INTELLECTUAL AND BEHAVIOURAL FUNCTIONING IN BOYS WITH DUCHENNE MUSCULAR DYSTROPHY: NEUROPSYCHOLOGICAL TESTING AND CORRELATION WITH GENOTYPE.

Thesis document submitted to the School of Adolescent and Child Health of the University of Cape Town in partial fulfilment of the requirements for the degree Masters in Philosophy (Paediatric Neurology)

By

Kirsten Ann Mary Donald

Student number DNLKIR001

MBChB, DCH (SA), MRCPCH (UK), FCPaed (SA)

SEPTEMBER 2008

Supervisor [s]: **Prof Jo Wilmshurst**
Dr Kevin Thomas

Faculty of Health Sciences
UNIVERSITY OF CAPE TOWN

DECLARATION

I, *Kirsten Ann Mary Donald* hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

_____

Kirsten Ann Mary Donald

_____

Date

TABLE OF CONTENTS

Declaration	2
List of tables and figures	4
Abbreviations	6
Acknowledgements	7
Abstract	8
Introduction	11
Literature Review	13
Aim and specific Objectives	26
Methods	27
Results	35
Discussion	67
Recommendations	74
References	76
Appendices	83

LIST OF TABLES AND FIGURES

Table 1: Background Characteristics of DMD and control group for cognitive evaluation 35
Table 2: *GMDS* results for affected and control groups 38
Table 3: Syndromes of behaviour 48
Table 4: *Internalising*, *Externalising problems* and *Total Problems* 63
Table 5: *Total Behaviour Problems* in boys receiving steroids and those not receiving steroids. 66
Table 6: Means for *Total Behavioural* problems 66

Figure 1: Genomic organisation of the dystrophin gene, located in Xp21 15
Figure 2: Performance on *General Quotient (no MS)* 39
Figure 3: Performance on *General Quotient (average MS)* 40
Figure 4: Performance on *Personal Social* subscale 41
Figure 5: Performance on *Hearing and Speech* subscale 42
Figure 6: Performance on *Hand and Eye Co-ordination* subscale 43
Figure 7: Performance on *Performance* subscale 44
Figure 8: Performance on *Practical Reasoning* subscale 45
Figure 9: DMD boys vs. international norms on *Somatic Complaints* 49
Figure 10: DMD boys vs. international norms on the *Anxious/Depressed* syndrome 50
Figure 11: DMD boys vs. international norms on the *Withdrawn/Depressed* syndrome 51
Figure 12: DMD boys vs. international norms on *Attention Problems* 52
Figure 13: DMD boys vs. international norms on *Aggressive Behaviour* 53
Figure 14: DMD boys vs. international norms on *Social Problems* 54
Figure 15: DMD boys vs. international norms on *Thought Problems* 55
Figure 16: DMD boys vs. international norms on *Rule-breaking Behaviour* 56
Figure 17: DMD boys' performance on syndromes of behaviour subscale 58
Figure 18: DMD boys vs. international norms for *Internalising Problems* 60

Figure 19: DMD boys vs. international norms for *Externalising Behaviours* 61
Figure 20: DMD boys vs. international norms for *Total Problems* 62
Figure 21: DMD boys' performance on *Internalising vs. Externalising Problems* subscale 64

Box 1: IQ interpretations 68

ABBREVIATIONS

ADHD: Attention Deficit Hyperactivity Disorder
ASD: Autistic Spectrum Disorder
BMD: Becker Muscular Dystrophy
CBCL: Child Behaviour Checklist
CP: Cerebral Palsy
DMD: Duchenne Muscular Dystrophy
DNA: Deoxyribonucleic acid
FIQ: Full-scale Intelligence Quotient
GMDS: Griffiths Mental Development Scales
GQ: General Quotient
HECS: Hand-eye Co-ordination subscale
HSS: Hearing and Speech subscale
IQ: Intelligence Quotient
kDa: Kilodalton
MLPA: Multiple Ligase-dependant Probe Amplification
MS: Motor subscale
OCD: Obsessive –compulsive Disorder
PCR: Polymerase chain reaction
PRS: Practical Reasoning
PS: Performance subscale
PSS: Personal and Social subscale
RNA: ribonucleic acid
SD: Standard deviation
UCT: University of Cape Town

ACKNOWLEDGEMENTS

I wish to thank my supervisors Professor Jo Wilmshurst and Dr Kevin Thomas for their guidance and encouragement.

Beyond her role as supervisor, I would like to thank Jo Wilmshurst for her tireless energy in motivating and supporting an environment which allows clinical research in a hugely busy service. I would also like to thank the other members of the neurology and development team for their support and encouragement: Dr Alvin Ndondo, Dr Gill Riordan, Dr Veruschka Ramanjam and Dr Kathie Walker.

Thanks also goes to the paediatric neurology department administration and nursing staff at Red Cross for help with obtaining patient details and a host of other administrative tasks which come up on a project such as this.

To Nancy Malgas of the University of Cape Town psychology department who spent many hours assisting me with statistical analysis.

To my husband for his encouragement, as well as his patience and help with mastering the computer.

To the Muscular Dystrophy Foundation of South Africa for their generous financial support and School of Child Adolescent Health for their research grant.

Finally my thanks go to the patients and their guardians who partook in testing taking many hours during the completion of this task.

ABSTRACT

Introduction

Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy seen in paediatric practice. The condition affects approximately 1 in 3300 live male births and occurs across all ethnic groups. It is an X-linked recessive disorder characterized by progressive muscle weakness and degeneration of skeletal muscle.

The spectrum of central nervous system manifestations of DMD is less well described than its musculoskeletal aspects. Although international studies have reported intellectual function ranging from above-average to severe intellectual disability, they have consistently found the average full-scale IQ of affected boys to be reduced by approximately one standard deviation. Fewer reports are available for DMD boys in the pre-school age group. There is also limited data on the behavioural profile of boys with this condition. No material on these aspects of DMD in South African children has been published to date.

This pilot case control study aimed to determine the neurocognitive and behavioural phenotype of a cohort of South African children with a confirmed diagnosis of Duchenne muscular dystrophy as compared to the profile of a matched control cohort of children.

Method

The sample consisted of a group for cognitive testing (5 pre-school boys with DMD and 4 suitably matched controls) and a larger group with a bigger age-range for behavioural assessment (11 boys). The cognitive measure used was the Griffiths Mental and Development Scales. The tool used for behavioural assessment was the Achenbach Child Behaviour Checklist (Parent Questionnaire). Testing was conducted in the paediatric neurology department at the Red Cross Children's Hospital, Cape Town.

Results

Even with the removal of the motor scale from the scores of the Duchenne boys, the General Quotient scores were significantly lower than their normally developing counterparts, where the mean $(M)_{DMD} = 78.90$; $M_{CONT} = 106.20$; $p = 0.027$ with effect size = 1.89. The group displayed significantly poorer performance in the hand-eye co-ordination subscale in relation to their controls ($p=0.03$). Three out of the 5 remaining subscales approached significance with a p-value of 0.05.

The results of parental reports on the behaviour of the DMD boys in our group reveal higher rates of general behavioural problems (54.5%) than normative data. This figure is slightly higher than reported in previously conducted studies on general behavioural problems in boys with DMD. This may be due to the exposure of the majority of our children to significant socio-economic stressors. The discrepancy may, however, also be an erroneous finding as a result of our very small sample size.

Conclusion and Recommendations

The cognitive profile of the pre-school group of boys with DMD as compared to controls is in keeping with previously reported international figures. The profile of the boys in the behaviour group was also in line with other, larger studies although the specific behaviour syndrome profile displayed some differences to a recent report of a cohort of American boys with DMD.

Current practice in the ongoing care of boys with Duchenne muscular dystrophy at Red Cross Children's Hospital does not routinely include cognitive evaluation or behavioural screening. This study suggests that boys with this condition are at greater risk for problems in both areas and that it may be of benefit to identify the

children with these problems so they can be managed with early intervention specific to their special needs.

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy seen in paediatric practice. The condition affects approximately 1 in 3300 live male births and all ethnic groups. It is an X-linked recessive disorder characterized by progressive muscle weakness and degeneration of skeletal muscle. Although there is a wide spectrum of severity, most boys with the diagnosis of DMD will have lost ambulation by 13 years of age and premature death generally occurs in the second or third decade. The central nervous system manifestations of the condition are less well described.

Since molecular genetic testing became available in South Africa, all patients suspected to have DMD attending the Neuromuscular Service at Red Cross Children's Hospital, have been offered screening for deletions (or point mutations) of areas of their dystrophin gene as a routine part of their diagnostic workup. We have previously created a database of children attending our institution with the diagnosis of DMD. For the majority of children the diagnosis was confirmed on either genetic testing (demonstration of deletion or point mutation in appropriate region) or muscle biopsy (demonstrating near complete absence of dystrophin at the muscle fibre sarcolemma on immunohistochemical staining).

Although cognitive problems are extensively reported internationally (if still imperfectly defined), there is limited published data on the cognitive profile of boys with DMD in South Africa and nothing attempting to correlate the genetic diagnosis with intellectual functioning. It can be postulated that these children may be at a greater risk for developmental problems compared to boys with DMD living in first world countries, mainly because of unfavourable socio-economic circumstances and access to fewer service resources (Richter & Grieve, 1991). Regular developmental evaluation should be regarded as an essential component of the overall care of children with DMD, especially in this social

context. However, in developing countries such as South Africa resources to undertake such evaluations are scarce and they may not occur routinely.

There is limited published data on the prevalence of behavioural problems or neuropsychiatric co-morbidities amongst children suffering from DMD in first world settings. There remains nothing on children who live in developing countries and are already at risk for behavioural problems (as well as learning difficulties) as a result of their deprived social circumstances (Donald & Dawes, 1994). The neurology team[1] felt it would be of value to investigate this further in our cohort of boys with DMD.

This study was an opportunity to describe the genetic, cognitive and behavioural profile of a cohort of boys living with DMD in South Africa. This may enable clinicians to identify a subgroup of children with DMD who are particularly at risk of learning difficulties and/or behavioural problems and as such should be managed with early intervention specific to their special needs.

[1] "The neurology team" refers to the paediatric neurology team at Red Cross Children's Hospital as detailed in the acknowledgements

LITERATURE REVIEW

GENETICS

Duchenne muscular dystrophy is known to have X-linked recessive inheritance and the actual gene has been mapped in detail. The gene is located at Xq21.2 and is the largest gene characterized to date, comprising nearly 0.1% of the entire human genome (Anderson, Head & Rae, 2002). Ninety-nine percent of the 2.4 MB dystrophin gene consists of introns with 86 exons that encode 14KB of deoxyribonucleic acid (DNA). Its main product is a 427 kilodalton (kDa) protein known as dystrophin. This is primarily expressed in skeletal and cardiac muscle with small amounts expressed in the brain (Nudel, Zuk & Einat, 1989). There are three active and unique promoters at the 5' end of the gene. These promotors all produce a full-length 427kDa isoform, with the same number of exons, but which vary by a few amino acids at the amino terminus and are preferentially (but not exclusively) expressed in 3 different sites (brain, muscle and Purkinje cerebellar neurons). **(Figure 1)** The tissue-specific expression of these isoforms is believed to account for aspects of extra-muscular disease. The transcript from the M-promoter is found in muscle, glia and the vascular endothelium of vessels in the central nervous system. The transcript from the B-promoter is found in the hippocampus and cerebral cortex and the transcript from the P-promoter seems to be found only in the cerebellar Purkinje cells and fetal cerebral cortex. Of the smaller isoforms, Dp260 is the largest and is localised to eye photoreceptor cells. Dp140 is found in the brain, kidney and developing foetal central nervous system. Dp116 is found in peripheral nerves, Schwann cells and in the foetal striatum. Dp71 is present in the ventral neural tube and embryonic mid- and hindbrain (Blake & Kroger, 2000).

DMD is caused by mutations that change the reading frame of the dystrophin transcript, resulting in premature stop codons and instability of the transcript.

The dystrophin gene has a high mutation rate and approximately a third of new cases of DMD are a result of new mutations (Anderson, Head & Rae, 2002). The most common gene mutation in DMD is a deletion, accounting for 65% of mutations. The remainder is made up of either duplications (5%) or point mutations, small deletions or insertions (35%) (Dellafave & McNally, 2007). It is therefore possible in the majority of cases (>65% in most centres) to identify not only the presence of a gene deletion, but also the exact position and size of that deletion. Deletions (and less commonly duplications) can occur anywhere in the gene. There are, however two well recognized deletion "hotspots". One is positioned closer to the 3' end of the gene and the other at the 5' end. Deletions in the first region generally include exons 45-55 and in the second region include exons 2-19. These "hotspots" have allowed the widely used multiplex polymerase chain reaction (PCR) technique to identify 98% of deletions by screening only 19 exons. The University of Cape Town (UCT) genetics laboratory has used this method from the early 1990s until late 2007 when the newer Multiple Ligase-dependant Probe Amplification (MLPA) technique was introduced (Niepieklo, 2008).

There is not a direct relationship between the size of the deletion and the severity of muscle disease (Koenig, Beggs & Moyer, 1998). However the position of the deletion (specifically if it disrupts the reading frame) seems to have an effect on the resulting phenotype (Muntoni, Torelli & Felini, 2003). Certain boys with intellectual disability at the severe/profound end of the spectrum have been shown in some studies to be more likely to have a mutation in specific areas of the gene (predominantly the 3' end). (Bushby, Appleton, Anderson, Welch & Gardner Medwin, 1995)

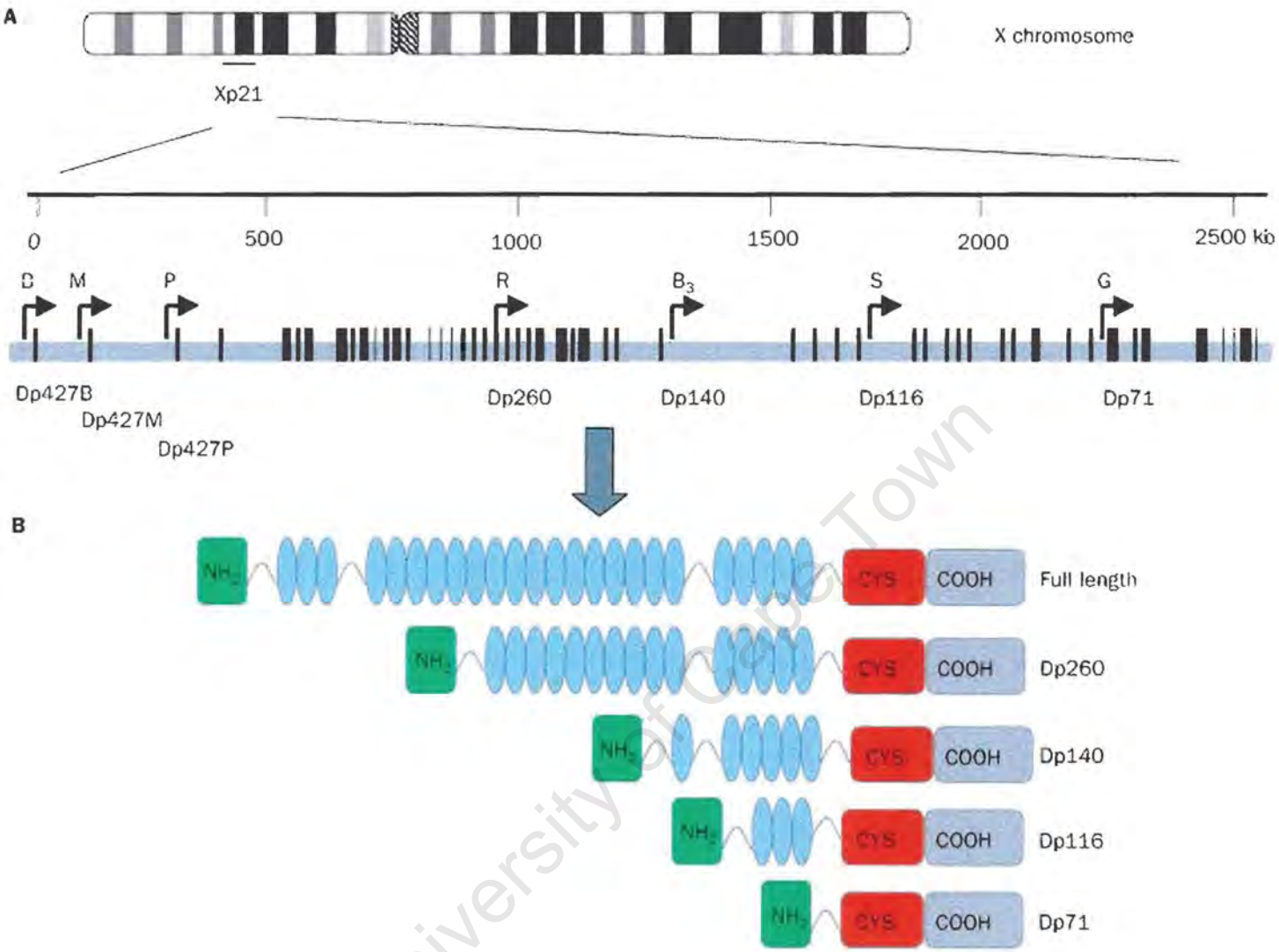


Figure 1. A: Genomic organisation of the dystrophin gene, located in Xp21. The black vertical lines represent the 79 exons of the dystrophin gene distributed over about 2·5 million bases. The arrows indicate the various promoters: in particular are brain (B), muscle (M), and Purkinje (P) promoters; R, B3, S, and G represent the Dp260 (retinal), Dp140 (brain3), Dp116 (Schwann cells), and Dp71 (general) promoters.

