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A MOLECULAR APPROACH TO HUNTINGTON DISEASE IN SOUTHERN AFRICA

A thesis submitted to the

UNIVERSITY OF CAPE TOWN

in fulfilment of the requirements

for the degree of

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JULY, 1990.

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University of Cape Town

DECLARATION:

This study was performed from 1987 to 1990 under the supervision of Professor P Beighton of the Department of Human Genetics, University of Cape Town.

The linkage study of Huntington disease in a black African family was completed by the author in Vancouver, Canada, during 1989 under the guidance of and in collaboration with Professor M Hayden of the Department of Medical Genetics, University of British Columbia.

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

Leslie J. H. L Greenberg
July, 1990.

DEDICATION:

To IVAN, for making so much possible.

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ABSTRACT

July, 1990

A Molecular approach to Huntington disease in Southern Africa.

Leslie Greenberg, MRC Unit for Inherited Skeletal Disorders, Department of Human Genetics, University of Cape Town Medical School, Observatory, Cape Town, SOUTH AFRICA.

The discovery of a polymorphic DNA probe (G8) showing genetic linkage to Huntington disease (HD) has facilitated presymptomatic testing and antenatal diagnosis of HD. Before implementation of a genetic service of this nature in Southern Africa (SA) however, several problems needed to be resolved. These include the degree of heterozygosity of certain G8 marker loci and the suitability of these markers for linkage analysis, the possibility of non-allelic mutations causing the HD phenotype and the moral, ethical and psychological problems that might arise from the practical application of such a preclinical test.

Ten SA HD families originating from different backgrounds have been studied for linkage between HD and the D4S10 locus (G8). Allele frequencies for markers at the D4S10 locus have been established in different non-Caucasoid population groups and linkage has been demonstrated in SA families using the HindIII, EcoRI and MspI polymorphisms, detected by G8. A maximum lod score of 8.14 at a recombination fraction (0) of 0.00 has been calculated using a combined haplotype of the HindIII and MspI polymorphism.

During the course of the investigation a hitherto unreported genetic DNA variant was detected, using a subclone of G8, in a SA kindred of mixed ancestry. In addition there was evidence of possible recombination within the D4S10 locus in this one family.

An intensive linkage study in a SA black HD family was undertaken using markers on chromosome 4 known to be linked to HD. A lod score of 1.16 at a θ of 0.00 was calculated using eight probes from different loci on the short arm of chromosome 4.

A questionnaire survey was conducted to assess the understanding and attitudes of at-risk individuals in Cape Town (CT) to predictive testing and antenatal diagnosis. The data obtained strongly indicated the need for a well-organised testing protocol with adequate pre-test counselling.

From the results obtained during this project, a protocol was designed for the implementation of a molecular genetic predictive service for HD in CT based on the specific needs of the persons concerned.

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To my colleagues in the laboratory Robea Ballo, Marlene Goodhart, Sue Schultz, Glynnis Schutte, and Dr Raj Ramesar without whose willing assistance, technical expertise, friendship and constant support this study would not have been possible. A special word of thanks go to my dear friends and colleagues Sue Ponzi and Laurienne Campbell whose technical guidance and advice, constant support and encouragement and faith in me enabled me to persevere even when everything went wrong!

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ABBREVIATIONS AND SYMBOLS

AID	artificial insemination donor
BSA	bovine serum albumin
°C	degree Celsius
CAT	computerized axial tomography
cM	centiMorgan
CT	Cape Town
CVS	chorionic villus sampling
DNA	deoxyribonucleic acid
GSH	Groote Schuur hospital
HC	Huntington's chorea
HCl	hydrochloric acid
HD	Huntington disease
hr	hour/s
HSSA	Huntington Society of South Africa
IHA	International Huntington Association
IVF	in vitro fertilisation
kb	kilobase/s
lod score	The $\log_{10}$ of the likelihood ratio
Medunsa	Medical University of Southern Africa
min	minute/s
ml	millilitre
µl	microlitre
µg	microgram
NaCl	sodium chloride
NaoH	sodium hydroxide
PIC	polymorphism information content
PET	positron emission tomography
RFLP	restriction fragment length polymorphism
RSA	Republic of South Africa

SA	Southern Africa/ Southern African
SASHG	Southern African society of Human Genetics
SDS	sodium dodecyl sulphate
SPECT	single photon emmission computed tomography
SSC	0.15M NaCl and 0.015M trisodium citrate
TOP	termination of pregnancy
Tvl	Transvaal
UCT	University of Cape Town
V	volts
WFN	World Federation of Neurology Research group on Huntington's Chorea.
w/v	weight/ volume
X^2	chi-square
θ	recombination fraction
□	unaffected male
○	unaffected female
■	affected male
●	affected female
◇	sex unspecified
⊠	male - deceased
⊘	female - deceased

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SECTION 1

INTRODUCTION

Chapter 1 AIMS, OBJECTIVES AND BACKGROUND REVIEW:

Chapter 2 HUNTINGTON DISEASE: HISTORY, CLINICAL ASPECTS AND EPIDEMIOLOGY:

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CHAPTER 1 AIMS, OBJECTIVES AND BACKGROUND REVIEW

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CHAPTER ONE

AIMS, OBJECTIVES AND BACKGROUND REVIEW

1.1 INTRODUCTION

Interest in Huntington disease (HD) in Southern Africa (SA) was generated in 1977 when M Hayden undertook a national survey of the disorder in South Africa. This condition is one of the best described genetic disorders and the HD locus was the first autosomal locus to be mapped to a specific chromosome using molecular markers. In recent years the question of presymptomatic prediction for the at-risk person has become a very controversial and emotionally charged topic. As a result, the literature on the disease is now voluminous.

The condition was previously known as Huntington's Chorea (HC). However the designation Huntington disease is now used to eliminate the confusion of the limiting term 'chorea '. In addition, according to accepted practice, the possessive form of the eponym has been discarded. Throughout this thesis, the abbreviation 'HD' will be used.

In order to give a perspective on the subject, a brief overview of the problems in establishing a genetic service for HD in SA is given in Chapter 1. The aspects mentioned in this chapter are discussed in greater detail in the thesis and are appropriately referenced therein.

1.2 AIMS AND OBJECTIVES OF THIS STUDY

The aims of this investigation are as follows;

1.2.1 The application of DNA markers for molecular linkage analysis and identification of the haplotypes segregating with HD in SA. Any recombinants that are found will be used in international collaborative studies for the localisation of the HD gene.

1.2.2 To investigate the occurrence of any non-random association between G8 marker alleles and HD.

1.2.3 To establish allele frequencies of some G8 markers in three different population groups of the RSA.

1.2.4 To determine if non-allelic heterogeneity exists in HD families with different genetic backgrounds.

1.2.5 To objectively assess the attitudes of at-risk persons in Cape Town towards predictive testing and antenatal diagnosis.

1.2.6 On the basis of the results obtained from 1.2.4 to 1.2.5 to establish a programme for predictive testing and antenatal diagnosis of HD in Cape Town.

1.3 DEMOGRAPHIC DATA

1.3.1 Area studied:

Collection of data for this study was made throughout the Republic of South Africa and Namibia. South Africa is divided into four major provinces; the Cape, Transvaal, Natal and Orange Free State; the Black 'Homelands' and independent Black States; Transkei, Ciskei, Venda and Bophuthatswana (Figure I.1).

1.3.2 Population:

One of the objectives of this study was to establish allele frequencies at the D4S10 locus in the black, white and mixed ancestry population groups in SA in order to design and implement a presymptomatic testing service for all SA HD families. To accomplish this, it was necessary to group or classify the people of SA according to their genetic origins. Such classification had an innocuous biological basis and was used only in a scientific and genetic context.

In this thesis, ten families with a history of HD were studied and belong to three ethnic groups; the indigenous black people of the Transvaal, the Cape population of mixed ancestry, and the SA whites of European origin.

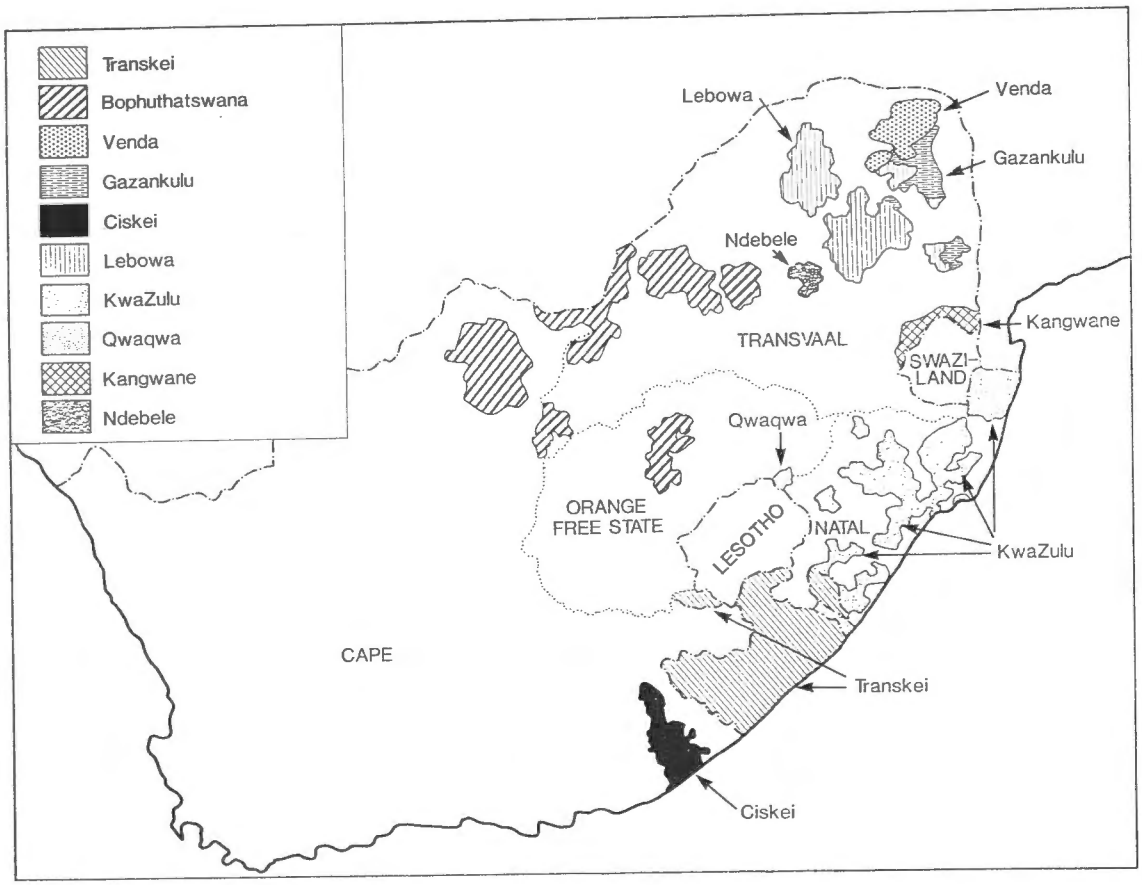

FIGURE I.1

A map of South Africa.

The three indigenous black peoples that were investigated live in the Northern Transvaal (Tvl) and are each a linguistic subgroup of the Bantu-speaking peoples of SA, to which all the SA Negro tribal groups or chiefdoms belong. In accordance with current convention, the term 'tribal group' is used throughout this thesis.

The Tswana are of Western Sotho origin, the Pedi of Eastern Sotho origin and the Venda who according to van Warmelo (1962)

> " this group is distinguished by a language peculiar to themselves, though reminiscent of both Sotho and Karanga, and by a culture sufficiently characteristic to separate them clearly from other Southern Bantu."

Since their languages have obvious similarities and two of the tribal groups have also been shown to cluster together in a genetic distance study based on the alleles at 14 loci (Jenkins, 1972), the results have been pooled as being representative of the SA black population of this region (Tvl).

The mixed ancestry population in the Cape Province of SA, where the study was undertaken, according to Botha and Pritchard (1972)

> " form an anthropologically distinct population group "

and

> " on historical grounds the origin of the Cape Coloured is found in three main ethnic groups: the Southern African races and immigrants from Asia and Western Europe."

The Southern African component of this mixed ancestry population includes the San (Bushman), Khoi-Khoi (Hottentot) and Bantu-speaking Negroes. Additional genetic endowment was provided by negro slaves imported from Madagascar and the West African coast, Asian immigrants from Ceylon, India, Java and Sumatra and Caucasians from Western Europe. On the basis of Botha and Pritchards' findings of blood group frequencies, the genetic makeup of the SA population of mixed ancestry is a compilation of approximately 34% Western European, 36% Southern African and 30% Asian genes.

A recent investigation of the polymorphism frequencies of HLA-D loci in South Africa confirmed that the SA of mixed ancestry population in the Cape are genetically intermediate between the SA black population in the Cape (the Xhosa tribal group) and the SA population of European origins (Oudshoorn, 1989).

The SA white population group is of Western European origin, mainly from Dutch, French and British stock.

In accordance with conventional usage the three ethnic groups in SA which were the subjects of this study are referred to as either SA of mixed ancestry or of black African or of European origins throughout this thesis.

The term SA Black in the frequency study is used to denote the broad group of Southern Africans of black origin from the Tvl region where the study of HD in black African families was undertaken.

1.4 MOLECULAR BACKGROUND

Huntington disease (HD) is a lethal neurodegenerative disorder characterized by progressive mental and physical disability. The condition is transmitted as an autosomal dominant trait and the symptoms, although devastating in severity, do not usually appear until the third to fifth decade of life. As a result of the late onset, most affected individuals have already procreated before they realise that they carry the gene for HD. Each child of an affected parent has a 50% chance of having inherited the gene and HD can thus be unwittingly transmitted from generation to generation in an affected family. Until recently there had been no reliable method of presymptomatic or prenatal diagnosis of HD and genetic counselling was based solely on family history and pedigree analysis.

In 1983 a DNA marker, G8, (D4S10 locus) was identified and found to be linked to HD in a number of families. By 1987, investigations worldwide using G8, had found linkage in 63 families from different genetic backgrounds.

The discovery of this molecular marker for HD was a significant breakthrough as it raised the possibility of developing a presymptomatic test for the disorder. This led to the establishment of pilot programmes aimed at preclinical detection and antenatal diagnosis of HD in Canada, the United States and other countries.

The molecular focus of the project which forms the subject of this thesis is the application of the currently available DNA markers for predictive testing in SA families with HD. The usefulness of any marker for linkage analysis is dependent on the frequency of the different alleles in the ethnic group in question, as allele frequencies may differ significantly in population groups of different origins.

It is therefore essential to establish the frequency of restriction fragment length polymorphisms (RFLPs) at the D4S10 locus in the different SA population groups in order to determine which markers will be informative. Furthermore, prior to implementing predictive and prenatal testing for at-risk individuals, it is necessary to estimate the recombination fraction between HD and the markers to be used in SA HD patients as the error rates in presymptomatic testing are critically dependent on the recombination fraction.

Southern Africa has the ideal situation for investigating the possibility of HD being a genetically heterogeneous disorder, by virtue of the diverse origins of its various ethnic groups. Affected black patients may have a unique form of HD as the gene for HD in Southern African whites is known to have originated in North-Western Europe; in general, few genes have reached the black African population from European sources. In this context, a comprehensive molecular investigation of a black African family with a history of HD was undertaken in this study.

1.5 SOUTHERN AFRICAN BACKGROUND TO HUNTINGTON DISEASE

In 1977, M.R.Hayden, who at the time was a research fellow in the Department of Human Genetics at the University of Cape Town (UCT), undertook a national survey of HD in the Republic of South Africa (RSA). His findings formed the subject of a Doctoral Thesis, a monograph and several publications. Extensive follow-up has been conducted subsequently, and by 1982 over 157 affected individuals in more than 50 supposedly unrelated families had been examined and diagnosed by this department.

Predictive testing for HD has to date been designed as a first world service. In SA the socio-economic situation is more complex and before offering this test to affected families, several difficulties will have to be overcome.

These include the ethical, psychological and sociological problems that might arise from the clinical application of such a preclinical test; taking into account the financial constraints upon medical services in Southern Africa.

By evaluating the results of research already being conducted worldwide and with careful consideration of the SA situation with special regard to the cultural differences and social attitudes of the different population groups, this study provides the foundation for the eventual establishment of a genetic predictive service for HD in SA. The successful formulation of a protocol for predictive testing for HD in SA could be used as a prototype for the establishment of similar services in developing countries and for numerous other late-onset genetic neurodegenerative disorders.

1.6 PLAN OF THE THESIS

The thesis is divided into four main sections: an introduction, the molecular study, the questionnaire survey and conclusions, practical applications arising from this project and recommendations.

Section One is an outline of the aims of the thesis and an historical background review, followed by a brief description of the SA situation with regard to HD.

Section Two deals with the molecular aspects of this study. There is a brief introduction to the molecular approach followed by the methods and materials used during the course of the project. There follows a detailed description of an intensive linkage study which was undertaken in SA black HD families. The results of the molecular study are followed by a discussion on each aspect of the study. A separate chapter is devoted to a new genetic variant which was detected during the course of the study and this is followed by a discussion on the complications arising from this finding.

Section Three deals with the questionnaire survey which was conducted in Cape Town; methodology and results are followed by a discussion on the results of the survey and the implications thereof. This is followed by the proposed programme for the implementation of predictive testing of HD in the Cape which was submitted to the Medical Superintendent of Groote Schuur Hospital as a result of this research project.

Section Four includes a concluding comment on each aspect of the study, as well as practical applications which have been implemented as a direct result of this project. Finally, recommendations are offered for the existing HD service and future research into HD in SA.

CHAPTER 2 HUNTINGTON DISEASE : HISTORY, CLINICAL ASPECTS AND EPIDEMIOLOGY

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CHAPTER TWO

HUNTINGTON DISEASE: HISTORY, CLINICAL ASPECTS AND EPIDEMIOLOGY

2.1 HISTORY OF HUNTINGTON DISEASE

Many references were made in early literature to a late onset hereditary disease characterized by involuntary movements and progressive mental deterioration. In 1872 George Sumner Huntington submitted his classical and only publication "ON CHOREA" to the Medical and Surgical Reporter, Philadelphia. Three generations of medical practitioners in George Huntington's family had treated the disease in families in East Hampton, Long Island, New York (Martin, 1984). George Huntington (Fig. II.1) based his article on his own observations and experiences as well as those of his father and grandfather (Critchley, 1984). In this publication Huntington described the clinical and genetic characteristics so accurately that his name has been associated with the disease ever since (Myers et al., 1985).

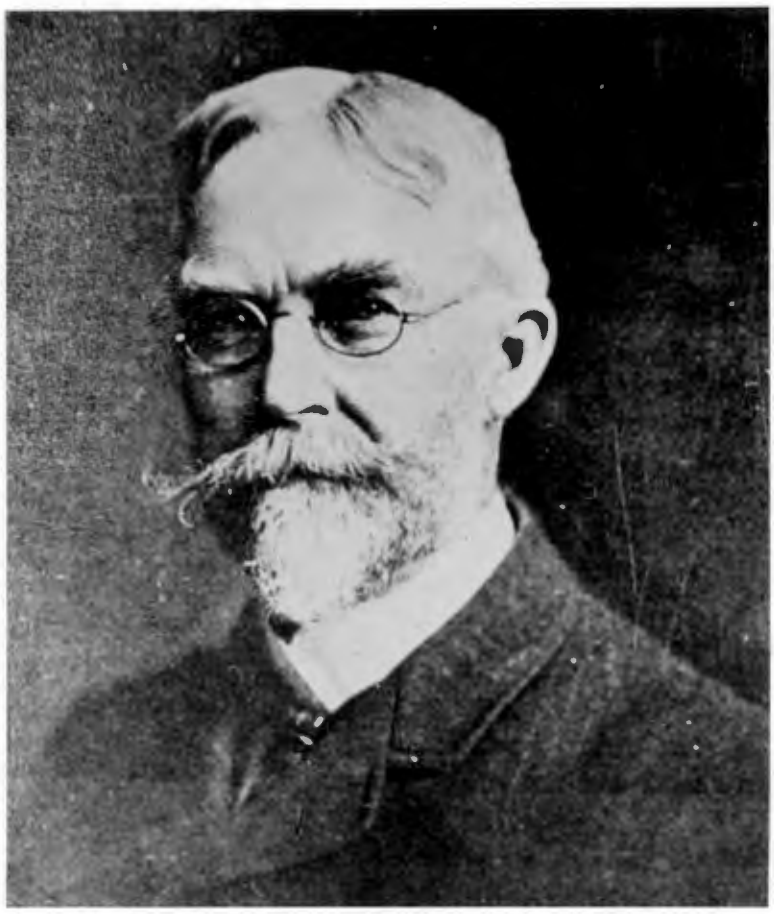

Figure II.1

Dr George Huntington after whom the disease is named.
From: Zabriskie E (1953) In: Webb Haymaker (ed) Founders of Neurology, 1st edn.
Courtesy: Charles C Thomas, Publisher, Springfield, Ill.
Russell N De Jong, Ann Arbor
Professor P Beighton: THE MAN BEHIND THE SYNDROME.

He wrote of the disease

> "and now I wish to draw your attention more particularly to a form of the disease which exists, so far as I know, almost exclusively on the east end of Long Island. It is peculiar in itself and seems to obey certain fixed laws."

Furthermore:

> " The hereditary chorea, as I shall call it, is confined to certain and fortunately a few families, and has been transmitted to them, an heirloom from generations way back in the dim past. It is spoken of by those in whose veins the seeds of the disease are known to exist, with a kind of horror, and not at all alluded to except through dire necessity, when it is mentioned of as that disorder."

(Huntington, 1872)

Regarding the clinical features he wrote:

> "There are three marked peculiarities in this disease:
>
> 1. Its hereditary nature.
> 2. A tendency to insanity and suicide.
> 3. Its manifesting itself as a grave disease only in adult life"

(Critchley, 1964).

2.2 CLINICAL FEATURES

The clinical features of HD have been well documented in early literature and recently, most extensively by M.R Hayden in his monograph HUNTINGTON'S CHOREA (Hayden, 1981). The main characteristics are outlined below for the sake of perspective.

The cardinal features of HD are chorea and dementia. The first symptoms usually occur in the third to fifth decade of life and both the motor abnormality and intellectual deterioration progress inexorably for 15 to 20 years. These manifestations are commonly accompanied by prominent psychiatric symptoms including severe depression. The physical symptoms include uncontrollable jerking and twitching movements of the head, neck, legs and arms, as well as abnormalities of gait. The chorea (dance-like movements), when present, is accompanied by constant flexing and extension of the fingers and tic-like grimaces of the face. As the disease progresses the abnormal movements become more pronounced. Eventually, sufferers are unable to walk or stand alone. Speech becomes slurred and this affects the patients' ability to communicate coherently. There is increasing difficulty with swallowing which results in feeding problems and eventual emaciation. This inability to swallow often leads to aspiration pneumonia or fatal choking on food in the terminal stages of the disease. Death usually occurs 15 to 20 years after the first clinical symptoms appear.

Mental changes are an integral feature of HD and lead to profound dementia as the disease progresses. Dementia was defined by Pearce and Miller (1973) as

> **"a symptom characterized by a decline of intellect and personality which reflect a disturbance of memory, orientation, the capacity for conceptual thought and often of affect."**

Furthermore they stated that

> **" Huntington's chorea is one of the best examples of a hereditary illness characterized by dementia."**

(Pearce, 1973).

Emotional disturbances can occur as early as ten years before the onset of physical manifestations. Following the availability of a presymptomatic test for HD it has been demonstrated that neuropsychological impairment may be present earlier than was previously recognised in individuals at risk for HD (Jason et al., 1988). The most common psychological symptom is depression, accompanied by sudden outbursts of anger, occasional uncontrollable violence, irritability, decreased attentiveness, change in work performance and general inadequacy in coping with day-to-day living (Koehler and Sass, 1984). The incidence of suicide and attempted suicide among at-risk individuals and patients in the early stages of the disease is higher than that of the general population (Schoenfeld et al., 1984a; Farrer, 1986).

As previously mentioned, HD is a late-onset genetic disease with the first clinical symptoms usually appearing in mid-life but there is also a juvenile form of HD with earlier onset and more rapid deterioration. This condition is not a separate clinical entity but rather a reflection of variable phenotypic expression (Farrer and Conneally, 1987).

There is no cure for HD and although no specific treatment will halt the progressive neurodegeneration, neuropharmacological medicinal agents may alleviate symptoms to some extent.

2.3 NEUROPATHOLOGY OF HD

The caudate nucleus of the brain is the region that appears to be primarily affected in HD. The neuropathological changes are widespread cell atrophy, particularly in the frontal cortex and in the small striatal cells of the caudate and putamen (Martin and Gusella, 1986). This selective premature cell death does not seem to be associated with any specific detectable biochemical defect, although experimental tissue culture studies have indicated a possible circulating neurotoxin which could be involved in the pathogenesis of HD (Perry et al., 1985 and 1987).

Ventricular dilation caused by the striatal atrophy may be demonstrated by Computerized Axial Tomography (CAT) in the advanced stages of the disease. Recent work with Positron Emission Tomography (PET) has thrown further light on the fundamental neuropathological process of HD (Mazziotta et al., 1987; Berent et al., 1988). It is now possible to accurately diagnose patients with minimal neurological or psychiatric symptoms and no obvious CAT changes using PET scan (Hayden et al.,1986). This technique may also be used to support information from DNA linkage studies for preclinical detection of HD, two to three years prior to the clinical onset of the disorder (Hayden et al.,1987a; Hayden et al., 1987c).

More recently, the use of Single Photon Emission Computed Tomography (SPECT) imaging of HD patients has revealed a remarkable reduction in regional uptake of cerebral blood flow markers in vivo (Reid et al., 1988). The authors claim that this method has advantages over PET scan and that the equipment is more accessible.

2.4 GENETICS OF HD

HD is inherited as a classical autosomal dominant trait. The gene for HD can be transmitted by either parent to the children, of either sex; each child of an affected parent has a 50% chance of inheriting the HD gene. The mutation is said to be completely penetrant and all HD gene carriers will ultimately manifest the disorder unless they die from an unrelated cause before the expected age of onset. The disease is reported to have amongst the lowest known mutation rates recorded for any inherited disorder (Stevens and Parsonage, 1969). A recent publication suggests that a new mutation has occurred at the HD locus in a large German family; if this is indeed the case, there is then a possibility of several distinct mutations at the HD locus (Wolff et al., 1989).

There is no evidence as yet for non-allelic genetic heterogeneity. All families studied worldwide who express the disease apparently carry mutations at the same locus on chromosome 4 (Conneally et al., 1989). However, there is considerable variation in the age of onset (Conneally, 1984a) as well as in the initial clinical presentation of the disorder. This variation in clinical expression may well be a consequence of differences in individual genetic background (epistatic phenomenae) (Folstein et al., 1983).

Nevertheless, in view of the fact that HD has been reported in most countries throughout the world, the possibility of allelic heterogeneity cannot be ruled out (Jenkins and Conneally, 1989; Sax et al., 1989).

The unexpected finding that individuals who are homozygous for the defective HD gene are not only viable but are clinically indistinguishable from sibs who are heterozygous adds further intrigue to the genetic makeup of HD (Wexler et al., 1987; Myers et al., 1989).

2.5 PREVALENCE OF HD

HD has been diagnosed throughout the world, but by far the majority of affected persons have been of Western European origin (Critchley, 1964). A classic example is that of the Spanish sailor in the mid-seventeenth century who is reputed to have brought the HD gene to Lake Maracaibo in Venezuela, which currently has the largest known HD family in the world (Wexler et al., 1987).
According to Hayden (1981), the first published results of epidemiological surveys were from England and Sweden. In his book, HUNTINGTON'S CHOREA, he provides a comprehensive summary of reported prevalence rates of HD in different countries. Table II-1 is a compilation of data from recent publications and gives prevalence figures from 1981-1990 which can be added to Table 3.1 on pages 34 and 35 of Hayden's monograph.

At a workshop held in Vancouver, Canada, in 1989, the World Federation of Neurology Research group on Huntington's Chorea estimated an overall prevalence of HD to be 4-6 affected individuals per 100,000 of the world population. Finland, SA (Blacks) and Japan were notable exceptions (see Table II-2), in that the incidence in these countries appeared to be much lower.

TABLE II-1

Geographic distribution and reported prevalences of Huntington disease. Compiled from publications dating from 1981-1990.

Area	Persons with HD	Population $X10^6$	Prevalence $X10^{-5}$	Reference
UNITED STATES				
Maryland	212	4.217	5.5	Folstein et al., 1987
South Carolina				
Whites			4.8	Wright et al., 1981
Blacks	9		0.97	
North American Indian	1 family			Morris et al., 1990
PERU	1 family			Morris et al., 1990
CANADA	>300			Fox et al., 1989
VENEZUELA	65	+-0.04	-+16000	Young et al., 1986
UNITED KINGDOM				
SWales	153	1.728	8.5	Quarrell et al., 1988
NWales	34	0.621	5.5	
Grampian region	198	0.44	9.94	Simpson and Johnston 1989
SOUTH AFRICA				
Blacks	8			Joubert and Botha 1988
FINLAND	26		0.5	Palo et al., 1987
SWEDEN	450	8	5.6	Palo et al., 1987
AALAND	3	0.02	1500	Palo et al., 1987
FRANCE (Pas-de-Calais)	158	3.9	4.04	Petit and Solomez 1985

TABLE II-2

Congress reports of the World Federation of Neurology Research Group on Huntington's Chorea. Vancouver, 1989

Area	Persons with HD	Population $X10^6$	Prevalence $X10^{-5}$	Reference
NORWAY	161	4.2	3.8	Heiberg 1989
ITALY	491		3.0	Frontali 1989
JAPAN			1.1	Kanazawa
NEW ZEALAND	180	3.33	5.4	Lintott 1989
SOUTH AUSTRALIA			7.0	Turner 1989

With an autosomal dominant inherited disease, it is known that each child of an affected parent is at 50% risk of having inherited the gene for the disorder. As HD is a late onset disease, at any given time, the number of individuals who are at high risk of having inherited the HD gene and therefore also at risk of possibly transmitting the disorder is considerably greater than the number who will eventually present with HD (Harper et al., 1979).

As it is now possible to identify the presymptomatic gene carrier for HD, an acceptable protocol for predictive testing for this disease must be drawn up. Counselling of HD families with regard to procreation should be non-directive and should be focused on helping individuals with their decisions. The possibility of achieving a general reduction in the future incidence of the disorder is of prime concern. It can now be suggested to younger individuals or couples that they delay their plans for childbearing in view of the possibility of a predictive test for HD becoming available. This would help them in making decisions of this nature (Harper, 1985b).

CHAPTER 3 SITUATION IN SOUTHERN AFRICA

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CHAPTER THREE

SITUATION IN SOUTHERN AFRICA

3.1 HUNTINGTON DISEASE IN SOUTHERN AFRICA

HD was initially considered to be relatively rare in SA and until 1977 there were very few cases of the disease reported in this country. Moreover, there were few publications emanating from the continent of Africa concerning HD. In a nation-wide project in 1977, numerous SA families with HD were investigated and documented by Hayden and his colleagues from the Department of Human Genetics, UCT (Hayden et al., 1980a). The prevalence of HD in SA at that time is shown in Table III-1. It was stated that these figures were probably underestimates of the true frequency of HD in SA. In the light of information accumulated in the past decade it has become apparent that the figures were relatively accurate and therefore represent a fair estimate of the current prevalence of HD in SA.

The prevalence of HD in the Cape Province in 1977 is shown in Table III-2 and can be compared with the number of living persons with HD at present within a 100km radius of Cape Town (Fig. V.I) based upon data obtained from our records in June 1990. These 1990 figures were derived from pedigree data which have been obtained from family members and have not been confirmed or medically verified.

For this reason the figures should be regarded with reserve but it is likely that they are reasonably accurate and therefore provide a perspective of the current situation.

It is generally accepted that for every affected person there are an estimated ten at-risk individuals in the immediate family (Hayden et al., 1980b). From the 38 kindreds in the Cape Town area it can therefore be assumed that there are approximately 800 individuals who are at risk for inheriting HD (Table V-2).

TABLE III-1

Prevalence of Huntington Disease in South Africa (1977) (after Hayden et al., 1980a).

	Number	Population x 10^3	Prevalence x 10^{-6}
Whites	97	4.367	22.2
Mixed ancestry	53	2.432	21.7
Black	3	16.647	0.1

TABLE III-2

Prevalence of Huntington disease in the Cape Province (1977) (after Hayden et al., 1980a) and living persons with Huntington disease in the Cape Province (1990).

