood-labile-irritable-aggressive subtype	Type 2.5.	*COMT and MAO-A*	Val158Met: *val* and *MAO-A* VNTR: *low*	Childhood abuse	?
Anxious subtype	?	*SERT and MAO-A*	5-HTT-LPR: *short* allele and *MAO-A* VNTR: *low*	Childhood abuse	?
Neurocognitive impairment	NA	*DRD4*	4R	?	22q11
Neurocognitive impairment + environmental insult	NA	*BDNF and ApoE*	Val66Met: *met* and ε4/ε2	Sexual abuse	?

The hypothetical model constructed above is indicative of the nascent potential of endophenotypic approach and the importance of characterising gene-environment interactions. Genetic knowledge is without peer in its potential for radically altering the practice of medicine but the obstacles, especially in the field of psychiatric genetics, are formidable and care should be taken not to over-estimate the probability of short-term success. Advances are likely to be incremental.

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Appendix A.

Background Information.

A..1. Linkage Analysis.

Linkage analysis, the traditional tool for identifying disease genes is based on the principle that the smaller the distance between two loci, the lower the probability that they will be separated by meiotic recombination events, and hence the greater the probability that the loci in question will be inherited as a unit (co-segregation). This probability is called the recombination fraction, θ. The identity and position of one of these loci – the hypothetical disease gene - is unknown. The information available for this "disease locus" is usually only binary: whether or not the individual is afflicted with the disorder. On the other hand, the position of the marker selected by the researcher is known and thus the degree to which the genetic marker and the disease phenotype co-segregate within families is indicative of the likelihood that the marker and the hypothetical susceptibility locus are linked.

In principle, θ is simply the observed number of recombinants divided by the total number of meioses in the sample: $\theta = R/(N+R)$. The problem is that only doubly heterozygous parents are informative for linkage and unless phase is known, by having full information on grandparents, it may be impossible to classify individual offspring as recombinant or non-recombinant (Thomas 2004). An additional difficulty is that the effects of a gene may not be fully penetrant and therefore the trait "genotypes" cannot be determined with any degree of certainty (Thomas 2004). These ambiguities are dealt with by the LOD (logarithm of the odds) score method which calculates θ based on all the possible combinations of genotypes for each individual (Thomas, 2004). The variables that influence this calculation - the mode of inheritance of the disorder (dominant or recessive), the disease allele frequency, the penetrance of the gene, and the phenocopy rate, have to be specified by the researcher.

In more formal language, the LOD score is the logarithm of a likelihood ratio test comparing the odds that the hypothetical disease gene and the marker are linked ($\theta<0.5$) against the null hypothesis that they are unlinked ($\theta=0.5$). In mathematical notation:

$LOD\ (\theta) = log10\ (L(\theta)/L(0.5))$.

LOD scores are derived from inheritance patterns within families but the scores from different pedigrees can be added together to increase the power of a study. Traditionally, a LOD score of 3, which provides odds of 1000:1 against the observed pattern linkage occurring by chance, is regarded as statistically significant. In statistical analyses the minimum acceptable risk of a false positive is usually 1 in 20 but in fact a LOD score of 3 corresponds to an approximate p value of 0.05 because of the low *a priori* probability of linkage which stands at about 1 in 50 given our complement of 46 chromosomes.

While a LOD score of 3 or more is suggestive of linkage, it is unclear what the threshold for significance should be when there is *a priori* evidence for linkage to a region and only a small number of markers are typed. One possibility is to carry out simulations using family structures in order to derive empirical p-values.

In a two-point linkage analysis a single marker locus is tested for linkage to a disease locus. Greater power can however be obtained by testing two or more markers for linkage to a disease locus, simultaneously (Thomas 2004). This is known as multipoint linkage analysis

Clearly the correct specification of parameters such as penetrance and allele frequency is important for the accuracy of linkage calculations. Unfortunately, however, the true values of many of these parameters are usually unknown which may lead to Type II errors (Thomas 2004). This led to the development of alternative but less powerful approaches that do not require the specification of a genetic model.

These model-free or non-parametric methods are based on the premise that affected relatives with a disorder are more likely to share genetic material in the vicinity of the

disease locus than expected by chance. The simplest form of non-parametric linkage analysis is the affected sib-pair (ASP) method in which identical by descent (IBD) allele-sharing (alleles inherited from a common ancestor) calculations are performed among pairs of siblings. On average, affected sib-pairs should share 0, 1 or 2 alleles in the proportions (¼, ½, and ¼), and any significant deviation from this ratio, supports the case for rejecting the null hypothesis of no linkage (Elston and Cordell 2001). The observed number of alleles shared IBD is calculated *(2*n2+n1)* and compared to the number of alleles shared under the null hypothesis of no linkage *(N)*. This gives the test statistic:

$Z = [(2n2+n1) - N]/\sqrt{(N/2)}$.

Identity by state (IBS) methodologies which simply examine whether two individuals share an allele regardless of whether it originated from a common ancestor, have also been developed. These approaches were later generalised to all affected relatives in the family (APM analysis) with the degree of IBD or IBS allele sharing compared to that expected under the null hypothesis of no linkage (Elston and Cordell 2001).

Sib-pair methodologies can also be applied to quantitative data, classically through the use of the Haseman-Elston algorithm. If a particular locus contributes to the variation in a quantitative trait then the more alleles shared IBD by sib-pairs or other relative pairs, the more similar the relatives should be phenotypically (Thomas 2004). To test this hypothesis, the difference between each sibling pair for the quantitative trait is squared and then regressed against the expected proportion of alleles shared IBD (Elston and Cordell 2001). The degree of allele sharing is denoted, π, and the squared trait difference, D^2. If D^2 decreases as π increases than this implies some degree of linkage to the relevant marker(s) (Elston and Cordell 2001; Thomas 2004).

A.2. Association Analysis.

If allele y of a particular gene is found more frequently than expected by chance in individuals with disease z then y is said to be associated with z. Here the allele frequency expected by chance is not determined by patterns of inheritance within a

family but by the frequency of y in the general population. Thus in theory, association is a population level phenomenon. There are two predominant reasons why an association between a genetic variant and a disorder occurs in the population.

The genetic variant may exert a direct causal effect such that carriers of the allele are more likely to develop the illness. In this case one would have to demonstrate that the allele in question has functional consequences - for example alteration of gene expression, translation (mRNA stability) or protein conformation. The second reason for association is linkage disequilibrium (LD).

LD is the phenomenon whereby particular variants of two genes are inherited together (or usually inherited together) by virtue of the fact that they lie close together on the chromosome and are therefore unlikely to be separated during meiotic recombination events. Here the assumption is that in the distant past a mutational event occurred in an individual, X, producing a new allele, K. This new allele would be passed on to some of X's children along with a large chunk of the surrounding chromosome. All of the genetic variants on this piece of chromosome would initially be in LD with K. Over the generations, however, many of these variants would become separated from K so that on average only those alleles in close proximity to K would still display evidence of LD. So the supposition inherent in LD mapping is that apparently unrelated individuals all share a common ancestor if one travels far enough back in time.

Theoretically patterns of LD in the genome can be exploited to map the loci predisposing to complex disorders. Markers in LD with each other provide redundant information and thus regions of high LD can be identified and key markers within these haplotype blocks genotyped and tested for association with the phenotype of interest (Cardon and Abecasis 2003; Clark 2003). It has been suggested in some quarters that 300 000 to 1000 000 SNPs may be enough to cover the entire genome (Gabriel et al. 2002) and these optimistic forecast have catalysed the launch of the NIH funded haplotype mapping (HapMap) project.

While elegant in theory, LD has not actualised its potential as an identifier of complex disease genes although it has facilitated the identification of Mendelian disease genes.

Firstly, the implicit assumption underpinning LD mapping is that the common disease - common variant (CDCV) assumption holds true. The CDCV hypothesis which has become almost *de rigueur* among geneticists holds that complex disorders such as psychiatric illness are common because of the high frequency of the predisposing alleles in the general population. These alleles may be necessary but are certainly not sufficient to cause the illness (Becker et al. 2004).

Even if most complex disorders are caused by the interactions of multiple genes and environmental factors, however, different alleles of the same gene may all predispose to the same illness, rendering the strategy of LD mapping ineffective (Terwilliger and Goring 2000). Potentially even more damaging is the possibility of widespread non-allelic heterogeneity with rare variants playing a causal role in disease (Weiss and Terwilliger 2000; Clark 2003).

Secondly, chromosomal distance is not a perfect predictor of the extent of LD. A marker that lies close to the disease locus can show weaker LD than a more distant marker (Nordborg and Tavare 2002). The reasons for this are manifold. A marker that has a young origin relative to the disease allele will display a greater degree of LD than a marker that is as old as, or older than the disease allele because the smaller the number of generations to the most recent common ancestor, the less opportunity there will have been for recombination to take place (Nordborg and Tavare 2002). Alternatively, natural selection for or against an allele can lead to extensive LD surrounding that variant (Nordborg and Tavare 2002). Recombination hot-spots are also scattered throughout the genome and levels of LD are likely to be significantly lower in these regions.

Thirdly, the patterns of LD vary widely between population groups making the extrapolation of results across studies problematic. The example that is usually described in the literature is that of ancestral African population groups which display significantly lower levels of LD than Caucasians. This has been attributed to the larger average size of the African population and the fact that only a subset of the gene pool was drawn from Africa during migration from the continent (Clark 2003).

Appendix B. Linkage Analysis Data.

Table B.1: Linkage Data - Chromosome 1.

Study.	Sample Characteristics.	Region of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value).
Reiss et al. (1997).	21 markers typed on 97 NIMH pedigrees (540 individuals: 32 SA, 232 BP1, 72 BP2, 88 MDE-R, 45 unaffected).	1p21.1 (73.1 Mb).	D1S1648	NA	NA	NA	NA	ASP analysis (SIBPAL). MOD score analysis (MODLINK)	MOD=1.94
Detera-Wadleigh et al. (1999).	GWS with 607 markers in 22 multiplex pedigrees (396 individuals).	1q32.2 (205.3 Mb) & 1q41 (206.1 - 211.1 Mb)	GATA124F08 (parametric). NPL: D1S471-S237.	Pairwise LOD score analysis (FASTLINK).	SA, BPI, BPII.	Dominant (P=0.85).	2.37.	Pointwise ASP (SIBPAL). Multipoint analysis (ASPEX & GENEHUNTER-PLUS -all).	ASP: 1.78 (0.0021). Multipoint: 2.67 (0.00022).