B: The domain composition of the various dystrophin proteins is indicated. The amino-terminal domain is followed by the spectrin like domain, the cysteine rich, and the carboxy-terminal domain.

Figure reproduced with personal permission from Prof F Muntoni as well as *The Lancet Neurology* (2007).

Very little has been published about the genetic profile of boys with DMD in South Africa. Ballo *et al* (1994) reported on a multi-center South African study based on a genetic service offered to families of boys with DMD or Becker muscular dystrophy (BMD). Molecular genetic screening was performed on DNA from 128 patients diagnosed 1987-1992. The technique employed in this study involved screening for deletions in the 'hotspot' areas in the 5' and 3' regions of the dystrophin gene. The affected boys were diagnosed clinically and the number with immunohistochemical confirmation was not recorded. It is therefore a possibility that a proportion of the individuals in the study were misdiagnosed. The authors found that 50% of DMD patients of European (n=16/32), Indian (n=12/24) or mixed ancestry (n=14/31) had identifiable deletions. However, only 22% of DMD patients of African ancestry (n=10/41) had identifiable deletions in their cohort. They concluded that the low deletion frequency seen in DMD patients of African ancestry suggested that they may have unique mutations not detectable using methods current at that time. Since 1992, however, many more deletion sites have been identified and the confirmation of diagnosis has become more defined.

Hallworth-Pillay and colleagues (2007) described a cohort of boys with DMD in Durban. Of the 53 patients with a diagnosis of DMD, 33 were deletion positive using standard PCR techniques (62%). Nineteen out of a total 29 boys of African ancestry (62%) were positive for deletions at one of the gene 'hotspots' (Hallworth-Pillay, Bill, Maduri, Mubaiwa & Rapiti, 2007). These figures present a similar frequency to those reported in the international literature to date and refute Ballo *et al*'s findings.

Hallworth-Pillay *et al* also reported a prevalence of 12 boys (18%) with "mental retardation" in their group of 53 boys with DMD and 15 with Becker muscular dystrophy. However, no definition of "mental retardation" was given and no mention was made of which formal measures used to categorise these boys as

suffering intellectual disability was made. Comparative commentary on this data is therefore not possible.

PATHOGENESIS

The muscle disease is caused by the absence of the dystrophin protein which plays a vital role in maintaining muscle membrane stability. It is a low-abundance protein and constitutes only 0.002% of total muscle protein (Menkes, Sarnat & Maria (eds), 2006. p985). However, the loss of dystrophin and consequent destabilisation of its associated protein complex results in the myofibres becoming vulnerable to contraction-induced damage through plasma membrane leakiness. As the illness progresses there are repeated episodes of necrosis and regeneration of muscle cells. Generally regeneration is inadequate and over time the number of muscle cells is reduced and fibre size variability increased. This correlates with the clinical progression of muscle weakness over time in boys with DMD. The clinical phenotype is related to the amount of functional dystrophin present rather than the size of the gene deletion (Koenig *et al*, 1998). Boys with a severe phenotype may demonstrate completely absent dystrophin on immunohistochemical staining of their muscle (McIntosh, Helms & Smyth(eds), 2003. p995).

The only non-muscle tissue in which dystrophin is significantly expressed is the brain. The level of dystrophin expressed in the brain is lower than in muscle (approximately 10% compared to levels in skeletal muscle) and the structure of brain-messenger ribonucleic acid (RNA) for dystrophin differs from the form expressed in muscle. This is a result of the promoter for cortical dystrophin being located at least 90KB upstream from the muscle promoter. Truncated dystrophins, such as the 140KDa Dp140 and the Dp71 are found in normal brain and seem to be preferentially expressed in certain areas of the brain as detailed above. Their exact role is as yet poorly defined.

Although Duchenne muscular dystrophy is now widely accepted as being associated with an increased risk of generally mild, non-progressive intellectual disability, no obvious or consistent neuropathology (either gross or histological)

has been identified in the brains of patients with the condition (Anderson *et al*, 2002).

Bresolin *et al* (1994) demonstrated decreased uptake of fluorodeoxyglucose in the cerebellums of a group of DMD boys. This pattern of hypometabolism was not demonstrated in children with other global physical disability (Spinal muscular atrophy type1), suggesting that cerebellar hypometabolism may not be related to the physical manifestations of DMD and may play a role in the cognitive profile. These findings are likely to be relevant given the fact that the cerebellum is known to be one of the major foci of dystrophin in the brain. Hinton and colleagues reported impairments in verbal working memory in a large group of Duchenne boys. It was noted that specific learning difficulties of this type in children in the general population may have an association with cerebellar abnormalities (Hinton *et al*, 2000).

Based on experimental work on the *mdx* mouse[2], Knuesel *et al* (1999) have reported a possible effect of the absence of dystrophin on the synaptic clustering of GABAa receptors. The authors demonstrated a reduction in the number of GABAa clusters in the *mdx* mouse cerebellum and hippocampus of approximately 50%. The hypothesis presented is that the extensive co-localisation of dystrophin with this subset of GABAa receptors in the postsynaptic densities represents a dependent relationship and suggest that this may play a role in the cognitive impairment seen in boys with DMD. (Knuesel, Mastrocola, Zuellig, Bornhauser, Schaub & Fritschy, 1999).

[2] A strain of mice arising from a spontaneous mutation (mdx) in inbred C57BL mice. This mutation is X chromosome-linked and produces viable homozygous animals that lack the muscle protein dystrophin, have high serum levels of muscle enzymes, and possess histological lesions similar to human muscular dystrophy. The histological features, linkage, and map position of mdx make these mice a worthy animal model of Duchenne muscular dystrophy.

DMD AND THE BRAIN

Intelligence

When Duchenne originally described the disorder in 1868 he reported 5 cases with some degree of cognitive impairment. Subsequent to this initial report there was some debate about whether this was a valid observation. Over the next 100 years conflicting reports in the literature (often single case reports) continued to confuse the issue. However, there is now general consensus, based on a large body of evidence, that boys suffering from DMD have an increased risk of intellectual disability-although most individuals are not intellectually disabled(Cotton, Voudaris & Greenwood, 2001; Smith, Sibert & Harper, 1990). A large meta-analysis of all studies undertaken to look at intellectual functioning in boys with this condition up to 2001 (1224 boys) was published by Cotton *et al* (2001). Despite small individual numbers in most of the studies, inconsistency regarding definitions of intellectual disability ("mental retardation") between groups, and different tools used to measure intellectual functioning, the overall finding was that the average Intelligence Quotient (IQ) of boys with DMD was lower than their non-affected counterparts. Mean full-scale IQ (FIQ) scores were shifted down approximately 1 standard deviation from the normal population (80.2). Thirty-four percent of boys had an IQ <70 and therefore fell into the formal category of intellectual disability. Of this group, 79.3% had mild learning difficulty (FIQ 50-70), 19.3% moderate (FIQ 35-50) and 1.1% severe intellectual disability (FIQ 20-35). These findings may represent the potential for major lifetime morbidity in affected boys.

Intellectual disability does not seem to be progressive, does not correlate with the severity of muscle disease and seems to involve verbal more than non-verbal intelligence (Bresolin, Comi, Felisari, Bardoni, Perani, Grass, Turconi, Mazzucchelli & Galloti, 1994). The pathogenesis of intellectual disability in DMD is not defined to date. Studies done over the last 30 years in different centres,

and using different tools, have demonstrated a variety of results as regards specific areas of learning disability. There does, however, seem to be increasing evidence that verbal and short term memory outcomes are selectively poorer than other areas of cognitive function (Cotton *et al* 2001; Wicksell, Kihlgren, Melin & Eeg-Olofsson, 2004). Specific areas of verbal skills which have been shown to be affected have included: a basic language deficit (Karagan & Zellweger, 1978), significant impairment in word reading ability (Leibowitz & Dubowitz, 1981), delay in reading abilities, comparable to those observed in developmental dyslexia (Dorman, Hurley & D'Avignon, 1988), specific deficit of verbal intelligence and verbal memory (Billard, Gillet, Barthez & Santini, 1992), reading disability similar to dysphonetic dyslexia (Billard, Gillet, Barthez, Hommet & Bertrand, 1998), decreased verbal span capacity (Hinton, De Vivo, Fee, Goldstein & Stein, 2004), as well as specific difficulties in receptive language skills as evidenced by difficulties in syntactic and grammatical comprehension, phonological analysis and discrimination (D'Angelo, Civati, Lorusso, Marini, Comi, Turconi, Fabbro & Bresolin, 2007).

Boys tested later on in the course of their disease seem to perform less well in measures of performance IQ. This is felt to be a reflection of their progressive physical disability and resultant decline in hand agility rather than a true progression of intellectual functioning (Cotton, Voudaris & Greenwood, 2005; Leibowitz & Dubowitz 1981).

The majority of papers published on intellectual functioning in DMD have been assessments of boys of school-going age. Limited data are available on the cognitive profile of preschool boys with DMD. Smith *et al* (1990) reported global developmental delay in a group of boys <6 years old with DMD. The areas most severely affected were the locomotor and language domains. Developmental quotients remained static in all domains except locomotor, in which performance decreased (Smith *et al*, 1990).

Studies from the United Kingdom (Bushby *et al* 1995), France (Moizard, Toutain, Berret, Billard, Marmin & Moraine, 1998; Moizard, Toutain, Fournier, Berret, Raynaud, Billard, Andres & Moraine, 2000) and Italy (D'Angelo *et al*, 2007) found that in boys with DMD, the presence of a deletion of genetic material in a particular area of the dystrophin gene (3' region or distal end) was associated with an increased prevalence of learning problems when compared to boys with the condition who had a deletion elsewhere on the gene. The hypothesis for this finding is that this particular area of the gene codes for the specifically expressed brain isoforms of the dystrophin protein and that the absence of these proteins translates into an increased risk of intellectual disability (Moizard *et al*, 1998; Moizard *et al*, 2000). More specifically, Moizard (2000) reported an association between the degree of intellectual disability and the presence of a mutation in the region of the dystrophin gene which codes for the Dp71 isoform.

Behaviour

It is widely accepted that children with *any* chronic illness or physical disability are at higher risk for emotional and behavioural problems than the general population in the course of their childhood (certain authors quote as much as 20-30% of this group of children) (Rutter, 1967). However, there is limited published data on the prevalence of behavioural problems or neuropsychiatric co-morbidities amongst children suffering from DMD, even in first world settings. There remains nothing on children who live in developing countries and are already at risk for behavioural problems (as well as learning difficulties) as a result of their deprived social circumstances (Donald & Dawes, 1994).

Leibowitz & Dubowitz (1981) were the first to publish a formal assessment of behaviour in boys with DMD. They administered Rutter A (55 boys) and B (52 boys) questionnaires to the parents and teachers respectively of a group of Duchenne boys. This cohort included some boys of pre-school age (5/55 or 9%), though the majority were all of school-going age. Thirty-three percent of their

group scored above the cut-off for these questionnaires (positive for abnormal behaviour). This is well above the percentage of normally developing boys who scored above the cut-off. No more detailed diagnoses were specified. The authors found no correlation between cognitive factors and the Rutter scores in this study. Cognitive function was assessed using the Weschler Pre-school and Primary Scale of Intelligence (WPPSI) for boys between 4 and 6 and a half years and the Weschler Intelligence Scales for Children-revised (WISC-R) for the older boys.

A recent survey performed in the Northern Region of the United Kingdom amongst school-age children (5-13 years) with neuromuscular disorders of varying types, demonstrated significantly more behavioural, social and communication problems in this group as compared to the physically healthy children (Darke, Bushby, Le Couteur & McConachie, 2006). Of their group of 82 children, 37 (45%) had a diagnosis of DMD, all of whom were male. The authors point out that this may have skewed their data as male gender is a well-recognised risk factor for mental health problems and their data was compared to the norms achieved in the general United Kingdom child population. Of the group as a whole, 41.5% scored above the cut-off on at least one of the three questionnaires that were used. On further analysis there was no significant difference found between the results of the whole group and those of the DMD boys analysed separately. The presence of learning difficulties was independently associated with an increased risk of behavioural, social and/or communication problems. Despite small numbers, they also reported that the prevalence of autistic spectrum disorders (ASD) was higher in the DMD group than in the general population (5.4%).

It is of interest that amongst this group of children parents reported very low use of services in relation to behavioural problems. Twenty-two percent of parents reported that they had not seen any professionals regarding their child's behavioural problems in the 6 months prior to their muscle clinic appointment. It

In a broader approach, Hendriksen & Vles, (2008) reported the prevalence of ASD as well as attention-deficit hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD) in a group of 351 males with DMD (drawn from America and the Netherlands). Information was collected from parents anonymously by means of a postal questionnaire. Findings were that 15.7% of the group had one or more of the three neuropsychiatric conditions under investigation. 11.7% percent had been diagnosed with ADHD as compared to 7% of the general population (p=0.007). Three point one percent had been diagnosed with an ASD as compared to 0.0016% of the general population (p=0.002). Four point eight percent had been diagnosed with OCD as compared to 2.3% of the general population (p=0.027). Although these figures are based on parent reports, only diagnoses made by appropriate professionals were included in the analysis. In addition, the figure reported for ASDs was comparable to that reported in the other studies discussed here. It is therefore likely that the postulated association of DMD with these neuropsychiatric conditions is valid. Due to the nature of the above study, no comment could be made on the relationship between the cognitive profile of these boys and their psychiatric condition.

The body of evidence to date for an association between DMD and behavioural and/or neuropsychiatric conditions is not great, but the increased prevalence of these comorbidities in the studies discussed above does suggest a relationship between the two. Further investigation into the area is definitely required- particularly in developing countries where little evidence at all is available.

AIM

To determine the neurocognitive and behavioural phenotype of a cohort of South African children with a confirmed diagnosis of Duchenne muscular dystrophy as compared to the profile of a matched control cohort of children.

SPECIFIC OBJECTIVES

1. To describe the baseline neurocognitive profile of boys with DMD (<7 years old) in the neuromuscular clinic at Red Cross Children's Hospital and compare to a control group.

2. To describe the behavioural profile of boys with DMD in the above clinic.

3. To look for an association between the genetic profile of the DMD boys and their neurocognitive performance

METHODS

STUDY DESIGN

This is a descriptive case control pilot study

SUBJECTS

Children to be included in the study were all those on the Red Cross Hospital DMD database who had a confirmed diagnosis of DMD (either with genetic or muscle biopsy confirmation), for whom we could obtain parental consent. Although DMD is the most common inherited neuromuscular condition in paediatric practice, it is still rare in absolute terms. Therefore, there were only 32 boys who fell into the group we could approach to be involved in the study (appropriate age range and positive diagnosis). Ages ranged from 3-16 years. In keeping with internationally reported clinic data, the majority of the boys known to the clinic were already of school-age.