	Number 1977	Population X 10^3 *	Prevalence X 10^{-6}	Number 1990	Population X 10^3**
Mixed ancestry	48	2.094	22.9	77	2.397
Whites	32	1.207	26.5	30	1.209
Blacks	2	1.674	0.5	0	1.402

* Based on the 1970 population census taking into account the average growth rate.

** Based on the 1985 population census, according to the Bureau for Information Statistical Services.

3.2 GENEALOGY OF HUNTINGTON DISEASE IN SOUTHERN AFRICA

In his thesis **"Huntington's Chorea in South Africa"** submitted in 1979, Hayden emphasised that there were several origins of the gene for HD in SA, the predominant sources being UK, Holland, France and other countries in Western Europe.

He wrote: **"The story of the origins of HD in SA is essentially a story of the multiple waves of immigration to this country, primarily from Western Europe, which were the results of different accidents of history such as the establishment of the seafaring companies, wars and the discovery of gold on the Witwatersrand"** (Hayden, 1979).

The prevalence figures for HD in the white Southern Africans of European origin and those of mixed ancestry were similar (Table III-1). This was not an unexpected finding in view of the Western European contribution to the genetic constitution of people from a mixed ancestry background. The HD gene in these two groups can therefore be presumed to be of the same origin. In contrast, the gene for HD in four Black familes reported in 1988 from the Northern Transvaal, possibly represents a new mutation as there is very little, if any, Caucasian admixture in Southern African blacks. The linkage analysis on a black African family with HD that was undertaken by the Department of Human Genetics, UCT, as part of a collaborative study is detailed in Chapter 6.

SECTION II

MOLECULAR STUDY

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Chapter 5 MATERIALS AND METHODS:

Chapter 6 LINKAGE STUDY OF HUNTINGTON DISEASE IN SA BLACK FAMILIES:

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CHAPTER FOUR

INTRODUCTION TO THE MOLECULAR APPROACH

4.1 HISTORY OF THE MOLECULAR BACKGROUND TO HUNTINGTON DISEASE:

In 1976 D C Wallace wrote

> **"Huntington's Chorea must be one of the most widely studied examples of Mendelian inheritance in Man and yet it remains one of the least well understood."**

Ten years later the following statement appeared in the Chicago Tribune, March 1986, in an introduction to gene therapy using recombinant DNA technology :

> **"The goal is to alleviate human disease - and to do it with a power that has heretofore been reserved for the Gods."** (Merz, 1986)

The past decade has seen great advances in the field of molecular genetics. The challenge now is use these advances to progress from the DNA level to the analysis of the corresponding gene product which, in the case of HD, has not yet been elucidated.

The initial step was taken in 1983 when James Gusella and his colleagues in Boston, Massachusetts discovered a molecular marker (G8) that cosegregated with HD. This marker could be used to follow the inheritance of the disorder in informative families and permitted early detection of the HD gene before the first clinical signs appeared (Gusella et al., 1983).

4.1.1 Localisation of the HD gene:

The original G8 marker was regionally localised to the terminal region of chromosome 4 by examining DNA from patients with Wolf-Hirschhorn syndrome, a birth defect involving heterozygous deletion of part of the short arm of chromosome 4 (Gusella et al., 1985). Subsequent fine mapping of the HD linked D4S10 locus, defined by the polymorphic DNA marker G8, using in-situ hybridisation (Landegent et al., 1986) with somatic cell hybrids, localised the HD gene to a terminal band of the short arm of chromosome 4 (4p16.1) (Magenis et al., 1986).

Since the initial breakthrough, many laboratories world-wide have been applying new molecular technology in an attempt to get closer to the HD gene locus. The primary aim is to find a marker distal to G8 that flanks the mutant gene. By identifying flanking markers that are closer to the HD gene the region of interest is more narrowly defined and there is less DNA to be sequenced in order to identify the gene itself.

Chromosome jumping (Richards et al., 1988), field-inversion gel electrophoresis (Pritchard et al.,1989a), and the construction of a NotI linkage library (Pohl et al., 1988) have all contributed to the isolation of new markers closer to the HD gene locus.

The most distal probe to G8, D4S90 (Youngman et al., 1988) is now being used as a starting point for identifying DNA fragments closer to the HD gene. Doggett et al (1989) have used D4S90, pulsed - field gel electrophoresis and a yeast artificial chromosome vector to construct a physical map that extends from the D4S90 marker to the telomere of chromosome 4. This map places the likelihood of the HD gene to within 325 kilobases of the chromosome 4p telomere.

Initially, no instances of genetic recombination were reported between the G8 marker and HD. However several have now been detected, placing G8 approximately four centimorgans (cM) from the HD gene locus with 95% confidence intervals of 2-4cM (Haines et al., 1986). The accuracy of using G8 in predictive testing is thus compromised by a 4% recombination frequency between G8 and the HD gene.

Until the gene is located, any recombination event identified between the marker used and the gene locus will provide an extra landmark for the mapping of new probes and the localisation of the HD gene (MacDonald et al., 1989b). In this context, international collaboration between researchers is essential, as each recombination event is unique and invaluable in this work.

A physical map of the chromosomal region 4p16.3, the area expected to contain the HD mutation, has recently been constructed using more than 20 probes (Bucan et al., 1990).

The ultimate goal, once the gene has been isolated and sequenced, is to use this genetic code as a template to convert the nucleotide sequence back to a protein sequence. This would be a major step towards unravelling the basic pathogenesis of HD. Knowledge of the HD gene sequence would enable the use of direct gene probing for prenatal and presymptomatic diagnosis and reduce the necessity for involved family studies.

4.1.2 Probes for linkage analysis:

Several new RFLPs have been identified for linkage analysis for predictive testing, using single copy probes from the expanded G8 locus. The polymorphism information content (PIC) of the expanded locus in 1987 was about 90% and 64 different haplotypes had been identified in families from diverse genetic backgrounds (Wasmuth et al., 1987).

By 1989, many more linked loci and as well as markers with the characteristics of VNTR probes (variable number of tandem repeats) had been identified near the HD gene. Robbins et al. (1989) reported that D4S95 was the most useful DNA marker for presymptomatic testing.

These advances increased the accuracy and applicability of linkage analysis and reduced the time and cost of presymptomatic and prenatal diagnosis for HD (MacDonald et al., 1989).

4.1.3 Immediate applications; prenatal diagnosis:

An extremely important application of the use of genetic markers is that prenatal diagnosis is now possible for informative individuals. An antenatal diagnostic service could be offered as soon as a predictive testing protocol is established (Hayden et al., 1987b).

A further practical application of the use of genetic markers is as follows: prenatal diagnosis could be offered where the result would determine either that the fetus has a low likelihood of having inherited the gene for HD or has the same risk as for the at-risk parent (Fahy et al., 1989). In other words, any test result obtained for the fetus would not alter the estimated likelihood of HD in the parent at risk (Fig.IV.I).

This approach is known as 'no news, good news testing' or exclusion testing. Prenatal exclusion testing can therefore be offered to a pregnant woman if either she or her partner has an affected parent (Quarrell et al., 1987; McIntosh et al., 1989). In general, this test is used when DNA is unavailable from crucial family members. The test is also an option for at-risk parents who do not wish to learn of their own HD status.

Some centres in fact have already embarked on pregnancy exclusion testing before implementing full predictive testing for HD. This approach allowed time for development and evaluation of a suitable protocol (Harper et al., 1988).

Bloch and Hayden (1987) pointed out two important consequences of this test that were not originally considered. The fact that the so called 'no news' outcome increases the risk of the fetus having inheriting the HD gene from 25% to the same risk as for the at-risk parent (around 50%) needs to be carefully explained to the persons choosing this option for prenatal diagnosis. In addition, a 'no news' test result 'links' the risk status of the fetus to that of the at-risk parent. For example, if the parent does not develop HD, then neither will the fetus. More recently Millan et al. (1989) reported on the additional problem of the laboratory performing the actual test having had access to too much information and so actually performing a predictive test on an individual who did not wish to be tested. The burden of knowing that someone has the gene for HD and not allowing this knowledge to influence decision making in any way, needs to be considered by laboratories offering prenatal diagnosis for HD. Furthermore, the possibility of presymptomatic testing being done on minors without their consent has also been identified as being an unexpected complication of exclusion testing (Quinn, 1989).

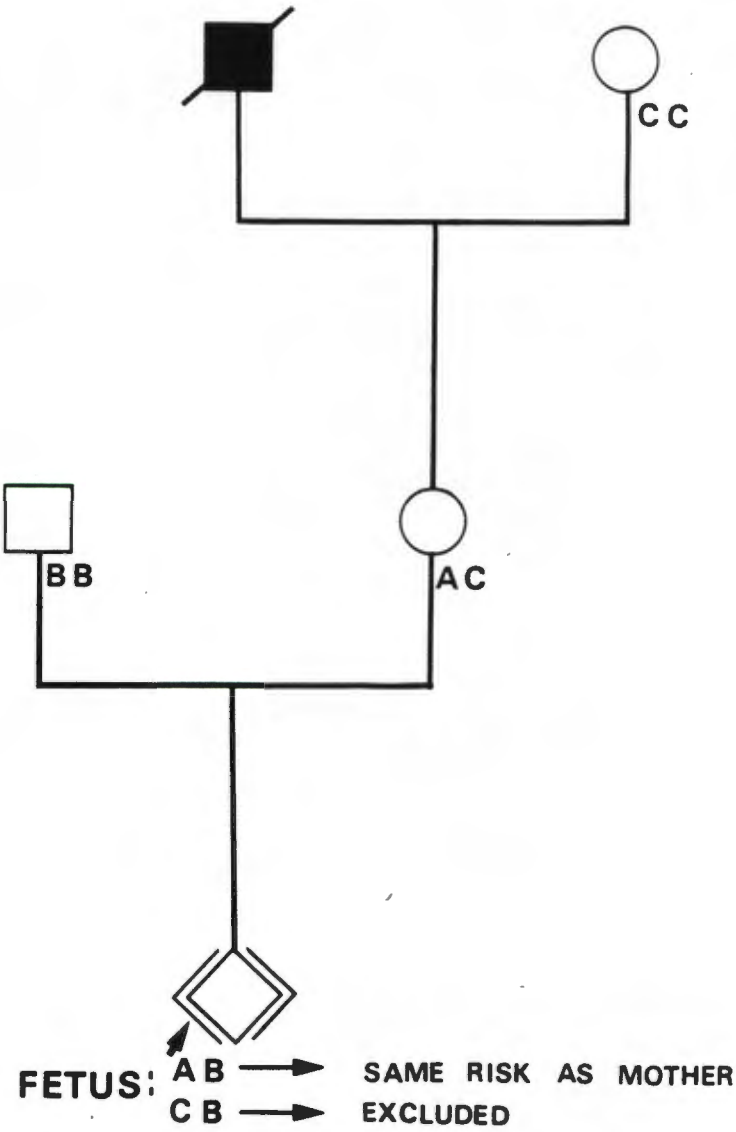

Figure IV.1

Principle of the prenatal exclusion test.

Information may be given concerning the fetus without altering the estimated likelihood of HD in the at-risk parent.

4.1.4 PET scans and predictive testing:

Finally, it has been suggested that at centres where the equipment is available, a PET scan of the caudate glucose utilization may help to confirm DNA studies in at-risk individuals or could be used for those persons in whom DNA studies are not helpful (Hayden et al., 1987a).

On the basis of their findings, Hayden's group suggested that in addition, PET may also provide an opportunity of detecting the condition when DNA results may be incorrect due to recombination. Abnormal striatal glucose utilization has been found to precede the onset of HD symptoms and it is thought that the first detectable signs of change occur only in the three years prior to the onset of the disease. Careful use of PET scans combined with DNA linkage analysis could therefore provide optimum results for presymptomatic testing of HD (Hayden et al., 1987c).
However, others have not found this method to be as successful (Young et al., 1987; Perlmutter and Raichle, 1987). Caution must therefore be exercised in advocating this technique which should only be utilised for predictive testing by those centres which have full confidence in their results.

4.1.5 Future applications:

Ultimately, provision could be made for some kind of gene therapy to alleviate the symptoms of the disease or prevent the onset thereof. It may also be possible to rectify the defect by means of genetic engineering. In future, therapeutic intervention following the early diagnosis of the presymptomatic gene carrier could possibly retard the inexorable neurodegenerative process (Shoulson et al., 1989).

4.2 HETEROZYGOSITY AT THE MARKER LOCUS

In order for a marker to be informative, the different genotypes must be easily discernable and it must be possible to distinguish the allele that segregates with the disease in a family from the one associated with the normal version of the gene (Maestri et al., 1987). The usefulness of any marker for linkage analysis is therefore dependent on the frequency of heterozygosity in the population group in question (Ikonen et el., 1990).

It was therefore essential to establish allele frequencies for RFLPs at the D4S10 locus in different population groups in SA in order to determine which markers would be informative.

4.3 QUESTION OF HETEROGENEITY AT THE GENE LOCUS

For the marker to be 100% reliable it is essential that the disease should not be genetically heterogeneous (i.e. alternative loci involved in the expression of the trait). To date, a total of 63 HD families worldwide have been examined for linkage with G8; a combined lod score of 87.69 at $\theta = 0.04$ (95% confidence interval 0.018-0.071) has been calculated, indicating that there is no evidence for non-allelic heterogeneity at the HD locus (Conneally et al., 1989). SA has the ideal situation for the examination of HD families of diverse genetic backgrounds, some from genetically isolated populations, in order to address this issue.

Genealogical investigations of the mixed ancestry group in SA are difficult to carry out. This is due to the lack of accurate documentation of the registration of births prior to 1895 and also to the lack of knowledge in this group of their antecedents' origins. However, due to the genetic constitution of this group it may be presumed that the gene for HD in the mixed ancestry population group had its origin in Western Europe. This assumption is based on the fact that it has been established that the gene for HD in the SA population of European origins almost certainly originated in Western Europe (Hayden et al., 1980c).

The black African peoples, in particular the Pedi, Tswana and Venda from the Northern Transvaal, where the black HD families live, are indigenous to SA. In general, few genes have reached these populations from European sources and it is very likely that this is a new origin of the HD gene and not the original North Western European gene. It is therefore possible that these individuals may have a unique form of the HD gene. The results of the linkage analysis of two black African HD families completed as part of a collaborative study are discussed in detail in Chapter 6.

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CHAPTER FIVE

MATERIALS AND METHODS

5.1 COLLECTION OF DATA

On the basis of a nation-wide project carried out by the Department of Human Genetics, UCT, in 1977, a South African Huntington disease Registry was established (Hayden and Beighton, 1982a). This data base contained details of affected persons and essential information pertaining to their families. The extensive data and documentation that had been accumulated and since maintained, was used in the current project as a starting-point for the initial contact and the tracing of families with a history of HD (Hayden et al., 1987d). Patients were appraised at a specialised HD clinic at Groote Schuur Hospital (GSH) where they and their families were seen on a regular basis by a Neurologist and a Medical Geneticist.

South Africa is a large, predominantly rural country and major population centres are separated by sparsely inhabited regions. The logistics of travelling to visit patients all over the country, the counselling of at- risk individuals and the collection of blood specimens from family members as well as language and cultural barriers posed enormous problems.

Although HD patients were seen and appraised in other major centres throughout the country, for the current project it was decided to concentrate on HD families living within a radius of 100km of Cape Town (Fig. V.1). Genetic nursing sisters from our Department undertook home visits in the Cape Town area to collect blood and counsel HD family members. However, families were often widely dispersed and vital key members were reported to be living in relatively inaccessible areas of the country; in these circumstances National Department of Health Genetic Sisters traced and visited the family members.

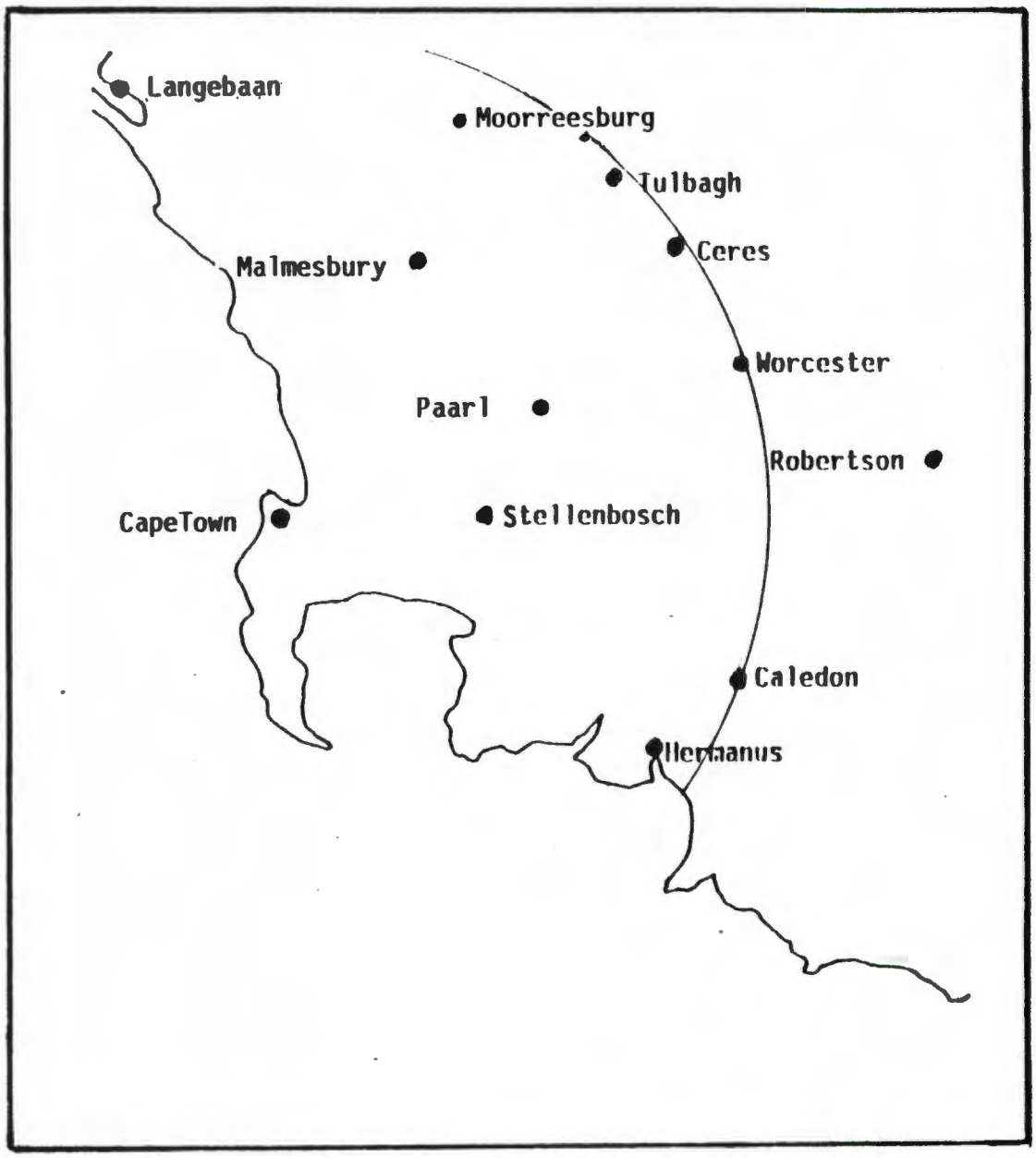

Figure V.1

Map of the Cape Province to indicate the area covered in the study.

5.2 DNA BANKING CENTRE

5.2.1 Evolution of the HD DNA banking centre:

In 1986 a DNA banking centre for HD was established at the Department of Human Genetics, UCT, similar in design and concept to the Canadian HD DNA banking centre (Anderson et al., 1986). A full time genetic co-ordinater was appointed for a six month period to establish contact with HD families in SA. Letters were sent to all the known HD families informing them about recent developments, with special reference to the new molecular technology and requesting their participation in establishing the DNA bank.

Affected patients who are crucial for family linkage studies may die prior to the establishment of a predictive service. To avoid family members subsequently being denied a presymptomatic test by virtue of the unavailability of DNA from deceased relatives, special appeals were made to affected individuals in HD families to donate blood for DNA banking, while the accuracy and reliability of the test was being assessed.

5.2.2 Approach to HD families:

In the information sheet that was distributed (Appendix I) it was explained that the 'bank' was a storage facility where blood specimens would be kept until such time as the test became available. The necessity for participation of the whole family, affected and unaffected members, was explained. It was emphasized at all times that no results would be given following the drawing of the blood specimen and that some of the DNA would be subjected to preliminary analysis while the balance would be stored until such time as linkage studies could be performed.

Illiteracy was a problem for some persons in understanding the information sheets and oral explanations using cultural and societal precepts and simple diagrams were used to help clarify difficult concepts.

In HD, confidentiality is of utmost importance and affected families were assured that personalised information of this nature would be maintained in the strictest confidence. Subjects were requested to sign a consent form indicating that they understood these procedures (Appendix II).

As far as possible, patients and their families were examined by a clinician who was experienced in the diagnosis of HD, before blood was drawn for the DNA bank. Comprehensive family histories and clinical details were obtained for the purpose of compiling accurate pedigrees.

5.2.3 Professional contact and general education to increase awareness of HD:

DNA Banking Centre Information Sheets (Appendix III) were sent to general practitioners known to have been involved with the management of HD families, requesting them to obtain blood specimens for the banking centre. Mental Health hospitals throughout the country were approached and sent the relevant information in an attempt to trace new patients. The author also lectured to nursing sisters, social workers, lay organisations and Psychiatrists about the concept of predictive testing and its implications in order to stimulate interest and raise the awareness of HD.

In June 1987, the first meeting of the Southern African Society of Human Genetics was held at Rustenburg, Transvaal. The author read a paper detailing the progress in the establishment of the DNA banking centre, as well as the current work being undertaken with newly acquired molecular probes (Appendix IV). It was proposed at this meeting that the Department of Human Genetics at UCT should be responsible for preparing for linkage studies of HD in SA.

It was agreed that all centres would send DNA specimens to our laboratory for banking and molecular workup (Fig. V.2). In turn, each centre would be responsible for the counselling and management of patients in their own region.

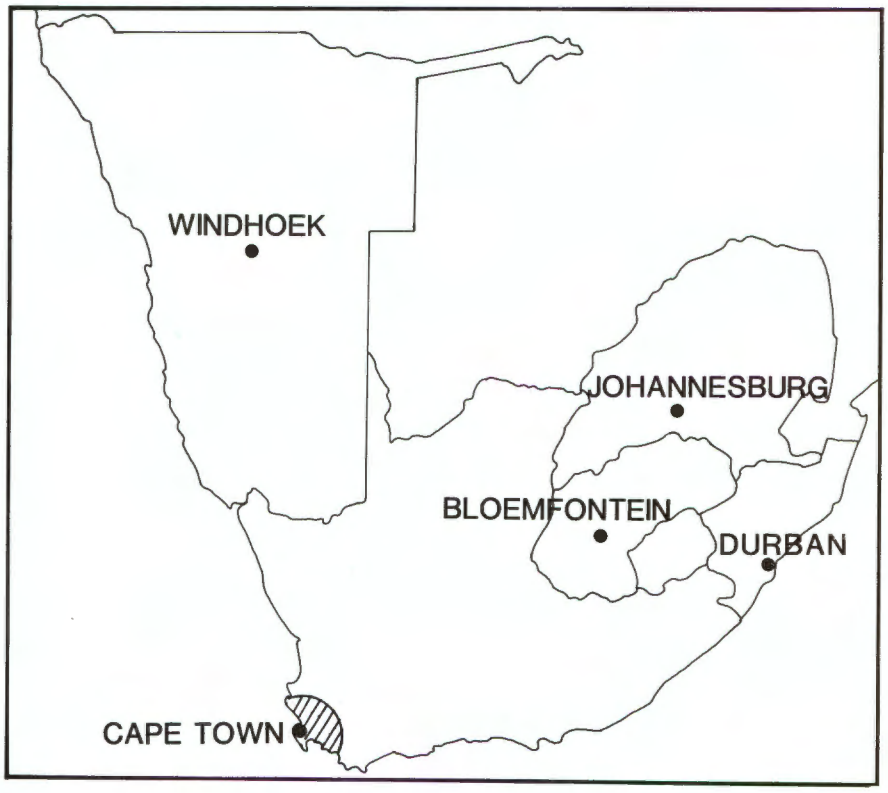

Figure V.2

Map of South Africa and Namibia with the major centres indicating the extent of the DNA banking service area.

Due to the logistic problems of servicing the whole country from one centre, it was subsequently agreed that a similar centre be established in the Transvaal. DNA stored at either centre, would be released reciprocally upon receipt of written consent from the person concerned.

Joubert and Botha (1988) reported on four black families with HD in the Northern Transvaal and our Department was approached to undertake a collaborative study. Due to the small number black persons known to have HD, this large series of black African HD patients offered a unique opportunity for linkage analysis. A field trip was undertaken into the Lebowa area by the author and a Medical Geneticist from the Department of Human Genetics, UCT, to visit these families and collect blood for DNA isolation and storage (Fig V.3). An assessment was made of these affected individuals' needs and also their attitudes to HD. Liaison was established with local social workers for the purpose of referral should any questions or problems arise at a later stage.

To date, at the UCT DNA Banking Centre, 45 families from different parts of SA from whom DNA has been collected, isolated and stored have been documented (Table V-1 and Table V-2). During the establishment of this service close liaison was maintained between the laboratory and medical staff, departmental nursing sisters and State Health Genetic services. The co-operation of all concerned at both local and national levels ensures that the service runs efficiently.

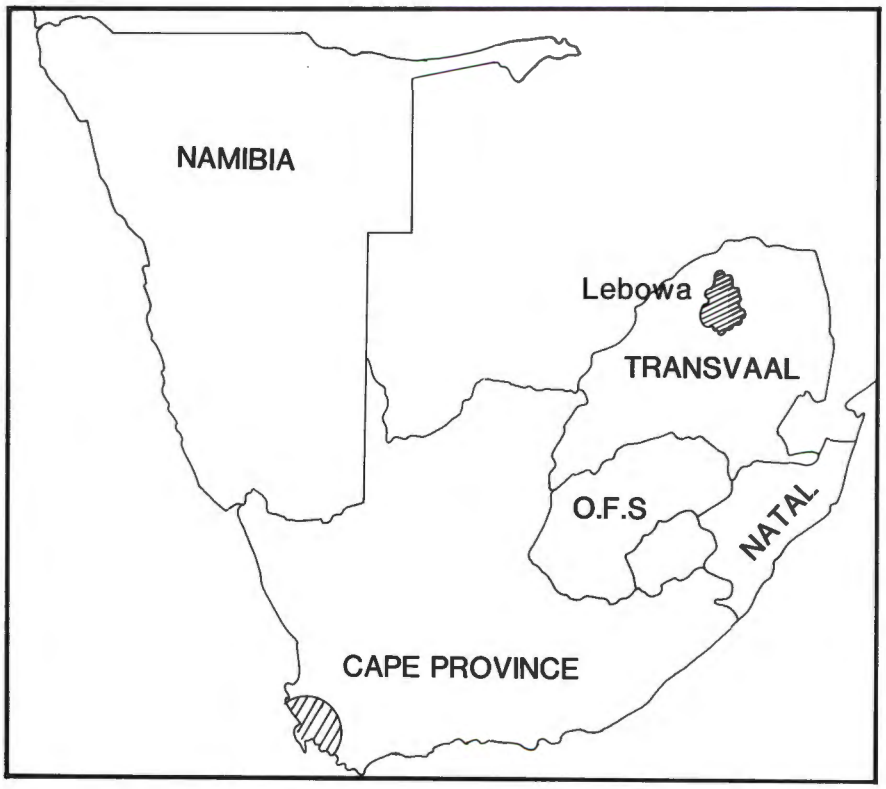

Figure V.3

Map indicating the Lebowa region where the study of SA black HD families was undertaken.

TABLE V-1.

DNA Banking Centre: National figures for 1990.

Ethnic group	Total	HD patients	Unaffected family members	Spouses
Mixed ancestry	223	60	132	31
Whites	110	30	61	19
Tswana	22	2	17	3
Pedi	21	5	12	4
Venda	17	1	14	2
TOTAL	393	98	236	59

30 June 1990.

TABLE V-2.

DNA Banking Centre: Figures for the Cape Province for 1990.

Ethnic group	Total	HD patients	Unaffected family members	Spouses
Mixed ancestry	212	57	125	30
White	87	23	49	15
TOTAL	299	80	174	45

30 June 1990.

5.3 COLLECTION OF BIOLOGICAL MATERIAL

Blood samples (30ml) for DNA isolation were collected from consenting individuals by venepuncture into vacutainer tubes containing EDTA. When possible, 10ml was processed within four days after being stored at 4° C and DNA was purified using standard laboratory techniques (Vandenplas et al.,1984). The balance of the frozen blood was stored at -20° C to be isolated separately as a precaution against laboratory error. DNA was stored at -70° C in two aliquots from the different isolations. DNA samples that were currently being used for restriction were stored at -20° C. Blood which was unable to be processed immediately was stored in 10ml aliquots at -70° C and isolated at a later date. Samples collected from other centres were transported without delay by air and stored at 4° C until they reached the laboratory.

In 1987 it was decided to increase the amount of blood taken at the time of collection by an amount of 10ml for the purpose of paternity testing, as some instances of questionable paternity had arisen from the linkage data. These samples were sent to tissue typing laboratories for testing, using different blood group systems, including HLA tissue typing (Du Toit et al., 1989) and the results were stored in their records. Information obtained from such testing was never divulged and was used strictly to elucidate questionable linkage results.

The necessity for extra blood specimens can now be overcome with the use of the new VNTR probes using the same principle as or in conjunction with DNA fingerprinting (Jeffries et al., 1985).

When the father of an HD patient died suddenly, it was essential for us to obtain DNA from the deceased to perform linkage analysis on the family. Permission was obtained from the family and a fibroblast cell line was established from explants of a skin biopsy specimen taken from the deceased person and DNA was isolated. Cells are now stored in liquid nitrogen for further culture if required. (For method see chapter 5.6.5)

Recombination events identified between molecular markers and the HD gene locus are of vital importance for the physical localisation of any new probes. In addition, the use of pulsed field gel electrophoresis requires large fragments of unsheared DNA. Consequently it was decided to immortalize cell lines from all HD patients. To date lymphocytes from five patients have been transformed, using standard techniques and are stored in liquid nitrogen.

5.4 FAMILIES SELECTED FOR LINKAGE ANALYSIS

The ten HD families selected for linkage analysis were primarily chosen on the basis of family size. Those with the greatest number of living HD patients, manifesting in two successive generations, were given preference (Table V-3).

Six families were of mixed ancestry, two of European origin (Dutch and French/Mauritian stock) and two were of SA black origin (Pedi and Tswana).

The prerequisites for family selection that became apparent during the study were:

i. The number of informative matings

ii. The frequency of heterozygosity of the markers in affected individuals

iii. The family structure and availability of relatives for genotyping.

In the absence of some of these prerequisites it is essential to be able to infer the G8 haplotypes in untyped individuals and to establish phase in affected individuals.

All affected individuals included in the study were examined by a clinician experienced in the diagnosis of HD. Individuals with an equivocal clinical status were regarded as being 'at-risk' and not as being affected. Children under the age of 16 years were only typed where necessary to establish the parental haplotypes. In instances where a parent was deceased, genotypes were inferred from those of the progeny.

TABLE V-3.

HD Families selected for linkage analysis

Family	Ethnic group	Total no. of family members	Persons reputed to be affected	Deceased HD patients	DNA obtained from HD patients
MA1	mixed ancestry	61	13	7	5
MA2	mixed ancestry	140	19	8	10
MA3	mixed ancestry	110	14	9	6
MA4	mixed ancestry	64	7	4	4
MA5	mixed ancestry	23	4	2	2
MA6	mixed ancestry	40	4	1	3
P11	Pedi	57	10	7	4
T9	Tswana	33	6	4	2
E20	European (French)	31	6	3	3
E40	European (Dutch)	22	7	5	2

There were living HD patients available for study in two successive generations in family MA1, but there were no direct instances of parent to child transmission of the disorder in the five living HD patients.

In family MA2 there were three instances of meiotic transmission of HD in the ten patients available for study.

Families MA3, MA4 and MA6 all had living HD patients available for study in two successive generations but no instances of direct meiotic transmission of the disease in the affected individuals. In MA5 only two HD sibs were available for study.

One of the two black African families in the study, P11, had DNA available from HD patients in two successive generations while the other, T9, only had DNA available from two HD sibs.

In both SA families of European origins (E20, French; E40, Dutch) the only HD patients available for study were from the same generation.

The numbers of HD patients, unaffected family members, at-risk individuals and spouses who were included in the study are shown in Table V-4 and the pedigrees of all the families in the study can be seen in Figs VII.3 and VII.4.