Morissette et al, (1999)	GWS with 332 markers typed in a large pedigree from Quebec (114 individuals: 39 BPI, 3 SA, 5 BPII, 9 MDE-R).	1	D1S229	Two-point analysis (FASTLINK)	SA, BPI, BPII, MDE-R.	D12S87: Dominant (AF=0.02; P=0.30[<25yrs]: 0.54[25-35]; 0.68[35-45]; 0.75[>45]; PH=0.0001-0.02). D12S339: Recessive (AF=0.17; P=as above; PH=as above).	NS	SimAPM & SimIBD (ANALYZE).	1.99 (0.0012).
Ewald et al. (2002).	GWS with 613 markers in two large Danish Caucasian pedigrees.	1p31.1 (68-77.4 Mb)	D1S216: D1S2829.	Affecteds-only two-point analysis (MLINK)	SA, BPI, BPII, MDE-R.	Dominant (AF=0.01; P=0.65; PH=0.02).	2.75	NPL multipoint analysis (GENEHUNTER - ... pairs).	11.91 (0.00079) 104cM from p-terminus.
Ekholm et al, (2003).	GWS with 389 markers typed in 41 families (37 nuclear & 4 extended pedigrees containing 153 affected individuals) from an isolated Finnish population.	1q31.3 (195 Mb)	D1S1660	NA	SA, BPI, BPII, MDE-R.	Recessive	NA	Two-point ASP analysis (SIBPAIR).	Z(max)=1.8

McInnes et al. (2003).	245 markers typed in 65 pedigrees (573 subjects 7 SA; 129 BPI; 97 BPII69 MDE-R).	1q41 (216 Mb)	D1S549	NA	SA, BPI, BPII, MDE-R.	NA	NA	Genome-wide multipoint analysis (GENEHUNTER PLUS).	2.27 (0.01).
Fallin et al. (2004).	GWS with 382 markers typed in 41 Ashkenazi Jewish families (54 ARPs & 38 ASPs).	1q23.3 (157.6 Mb)	D1S484	Affecteds-only heterogeneity (HLOD) analysis (GENEHUNTER)	BPI, BPII	Dominant (AF=0.005; P[homozygotes]=0.65; [heterozygotes]=0.65; PH=0.0096).	1.73	Affected relative pair (ARP) analysis (GENEHUNTER).	2.464 (0.0072).
Kohn et al, 2004.	359 microsatellite markers on 7 chromosomes in a genetically isolated Muslim village (52 psychotic patients & 42 unaffecteds).	1p36 (148 Mb	D1S434; D1S57; D1S436	Linkage disequilibrium mapping (IBD haplotype sharing)	Schizophrenia spectrum	NA	2.361.	NA	NA
MacGregor et al. (2004).	34 markers across chromosome 1 were typed in 22 extended Scottish pedigrees (398 individuals).	1q42.2 (227.1 Mb)	D1S103.	Two point parametric analysis (FASTLINK). Multipoint variance components analysis (SOLAR).	SA, BPI, BPII & MDE-R.	Recessive	2.63 2.77 (Multipoint VC).	NA	NA
Shink et al,	GWS with 380	1q23.3	D1S484 &	Two-point analysis	SA, BPI, BPII.	D1S484: Dominant	1.14 & 1.23	NA	NA

(2004).	polymorphic markers typed in 20 families from eastern Quebec (77 BPI, 28 BPII, 43 MDE-R, 45 MDE-S, 68 misc, 133 unaffected).	(157.6 Mb) & 1q31.3 (195.4 Mb)	D1S413	(MLINK).	MDE-R (D1S484 only)	(AF=0.02; P=0.5; PH<0.01) D1S413: Recessive (AF=0.15; P=0.5; PH<0.01)			
Hamshere et al. (2005).	GWS with 426 markers in 24 UK families SA disorder.	1q42.2 (231 Mb).	D1S2800	NA	Schiz, SA, BP 1.	NA	NA	Multipoint analysis (Mapmaker/Sibs)	3.54

Table B.2: Linkage Data - Chromosome 2.

Study.	Sample Characteristics.	Region of Linkage	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value)
Detera-Wadleigh et al. (1999).	GWS with 607 markers in 22 multiplex pedigrees (396 individuals).	2pter & 2p24.2-p25.1 (11.6-17.4 Mb)	D2S2976 & D2S1400 to S1360.	NA	SA. BPI. BPII. & MDE-R (2pter only)	NA	NA	Multipoint analysis (ASPEX).	2.0 (0.0012) & 1.12 (0.012).
Bennett et al. (2002).	GWS with 398 markers in 151 UK nuclear families (154 narrowly defined sibling pairs: 258 broadly defined sibling pairs).	2q37.3 (242-6.9 Mb).	D2S125.	NA	BPI.	NA	NA	Multipoint (MAPMAKER/SIBS) analysis.	MLS=1.70
Zubenko et al. (2002).	6 markers typed in a 15cM region on chromosome 2. 81 families with recurrent unipolar depression	2q33.3 (208 Mb)	D2S2321 & D2S2208.	NA	Major & minor mood disorders.	NA	NA	Single and multipoint affected-relative pair analyses (LODPAL).	Single-point: S2321=4.791; S2208=5.097. Multipoint: S2321=6.331

	(81 probands, 407 1st degree relatives, 835 extended relatives).								& S2208= 6.866. Both analyses significant in female pairs only.
McInnes et al. (2003).	245 markers typed in 65 pedigrees (573 subjects 7 SA; 129 BPI; 97 BPII 69 MDE-R).	2p13.2 (72.2 Mb) & 2p22.3 (36.2 Mb)	D2S99 & D2S1788.	NA	SA, BPI, BPII & MDE-R (for 2p22 only).	NA	NA	Genome-wide multipoint analysis (GENEHUNTER PLUS).	2.54 (0.007) & 1.75 (0.04).
Zubenko et al. (2002).	Genome-wide scan [illegible] families with recurrent depression.	2q33.3 [illegible] & 2q37.1 (232 Mb)	D2S2321 & D2S427	NA	Major [illegible]	NA	NA	Multipoint ARP analysis [illegible]	Max LOD: [illegible] & 6.63 (<0.0001).
Maziade et al. (2004).	21 multigenerational pedigrees from eastern Quebec. 7 predominantly schiz families: 6 BP pedigrees. & 8 mixed pedigrees. 480 individuals in total.	2q14.1 (114.3 Mb) & q22.3 (148.2 Mb)	D2S121 & D2S298.	Multipoint affected-only (FASTLINK).	Broad model including BP & Schiz.	Dominant (AF=0.01; P=0.7; PH=0.2).	Z=2.76.	NA.	NA.
Middleton et al,	GWS using 11 190 SNPs in 25 extended	2p22.2 (38Mb)	Not given.	NA	Not given.	NA	NA	Multipoint NPL linkage analysis (MERLIN).	Z=3.83 Z=2.98

(2004).	pedigrees from the Azores and Madeira (233 individuals).	2p16.2 (57Mb) 2q22.3 (145Mb)							Z=3.09
Park et al, (2004).	GWS with 343 markers. 40 pedigrees from Israel and the USA with 373 individuals. 68-79 psychotic individuals.	2p13.2 (73 Mb)	D2S1394.	NA	Psychotic individuals.	NA	NA	Multipoint ASP analysis (MAPMAKER/SIBS).	1.34
Venken et al. (2005).	GWS with 350 markers using 9 [illegible] families from an isolated northern Swedish population (171 individuals: 3 SA, 19 BP1, 6 BP2, 18 MDE-R, 116 unaffected).	2q31-q36.3 (172.9 Mb: 217.5 Mb; 221.8 Mb: 230.5 Mb)	D2S326. D2S2248. D2S12[illegible] D2S396.	Genome-wide multipoint affected-[illegible] (GENEHUNTER)	Broad model.	Dominant (AF=0.01: PH=0.01[BPI]: [illegible] 0.05[MDE-R])	2.35.	Non-parametric affected-only analysis (GENEHUNTER).	NPL=2.93 (0.0085).

Table B.3: Linkage Data - Chromosome 4.

Study.	Sample Characteristics.	Region of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value).
Blackwood et al. (1996).	17 markers in a single family (120 individuals: 11 BP, 16 MDE-R).	4p16.1 (7 Mb)	D4S394	Two-point analysis (MLINK)	Both narrow & broad models.	Dominant (AF=0.007; P=age dependent - 0.046-0.28; PH=0.1)	4.1	NA	NA
Detera-Wadleigh et al. (1997)	26 markers typed in 97 NIMH families (540 individuals).	4p15.2 (26.9-27.3 Mb) & 4q23 (99.8 Mb)	D4S2397; D4S391 & D4S1647	NA	SA, BPI, BPII & MDE-R (for 4q only).	NA	NA	Single locus ASP analysis (SIBPAL).	p=0.023; 0.027 & 0.036
Adams et al. (1998).	GWS with 214 markers in one pedigree (87 individuals: 5 BP, 1 SA, 5 MDE-R).	4q35.2 (190.4 Mb)	D4S1652	Two-point analysis (SLINK)	SA, BP, MDE-R.	Dominant (AF=0.035; P=age-dependent: 0.18-0.90).	2.20	NA	NA
Asherson et al. (1998).	7 markers typed in 24 families (22 from the UK & 2 from Japan) with schiz & affective	4p15.3 (13.4 Mb).	D4S403.	Two point analysis (LINKAGE).	Schiz. SA & psychosis	Dominant (AF=0.02: P=0.99 – homozyg & 0.95 -heterzyg; PH=0.001) &	1.12	NA	NA

	disorders (191 subjects)					Recessive (AF=0.01; P=1 - homozyg. 0 - heterozyg. PH=0)			
Ewald et al. (1998).	16 markers (4pter-4p12) typed in two large Danish families.	4p16.1 (7.1 Mb)	D4S394	Two-point analysis (MLINK)		Recessive (AF=0.1; P=0.60; PH=0.02)	2.0	IBD sharing among affected relative pairs analysis (Sim-IBD).	NS
Detera-Wadleigh et al, (1999).	GWS with 607 markers in 22 multiplex pedigrees (396 individuals).	4p15.1 (31-35.5 Mb)	D4S2632 (parametric) & D4S2408-S2632 (NPL)	Pairwise analysis (FASTLINK)	SA, BPI, BPII, & MDE-R (NPL only)	Dominant (P=0.5)	3.24 for one pedigree only.	Multipoint analysis (ASPEX).	1.77 (0.0022).
Friddle et al. (2002).	50 families (470 individuals; 28 BPI; 75 BP II, 53 MDE-R).	4q35.1 (185.5-189.4 Mb)	D4S408 & D4S426	Multipoint HLOD analysis (GENEHUNTER)	BP I & BP II	Recessive (AF=0.1; P= 0.63-0.85; PH=0.1-0.19)	2.11	NA	NA
Bennett et al. (2002).	GWS with 396 markers in 151 UK nuclear families (154 narrowly defined sibling pairs; 258 broadly defined sibling pairs).	4p14 (40.2 Mb) & 4q13.2 (70.7 Mb)	D4S405 & D4S392.	NA	BPI.	NA	NA	Multipoint (MAPMAKER/SIBS) analysis.	MLS=0.79 & 0.78.
Badenhop et al. (2003).	55 Australian pedigrees (674 individuals, 214 affecteds).	4q35.2 (190.4 Mb)	D4S1652.	Two-point analysis (ANALYZE)	SA, BPI, BPII, MDE-R	Dominant (AF=0.035; P=0.18-0.90 by age; PH=0.05).	3.19	Affected sib-pair analysis.	Mean IBD sharing 0.66 (0.0009).
Ewald et al, (2003)	GWS with 613 markers in two large Danish	4p16.1 (7.1 Mb)	D4S394.	Two-point analysis (MLINK)	SA, BPI, BPII,	Recessive (AF=0.1; P=0.65; PH=0.02).	1.97	NPL multipoint analysis	NS.