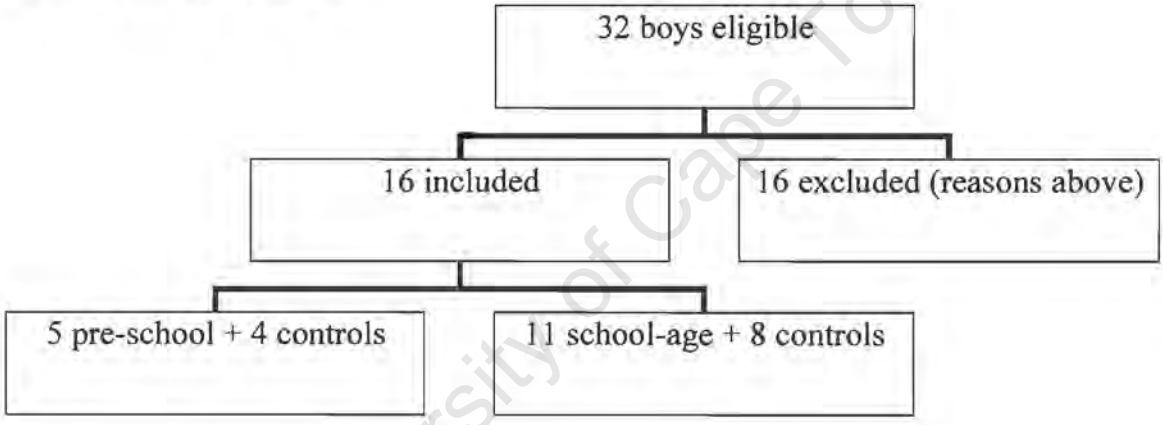
Of the 32 eligible boys only 16 were ultimately included. Reasons for exclusion included:

1) Refusal by parents or boys themselves
2) Uncontactable (patients who had dropped out of the system and whom we were unable to contact by telephone or via the local muscular dystrophy society)
3) Too unwell to take part
3) Deceased

Boys were also to be excluded if they clearly had another pathology which would impact on their neurocognitive functioning (for example a history of significant head injury or bacterial meningitis). There were no children in the study or control group who needed to be excluded for these reasons.

In addition there were controls matched for age and sex (if possible) and as closely as possible for socioeconomic background and schooling (where relevant). The controls were recruited from the child's home locality, for example a school friend, family member or neighbour of the same sex (where possible) and within 6 months of age. The control group was recruited with the help of the patients attending the clinic in an attempt to find children from the same community. They were not accessed through the education system.

The breakdown of children for cognitive testing is represented in the diagram below. The 11 boys who were included in the behavioural assessment were drawn from both groups.



ETHICAL CONSIDERATIONS AND CONSENT

Genetic (DNA) testing is part of routine clinical practice in the care of children with DMD at our institution. Testing is performed by an accredited laboratory (Department of Human Genetics, UCT medical school). Muscle biopsy confirmation is offered if a positive diagnosis is not achieved by genetic methods.

Pre-school children with DMD are not at present routinely offered formal neuro-developmental assessment unless a problem has been identified during clinic visits or parents have voiced concern. Children of school-going age in our clinic are followed up outside of our institution (for educational purposes) by Department of Education psychologists via their schools. Consent for

neuropsychological testing in this study was requested from parents of children eligible to enter the study for the neuro-cognitive testing.

Consent took the form of a verbal explanation by one of the investigators (interpretation into first language as required) and the provision of written material for both families of patients (Appendix A) and controls (Appendix B). The consent form was available in the family's first language (English, Xhosa or Afrikaans). In addition to consent from parents, verbal assent was sought from the boys themselves for children 7 years and older.

Assent from the boys was approached in the following way:

1) An explanation was given: that some boys in other countries who have the same condition, have certain types of problems at school. If they help us by doing these tests of learning and development, it may help us find out whether this is true for boys like themselves here in Cape Town.

   We also explained that if there are problems we would (with their permission), let their schools know so that extra help with their school work could be arranged.

2) We stressed that participation was entirely their choice and if they didn't feel happy taking part that this would be respected. It was further explained that even if they agreed up front, they could withdraw at any time should they feel uncomfortable

3) It was detailed that parents could phone/e-mail the principal investigator at any stage to discuss any aspect of their involvement (telephone number on the consent form of which the parents had a copy).

The study protocol was approved by the Red Cross Hospital Research Committee and to the University of Cape Town Research Ethics Committee prior to commencing testing.

MEASURES

Demographic data

This included a history on social circumstances (family income), birth background, schooling history (of parents and patient) and significant previous medical history at enrolment. Family income was assessed using the hospital categorization of family income for fee allocation purposes. This categorization does rely on accurate reports from parents on the family income on registration at the hospital.

Cognitive and Behavioural tools

A neurocognitive battery of tests was performed on each child with the disorder as well as each child in the control group. In the pre-school age children (range 3-7years) this took the form of the *Griffiths Mental Developmental Scales* (testing performed by principal investigator). The cut-off of 7 years was chosen as children in South Africa start formal schooling the year they turn 7.

School age children (7+ years) were tested by post graduate psychology student under the direct supervision of Dr Kevin Thomas, PhD, senior lecturer in the department of Psychology, University of Cape Town. The detailed results of this group have been presented in an honours thesis (Mathema, 2007).

Although serial neuropsychological testing is the most reliable measure of intellectual disability, the diagnosis may be based on neurological and developmental observations and parental report. Standardized developmental screening checklists that include both motor and language skills are adequate for this purpose (Holt, 1977).

The *Griffiths Mental Development Scales* (GMDS) was administered to the pre-school group. This scale is a comprehensive assessment of the different aspects of normal infant and child development that has been validated (but not yet standardised) for use in children of different language groups (English, Afrikaans and Xhosa) in South Africa (Allan, Liuz & Foxcroft 1992). It is suitable for use up to 8 years of age.

Subscales consist of the following areas:

A. Locomotor Subscale: This evaluates the gross motor function. It observes physical development in young children and includes the ability to run fast, to bounce and catch a ball, to jump off stairs and skipping.

B. Personal-Social Subscale: This area assesses personal and social development and includes ability to give home address and to dress/undress him/her self.

C. Hearing and Speech: This is the most intellectual of the scales and gives opportunity for the study of the growth and development of language. Items include naming of colours, comprehension of the use of items, opposites and repetition of sentences with 6-16 syllables.

D. Eye and Hand Co-ordination Subscale: This subscale consists of items relating to the handwork and visual ability of the child. Items include drawing and threading beads. This includes assessment of fine motor function.

E. Performance Subscale: These tests enable the examiner to observe and measure skills in manipulation, speed of working and precision. It is done with the use of form boards and pattern making.

F. Practical Reasoning: This looks at the earliest indications of arithmetical comprehension and the realisation of the simplest practical problems. It indicates the child's ability to benefit from formal schooling.

All the children in the younger group were either English or Afrikaans speaking and the GMDS was administered in their home language by the principal investigator. No external translator was required.

The *Child Behaviour Checklist (CBCL)* (Achenbach, 2001) was designed to assess behavioural problems and social competencies in children as reported by their parents. Two versions of this instrument exist: one for children 18 months to 5 years and one for 6-18 year olds. The checklist is composed of 113 items each scored on a 3-step scale: 0=not true, 1=somewhat true and 2=very true. In addition to the 8 syndrome report generated, the instrument provides 3 more general scores: a total score, a score on internalising behaviours (fearful, shy, anxious and inhibited) and a score on externalising behaviours (aggressive, anti-social and under-controlled). The recommended t-score transformation of the raw behaviour scores was used. These adjust for age and gender differences in behaviour scores of normative samples. A T-score of >63 in the general scores or >69 on the individual syndrome subscales represents clinically meaningful symptoms.

The *CBCL* was completed by the children's parents to assess behaviour and, when possible, by the parents of control children. Unfortunately the majority of the control children attended the testing session without a parent and so in these cases, it was not possible to get the behaviour questionnaire completed. As a result, the control group was too small for meaningful statistical comparison to the group of boys with DMD.

The Achenbach Child Behaviour Checklist has been extensively validated across wide cultural and socioeconomic spectrum (Ivanova, Achenbach & Dumenci *et al* 2007). The official validated cut-off scores used for this measure were employed to calculate the significance of clinical symptoms (Achenbach, 2001).

Full confidentiality was observed with respect to the results of the tests. This was clearly specified in the consent forms for families of both affected children and that of controls.

Every effort was made to ensure that the children were comfortable and as relaxed as possible during the duration of testing. They were allowed to withdraw at any time were they to become uncomfortable, but none exercised this option.

Testing occurred at the Red Cross Children's Hospital in the Neurology or Developmental Service consulting rooms during mornings/afternoons when the rooms were not in use for established clinics. Families were given transport money for this visit and each child received a non-monetary incentive.

Follow up

Following testing, parents were given a copy of the report of the results of the neurocognitive battery as well as a face-to-face counselling session explaining the results and the educational implications for their child. All the parents in this group elected to discuss the results personally with their child's school/pre-school following feedback.

Genetic techniques

From the early 1990's until 2007, the Department of Human Genetics at UCT tested DNA for the condition by screening the following exons within the DMD gene using the Multiplex PCR deletion screen:

5' exons screened: PM (promoter), 3, 4, 6, 8, 9, 11, 12, 13, 17, 19, 25, 32, 34
3' exons screened: 42, 43, 45, 48, 49, 50, 51, 52, 60

This method involved amplification of exons in the two deletion hotspots of the DMD gene with a set of three multiplex (amplification of more that one target) PCRs. Approximately 80% of deletions were identified , but no duplications, point

mutations or deletions of exons outside of the hot-spot regions. Amongst the 41 boys with confirmed DMD in the Red Cross Hospital database, 19 are mutation-positive and 22 are mutation-negative (ie deletion prevalence in our population prior to the introduction of the MLPA technique was 46%).

From late 2007 onwards the more detailed MLPA technique was used. DNA from boys who had previously tested negative for a deletion was retested using this extended method. The MLPA is a commercially available kit for detection of deletions or duplications of exons of the DMD gene. Two sets of molecular probe-mixes include probes for all of the exons in the gene, not just those in the deletion hotspots. The product resolution is done by capillary electrophoresis using the ABI3100 Gene Analyser. The deleted exons produce no product/signal. Detection of duplications is possible through dosage analysis of the results data, where the output files are put through a statistical analysis program which quantitatively compares the strength of the test signals to those of the controls. This method also allows for determination of carrier status in females who have offspring or relatives with exon deletions or duplications. The DMD MLPA does not pick up point mutations.

RESULTS

INTELLIGENCE

A total of 5 boys and their 4 controls fell into the younger category for cognitive evaluation and were tested for developmental delay using the GMDS. The ages of the affected boys ranged from 4 years 7 months to 6 years and 8 months. The age of the control in each case, was within 6 months of the DMD-affected boy with whom he was paired **(Table 1).**

Table 1: Background Characteristics of DMD and control group for cognitive evaluation

	DMD n=5	**Control n=4**
Child Age (mean)	73months	69.5months
Ethnic Background		
African	n=0	n=0
Mixed	n=3	n=2
European	n=2	n=2
Parental education		
Mother		
<Gr 7	n=0	n=0
Gr 8-10	n=1	n=2
Gr 12	n=3	n=1
>Gr 12	n=1	n=1
Unknown		
Father		
<Gr 7	n=0	n=0
Gr 8-10	n=1	n=1
Gr 12	n=1	n=2
>Gr 12	n=1	n=1
Unknown	n=2	n=0

The demographic data for this group was based on family income as measured by the hospital fee categorisation structure. This measure does have disadvantages as families may under-report their income in order to avoid paying

higher fees. Of the 5 patients who participated in the cognitive evaluation, 2 (40%) fell into the very lowest income category. Of the 11 boys who participated in the behaviour evaluation, 8 (63%) fell into the lowest income category (family income <R4800 per month). As a indication of the income of children attending RCCH in general, records show that 86% of families over an 18month period (January2005-June2006) reported an income in the lowest bracket.

As DMD is a disorder affecting muscle strength, it was felt to be appropriate to remove the gross motor subscale from the analysis and instead calculate the general developmental quotient excluding the loco-motor subscale. A value using the average motor score for the age of the children was also included for comparison.

The group of children in this younger age bracket is very small. Although the background characteristics of the DMD and control groups as a whole were similar, there were huge variations in the background and parental education between individuals within the two groups. The combined scores of the DMD and control group are presented in table form **(Table 2)**. However, the results needed to be interpreted with extreme caution as a result of these factors.

Statistical analysis

An initial exploration of normality revealed that the variables were not normally distributed. Therefore, non-parametric tests were employed for this analysis. The Mann-Whitney U test was used to explore between group differences in neuropsychological subtest performances on the GMDS. Effect sizes were reported for analysis of neuropsychological performance scores.

The Mann-Whitney U test revealed that the DMD children show a statistically lower performance than the healthy controls in the following areas:

General intellectual ability (GQ (no MS)) was statistically significant, where M_{DMD} = 78.90; M_{CONT} = 106.20; p = 0.027 with effect size = 1.89. The spread of performance within the group is described in Fig 2(a), whereas fig 2(b) illustrates the group differences on this measure. Statistical significance between the groups was also achieved for general intellectual ability (GQ (average MS)), where M_{DMD} = 82.48, M_{CONT} = 105.15; p = 0.027 with effect size = 1.88. The spread of performance within the group is described in Fig 3(a), whereas fig 3(b) illustrates the group differences on this measure.

There were also statistically significant differences between groups on some of the individual subscales. Differences between groups on performances in the Personal & Social subscale showed a strong trend towards significance, where mean $(M)_{DMD}$ = 82.22; M_{CONT} = 96.63 ; p = 0.05 with effect size =1.46 The spread of performance within the group is described in figure 4(a), whereas figure 4(b) illustrates the group differences on this measure.

Differences between groups in the Hearing & Speech subscale (HSS) also approached statistical significance, where M_{DMD} =79.58; M_{CONT} = 109.30; p = 0.05 with effect size = 1.83. The spread of performance within the group is described in figure 5(a), whereas figure 5(b) illustrates the group differences on this measure.

Performance subscale performances between groups approached significance, where M_{DMD} = 82.26; M_{CONT} = 115.35; p = 0.05 with effect size = 1.16. The spread of performance within the group is described in figure 7(a), whereas figure 7(b) illustrates the group differences on this measure.

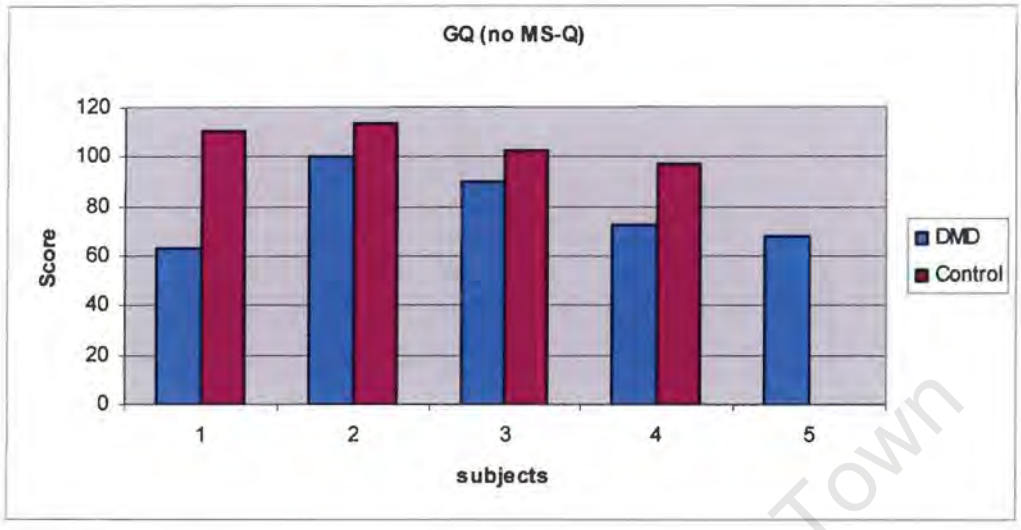
There was no clear difference between performances of the two groups in the practical reasoning subscale. The spread of performance within the group is described in figure 8(a), whereas figure 8(b) illustrates the group differences on this measure.