In order to ensure and maintain confidentiality at all times, all individuals genotyped were coded from the start of the study and referred to by their laboratory numbers.

In the pedigrees presented in this thesis, the sex and order of birth have been changed to maintain anonymity. All participants were assured that their DNA would be stored in the DNA bank and that only a portion would be used for research purposes. All results are regarded as research data and will not be used for any predictive testing.

Once an acceptable programme for presymptomatic testing of HD in SA has been established and implemented, fresh DNA samples will be obtained from those individuals who are deemed to be strictly necessary for linkage analysis within a family. In the event of DNA stored in the bank being unobtainable for a predictive test for any reason, written consent will have to be obtained from the individual for its release and use in presymptomatic testing.

During the course of the study, 42 HD patients were genotyped representing 43% of all the HD patients whose DNA is stored in the HD DNA banking centre (Table V-5). In five cases DNA samples were used from HD patients who had died before linkage analysis was performed, emphasizing the necessity for DNA banking from all HD family members for future use in presymptomatic testing.

TABLE V-4.

Totals of SA HD family members genotyped in this study.

Family	Ethnic group	HD patients	Unaffected family members over 60 years	At-risk individuals used for genotype assignment	Spouses
MA1	mixed ancestry	5	5	10	2
MA2	mixed ancestry	10	2	15	2
MA3	mixed ancestry	6	3	7	3
MA4	mixed ancestry	4	2	7	4
MA5	mixed ancestry	2	0	6	2
MA6	mixed ancestry	3	0	4	3
P11	Pedi	4	0	11	4
T9	Tswana	2	2	4	2
E20	European (French)	3	0	3	3
E40	European (Dutch)	2	1	4	2
		41	15	71	27

Total no. of individuals genotyped in the study = 154

TABLE V-5.

Number of HD patients genotyped during the study compared with the number of patient blood stored in the DNA bank.

Ethnic group	HD patients' DNA stored in bank	HD patients' DNA genotyped in study	%
Mixed ancestry	60	31	52%
European origins	30	5	17%
Tswana	2	2	100%
Pedi	5	4	80%
Venda	1	0	0%
Totals	98	42	43%

5.5 DNA PROBES USED IN THE CAPE TOWN STUDY

Initially, in 1985, DNA was isolated from patients with HD and at-risk individuals from two large families in Cape Town (MA1 and MA2). DNA was digested with restriction enzymes HindIII and EcoRI, restriction fragments were electrophoretically separated on 0.6% agarose gels and Southern blots were prepared. These blots were sent to the laboratory of James Gusella in Massachusetts where they were hybridized to two subclones of the original G8 probe (pK082 and pK083). Linkage was found in both these families and the results were presented at a local congress as part of a collaborative study (Goldblatt et al., 1986).

In 1986 we obtained the G8 probe and three of its sub clones (pK081, pK082 and pK083) from the Boston group. This DNA fragment (G8), now designated D4S10, had been mapped to the terminal region of the short arm of chromosome 4, thus localising the HD gene to bands 4p16->p15 (Zabel et al., 1986). Fig. V.4.

Linkage analysis using these probes was initially undertaken in three large families of mixed ancestry (MA1, MA2, and MA3). The subclones of G8 that were used were pK081 and pK083 which both recognise EcoRI polymorphisms and pK082 which identifies the HindIII polymorphism (Table V-6).

RESTRICTION MAP OF THE PROBES USED IN THE CAPE TOWN STUDY

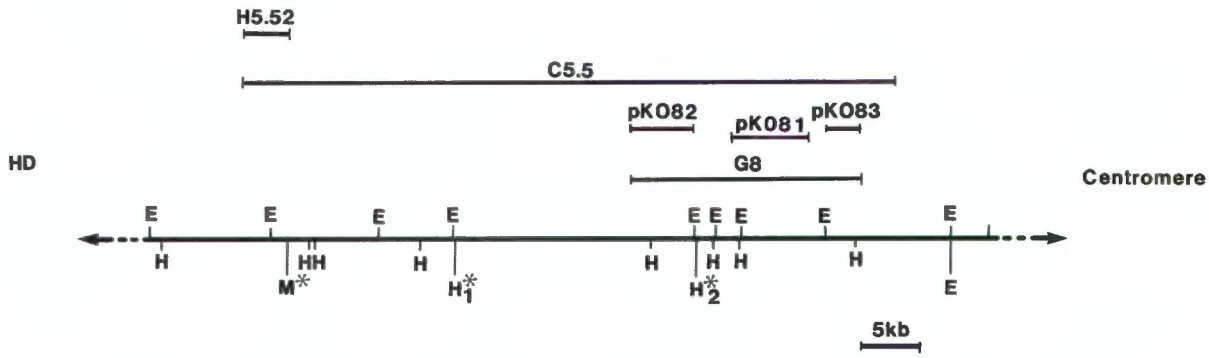

Figure V.4

Restriction map indicating the polymorphic sites in the D4S10 locus.
E=EcoRI H=HindIII M=MspI.

The relevant polymorphic sites used in this study have been indicated by an asterisk (*).HindIII RFLPs detected with genomic clone pK082, MspI with H5.52 and EcoRI with pK083 and pK081; pK081 is indicated with a dotted line as the exact position of this probe has not been determined (Gusella, personal communication).

Reproduced in part from Skraastad et al. (1989).

TABLE V-6.

Probes used in the Cape Town study

G8 Subclone Probe name	Restriction enzyme	Fragment size(kb)	Reference
pK082	HindIII site 1 site 2	17.5 / 15 4.9 / 3.7	Gusella et al. 1983
pK081	EcoRI	6.0 / 5.5	Gusella et al. 1983
pK083	EcoRI	14 / 9	Gusella et al. 1983
H5.5	MspI	3.5 / 2.8	Skraastad et al. 1989

Difficulty was experienced with double heterozygote parents and homozygosity of the marker in the affected individual. Often the disease appeared to be segregating with the more frequent marker and was thus uninformative (Fig VII.4; MA1,MA2, and MA3).

M Skraastad et al., (1986) reported the isolation of a cosmid, C5.5, which increased the heterozygosity in the Dutch population from 68% to 96%. In 1987 we obtained the Dutch group's subclones H5.52 and F5.53 which had proven most informative for them. As the majority of our Afrikaner families originated from Holland in the 17th century, we anticipated a similar polymorphism frequency in some of our SA population groups (Bakker et al., 1987) (Tables VII-2 and VII-6).

The subclone H5.52 is a BglII clone from one end of cosmid C5.5 which is, in turn, a 5.5kb EcoRI fragment of the original G8 probe found 48kb from the EcoRI RFLP on the centromeric end of G8 (Skraastad et al.,1989). (Fig. V.4) This probe proved to be most useful and increased the informativity in two SA families (Table VII-19; MA1 and MA3).

The position of these subclones relative to the original G8 probe and the relevant restriction sites are indicated in Fig. V.4. Results of our linkage studies substantiate the orientation of these restriction sites (Chapter 8.2).

5.6 GENOMIC DNA HYBRIDIZATION

In my investigations the following standard techniques (Vandenplas et al., 1984) were used, with some modifications

- preparation of DNA from EDTA anticoagulated blood
- restriction enzyme digestion
- electrophoretic fragment separation
- Southern blotting
- filter hybridization
- autoradiography

The changes made to these techniques during the course of this study include:

5.6.1 'Blotto' was used in place of Denhart's solution.

5.6.2 Hybond N membrane (Amersham) was used in place of nitrocellulose to facilitate multiple rehybridization of the filters.

5.6.3 A year after the project began, Amersham, the suppliers of Hybond N membrane, changed the blotting method to ensure the transfer of DNA fragments > 10kb in size. This involved acid nicking and alkali blotting.

5.6.4 It was found that very poor results were obtained using probe H5.52 and the standard nick translation method. Oligolabelling of the plasmid insert DNA was routinely performed with this probe using the method adapted by Chris Mathew (Personal communication).

5.6.5 Modified method for DNA isolation from cultured fibroblasts.

The modified methods that were used are as follows:

5.6.1 ' Blotto' hybridization (Johnson D et al., 1984)

Filters were prehybridized for 30 min at 65° C in 3 X SSC (1 X is 0.15M NaCl, 0.015M trisodium citrate), 60 min at 65° C in 3 X SSC and 0.25% powdered milk (Carnation low fat = 'Blotto') and then for 30 min at 65° C in 3 X SSC, 0.25% powdered milk and 0.1% SDS (sodium dodecylsulphate). The denatured labelled probe was then added to 5 - 8ml of this final prehybridization solution and incubated in a heat-sealed plastic bag in a shaking waterbath for at least 48 hrs at 65° C. Post hybridization washes also included 0.25% powdered milk in place of Denhart's solution. The final stringency washes were routinely performed in 0.1 X SSC and 0.1% SDS (w/v) at 65° C for 1 hr (2 changes of stringency washes).

5.6.2 Removal of probe and re-use of blots. (Amersham protocol)

Membranes were not allowed to dry after hybridization and washing. They were stored at -20° C until they were 'stripped' of the previously used probe. To remove the probe the filters were incubated for 30min in 0.4M NaOH in a shaking waterbath at 45° C, transferred to 0.1 X SSC, 0.1% (w/v) SDS and 0.2M Tris HCl pH 7.5 and left shaking at 45° C for a further 30 min. Following the 'stripping', the blots were autoradiographed to check that the probe had been successfully removed. The filters were then prehybridized and hybridized with a new probe as before.

5.6.3 Acid nicking of DNA fragments >10kb and alkali blotting (Amersham recommended protocol)

After electrophoresis for 24hrs at 45V to separate the larger fragments, the 0.6% agarose gel was placed in 0.25M HCl for 10min. The gel was then rinsed in distilled water, placed in denaturing buffer, completely covering the gel and shaken gently at room temperature for 30 min. It was then lifted out of the denaturing solution, blotted to remove any excess liquid and equilibrated in an alkali transfer buffer (0.25M NaOH and 1.5M NaCl) for 15min. A capillary blot (standard Southern blot) was then set up, using alkali transfer buffer in the place of the usual 20 X SSC.

5.6.4 Oligolabelling of the plasmid insert DNA for probe H5.5.

The solutions used were all from the Amersham multiprime DNA labelling systems kit.

50ng of probe in distilled water was boiled for 3min to denature the DNA and then placed on ice for 10min.

Reagents were added to the DNA in the Eppendorf tube in the following order:

Multiprime buffer	11µl
BSA	4µl
32 P dCTP 50µCi (3000 Ci/mmol)	5µl
Polymerase	2µl
Total reaction vol.	50µl

The reaction mixture was left overnight at room temperature in a lead pot. The following day the reaction mixture was extracted with an equal volume of phenol:chloroform and the free nucleotides were separated by chromatography on a small column of Sephadex G-50 as per usual for nick translation of DNA probes.

5.6.5 DNA isolation from Fibroblasts

2 X 25ml Flasks of confluent fibroblasts were trypsinized and re-suspended in 0.5ml PBS (Phosphate buffered saline). The cell suspension was then homogenized after the addition of 3 mls lysis buffer and the same procedure followed as for lymphocytes. Roughly 100µg DNA was recovered from one isolation.

5.7 LINKAGE ANALYSIS

The basis for linkage analysis studies is that two loci which are located close to each other on the same chromosome tend to co-segregate. Independent segregation is observed where recombination events occur between loci. The chance of recombination increases with the distance between the loci.

As previously stated, international studies of genetic linkage between HD and G8 have generated a combined lod score of 87.69 at $\theta = 0.04$ (95% confidence interval 0.018-0.071). This means that the accuracy of using G8 in predictive testing is compromised by a 4% recombination frequency between G8 and HD (Conneally et al., 1989). Prior to implementing predictive and prenatal testing for at-risk individuals in SA, the recombination fraction between HD and the markers in SA HD patients needed to be calculated as the error rates in presymptomatic testing would be critically dependent on this recombination fraction.

The computer program LIPED (Ott, 1974) was used to analyse the genetic linkage data with a modification for age dependent penetrance (Table V-7). No allowance was made for the possibility that age of onset has a familial basis (Farrer and Conneally, 1985).

The lod scores between the markers and the HD locus were calculated using recombination fractions from 0.00 to 0.40. Individual lod scores were established for each marker as well as for a combined haplotype for the ten families studied (Tables VII-18 to VII-22).

Confidence limits were estimated by the method recommended by Conneally et al. (1985) and the computer program HOMOG was used to test for heterogeneity (Ott, 1985). The HD frequency was taken as 0.0001 and the marker frequencies were set according to the population group of the HD family analysed.

All living HD patients, their parents, unaffected sibs over the age of 60 years and at-risk individuals whose genotypes were essential for assigning haplotypes were included in the analysis. Whenever the inclusion of an at-risk individual under the age of 60 years resulted in a large negative lod score at all the values of θ, these individuals were excluded from the linkage analysis.

Liability codes for age dependent penetrance.

CODE	AGE	BIRTHYEAR	PENETRANCE
1	0-27	63-90	.02
2	28-32	62-58	.05
3	33-36	57-54	.1
4	37-41	53-49	.3
5	42-46	48-44	.4
6	47-51	43-39	.5
7	52-56	38-34	.6
8	57-62	33-28	.7
9	63-67	27-23	.8
10	68 >	22 <	.9

* As of 31/12/1989.

CHAPTER 6 LINKAGE STUDY OF HUNTINGTON DISEASE IN SA BLACK FAMILIES.

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CHAPTER SIX

LINKAGE STUDY OF HUNTINGTON DISEASE IN SA BLACK FAMILIES.

6.1 BACKGROUND TO HUNTINGTON DISEASE IN BLACK FAMILIES

HD is uncommon in African and American black persons (Hayden, 1981). Apart from a report from Maryland in 1987 (Folstein et al., 1987), where the prevalence of HD in blacks was found to be unexpectedly high, there have been few descriptions in the past decade of HD involving black families (Wright et al., 1981). In particular, there has been only one report with eight confirmed cases of HD in African blacks (Joubert and Botha, 1988). Furthermore there is little published on genetic linkage in black HD families (Folstein et al.,1985; Bird et al., 1986; Folstein et al., 1985).

Due to this low frequency of HD in black persons, together with the necessity mentioned in chapter 4.3 for addressing the question of heterogeneity at the HD locus, a collaborative project was undertaken involving M Hayden, University of British Columbia, Canada and J Joubert, Medunsa and Ga-Rankuwa Hospital, Pretoria, RSA. The purpose of this study was to establish linkage in one of the three black African families reported in 1988 (Joubert and Botha, 1988). Numerous markers on chromosome 4, currently being used for predictive testing of HD in Canada (Hayden et al., 1988a) were used in this investigation. This study was the first investigation in Africa of a black kindred with HD using molecular linkage analysis.

The SA black HD family from the Lebowa region (Fig V.3) that was intensively investigated in this study can be seen in Fig VI.1. Fig VI.2 is the pedigree of this indigenous black African HD family (P11).

Figure VI.1

A South African black HD family in Lebowa

6.2 CLINICAL ASPECTS

Clinical data have already been published on this HD family (Joubert and Botha, 1988). In the present investigation, four affected family members were examined and clinically diagnosed.

(It should be noted that in the publication HUNTINGTON DISEASE IN SOUTH AFRICAN BLACKS (Joubert and Botha, 1988) the pedigrees published of families 1-3 refer to families 2-4 in Table V of the article. The pedigree of family 2 is the one referred to in this thesis.)

There were no remarkable differences noted on physical examination between these four HD patients and patients seen at the HD clinic at Groote Schuur hospital (from all different SA populations).

The age of onset in the four HD patients examined were 46, 45, 48 and 37 years (Fig VI.2: individuals III.3, III.6, III.13 and IV.25 respectively).

The unaffected sister in generation III (III.9) was 60 years old at the time of the study and was included in the linkage analysis.

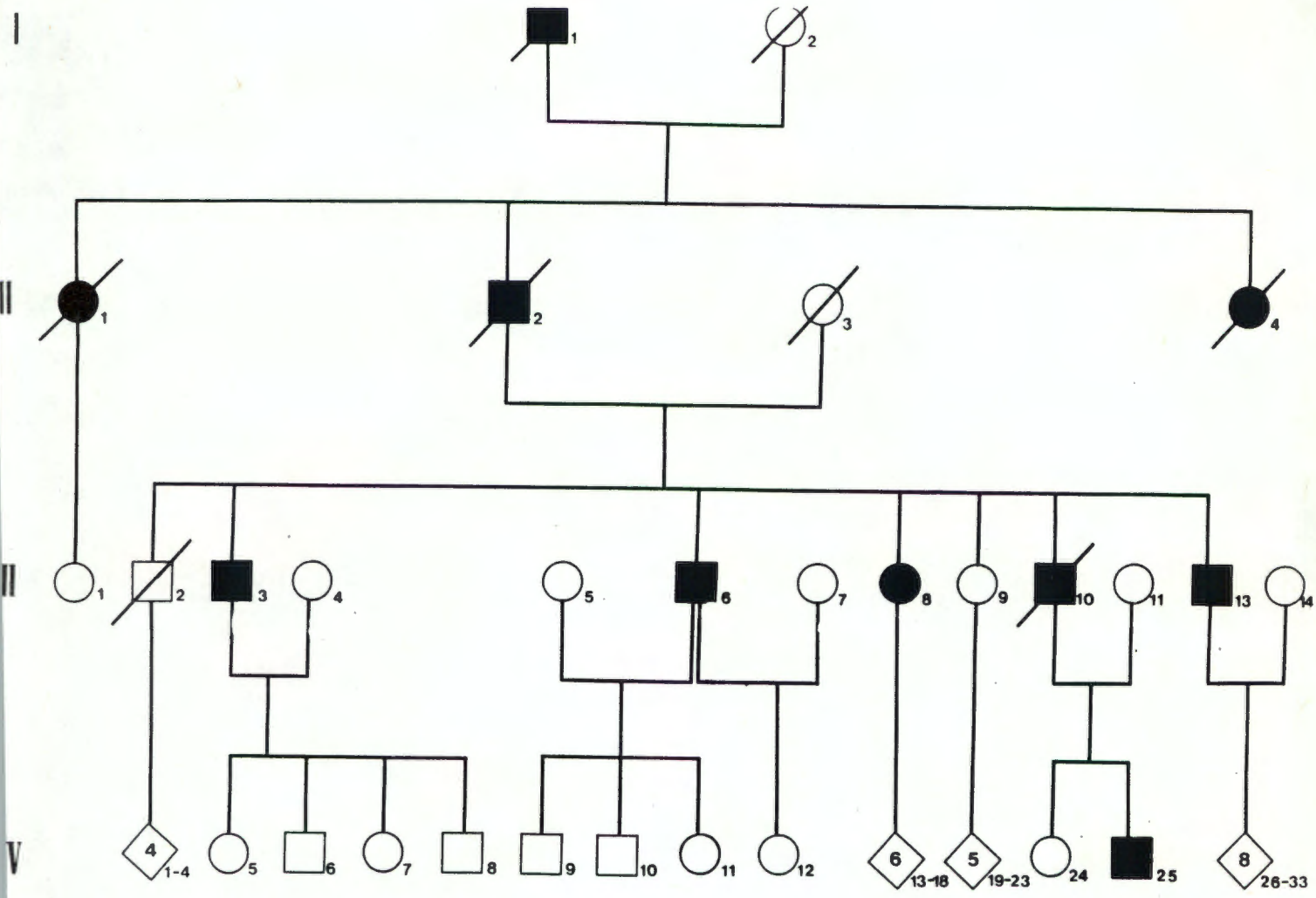

Figure VI.2

Pedigree of the SA black HD family (P11) in the extended study.

Squares represent males and circles females. Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.

6.3 EXTENDED LINKAGE ANALYSIS OF A BLACK AFRICAN HD FAMILY

DNA from this family was isolated from whole blood in Cape Town and taken by the author to Canada. Additional endonuclease restrictions, electrophoretic separation of DNA size-fractions in agarose gels of different concentrations according to the different expected RFLPs and Southern blotting were performed by the author in the Canadian laboratory. Oligolabelling, hybridization and autoradiography were carried out according to standard laboratory procedures routinely employed in Canada with very little variation from the methods used and described in this thesis.

The probes used for this extended linkage study were the subclones of the original G8 probe used throughout the course of the project as well as five newer probes found to be more tightly linked to HD and currently being used in predictive testing of HD in Canada (Table VI-1 with appropriate references)(Fig VI.3).

Computations for linkage analysis were performed in Cape Town together with the rest of the study using the LIPED program and marker frequencies determined for this population group established as part of the collaborative study (unpublished data).

Individual lod scores between the markers and the HD locus were calculated for each marker as well as for a combined haplotype of the eight markers used (Fig VII.5).

DNA MARKERS ON CHROMOSOME 4 USED IN THE STUDY OF A BLACK HD FAMILY.

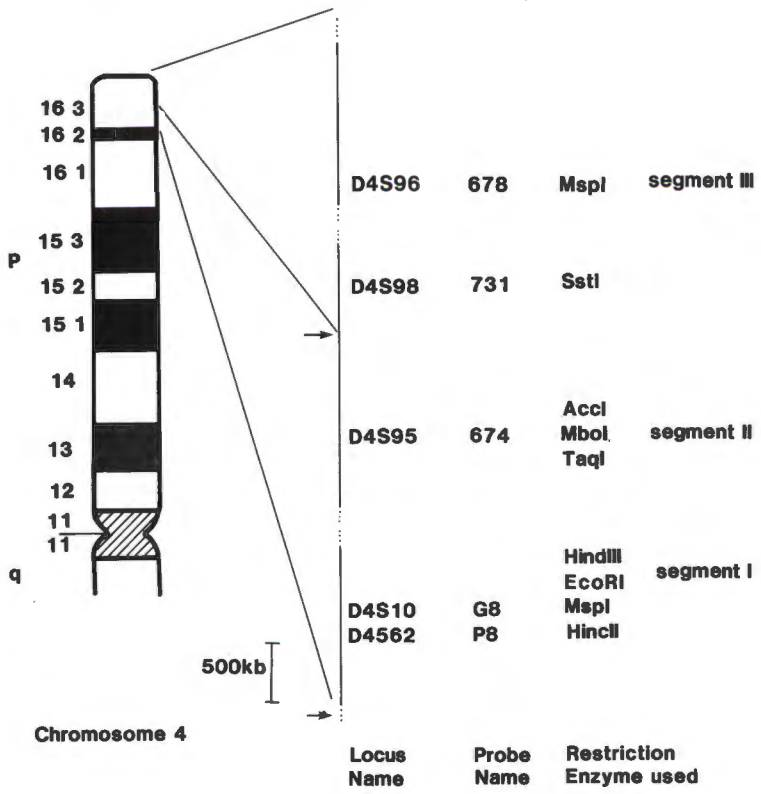

Figure VI.3

Diagrammatic map showing the DNA markers used in the extended study of a black African HD family. Probes, enzymes and references as in Table VI-1.

Reproduced in part from MacDonald et al. (1989).

TABLE VI-I

Probes used in the study of a black African HD family.

PROBE	LOCUS	ENZYME	REFERENCE
pK082	D4S10	HindIII	Gusella et al., 1983
pK083	D4S10	EcoRI	Gusella et al., 1983
H5.52	D4S10	MspI	Skraastad et al., 1989
731	D4S98	SstI	Whaley et al., 1988
p8	D4S62	HincII	Hayden et al., 1988b
678	D4S96	MspI	Smith et al., 1988
674	D4S95	TaqI	Wasmuth et al., 1988
		AccI	Wasmuth et al., 1988
		MboI	Wasmuth et al., 1988

CHAPTER 7 RESULTS AND DISCUSSION:

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CHAPTER SEVEN

RESULTS AND DISCUSSION:

7.1 ALLELE FREQUENCIES AT THE D4S10 LOCUS

The polymorphic DNA fragment sizes, allele frequencies and polymorphism information content (PIC) values at the D4S10 locus in unrelated normal individuals from three different population groups in SA are presented in Tables VII-1, VII-2, VII-3, VII-4 and VII-5. These results are compared with published data from Britain, Holland, USA, Germany, Finland and Scotland in Table VII-6. The individual findings of the three black African tribal groups that were studied (Tswana, Pedi and Venda) are shown in Table VII-3 (their combined frequency is representative of a broad group of the indigenous black African peoples of the Northern Transvaal).

TABLE VII-1.

Polymorphisms at the D4S10 locus.

Probe	Enzyme	Fragment Size(kb)	Allele	Reference
pK082 (Site 1)	HindIII	17.5 15	HI H2	Gusella et al.,1983
pK082 (Site 2)	HindIII	4.9 3.7	h1 h2	"
pK081	EcoRI	6.0 5.5	E1 E2	"
pK083	EcoRI	14 9	e1 e2	"
H5.5	MspI	3.5 2.8	M1 M2	Skraastad et al.,1989

TABLE VII-2.

Allele frequencies of D4S10 in the SA population groups of black African, mixed ancestry and European origins.

Probe Enzyme	Allele	Allele frequency in different populations		
		SA Black (Tvl)	SA of Mixed ancestry	SA of European origins
pK082 (Site1) HindIII	H1	(81) 0.74	(50) 0.83	(47) 0.90
	H2	(27) 0.26	(10) 0.17	(5) 0.10
N		108	60	52
PIC		0.310	0.242	0.163
pK082 (Site2) HindIII	h1	(56) 0.52	(19) 0.32	(7) 0.13
	h2	(52) 0.48	(41) 0.68	(45) 0.87
N		108	60	52
PIC		0.375	0.341	0.201
pK081 EcoRI	E1	(23) 0.24	(33) 0.57	(50) 0.96
	E2	(73) 0.76	(25) 0.43	(4) 0.04
N		96	58	54
PIC		0.298	0.370	0.074
pK083 EcoRI	e1	(19) 0.20	(19) 0.32	(36) 0.62
	e2	(77) 0.80	(41) 0.68	(22) 0.38
N		96	60	58
PIC		0.269	0.341	0.360
H5.5 MspI	M1	(16) 0.17	(15) 0.28	(28) 0.42
	M2	(76) 0.83	(39) 0.72	(36) 0.58
N		92	54	64
PIC		0.242	0.322	0.369

N = Number of Chromosomes

PIC = calculated according to the method recommended by Botstein et al. 1980.

TABLE VII-3.

Allele frequency of D4S10 in the Pedi, Tswana and Venda.

Probe Enzyme	Allele	Allele frequencies			
		Pedi	Tswana	Venda	SA Black (Tvl)
pK082 (Site1)	H1	(45) 0.77	(18) 0.69	(18) 0.75	(81) 0.74
	H2	(13) 0.23	(8) 0.31	(6) 0.25	(27) 0.26
N		58	26	24	108
PIC		0.292	0.336	0.305	0.311
pK082 (Site2)	h1	(30) 0.52	(12) 0.46	(14) 0.58	(56) 0.52
	h2	(28) 0.48	(14) 0.54	(10) 0.42	(52) 0.48
N		58	26	24	108
PIC		0.373	0.373	0.369	0.375
pK081	E1	(14) 0.27	(4) 0.20	(5) 0.21	(23) 0.23
	E2	(38) 0.73	(16) 0.80	(19) 0.79	(73) 0.77
N		52	20	24	96
PIC		0.317	0.269	0.277	0.299
pK083	e1	(9) 0.17	(5) 0.25	(5) 0.21	(19) 0.20
	e2	(43) 0.83	(15) 0.75	(19) 0.79	(77) 0.80
N		52	20	24	96
PIC		0.242	0.305	0.277	0.269
H5.5	M1	(10) 0.19	(0) 0.00	(5) 0.21	(15) 0.13
	M2	(38) 0.81	(20) 1.00	(19) 0.79	(77) 0.87
N		48	20	24	92
PIC		0.260	0.0000	0.277	0.242

(SA Black (Tvl) =Pedi,Tswana and Venda together as one group)

N = Number of Chromosomes

PIC = calculated according to the method recommended by Botstein et al. 1980.

There are two polymorphic HindIII sites identified at the D4S10 locus by the pK082 subclone of G8. At each site there are two possible alleles. The combination of the alleles at these sites can be combined as haplotypes named A,B,C, and D according to the nomenclature of Gusella (Gusella et al., 1983) (Fig.VII.1a). The haplotype frequencies and PIC values calculated for the three population groups studied in SA are as shown in Tables VII-4 and VII-5 and are compared with published results from Britain (Youngman et al., 1986).

Figure VII.1

Southern blots showing the RFLPs associated with the G8 subclones used in the Cape Town study.

DNA samples digested with

a) HindIII and hybridized to pKO82.
b) EcoRI and hybridized to pKO81.
c) EcoRI and hybridized to pKO83.
d) MspI and hybridized to H5.52.

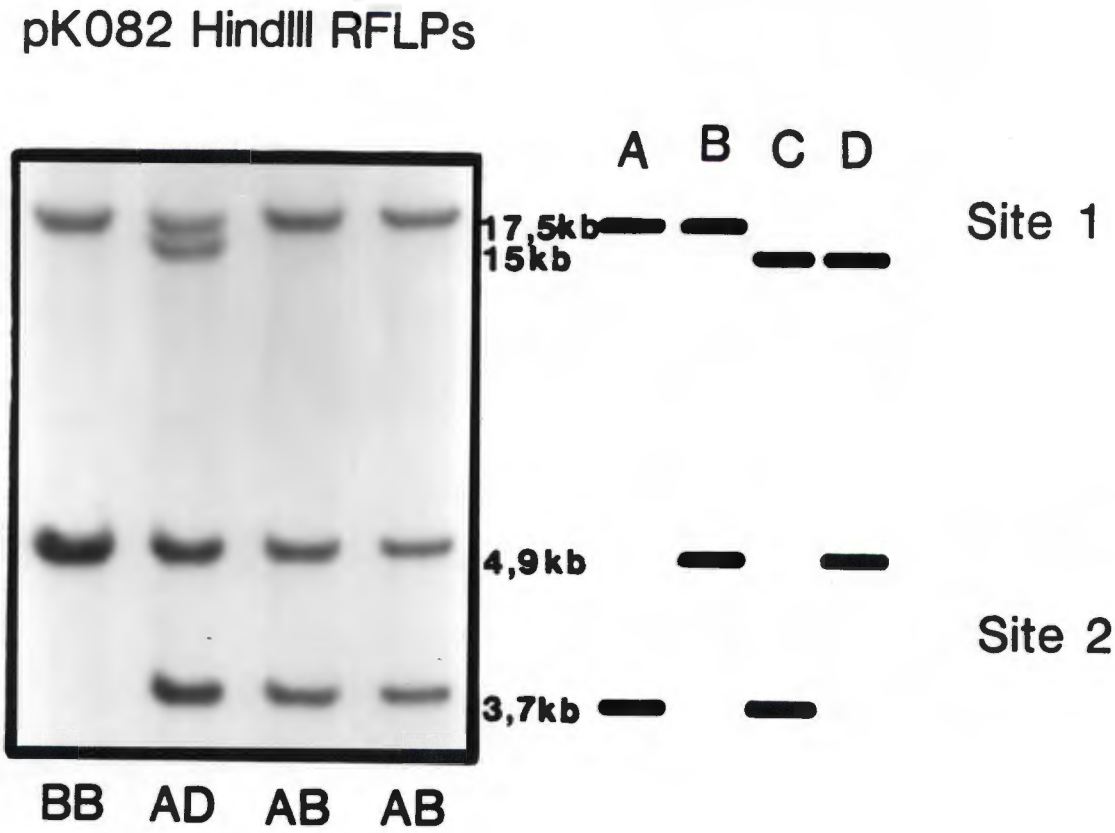
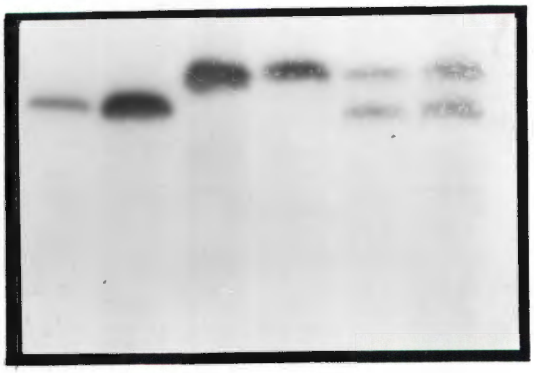


Figure VII.1a

Southern blot showing the hybridization of pK082 probe to HindIII-digested genomic DNA. Haplotypes were determined from the alleles seen for each HindIII RFLP (sites 1 and 2). (Gusella et al., 1983).

pK081 EcoRI RFLPs



6.0kb E1
5.5kb E2

E 22 22 11 11 12 12

Figure VII.1b

Southern blot showing the hybridization of pK081 probe to EcoRI-digested genomic DNA. The EcoRI 6.0kb and 5.5kb polymorphic bands are indicated by E1 and E2 respectively. (Gusella et al., 1983).

pK083 EcoRI RFLPs

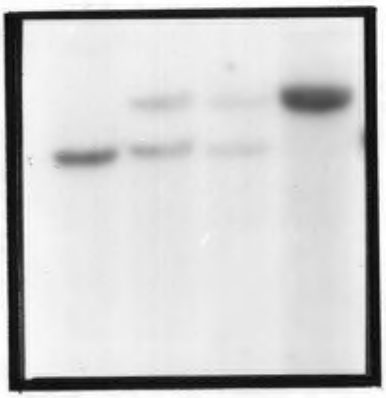


Figure VII.1c

Southern blot showing the hybridization of pK083 probe to EcoRI-digested genomic DNA. The EcoRI 14kb and 9kb polymorphic bands are indicated by e1 and e2 respectively. (Gusella et al., 1983).