	Caucasian pedigrees.				MDE-R.			(GENEHUNTER - all pairs).	
Ekholm et al, (2003).	GWS with 389 markers typed in 41 families (37 nuclear & 4 extended pedigrees containing 153 affected individuals) from an isolated Finnish population.	4q32.1 (158.7 Mb).	D4S1629	Two point analysis (MLINK).	SA. BPI. BPII. MDE-R.	Dominant (AF=0.05; P=0.9; PH=0.045). Recessive model for non-parametric ASP.	Z(max)=2.6	NA.	NA.
Nelmes et al. (2003).	245 markers typed in 65 pedigrees (573 subjects 7 SA; 129 BPI; 97 BPII; 69 MDE-R).	4q32.1 (158.7 Mb) & 4q35.2 (188.4 Mb)	D4S1629 & D4S3051	NA	SA. BPI. BPII. MDE-R.	NA	NA	Genome-wide multipoint analysis (GENEHUNTER PLUS).	2.80 (0.004) & 2.43 (0.01).
Willour et al, (2003).	21 markers typed on chromosome 4 in 56 NIMH pedigrees (354 individuals: 139 BP1; 41 BP 2; 43 MDE-R).	4p14-13 (39.1-42.5 Mb) & 4q32 (158.7 Mb).	D4S335 to D4S2329 & D4S1629.	NA	BP1; BP2.	NA	NA	Affecteds-only multipoint analysis (GENEHUNTER PLUS).	2.16 & 2.49
Als et al. (2004).	34 markers on chromosome 4 typed in 11 schizophrenics, 17 bipolars & 44 controls	4p16.1 (8.3-10.3 Mb)	Haplotype of D4S2923; D4S2928; D4S1582.	Case-control linkage disequilibrium mapping	NA	NA	p=0.06.	NA	NA

	from the Faroe Islands in order to examine haplotype sharing among distantly related patients.			(CLUMP).					
Middleton et al. (2004).	GWS using 11 190 SNPs in 25 extended pedigrees from the Azores and Madeira (233 individuals).	4q22.1 (91Mb).	Not given.	NA	Not given.	NA	NA	Multipoint NPL linkage analysis (MERLIN).	Z(max)=2.97.
Vanhon et al. (2005).	GWS with 359 markers using 9 multigenerational families from an isolated northern Swedish population (171 individuals: 3 SA, 19 BP1, 6 BP2, 18 MDE-R, 116 unaffected).	4q34.3 (179 Mb).	D4S415	Two-point affected-only (MLINK).	Broad model.	Recessive (AF=0.1; P=2; PH 0.01[BPI]; 0.02[BPII]; 0.05[MDE-R])	1.46.	NA	NA
Lambert et al. (2005).	395 ASPs. 17 regions of interest typed with 198 markers at a resolution of 4.8cM.	4q13.3 (70.7 Mb).	D4S392.	NA	BPI, BPII, MDE-R.	NA	NA	Two-point ASP analysis (GENEHUNTER)	3.30

Table B.4: Linkage Data - Chromosome 10.

Study.	Sample Characteristics.	Region of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value)
Blouin et al. (1997).	33 markers typed on 97 NIMH pedigrees (540 individuals: 32 SA, 232 BP1, 72 BP2, 88 MDE-R, 45 unaffected).	10q22.2 (75 Mb).	D10S188.	ASP analysis (SIBPAL). MOD score analysis (MODLINK)	SA, BP1, BPII.	NA	MOD=3.47	NA	NA
Detera-Wadleigh et al. (1999).	GWS with 607 markers in 22 multiplex pedigrees (396 individuals).	10q25.3 (118.6 Mb).	D10S187	Pairwise LOD score analysis (FASTLINK).	SA, BP1, BPII, MDE-R	Recessive (P=0.85).	1.69.	NA	NA
Foroud et al. (2000) *a*.	GWS with 365 markers in 97 NIMH families of mixed ethnicity (540 individuals: 232 BP1, 32 SA, 72 BPII, 88 MDE-R).	10p12.31 (19.5 Mb) & 10p11.23 (30.5 Mb).	D10S1423 & D10S1426	NA	SA, BP1, BP2 (S1423 only).	NA	NA	Non-parametric affected relative pairs method (GENEHUNTER).	2.5 & 1.5.
Cichon et al. (2001).	33 markers typed in 75 families from Germany, Israel & Italy. 444	10q26 (129.4 & 125.2 Mb).	D10S217 & D10S587.	Affected only two-point analysis	SA, BP1, BPII, MDE-R.	Dominant (AF= 0.9; P=0.5; PH=0.032).	2.86 & 1.48. ASP: S217 - p=0.019;	Affected-only NPL multipoint analysis (GENEHUNTER –	Z=3.12 (0.0013)

	individuals (2 Schiz: 126 BP1: 40 BP2: 14 SA: 40 MDE-R: 271 unaffected).			(MLINK) and ASP (GAS)			S537 - p=0.045.	relative pair).	
Kelsoe et al. (2001).	Genome-wide scan using 443 markers in 20 US families (164 individuals).	10q26.2 (129.1 Mb)	D10S1223	Two-point LOD score analysis (LIPED)	BPI only.	Recessive (AF=0.01; P=0.50; PH=0.05).	2.27.	NA	NA
Bennett et al. (2002).	GWS with 398 markers in 151 UK nuclear families (154 narrowly defined sibling pairs; 258 broadly defined sibling pairs).	10p14-12 (10.6-18.8 Mb) & 10q11.23 (51.8 Mb).	D10S547, D10S548 & D10S196	NA	BPI.	NA	NA	Multipoint (MAPMAKER/SIBS) analysis.	MLS=0.98 & 0.85.
Ewald et al. (2003).	GWS with 613 markers in two large Danish Caucasian pedigrees.	10q26.2 (129.4 Mb)	D10S217	Two-point analysis (MLINK)	SA, BPI, BPII.	Dominant (AF=0.004; P=0.60; PH=0.005).	2.1	NPL multipoint analysis (GENEHUNTER - all pairs).	NS
McInnes et al. (2003).	245 markers typed in 65 pedigrees (573 subjects 7 SA: 129 BPI: 97 BPII69 MDE-R).	10q26.2 (129.1 Mb).	D10S1223	NA	SA, BPI, BPII.	NA	NA	Genome-wide multipoint analysis (GENEHUNTER PLUS).	2.12 (0.02).
Zubenko et al. (2003).	Genome-wide scan with 392 markers in 81 families with recurrent depression.	10p14 (9.3 Mb) & 10q26.13 (126.1 Mb)	D10S1412 & D10S1656	NA	Major depression.	NA	NA	Multipoint ARP analysis (SAGE)	Max LOD: 4.70 (<0.05) & 5.39 (<0.001).
Maziade et al. (2004).	21 multigenerational pedigrees from eastern Quebec. 7 predominantly schiz families; 6 BP	10p13-12 (16.6-23.6 Mb).	D10S245; D10S674.	Multipoint affected-only (FASTLINK).	BP1.	Dominant (AF=0.01; P=0.7; PH=0.2).	Z=2.66.	NA	NA

	pedigrees, & 8 mixed pedigrees. 480 individuals in total.								
Park et al, (2004).	GWS with 343 markers. 40 pedigrees from Israel and the USA with 373 individuals. 68-79 psychotic individuals.	10q22.3 (80.4 Mb) & 10q26.3 (132.4 Mb) (ASP only)	D10S2327 D10S169 (10q26).	Two-point affected only (MLINK)	Psychotic individuals.	Recessive (AF=0.1; P=0.8; PH=0.0001).	1.60 (10q22).	Multipoint Affected Sib-Pair (MAPMAKER/SIBS).	1.51. 1.18 (10q26)
Shink et al. (2004).	GWS with 380 polymorphic markers typed in 20 families from eastern Quebec (77 BPI, 26 BPII, 43 MDE-R, 45 MDE-S, 68 misc, 133 unaffected).	10q21.2 (67.1 Mb)	D10S589	Two-point analysis (MLINK).	SA, BPI, BPII	Dominant (AF=0.02; P=0.5; PH<0.01).	1.45	NA	NA
Lambert et al. (2005).	395 ASPs. 17 regions of interest typed with 198 markers at a resolution of 4.8cM.	10p12.1 (26.6 Mb)	D10S197.	NA	BPI, BPII, MDE-R.	NA	NA	Two-point ASP analysis (GENEHUNTER)	3.18

Table B.5: Linkage Data - Chromosome 12.

Study.	Sample Characteristics.	Region of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD.	Non-Parametric Analysis.	LOD (p-value).
Craddock et al, (1994).	Pedigree in which five individuals have both affective disorders and Darier's disease.	12q23-q24.1	ATP2A2 (Darier's Locus).	Maximum likelihood methods.	Affective Disorder.	Dominant (AF=0.005; P=1).	2.11	NA	NA
Dawson et al. (1995).	Seven markers spanning the Darier's disease locus in 45 European pedigrees.	12q23-24.1	PLA2	Two-point analysis (MLINK)	Bipolar disorder	Dominant (AF=0.009 P=0.5; PH=0.1)	HLOD=3.52	Sib-pair analysis (ESPA)	NS.
Rice et al. (1997).	11 markers typed on 97 NIMH pedigrees (540 individuals: 32 SA, 232 BP1, 72	12q21.3 (83.4 Mb)	D12S379.	MOD score analysis (MODLINK).	SA, BPI, BPII, MDE-R.	NA	MOD=1.89	NA	NA

	BP2, 88 MDE-R, 45 unaffected).								
Ewald et al. (1998).	Two large Danish families.	12q24.31 (124.9 Mb)	D12S1639		BPI, BPII	Dominant	3.37	SimIBD	p=0.005
Morissette et al. (1999)	GWS with 332 markers typed in a large pedigree from Quebec (114 individuals; 39 BPI, 2 SA, 5 BPII, 9 MDE-R).	12q23-q24 & 12p11.22 (30.39 Mb)	D12S86 – D12S82 & D12S87 – D12S339	D12S87 – D12S339; Two-point analysis (FASTLINK)	SA, BPI, BPII, MDE-R.	D12S87: Dominant (AF=0.02: P=0.30[<25yrs]; 0.54[25-35]; 0.68[35-45]; 0.75[>45]; PH=0.0001-0.02). D12S339: Recessive (AF=0.17; P=as above; PH=as above).	1.61 & 0.74.	Non-parametric multipoint analysis (GENEHUNTER).	D12S86: 2.5 – D12S82: 3.92
Detera-Wadleigh et al. (1999).	GWS with 607 markers in 22 multiplex pedigrees (396 individuals).	12q24.2 (114.5 Mb)	D12S1343-S2070	NA	SA, BPI, BPII, MDE-R.	NA	NA	Multipoint analysis (ASPEX).	1.24 (0.0084).
Degn et	17 markers spanning	12q24.3	D12S1614	Single and two-	BPD	NA	p<0.01.	NA	NA

al. (2001).	24 cM in 14 patients with bipolar disorder and 43 controls descended from a common Faroese ancestor.	(124.4-126.7 Mb)	& D12S1675.	marker haplotype analysis (CLUMP)					
Turecki et al. (2001).	Genome-wide scan in 31 families with an excellent response to lithium.	12q23.1 (97 Mb)	D12S1300	Parametric analysis (MLINK)	BPI, BPII, MDE-R.	"Intermediate" (AF=0.024; PH=0.005-0.009)	1.27	NA	NA
Baier et al, (2002).	GWS with 388 markers in 5 schizophrenia and 3 bipolar pedigrees (50 individuals: 27 affected & 23 unaffected).	12q24.3 (124.3-128.9 Mb)	D12S97 & D12S342.	Parametric analysis (GENEHUNTER).	Schiz, SA. BPI, BPII. MDE-R.	Dominant (AF=0.05; P=0.7; PH=0.01).	0.97 & 1.24.	Multipoint analysis (GENEHUNTER-all).	2.43 (0.0115) & 1.91 (0.03).
Bennett et al, (2002).	GWS with 398 markers in 151 UK nuclear families (154 narrowly defined sibling pairs; 258 broadly defined	12q21.2 (76.4 Mb).	D12S326	NA	BPI.	NA	NA	Multipoint (MAPMAKER SIBS) analysis.	MLS=0.77

	sibling pairs).								
Jones et al. (2002)	Family cosegregating Darier's disease and affective disorders.	12q23-q24.1.	ATP2A2 (DD locus).	Two point analysis (MLINK).	All affective disorders.	Dominant (AF=0.03; P=1; PH=0).	3.58	Affecteds only analysis (GENEHUNTER).	13.51
Abkevich et al. (2003).	GWS with 628 markers in 110 Utah pedigrees with major depression.	12q23.1 (97 Mb).	D12S1300	Multipoint analysis (MCLINK).	Males with depressive spectrum illness.	Dominant (AF=0.003; P=0.54).	HLOD=4.6 (0.00003)	NA	NA
Curtis et al. (2003).	Genome-wide scan of multiplex families affected with bipolar disorder.	12q24.31 (124.4 Mb).	D12S342				2.9		
Ewald et al. (2003).	GWS with 613 markers in two large Danish Caucasian pedigrees.	12q24.3 (124.3 Mb)	D12S1639; D12S342; D12S1658.	Two-point analysis (MLINK)	SA, BPI, BPII.	Dominant (AF=0.004; P=0.60; PH=0.005).	3.42	NPL multipoint analysis (GENEHUNTER - all pairs).	2.29 (0.013).
Ekholm et al. (2003).	GWS with 389 markers typed in 41 families (37 nuclear & 4 extended pedigrees containing	12q23.	PAH	NA	SA, BPI, BPII. MDE-R.	Recessive	NA	Two-point ASP analysis (SIBPAIR).	Z(max)=2.0