Table 2: *GMDS* results for affected and control groups: means and standard deviations (in parentheses)

	DMD (age equivalence in months) N=5	QUOTIENT	CONTROL(age equivalence in months) N=4	QUOTIENT	p-level (Quotients)
GQ (no MS)	56.84 (10.19)	78.9 (15.67)	73.22 (9.72)	106.20 (7.54)	P=0.02
GQ (MS)	59.62 (9.42)	82.48 (13.06)	72.63 (10.06)	105.15 (6.32)	P=0.02
PSS	59.80 (10.42)	82.22 (8.19)	66.75 (10.04)	96.63 (8.67)	P=0.05
HSS	56.90 (8.68)	79.58 (18.83)	76.13 (15.02)	109.30 (3.32)	P=0.05
HECS	54.30 (13.58)	74.76 (15.61)	71.38 (16.85)	102.23 (8.74)	P=0.02
PS	58.60 (10.94)	82.26 (20.97)	82.25 (15.88)	115.35 (34.52)	P=0.08
PSR	54.60 (14.09)	75.78 (21.61)	70.50 (7.78)	102.48 (18.57)	P=0.14

Figure 2: Performance on *General Quotient (no MS)*: (a) individual and (b) group

2(a)



2(b)

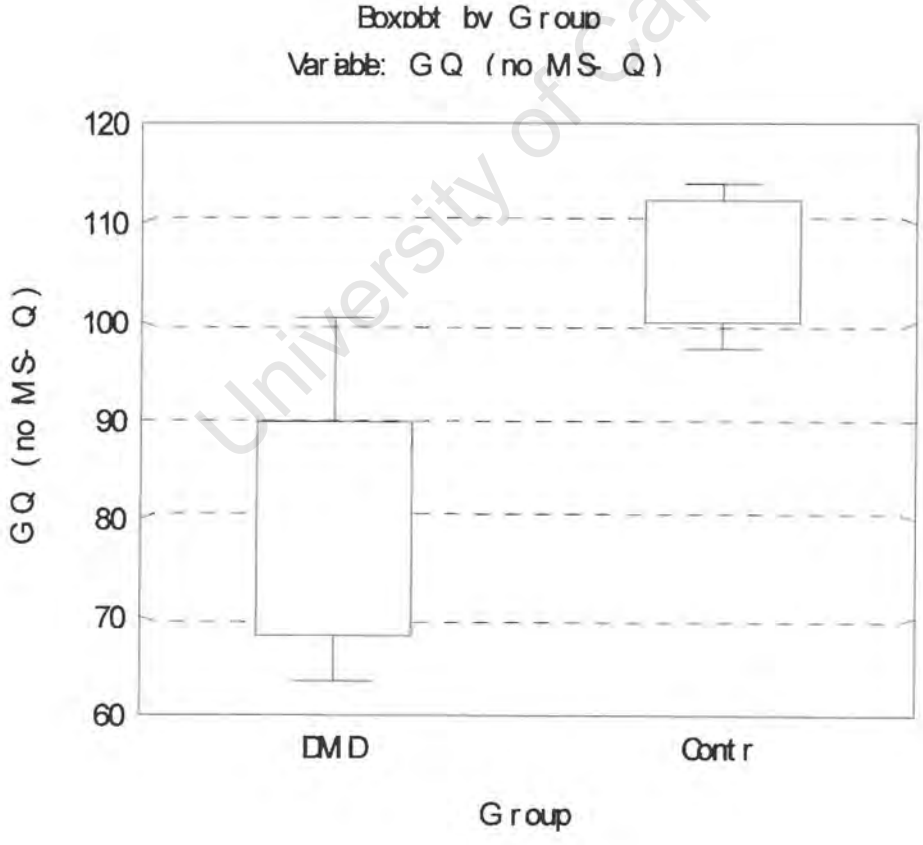
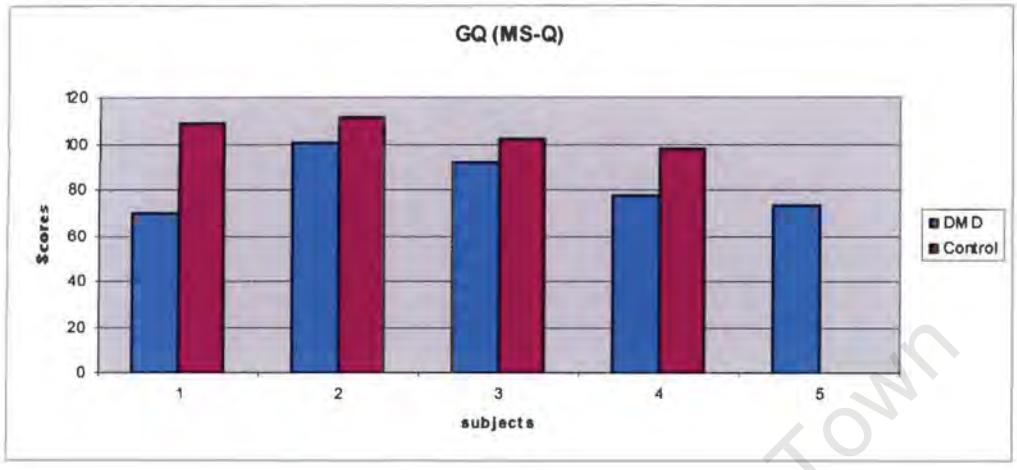


Figure 3: Performance on *General Quotient (average MS)*: (a) individual and (b) group

3(a)



3(b)

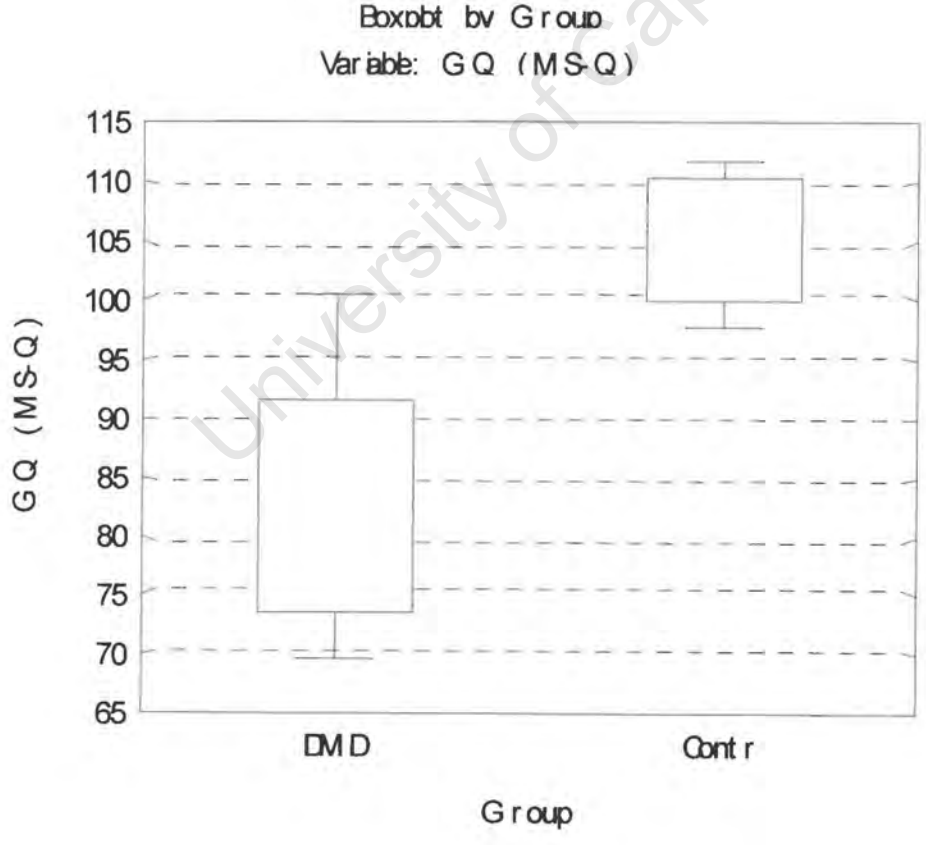
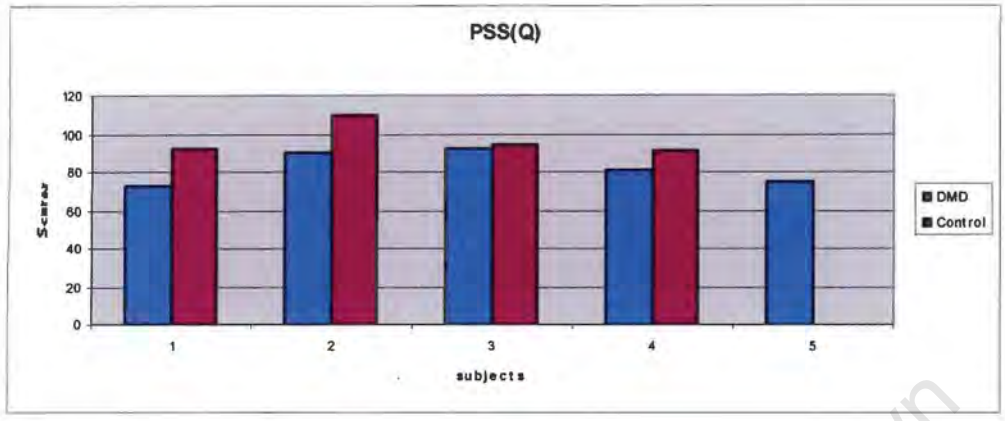


Figure 4: Performance on *Personal Social* subscale: (a) individual and (b) group

4(a)



4(b)

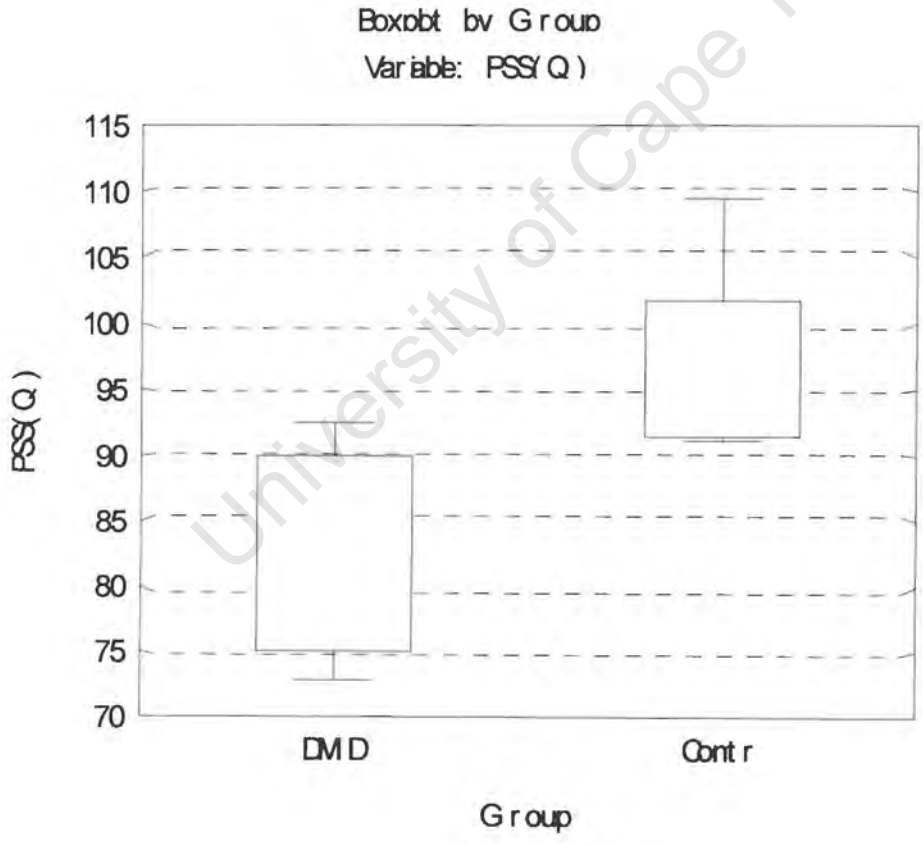
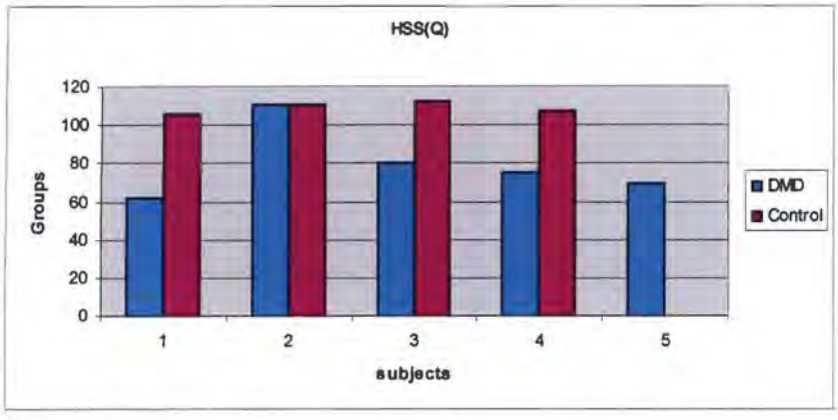


Figure 5: Performance on *Hearing and Speech* subscale: (a) individual and (b) group

5(a)



5(b)

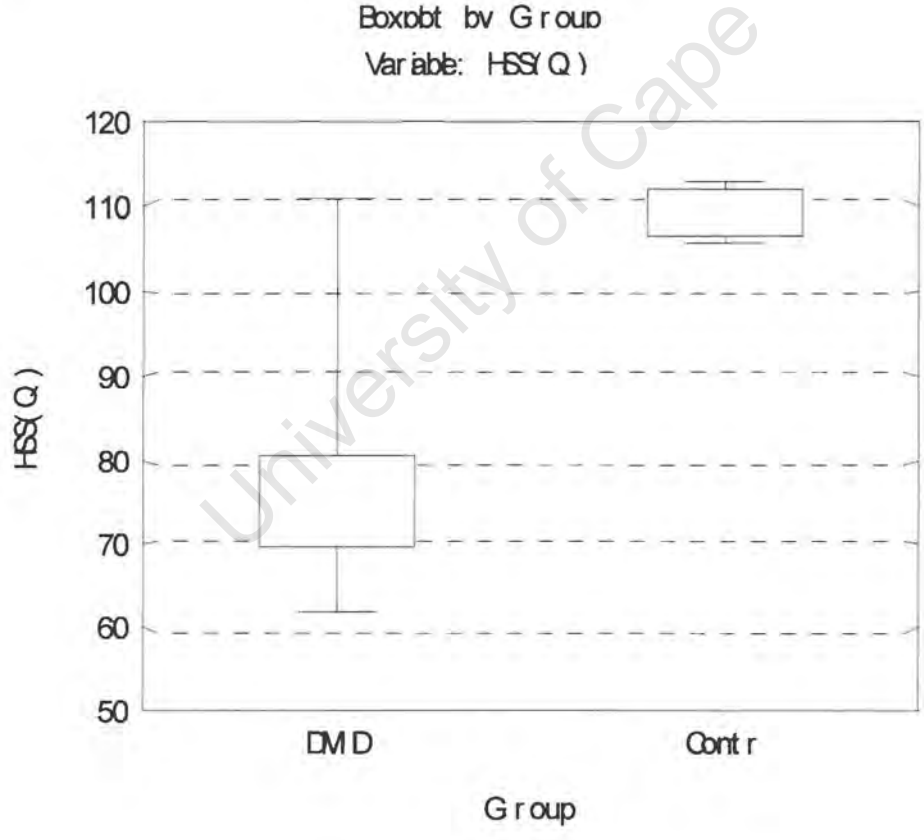
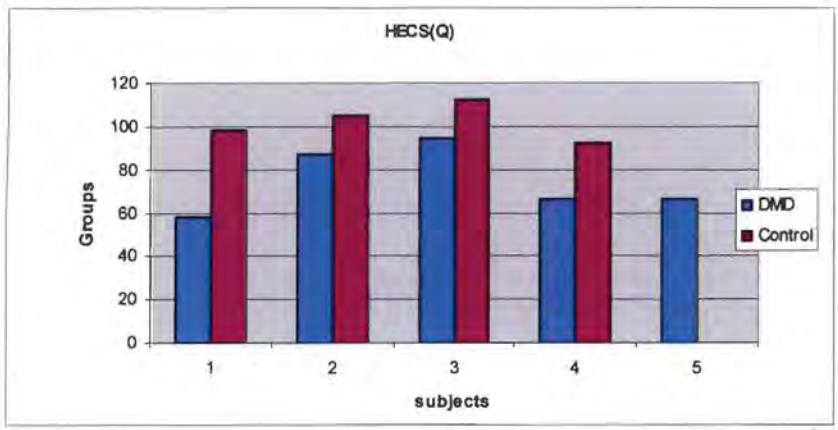


Figure 6: Performance on *Hand and Eye Co-ordination* subscale: (a) individual and (b) group

6(a)



6(b)