H 5.52 MspI RFPLs

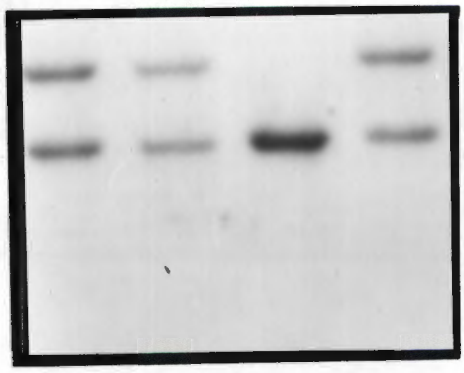


Figure VII.1d

Southern blot showing the hybridization of H5.52 probe to MspI-digested genomic DNA. The MspI 3.5kb and 2.8kb polymorphic bands are indicated by M1 and M2

TABLE VII-4.

Haplotypes at the G8 locus.
in the SA population groups studied.
(HindIII polymorphic sites)

There are 2 polymorphic HindIII sites identified at this locus by the pK082 subclone of G8. At each site there are two possible alleles. + and - denotes the presence and absence, respectively, of the HindIII restriction site. The combination of the alleles at these sites is the haplotype: A,B,C and D according to the nomenclature of Gusella (Figure VII.Ia).

Haplo-types	Site 1	Site 2	Frequencies.			
			SA Black (Tvl) N=108	SA of Mixed ancestry N=60	SA of European origin N=52	British N=102
A	13(-)	3.7(+)	0.40	0.59	0.72	0.66
B	13(-)	4.9(-)	0.41	0.28	0.13	0.09
C	11(+)	3.7(+)	0.09	0.09	0.13	0.22
D	11(+)	4.9(-)	0.10	0.04	0.03	0.03
PIC			0.588	0.501	0.429	

N = number of chromosomes
British haplotype frequencies according to published data Youngman et al. 1986

PIC = calculated according to the method recommended by Botstein et al. 1980.

Haplotypes at the G8 locus in the Pedi Tswana and Venda. (HindIII polymorphic sites)

There are two polymorphic HindIII sites identified at this locus by the pK082 subclone of G8. At each site there are two possible alleles. + and - denotes the presence and absence, respectively, of the HindIII restriction site. The combination of the alleles at these sites is the haplotype: A,B,C and D according to the nomenclature of Gusella (Figure VII.Ia).

Haplo-types	Site 1	Site 2	Frequencies.			
			Pedi N=50	Venda N=22	Tswana N=24	SA Black(Tvl) N=96
A	13(-)	3.7(+)	0.38	0.41	0.42	0.40
B	13(-)	4.9(-)	0.44	0.45	0.29	0.41
C	11(+)	3.7(+)	0.10	0.00	0.16	0.09
D	11(+)	4.9(-)	0.08	0.14	0.13	0.10
PIC			0.639	0.527	0.644	

N = number of chromosomes
(SA Black(Tvl) =Pedi, Tswana, Venda together as one group)

PIC = calculated according to the method recommended by Botstein et al. 1980.

TABLE VII-6.

Reported allele frequencies of D4S10 from Scotland, UK, Holland, Germany, Finland and USA.

		Scottish Holloway et al 1989 N=64	British Youngman et al 1986 N=102	Dutch Skraastad et al 1989	German Riess et al 1989 N=54	Finnish Ikonen et al 1990 N=100	USA Farrer et al 1988 N=46
pK082	H1	0.80	0.75	0.75	0.705	0.73	0.79
Site 1	H2	0.20	0.25	0.25	0.295	0.27	0.21
pK082	h1	0.08	0.125	0.16	0.06	0.12	0.19
Site 2	h2	0.92	0.875	0.84	0.94	0.88	0.81
pK081	E1		-	-	-	-	-
	E2		-	-	-	-	-
pK083	e1	0.44	0.50	0.47	0.59	-	0.60
	e2	0.56	0.50	0.53	0.41	-	0.40
5.5	M1	-	-	0.40	0.41	0.52	-
	M2	-	-	0.60	0.59	0.48	-

N = number of chromosomes

The chi-square test for goodness of fit revealed no statistical significant deviation from the Hardy-Weinberg distribution in the SA population groups studied (Tables VII-7 to VII-16) (Swinscow, 1977).

Genotype frequencies of the HindIII/pK082 RFLPs (alleles H1 and H2) in the SA population groups studied.

Population group	Allele		Genotype						X^2
	H1 17.5kb	H2 15kb	H1H1	H2H2	H1H2	H1H1	H2H2	H1H2	
			Observed (O)			Expected (E)			
SA Black(.74) Tvl N=108	81	27 (.26)	31	4	19	29.5	3.6	20.7	0.246
SA of Mixed(.83) ancestry N=60	50	10 (.17)	21	1	8	20.66	0.87	8.46	0.45
SA of European origin N=52((.90)	47	5 (.10)	21	0	5	21	0.26	4.68	0.26

N = Number of chromosomes,

X^2 = chi square test at 1 degree of freedom.

Genotype frequencies of the HindIII/pK082 RFLPs (alleles H1 and H2) in the Pedi, Venda and Tswana from the Tvl.

Population group	Allele		Genotype						X^2
	H1 17.5kb	H2 15kb	H1H1	H2H2	H1H2	H1H1	H2H2	H1H2	
			Observed (O)			Expected (E)			
Pedi N=58	45 (.77)	13 (.23)	18	2	9	17.1	1.5	10.3	0.38
Tswana N=26	18 (.69)	8 (.31)	6	1	6	6.1	1.2	5.6	0.56
Venda N=24	18 (.75)	6 (.25)	7	1	4	6.75	0.75	4.5	0.13
SA Black (Tvl) N=108	81 (.74)	27 (.26)	31	4	19	29.5	3.6	20.7	0.24

SA Black(Tvl) = Pedi, Tswana and Venda as one group

N = Number of chromosomes,

X^2= chi square test at 1 degree of freedom.

Genotype frequencies of the HindIII/pK082 RFLPs (alleles h1 and h2) in the SA population groups studied.

Population group	Allele		Genotype						X^2
	h1 4.9kb	h2 3.7kb	h1h1	h2h2	h1h2	h1h1	h2h2	h1h2	
			Observed (O)			Expected (E)			
SA Black Tvl N=108	56 (.52)	52 (.48)	12	9	33	14.6	12.4	27	2.72
SA of Mixed ancestry N=60	19 (.32)	41 (.68)	3	14	13	3	13.87	13.05	0.00
SA of European origin N=52	7 (.13)	45 (.87)	0	19	7	0.44	19.68	5.88	0.67

N = Number of chromosomes,

X^2 = chi square test at 1 degree of freedom.

Genotype frequencies of the HindIII/pK082 RFLPs (alleles h1 and h2) in the Pedi, Venda and Tswana from the Tvl.

Population group	Allele		Genotype						X^2
	h1 4.9kb	h2 3.7kb	h1h1	h2h2	h1h2	h1h1	h2h2	h1h2	
			Observed (O)			Expected (E)			
Pedi N=58	30 (.52)	28 (.48)	6	4	19	7.8	6.7	14.1	3.18
Tswana N=26	12 (.46)	14 (.54)	3	4	6	2.7	3.7	6.4	0.07
Venda N=24	14 (.58)	10 (.42)	3	1	8	4	2.1	5.8	1.65
SA Black (Tvl) N=108	56 (.52)	52 (.48)	12	9	33	14.6	12.4	27	2.72

SA Black (Tvl) = Pedi, Tswana and Venda as one group
N = Number of chromosomes,
X^2 = chi square test at 1 degree of freedom.

Genotype frequencies of the EcoRI/pK081 polymorphisms in the SA population groups studied.

Population group	Allele		Genotype						X^2
	E1 6.0kb	E2 5.5b	E1E1	E2E2	E1E2	E1E1	E2E2	E1E2	
			Observed (O)			Expected (E)			
SA Black Tvl N=96	23 (.24)	73 (.76)	1	26	21	2.8	27.72	17.5	1.95
SA of Mixed ancestry N=58	33 (.57)	25 (.43)	9	5	15	9.4	5.4	14.2	0.1
SA of European origin N=54	50 (.96)	4 (.04)	25	2	0	24.88	0.04	2.1	0.06

N = Number of chromosomes,
X^2 = chi square test at 1 degree of freedom.

Genotype frequencies of the EcoRI/pK081 polymorphisms in the the Pedi Venda and Tswana from the Tvl.

Population group	Allele		Genotype						X^2
	E1 6.0kb	E2 5.5kb	E1E1	E2E2	E1E2	E1E1	E2E2	E1E2	
			Observed (O)			Expected (E)			
Pedi N=52	14 (.27)	38 (.73)	1	13	12	1.9	13.85	10.14	0.81
Tswana N=20	4 (.20)	16 (.80)	0	6	4	0.4	6.4	3.2	0.63
Venda N=24	5 (.21)	19 (.79)	0	7	5	0.52	7.49	3.96	0.82
SA Black Tvl N=96	23 (.24)	73 (.76)	1	26	21	2.8	27.72	17.5	1.95

SA Black(Tvl) = Pedi, Tswana and Venda as one group

N = Number of chromosomes,

X^2 chi square test at 1 degree of freedom.

Genotype frequencies of the EcoRI/pK083 polymorphisms in the SA population groups studied.

Population group	Allele e1 14kb	Allele e2 9kb	Genotype e1e1 Observed (O)	e2e2	e1e2	e1e1 Expected (E)	e2e2	e1e2	X^2
SA Black Tvl N=96	19 (.20)	77 (.80)	0	29	19	1.9	31.02	15	3.08
SA of Mixed ancestry N=60	19 (.32)	41 (.68)	2	13	15	3.07	13.87	13.06	0.71
SA of European origin N=58	36 (.62)	22 (.38)	12	5	12	11.1	4.2	13.7	0.43

N = Number of chromosomes,
X^2 = chi square test at 1 degree of freedom.

Genotype frequencies of the EcoRI/pK083 polymorphisms in the the Pedi, Venda and Tswana from the Tvl.

Population group	Allele e1 14kb	Allele e2 9kb	Genotype e1e1 Observed (O)	e2e2	e1e2	e1e1 Expected (E)	e2e2	e1e2	X^2
Pedi N=52	9 (.17)	43 (.83)	0	17	9	0.75	17.91	7.28	1.18
Tswana N=20	5 (.25)	15 (.75)	0	5	5	0.625	5.62	3.8	1.073
Venda N=24	5 (.21)	19 (.79)	0	7	5	0.53	7.49	3.96	0.83
SA Black Tvl N=96	19 (.20)	77 (.80)	0	29	19	1.9	31.02	15	3.08

SA Black(Tvl) = Pedi, Tswana and Venda as one group
N = Number of chromosomes,
X^2 = chi square test at 1 degree of freedom.

Genotype frequencies of the MspI/H5.5 polymorphisms in the SA population groups studied.

Population group	Allele		Genotype						X^2
	M1 3.5kb	M2 2.8kb	M1M1	M2M2	M1M2	M1M1	M2M2	M1M2	
			Observed (O)			Expected (E)			
SA Black (Tvl) N=92	15 (.17)	77 (.83)	1	32	13	1.3	31.6	12.98	0.02
SA of Mixed ancestry N=54	15 (.28)	39 (.72)	3	15	9	2.1	13.99	11.07	0.84
SA of European origin N=64	28 (.42)	36 (.58)	6	10	16	5.7	10.76	15.68	0.12

N = Number of chromosomes,
X^2 = chi square test at 1 degree of freedom.

Genotype frequencies of the MspI/H5.5 polymorphisms in the Pedi, Venda and Tswana from the Tvl.

Population group	Allele M1 3.5kb	Allele M2 2.8kb	Genotype M1M1 Observed (O)	Genotype M2M2 Observed (O)	Genotype M1M2 Observed (O)	M1M1 Expected (E)	M2M2 Expected (E)	M1M2 Expected (E)	X^2
Pedi N=48	10 (.21)	38 (.79)	1	15	8	0.87	15.75	7.44	0.09
Tswana N=20	0	20 (1.0)	0	10	0	0	10	0	0.00
Venda N=24	5 (.21)	19 (.79)	0	7	5	0.53	7.49	3.96	0.83
SA Black (Tvl) N=92	15 (.17)	77 (.83)	1	32	13	1.3	31.6	12.98	0.02

SA Black(Tvl) = Pedi, Tswana and Venda as one group
N = Number of chromosomes,
X^2 = chi square test at 1 degree of freedom.

The percentage of heterozygosity for the RFLPs in each population group in this study is shown in Table VII-17. The greater degree of heterozygosity for the EcoRI RFLPs in the SA population of mixed ancestry is possibly due to the genetic admixture of European and African peoples (Table VII-2).

In the ten SA HD families studied, 45% of all individuals were heterozygous for at least one of the four polymorphisms used. In the normal SA population as a whole, 104 unrelated individuals were examined from different population groups, of whom 46% were found to be heterozygous. By combining the HindIII and MspI polymorphisms 77% of all individuals haplotyped were heterozygous at the G8 locus.

TABLE VII-17.

Percentage heterozygosity for the RFLPs used in this project

	SA Black (Tvl)	SA of Mixed ancestry	SA of European origin
HindIII (haplotype)	80	59	46
EcoRI (pK081)	40	53	7
EcoRI (pKo83)	36	50	41
MspI (H5.52)	25	41	47

7.2 DISCUSSION ON THE ALLELE FREQUENCIES ESTABLISHED FOR THE DIFFERENT SA POPULATIONS.

Allele frequencies for the RFLPs at the D4S10 locus in unrelated normal individuals from three different population groups in SA have been established. This was the first comparative study of the G8 allele frequencies in different non-Caucasoid population groups. In most instances, the allele frequencies of the SA population group of European origin were in the same range as those reported for Caucasians. There were, however, differences between the various SA population groups which would influence the choice of markers when initially attempting to establish linkage in an HD family. For example, it would not be advisable to use the pK08I probe on a family of European origin in SA as the 5.5kb fragment was rarely seen in this population group (Table VII-2). In addition, the minor alleles in the European population for the EcoRI RFLPs were the major alleles in the black African population (Table VII-2).

PIC values of greater than 0,5 are considered to be 'highly informative', values between 0,25 and 0,5 as 'reasonably informative' and values less than 0,25 as only 'slightly informative' as defined by Botstein et al. (1980). The calculated PIC values for the HindIII RFLP haplotypes were ' highly informative' for the SA of mixed ancestry and black origins whereas the value calculated for the SA of European origins was 'reasonably informative'(similar to the value reported by Youngman et al. (1980) for the British population). In general , the calculated PIC values for the other 3 RFLPs were all 'reasonably informative' apart from that of the pK08I locus in the SA of European origin.

Furthermore, the frequencies of the B and D haplotypes at the HindIII polymorphic site were remarkably higher in the black African population; this finding must be taken into account when addressing the question of linkage disequilibrium in SA HD families (Table VII-4).

The apparent low frequency of the 3.5kb RFLP (M1) in the Tswana tribal group warrants comment (Table VII-3). Although the number of unrelated normal individuals studied was small, it is interesting to note that in the ten people studied (20 chromosomes) every individual was homozygous for the 2.8kb allele (M2). However, in the Tswana HD family in this study (Fig. VII.3. T9), seven individuals were heterozygous for this RFLP at the G8 locus. It can therefore be concluded that the low frequency of M1 is probably the result of the small number of unrelated normal individuals who were studied. Examination of additional normal unrelated Tswana individuals would confirm or refute this observation.

The lod scores for the individual polymorphisms at various recombination fractions are shown in Tables VII-18, VII-19, VII-20 and VII-21 (given to two decimal places as recommended by Conneally et al., 1985). In summing lod scores across pedigrees, it has been assumed that the same HD locus was involved in all cases as there was no significant evidence in favour of heterogeneity for the families studied according to the test applied.

It was decided after studying the individual lod scores to determine haplotypes by combining the MspI and HindIII RFLPs. The lod scores for these haplotypes are shown in Table VII-22. A maximum lod score of 8.14 at a O of 0.0 was calculated, thus confirming linkage between HD and G8 as was originally found by Gusella in 1983. The maximum likelihood estimate of the recombination fraction was 0.00, with a confidence limit of 0.00-0.058 using the combined haplotypes.

A simple method of constructing confidence intervals recommended by Conneally et al. (1985) was used (Fig VII.2). As the lod score for the combined haplotypes (MspI and HindIII) peaked at a recombination fraction of 0.00, the lod scores were plotted against O values of 0.00 to 0.20. The point of intersection at the lod score of 7.14 which was 1 lod score below the maximum lod score obtained in this study, resulted in a O value of 0.058. The confidence interval is therefore 0.00, 0.058.

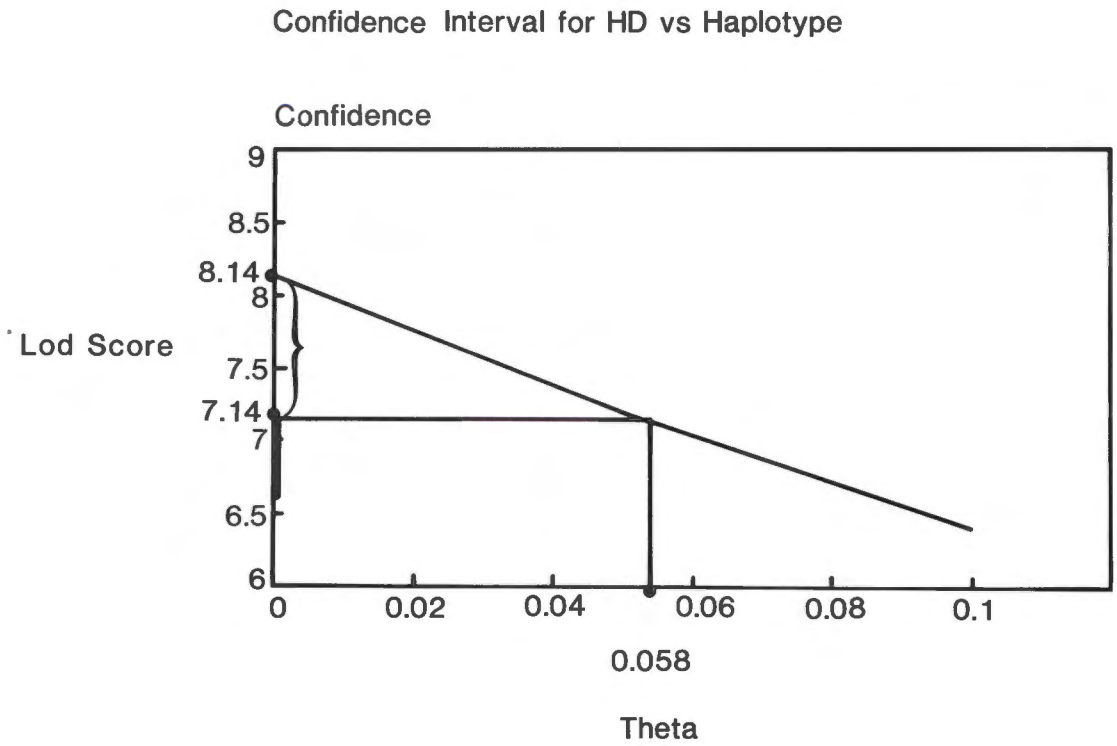


Figure VII.2

Construction of the confidence interval in this linkage study.
θ=0.058
Confidence interval = (0.00, 0.058)

TABLE VII-18.

Individual family lod scores: HD versus HindIII polymorphisms.

Family	Recombination fraction (θ)						Posterior probability of linkage
	0.00	0.05	0.1	0.2	0.3	0.4	
MA1	0.42	0.38	0.35	0.28	0.20	0.11	1.00
MA2	1.04	0.96	0.87	0.68	0.47	0.24	1.00
MA3	0.06	0.05	0.04	0.02	0.01	0.00	1.00
MA4	1.07	0.95	0.82	0.55	0.29	0.08	1.00
MA5	0.12	0.10	0.08	0.05	0.02	0.01	1.00
MA6	0.29	0.24	0.20	0.14	0.08	0.04	1.00
T9	0.09	0.10	0.09	0.07	0.04	0.01	1.00
P11	-0.17	-0.15	-0.13	-0.09	-0.04	-0.01	1.00
E20	0.22	0.19	0.17	0.13	0.08	0.04	1.00
E40	0.50	0.43	0.36	0.23	0.11	0.03	1.00
Total	3.64	3.25	2.86	2.05	1.26	0.55	

TABLE VII-19.

Individual lod scores: HD versus MspI polymorphisms.

Family	Recombination fraction (θ)						Posterior probability of linkage
	0.00	0.05	0.1	0.2	0.3	0.4	
MA1	1.42	1.31	1.17	0.86	0.51	0.17	1.00
MA2	0.70	0.58	0.47	0.29	0.14	0.04	1.00
MA3	1.93	1.74	1.53	1.10	0.65	0.21	1.00
MA4	0.00	0.00	0.00	0.00	0.00	0.00	1.00
MA5	0.43	0.37	0.32	0.20	0.10	0.03	1.00
MA6	0.28	0.23	0.19	0.12	0.08	0.03	1.00
T9	-0.14	-0.06	-0.02	0.02	0.02	0.01	1.00
P11	0.16	0.12	0.08	0 03	0.01	-0.00	1.00
E20	0.15	0.13	0.11	0.08	0.05	0.03	1.00
E40	-0.01	-0.01	-0.01	-0.00	-0.00	-0.00	1.00
Total	4.92	4.40	3.86	2.71	1.56	0.52	

TABLE VII-20.

Individual lod scores: HD versus EcoRI (pK081) polymorphisms.

Family	Recombination fraction (θ)						Posterior probability of linkage
	0.00	0.05	0.1	0.2	0.3	0.4	
MA1	-0.23	-0.14	-0.08	-0.02	0.00	0.00	1.00
MA2	0.26	0.22	0.18	0.11	0.05	0.01	1.00
MA3	0.17	0.14	0.11	0.06	0.03	0.00	1.00
MA4	0.01	0.01	0.01	0.01	0.00	0.00	1.00
MA5	0.18	0.15	0.12	0.07	0.03	0.01	1.00
MA6	0.27	0.23	0.19	0.13	0.08	0.04	1.00
T9	0.27	0.23	0.19	0.11	0.05	0.01	1.00
P11	0.00	0.01	0.01	0.01	0.00	0.00	1.00
E20	-0.00	-0.00	-0.00	-0.00	-0.00	-0.00	1.00
E40	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Total	0.94	0.84	0.73	0.48	0.25	0.08	

TABLE VII-21.

Individual lod scores: HD versus EcoRI (pK083) polymorphisms

Family	Recombination fraction (θ)						Posterior probability of linkage
	0.00	0.05	0.1	0.2	0.3	0.4	
MA1	0.21	0.19	0.17	0.11	0.06	0.02	0.86
MA2	-1.18	-0.97	-0.66	-0.25	-0.07	-0.01	0.21
MA3	1.28	1.16	1.03	0.76	0.46	0.15	0.98
MA4	-0.02	-0.02	-0.01	-0.00	0.00	0.00	0.79
MA5	0.02	0.01	0.01	0.00	0.00	0.00	0.80
MA6	0.08	0.06	0.05	0.02	0.01	0.00	0.82
T9	0.19	0.15	0.12	0.06	0.02	0.01	0.86
P11	0.68	0.67	0.59	0.33	0.16	0.04	0.94
E20	-0.06	-0.09	-0.10	-0.10	-0.07	-0.04	0.77
E40	0.51	0.44	0.37	0.23	0.11	0.03	0.92
Total	1.69	1.61	1.55	1.16	0.68	0.21	

TABLE VII-22.

Individual lod scores: HD versus combined HindIII and MspI polymorphisms.

Family	Recombination fraction (θ)						Posterior probability of linkage
	0.00	0.05	0.1	0.2	0.3	0.4	
MA1	1.37	1.32	1.23	0.98	0.63	0.25	1.00
MA2	2.10	1.86	1.61	1.10	0.62	0.24	1.00
MA3	2.33	2.11	1.87	1.36	0.81	0.27	1.00
MA4	0.79	0.70	0.61	0.40	0.20	0.05	1.00
MA5	0.36	0.31	0.27	0.17	0.09	0.02	1.00
MA6	0.30	0.25	0.21	0.14	0.08	0.04	1.00
T9	-0.02	-0.03	-0.03	-0.02	-0.01	-0.00	1.00
P11	0.00	0.01	0.00	-0.01	-0.02	-0.01	1.00
E20	0.20	0.16	0.12	0.06	0.02	0.00	1.00
E40	0.73	0.62	0.51	0.30	0.13	0.03	1.00
Total	8.14	7.30	6.39	4.47	2.55	0.09	

On examination of the RFLP haplotypes in eleven spouses from the six families of mixed ancestry, representing the general population of mixed ancestry in SA, five different haplotypes could be identified (Table VII-23). The most frequent haplotype associated with the disease in four of the six HD families of mixed ancestry did not represent the most common haplotype in the general population. In fact, only two of the five different haplotypes appeared to be linked with HD. The HD gene appeared to be cosegregating with haplotype V more often than expected (Fisher's p=0.0034) which is suggestive of non-random association in these SA HD families of mixed ancestry.

Pedigrees and some of the genotypes of the ten families studied are shown in Fig VII.3 and Fig VII.4.

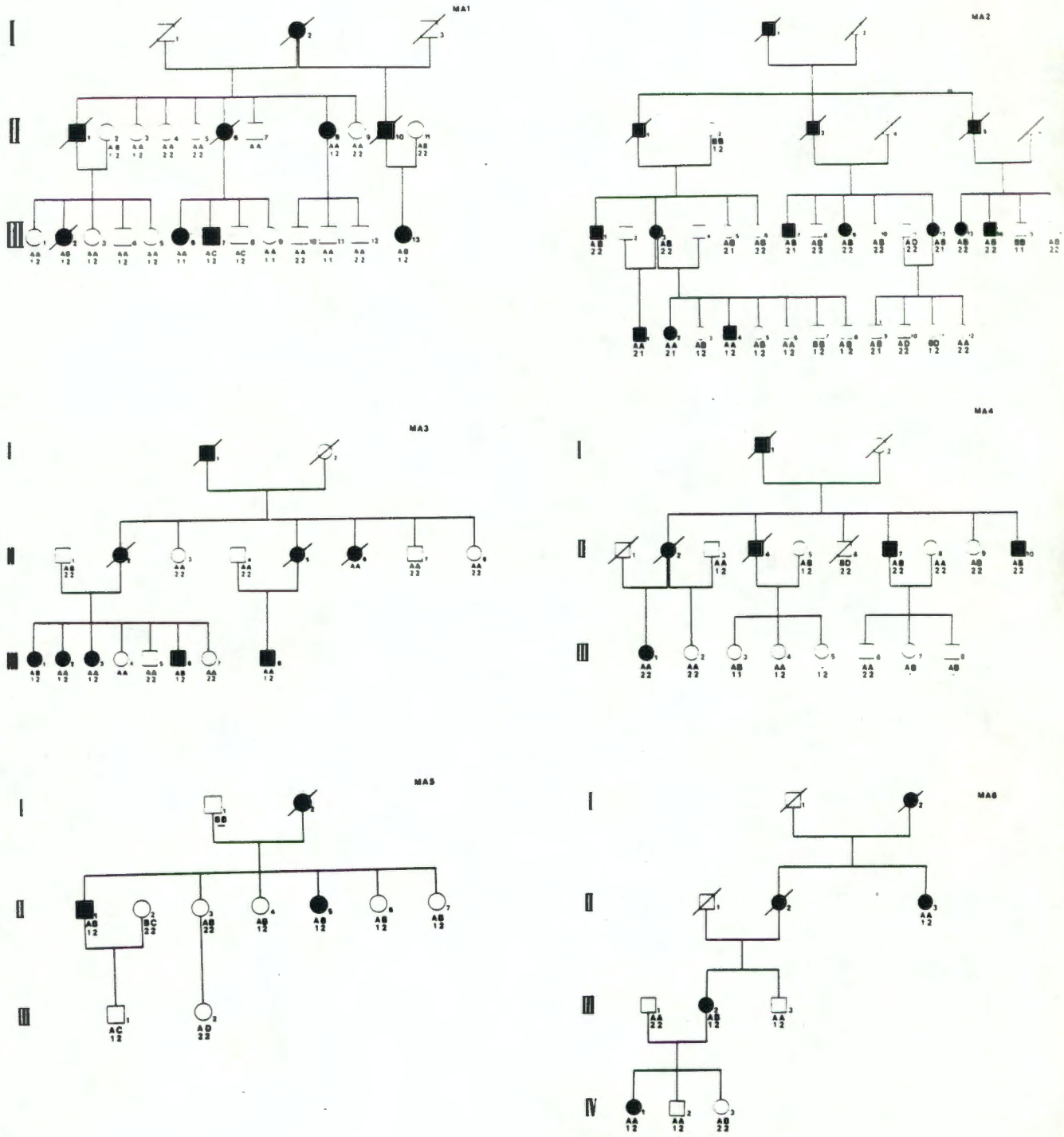
Figure VII.3

Pedigrees of the ten SA HD families studied with haplotypes of the combined MspI and HindIII RFLPs.

Key to Figure VII.3

The HindIII polymorphic sites are combined as haplotypes designated by A,B,C,and D according to the nomenclature of Gusella (Gusella et al.,1983). The MspI 3.5kb and 2.8kb polymorphic bands are indicated by 1 and 2 respectively.

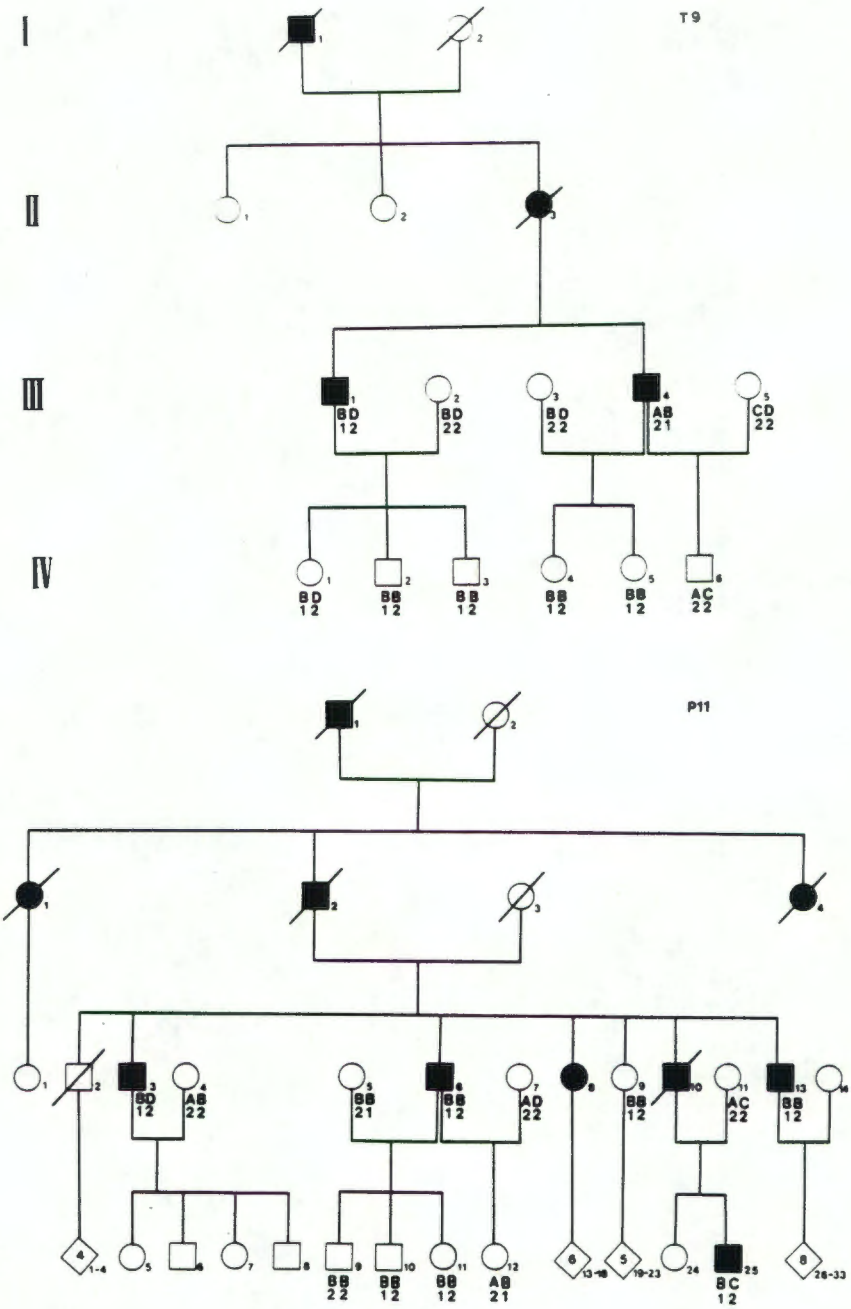
Squares represent males and circles females. Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.