	153 affected individuals) from an isolated Finnish population.								
McInnes et al. (2003).	245 markers typed in 65 pedigrees (573 subjects 7 SA: 129 BPI: 97 BPII69 MDE-R).	12q23.1 (99 Mb).	D12S69.	NA	SA. BPI. BPII. MDE-R.	NA	NA	Genome-wide multipoint analysis (GENEHUNTER PLUS).	2.08 (0.02).
Maziade et al, (2004).	21 multigen rational pedigrees from eastern Quebec. 7 predominantly schiz families: 6 BP pedigrees: & 8 mixed pedigrees. 480 individuals in total.	12q23.1 (99.4-101.4 Mb)	D12S332: D12S1030.	Multipoint affected only (FASTLINK).	BPI. BPII: MDE-R.	Recessive (AF=0.01; P=0.7; PH=0.2).	Z=3.53.	Multipoint affected-only parametric linkage analysis (FASTLINK) using a broad BP affection status model & recessive mode of inheritance.	
Shink et al, (2004).	GWS with 380 polymorphic markers typed in 20 families from eastern Quebec (77	12q24.31 (123.2 Mb)	D12S378	Two-point analysis (MLINK).	SA. BPI, BPII. MDE-R.	Recessive (AF=0.15; P=0.5: PH<0.01).	3.35	ASP analysis (ASPEX)	5.05 (<0.0001)

	BPI. 28 BPII. 43 MDE-R, 45 MDE-S, 68 misc. 133 unaffected).								
Green et al. (2005).	45 markers spanning 1.47Mb region (cpt.) in two Caucasian pedigrees with Bipolar Disorder and Darier's Disease.	12q23.3-12q24.2 (106.7-111.3 Mb)	D12S1127 to D12S1646.	Multipoint parametric analysis (GENEHUNTER)	BPI. BPII. MDE-R.	Dominant (AF=0.01; PH=0.1)	4.47.	NA	NA
Venken et al, (2005).	GWS with 580 markers using 9 multigenerational families from an isolated northern Swedish population (171 individuals: 3 SA, 19 BP1, 6 BP2, 18 MDE-R. 116 unaffected).	12q21.2-12q23.3 (98-102.7 Mb).	D12S320, D12S346; D12S78	Two-point affected-only (MLINK)	Broad model.	Recessive (AF=0.1; P=?; PH=0.01[BPI]; 0.02[BPII]; 0.05[MDE-R]).	1.65.	NA	NA

Table B.6: Linkage Data - Chromosome 13.

Study.	Sample Characteristics.	Region of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value).
Wadleigh et al, (1999).	22 multiplex pedigrees (396 individuals).	(100.3 Mb)	(parametric) & D13S1271-S779 (NPL).	score analysis (FASTLINK).	BPII				
C Liu et al, (2001).	Fine mapping of 13q32 with 9 markers. 22 NIMH multiplex families of Caucasian obesity (~7. individuals).	13q32.3 (100.3 Mb).	D13S779 to D13S225.	NA	SA, BPI, BPII, MDE-R.	NA	NA	Multipoint analysis (ASPEX).	3.25 (0.00005).
Kelsoe et al. (2001).	Genome-wide scan using 443 markers in 20 US families (164 individuals)	13q22.1-q33.3 (95-106.7 Mb)	D13S154 & D13S796	Two-point LOD score analysis (LIPED)	BPI, BPII & MDE-R.	Recessive (AF=0.01; P=0.50; PH=0.05) & Dominant (AF=0.01; P=0.85; PH=0.05).	2.4 & 2.34.	NA	NA
McInnes et al, (2003).	245 markers typed in 65 pedigrees (573 subjects 7 SA: 129 BPI: 97 BPII69 MDE-R).	13q13.2 (32.9 Mb)	D13S1493.	NA	SA, BPI, BPII, MDE-R	NA	NA	Genome-wide multipoint analysis (GENEHUNTER PLUS).	2.53 (0.009).
Potash et al. (2003).	65 families with bipolar disorder (129 BPI; 97 BP 2; 69 MDE-R). 10 families with psychotic features.	13q31.1 (81.6 Mb)	D13S317	NA	SA, BPI, BPII, MDE-R.	NA	NA	NPL analysis testing IBD allele sharing among all affected individuals (GENEHUNTER) and sibling	3.56 (0.003) – GENEHUNTER & 2.53 (0.0006) – SIBIBD.

								pairs (ASPEX SibIBD)	
Shaw et al. (2003).	12 markers spanning 13q32-34 typed in 32 pedigrees (194 individuals; 43 BP1, 26 BP2, 49 MDE-R).	13q32.1 - 13q32.3 (95.1-105 Mb)	D13S154 & D13S225	Two point analysis (MLINK) & multipoint analysis (LINKMAP)	SA, BPI, BPII & MDE-R (D13S225 only)	Dominant (P=0.85)	2.29 & 1.65 (2-point), & 3.3% (multipoint)	Two-point analysis (MERLIN) & multipoint analysis (MERLIN)	0.65 (0.04) & 1.36 (0.006). Multipoint - 1.83 (0.002).
Maziade et al. (2005).	21 multigenerational pedigrees from eastern Quebec, 7 predominantly Schiz families, 6 BP pedigrees, & 8 mixed pedigrees. 480 individuals.	13q13.3-q21.1 (37.5 – 62.1 Mb)	D13S1491; D13S1247; D13S325.	Multipoint affected-only (FASTLINK).	BPI	Dominant (AF=0.01; P=0.7, PH=0.2)	2.27.	NA	NA
[illegible] (2004).	[illegible] pedigrees from Israel and the USA with 373 individuals 68-79 psychotic individuals.	[illegible] (100.3 Mb)	[illegible]	[illegible] affected only (MLINK)	[illegible] individuals.	[illegible] P=0.8; PH=0.0001).	[illegible]	[illegible] (MAPMAKER/SIBS).	[illegible] 55.
[illegible] al. (2005).	[illegible] CNG families & 72 quads & 28 trios from NIMH families.	[illegible] (100.3 - 102.3 Mb).	[illegible] between D13S225 & D13S280.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] (ASPEX SibIBD)	[illegible] significance.

Table B.7: Linkage Data – Chromosome 15.

Study.	Sample Characteristics.	Region of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; **P=Penetrance; PH=Phenocopy Rate).**	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value).
[illegible]	[illegible] families with an excellent response to lithium.	[illegible] Mb)	[illegible]	[illegible]	[illegible]	[illegible] (AF=[illegible] P(female)=0.65; PH(M)=0.005. PH(F)=0.005).	3.43	[illegible]	NA/[illegible]
Zubenko et al, (2003).	Genome-wide scan with 392 markers in 81 families with recurrent depression.	15q14 (36.8 Mb)	D15S1012	NA	Major depression.	NA	NA	Multipoint ARP analysis (SAGE)	Max LOD: 1.96 (NS).
Maziade et al. (2004).	Genome-wide scan with 607 markers in 21 multiplex families from Quebec	1. 15q12 (23.2 Mb) & 2. 15q26.3 (95.8 Mb)	1.D15S1022 & 2.D15S1014.	affected's only analysis (FASTLINK) & 2. multipoint affected's only (FASTLINK).	MDF-R. 2. SZ, BPI, SAD	[illegible] P=0.7; PH=0.2) & 2. Recessive (AF=0.1; P=0.7; PH=0.1).	Z=4.59 2. Z=4.55.	NA	NA
Park et al. (2004).	GWS with 343 markers. 40 pedigrees from Israel and the USA with 373 individuals. 68-79 psychotic individuals.	15q26.1 (90.3 Mb).	D15S652	Two-point affected only (MLINK)	Psychotic individuals.	Dominant (AF=0.01; P=0.8; PH=0.0001).	1.96	Multipoint Affected Sib-Pair (MAPMAKER/SIBS)	2.62

Table B.8: Linkage Data - Chromosome 16.

Study.	Sample Characteristics.	Region of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value).
McInnes et al. (1996).	GWS with 473 markers in two large Costa Rican pedigrees.	16p	D16S521	Two-point analysis	BPI	Dominant (AF=0.003; P=0.81-0.9; PH=0.67).	1.46	NA	NA
Edenberg et al. [illegible]	97 NIMH pedigrees (540 individuals: 232 BP1; 32 SA; 72 BP2; 88 MDE-R).	16p13.3 (13.6Mb).	D16S2619; D16S749.	NA	SA, BPI, BPII.	NA	NA	Affected sibling pair method (SIBPAL).	1.9 (p=0.006).
Berrettini et al. (2000). α	GWS with 345 markers in 97 NIMH families of mixed ethnicity (540 individuals: 232 BPI, 32 SA, 72 BPII, 88 MDE-R).	16	D16S749	NA	SA, BPI, BP2, MDE-R.	NA	NA	Non-parametric affected relative pairs method (GENEHUNTER).	1.7
Baller et al. (2002).	GWS with 388 markers in 5 schizophrenia and 3 bipolar pedigrees (50 individuals, 27 affected & 23 unaffected).	16q	D16S289	Parametric analysis (GENEHUNTER).	Schiz, SA, BPI, BPII, MDE-R.	Dominant (AF=0.05; P=0.7; PH=0.01).	- 0.41	Multipoint analysis (GENEHUNTER-all).	1.76 (0.047).
Dick et al,	56 NIMH families (353	16p13.12	D16S2619	NA	BP1, BP2,	NA	NA	Non-parametric multipoint	2.13 (ARP)

(2002).	individuals: 143 BP 1; 41 BP 2; 44 MDE-R).	(13.6Mb).			& MDE-R			affected relative pair (GENEHUNTER-PLUS-all) & affected sibling pair (ASPEX sib_ibd/sib_phase) analysis.	& 1.69 (ASP).
Ekholm et al. (2003).	GWS with 389 markers typed in 41 families (37 nuclear & 4 extended pedigrees containing 153 affected individuals) from an isolated Finnish population.	16p12.1 (26 Mb).	D16S769	Two point analysis (MLINK).	SA, BPI, BPII, MDE-R.	Dominant (AF=0.05; P=0.9; PH=0.045). Recessive model for non-parametric ASP.	2.5	NA	NA
Ewald et al. (2004).	GWS with 613 markers in two large Danish Caucasian pedigrees.	16p13.3 (3.7-9.3Mb).	D16S510.	Two-point analysis (MLINK).	SA, BPI, BPII, MDE-R.	Recessive (AF=0.1; P=0.05; PH=0.2).	2.23	NPL multipoint analysis (GENEHUNTER - all pairs).	NS
Maziade et al. (2004).	21 multigenerational pedigrees from eastern Quebec. 7 predominantly Schiz families; 6 BP pedigrees, & 8 mixed pedigrees. 480 individuals	16p12.3 (10-19.3 Mb).	D16S410; D16S3041.	Multipoint affected-only (FASTLINK).	BPI.	Recessive (AF=0.01; P=0.7; PH=0.2).	MOD=4.05	NA	NA
Maziade et al. (2004).	21 multigenerational pedigrees from eastern Quebec. 7 predominantly schiz families; 6 BP	16q12.2 (53.3 Mb)	D16S3253.	Multipoint affected-only (FASTLINK).	BPI; BPII; MDE-R.	Dominant (AF=0.01; P=0.7; PH=0.2).	Z=2.19.	NA	NA

	pedigrees. & 8 mixed pedigrees. 480 individuals								
Middleton et al. (2004).	GWS using 11 190 SNPs in 25 extended pedigrees from the Azores and Madeira (233 individuals).	16p12.3 (26Mb).	Not given.	NA	Not given.	NA	NA	Multipoint NPL linkage analysis (MERLIN).	Z=2.89

Table B.9: Linkage Data - Chromosome 22.