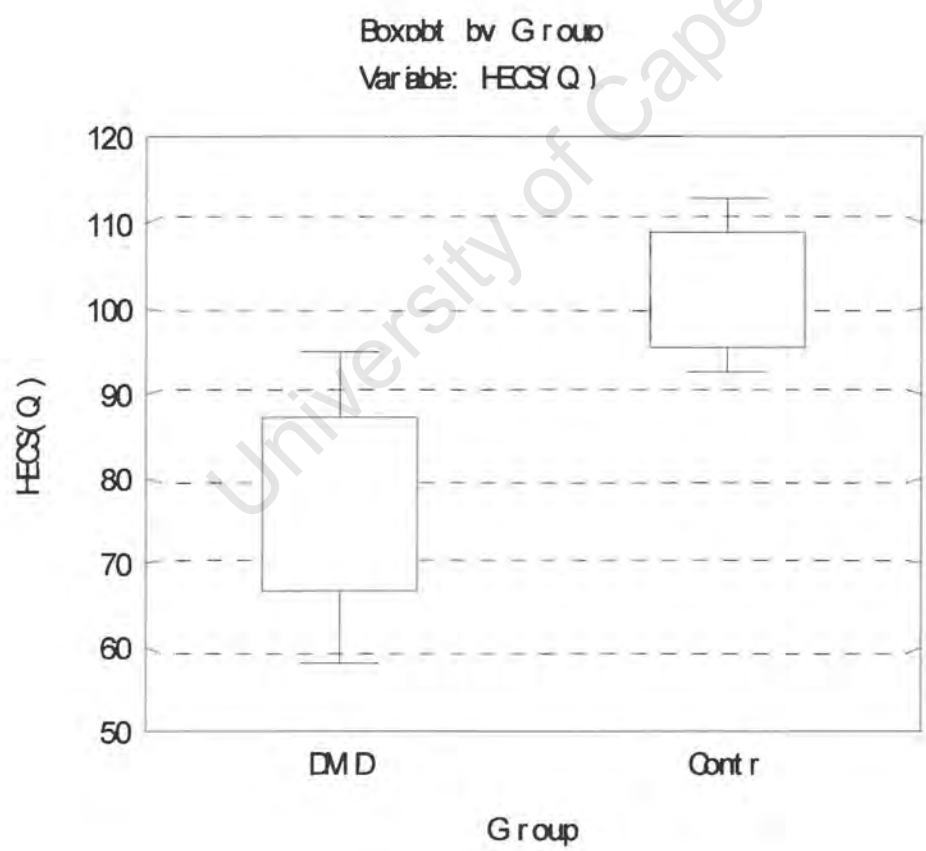
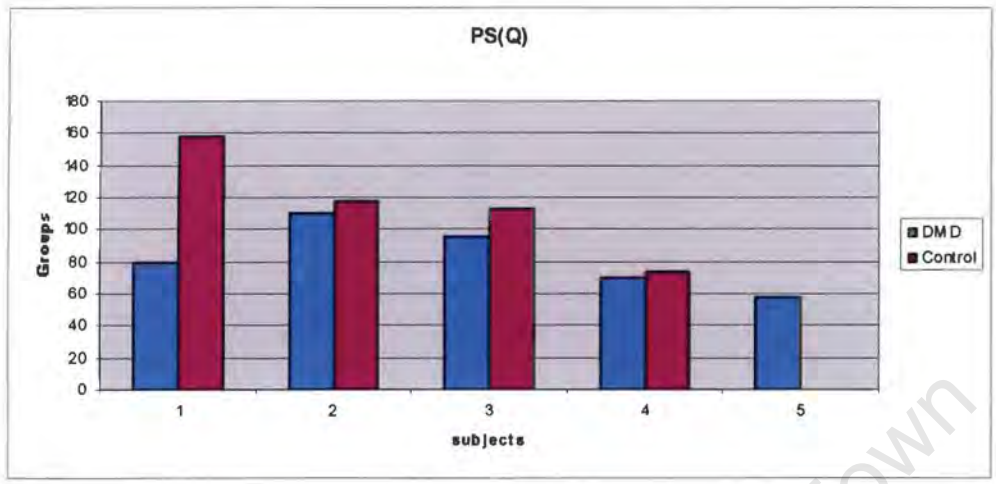


Figure 7: Performance on *Performance* subscale: (a) individual and (b) group

7(a)



7(b)

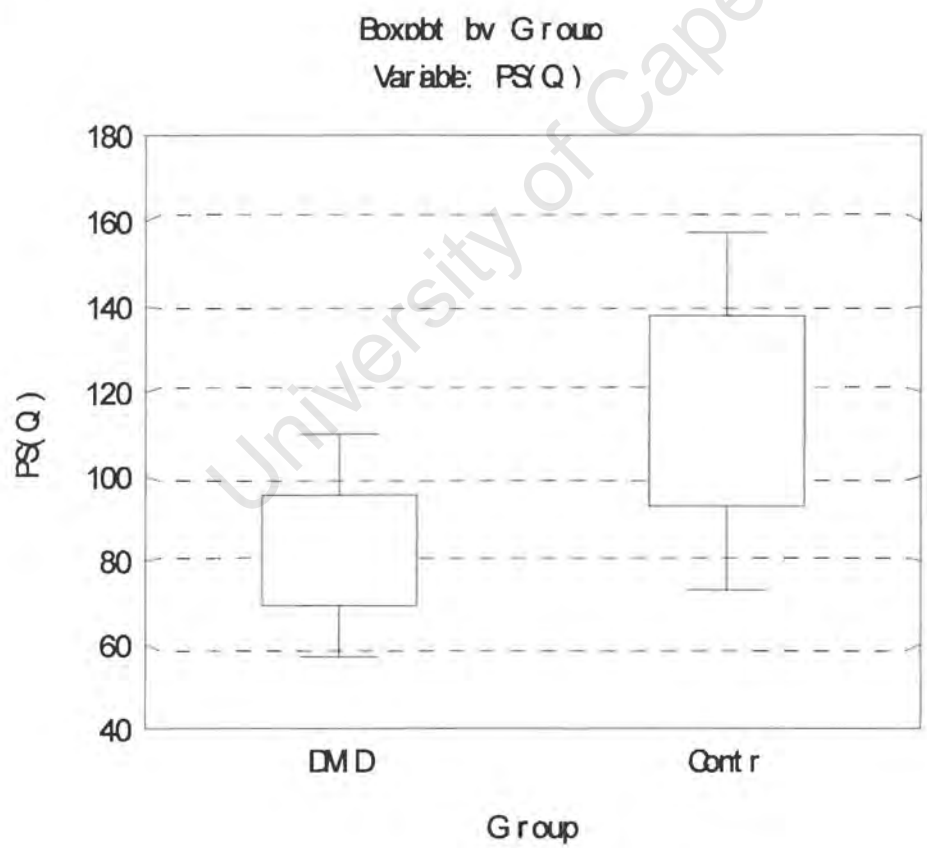
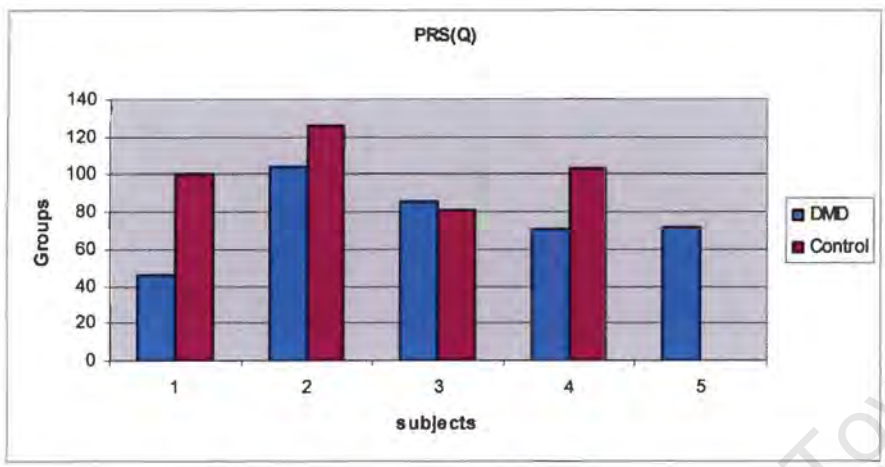
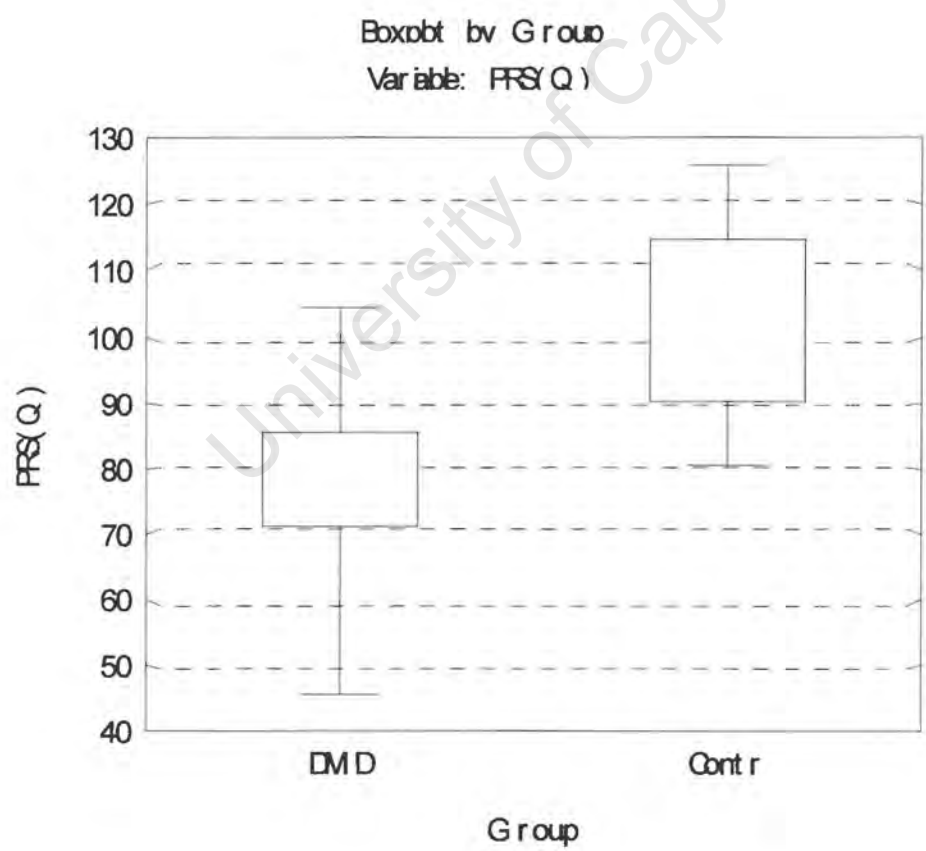


Figure 8: Performance on *Practical Reasoning* subscale: (a) individual and (b) group

8(a)



8(b)



Genetics correlation

Of the 5 boys in the preschool group, two tested positive for a deletion (both in the 3' hotspot or distal). The first boy with this distal deletion was 1 of 2 boys who fell into the mild intellectual disability range, however the other boy who was deletion positive scored the highest GQ in the group. The remaining three boys were DNA negative. The numbers in this group are unfortunately too small and the variance in performance between individuals too great to make any meaningful statistical associations between gene deletion status and degree of intellectual disability.

BEHAVIOUR

A total of 12 DMD boys had a primary caregiver present to complete the Child Behaviour Checklist (CBCL). One form had to be excluded from the analysis as too many items had been omitted to generate a valid score. The ages ranged from 4 years 5 months to 16 years and 7 months. Two boys fell into the younger group and were assessed using the 18 month to 5 year checklist and the remaining 9 boys were assessed using the older CBCL. The behavioural data for pre-school and school-age boys was analysed together due to very small numbers. The syndrome structure of the reports are different for the 18 month to 5 year olds and the 6 to18 year olds, so the 5 behavioural syndromes which overlapped both age-groups were used for intra-group analysis. Unfortunately very few parents attended the sessions with the control children and so there were inadequate numbers to make comparison with the DMD group useful. Therefore the international norms for age and sex had to be used (Achenbach, 2001). These figures could be used for descriptive comparison, but statistical significance could not be calculated due to huge number discrepancy between our small cohort and the groups used for norming this tool.

As for the cognitive results, an initial exploration of normality revealed that the variables were not normally distributed. Therefore, non-parametric tests were employed for this analysis. Wilcoxon's matched pairs test was used to assess between subjects (same group) differences in scores on the CBCL.

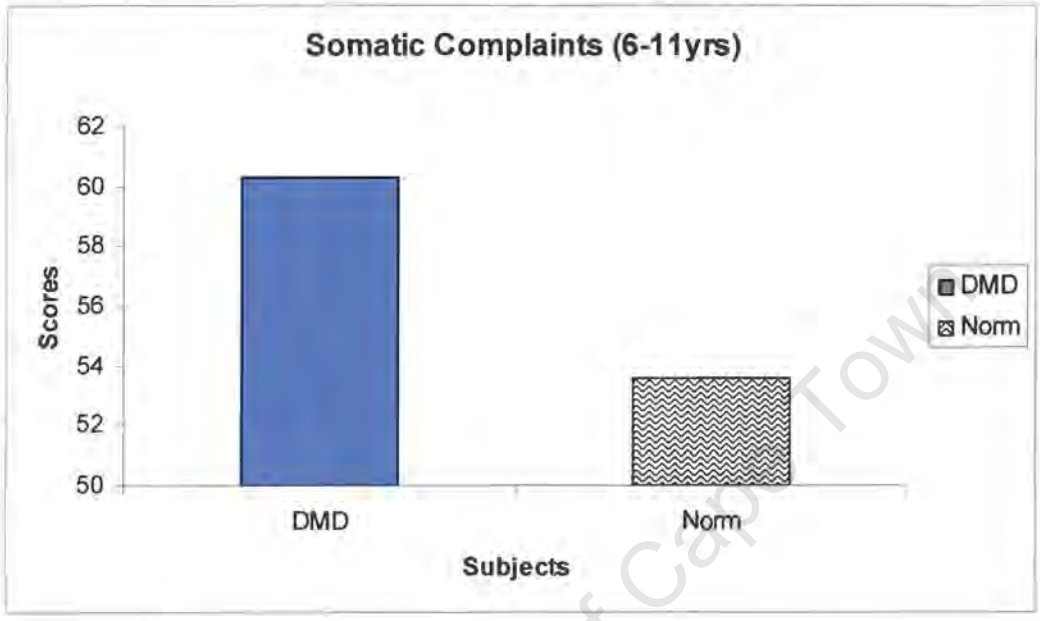
The Wilcoxon matched pairs test revealed the following behavioural characteristics for DMD children: No statistically significant differences in performance between syndromes of behaviour were found. None of the mean values for the whole group of DMD boys for the following behavioural syndromes fell into the category of clinically important (a score of >69) **(Table 3)**. However, when compared to norms, the DMD boys scored higher on every syndrome than the norm group. As normed data was only available in two age groups for this tool, we were forced to split the group further for descriptive comparison. There were 3 boys in the younger group and 6 in the older group **(figures 9-16).** Inferential statistics were not possible on comparisons to norms on the CBCL. The difference in sample size of the research group and the normative group (11 vs 167) was too large, therefore statistical significance tests did not make sense. The trends described in these figures require investigation with larger numbers in order to allow more meaningful statistical comparisons.