Key to Figure VII.3

The HindIII polymorphic sites are combined as haplotypes designated by A,B,C,and D according to the nomenclature of Gusella (Gusella et al.,1983). The MspI 3.5kb and 2.8kb polymorphic bands are indicated by 1 and 2 respectively.

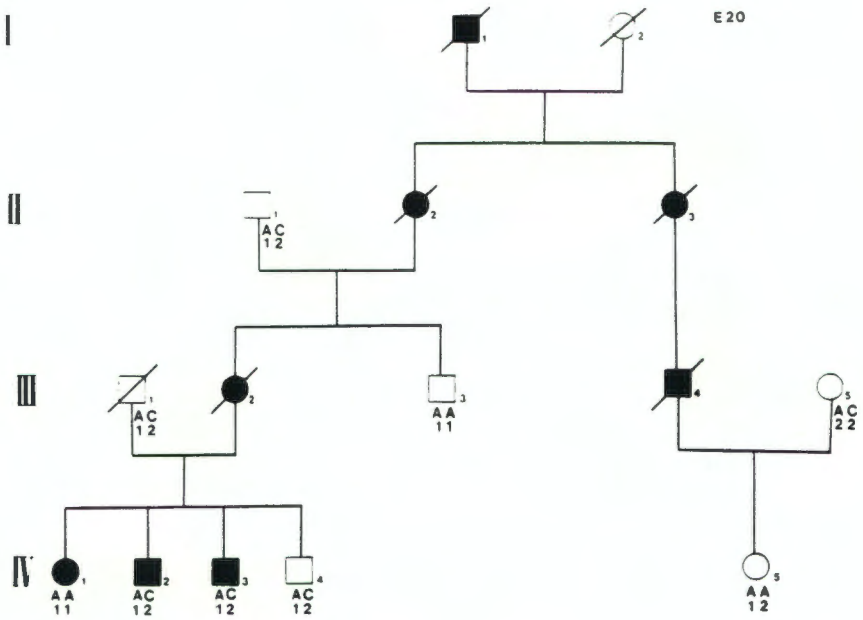
Squares represent males and circles females. Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.

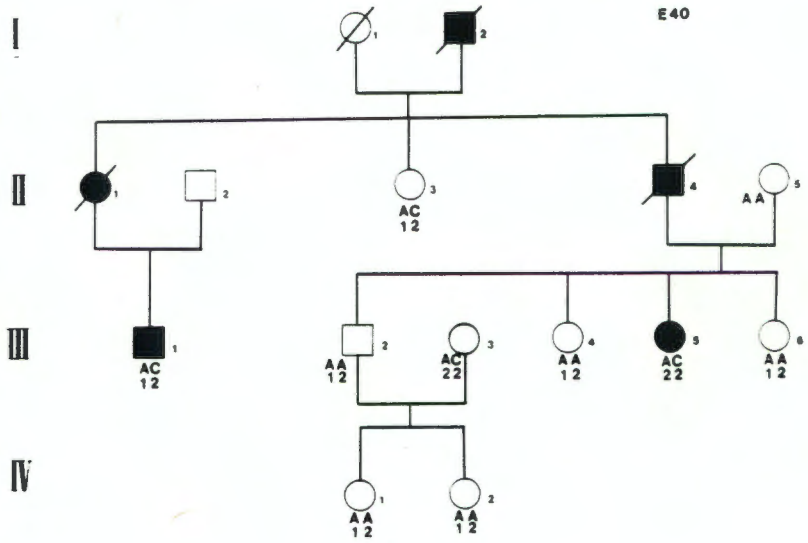


Key to Figure VII.3

The HindIII polymorphic sites are combined as haplotypes designated by A,B,C,and D according to the nomenclature of Gusella (Gusella et al.,1983). The MspI 3.5kb and 2.8kb polymorphic bands are indicated by 1 and 2 respectively.

Squares represent males and circles females. Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.





Key to Figure VII.3

The HindIII polymorphic sites are combined as haplotypes designated by A,B,C,and D according to the nomenclature of Gusella (Gusella et al.,1983). The MspI 3.5kb and 2.8kb polymorphic bands are indicated by 1 and 2 respectively.

Squares represent males and circles females. Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.

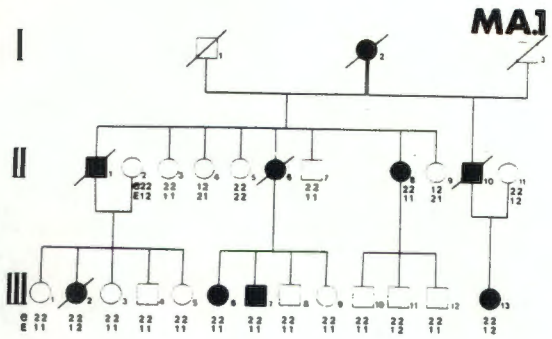
Figure VII.4

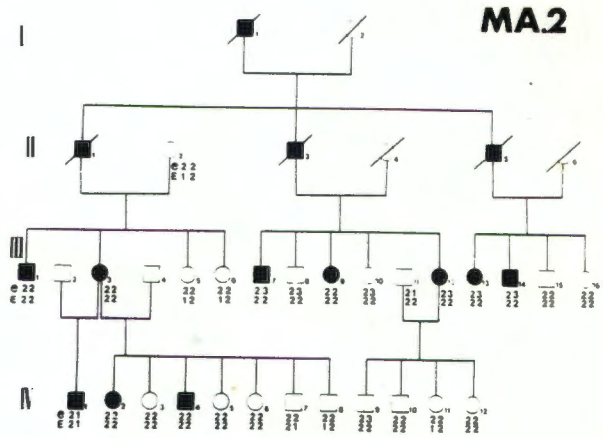
Pedigrees of the ten SA HD families studied with the EcoRI RFLPs.

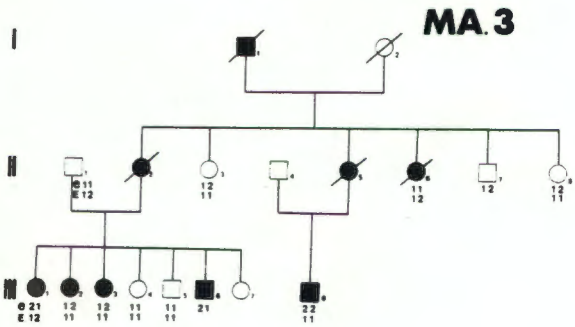
Key to Figure VII.4

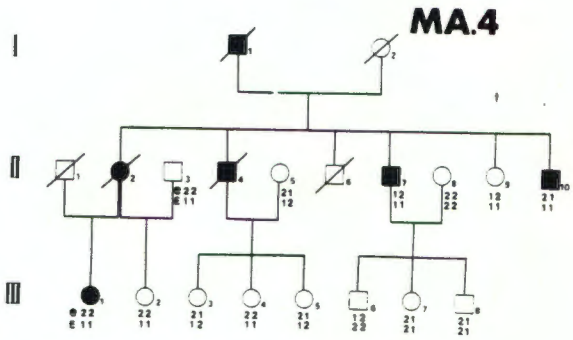
The EcoRI/pK081 6.0kb and 5.5 kb RFLPs are depicted by E1 and E2 respectively. The EcoRI/pK083 14kb, 9kb and 11kb RFLPs are depicted by e1, e2 and e3 respectively.

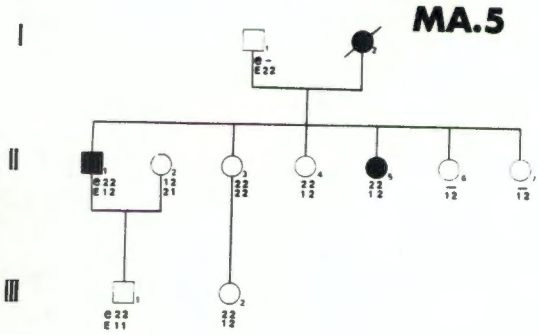
Squares represent males and circles females.Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.

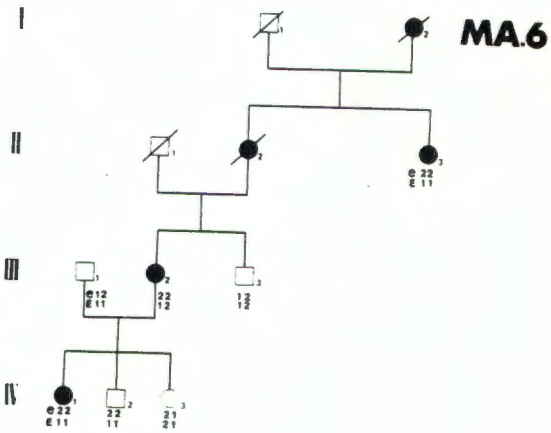








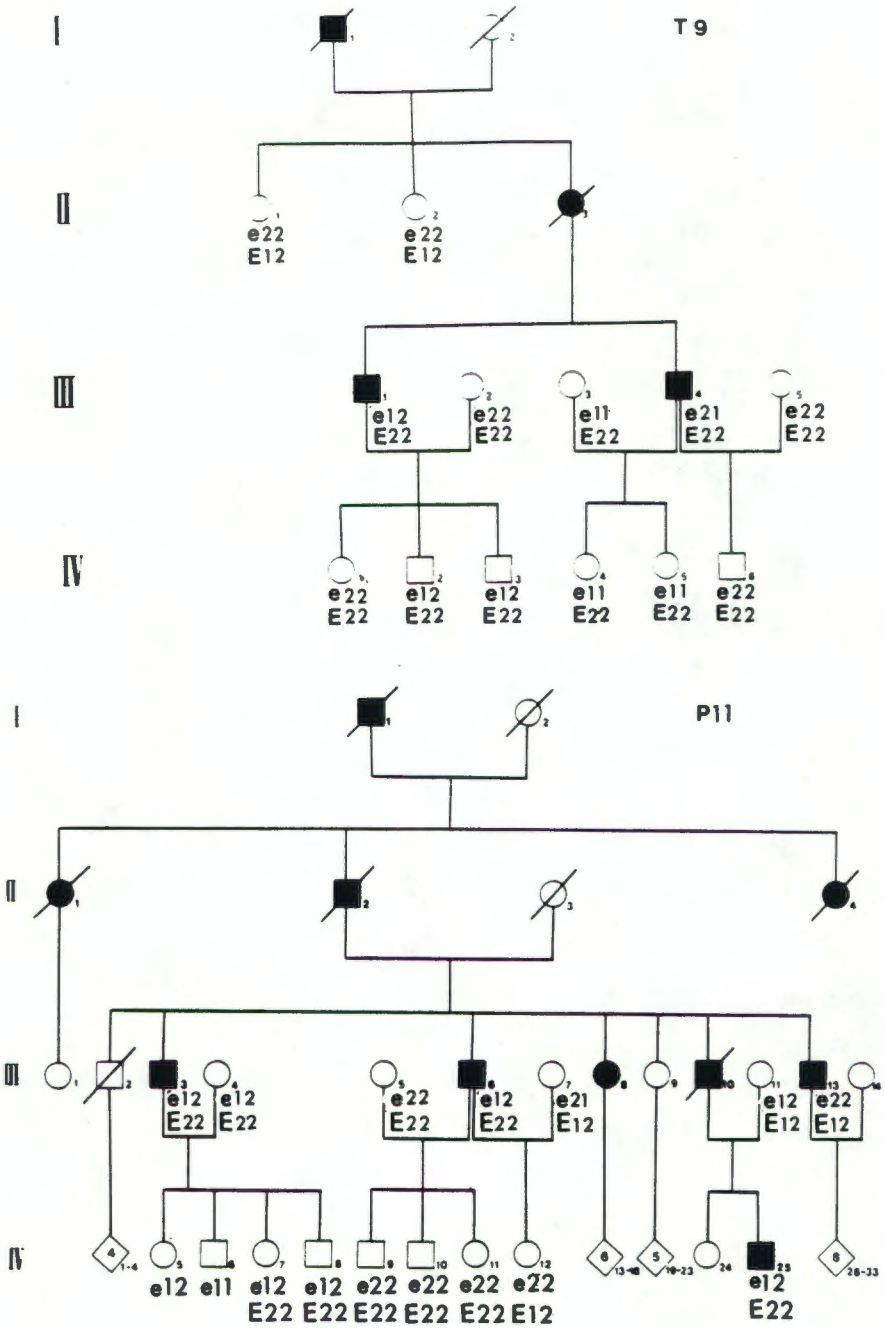




Key to Figure VII.4

The EcoRI/pK081 6.0kb and 5.5 kb RFLPs are depicted by E1 and E2 respectively. The EcoRI/pK083 14kb, 9kb and 11kb RFLPs are depicted by e1, e2 and e3 respectively.

Squares represent males and circles females.Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.



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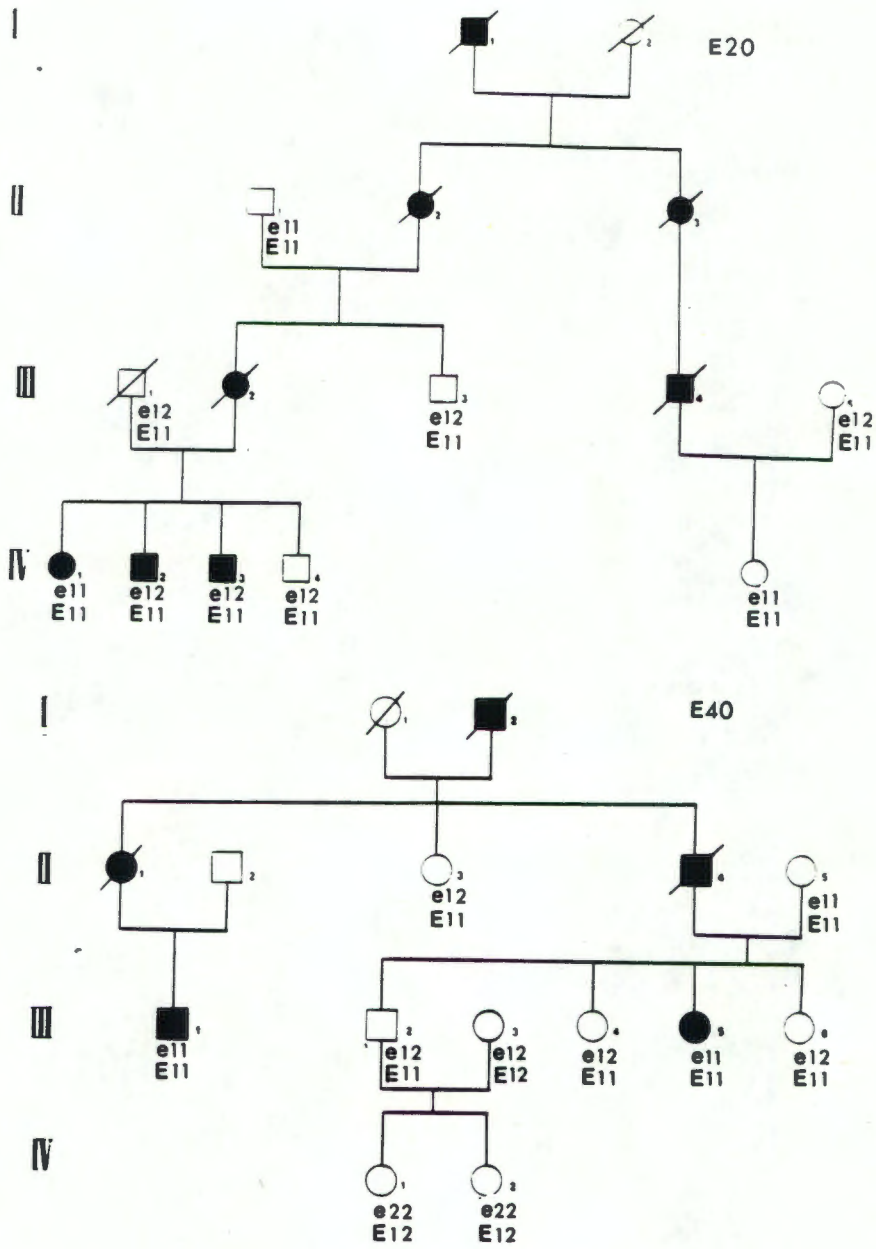
Squares represent males and circles females.Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.



Key to Figure VII.4

The EcoRI/pK081 6.0kb and 5.5 kb RFLPs are depicted by E1 and E2 respectively. The EcoRI/pK083 14kb, 9kb and 11kb RFLPs are depicted by e1, e2 and e3 respectively.

Squares represent males and circles females.Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased.

TABLE VII-23.

(a) Haplotypes in the SA population of mixed ancestry and those associated with HD.

The distribution of the combined HindIII and MspI polymorphisms in the general SA population of mixed ancestry. Associated HindIII and MspI haplotypes in 6 HD families of the same ethnicity. (H5.5probe M1 = 3.5kb allele M2 = 2.8kb allele)

Probe (Restriction Enzyme)	Haplotype I	II	III	IV	V
pK082 (HindIII)	A	B	B	D	A
H5.5 (MspI)	M2	M2	M1	M2	M1
General population of mixed ancestry (22 chromosomes)	13	6	1	1	1
Haplotype associated with HD in 6 SA HD families of mixed ancestry	2				4

(b) Haplotypes in the individual families from mixed ancestry, associated with HD.

Haplotypes of 3 HD patients in MA1	1		1	4
Haplotypes of 7 HD patients in MA2	7	4	1	2
Haplotypes of 5 HD patients in MA3	3	2		5
Haplotypes of 3 HD patients in MA4	4	2		
Haplotypes of 2 HD patients in MA5		2		2
Haplotypes of 3 HD patients in MA6	2	1		3

7.4 DISCUSSION ON THE LINKAGE STUDY OF SA HD FAMILIES.

Genetic linkage has been confirmed in ten SA HD families from different ethnic backgrounds at the D4S10 locus using HindIII, EcoRI and MspI RFLPs. Eight of the ten families investigated were informative with the markers used in the study while in the other two phase could not be established. The individual lod scores were low due to the family structure and number of living affected persons and did not appear to be as a result of the detection of recombinants.

The estimated recombination fraction using the combined haplotypes in this study was very low since no apparent recombination events were observed. Together with the confidence limit of 0.058, this information can be usefully applied when a predictive service is introduced in SA.

Although the number of HD patients of mixed ancestry in this study was relatively small (23 in six families), the possible non-random association between the HD gene and the MspI/HindIII haplotypes in the SA HD patients of mixed ancestry would imply a founder effect. Further examination of HD patients from this population is necessary to confirm or refute this observation.

A major limitation of using linkage analysis for predictive testing is the family structure (Harper and Sarfarazi, 1985a). DNA from 45 SA families in which a history of HD has been documented is being stored at the University of Cape Town DNA banking centre (Greenberg 1989). In 66% of these families there are not sufficient living affected individuals to establish linkage with G8.

Confirmation of the above findings could be usefully applied on the introduction of predictive service for HD. The use of linkage disequilibrium data could allow substantial risk modification for some SA HD families of mixed ancestry. In addition, in certain instances, predictive testing could be performed on an at-risk individual without DNA being available from an affected relative (Beaudet et al., 1989). However, as disequilibrium data for one ethnic group may differ drastically from data for others, this finding, if confirmed, would be of benefit only to the Cape population of mixed ancestry (Ikonen et al., 1989).

When a predictive testing service is eventually established in SA, two additional markers that have recently been obtained will be used. D4S95 (Wasmuth et al., 1988) and D4S96 (Smith et al., 1988) are presently being investigated for heterozygosity in SA in a collaborative study with M Hayden, Vancouver.

Apart from the fact that these markers are more tightly linked to HD, the use of these additional markers should decrease the chance of individuals being uninformative due to homozygosity at the marker locus. Furthermore, the combined use of more DNA markers would increase the informativeness of family structures previously thought to be inadequate for linkage analysis when DNA markers only from the G8 region were used (Hayden et al., 1988a).

7.5 RESULTS OF THE LINKAGE STUDY OF A BLACK AFRICAN HD FAMILY.

The individual RFLP lod scores for the different probes used in this investigation are listed in Table VII-24. In Table VII-25 the linkage analyses of the combined haplotypes (Fig.VII.5) using eight markers is compared with that of the study using only two markers in the G8 region (Table VII-22:P11).

The improved lod score of 1.16 at a θ of 0.00 is better than the initial lod score of 0.00 at θ=0. The limited number of living affected individuals in this family however precludes further studies to confirm or refute linkage in this family.

The probe 678 (D4S96) was uninformative in this family study as all the individuals investigated with this probe were homozygous at the marker locus (for the 1.4kb allele). Additional investigation of 25 unrelated Pedi, Tswana and Venda individuals also revealed no heterozygosity at this marker locus (unpublished data). It is noteworthy that this MspI restriction site does not appear to be present in the all the individuals investigated from the SA black population (Tvl) in this study. In addition, there was a low frequency of the 3.5kb RFLP in the Tswana tribal group (also a MspI restriction site) reported in Chapter 7.2.

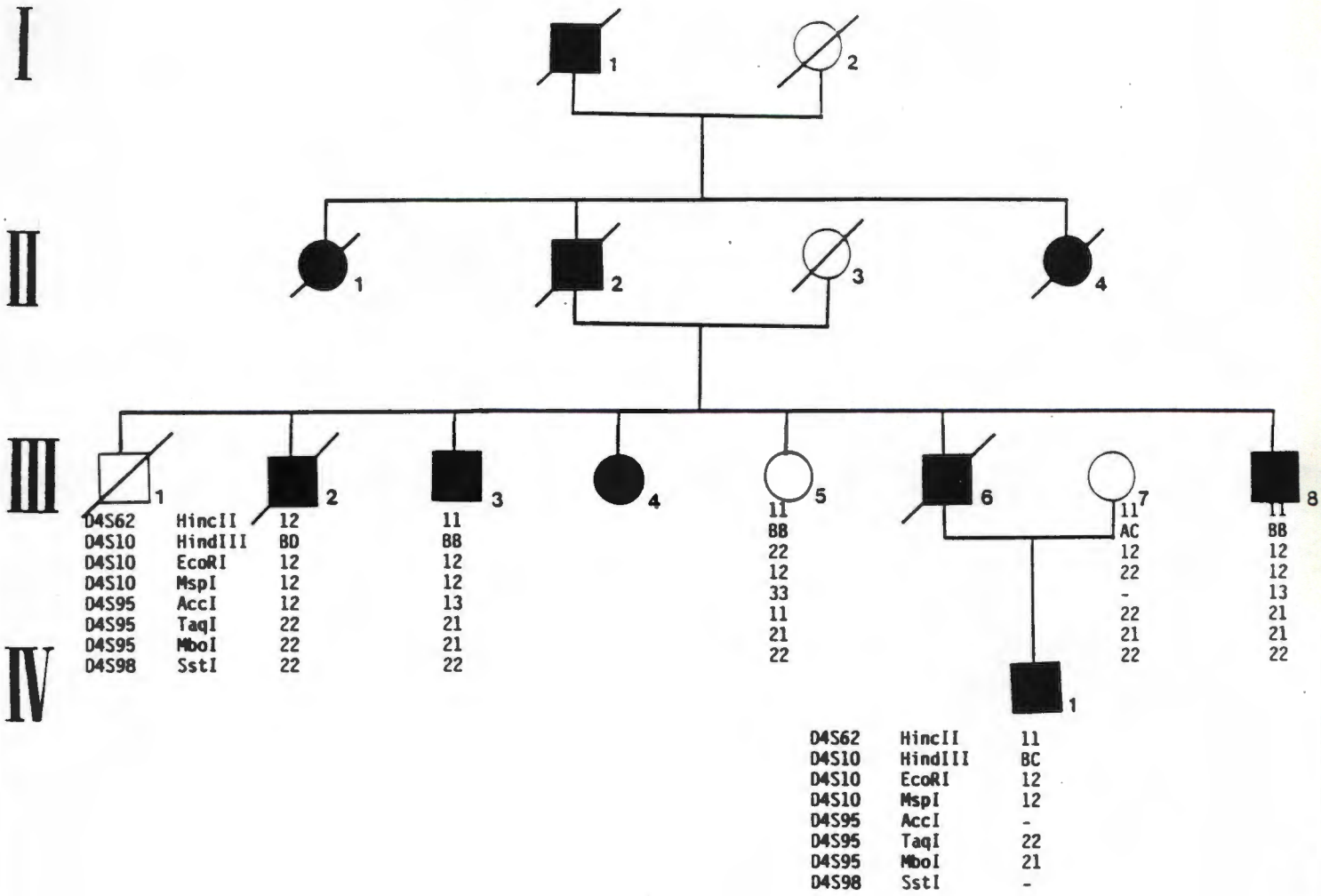


Figure VII.5

Condensed pedigree of the SA black HD family (P11) with haplotypes of the 8 markers used on chromosome 4.

Genotypes of the markers used on chromosome 4 known to be linked to HD as listed, with references, in Table VI-1. Haplotypes were determined by analysing DNA of the 4 individuals in generation III and their offspring and assigning haplotypes for the deceased parents. Individual III-6's haplotype was inferred from that of his affected son (IV-1).

TABLE VII-24.

Individual RFLP lod scores for recombination fractions 0 to 0.40 HD versus HincI, HindIII, EcoRI, MspI, AccI, TaqI, MboI, SstI polymorphisms.

Enzyme	Recombination fraction (θ)								
	0.00	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4
HincII	0.18	-0.17	-0.15	-0.13	-0.10	-0.07	-0.05	-0.03	-0.01
HindIII	-0.17	-0.15	-0.13	-0.11	-0.09	-0.07	-0.05	-0.03	-0.01
EcoRI	0.66	0.57	0.49	0.40	0.32	0.24	0.16	0.09	0.04
MspI	0.16	0.12	0.08	0.05	0.03	0.02	0.01	0.00	-0.00
AccI	0.56	0.48	0.40	0.32	0.25	0.18	0.12	0.07	0.03
TaqI	0.56	0.49	0.41	0.33	0.26	0.19	0.13	0.07	0.03
MboI	0.28	-0.25	-0.22	-0.17	-0.13	-0.09	-0.06	-0.03	-0.02
SstI	-0.00	-0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE VII-25.

Haplotype lod scores for recombination fractions 0.00 to 0.40

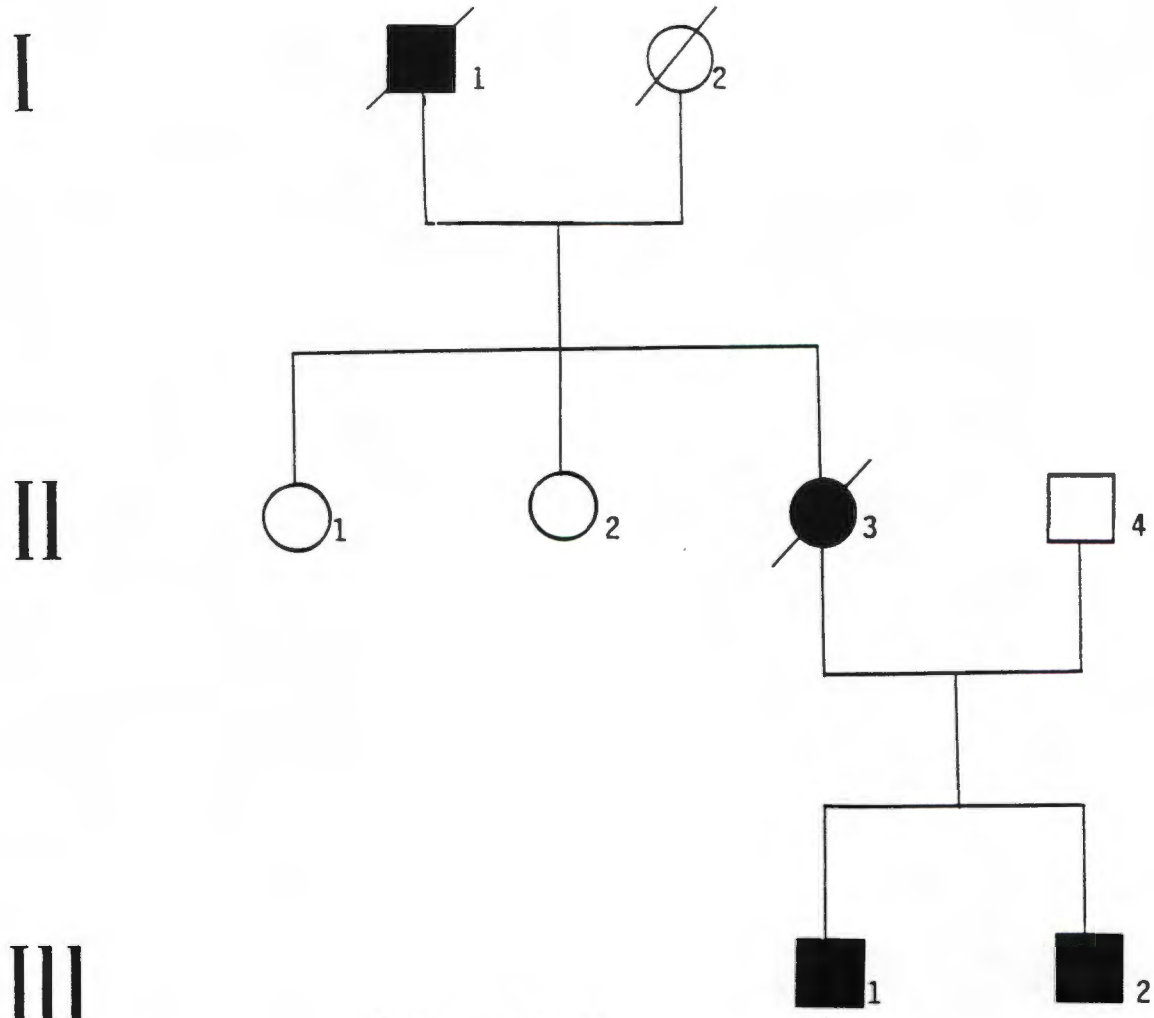
Haplotype	Recombination fraction (θ)								
	0.00	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4
Hap8	1.16	1.03	0.89	0.75	0.60	0.45	0.31	0.18	0.08
Hap2	0.01	0.01	0.01	-0.00	-0.01	-0.02	-0.02	-0.01	-0.01

Hap8 = HD versus combined HincI, HindIII, EcoRI,MspI,AccI,TaqI, MboI,SstI RFLPs
Hap2 = HD versus combined HindIII and MspI RFLPs

7.6 ADDITIONAL GENOTYPING OF OTHER BLACK HD PATIENTS.

As a result of the extended linkage study on the one black African HD family (P11), the combined haplotype that appeared to be associated with HD in this family was 1-B-1-1-1-2-2-2 (HincII, HindIII, EcoRI, MspI, AccI, TaqI, MboI, SstI) (Fig. VII.5). The second black African HD family (T9) in the study was too small to establish linkage, nevertheless the two affected individuals were genotyped with the eight probes used in the extended study of the other black African family (Fig VII.4 and Fig VII.5).

The genotypes of these two individuals with AccI and TaqI are 33 and 11 respectively. This implies that, assuming linkage with the all markers used on chromosome 4 and HD, and that there have been no recombination events between the markers used and HD in any of the individuals in this study, the genotypes co-segregating with HD appears to be different in family T9 to that of P11.



D4S62	HincII	11	--
D4S10	HindIII	BD	AB
D4S10	EcoRI	12	21
D4S10	MspI	12	21
D4S95	AccI	33	33
D4S95	TaqI	11	11
D4S95	MboI	11	21
D4S98	SstI	22	22

Figure VII.6

Condensed pedigree of the SA black HD family (T9) with additional genotyping.