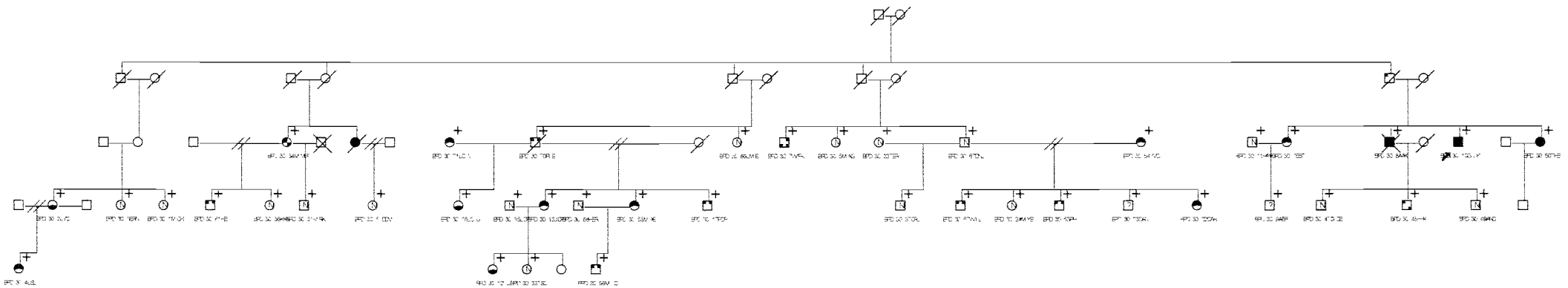
Study.	Sample Characteristics.	Regions of Linkage.	Markers Showing Linkage.	Parametric Analysis.	Affection Status Model.	Inheritance Model (AF=Disease Allele Frequency; P=Penetrance; PH=Phenocopy Rate).	LOD (p-value).	Non-Parametric Analysis.	LOD (p-value).
Edenberg et al. (1997).	6 markers typed in 97 NIMH pedigrees (540 individuals: 232 BP1; 32 SA; 72 BP2; 88 MDE-R).	22q11.2 (24.1Mb).	D22S533.	NA	SA, BPI, BPII, MDE-R.	NA	NA	Multipoint linkage analysis (ASPEX).	2.46
Detera-Wadleigh et al. (1999).	GWS with 607 markers in 22 multiplex pedigrees (306 individuals).	22q12.1 (27.1Mb).	D22S689-S685	NA	SA, BPI, BPII, MDE-R.	NA	NA	Multipoint analysis (ASPEX).	2.1 (0.00094).
Kelsoe et al. (2001).	Genome-wide scan using 443 markers in 20 US families (164 individuals).	22q12.3 (34.7 + 34.8Mb).	D22S278 & D22S683	Two-point LOD score analysis (LIPED)	BPI only	Dominant (AF=0.01; P=0.85; PH=0.05).	3.84 & 2.39.	NA	NA
Turecki et al. (2001).	Genome-wide scan in 31 families with an excellent response to lithium.	22q11.2 (16.2 Mb).	D22S420	MLINK	BPI, BPII, MDE-R.	Recessive (AF=0.16; P(M)= 0.18, P(F)= 0.33; PH=0.005-0.009).	1.91	SimIBD	NS
Potash et	65 families with bipolar	22q12.3	D22S277	NA	SA, BPI,	NA	NA	NPL analysis testing IBD	3.32 (0.005) –

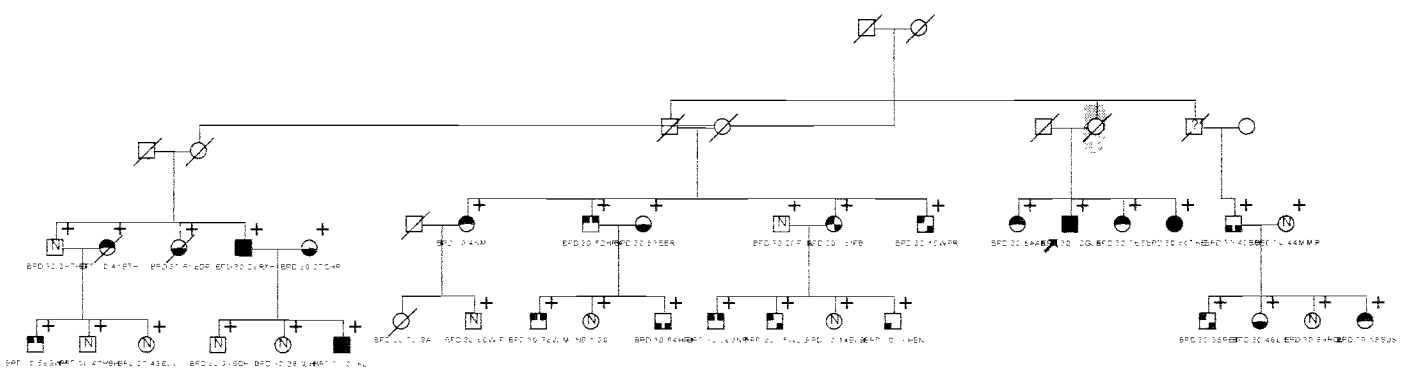
al. (2003).	disorder (129 BP1; 97 BP 2; 69 MDE-R). 10 families with psychotic features.	(34.6Mb).			BPII. MDE-R.			allele sharing among all affected individuals (GENEHUNTER) and [illegible] SIBIBD.	GENEHUNTER & 3.06 (0.00009) – SIBIBD.
(2004).	polymorphic markers typed in 20 families from eastern Quebec (77 BPI, 28 BPII, 43 MDE-R, 45 MDE-S, 68 misc, 133 unaffected).	(45.6 Mb).		analysis (MLINK)	BPII.	P=0.5; PH<0.01).			
Hamshere et al. (2005).	GWS with 426 markers in 24 UK families SA disorder.	22q11.1 (16 Mb).	D22S420	NA	Schiz. SA. BP I.	NA	NA	Multipoint analysis (Mapmaker/Sibs)	1.96.

Appendix D. Pedigree of Family 30.

The two branches of the family are shown separately for clarity.

+ indicates that the individual has been genotyped.





Appendix C.

Table C.1. Primer Sequences and Markers used in the Linkage Analysis.

Region	Location (Mb)	Marker	Forward Primer (5'-3')	Reverse Primer (5'-3').
1q31.3	194.8	D1S1660	tgctatctctcaccagtga	gtctgaagttcatgggaacg
1q32.1	199.3	D1S2655	agggtccccaaagagccttc	atggcagcacatcctgcttc
1q32.1	200.2	D1S1678	actttctcacatgaccacagg	cagcgagactctgtcaaaaa
2q33.3	208.1	D2S2321	ccatgtggtgtttggcag	gagcagaattgcagggc
2q33.3	208.5	D2S2208	ctatttgtaaacatggcggga	agctaagtacctgctcaggaaa
4p16.1	7.1	D4S394	ccctgagcatcctgacttc	gagtgagccccctgtactcca
4p16.1	7.8	D4S2983	tgtccagtggcaggg	ggtcgcattcattcgc
4p16.1	10.4	D4S1582	atcagggttctccacacaaa	ttggttgaaacttgtggatataaa
10q23.3	92.4	D10S1753	ctgctgccaccaacctaa	caagtggagactcgatgaca
10q23.3	92.6	D10S564	tgggaatgtgtctttatcca	gctctaacatagaggccagat
10q23.3	92.6	D10S1171	gggttgaataaacatgcatg	gaatggggatgcagctaaa
12q24.2	117.6	D12S86	agctagtctggcatgagcag	ctatcccctgatgatctccc
12q24.3	123.4	D12S1612	tcagccccctgtctcac	gcatccttgatgtccc
13q32	97.8	D13S1298	gattgttttatattctctgcttttc	cctaccagtgggacttttc
13q32	98	D13S1271	ccgagattgcaccactg	gcggtttttgccattaaag
13q32	98.5	D13S1284	gaggtgtccatcaatgc	tgtggctgcaaatgac
15q11.2	23.2	D15S122	gataatcatgcccccca	cccagtatctggcacgtag
15q12	25.5	D15S1002	gtatcccaaggccatacccct	ctcttgctagagacagcagg
15q13.1	27.6	D15S1048	agccgtctttgtgcca	tgcagccactgtggaa
16p13.3	1.6	D16S3024	acatgctgtgccacct	agctgccagtatatggagga
16p13.3	4	D16S3027	atattggcatctgggg	ccagcatgagttgcttt
16p13.2	7.1	D16S3088	ctctgaataggggtggggatg	aaggaaatctgggggtgtacg
22q11.2	24.3	D22S421	ctgctgcccctaacatatcac	ggccaggagtgtctgaatttta
22q11.2	24.3	D22S315	tgcctattaaactctccactcctta	gcattatgattcatttctcacaga
22q11.2	24.5	D22S1164	atccagacactgcctctc	catcagaagccatttgc

Table C.2. Genetic Variants and Primer Sequences used in the Association Analysis.

Variant	**PCR Annealing Temperature (°C)**	**Forward Primer (5'-3')**	**Reverse Primer (5'-3')**
5-HTT-LPR	59.2	CTTGTACTTGGAGGAACTGACC	GAGGCAGCAGACAACTGTG
SERT VNTR	55	GTCAGTATCACAGGCTGCGAG	TGTTCCTAGTCTTACGCCAGTG
DRD4-48R	55	GCGACTACGTGGTCTACTCG	AGGACCCTCATGGCCTTG
DRD4-120Ins/Del	59.2	CCATCCTGGGAGAGAAGAAAC	CAAAGGTCTGGAGGACACG
COMT V158M	56-51 TD	ACTGTGGCTACTCAGCTGTG	CCTTTTTCCAGGTCTGACAA
BDNF V66M	58	CAACAGGCTTGACATCATTGGCTG	CCGACATGTCCACTGCAGTCTTTT
DRD2 Taq	55	CCGTCGACGGCTGGCCAAGGTGTCTA	CCGTCGACCCTTCCTGAGTGTCATCA
DAT	59.2	TG(CT)GGTGTAGGGAACGGCCTGAG	CTTCCTGGAGGTCACGGCTCAAGG
Notch4	57	GGAAACAGCTCAGACGTGAG	CCACTGAACATCCTCCTAAG
MAO-A VNTR	57	ACAGCCTGACCGTGGAGAAG	GAACGGACGCTCCATTCGGA
APOE	67	TCCAAGGAGCTGCAGGCGGCGCA	GCCCCGGCCTGGTACACTGCCA
Prion Met129Val	54.5	AACGTCGGTCTCGGTGAAGT	TCAAGGAGGTGGCACCCACA

Table C.3. Restriction Digests of Single Nucleotide Polymorphisms.