Table 3: Syndromes of behaviour (N=11)

	Mean	SD	%t-score>69	t-score range
Anxious/Depressed	64.91	12.31	36	50-83
Withdrawn/Depressed	64.73	13.15	27	50-96
Somatic Complaints	63.82	12.82	45	50-79
Attention Problems	64.64	10.95	18	50-92
Aggressive Behaviour	64.82	13.91	27	50-98
Thought Problems (N=9)	65.67	10.07	44	51-79
Rule breaking (N=9)	59.89	8.28	11	50-73
Social Problems (N=9)	67.56	9.34	22	50-88

Figure 9: Comparison of DMD boys vs. international norms on the *Somatic Complaints* syndrome: (a) 6-11years and (b) 13-18 years

9(a)



9(b)

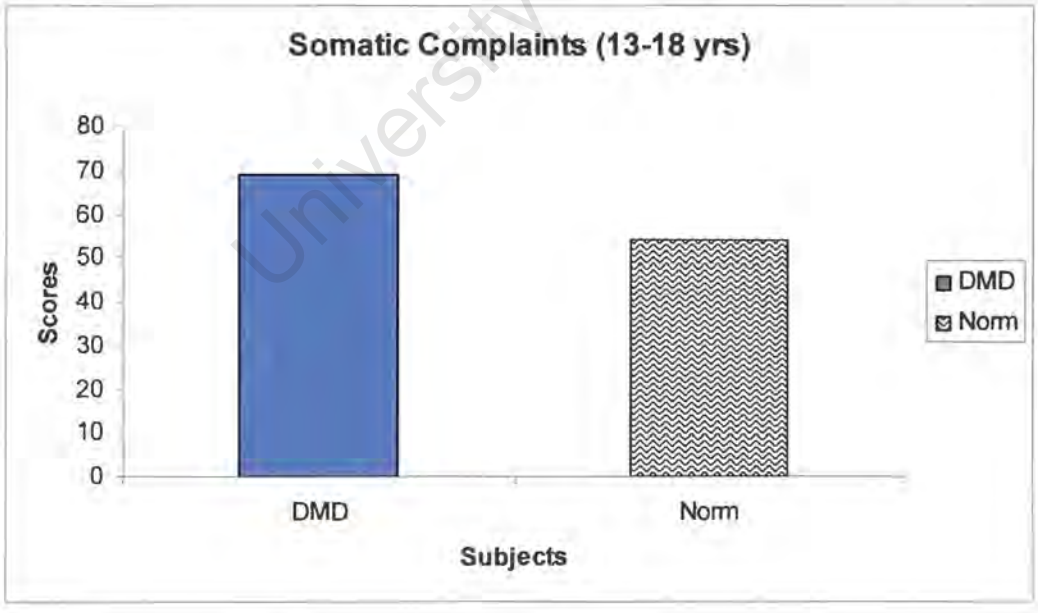
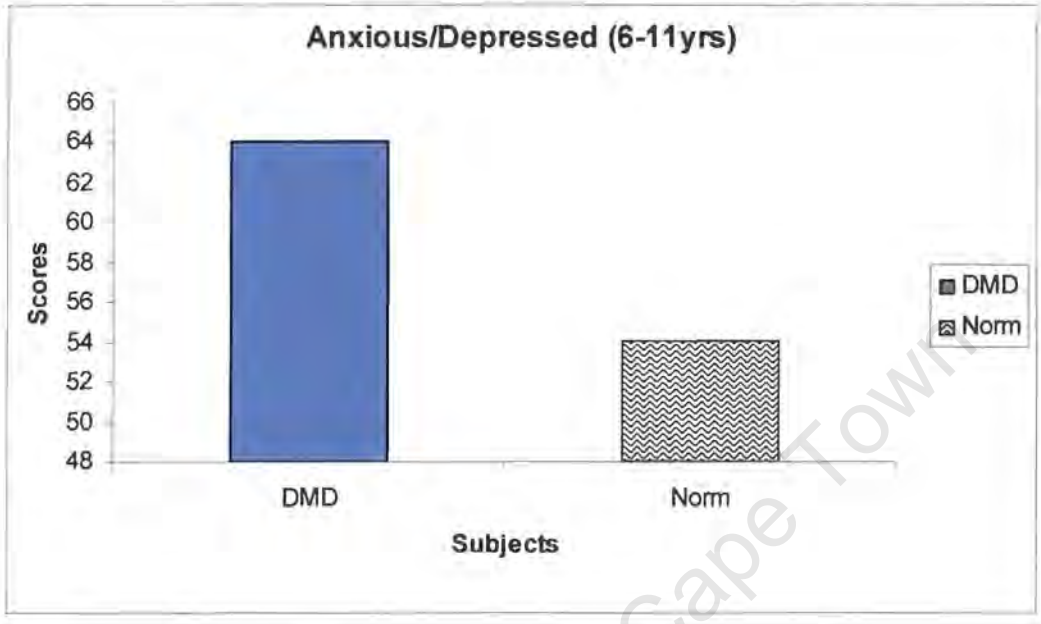


Figure 10: Comparison of DMD boys vs. international norms on the *Anxious/Depressed* syndrome: (a) 6-11years and (b) 12-18 years

10(a)



10(b)

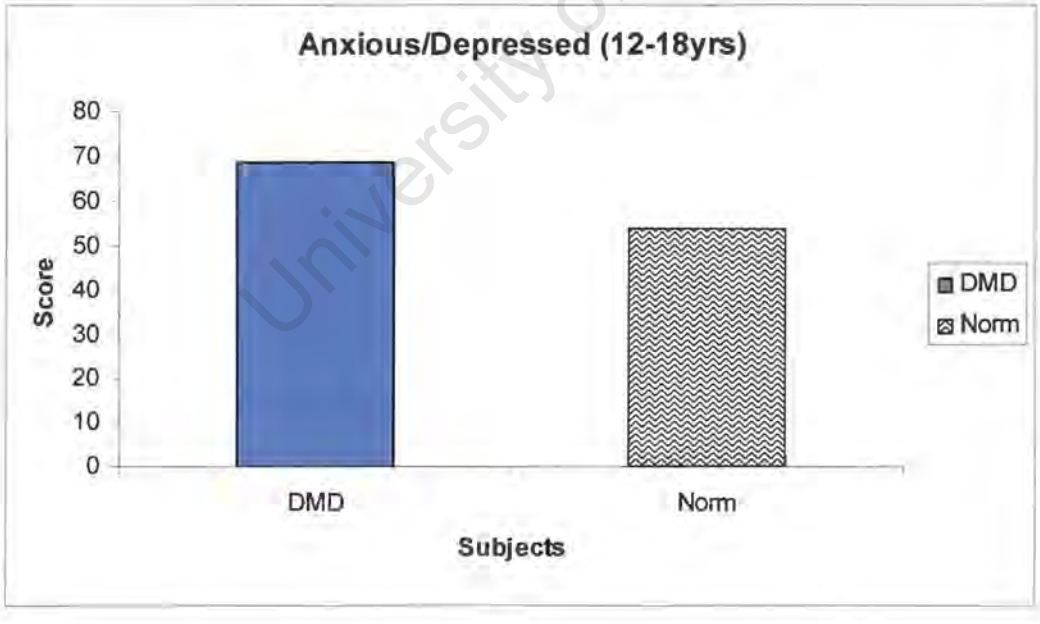
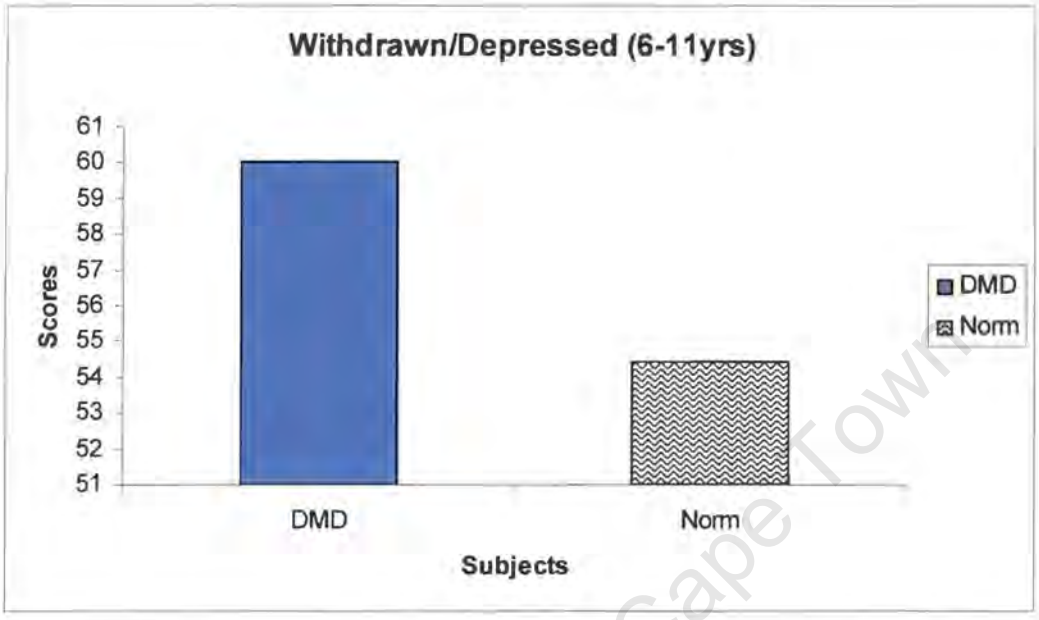


Figure 11: Comparison of DMD boys vs. international norms on the *Withdrawn/Depressed* syndrome: (a) 6-11years and (b) 12-18 years

11(a)



11(b)

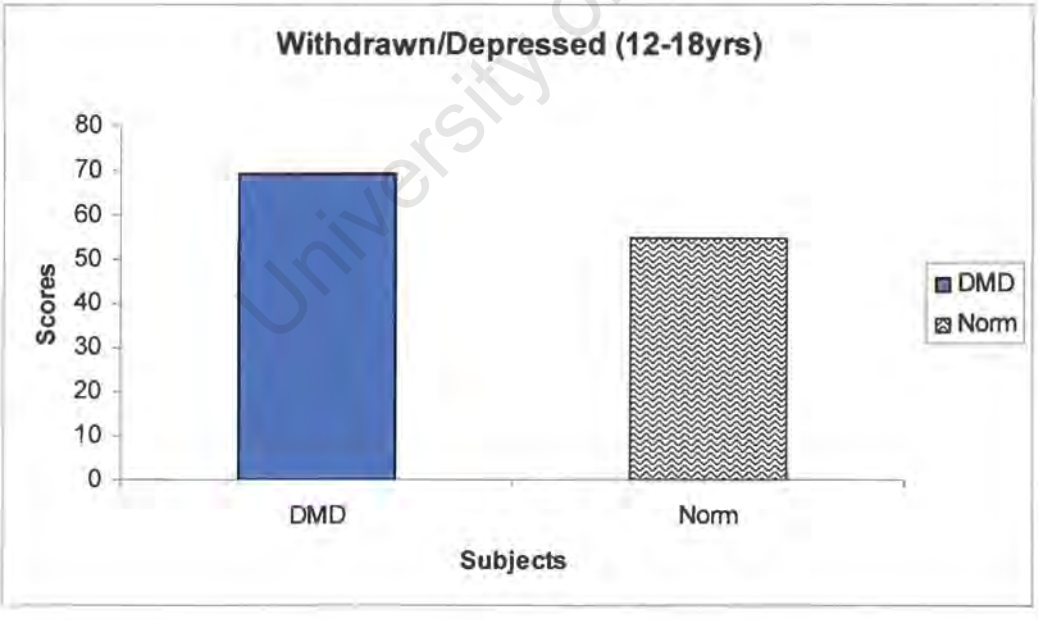
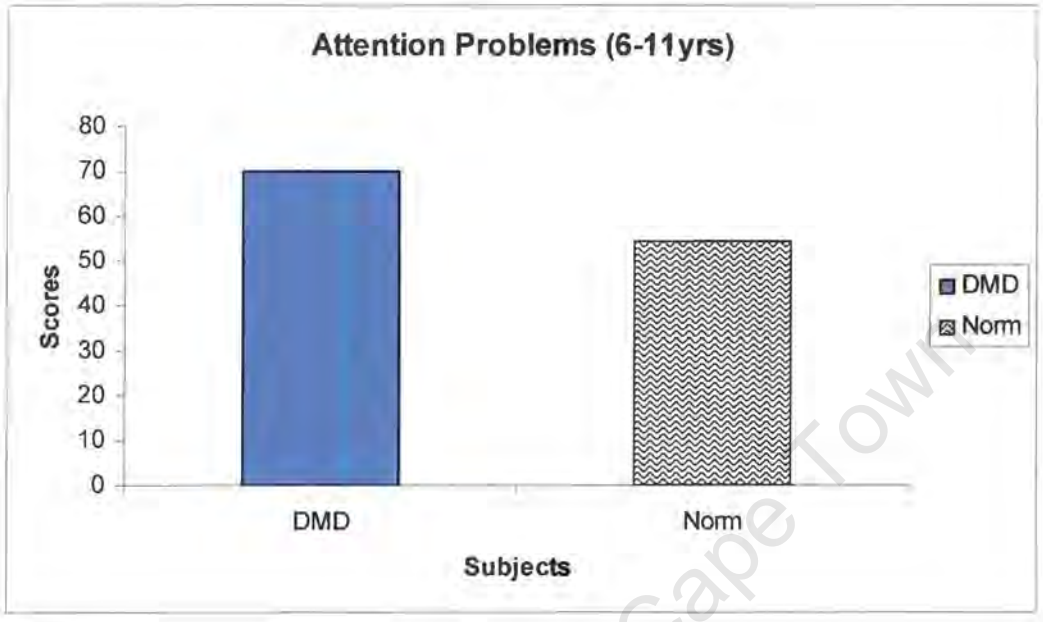


Figure 12: Comparison of DMD boys vs. international norms on the *Attention Problems* subscale: (a) 6-11years and (b) 12-18 years

12(a)



12(b)

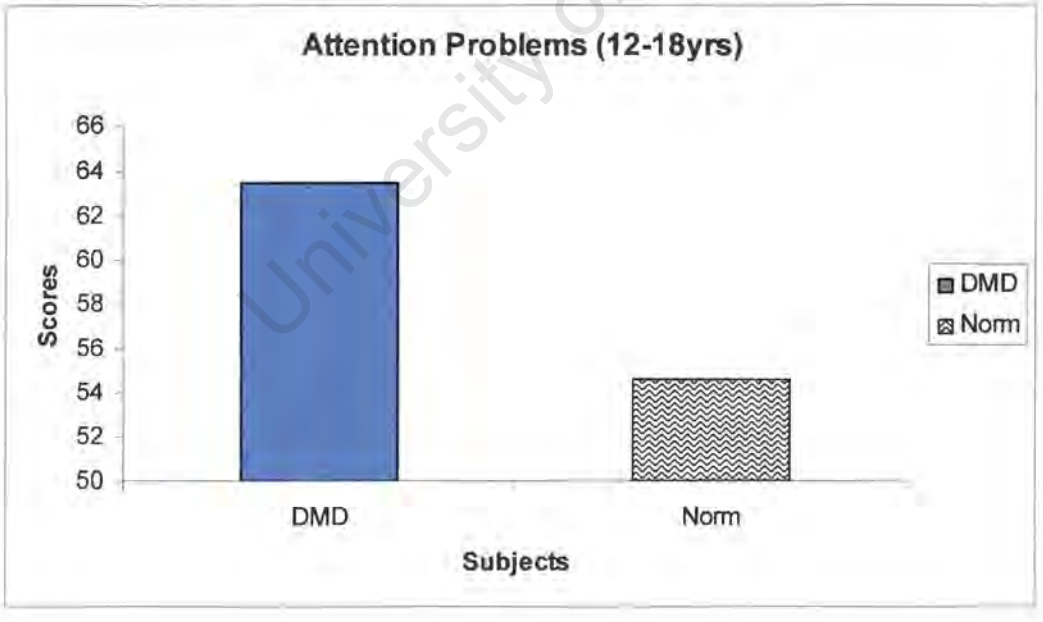
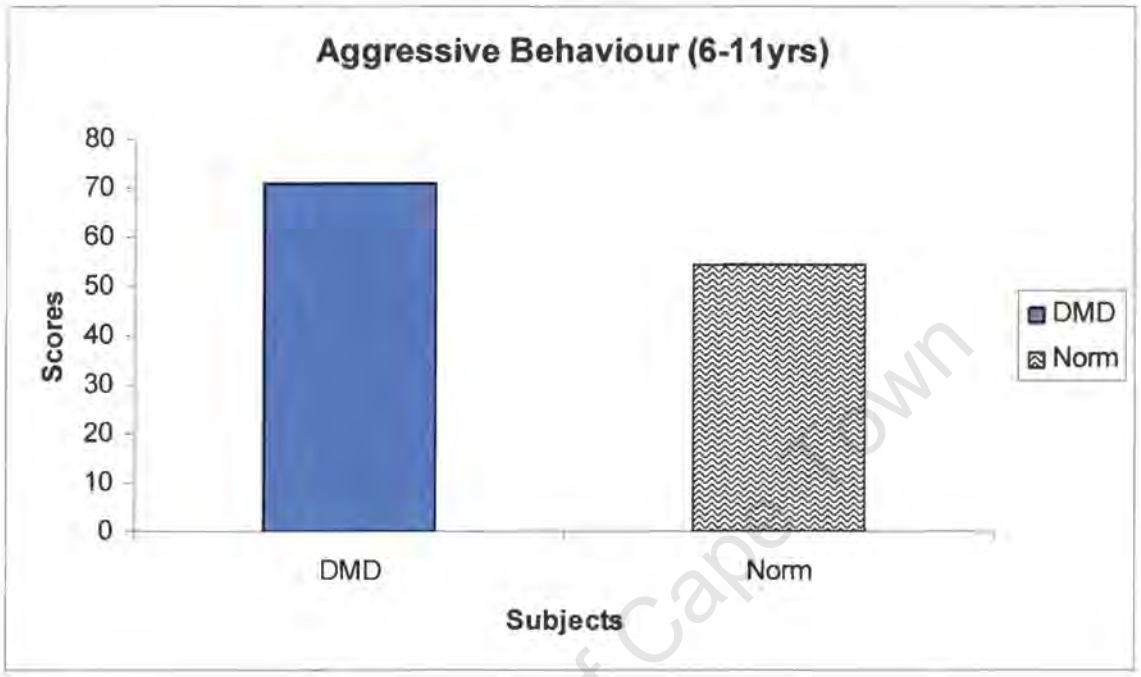


Figure 13: Comparison of DMD boys vs. international norms on the *Aggressive Behaviour* syndrome: (a) 6-11years and (b) 12-18 years

13(a)



13(b)