Genotypes of the markers used on chromosome 4 known to be linked to HD as listed, with references, in Table VI.1.

7.7 DISCUSSION ON THE MOLECULAR STUDY OF SIX BLACK AFRICAN HD PATIENTS.

The limited number of living affected individuals in the Pedi (P11) family precludes further linkage studies to improve the lod score which is at present not proof of linkage. If tight linkage with the markers on chromosome 4 and HD is assumed and no recombination has occured between HD and any of the markers used, the genotyping of the additional black African HD patients indicates that HD is associated with different RFLPs in the two black African families.

Furthermore, in both black African HD families, the disease does not appear to be associated with the same haplotype as in the SA HD families of mixed ancestry or European origins (Fig. VII.3).

The lod scores obtained in this study are low and can only be improved on further examination and genotyping of additional HD patients. However, the results do imply that the gene for HD is associated with different RFLPs in SA HD families from different population groups.

Theilman et al. (1989a), Snell et al. (1989) and more recently Dodge et al. (1990) have reported evidence for non-random association between D4S95 and HD. Furthermore, it has been found that the AccI allele system is a difficult restriction site to diagnose as there have been reports of methylation at the D4S95 locus (Pritchard et al., 1989b ; Theilman et al., 1989b). It is therefore not possible to compare results from different countries without establishing allele frequencies for each population group and thereby standardizing data for each laboratory when attempting to establish evidence for linkage disequilibrium.

Following the additional genotyping of the other black HD patients it was decided to genotype eight patients from families of mixed ancestry with D4S95 and TaqI RFLPs. The preliminary results of this additional study have not changed the linkage data on the families investigated with TaqI RFLPs and D4S95. Further investigation with this marker is necessary as the numbers are still too few to be of any significance.

CHAPTER 8 A NEW GENETIC VARIANT DETECTED DURING THIS STUDY.

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8.2 Discussion on the new variant and evidence of possible recombination within the D4S10 locus..... 151

CHAPTER EIGHT

A NEW GENETIC VARIANT DETECTED DURING THIS STUDY

8.1 THE NEW ECORI/PK083 VARIANT.

During the course of this study a hitherto unreported genetic variation was detected with the pK083 probe when an unexpected 11kb EcoRI restriction fragment was identified in a nuclear section of a family of mixed ancestry. This variant may represent a polymorphism but is of a very low frequency and so would be of little value in linkage analysis. It caused considerable confusion in the linkage analysis of this SA HD family (MA2) which will be discussed in detail in the following chapter.

8.1.1 The family and the variant:

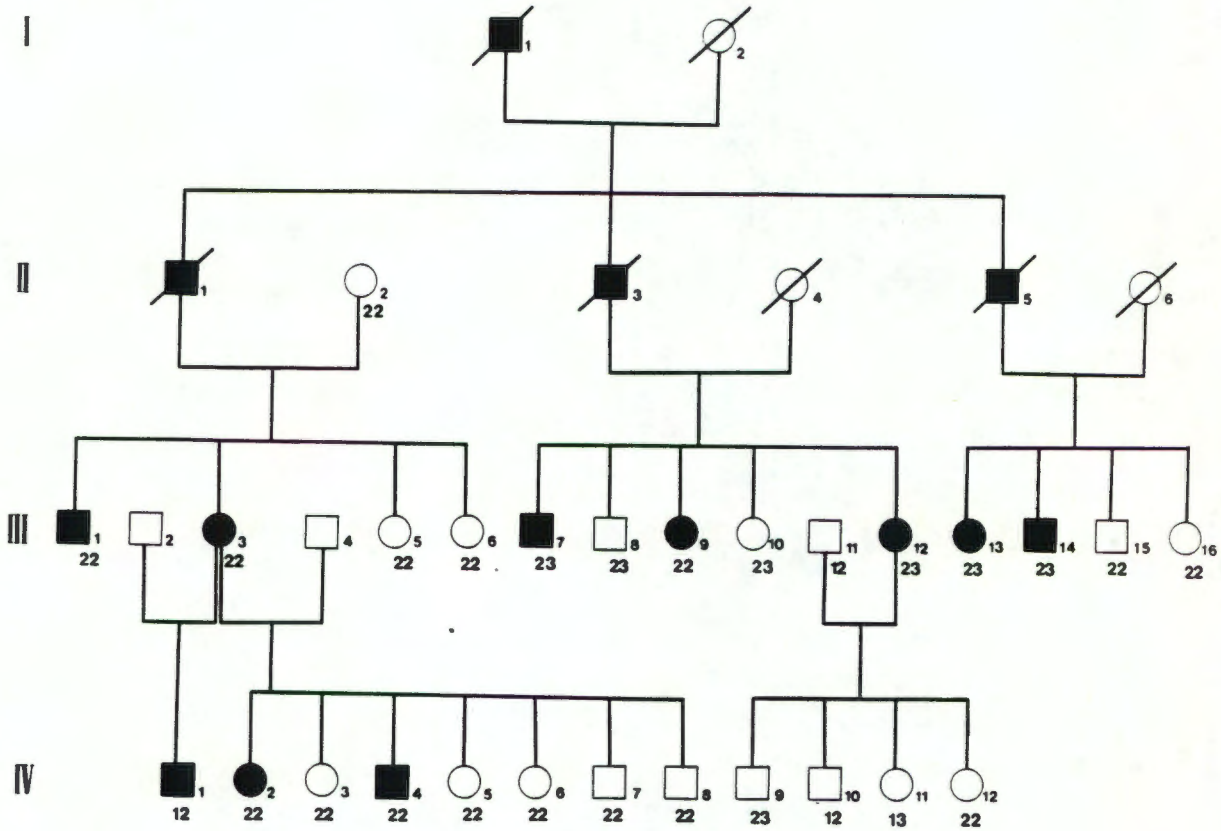
MA2 is a large SA family of mixed ancestry with a history of HD (Fig VIII.1). All individuals in generations III and IV were under the age of 60 years at the time of the study and were therefore NOT included in the computation of the linkage data. The ages of onset of the HD patients in this family were all between 30 and 40 years or younger.

On examination of the EcoRI genotypes indentified with the pK083 probe an affected female (Fig.VIII-1;111.12), her affected brother (III.7), two unaffected sibs (III.8 an III.10), two cousins (III.13 and III.14) as well as two unaffected children (IV.9 and IV.11) had inherited the new 11kb (identified as 3 on Fig.VIII.1) variant.

The Mendelian inheritance of the rare 11kb polymorphic band identified with pK083 and EcoRI is illustrated in an autoradiograph in Fig. VIII.2.

Figure VIII.1

Pedigree of a large SA family of mixed ancestry in which the new variant was detected.



Key to Figure VIII.1

Squares represent males and circles females. Symbols for affected persons are solid and a diagonal line through the symbol indicates deceased. G8 genotypes are depicted by 1,2 and 3 indicating the 14, 9 and 11 kb bands respectively (EcoRI/pK083 RFLPs).

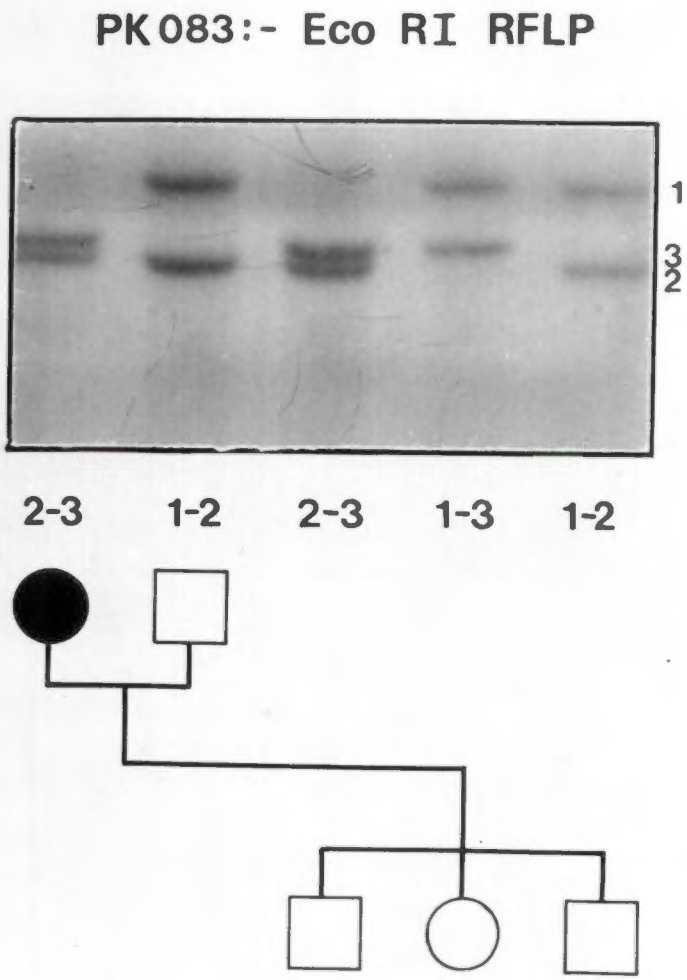


Figure VIII.2

Autoradiograph of the new variant detected during this study.

Key to Figure VIII.2

1, 2 and 3 represent the 14, 9 and 11kb bands respectively (EcoRI/pK083 RFLPs).

During the course of this project, a total of 250 people from different genetic backgrounds were genotyped with this polymorphic system and only the nine individuals reported here have the variant band (a total of 135 SA individuals of mixed ancestry were genotyped of whom 46 were unrelated controls from the general population).

8.1.2 Linkage data:

Linkage analysis of this RFLP system revealed that in individuals 1-6 in generation III and 1-8 in generation IV, HD appeared to be inherited with the 9kb fragment (indicated by 2 on Fig.VIII.1). However, taking into account that there was a new variant detected in this family, it appeared that in the rest of the pedigree, HD was associated with the rarer allele, with one notable exception (III-9). This assumption was based on the fact that, due to the rarity of the 11kb band, it was less likely that the originals (individuals from the general population) in generation II (II-4,II-6) would bothhave had this rare allele (unless they were related) and it was therefore more probable that the two affected males in that generation had had the 11kb band (II-3, II-5).

Linkage data of the computer analysis of these results in Table VIII-I indicated a negative lod score for all values of θ, when only the affected individuals, as well as at-risk persons over the age of 60 years were used in the linkage analysis. This result indicated the possibility of recombination between HD and pK083, the significance of which is discussed in 8.2.

TABLE VIII-1.

Linkage Data of family with new variant and possible recombination within D4S10.

θ	lod-score EcoRI RFLPs	lod-score minus III-9 EcoRI RFLPs	lod-score combined haplotypes of MspI & HindIII RFLPs
0.4000	-0.00	0.04	0.24
0.3000	-0.07	0.10	0.62
0.2000	-0.26	0.09	1.10
0.1000	-0.66	-0.10	1.61
0.0500	-0.97	-0.35	1.86
0.0100	-1.16	-0.81	2.09
0.0000	-1.17	-1.07	2.10

8.2 DISCUSSION ON THE NEW VARIANT AND THE POSSIBILITY OF RECOMBINATION WITHIN THE D4S10 LOCUS.

The new rare variant revealed with pK083 and EcoRI was only detected in a single SA HD family.

Initially it was thought that the variant had increased the informativity of the probe in this family, but on examination of the pedigree (Fig VIII.1), it was apparent that all individuals genotyped from generation III. 1-6 and generation IV.1-8 were homozygous at the marker locus (with the exception of IV-1).

Assuming that HD is linked to pK083, the disease must have been associated with the 9kb allele (indicated as 2 in Fig. VIII.1) in this family, but the affected individuals were uninformative at the marker locus. With the appearance of the new variant in generation III-7,8,10,12,13,14 and generation IV-9,10,11 and continuing with the assumption that HD was associated with the 9kb allele, it followed that this system would be informative in the heterozygous affected individuals (III-7,12,13,14).

Computation of the linkage data indicated that the above assumption was in fact incorrect and there were indications that recombination could have occurred between pK083 and HD in this family (Table VIII-1).

The negative lod score at all values of θ could be indicative of recombination assuming that the new variant first appeared in generation I or II in the pedigree and that individuals II.4 and II.6 were not related and therefore less likely for both having had the new variant(Fig VIII.1).

Furthermore when other more distal markers from G8 were used, a postive lod score of 2.09 at $\theta = 0.00$ was calculated (Table VII-22, MA2), thus indicating that recombination had possibly occurred within the G8 locus. In addition, by removing the one obvious recombination event (Fig VIII.1; III-9), the lod score peaked at a recombination fraction of $\theta=0.3$. (Table VIII-1). These results implied that there was more than one possible recombination event within the D4S10 locus in this family.

There had been previous reports of recombination within the D4S10 locus and the results of the present study substantiated the orientation of the restriction sites suggested in Fig.V.4 (Youngman et al., 1986, Gilliam et al., 1987, Hollaway et al., 1989, Curtis et al., 1989, Skraastad et al., 1989).

This family study demonstrates the importance of using multiple markers at the D4S10 locus and it is now advisable to use newer markers that are closer to the HD locus for presymptomatic testing for HD (Hayden et al., 1988a). This experience also emphasises the necessity for computation of genotype results in linkage analysis especially when this type of analysis is to be used for eventual predictive testing.

SECTION III: QUESTIONNAIRE SURVEY

CHAPTER 9 INTRODUCTION TO THE CAPE TOWN QUESTIONNAIRE SURVEY:

CHAPTER 10 METHODOLOGY:

CHAPTER 11 RESULTS, DISCUSSION AND PROGRAMME PROPOSAL:

SECTION III

QUESTIONNAIRE SURVEY

Chapter 9 INTRODUCTION TO THE CAPE TOWN QUESTIONNAIRE SURVEY:

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CHAPTER NINE

INTRODUCTION TO THE CAPE TOWN QUESTIONNAIRE SURVEY:

9.1 REVIEW OF THE ETHICAL ISSUES INVOLVED WITH PREDICTIVE TESTING

Essentially, the situation brought about by the availability of presymptomatic testing for HD, is as follows: the genotype of some clinically 'normal' individuals can now become known well in advance of the signs and symptoms which are the usual indicators of the disease. These at-risk persons will thus be put in a unique category in that their fate could be revealed to them many years before they would normally have been aware of it. Once presymptomatic testing for HD becomes available and publicised there is the danger that there might be pressure on the at-risk person to undergo testing, not only for their own self knowledge concerning their risk status, but to inform others who might be affected thereby. Family, potential marriage partners, employers, business partners and insurance agencies would all be privy to information which should be private and personal. The question arises as to whether pension funds and prospective employers for example have the right to demand presymptomatic testing for HD prior to entering into a contract with someone at risk (Lamport, 1987). In this context, according to a special article in a recent SA medical journal (Taitz, 1990) it would appear that currently in SA it would not be an unlawful breach of the rule of medical confidentiality if a medical professional were to divulge information for the public good, even against the patient's wishes. Confidentiality however, is not absolute and each case must be considered on its own merits (Benatar, 1990).

Furthermore there is the danger that insurance companies could insist on presymptomatic testing for at-risk individuals and could either deny them cover on the basis of the results or load the premiums accordingly. Applications for mortgage loans and medical- aid insurance could be jeopardized by an increased risk result of a presymptomatic test for HD.

A new aspect of the basic problem of balancing the needs and rights of the individual with the demands and interests of society has become apparent. As society is always stronger in this conflict, special care must be taken to safeguard the legitimate interests of the individual. The rights of the individual must at all times be protected and should not be discarded in preference to other interests (Kolata, 1986).

A.E.H Emery published a paper in 1980 titled

'Whether or not predictive tests'.

He wrote

> " In my experience, having discussed this matter with many at risk individuals, most would prefer to live in the hope of being unaffected rather than risk being told that there is a certainty of becoming affected."

Furthermore:

> " Taking all these considerations into account, is it justified to pursue research on predictive testing? My feeling is that this would only be justified once an effective treatment has been found; then, many of the problems now associated with predictive tests would disappear. The main concerns of research in this disorder should be furthering our understanding of the cause and above all, finding an effective treatment."

These sentiments were also expressed by others prior to the availability of a predictive test.(Stevens, 1971; Stern and Eldridge, 1975; Thomas, 1982).

The discovery of a molecular marker for HD and the definite possibility of a 96% accurate predictive test for certain individuals, has resulted in numerous surveys being undertaken worldwide to ascertain the attitudes of at-risk individuals towards presymptomatic testing. The general consensus is that most people would in fact, want to know their risk status, though some stated that they still had certain reservations and would prefer to wait until the test was 100% accurate (Evers-Kiebooms et al., 1987; Markel et al., 1987; Kessler et al., 1987; Mastromauro et al., 1987; Meissen and Berchek., 1987).

There is a growing opinion amongst professionals that the medical profession should not become submerged in the new technology and that predictive testing should be withheld until something positive could be offered to those for whom the risk is increased (Robert, 1985; Shaw, 1987; Smurl and Weaver, 1987). However, consideration must be given to the fact that there are possible legal aspects regarding counsellors obligations to provide the test. (Bains, 1986).

In any event there are at-risk individuals who maintain that the uncertainty of whether or not they will eventually develop the disease is too much to bear. Some have indicated that they would rather know that they had inherited the gene for HD and so end the ambiguity. Possession of this knowledge would enable them to prepare and plan their lives accordingly. They would be able to make decisions regarding marriage and procreation, long term business and/or career planning and about informing children of <u>their</u> risk status.

In addition, preparation could be made for the care of the patient and the maintenance of the family after the onset of the disease (Dalby, 1986; Crauford and Harris, 1986).

As one person explained:

> **"I need to know whether retiring will mean buying a motor home or hiring a nurse."**

A woman, aged 32, taking part in the predictive testing programme in the UK said that she needed to know what her risk status was, as, amongst other things, she wanted to learn to drive. She said she realised that it would not be advisable to do so if the test were to be positive (personal communication).

In general, it would appear that people at risk were more concerned about finding out if they were gene carriers and how they would live with the results rather than how society would use this information against them.

Finally, there is the danger of introducing a new psychiatric illness; one where an individual knows that he has a 96% chance of developing a dreadful, lethal, incurable condition sometime in the future. This knowledge could limit and drastically alter his entire adult life and not just the last ten to fifteen years after the onset of the disorder (Levine, 1986).

As genetic testing finally leaves the realm of the hypothetical, there are several issues that now have to be faced. A number of these are listed in the proposed protocol in the appendix (Appendix V). Based on the experiences of a number of pilot studies and following a great deal of debate internationally, a consensus has emerged that implementation of such a prediction service is totally dependent on the availability of extensive psycho-social support for HD families. Sophisticated professional counselling is necessary to make the at-risk person aware of the options available and the implications thereof in order to reach an informed decision about predictive testing (Bird, 1985). Appropriate and continuing support which helps individuals to cope with the consequences of their decisions and to enable optimal adjustment to the situation are fundamental to a service of this type.

The social dilemmas created by the test are at least as important as its scientific ramifications; stumbling blocks are likely to arise as similar techniques are applied to other diseases in the future. By maintaining the highest possible degree of preparedness and providing continuing support and availability of therapeutic services, the negative consequences that might result from presymptomatic testing can possibly be avoided or the effects thereof, minimised.

Evers-Kiebooms et al. (1989) aptly stated

> " It is clear that interdisciplinary collaboration between geneticists, psychologists, social workers and psychiatrists will be necessary to ensure that this new diagnostic possibility will allow the testees to integrate this experience positively in the fulfilment of their future lives."

HD families need to be reassured that it is the condition, not the HD patient, that we wish to eradicate Essentially, at-risk individuals need to be given the opportunity to make informed decisions about their lives in an environment of caring, support and understanding.

The major goal of presymptomatic testing for HD should be to enable at-risk individuals to enhance the quality of their lives. In the long run, it is possible that the gene frequency will decrease, which would be an added bonus (Hayden et al., 1987e).

9.2 INTERNATIONAL EXPERIENCE WITH PRESYMPTOMATIC TESTING

There have been many reports emanating worldwide regarding the attitudes of patients and their relatives towards predictive testing of HD. A list of some articles published from different countries in the past decade is shown in Table IX-1. In each case, the more recent or most relevant publication has been cited.

It was reported in 1984 that 73% of persons from HD families canvassed in Boston, before the discovery of the linked marker, indicated that they would take a presymptomatic test should it become available (Schoenveld et al., 1984a). By 1986, once the test was already being offered in certain centres, this figure decreased to 62%, which was a significant change in attitude (Mastromauro et al., 1986). Subsequently, similar findings have been reported by other centres that have already started presymptomatic testing: the proportion of persons who eventually enrolled in the predictive programmes was much smaller than was expected on the basis of previously expressed intentions to undergo the test. In addition, during the pilot predictive testing project in Boston between 1986 and 1988, 19 of the 47 persons (i.e.40%) who requested a presymptomatic or prenatal genetic linkage test voluntarily withdrew from the programme after extensive pre-test counselling (Meissen et al., 1988).

It is further accepted that probably 40% of this group will not have an appropriate family structure to be able to perform the present linkage test (Farrer et al.,1988).

Based on these findings and the experiences of other similar programmes, (Quaid et al., 1989), it can be concluded that only a small proportion of individuals, who are at risk of having inherited the HD gene, will eventually receive presymptomatic test results.

TABLE IX-1.

Published reports of attitudes towards presymptomatic testing for Huntington Disease from different countries (1979-1989).

Country	Authors	Year of publication	Title
UK London	Barette and Marsden	1979	Attitudes of families to some aspects of Huntington's chorea.
Australia Melbourne	Teltscher and Polgar	1981	Objective knowledge about Huntington's disease and attitudes towards predictive tests of persons at risk.
Wales South Wales	Tyler and Harper	1983	Attitudes of subjects at risk and their relatives towards genetic counselling in Huntington's chorea.
France North West	Pettit and Salomez	1985	Huntington's disease, interest of clinical and epidemiologic data for genetic counselling.
USA California	Kessler et al.	1987	Attitudes of persons at risk for Huntington disease towards predictive testing.
USA Kansas	Meissen and Berchek	1987	Intended use of predictive testing by those at risk for Huntington disease.
Belgium	Evers-Kiebooms et al.	1987	Attitudes towards predictive testing in Huntington's disease: a recent survey in Belgium.
USA Michigan	Markel et al.	1987	At-risk persons' attitudes toward presymptomatic and prenatal testing of Huntington disease.
USA New England	Mastromauro et al.	1987	Attitudes towards presymptomatic testing in Huntington disease.
Canada British Columbia	Fox et al.	1989	Predictive testing for Huntington disease: Description of a pilot project in British Columbia.
UK Manchester	Crauford et al.	1989	Uptake of presymptomatic predictive testing for Huntington's disease.
USA Maryland	Quaid et al.	1989	Knowledge, attitude, and the decision to be tested for

Since the inception of predictive testing for HD, parents have requested presymptomatic testing for their children for various reasons but it has been strongly urged that the testing should not be offered at the request of a third party (Morris et al., 1988; Bloch and Hayden, 1990). Following widespread discussion amongst professionals and lay groups it has been internationally agreed upon that children should not be tested (Harper et al.,1990; Went, 1990).

In conclusion, although there was much concern for the adverse consequences of predictive testing (Kessler, 1987a) there have been favourable reports to date as to the beneficial effects of two very well-organized presymptomatic testing programmes (Bloch et al., 1989; Brandt., 1989).

9.3 THE SOUTH AFRICAN SITUATION AND PREDICTIVE TESTING.

9.3.1 A background to the SA situation:

Predictive testing has to date been designed as a first-world service. In SA, however, which is a mixture of first and third-world, the situation is more complex; the issues involved in the practical implementation of such a service are considerably different to those in Western Europe and North America. These include the current political and socio-economic situation in SA, its multi-ethnic society and the physical size of the country. Cultural differences and social attitudes have a direct influence on the development of such a service in developing countries.

Careful examination of cross-cultural ethics is critical to the SA situation as the basic ethical principles that guide human investigation need to be interpreted and applied within the different cultural settings.

Due to the socio-political situation in the country there is a general low standard of education. Consequently, the overall awareness of HD and more specifically the lack of insight as to the immediate and longterm implications for a presymptomatic HD gene carrier, is a major concern.

9.3.2 The facilities available for investigation and management of HD in Cape Town:

In 1987, at the inception of this project, there were and still are very limited facilities available for HD patients and their families in Cape Town. There was an HD out-patient clinic at Groote Schuur hospital (GSH) where patients were seen on a regular basis for neurological assessment and management. Two Provincial psychiatric hospitals, Valkenberg and Lentegeur, had a number of HD in-patients who were in the advanced stages of the disease. Although these hospitals are State-run, due to the high cost of keeping a patient in an institution, there was very limited space available for patients in these institutions. For example, the cost of keeping one patient in an institution has doubled since 1981 and the current cost is quoted as being R15,000.00 per year per patient (Smart, 1981; 1990 figure, personal communication). Private nursing services were very expensive and were therefore not generally affordable by the majority of the Cape Town HD families. In addition there was the Cape Mental Health Society (State subsidised) and a newly established HD lay society where HD family members were referred for support.

HD and its problems are a financial burden for the affected individual, the family involved and the community as a whole. The tremendous cost of hospitalisation, medication, social support and loss of savings cannot be carried or absorbed personally in a third world setting. For these reasons, the cost of a programme for the prevention of a disorder such as HD must be carefully assessed and considered.

9.3.3 Implications for HD in the third world:

Firstly, the social implications and understanding of the disease, for the patient, his family and the community at large, have to be very carefully examined. HD is a family disease; the patient is the nucleus around whom approximately ten people revolve, each suffering from the far reaching social consequences of the disorder. The affected person is the first one who attracts medical attention to the family, however it is often the unaffected spouse who is more likely to need the most assistance in coping (Hayden et al., 1980b).

In some HD families, at-risk persons have gone ahead with marriage and procreated, having kept secret their genetic history from their spouses. In this way denial propagates the disease. Ignorance or inadequate information of HD has, on the whole, produced more problems than has the knowledge of the disorder and its genetic risks (Harper et al., 1979). In a third world situation where the emphasis is on primary care, and the levels of education are low, this situation is aggravated. HD is not just the passing on of a gene unwittingly from affected parent to child, but is also a frightening and inaccurate store of knowledge combined with a panic stricken strategy for living. This combination of factors creates a vicious circle of disease, fear, ignorance and further disease.

Of vital importance in HD families is often the attitude of the non-affected parent because this affects the family as a whole (Korer and Fitzsimmons, 1985). It is therefore essential that the spouse be correctly counselled and well educated about the disease and the predictive test (Tibben et al., 1990). Once again, this demands a well organised programme with highly trained specialist counsellors which is not practicably possible in the SA setting at present.

9.3.4 The feasibility of predictive testing in SA:

In order to establish a predictive testing service in SA, existing auxiliary medical services and other disciplines will have to be co-opted. The primary task will be to increase the awareness of HD and impress on those concerned the implications of the predictive test. With a better understanding of the disorder, families will feel free to use the available community resources without the deplorable stigma of the disease (Kenen and Schmidt, 1978). Maximum utilization has to be made of all available resources recognising that there are minimal tertiary education centres and limited media resources.

Finally, the logistics of back-up services, particularly in the rural areas, which necessitate the involvement of highly trained medical personnel such as neurologists, psychiatrists, psychologists, geneticists and social workers are major problems in developing a predictive service of this nature in the third world. The diverse social and ethnic background differences and the gross lack of adequate counselling facilities, even in urban areas, are also contributory factors to these problems.

The size of the country, the complexity of the socio-political situation and the organisation of medical health services in SA provide a real challenge in devising an acceptable and reliable predictive testing service. The potential of the proposed service extends well beyond this one disease. There are other late onset genetic neurodegenerative disorders such as the cerebellar ataxias and familial Alzheimer disease, for which diagnostic genetic linkage will probably be available in the next few years. The implementation of a prediction service for HD in SA should provide guidelines for similar services in other situations.

With the financial constraints on medical services in SA during the past few years, the emphasis has been on primary medical care. The dilemma of sacrificing health care finance for individuals faced with third world diseases such as Tuberculosis, Kwashiorkor and Leprosy in favour of finance for first world medical practice (genetic services and predictive testing) is one that is often faced by medical researchers in SA.

9.4 AIMS OF THE CURRENT CAPE TOWN QUESTIONNAIRE SURVEY

9.4.1 Previous experience:

During the course of the aforementioned national survey to identify all patients with HD in SA (Hayden et al., 1980a), HD family members were asked whether they would themselves have a presymptomatic test should it become available. The results presented in Table IX-2 show the responses to the possible utilisation of a hypothetically 100% safe and reliable predictive test. At that time 50.75% of the at-risk individuals approached indicated that they would take such a test should it become available. It was noted that there was a tendency towards a lower acceptability of such a hypothetical test in the SA population of mixed ancestry (Hayden, 1979 Page 286).

TABLE IX-2.

Responses to the possible utilization of a hypothetical predictive test. Reproduced in part from Hayden, 1979.

At-risk persons	Yes	No	Uncertain	Total
White	33	10	13	56
(%)	(59%)	(18%)	(23%)	
Mixed ancestry	34	23	19	76
(%)	(45%)	(30%)	(25%)	
	67	33	32	132

9.4.2. Aims of the present SA survey:

In 1987, when HD predictive testing was already available in certain countries, a questionnaire survey was conducted by the author to assess the attitudes and eventually the needs of at-risk individuals towards such testing. This survey was adapted to the needs of the very diverse population and was designed to assess the subjects' socio-economic characteristics; their understanding of the genetic, scientific and therapeutic aspects of HD and their attitudes towards and acceptance of predictive testing.

The main objective of the survey was to evaluate the knowledge and expectations of at-risk individuals to predictive testing and prenatal diagnosis in order to provide adequate psycho-social support systems catering for the needs of the different members of the community.

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CHAPTER TEN

METHODOLOGY:

CHAPTER 10.1 QUESTIONNAIRE DESIGN:.

A proposal for the intended survey was submitted to and approved by the Groote Schuur hospital medical faculty ethics committee before commencement.

There were four parts to the questionnaire. Sections A and B related to personal details of the subjects and their knowledge of HD, Sections C and D related to the subjects' attitudes towards future predictive testing (Appendix VI).

Section A of the questionnaire entitled 'PERSONAL INFORMATION' was designed to appear only to address the sociodemographic characteristics of the individual. However, questions regarding the participants' personal details were followed by four very important additional groups of questions. This design was intended to make the respondents feel completely at ease and not aware that they were being 'tested' at this stage.

The reason for this approach was as follows:- during the designing of the questionnaire it was discovered, although a prerequisite for participation was that the subjects should be aware of their risk status, there was no other way for the investigator to confirm this fact. It followed that it was not possible to ask people how they felt about the possibility of a change to their risk status (i.e. a predictive test) if they were not already aware of their present risk status.

It must be emphasized that this questionnaire was never intended to educate individuals regarding their risk status, but to assess their understanding of the disease, acceptance of their own risk status and knowledge of the predictive test. The primary aim of Section A of the survey was to inform the testers about the testees knowledge of their risks.

The vital additional questions included in Section A were designed to assess the subjects':

i. Exposure to HD (objective knowledge of the disease) Table X-1a.

ii. Intentions regarding future procreation (subjective attitude to HD) Table X-1b.

iii. Knowledge of the statistical risk and acceptance of their own risk status (acknowledgment and acceptance of risk) Table X-1c.

iv. Knowledge about predictive testing for HD (acknowledgement and acceptance that there was a possibility of changing their present risk status) Table X-1d.

Section B entitled 'KNOWLEDGE OF HD' was designed to assess the subjects'

i. Awareness of the symptoms and progress of HD.

ii. Knowledge of the genetics of HD.

iii. Source of knowledge about HD and the available resources.

Section C was designed as a formal 14 point questionnaire based on a hypothetical question: **If a test was available to diagnose HD long before the onset of symptoms; Would you.....?**

This section was formulated to take the individual through the various options, following presymptomatic testing, in order to assess:-

i. Their reasons for wanting the test.

ii. Their attitudes towards predictive testing for themselves (Subjective).

iii. Their attitudes towards predictive testing for others (Objective).

iv. Their perceptions of how they would feel about the results and their intentions following testing.

Table X-1.

Questions from Section A to assess the subjects' objective knowledge of and attitudes to HD as well as their acknowledgement of their own risk status and knowledge of predictive testing.