SNP	**Restriction Enzyme**	**Digestion Temp (°C)**	**Resolved on ...**
COMT V158M	HSP92 II	37	2% Agarose
BDNF V66M	Bbr PI	37	6% PolyAcrylamide
DRD2 Taq	TaqIA	65	2% Agarose
ApoE ε	CFO I	37	6% PolyAcrylamide
Prion	BSA AI	37	2% Agarose

Appendix E. Personality Questionnaires.

(1). Cloninger's Biopsychosocial Model of Personality: The Temperament and Character Inventory (Cloninger et al. 1994).

The Temperament and Character Inventory (TCI) is a self-report inventory that measures seven discrete dimensions of personality. Four of these scales measure putatively heritable or largely heritable temperaments, while the other three dimensions measure socioculturally determined character traits (Cloninger et al. 1994). The TCI has been shown to possess adequate reliability. The internal consistency coefficients (the average of the single test item inter-correlations) of the seven dimensions range between 0.7 and 0.89, while the test-retest (the temporal stability of the test over multiple administrations) correlations range between 0.51 and 0.84 (Cloninger et al. 1994).

Cloninger et al. (1993) have developed a psychobiological model of personality that attempts to differentiate heritable, genetic factors or temperaments from so-called character traits which develop later on through a distillation of life experiences. Cloninger et al. (1993) identify four temperaments - novelty seeking; harm avoidance; reward dependence and persistence - which putatively reflect biases in the way the brain processes information and therefore responds to perceptual stimuli.

Novelty seeking (NS) is a description of the nervous system's inclination to *initiate* exploratory behaviour. High scorers will be quick tempered, excitable, curious, impulsive, and extravagant. Harm avoidance (HA), on the other hand, is a description of the nervous system's proclivity for *inhibiting* or ceasing certain behaviours. High scores on this dimension are associated with traits such as anxiety, shyness, fear of uncertainty and fatigability (Cloninger 1991). Reward dependence (RD) is described by Cloninger et al. (1993) as a neural bias for *maintaining* current behaviours. High scorers depend on the approval of others, and therefore display traits such as sentimentality, warmth and social attachment (Cloninger 1991). Persistence is a bias for *maintaining* behaviour despite frustration and fatigue, and is a measure of diligence, industriousness, degree of ambition, perfectionism and perseverance

(Cloninger et al. 1994) Persistence was originally subsumed under the reward dependence dimension. However because it was found to be largely independent of other aspects of reward dependence Cloninger et al. (1993) have labelled it as a fourth independent temperament.

Cloninger et al. (1993) have proposed that character (as opposed to temperament) traits develop as a result of learning and involve the reorganisation of self-concepts to allow the individual to adapt to the demands of the environment. It is important to note however, that temperament also influences the individual's development of character traits. Life experiences interact with the underlying temperament to forge character (Cloninger et al. 1999). For example, people with an obsessive temperament are more likely to display a melancholic or dependent character organisation while individuals with an independent temperament profile have a greater probability of displaying an autocratic character. The three character traits - self-directedness (SD); cooperativeness (C), and self-transcendence (ST) - reflect acceptance of the individual self, acceptance of others, and acceptance of the world, or nature in general, respectively. SD taps traits such as responsibility, reliability, and surgency. Cooperativeness is a measure of helpfulness, compassion, social tolerance and adherence to ethical principles, and ST reflects traits such as imagination, patience, and wisdom (Cloninger et al. 1994).

(2). The Hypomanic Personality Questionnaire (Eckblad and Chapman 1986).

The Hypomanic Personality Scale (HPS) is a 48 item self-report instrument that measures traits associated with bipolar disorder (Eckblad and Chapman 1986; Kwapil et al. 2000). These traits describe people who are cheerful, optimistic, extroverted, self-confident, irritable, rude, reckless and irresponsible (Akiskal 2001; Meyer and Keller 2002).

The Hypomanic Personality Scale appears to identify individuals at risk for the development of bipolar disorder (Meyer and Keller 2002). Eckman and Chapman (1986) found that high scorers suffered from a greater number of mood disorders, were more likely to display psychotic symptoms, were at an increased risk for drug

abuse and had lower psychosocial functioning than controls. Kwapil et al. (2000) carried out a longitudinal study of HPS test-takers and found that 25% of the high scoring group compared to 0% of the low-scoring group, had developed bipolar disorder at follow up, 13 years later. *Meyer et al.* (2001) found that scores on the HPS were predictive of depressive symptomatology at a 5 year follow up.

Although the HPS was originally normed on undergraduate students at the University of Wisconsin-Madison, and may vary with age, ethnicity and social-economic status (Meyer and Keller 2002), Klein (1996) has argued that the scale is cross-culturally valid.

(3). The Affective Neuroscience Personality Scale (Davis et al. 2003).

The leitmotif running through the work of Panksepp and colleagues is the notion that emotions can only be understood by analysing their adaptive significance and studying the evolutionary conserved, innate neuroanatomical circuits that generate them. Davis et al. (2003) hypothesise that variation in personality can be explained by reference to the activity of six discrete affective systems in the brain: Anger, Care, Fear, Play, Sadness, and Seek. These sub-cortical emotional systems which are putatively characteristic of the mammalian brain, help elucidate both affective tendencies or temperaments, and "higher" level psychodynamic or personality structures which grow out of these evolutionary older emotional biases (Davis et al. 2003). In fact, Davis et al. (2003) hypothesise that their six affective dimensions form the foundation of the now ubiquitous five factor personality model (FFM) developed by Costa and McCrae (1992). For heuristic reasons they also introduce another "higher emotional attribute", spirituality (Davis et al. 2003).

Table E.1: The ANPS: Description of the Seven Scales (Adapted from Davis et al. 2003).

Scale.	**Description.**
Playfulness.	Having fun and being generally joyful vs. being serious.
Seeking.	Feeling curious, exploring, striving for solutions.
Caring.	Nurturing, being drawn to young children or animals, empathy, and soft-heartedness.
Fear.	Feelings of anxiety and tension, losing sleep, ruminating about future decisions.
Anger.	Easily irritated and frustrated; expression of anger verbally or physically.
Sadness.	Feelings of loneliness and distress, crying and thinking about loved ones often.
Spirituality.	Feeling connected to humanity and creation, searching for meaning and inner peace.

The Seeking System.

The *seeking* system, which is activated by endogenous need detectors or cues in the environment that signal reward, controls the appetitive motivational system, that is the impulse to search, investigate and make sense of the environment (Panksepp 1998). This enables animals to survive and perpetuate the species by obtaining resources such as food, warmth, and sex. Feelings associated with the activation of this system include excitement, curiosity and general energisation. The *seeking* system is made up of mesolimbic and mesocortical DA tracts which ascend from the ventral tegmental area (VTA) of the brain stem through the lateral hypothalamus to the nucleus accumbens and cerebral cortex (Panksepp 1998). These DA tracts are activated in a tonic fashion and this relative degree of stability means that appetitive drive can be

considered to be a personality trait. Panksepp (1998) hypothesises that activation of the *seeking* system facilitates learning and enhances reactivity but over-activation leads to psychotic disorders such as schizophrenia or mania, perhaps explaining why anti-psychotic drugs exert their effect by reducing DA activity at D2 receptors and psychostimulants like cocaine sometimes induce paranoia. Conversely, underactivity of the system is hypothesised to contribute to depression.

The Anger or Rage System.

Anger is adaptive in the sense that it propels organisms to compete for resources when expected or desired rewards or not obtained. Anger is also precipitated by restrictions placed on an individual's freedom to act or by pain or irritation of the bodily surface, and here the adaptive value of the emotion is self-evident. The probability of experiencing anger is enhanced by activation of the *Seeking* system since high expectations of rewards will not always be met, and these two systems may therefore act in a mutually inhibitory manner. Feelings of anger are hypothesised to be generated by a circuit which runs from the medial amygdaloid areas via the stria terminalis to the medial hypothalamus, and from there to the periaqueductal gray matter (PAG). The neurochemistry of this system has not been well elucidated but Substance P and serotonin may play an important modulatory role.

The Fear System.

The ability to detect and anticipate dangers in the environment is key to any animal's survival. In mammals, this adaptive problem has been solved through the evolution of a neural circuit that coordinates the physiological, perceptual and behavioural responses to threats (Panksepp 1998). Activation of this circuit which originates in the lateral and central amygdala, runs through the anterior and medial hypothalamus, and extends to the PAG, is associated with the emotions of anxiety and fear and parallel signs of autonomic arousal such as sweating and tachycardia. In humans, anxiety is not only precipitated by exogenous events, but emerges from internally generated memories or pathology such as epileptic foci. Panksepp (1998) hypothesises that trauma or other adverse experiences may sensitise this deep subcortical circuit,

leading to a chronic hyperemotional state that is refractory to higher cognitive influence.

Sadness.

A related, but independent circuit mediating panic or separation distress runs from the preoptic area and bed nucleus of the stria terminalis down through the dorsomedial thalamus and PAG. Activation of this pathway leads to emotions such as loneliness, grief, and depression accompanied by parasympathetic autonomic symptoms such as tightness in the chest, a lump in the throat, and the desire to cry (Panksepp 1998). Over-arousal of this circuit is hypothesised by Panksepp (1998) to mediate the clinical phenomenon of panic attacks.

Play.

The capacity for rough-and-tumble play is common to all mammals and has been hypothesised to improve social cooperation and bonding, facilitate the learning of both social and cognitive skills, and promote the development of social rank and leadership (Panksepp 1998). The neuroanatomical and neurochemical basis of these putative play circuits remain a matter of speculation but preliminary evidence suggests that various posterior thalamic nuclei mediate ludic phenomena, in part through the modulatory activity of the opioid system (Panksepp 1998). Panksepp (1998) hypothesises that underarousal of the play circuit is associated with depression, while overactivity of this emotional system might provoke symptoms of mania

Care.

The mammalian care circuit is hard-wired to promote the nurturant behavior of mothers and in the case of humans, fathers towards their offspring, a phenomenon that clearly enhances the survival of their progeny (Panksepp 1998). The circuit probably incorporates many subcortical structures including the pathway from the preoptic area to the habenula of the brain-stem, and beyond through the hypothalamic tract to the ventral tegmental dopaminergic system (Panksepp 1998). The activity of this system appears to be mediated by neurotransmitters such as oxytocin, the opioids, and prolactin which induce feelings of acceptance, nurturance and love.

Psychometric Characteristics.