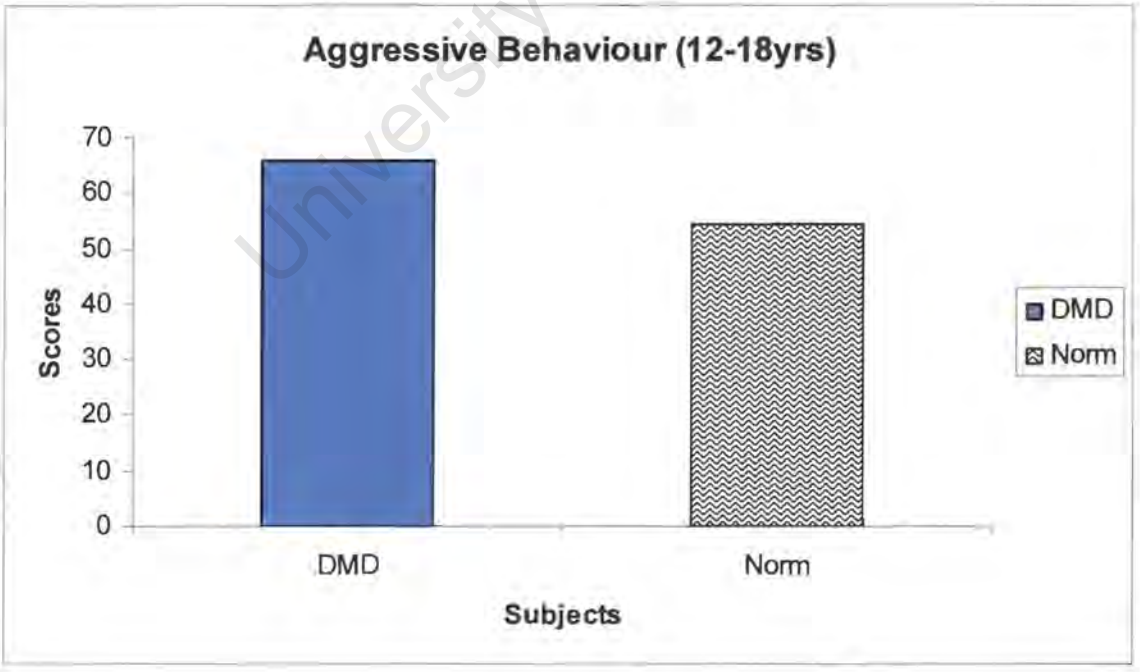
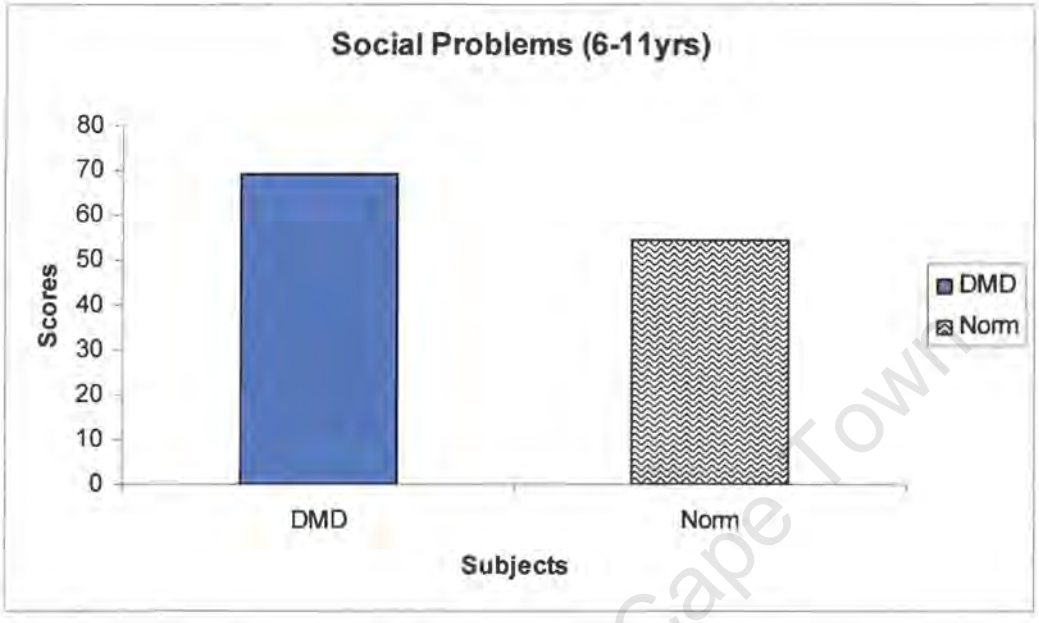


Figure 14: Comparison of DMD boys vs. international norms on the *Social Problems* syndrome: (a) 6-11years and (b) 12-18 years

14(a)



14(b)

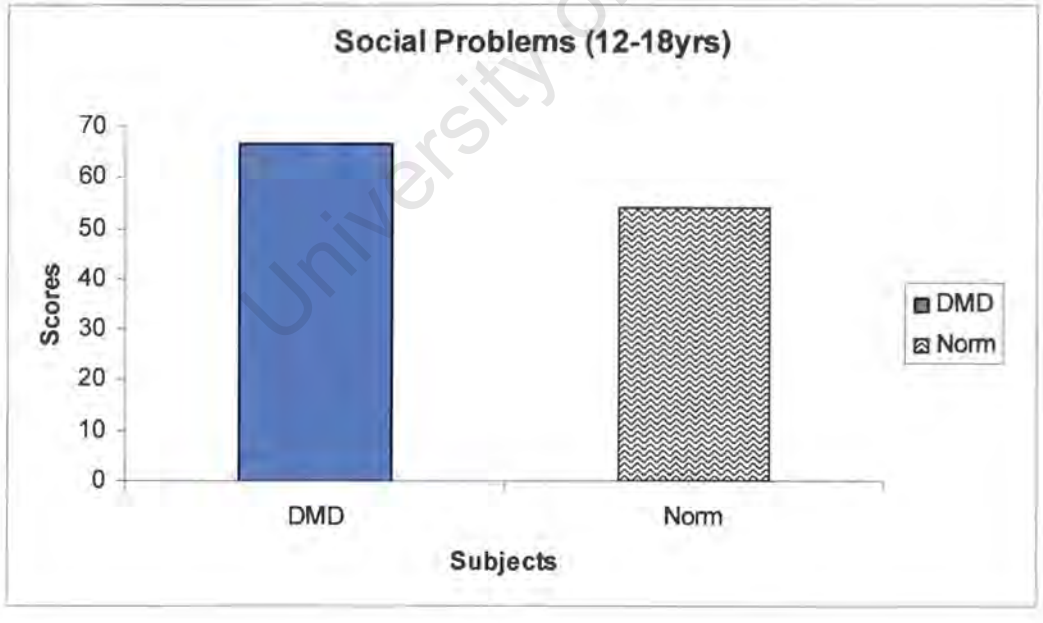
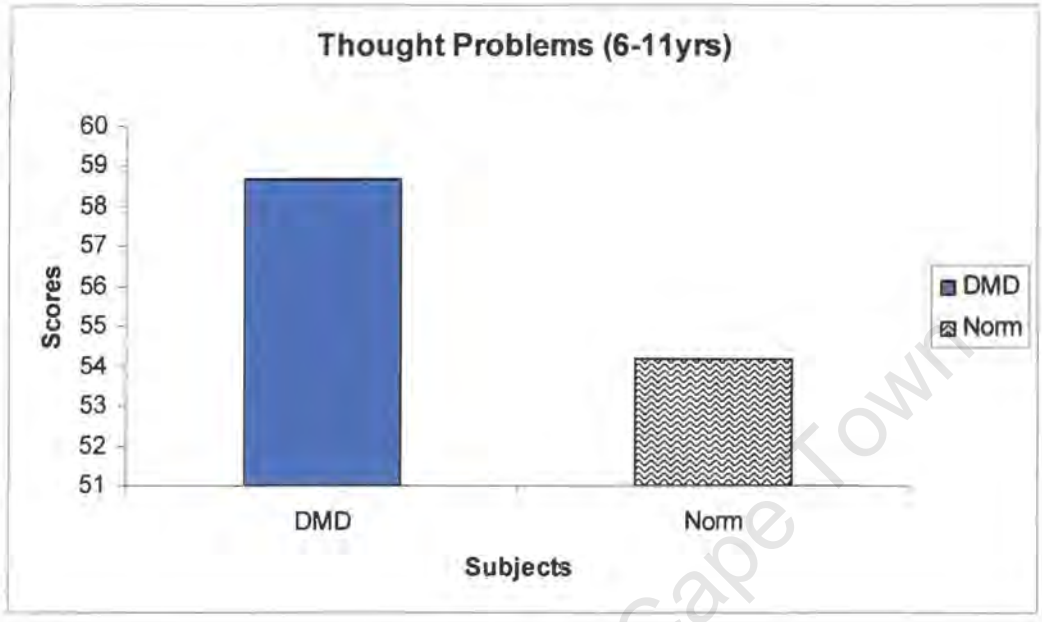


Figure 15: Comparison of DMD boys vs. international norms on the *Thought Problems* syndrome: (a) 6-11years and (b) 12-18 years

15(a)



15(b)

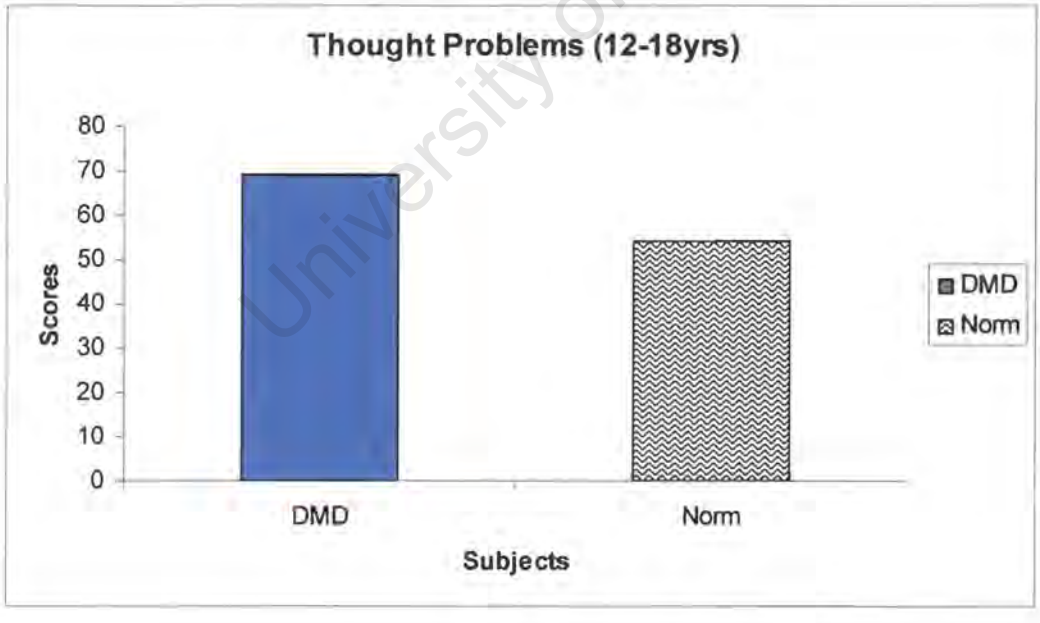
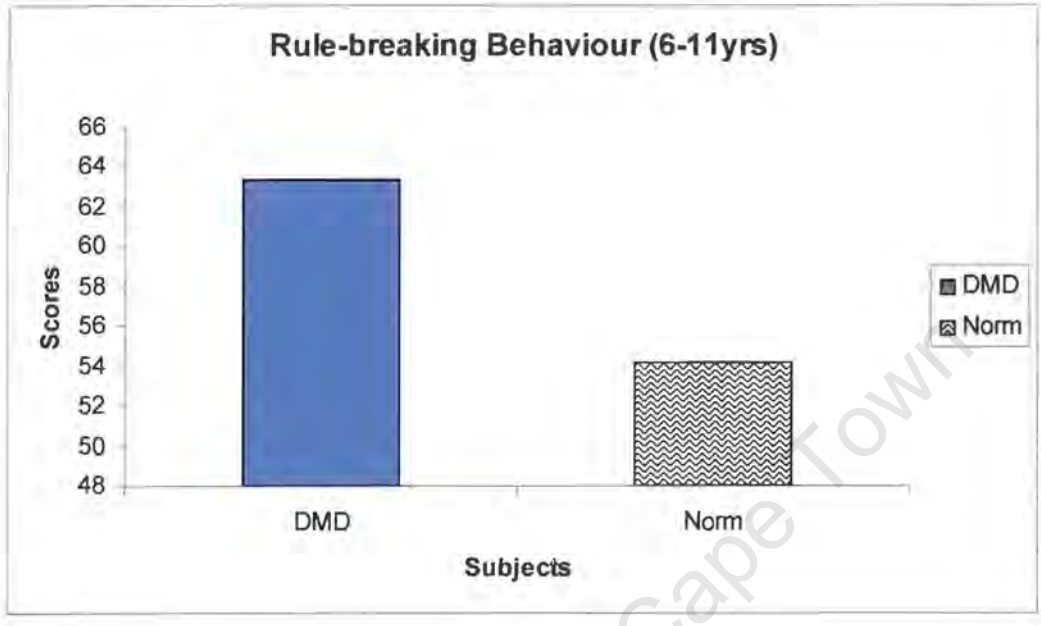
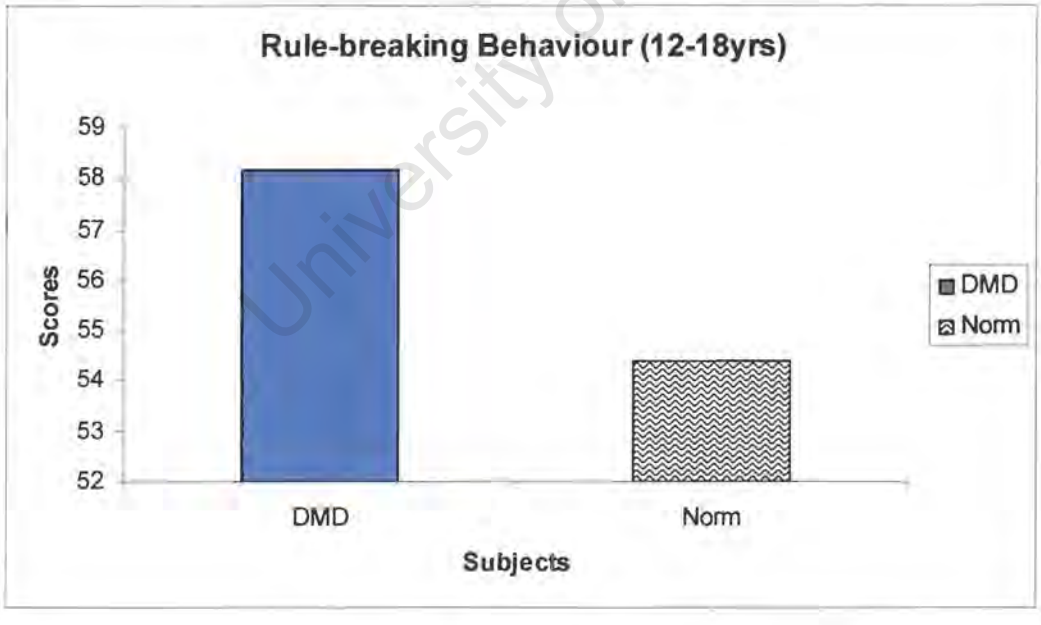


Figure 16: Comparison of DMD boys vs. international norms on the *Rule-breaking Behaviour* syndrome (a) 6-11years and (b) 12-18 years

16(a)