---

a) HAVE YOU LIVED WITH OR CARED FOR THE HD PATIENT?
(or any other contact)
IF YES, FOR HOW LONG?
HOW LONG HAS THE PATIENT HAD HD?
WHAT DO YOU CONSIDER TO BE THE BIGGEST PROBLEM FOR:
i) the patient with HD?
ii) the family?

b) DO YOU INTEND TO HAVE ANY/MORE CHILDREN?
IF YES, HOW MANY?
IF NO, WHY NOT?

c) HOW ARE YOU RELATED TO THE HD PATIENT?
ARE YOU AT RISK OF INHERITING HD?
IF YES, WHEN DID YOU LEARN THIS? (what age)
WHERE AND HOW?

d) DO YOU KNOW OF A TEST THAT SHOWS WHETHER YOU WILL GET HD?

Section D was a repeat of Section C and was to be completed at a later date, following counselling. The intention being that this would enable analysis of the at-risk individuals' attitudes towards predictive testing both before and after appropriate counselling.

10.2 ENROLMENT OF PARTICIPATING VOLUNTEERS

Family members of patients seen at the HD clinic at Groote Schuur Hospital and members of the Huntington Society of South Africa, a lay organisation, were informed of the questionnaire survey and invited to participate in the programme.

Individuals who volunteered to complete the questionnaire were only accepted to participate in the survey if they met all the following criteria:-

i. had prior knowledge of their risk status.

ii. had had direct contact with someone with HD and knew about the progressive nature of the disease.

iii. were 18 years or older.

Volunteers were seen either at the HD clinic or at the Department of Human Genetics, UCT. The interviews were conducted on an individual basis by the author and a medical genetics counsellor. The questionnaire was explained to the subject, and Sections A and B were completed. After assessment of the responses, certain participants were asked to complete Section C at a second interview and any queries arising therefrom were answered by the medical genetics counsellor. A further interview was to be arranged for the subject to complete Section D at a later date.

A research number was granted so that participants would not have to pay for the use of hospital facilities whilst participating in the survey. A special clinic was established to handle any psychological problems which might have arisen from participation in the survey.

All participants were issued with a survey number and only the author had access to the key. Confidentiality was guaranteed and maintained throughout the programme. The questionnaires were translated into both official languages (English and Afrikaans) and made available to the participants in their language of preference.

10.3 COMPLICATIONS:

A major problem in the recruitment of volunteers for participation in the survey was that young, unaffected individuals could be seriously affected psychologically in the long term if they were unaware of the chances of developing the disease.

For this reason, it was stipulated that subjects be 18 years or older and should have acknowledged that they were aware of their risk status before they could be approached to complete Section C and D of the questionnaire. These criteria were strictly adhered to and at no time were individuals canvassed or coerced to participate. The first part of the survey (Section A and B) was not intended to inform people about their risk status but primarily to assess whether they were aware that there was a risk. Three participants were illiterate and their responses were recorded by the author verbatim.

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CHAPTER ELEVEN

RESULTS, DISCUSSION AND PROGRAMME PROPOSAL:

11.1 SURVEY DATA

Forty requests to participate in the survey were made to HD family members in the greater Cape Town (CT) area; 30 people were approached by the Huntington Society of South Africa (HSSA) and only 10 people volunteered from this group. A further 10 volunteers were recruited at the HD clinic.

Of the 20 respondents who completed Sections A and B of the questionnaire, 15(75%) were female and 5 (25%) were male; 15 (75%) were of mixed ancestry and 5 (25%) from European origins. The minimum age of the at-risk group was 19 years and the maximum age 68 years. According to the individuals' family history, the genetic risk status for having inherited the gene for HD for 17 (85%) of the participants was 50% while 3 (15%) were at 25% risk. Neither the age of the participant at the time nor the age of onset of HD in the family were taken into account in estimating the risk status. The sociodemographic characteristics of this sample are summarised in Table XI-1.

The responses to certain questions in both Sections A and B indicated that there was an element of denial in the SA at-risk population. These questions together with the replies are shown in Tables XI-2 and XI-3.

TABLE XI-2.

Questions and replies regarding the subjects' knowledge of the genetics of HD and their own risk status.

Questions	Replies					
	SA of Mixed ancestry N=15			SA of European origin N=5		
	Yes	No	Don't Know	Yes	No	Don't Know
1. ARE YOU AT-RISK OF INHERITING HD?	6 (40%)	5 (33%)	4 (27%)	4 (80%)	1 (20%)	- (0%)
2. WHAT ARE THE CHANCES THAT A PARENT WITH HD WILL PASS THE CONDITION ON TO HIS CHILDREN? (Subject either knew or was not sure)	10 (67%)		5 (33%)	5 (100%)		
3. DO YOU KNOW OF A TEST THAT SHOWS WHETHER YOU WILL GET HD?	3 (20%)	6 (40%)	6 (40%)	3 (60%)	1 (20%)	1 (20%)

(N = number)

TABLE XI-3.

Question regarding the subjects' knowledge of the predictive test.

Question	Respondents from HC clinic			Respondents from Lay society		
	Yes	No	Don't know	Yes	No	Don't know
DO YOU KNOW OF ANY TEST THAT SHOWS WHETHER YOU WILL GET HD?	1 (10%)	4 (40%)	5 (50%)	5 (50%)	3 (30%)	2 (20%)

From the results in Table XI-2 (question 2) it was evident that although 75% of the participants were aware of the genetic implications of being a member of an HD family (15 of the 20), only 50% acknowledged that they were at risk (10 of the 20)(Table XI-2, question 1).

However, ALL the respondents were at high risk of having inherited the gene for HD (Table XI-1). In addition, six individuals of the total survey sample implied that they were aware that there was a possible predictive test. Upon further analysis of the responses to the question relating to their knowledge of the predictive test, it became apparent that there was a remarkable difference between the group that was recruited through the HD clinic to that from the lay society (Table XI-3). Very few individuals who responded from the HD clinic (10%) acknowledged that they definitely knew of a test, whereas 50% of the respondents from the lay society acknowledged the possibility of a test. In response to the question inquiring from which source individuals had gained most of their knowledge about HD and with reference to the question regarding whom they would approach should they require more information about HD, there was no significant difference between these two groups (Table XI-4).

Only four individuals were approached to complete Section C of the questionnaire. Selection was based on the individuals' overall knowledge of the disease and their awareness of their own risk status.

The demographic characteristics of this group are tabulated in Table XI-5. It is not possible to analyse these results statistically due to the small number and the diversity of the four persons and an attempt to standardise the results would render them meaningless. Because of these limitations and in order provide a perspective of the SA situation, some of the actual answers given are tabulated in Table XI-6 and the results are discussed in the following section (Chapter 11.2).

Owing to the small number of participants in the final survey, it was felt that Section D of the questionnaire was superfluous and was therefore abandoned.

TABLE XI-4.

Questions and replies regarding individuals' source of information about HD and whom to approach.

Question	Respondents from HD clinic				Respondents from lay society			
	m.	f.	HSSA.	other	m.	f.	HSSA.	other
FROM WHICH SOURCE DID YOU GAIN MOST OF YOUR KNOWLEDGE OF HD?	6	0	1	3	6	3	1	0
WHO WOULD YOU APPROACH SHOULD YOU REQUIRE MORE INFORMATION ABOUT HD?	9	0	0	1	7	0	2	1

m = Medical, f = Family/friends,

HSSA = Huntington Society of South Africa

other = Books or own experience.

TABLE XI-5.

Sociodemographic details of the four subjects who completed Section C of the questionnaire survey.

	SA of Mixed ancestry N=2 at 50% risk	SA of European origin N=2 at 50% risk
Males:Females	0:2	1:1
Age in years	68, 41	41, 29
Number of children	1, 4	2, 2
Education level		
High school	2	0
Tertiary level	0	2

(N = number)

TABLE XI-6.

Questions relating to the four participants' attitudes to the possible results of predictive testing and their actual responses.

Questions	Replies
i. HOW WOULD YOU FEEL IF YOUR TEST WAS POSITIVE?	" Satisfied knowing my family and husband will stand by me."
	" It would be like the end of the world."
	" Challenged."
	" Would have to accept the fact."
ii. HOW WOULD YOU FEEL IF YOUR TEST WAS NEGATIVE?	" Happy."
	" Relieved."
	" Relieved."
	" Extremely relieved."
iii. WOULD YOU REQUIRE HELP IN COPING WITH THE RESULTS EITHER WAY? IF SO WHO WOULD YOU GO TO?	" No, but if it is necessary that I will have to be sent to hospital, I would prefer to be near home."
	" The HD clinic."
	" My family/ someone who knows about HD."
	" HD society and Genetics clinic."

11.2 DISCUSSION OF RESULTS

After careful analysis of the replies obtained in the survey and as a result of the attitudes of SA HD patients and their families to HD, as experienced at the routine HD clinic at GSH, it was apparent that the problems of day-to-day survival were more immediate than events a generation away.

Although the sample size in this study was small (20), this number was in itself significant, considering the number of at-risk individuals in the region where the survey was conducted. As stated in Chapter V, it was estimated that there were approximately 890 individuals from the 42 kindreds in the Cape Town area who were at risk for inheriting the gene for HD. Based on pedigree analysis and information obtained from patients and family members, a conservative estimate indicated that there were a minimum of 458 at-risk individuals living within 100km radius of Cape Town at the time of the survey.

The Huntington Society of SA (HSSA) was established in CT in 1986. The aim of this lay organisation is to act as a support group for HD families and to educate people about the disease. Although concerted efforts have been made to contact all known HD families, the HSSA currently has a membership of only 60. Despite the fact that specific mention was made of the questionnaire survey at three separate meetings of this association, only ten people from this group eventually volunteered to participate.

The HD clinic is a special clinic at GSH run jointly by the Departments of Neurology and Human Genetics. HD patients and their families are seen on a regular basis for neurological assessment and management. Patients are re-evaluated every three months and are often accompanied by carers: generally the spouse or a first degree relative.

Of the 89 HD patients in the Cape Province documented in Table III-3 only 15 attended the clinic regularly between January 1987 and December 1988 (>6 visits), whereas a total of 30 patients were seen over that period. Twenty-two of the 89 patients were institutionalised, four of whom were brought regularly to the clinic; of the two HD patients that are serving prison sentences, one was seen on a regular basis at the clinic.

These facts imply that there was a certain reticence amongst CT HD families to appeal for medical or group assistance. This reluctance in requesting help was probably as a result of the stigma attached to the disease (Kenen, 1978). The desire for privacy was therefore respected and no other attempts were made to canvass individuals in any way.

The ethnic distribition of the participants in the SA questionnaire survey was similar to that observed at the HD DNA banking centre (Table V-2). The disproportionate ratio of male:female (chi-square exceeds the critical value at $p = 0.05$) was probably due to the fact that the majority of carers, who were canvassed at the HD clinic, were female.

With regard to the participants' exposure and resulting objective awareness of the symptoms and progress of the disorder, the majority (80%) were very well aware of the features of HD. All 17 (Table XI-1) who were at 50% risk of having inherited the gene for HD had previously or were presently caring for a relative with HD at the time of the survey. The three individuals who were at 25% risk had had little exposure to HD but were well informed about the disease and knew about the genetics of HD. No significant information was obtained regarding the influence of HD on the intentions of at-risk individuals regarding procreation. Seventeen participants already had had children and gave advanced age and family size as their reasons for not wanting more.

One person who had no children replied that he did not want any, but failed to indicate why. Two individuals failed to answer both questions (desire to have children and reason why if no).

It was distressing to learn of the limited extent of the individuals' awareness of their own risk status. Judging from the replies tabulated in Table XI-2 it would appear that certain individuals had an inability or unwillingness to apply their genetic knowledge of HD to their own situations. The implications of the apparent lack of knowledge of the predictive test was indicative of denial, as all individuals who participated in the survey, had had blood taken for the HD DNA banking centre. At that time, each individual was informed of the possibility of a predictive test as this was the whole rationale for banking DNA (Chapter 5.2.). In addition, when the questionnaires were distributed the reason stated for the survey was to design a suitable protocol for the implementation of predictive testing of HD in SA. Denial, as a defence mechanism which can be defined as pathological, is not an unique observation; Martindale (1987) reported on pathological defences seen in some at-risk individuals as well as the defences adopted by some professionals coming in contact with HD families. However, the results in Table XI-3 indicated that 50% of the respondents from the lay society acknowledged that they knew about the test whereas 90% of those from the HD clinic either denied any knowledge of the test or indicated that they did not know. It is clear that repeated exposure to the concept of predictive testing as a result of joining a support group and the willingness to be identified as an at-risk person influenced the awareness and acceptance of the respondents from the lay society to the test.

The four respondents who completed Section C of the questionnaire all indicated that they would take the test, irrespective of whether it was 96% accurate or not and that they would all recommend that others take the test.

Furthermore, they all stated that they would have their own children tested and suggested testing children at an early age, preferably in their teens.
All four respondents already had had even children and were not planning any more due to advanced age and family size. However, two indicated that, hypothetically, if, at an earlier age, had the test been available, they would still have had children if their test had been positive. Two replied that they would not have procreated. Three of the four respondents would have had a prenatal test but one of these would not have had the pregnancy terminated if the test had been positive. One respondent indicated that she would neither have had a prenatal test nor opted to terminate.

These responses implied that the introduction of predictive testing for HD would significantly affect life decisions for the four respondents who participated in Section C this survey.

No SA Blacks participated in this questionnaire survey as the only black African families with HD in SA known to us were all in the Transvaal, which was out of the study area. However, the author noted when on the field trip to Lebowa, that the main concern of HD family members was for the social security benefits that could be obtained for the HD patient and that there was very little concern for the hereditary aspect of the disorder. Whilst they knew that the disease was **'in the family'** it was seen as **'an act of God'** and accepted as such.

Recently, in the context of a discussion on AIDS it was stated that there were differences in opinions on counselling between Western and African health workers. Dr M V Gumede from the Kwamashu Polyclinic, Zululand, Natal is quoted as saying that :

> **"death holds no terror in the black community. AIDS is grouped among 'Izishawa' in Zulu, understood in the West as 'Acts of God'. No extraordinary counselling measures were therefore needed"** (MRC news, 1988).

The same dictum would probably apply to HD.

The results and observations made during the course of the survey indicate that the implementation of a presymptomatic testing programme for HD in SA is a difficult task. Clearly, the first priority will be to ensure adequate pre-test counselling with the main emphasis on education and enlightenment. In order to accomplish this successfully, the available professional and community resources need to be identified, educated and informed of the problems relating to HD to enable them to provide psychosocial support for HD families. An integrated professional team approach to counselling for HD needs to be established (Fig XI.1). The natural consequence would be to utilise these facilities as a follow-up support system to deal with the psychological impact of predictive testing.

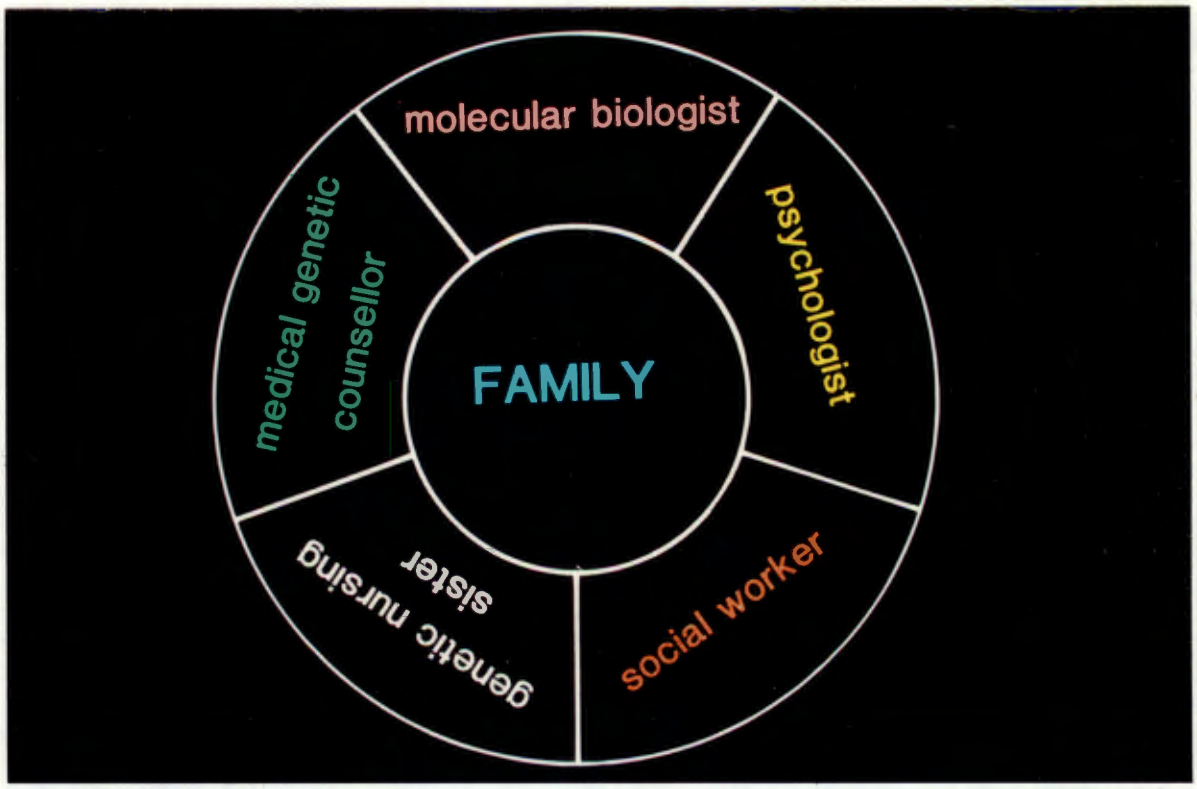

Figure XI.1

Schematic representation of the combined professional team approach to counselling for HD.

11.3 REVIEW OF THE ORIGINAL DEVELOPMENT PROGRAMME

In 1987 a proposed protocol for the development and implementation of a predictive testing service for HD in SA was drawn up (Appendix V.). The technological aspects, questionnaire survey, education programme and the establishment of a counselling service already completed are detailed in this thesis. As previously stated, it was found advisable to restrict the study to the Cape Town area and the eventual proposal was designed for this area of study and submitted to the local hospital authorities.

11.4 PROPOSALS FOR THE IMPLEMENTATION OF PREDICTIVE TESTING FOR HUNTINGTON DISEASE IN THE CAPE.

In 1989 a proposal was submitted to the Medical Superintendent of Groote Schuur Hospital (GSH) for the implementation of a predictive testing service for HD in the Cape.

The submitted proposal included details of the completed service-orientated development and stated the major requirements for the inception of the comprehensive service. These included a full-time laboratory technologist and a GSH social worker allocated to the programme for one day each week. In addition it was stressed that it was necessary for a high level committee to be convened to establish policy on the moral and ethical issues which related specifically to SA.

As this proposal represented the culmination of all the work outlined in this research project, it is presented here.

PROPOSAL FOR THE IMPLEMENTATION OF PREDICTIVE TESTING FOR HUNTINGTON DISEASE IN THE CAPE

1. INTRODUCTION

Huntington Disease (HD) is an autosomal dominant neuropsychiatric disorder that usually manifests in mid life with abnormal involuntary movements, personality change and dementia. In the past, genetic counselling for the statistical risk of inheriting HD was based solely on family history and pedigree analysis. Now, prediction can be made, with approximately 96% accuracy, in informative families, using molecular technology and linkage analysis.

Following a great deal of debate in overseas centres, a consensus has emerged that implementation of such a prediction service is totally dependent upon the availability of extensive psycho-social support for HD families. Sophisticated professional counselling is necessary to make the 'at-risk' person aware of the options available and implications thereof in order to reach an informed decision about predictive testing. Appropriate and continuing support to help individuals cope with the consequences of their decision are fundamental to a service of this type.

The Department of Human Genetics, UCT, is now in a position to offer a molecular predictive testing service for Huntington Disease. In view of the financial implications and complex ethical and moral issues concerning presymptomatic testing and antenatal diagnosis of a disease for which there is no cure and very little treatment, any decision to implement predictive testing must rest with higher authority.

2. MOLECULAR SERVICE:

The current molecular test for HD involves linkage analysis using restriction fragment length polymorphisms (RFLPs) that are closely linked to the HD gene locus. This type of analysis requires the use of blood samples from numerous family members. (It is preferable that specimens are obtained from three generations of living affected individuals in order to establish which variation in the marker is co-inherited with the abnormal gene in each family)
Interest in this disorder in South Africa was generated in 1977 when a national survey was undertaken by the Department of Human Genetics, UCT.

A collaborative Huntington disease clinic was subsequently established at GSH and thereafter, service orientated development was undertaken in our molecular laboratory using resources from the Cape Provincial Administration (CPA), UCT and the Medical Research Council (MRC).
A perspective of the magnitude of the problem can be gained from the following statistics:

HD IN THE CAPE TOWN AREA		DNA BANK: (70% Cape Province).
AT RISK	485	203
AFFECTED	80	67
FAMILIES	40	37

3. EXISTING SERVICE:

A 'Core Team' has been established which will be available to provide the counselling service and the technology for HD predictive testing. This team consists of:

A medical geneticist to undertake the counselling.

A molecular scientist who has established the DNA test in our molecular laboratory.

A psychologist (i) to organise and carry out the psychological tests necessary in patient selection.
(ii) to be available for any psychological problems that may arise both pre- and post test and for long term support of patients, if necessary.
(iii) to be available for the ongoing support service of 'at-risk' individuals who do not wish to take the test but require psychological counselling.

A genetic nursing sister (i) to organise the appointments and be a link between patient and team
(ii) to collect the necessary blood specimens from patients and key relatives.

A neurologist for neurological assessment and management of the HD patients and to exclude early evidence of HD already manifesting and so preclude the patient from unnecessary testing.

4. REQUIREMENTS FOR THE IMPLEMENTATION OF A PREDICTIVE TESTING SERVICE FOR HD.

The initial stages of development have been completed and we are in a position to offer a molecular predictive service for HD.

A definite decision is now necessary as to the advisability and practicability of offering this costly test in SA. The majority of our patients are from the low literacy, low education, low income population, and they will have to make 'informed decisions' about extremely difficult, complex and emotive issues. The question arises as to whether the programme is feasable in view of the present shortage of available resources and skilled personnel.

Staff still required and key specialists needed mainly for consultation and advice if and when specific problems arise.

a) A full-time technologist to carry out the DNA analyses.

b) A Social Worker (1 day per week) Huntington disease is a family disease and we are often required to call for assistance from social workers to help family members cope with day-to-day problems associated with HD. The social workers at GSH have many commitments and it is often not possible for them to attend to HD problems immediately. With the implementation of predictive testing a specific social worker would have to be allocated to HD to handle these day-to-day problems as well as to be familiar with possible problems that can arise as a result of predictive testing. Psychosocial support after testing is an essential pre-requisite for a predictive testing protocol.

c) A Psychiatrist who will be interested and prepared to be involved when required. (available for consultation: 1 session per week)

d) An Obstetrician/Gynaecologist (available for consultation : 1 session per week)

- for discussion on prenatal diagnosis with exclusion option
- for prenatal testing; chorionic villus sampling(CVS) and amniocentesis and for discussion on what parenting options we may offer, e.g. artificial insemination donor (if male carrier), donor egg in vitro fertilization(IVF) (if female carrier), adoption.
- for termination of pregnancy (TOP) when indicated
- for contraceptive advice and sterilization

e) Psychologist At present our team psychologist, has one session per week allocated to HD. If predictive testing is implemented, he may need more time allocated for the service.

5. SPECIFIC PROBLEMS AND NEEDS

If the decision is made to provide ancillary staff and to implement the service, it will be necessary to convene a high level committee to establish policy on moral and ethical issues which relate specifically to Southern Africa.

Examples of some of the issues that will have to be resolved are:-

(i) The ethics of presymptomatic and antenatal diagnosis of a progressive lethal condition that may only manifest in the third and fourth decade of life and for which there is no cure and much stigmatization.

(ii) The ethics of exclusion prenatal diagnosis; does one insist on prior agreement to termination of pregnancy if there is no new information following testing (no exclusion)? Problem of victimization of child if the parent then manifests HD.

(iii) Legal rights of counselling team to reject someone from the test programme as being 'unsuitable' on psychological grounds that may raise the issue of the team being 'paternalistic' in judgment.

(iv) Who has the 'right' to the results? Only the 'at-risk' individual? The spouse? The parents? Other siblings?
What about life insurance disclosure? What about employers? What if patient (positive) is a pilot, a surgeon, an accountant, etc. ?

(v) Other 'rights' - does the spouse have the right to insist that an at-risk partner have the test against their will? Can vital key relatives, whose blood is needed for an informative test and who refuse, be coerced to give blood?

(vi) The ethics of using stored DNA - given purely for research and the DNA bank - in situations where relatives refuse to co-operate.

(vii) What is the legal age of consent? Can a 16 year old married woman request the test?

In addition, it will be essential to establish a small standing committee to whom specific problems can be addressed if and when they arise.

SECTION IV: CONCLUSIONS, APPLICATIONS AND RECOMMENDATIONS

CHAPTER 12 CONCLUDING COMMENTS:

CHAPTER 13 PRACTICAL APPLICATIONS RESULTING FROM THIS PROJECT:

CHAPTER 14 RECOMMENDATIONS:

SECTION IV

CONCLUSIONS, PRACTICAL APPLICATIONS AND RECOMMENDATIONS

Chapter 12 CONCLUDING COMMENTS:

Chapter 13 PRACTICAL APPLICATIONS RESULTING FROM THIS PROJECT:

Chapter 14 RECOMMENDATIONS:

CHAPTER TWELVE

CONCLUDING COMMENTS

12.1 THE RFLP FREQUENCY STUDY

The determination of RFLP frequencies at the D4S10 locus in different population groups is essential in order to establish which markers will be informative for predictive testing. It is noteworthy that this was the first study to be undertaken concerning G8 allele frequencies in non-Caucasoid population groups.

When a predictive testing service for HD is eventually established in SA, additional markers closer to the disease locus than G8 will be used. Allele frequencies are presently being established by the author for D4S90 (Youngman et al., 1988) as well as two other markers that are being investigated for heterozygosity in SA in a collaborative study with the author and M Hayden, Vancouver (Chapter 6).

12.2 THE LINKAGE STUDY:

During the four year period of this project, 22 probes on chromosome 4, known to be linked to HD, were acquired by the Department of Human Genetics, UCT. All these probes were isolated, purified and tested by the author and individuals from the initial three families that were selected for linkage studies (Chapter 5.4) were extensively investigated with these probes immediately following their acquisition. Some individuals were genotyped with ten different markers which required at least 100 µg of DNA. It was not considered advisable to complete genotyping all individuals from the ten HD families in the study with more probes as sufficient DNA was not available for development in all these families. The DNA bank requires a minimum amount of 100 µg of DNA for storage at any one time. The utilisation of more DNA would have depleted our stocks needed for predictive testing and it was therefore decided to restrict the project to the use of G8 probes only. Until such time as all HD patients lymphocytes have been transformed and are safely stored in liquid nitrogen, the amount of DNA available for research will remain limited.

The results obtained from the study of the four probes that were selected for this project demonstrated that for accurate linkage analysis, it would be essential to use more markers, preferably ones closer to the HD locus. By using more markers, the individual informativeness will be improved with the use of haplotypes as was demonstrated by the family study with the new variant (Chapter 8). Also, the possibility of error in predictive testing, due to recombination between the markers and HD will be reduced by using markers closer to the HD gene locus. The use of additional markers will also result in a larger number of families becoming informative for linkage analysis.

The use of additional markers in the SA black HD families indicated that, assuming linkage to the markers used, the HD gene in the two SA black families is not associated with the same markers.

Furthermore, the possible positive genetic evidence in favour of a founder effect for HD in the SA population of mixed ancestry has very important implications for predictive testing of HD for families of this group. The use of linkage disequilibrium data could allow substantial risk modification for certain individuals and reduce the necessity for extended family linkage analysis (Chapter 7.4).

12.3 THE HETEROGENEITY QUESTION

If different gene loci were to be found to cause HD (i.e. HD being heterogeneous), then the RFLPs identified with the markers on chromosome 4 would not be helpful in all HD families. To date, there have been no families in which non-linkage has been demonstrated.

The SA black HD families investigated in this study were not large enough to obtain a positive lod score (>3) to confirm linkage. However, according to Ott's A test, there was no significant evidence in favour of heterogeneity for the ten SA families originating from different backgrounds that were studied (Chapter 7.3).

12.4 THE QUESTIONNAIRE SURVEY

Although the questionnaire survey was originally intended for fact-finding, it was also responsible for enlightening many HD families about the genetic implications of the disease. The long-term impact of this knowledge on their lives was not easy to assess at that stage. In view of continued contact with the families and their increased awareness of the disease, it was felt necessary to offer counselling in advance of the predictive test. A special HD at-risk clinic was established in addition to the existing HD clinic for the medical management of HD patients. One of the reasons for this special clinic was that additional counselling of at-risk individuals at the existing HD clinic was very distracting for all concerned. It is possible that HD family members who have not yet seen an HD patient in the advanced stages of the disease could be unduly alarmed by suddenly being confronted with severely affected HD patients before appropriate counselling. HD family members are now referred to the at-risk clinic for counselling by a specially appointed psychologist who is familiar with the problems associated with the disease.

The most striking finding of the survey was that in general, at-risk individuals in Cape Town were well informed about HD and were knowledgeable about the genetics of the disorder. However, there was an unwillingness or inability to apply this theoretical knowledge to their own situation. Furthermore, there was evidence that many individuals denied knowledge of the possibility of a predictive test.

CHAPTER 13 PRACTICAL APPLICATIONS RESULTING FROM THIS PROJECT.

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CHAPTER THIRTEEN

PRACTICAL APPLICATIONS RESULTING FROM THIS PROJECT

13.1 THE ESTABLISHMENT OF A SPECIAL AT-RISK CLINIC.

The necessity for establishing a special at-risk counselling clinic has been discussed (Chapter 12.4). This clinic is now available to at-risk HD family members and has proven to be most successful.

The clinic is run jointly by the Department of Human Genetics and the Department of Psychiatry, GSH. The staff comprises a psychologist, a medical genetic counsellor and a genetic nursing sister. There is also a psychiatrist on call for consultation.

13.2 THE FOUNDATION OF THE HUNTINGTON SOCIETY OF SOUTH AFRICA (HSSA).

In 1986, the Huntington Society of SA (HSSA) was founded as a direct result of the establishment of the HD DNA banking centre. The aim of this lay society is to provide HD families an opportunity of meeting with one another to share experiences, discuss problems and act as a support group. In addition, it is their aim to educate the community at large as well as medical and professional health carers about the disease. The society has been very successful in its endeavours and is a member of the International Huntington Association (IHA). In 1989, two of their delegates attended the 8th International Meeting of the IHA in Vancouver, Canada. The author has addressed this lay organisation on a number of occasions and has had continued contact with many of their members.

13.3 SURVEY OF THE SERVICE NEEDS AND PROBLEMS OF HD PATIENTS AND THEIR FAMILIES IN CAPE TOWN.

As a result of the questionnaire survey during the course of this project and the need to establish what services were available currently in Cape Town (CT) for HD families, the Department of Community Health (UCT) undertook a survey in 1988 of the service needs and problems of HD patients and their families in CT (Kvalsvig and Blockman, 1988).

Their survey identified the available services as the HD clinic at GSH, the HD lay society, the two local Psychiatric Hospitals (Valkenberg and Lentegeur), the Cape Mental Health Society and private nursing services. The general overall impression gained during this survey was that the existing services were not being fully utilised by the HD community, mainly because of fear of stigmatization, lack of communication and knowledge about the these services as well as socioeconomic pressures.

It was found that many HD families were from the lower socioeconomic groups and could only afford services that were state-subsidised. There was a very poor understanding of existing services and it was felt that social workers were needed to direct individuals to these services, develop new resources and assist with the identification of problems facing HD patients and their families. The HD community requires constant support and guidance to help them overcome and cope with their feelings of despair and isolation, their frustration at the inaccessibility of the outside world, their fear of being a burden to others and the never ending struggle of day-to-day living. The one statement made by all was **"No one really understands what it is like".**

13.4 HD WORKSHOP AND LECTURES.

An HD workshop, organised by the Department of Human Genetics, UCT was held in conjunction with the Second Congress of the Southern African Society of Human Genetics in Cape Town, 1988. This served as a very successful educational programme for the health workers, genetic state nurses and concerned medical geneticists who attended the Congress.

As a result of the workshop, lectures and presentations given throughout the country by the author, on various aspects of this project, a new awareness and concern has been created for HD. Consequently there is a better general understanding of the disease and the associated social problems it creates (Abstracts of papers read at various national and international meetings on aspects of this project are in the appendix).