Internal consistency reliability (Cronbach's alpha) ranges from 0.65 to 0.86 for the various scales. The negative emotion scales (anger, fear and sadness) tend to correlate with each other, while spirituality is related to scores on the positive emotion scales, which also correlate with each other (seek, care, and play) (Davis et al. 2003). A factor analysis confirmed the existence of the positive and negative emotional factors which are hypothesised to show equivalence to Gray's (1987) Behavioural Inhibition and Activation Systems (Davis et al. 2003). Scores on the ANP scales correlate highly with some of the FFM dimensions: Play and Extraversion (r 0.46), Seeking and Openness to Experience (r 0.47), Care and Agreeableness (r 0.50), and Fear (r=0.75), Anger (r=0.65), and Sadness (r=0.68) with Neuroticism (Davis et al. 2003). It is hypothesised by the authors that Fear, Sadness and Anger all inter-correlate highly with each other and with the FFM Neuroticism score because humans are unable to distinguish well between the feelings of anxiety, distress or sadness, and irritation (Davis et al. 2003).

(4). *The Temperament Assessment of Memphis, Pisa, Paris, and San Diego Auto-questionnaire (TEMPS-A) (Perugi and Akiskal, 2002).*

The TEMPS-A was discussed in a fair amount of detail in section 3.2, and the details will not be unnecessarily repeated here. In short, the TEMPS-A is a 110 item questionnaire consisting of 5 subscales (DT, CT, HT, IT and AT) of 21 items (see Table 3.2, below). Each item requires the respondent to circle true or false in response to a statement. An affirmative response to 11 or more items is indicative of an elevated score on that particular subscale (Perugi and Akiskal 2002).

Table E.2. A Description of the Temperaments Measured by the TEMPS-A. (Akiskal and Mallya 1987, p71-72).

Temperament.	**Description.**
Depressive	Gloomy, pessimistic and incapable of fun; quiet, passive and indecisive; broody and given to worry; lack of self-confidence; conscientious; self critical and self reproaching; preoccupied with failure and negative events.
Hyperthymic	Irritable, cheerful, exuberant, naïve, overconfident, bombastic, loquacious, extroverted, warm, meddlesome, uninhibited; haughty; stubborn; insubordinate.
Irritable	Moody, irritable, hot-tempered, paranoid, brooding, hypercritical and complaining, obtrusive, dysphorically restless and impulsive.
Cyclothymic	Introverted self-absorption alternating with people seeking; decreased verbal output alternating with talkativeness; unexplained tearfulness alternating with jocularity; pessimism alternating with optimism; low self-esteem alternating with grandiosity; mental confusion alternating with creative thinking.

(5). *The Schizotypal Traits Questionnaire* (Claridge and Broks, 1984).

The Schizotypal Traits Questionnaire is composed of two separate scales, the 37 item Schizotypal Personality Scale (STA) and the 18 item Borderline Personality Scale (STB). A principal components analysis carried out by Rawlings et al. (2001) produced a four factor solution: magical thinking, paranoia, unusual perceptual experiences, and social anxiety and sensitivity to criticism. The STB loaded on two factors: feelings of purposelessness and self-destructiveness and impulsive antisocial urges (Rawlings et al. 2001).

Additional Tests Administered.

(6). *The Beck Depression Inventory (BDI)* (Beck and Steer 1993).

The BDI is a 21-item self-report inventory that was designed to detect and measure the degree of severity of depressive symptoms in adolescents and adults (Beck and Steer 1993). The BDI has been shown to be a reliable (internal consistency = 0.86) and valid measure of depression (Piotrowski 1996) and is thus widely used in research studies (Startup et al. 1992). Test-retest reliabilities range from 0.74-0.93, and no gender or ethnic differences have been reported in American populations (Lezak 1995). One caveat is that the intention of test is transparently reflected in each item and this makes the test open to manipulation or problematic in the testing of patients who deny their distress (Lezak 1995).

(7). *The Altman Self-Rating Mania Scale (ASRM)* (Altman et al. 1997).

The ASRM consists of 5 groups of statements covering mood, self-confidence, sleep, talkativeness, and activity level, respectively. The score for each group of statements ranges from 0-4 depending on the severity of the symptom in question so that the total ASRM score ranges between 0 and 20. Scores greater than 5 are considered to be indicative of hypomania or mania (Altman et al. 1997). This cut-off point correctly classified 85.5% of patients with mania and 87.3% of non-manic individuals in Altman et al.'s, (1997) test sample.

Although there is a school of thought that manic patients do not have the requisite insight to produce valid self-reports (Platman et al. 1969), this has been disputed by Bauer et al. (1991), Shugar et al. (1992), and Altman (1998) who argue that self-rating measures of mania are highly correlated with clinical ratings of mania. The ASRM shows good test-retest reliability (r 0.86-0.89) and concurrent validity as evinced by significant correlations (r = 0.718 and r 0.760) with the Mania Rating Scale and the Clinician Administered Rating Scale for Mania respectively (Altman et al. 1997).

Appendix F. Neuropsychological Tests.

(1). *The Digit Span* (Forward and Reverse).

The Digit Span is really two tests in one. The Digits Forward is a test of concentration and attention, or as Lezak (1995) phrases it, "freedom from distractibility". The examiner reads a list of numbers to the patient, whose task it is to repeat back each sequence exactly as it is given. The list of digits read to the patient becomes progressively longer and the task increasingly difficult until the patient fails or 8 consecutive digits are recalled correctly.

The Digits Backwards is administered in exactly the same way except that this time the subject is required to repeat back the list of digits in the reverse order. This requires the individual to keep the relevant numbers in mind and "juggle" them around. In other words, it is a measure of working memory (Lezak 1995). The reverse digit span test is sensitive to both frontal cortex, and left and right parietal lobe damage. The "online" manipulation of data which is usually equated with working memory, involves recruitment of regions of the dorsolateral prefrontal cortex, especially the left dorsolateral convexity (Smith and Jonides 1999)

(2). *The Controlled Oral Word Association Test* (COWAT).

This task requires the individual to name as many words as possible that begin with a particular letter of the alphabet. The three letters commonly used are F, A, and S, hence the alternative name, the FAS test. A time limit of one minute per letter is imposed. Proper nouns, names and identical words with different suffixes are not allowed. The score is the total number of valid words obtained by the individual over the three trials.

The COWAT measures expressive language function or verbal flueney, but it also loads on factors such as vocabulary, reading-writing and mental operations (Johnstone et al. 2000). Variables such as age and education level influence task performance, and Lezak (1995) urges caution in the interpretation of scores of subjects without an

high school education. Test-retest reliability varies from 0.7 to 0.88 depending on the testing interval (Johnstone et al. 2000).

Ramier and Hecaen 1978, quoted in Cavedini et al. (1999) correlate task performance with the left frontal lobe anterior to Broca's area, although more recent reports indicate that right or bilateral frontal lobe lesions may also depress COWAT scores (Johnstone et al. 2000). Frith et al. (1991) carried out positron emission tomography (PET) scans of individuals who were required to generate words, and demonstrated an increase in activity of the left dorsolateral prefrontal cortex, and bilateral decreases in activity of the superior temporal cortices. Other researchers postulate that the FAS is primarily a measure of generativity, and performance is therefore maximally sensitive to lesions of the deep ventromesial white matter and to a lesser extent the supplementary motor cortex (Solms 2005, personal communication).

(4). *The Rey Complex Figure Test* (RCF).

The RCF is a measure of visual perception and memory, constructional praxis, and planning (Helmes 2000). The complexity of the test makes it difficult to interpret in isolation from performances on other neuropsychological tasks (Helmes 2000) and therefore scores from other tasks in the battery were used to facilitate the interpretative process.

The major advantage of the RCF is its availability and ease of administration. Subjects are given a blank sheet of paper and asked to copy the figure, unaware of the recall task that awaits them. The study participants were asked to recall the figure of Rey 30 seconds after completion of the copy trial. The scoring method of Taylor (1959) as described by Lezak (1995) was used for both the copy and the recall trials of the RCF. This scoring method takes into account both the presence of a particular aspect of the diagram, and its relative position within the whole of the design. Thus for each of the 18 elements of the diagram, the possible score ranges from 0-2, allowing for a maximum of score 36 points.

The different cognitive processes tapped by the RCF are discussed below.

(a). Visual-Spatial Perception.

Distortions or omissions in the subject's copy may be indicative of either left or right hemisphere pathology (Helmes 2000). Patients with damage to the right parietal-occipital lobe are unable to process the overall organisation or "gestalt" of the figure, resulting in distorted drawings, while left parietal-occipital lobe lesions tend to produce diagrams with a paucity of details (Lezak 1995). This pattern of deficits is usually seen even more clearly in the more taxing recall trial.

(b). Visual Memory.

In order to remove the effects of the copy trial on memory performance and obtain the "cleanest" estimate of visual memory possible, it was decided to use the "Percent Recall" score (recall score/copy score x 100) method devised by Snow (1979) as quoted by Lezak (1995). The only caveat attached to this method is that sometimes the copy performance of the patient can be so low that the recall score cannot be much lower, artificially inflating the memory score (Lezak 1995). Recall performance is particularly sensitive to right or bilateral temporal lobe lesions (Helmes 2000).

(c). Praxis.

A successful copy of the RCF requires intact fine motor control, visual-spatial perception and a reasonable degree of organisational ability (Helmes 2000).

(d). Planning.

The RCF test is also sensitive to "executive function" because a drawing strategy that entails reconstruction of the overall design configuration followed by inessential details, will aid recall. Besides planning and strategic thinking deficits, frontal lobe patients may lose track of the elements of the drawing that have already been completed, resulting in repetitions or omissions (Lezak 1995). Perseveration is also seen although this is less common (Lezak 1995).

Memory and copy performance on the Rey shows an age related decline that begins in middle adulthood, and accelerates in by the seventh decade of life (Spreen and Strauss 1998). In contrast to the RAVLT, males tend to outperform females by one or two points (Rosselli and Ardila 1991). Inter-rater reliability scores are excellent and range from 0.91 to 0.98, while test-retest reliability scores vary between 0.60 and 0.76 (Lezak 1995). Performance on the copy and recall trials of the RCF is influenced by both education levels (r=0.43 and r=0.37) (Rosselli and Ardila 1991) and intellectual ability (Lezak 1995)

(6). The Stroop Colour Word Test (Trenerry et al. 1989).

The Stroop Neuropsychological Screening Test (SNST) is based on the idea that it takes longer to read a printed colour name when the ink in which the word is printed is different to the colour that the collection of letters specify (Lezak 1995). The individual is initially required to read a list of words (green, blue, red, tan) as quickly and as accurately as possible, and then asked to specify the colour of the ink that each of these words is written in. The former task has been dubbed the "control condition" of the Stroop, and the latter task the "interference condition" of the test.

The Stroop is a primarily a measure of the ability to inhibit over-learnt or prepotent responses in the face of conflicting information (Roberts Jr and Pennington 1996), an ability which has been localised to the dorsolateral prefrontal cortex, anterior cingulate gyrus (Posner and Raichle 1994), the left inferior frontal gyrus (Taylor et al. 1997), and the orbital-basal cortex (Solms 2005, personal communication).

Stroop theorised that his now eponymous interference effect was due to differential practice: people are trained from an early age to associate words with reading, not colours (MacLeod 1991). Stroop's explanation has proved to be robust, and is compatible with two more modern accounts of the Stroop effect: relative speed of processing and automaticity (see MacLeod 1991 for a review). The relative speed of processing view is based on the idea that words are read faster than colours causing interference at a limited capacity buffer when the colour-word response in required. According to the automaticity hypothesis, the naming of colours requires much more

attentional resources than the naming of words which is automatic, and hence less colour-words than words are named during the testing period (MacLeod 1991).