13.5 CO-ORDINATION OF EXISTING SERVICES.

As a result of this research undertaking, two other Departments at GSH and UCT as well as the Department of National Health and Population Development, both on a regional and national level, have become increasingly involved with HD patients and the problems experienced by their families. The survey undertaken by the Department of Community Health (UCT) has been discussed (13.3) as well as the collaborative clinic established by the Department of Human Genetics (UCT) and Department of Psychiatry (GSH) (13.1). The interest in HD taken by the Department of National Health and Population Development and their financial and service commitment to the banking of DNA, must be maintained and is vital for the continuation of the HD DNA banking service.

At a local level, the Department of National Health and Population Development formed a Co-ordinating Committee for Genetic Services in the Western Cape and a special meeting was held on the 6th March 1989, with the Department of Human Genetics (UCT) to discuss the co-ordination of services for HD in CT. At this meeting, the Department of Community Health (UCT) and the Community Mental Health Nursing Services undertook to establish a co-ordinating mechanism for dealing with HD families and their problems in the community.

13.6 dBASE III MANAGEMENT SYSTEM FOR HD DNA BANKING.

In 1988, a dBase III management system was installed on a microcomputer in the laboratory of the Department of Human Genetics, UCT, in accordance with the system recommended by Sarfarazi et al. (1987) and the system used at the Canadian DNA banking centre at the University of British Columbia, Vancouver (Amy Hedrik, personal communication). Details of all persons whose blood was taken for the HD DNA banking centre were stored using this program. The program and its data were maintained by the author and a constant check kept on the amount of DNA stored in the bank for the period that the developmental molecular programme was being completed. Whenever the level of DNA stored in the bank dropped to less than 100ug of DNA, genetic nurses throughout the country were notified to obtain more blood for banking.

Confidentiality remains an important aspect of HD DNA banking and only authorised staff members are allowed access to the HD data on the microcomputer. A copy of the patient information stored on the computer is given in the appendix (Appendix X).

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CHAPTER FOURTEEN

RECOMMENDATIONS AND FUTURE RESEARCH

14.1 RECOMMENDATIONS FOR THE EXISTING SERVICE

The results of the survey conducted by the Department of Community Health (Chapter 13.3) indicated that the facilities available to HD patients and their families are presently concerned mainly with the medical care of the patients. The emphasis should now be concentrated on helping family members to cope with the problems of caring for the HD patient, informing them of the existing services and helping at-risk individuals to come to terms with their own risk status.

There are a number of problems that have been identified by the aforementioned survey that need to be dealt with immediately, such as the lack of transportation, availability and accessibility of disability grants and full utilization of existing services.

The lack of transportation for patients and at-risk individuals has made certain services inaccessible to HD families. An immediate possible solution would be for social workers to visit HD families at home. This would provide them with the opportunity to deal individually with family problems, applications for disability pensions, institutional placement and help with temporary care to relieve family stress. In addition, state-subsidised home nursing services could alleviate the immediate pressures and stresses caused by caring for HD patients at home. Such services at present are privately run and are therefore out of reach of many financially crippled HD families.

Finally, by educating family members and the community at large, the stigma associated with HD would be minimised, thus enabling at-risk individuals to make informed, voluntary decisions about their lives and predictive testing for HD in an atmosphere of support and understanding.

A committee consisting of representatives of the International Huntington Association (IHA) and the World Federation of Neurology (WFN) issued a policy statement on the HD molecular genetics predictive test (Went L, 1990 and World Federation of Neurology statement, 1989). The document provides some realistic ethical and moral principles based on the current knowledge and techniques in molecular genetics and will be used as a guideline for the eventual implementation of predictive testing for HD in SA.

14.2 FUTURE RESEARCH

14.2.1 The linkage data indicating a possible founder effect for HD in the SA population of mixed ancestry needs further investigation. Haplotyping of further HD patients of mixed ancestry to determine whether the HD gene is cosegregating with the same haplotype, more frequently than would be expected if the genes were randomly distributed in the population, would help to confirm or refute this observation. The implications of the finding of a founder effect have been discussed and would influence the eventual approach to predictive testing for HD in a SA family of mixed ancestry.

14.2.2 Once the HD gene has been cloned, it would be useful to investigate affected individuals from different origins at the intragenic level to determine whether there is true molecular heterogeneity or homogeneity for HD.

14.2.3 Hayden et al. (1982) commented on the high frequency of juvenile HD in SA. Although the juvenile form of the condition is not a separate clinical entity but rather a reflection of the variable phenotypic expression (van Dijk et al., 1986), molecular investigations at the intragenic level of these individuals might reveal allelic heterogeneity.

In addition, two recent reports on genomic imprinting being a possible mechanism for the parental origin effect in juvenile HD would require a study of the methylation profile of the parental alleles in these families in order to examine this proposal (Reik, 1988; Ridley et al., 1988).

We have been approached to participate in a collaborative study with the Vancouver HD research group to determine whether there is any alteration in DNA methylation which may account for early onset of HD. For this project, DNA from our banking centre will be sent to M. Hayden, Vancouver for specialised molecular investigation.

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APPENDIX:

APPENDIX	I.	Information sheets sent to HD families.
APPENDIX	II.	Consent forms for DNA banking.
APPENDIX	III.	Information sheets sent to Doctors and institutions.
APPENDIX	IV.	Abstract of paper read at the SASHG meeting, Rustenburg (1987).
APPENDIX	V.	Copy of the original proposed protocol for the implementation of predictive testing for HD.
APPENDIX	VI.	Questionnaires.
APPENDIX	VII.	Abstract of paper read at the IHA meeting, Genoa (1987).
APPENDIX	VIII.	Abstract of paper read at the SASHG meeting, Durban (1989).
APPENDIX	IX.	Abstract of the poster presented at the Clinical Genetics meeting, Boston (1989).
APPENDIX	X.	Computer form of HD dbase file

UNIVERSITY OF CAPE TOWN

Department of Human Genetics

University of Cape Town · Medical School, Observatory, 7925
Telephone 47-1250 Ext. 297
Professor Peter Beighton, M.D., Ph.D., F.R.C.P., D.C.H.

DNA BANKING CENTRE FOR HUNTINGTON'S CHOREA

INFORMATION SHEET

Huntington's chorea (HC) is a dominantly inherited disorder. This means that each child of an affected parent has a 50-50 chance of inheriting the condition. These children are therefore 'at risk' of developing HC. Previously there have been no reliable markers to determine which of these 'at risk' children will indeed have the condition. However, a test has recently been developed in the USA which will determine which of the 'at risk' family members will probably develop Huntington's chorea and who are therefore at risk of passing the condition on to their children.

Local research is being concentrated on finding out how accurate this test will be. It involves looking at the DNA (a normal component in the blood) of blood specimens from affected and unaffected members of a family, as well as their spouses (husbands/wives). The DNA is isolated from the blood specimen and can be stored in a special refrigerator indefinitely. The test, in its present form cannot be done on individuals alone and blood specimens from affected and unaffected family members are required. This research is conducted with strict concern for confidentiality and all specimens are coded so that only the scientists directly involved in the laboratory work are aware of the donor identity.

Hopefully, this research will provide a reliable test for family members at risk of developing HC. For this reason, we have established a storage facility, or "DNA bank", where blood specimens can be kept until such time as the test is available.

In this way blood from numerous family members, who are crucial to the accuracy of the test, can be stored for an indefinite period while we assess the accuracy and reliability of this method. This

will / ...

We will avoid the possibility of the test being denied to "at risk" individuals in the future because certain family members are not available for various reasons.

If you or other members of the family wish to use the services of the unique blood storage facility, or would like further information in this regard, please contact :-

Sister S Beatty or Mrs J Greenberg
Department of Human Genetics
Medical School
University of Cape Town
Observatory 7925
Cape Town
Telephone (012) 47-1250 Ext 304 or 456

IT MUST BE EMPHASISED THAT AT THIS TIME THE LABORATORY WORK IS STILL AT THE DEVELOPMENT STAGE, THEREFORE NO RESULTS WILL BE AVAILABLE.

Also available in Afrikaans
Ook verkrygbaar in Afrikaans

UNIVERSITY OF CAPE TOWN

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Professor Peter Beighton, M.D., Ph.D., F.R.C.P., D.C.H.

DNA BANKING CENTRE FOR HUNTINGTON DISEASE

INFORMATION SHEET

The finding of a DNA marker linked to the gene for Huntington Disease (HD) offers the prospect for preclinical testing and ante-natal diagnosis in the near future. This test will enable persons at risk for HD to determine whether or not they have a high probability of being presymptomatic carriers of this disease.

HD is inherited as an autosomal dominant trait, therefore each child of an affected parent has an even chance (50-50) of inheriting the gene. Recent evidence suggests that the HD gene is located on chromosome No.4. A "marker" has been identified that is so close to the location of the HD gene that it tends to be inherited along with this gene. In all the families investigated thus far, coinheritance of the marker and the HD gene occur in 98% of cases. Thus, there is about a 2% error with this test; this is due to crossing over of DNA between chromosomes during meiosis and is directly related to the distance between the marker and the gene.

The marker that is near the HD gene has different forms and for this reason it is essential to establish which form of the marker is inherited with the HD gene in a particular family. Thus, family studies are needed involving affected and unaffected (at negligible risk of developing HD) family members. If the affected persons have identical forms of the marker on each chromosome, it is not possible to determine which chromosome has the HD gene. For this reason it must be emphasised that this test will not be of use in all families.

Many affected individuals who are crucial for family linkage studies may die prior to the availability of the test. To avoid family members subsequently being denied this test by virtue of the unavailability of DNA from deceased relatives, a DNA Banking Centre for Huntington disease in South Africa has been established.

Specifically, blood is being stored from:

i) all affected persons
ii) their spouses
iii) persons at risk of HD but having a low probability of developing this disease in the future because of their advanced age
iv) persons at risk who are ill.

2/..

For the past three years blood specimens have been collected from affected and unaffected members of Huntington disease families for the DNA Banking Centre. The purpose of this banking centre is to store DNA from Huntington disease family members for possible future linkage studies for presymptomatic testing of Huntington disease. At present no such test is available in Cape Town and due to the fact that people have had blood taken for DNA banking and the possibility of the test has been mentioned to them, we are receiving numerous telephone calls from families requesting results.

It is therefore essential to explain to the families that NO tests have been done in Cape Town and that the DNA is simply being stored here.

One of the biggest problems is that people are under the impression that the presymptomatic test is a diagnostic test. Essentially, all that the test could in fact do is alter the individual's risk status. If there is any question of possible onset of the disease this is a clinical diagnosis and the person must be referred to a Neurologist for assessment.

Should you require more detailed information in this regard, please contact Mrs J Greenberg at the above address.

Also availbale in Afrikaans

Ook verkrygbaar in Afrikaans

COVERING LETTER TO DOCTORS

UNIVERSITY OF CAPE TOWN

Department of Human Genetics

University of Cape Town · Medical School, Observatory, 7925
Telephone 47-1250 Ext. 297
Professor Peter Beighton, M.D., Ph.D., F.R.C.P., D.C.H.

DNA BANKING CENTRE FOR HUNTINGTON'S CHOREA

Dear Dr

We are establishing a DNA Bank for Huntington's Chorea for the reasons outlined in the information sheet. We understand that family, ... who are in your care, have a history of Huntington's Chorea. We have contacted a member of this family,....., with regard to obtaining blood from the following family members:-

1.

2.

3.

4.

5.

Information on the purpose of the DNA Bank has been sent to these family members.
We would appreciate your assistance in obtaining blood specimens from these persons, who will be contacting you in the near future. The DNA will be extracted from the blood, and then stored in our laboratory until such time when the test for Huntington's Chorea is available and is requested by "at risk" persons in the family.

You have our assurance that strict confidentiality will be maintained. In view of the ethical issues which are involved, it is necessary for consent forms to be signed (enclosed). If you have any questions please do not hesitate to contact us.

Yours sincerely

Also available in Afrikaans

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SISTER GAIL ROSS
Research Assistant
DNA Banking Centre for Huntington's Chorea

UNIVERSITY OF CAPE TOWN

Department of Human Genetics

University of Cape Town · Medical School, Observatory, 7925
Telephone 47-1250 Ext. 297
Professor Peter Beighton, M.D., Ph.D., F.R.C.P., D.C.H.

re : HUNTINGTON'S CHOREA IN SOUTH AFRICAN BLACKS

Our department recently openend a DNA Banking Centre for Huntington's chorea. Our main aim is to store blood specimens indefinitely from persons affected with Huntington's chorea, as well as their at risk family members for the purpose of genetic analysis when the presymptomatic/predictive test is generally available in South Africa. We currently have a research programme assessing the applicability of this test to the local families and foresee this screening service becoming available in the near future. It is important to store these blood specimens because the future unavailability of family members, specifically affected individuals, would reduce the information obtained and thus deny the at risk individuals the opportunity of having their genetic status determined.

To date we have concentrated mainly on the Cape White and mixed ancestry families due to their accessibility. Indeed, previously Huntington disease was reported as being extremely rare in South African Blacks, however we have recently encountered some affected individuals in the Transvaal. It would be extremely important to clarify this situation for the purpose of counselling these families and future planning of this nationwide service.

We would greatly appreciate your assistance in providing us with information on the numbers of affected Black patients with Huntington's chorea seen at your hospital, either in the neurology or psychiatry departments. We assure you that we have strict protocols to insure confidentiality and individuals are only documented in code with access limited to a few senior members of the banking programme.

I have also enclosed a few information brochures with details of our DNA Banking Centre. I would be grateful if this could be circulated amongst your hospital neurologists.

Many thanks.

Yours sincerely,

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G A ROSS (Mrs)
Research Assistant
DNA Banking Centre for Huntington's chorea

DNA BANKING CENTRE FOR HUNTINGTON'S CHOREA

CONSENT FORM

I, the undersigned ....., being over 21 years of age agree to having a specimen of my blood drawn and stored in the DNA Banking Centre for Huntington's Chorea.

I understand that a needle will be inserted into a vein and that 30cc of blood will be removed. DNA will later be extracted from this blood, subjected to preliminary analysis and then stored in the laboratory. I understand that no results will be given to me at the present.

Donor's Signature : .....

Witness 1. : .....

2. : .....

Date : .....

If donor is a minor or unable to give consent :
(To be completed by curator/legal guardian)

I have read the above protocol and understand the nature of the procedure.

I hereby give consent on behalf of .....

Curator/Legal Guardian .....

Capacity : .....

Witness 1. : .....

2. : .....

Date : .....

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APPENDIX IV: Abstract of paper read at the Ist meeting of the SASHG, Rustenburg (1987).

PREDICTIVE TESTING FOR HUNTINGTON DISEASE IN SOUTH AFRICA

L.J.Schram (now Greenberg), J.Goldblatt, P.Beighton, G.Wallis.

MRC Unit for Inherited Skeletal Disorders, Department of Human Genetics, UCT Medical School.

Huntington's disease (HD) is a fatal, late onset, progressive neurodegenerative disorder of autosomal dominant inheritance.

The recent discovery of a polymorphic DNA probe (G8) showing genetic linkage to HD has facilitated predictive testing and antenatal diagnosis of HD. However, before the implementation of a genetic service of this nature in South Africa several problems need to be resolved. These include :-

i) the degree of heterozygosity at the G8 locus and its suitability for linkage analysis

ii) the exclusion of non-allelic heterogeneity for HD in the local kindreds

iii) the provision of adequate psycho-social support systems for 'at-risk' individuals on implementation of the service.

A DNA banking centre for HD has been established in the Department of Human Genetics at UCT and 280 blood specimens from 30 families with HD have been collected. Different ethnic groups are being screened to determine the RFLP frequencies and the estimated degree of heterozygosity at the G8 locus. Preliminary linkage analysis in families of different ethnic backgrounds has not suggested non-allelic heterogeneity. A questionnaire survey has been initiated to ascertain the attitudes of persons at risk for HD towards the advent of predictive and antenatal testing and to assess their psycho-social needs.

Analysis of the data concerning heterozygosity of the probes, heterogeneity and attitudes of the 'at-risk' individuals will facilitate the future implementation of a genetic service of this nature in South Africa.

APPENDIX V: Copy of the original proposal.

PROPOSED PROTOCOL FOR IMPLEMENTATION OF PREDICTIVE TESTING FOR HD IN SA.

I. TECHNOLOGY:

(1) establish a DNA bank for HD at UCT Medical School.
(2) establish a programme to offer CVS for prenatal diagnosis.
(3) establish contact with an overseas laboratory to assist with the interpretation of problematic results.

II.COUNSELLING SERVICE; PRE AND POST TEST.

In establishing this service it is envisaged that the following persons would be involved:

(1) MEDICAL GENETICIST :
a) to assess 'at-risk' individuals' knowledge about HD.
b) to explain the predictive test and its limitations to the individual; also its immediate and long term implications and any other information needed by the patient to reach an informed decision about his future.
c) to explain the options and consequences of prenatal diagnosis if requested.

(2) GENETIC SISTER :

a) to arrange appointments.
b) to assist in drawing up pedigrees.
c) to contact key relatives.
d) to collect blood for DNA studies.
e) to be the link between the patient, the medical technologist.
f) to provide long term follow up.

(3) NEUROLOGIST :

a) to examine the 'at-risk' individual for any manifestation of HD which would preclude predictive testing.
b) for management and long term follow up of HD patients.

(4) PSYCHIATRIST AND PSYCHOLOGIST :

a) to test mental and emotional stability of 'at-risk' individuals requesting predictive testing.
b) to assess and advise the counsellor regarding patient response to pre-test counselling.
c) to advise the counsellor in helping patients' coping with test results.
d) to provide crisis intervention where necessary.
e) to provide long term support if needed.

(V) SOCIAL WORKER : to be involved with family and patient support and home visits.

VI) OBSTETRICIAN/ GYNAECOLOGIST : contact to be established for advice when needed and referral of patients when necessary for:
a) prenatal diagnosis; CVS or TOP.
b) options about having children; AID, IVF or adoption
c) contraception and sterilization.

Structure and numberof counselling sessions both pre and post test to be decided on.

It is envisaged that a core team consisting of a Medical Geneticist, a Genetic Nurse, a Psychologist and a Social Worker be involved in each counselling session with a Neurologist and a Psychiatrist being available for consultation when needed.

III. QUESTIONNAIRE : to ascertain attitudes of 'at-risk' individuals towards predictive testing.
a) to be compiled based on similar existing questionnaires
b) venue to be arranged
c) research number to be obtained.

IV. EDUCATION : About HD and Predictive Testing.
a) Lay group - Huntington society.
b) State Health Nurses.
c) GPs.

V. DIFFICULTIES TO BE RESOLVED : Moral, ethical and legal issues.

a) limitations of the test - only 96% accuracy. There is a necessity for an appropriate family structure and involvement of numerous family members for linkage analysis. There is also the possibility of the individual not being informative with the markers used.
b) no cure - prediction of a lethal condition with stigmatization.
c) ethics of 'no news' 'good news' prenatal diagnosis.
d) rights of the spouse to insist on the 'at-risk' partner having the predictive test against their will.
e) rights of vital key relatives refusing to give blood for linkage studies.
f) question of confidentiality : (i) other relatives
(ii) life insurance
(iii) employer disclosure
g) the question of survivor guilt.
h) the problem of the inability of poorly educated individuals to make informed decisions.

SUMMARY

Ultimately, it is the role of the Genetic Counsellor to inform the patients as to the consequences and all the options of the test giving them the opportunity to make their own decisions in a setting that provides diverse and continuing support where necessary.

In the past, genetic counselling for the empiric risk of inheriting HD was based solely on family history and pedigree analysis. Now, prediction can be made with 96% certainty based on linkage analysis. It has been predicted that in the next two years the gene for HD will be isolated and characterized, which will ultimately lead to treatment and gene therapy.
This provides us with an ideal opportunity to prepare ourselves for the time when we will be able to offer predictive testing with 100% accuracy and without having to involve the extended family. To date, eight countries are already offering predictive testing and by close collaboration with these centres we will eventually reach the stage where we too will be able to offer the test. This will be done in a prepared, secure environment where "at- risk" individuals will be able to make informed decisions in the knowledge that there will be the necessary continuing support for them if they should need it.

IN CONCLUSION

Are we not in danger of introducing a new psychiatric disease? A condition where the individual knows that he has a 96% chance of developing a dreadful lethal incurable condition in the future. This knowledge can immediately alter his entire life drastically and not just the last 10-15 years after the onset of HD.

In view of the poor economic climate and state of medical services at the present, can the test be offered here in the near future, bearing in mind the considerable expense of medical personnel, technical expertise and back up services required?

SUGGESTION

A committee is needed to decide whether or not this test should be offered.
This committee should include;
Professor Beighton and representatives from the Department of Human Genetics
The Medical Superintendent.
Professor Benatar to deal with moral and ethical issues.
A Psychiatrist.
A Neurologist.
The Chief Social Worker.

HD CLINIC :Suggestions for improving the existing clinic.

At present this clinic performs the following functions :
(i) is mainly diagnostic and therapeutic
(ii) provides genetic counselling if needed
(iii) collects blood for the DNA bank.

There are additional needs to provide overall care for the HD patient.
These are as follows:
(a) A Social Worker to be attached to the clinic to deal with family problems, disability pensions, institutional placement and help with temporary care to relieve family stress.
(b) Access to :- Dieticians, Physiotherapists, Occupational Therapists, Speech Therapists, and Dentists.
(c) Day care or community centre to help family with everyday care and other problems.

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QUESTIONNAIRE

A: PERSONAL INFORMATION

Survey Number:

Sex: Ethnic group: Date of birth: Religion:

Occupation:

Level of education obtained:

Marital status: Date of marriage:

Number of children:

Year of birth: 1. 2. 3.

4. 5. 6.

7. 8. 9.

Number of grandchildren:

Year of birth: 1. 2. 3.

4. 5. 6.

7. 8. 9.

Do you intend to have any/more children ?

If yes, how many ? If no, why not ?

How are you related to the HD patient ?

Have you lived with or cared for the HD patient ? (or any other contact):

If yes, for how long ?

How long has the patient had HD ?

How old was the patient when it first started ?

What do you consider to be the biggest problem for

a) the patient with HD

b) the family

Are you at risk of inheriting HD ?

If yes, when did you learn this (what age) ?

Where and how ?

Do you know of any test that shows whether you will get HD ?

B. We would like to see how much you know about HD.

1. From which source did you gain most of your knowledge of HD ?

Family doctor
Specialist
Friends or relatives
Genetic counselling clinic
Books or films
Other (please specify)

2. Which part of the body is involved in HD ?

Heart Kidneys Brain

3. How is HD contracted ?

Virus infection Inherited Alcoholism/Drug abuse

4. What is the usual age group for HD to appear ?

24 years or less 25-44 yrs 45 years or over

5. What are the most common changes to the body in HD ?

Jerky movements and twisting
Gradual paralysis of arms and legs
Painful, swollen joints

6. Once the symptoms of HD appear, do they become:

Gradually better Gradually worse
Stay the same indefinitely Gradually worse, then better ?

7. What form of treatment is common for HD ?

X-ray treatment Physiotherapy
Surgery Drug treatment

8. Does HD affect both male and females equally ?

Yes No Don't know

9. What are the chances that a parent with HD will pass the conditi on to his children ?

25% chance 0% chance 50% chance

75% chance Not sure, but you know there is a risk

10. Can a family member who does not yet show features of HD pass th disease on to his/her children ?

Yes No Don't know

11. Are you aware of any research going on in the field of HD ?

Yes No Don't know

12. Who would you approach should you require information about HD f yourself or family members ?

C. PLEASE ANSWER THE FOLLOWING QUESTIONS

At present there is no cure for HD. If a test was available to diagnose HD long before the onset of symptoms:

1. Would you advise all people at risk to have such a test ? .....

2. Would you have the test done yourself ? .....

3. Would you be willing to have a child of yours undertake this test ?

.....

4. Would you discourage a child of yours from having such a test ? If so, why? .....

.....

5. How would you feel if your test was negative ? .....

.....

6. How would you feel if your test was positive ? .....

.....

7. Would you require help in coping with the result either way and if so, who would you go to ? .....

.....

8. At present, if the test was available it would only be 95% accurate. Would you still take the test ? .....

9. Would you only have the test if it was 100% accurate ? .....

10. If your test was positive for HD, would you have children ? .....

11. If your test was negative, would you have children ? .....

12. What age do you think it best for a child to have the test ? .....

13. Would you/your wife have a prenatal test if available ? .....

14. If this test was positive, would you/your wife have the pregnancy terminated (legal abortion) ? .....

D. ATTITUDES TOWARDS PREDICTIVE TESTS:

In the future a test will probably be available to you and other family members. This test, if informative, will tell you whether you will develop HD in the future or not but there still is no cure. This test will not be compulsory and will only be done if you ask for it, and all results when available will be kept strictly confidential.

Now that we have completed the questionnaire and discussed HD and the new test with you, please answer the following questions:

When the test becomes available:

1. Would you have the test done yourself ? .....

2. Would you advise all people at risk to have such a test ? .....
   .....

3. Would you be willing to have a child of yours undertake this test ?
   .....
   .....

4. Would you discourage a child of yours from having such a test ?
   If so, why ? .....
   .....

5. How would you feel if your test was negative ? .....
   .....

6. How would you feel if your test was positive ? .....
.....

7. Would you require help in coping with the result either way, and if so, who would you go to ? .....

8. At present, if the test was available it would only be 95% accurate. Would you still take the test ? .....

9. Would you only have the test if it was 100% accurate ? .....

10. If your test was positive for HD, would you have children ? .....

11. If your test was negative, would you have children ? .....

12. What age do you think it best for a child to have the test ? .....

13. Would you/your wife have a prenatal test if available ? .....

14. If this test was positive, would you/your wife have the pregnancy terminated (legal abortion) ? .....

APPENDIX VII: Abstract of paper read at the 7th IHA workshop and conference, Genoa (1987).

PREPARATION FOR PREDICTIVE TESTING FOR HD IN SOUTH AFRICA

LJ Schram [LJ Greenberg], P Beighton, J Goldblatt, G Wallis

At present we have about 85 South African families with Huntington's Disease documented by the Department of Human Genetics, University of Cape Town. A DNA banking centre has been established to obtain specimens from the family members and 330 bloods from 30 families with Huntington's Disease have been collected.

Most of these families are of European origin or mixed ancestry but in the rural Black populations, where Huntington's Disease is rare, there is no evidence of European admixture. For this reason the possibility of heterogeneity is being investigated.

Linkage analysis in South African families from different population groups has not as yet revealed any instances of discordance between the G8 locus and HD.

In anticipation of predictive testing for Huntington's Disease, various issues relating to its practical implementation in South Africa's multi-ethnic society have been addressed. Recommendations to date for the implementation of predictive testing have been based on studies of informed sophisticated societies. These guidelines are not generally applicable to the South African situation where social and ethnic background differences, the lack of counselling facilities and logistics of back-up services in rural areas are of major concern.

A questionnaire survey is currently underway to assess the understanding and attitudes of at risk individuals to predictive testing and antenatal diagnosis in order to provide adequate psycho-social support systems based on their needs. The Huntington's Disease Association of South Africa was founded at our instigation and assists in the dissemination of accurate information regarding HD to its members. Special clinics have been established for the counselling of at risk individuals. Data arising from this survey will be used for the preliminary planning and future implementation of a preventative programme for HD based on the specific needs of the persons concerned.

It is essential that these results be considered in the international discussion of predictive testing guidelines as they represent the third world situation.

APPENDIX VIII: Abstract of paper read at the 3rd meeting of the SASHG, Durban (1989).

'AT-RISK' PERSONS KNOWLEDGE OF AND ATTITUDES TOWARD HUNTINGTON DISEASE AND PREDICTIVE TESTING : RESULTS OF A SURVEY IN CAPE TOWN

GREENBERG L J

MRC Unit for Inherited Skeletal Disorders, Department of Human Genetics, UCT Medical School, CAPE TOWN.

Forty questionnaires were distributed to Huntington Disease (HD) families throughout the greater Cape Town area. 20 individuals, at risk of inheriting HD, volunteered to complete the in depth structured questionnaires to determine their knowledge about HD and assess their attitudes towards predictive testing. In terms of genealogical data, 17 (85%) were at 50% risk and 3 (15%) were at 25% risk of having inherited the gene for HD at the time of the survey. Of the 20 respondents, 15 (75%) were of mixed ancestry and 5 (25%) from the white population group.

The initial results of the survey indicate the limited extent of the individuals' knowledge about the genetics of HD and the implications for themselves. 10 (50%) were totally unaware of their own risk status while only 6 (30%) of these were unsure of the mathematical risk of inheriting the disorder if there was a history of HD in a family. Only 6 (30%) knew about the possibility of a predictive test.

It is evident that there is a strong element of denial in this 'at-risk' population group. Whereas individuals are informed about the genetics of the disorder they are often unable to apply this information to their own risk status. The anticipated demand for predictive testing, should it be available in South Africa, is much smaller than in previously published international studies. This situation is possibly due to the lack of awareness and basic knowledge of the implications of HD. The survey was probably responsible for drawing the attention of many HD families to the genetic implications of the disorder. The long term impact of this knowledge on their quality of life is not easy to assess at this stage.

The data indicates the need for a well organised programme designed to address the genetic, psychological and ethical issues that may arise in the use of presymptomatic testing for HD. Any prediction service of this nature must have adequate pre-test counselling with the main emphasis on education and enlightenment as well as follow-up support. Of utmost importance is the availablity of professional and community resources to deal with the issues and the final impact of predictive testing. It is clear that strategies need to be developed to care for otherwise healthy persons who can be given a diagnosis years before the onset of the condition. In HD, more than in any other genetic disorder, an integrated professional team approach to counselling is essential.

APPENDIX IX: Abstract of the poster presented at the March of Dimes Congress, Boston (1989).
CLINICAL APPLICATIONS OF MOLECULAR GENETICS.

PREPARATION FOR A MOLECULAR SERVICE FOR HUNTINGTON DISEASE IN SOUTHERN AFRICA.

Greenberg LJ, MRC Unit for Inherited Skeletal Disorders, Department of Human Genetics, University of Cape Town Medical School, South Africa.

Huntington Disease (HD) is a fatal, late onset, progressive neurodegenerative disorder inherited as an autosomal dominant trait.

The discovery of a polymorphic DNA probe (G8) showing genetic linkage to HD has facilitated presymptomatic testing and antenatal diagnosis of HD. Before the implementation of a genetic service of this nature in Southern Africa however, several problems need to be resolved. These issues include the usefulness or frequency of heterozygosity of the marker in the different South African population groups, the possibility of non-allelic mutations causing the HD phenotype and the ethical, psychological and sociological problems that might arise from the clinical application of such a preclinical test.

A DNA banking centre for HD was established in the Department of Human Genetics at the University of Cape Town in 1986 and 370 blood specimens from 39 families with HD have been collected. Different ethnic groups are being screened to determine the RFLP frequencies and the estimated degree of heterozygosity at the G8 locus. Other probes closer to the HD gene are also being applied to determine their suitability for linkage analysis in the various populations of this country.

Preliminary linkage analysis in families of different ethnic backgrounds has not suggested non-allelic heterogeneity.

A questionnaire survey has been undertaken to ascertain the knowledge and attitudes of persons at risk for HD in Cape Town in preparation for the advent of predictive and antenatal testing. The main objective of this undertaking is to provide adequate psycho-social support systems based on the specific needs of the persons concerned.

Predictive testing guidelines have been laid down by American and European investigators who are often unfamiliar with the culture, customs and economic pressures within developing countries. In terms of the ethical considerations related to HD a careful examination of cross-cultural ethics is critical and basic ethical principles need to be interpreted and applied within different cultural settings.

Analysis of the data concerning heterozygosity of the probes, heterogeneity and the attitudes of 'at-risk' individuals will facilitate the future implementation of a molecular genetic service for HD in Southern Africa.

APPENDIX X:

Computer form of the Huntington disease dBase file.

NAME	DOE JOHN
FAMNAME	ALLEMAN H16
BIRTHDATE	401110
SEX	M
ETHGROUP	E
NUMBERCHIL	5
CENTRE	CT
CLINIC	GSH HD
REASON	FAMILY HISTORY OF HD SHOWING SIGNS OF HD
EXAMINER	DR.SMITH
DIAGCODE	AFFECTED
DCONFIRM	Y
ONSETAGE	38
DIAGAGE	34
PARENTDNAN	H 110/86SED
SPOUSEDNAN	H 120/86ALI
BLOODDATE	860612
AMNTBLOOD	30
DNAISODATE	860816
CONC	150 A 3 30
DNAISODAT2	871107
CONC2	200 B 5 49
DNAISODAT3	871211
CONC3	200 F 5 47
DNAREMAIN	550 UG
ASOF	880110
DNASTORED	A 3 30
DNANUMBER	H 95/86JOH
FOLDERNO	34509878
LASTDATE	880110
DEATHDATE	0
DEATHAGE	0
RECORDNO	101