A review of the literature suggests that the Stroop test has been commonly used in the assessment of bipolar individuals (Albus et al. 1996; Paradiso et al. 1997; Van Gorp et al. 1998). The SNST is known to have excellent test-retest reliability (0.90), and has been shown to discriminate between brain-damaged and normal adults with 80-90% accuracy (Trenerry et al. 1989), making it a valid measure of frontal lobe function in adults. The Stroop is reported to be equally sensitive to "executive" and attention deficits (Shum et al. 1990; Hanes et al. 1996; Savitz and Jansen 2003). Patients with mild head injuries, schizophrenia, depression and dementia may also find this test difficult (Lezak 1995; Ponsford 2000). Although the weight of the data suggest that left frontal cortical lesions are associated with performance deficits on the Stroop (Ponsford 2000), right lateral prefrontal lesions have also been associated with performance errors (Vendrell et al. 1995).

(7). *The Rey Auditory-Verbal Learning Test* (RAVLT)

The RAVLT measures verbal learning and short-term verbal memory (Lezak 1995) although it also possesses an "executive" component, namely the ability to self-monitor and verify performance thus facilitating learning (Solms 2005, personal communication). Fifteen words are read aloud to the subject who is required to recall as many words as possible from the list. This serves as a measure of immediate memory. The procedure is repeated another 4 times.

The learning rate can be calculated by subtracting the number of words obtained in trial 1 from the number of words obtained in trial 5. The total learning score is calculated by adding together all the words obtained over the first 5 trials.

The subject is then required to remember a new list of words (trial 6). Trial 6 serves as a distracter, and can also be used to measure immediate memory. Proactive interference (recently learnt material interferes with the acquisition of new material) is estimated by dividing the number of words obtained in trial 6 by the number of words

obtained in trial 1 (Helmes 2000). After trial 6, the subject is once again required to recall the original list of fifteen nouns without the examiner re-reading the words.

Retroactive interference is calculated by dividing the number of words obtained in trial 7 by the number of words obtained in trial 5 (Helmes 2000). After a delay of about 20 minutes, during which time the WCST was administered, the subject was given a list of words containing the 15 target words, and required to identify them. Recognition of the target words is a measure of long-term memory storage and retrieval efficacy (Helmes 2000).

Table F.1: Summary of Scoring Method for the RAVLT

Measure.	**Calculation**
Immediate Memory.	Trial 1.
Proactive Interference.	Trial 6 – Trial 1.
Learning Rate.	Trail 5 – Trial 1.
Total Learning.	Sum of Trials 1-5.
Retroactive Interference.	Trial 7 – Trial 5.
Long Term Memory and Recall Efficacy.	Recognition Trial.

Memory problems in bipolar patients have been detected through the use of the RAVLT (Wolfe et al. 1987; Coffman et al. 1990). Frontal lesions appear to effect recall more than recognition, but left temporal lobe damage adversely affects performance on all task variables (Lezak 1995).

Age, gender and mental ability affect RAVLT scores. Elderly patients tend to perform at a lower level than their younger counterparts, and women outperform men because of their greater verbal dexterity (Lezak 1995). Test-retest correlations after a year-long hiatus have been reported to range from 0.38 to 0.70 (Snow et al. 1988 in Lezak 1995). The RAVLT has been successfully used in the assessment of patients with closed head injuries, Alzheimer's disease (Bigler et al. 1989), and general neurological and psychiatric patients (Rosenberg et al. 1984).

(8). *The Wisconsin Card Sorting Test* (WCST-64).

The WCST consists of four stimulus cards - 1 red triangle, 2 green stars, 3 yellow crosses, and 4 blue circles, respectively - and 2 packs of 64 cards each. The subject is asked to match each of the cards in the pack (or packs if the full 128 card version is used) to one of the 4 stimulus cards placed on the table. Each card can be matched according to 3 categories: colour, form or number. The subject is not told how to match the cards, but is given feedback after each trial as to whether the card was correctly matched. After 10 consecutively correct responses, the examiner changes the sorting category without informing the test-taker.

Four different scores were obtained from the WCST data:
(a). Number of categories achieved.
(b). Trials taken to first category. (An indication of how many trials the first category was achieved in).
(c). Failure to maintain cognitive set. (This was defined as getting 5 or more sorts in a row correct and then making a mistake).
(d). Percent perseverative errors. (The number of perseverative errors made divided by the total number of trials which in this case was 64).

The original test developed by Berg in 1948 was designed to assess cognitive flexibility, hypothesis testing and problem solving (Axelrod 2002). The WCST was quickly found to be sensitive to lesions of the frontal lobes, and became a ubiquitously used neuropsychological tool (Heaton et al. 1993). It is in fact regarded in some quarters as the best single measure of prefrontal lobe function (Morice 1990) although Anderson et al. (1991) assert that the WCST cannot differentiate frontal from non-frontal focal lesions. Frontal cortex dysfunction may interfere with the planning of tasks and the ability to generate and select alternative hypotheses (shifting cognitive set), traits which the WCST putatively measures (Lezak 1995). The WCST also differentiates individuals with diffuse brain damage from neurologically intact individuals (Axelrod et al. 1992).

The WCST often takes at least 30 minutes to administer (Axelrod et al. 1997), and thus a number of different administrative and scoring mechanisms have been introduced over time, including the abbreviated 64 card version (WCST-64) of the original 128 card test. Axelrod et al. (1992) compared the mean scores attained by 120 subjects on the WCST-64 and WCST-128 (the regular version), and found no statistically significant differences for any variable, providing evidence for the validity of the WCST-64. Kongs et al. (2000) have since published normative data for the WCST-64 which indicate that the abbreviated test is sensitive to frontal lobe dysfunction, and like the full version of the test, adequately differentiates clinical patients from controls. The WCST has also been used a number of times in the assessment of bipolar individuals (Coffman et al. 1990; Goldberg et al. 1993; Albus et al. 1996, and van Gorp et al. 1998).

Appendix G. The CTQ and DES .

The original, 70 item version of the Child Trauma Questionnaire (CTQ: Bernstein and Fink 1998) is a self-administered inventory that provides a retrospective assessment of childhood abuse and neglect. Therapists' ratings of abuse and neglect were used to demonstrate "good" criterion-related validity, while convergent and discriminant validity was demonstrated with a structured trauma interview. Test-retest reliability over a 2 to 6 month interval is putatively "excellent". In short, the CTQ appears to correctly detect abuse and neglect histories in both normal and clinical populations (Bernstein et al. 2003).

The abbreviated CTQ was designed as a rapid screening instrument for both clinical and non-referred populations. Like the original version of the questionnaire, the short 28 item version of the CTQ is composed of the following five clinical sub-scales: physical, sexual, and emotional abuse, and physical and emotional neglect. A three item denial/minimisation scale is also included to detect the under-reporting of maltreatment (Bernstein et al. 2003).

The CTQ short form performed equivalently across four diverse USA populations. In other words, despite differences in age, sex, ethnicity, socio-economic status, and psychopathology, individuals interpreted the items of the scale in a consistent manner (Bernstein et al. 2003). Correlations with therapist ratings of abuse were statistically significant for all the subscales ($p<0.01$), and ranged from 0.36 to 0.75 (Bernstein et al. 2003).

The Dissociative Experiences Scale (DES) is the most widely used self-administered measure of dissociation and was designed to measure dissociation in both normal and clinical populations (Bernstein and Putnam 1986) although it performs better in the latter (Wright and Loftus 1999). The DES is composed of three subscales – amnesia, depersonalisation-derealisation, and absorption. The 28 items were derived from clinical data, interviews, and discussions with experts in dissociation (Bernstein and Putnam 1986). The test-retest reliability and the internal reliability of the DES is 0.84 and 0.90, respectively (Bernstein and Putnam 1986). The DES has been shown to

differentiate between controls, subjects with various types of non-dissociative psychopathology, and subjects with dissociative identity disorder (Bernstein and Putnam 1986; Carlson and Putnam 1993). Individuals with post-traumatic stress disorder (PTSD) and dissociative identity disorder (DID) tend to produce the highest scores on the scale, followed by individuals with borderline personality disorder, schizophrenics, patients with mood disorders, and the general non-clinical population (Carlson and Putnam 1993). Gender, socioeconomic status, education level, and religious affiliation do not appear to influence DES scores (Carlson and Putnam 1993).

On the original DES, respondents are asked to make a mark on a 100mm line to indicate what percentage of the time they spend having a particular experience. One end of the line is labelled 0% and the other end 100%. The marking of the original DES can be time-consuming, and Carlson and Putnam (1993) thus introduced an alternative response set (0%, 10%, 20% ..... 100%), and named the new scale the DES II. The DES II has almost identical psychometric properties to its predecessor (Carlson and Putnam 1993; Wright and Loftus 1999), but is easier to administer. A score of 30 or above on the DES II is usually indicative of the presence of a severe dissociative disorder, but it should be noted that the DES cannot be used in isolation to make DSM-IV diagnoses, and should always be corroborated with a clinical interview (Carlson and Putnam 1993).

Appendix H. Explanation of the ANOVA Interaction Effect.

The author is indebted to Lize van der Merwe for providing the example used in this Appendix. In order to test for gene-environment interactions a mixed-model ANOVA statistic was used. For each statistical test, family of origin was entered into the model as a random-factor to control for the fact that individuals in the sample are related to each other. The following covariates were entered into the model: age, gender, ethnicity, self-reported depression and self-reported mania scores. The effect of the genotype was then tested, followed by the effect of the relevant CTQ variable. These are the main effects of the model. The interaction between the two main effects was then calculated. In order to explain what the interaction result means, refer to the interaction between the *SERT* 9R allele, sexual abuse and its effects on Memory below.

Table H.1. Mixed Model ANOVA: *SERT* 9R*sexual abuse

Fixed Effect	**Value**	**STD Error**	**D.F.**	**t-value**	**p-value**
Age	**-0.0267**	**0.0030**	**152**	**-8.743**	**0.0000**
Gender	**-0.2033**	**0.1018**	**152**	**-1.996**	**0.0477**
Ethnicity	0.0679	0.1163	42	0.583	0.6527
Altman (mania)	-0.0030	0.0174	152	-0.175	0.8612
Beck (depression)	**-0.0149**	**0.0066**	**152**	**-2.263**	**0.0250**
Sexual abuse	**-0.0345**	**0.0119**	**152**	**-2.890**	**0.0044**
***SERT* 9R**	**-0.7902**	**0.3865**	**152**	**-2.044**	**0.0426**
Sexual abuse*9R	**0.0733**	**0.0357**	**152**	**2.052**	**0.0419**

After adjusting for the covariates, each sexual abuse point (range 5-25) subtracts on average 0.0345 from the Memory score (Table H.1, column 2). Each allele 9 subtracts on average 0.7902 from memory. If both sexual abuse and an allele 9 is present 0.0733 x the sexual abuse score is added back, if there are 2 alleles then 0.146 (0.0733x2) x the sexual abuse score is added to the Memory score.

So if a person has a sexual abuse score of 20, and 2 9R alleles, he/she gets both 0.7902 x 2 = 1.5804 and (20 x 0.0345) 0.69 = 2.22 /04 subtracted from the Memory

score But also 20x2x0.0733=2.932 added back. The combined effect will be to add 0.6616 to the Memory score.

It is clearly impractical for all this information to be provided for every gene-abuse interaction covered in the thesis and therefore a short-hand will be used. For example the following paragraph illustrates how this interaction was described in the results section of the thesis:

"As far as Memory performance was concerned, both the 9R SERT VNTR variant (t=-2.044, p=0.0426) and sexual abuse (t=-2.890, p=0.0044) were associated with reduced memory scores. However, the 9R allele of SERT interacted with sexual abuse to produce higher Memory scores (t=2.052, p=0.0419)".