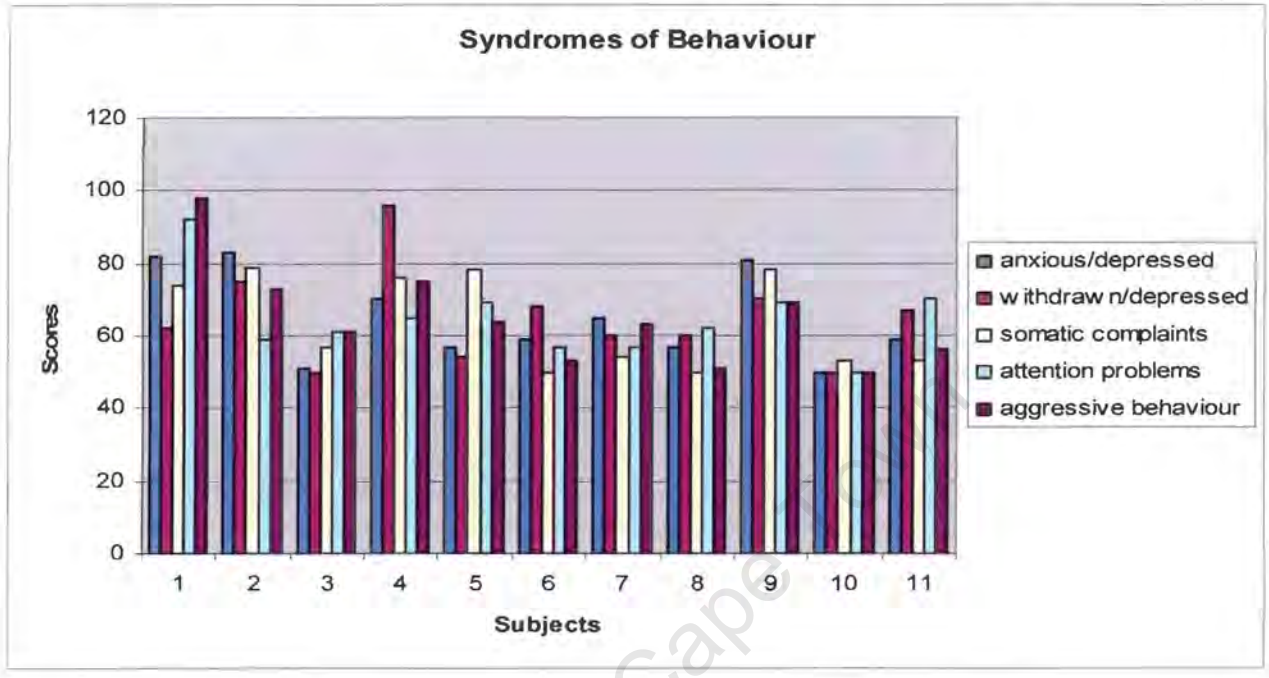
16(b)



The cut-off for categorising the behavioural score as clinically relevant for these categories is a t-score of 70 and above (>97th percentile for age and sex). A t-score of between 65 and 69 is considered borderline. To determine whether some problem behaviours are selectively reported in DMD, the percentage of boys scoring in the clinically significant range on the individual behaviour scales was determined. The CBCL is a continuous scale which measures behaviours that occur in normal populations, the cut-off for clinical significance is at the 97th percentile for age and sex. Looking at the scores of individual boys across the syndromes, the percentage of DMD boys scoring in the clinical range was highest in the following syndromes: 5 out of 11 boys (45.45%) scored in the clinically important range for the *Somatic Complaints* syndrome, 4 out of 9 boys (44%) scored in the clinically significant range for *Thought Problems* and 4 out of 11 boys (36.36%) scored in the clinically important range for the *Anxious/Depressed* syndrome **(Table 3)**.

Due to the lack of a control group in the analysis of the CBCL, it was not possible to report effect sizes.

Figure 17: DMD boys' performance on syndromes of behaviour subscale



The Diagnostic Statistic Manual -IV (DSM)-orientated scores are a derivative categorisation of the CBCL questions. Although similar to the syndromes described above, behavioural characteristics are clustered under the more familiar labels of the DSM-oriented terminology. These results have not been reported in detail as this level of analysis was not warranted given the very small numbers in our group and results using this categorisation did not add anything of substance to the results achieved using the original CBCL syndrome structure. The only exception to this was the intra-group analysis where Wilcoxon's matched pairs test revealed a significant difference between *Affective Problems and Oppositional Defiant Problems*, $p = 0.025$, with *Affective Problems* being the more severe.

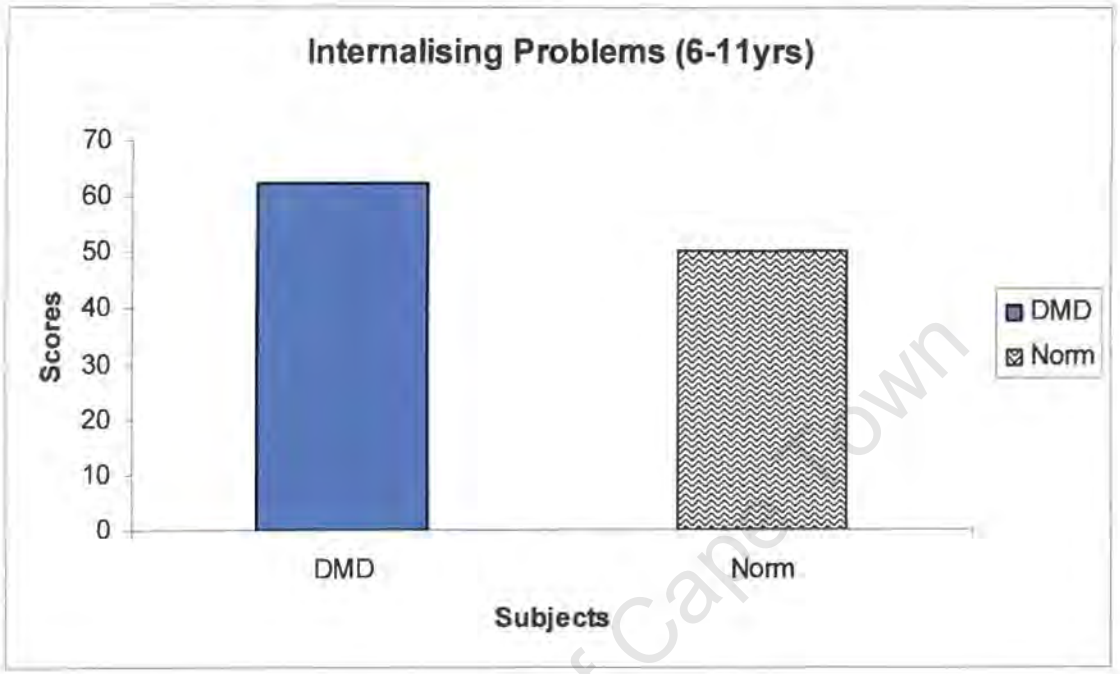
The CBCL report system allows for a simplification of the syndrome report into *internalising*-type behaviour problems, *externalising* –type behaviour problems and *total* behaviour problems in an individual child. The cut-off for categorising

the behavioural score as clinically relevant for these categories was a t-score of 63 and above (>97th percentile for age and sex). A t-score of between 60 and 63 is considered borderline. The mean score for *Internalising Behaviours* in this group of boys with DMD is 65.45 (SD 12.52) which falls into the clinical range. The mean t-score for *Externalising Behaviours* is 61.45 (SD 11.28) which falls into the borderline range **(Table 4).** Although the differences between scores on *Internalising vs. Externalising Behaviours* were not shown to be statistically significant, p = 0.109, it would be of interest to explore this trend with a larger cohort in the future.

Five of the 11 boys (45.45%) fell into the clinical range for *Internalising Behaviours.* Four out of the 11 boys (36.36%) fell into the clinical range for *Externalising Behaviours.* And overall 6 out of the 11 boys (54.54%) fell into the clinical range for *Total Problems* **(Table 4).** The DMD boys scored consistently higher than their norms for *Internalising, Externalising and Total behavioural problems* **(figures 18-20).**

Figure 18: Comparison of DMD boys vs. international norms for *Internalising Problems:* (a) 6-11years and (b) 12-18 years

18(a)



18(b)

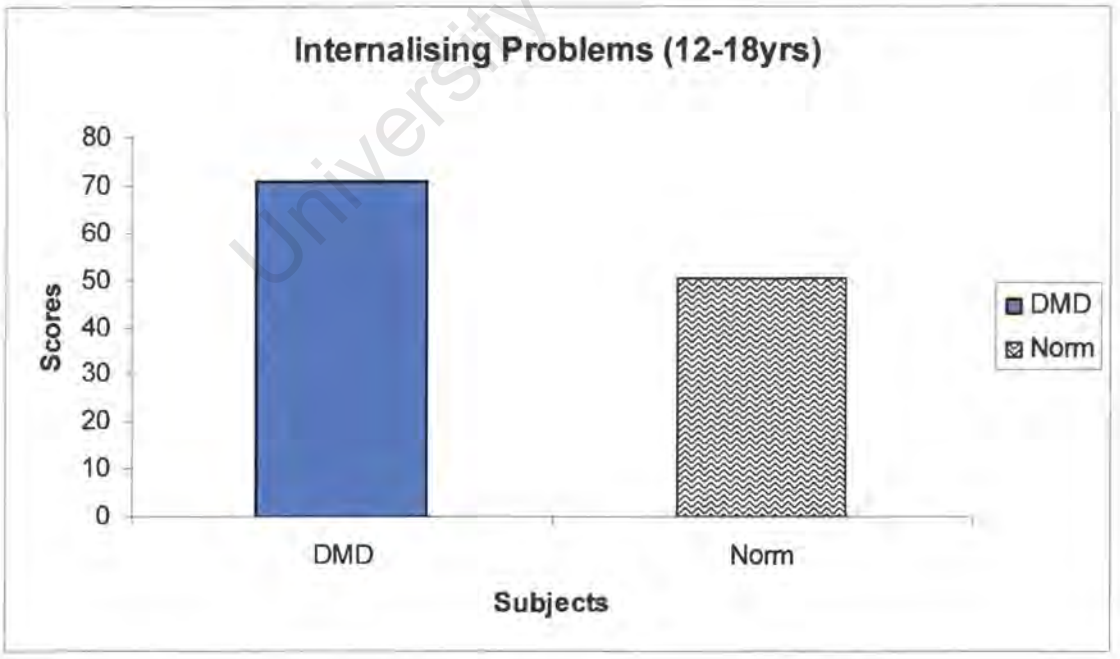
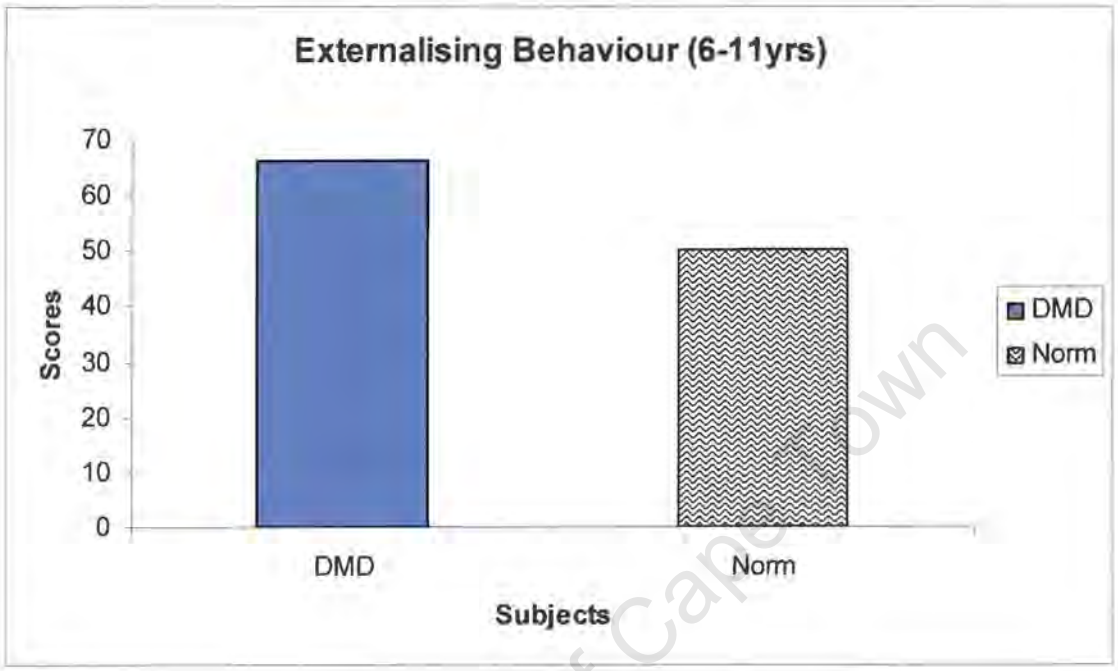


Figure 19: Comparison of DMD boys vs. international norms for *Externalising Behaviours:* (a) 6-11years and (b) 12-18 years

19(a)



19(b)

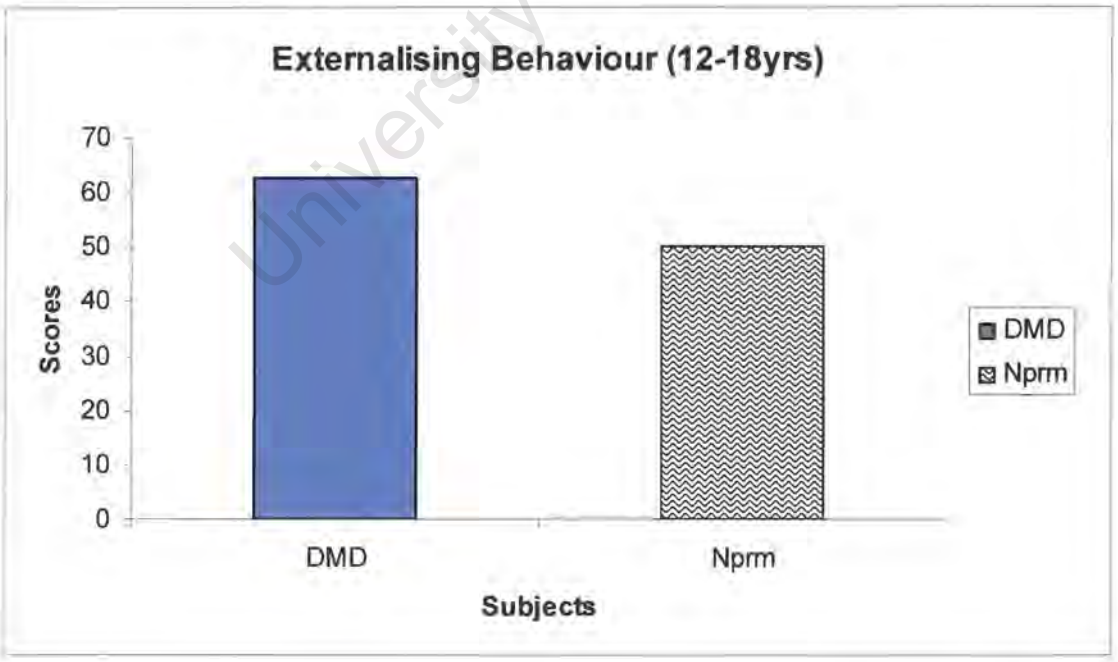
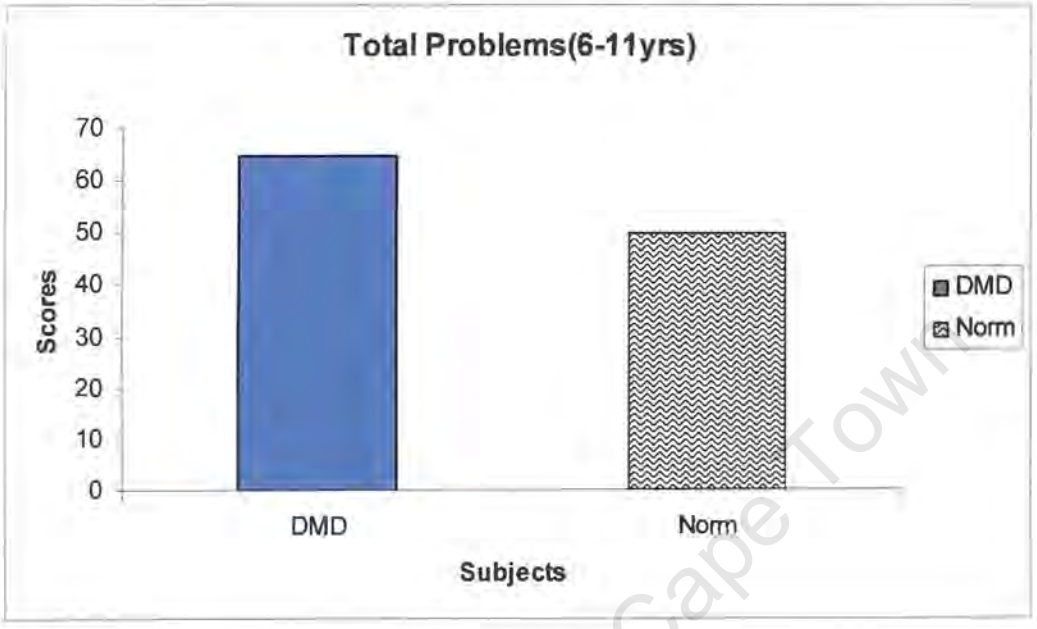


Figure 20: Comparison of DMD boys vs. international norms for *Total Behaviour Problems*: (a) 6-11years and (b) 12-18 years

20(a)



20(b)

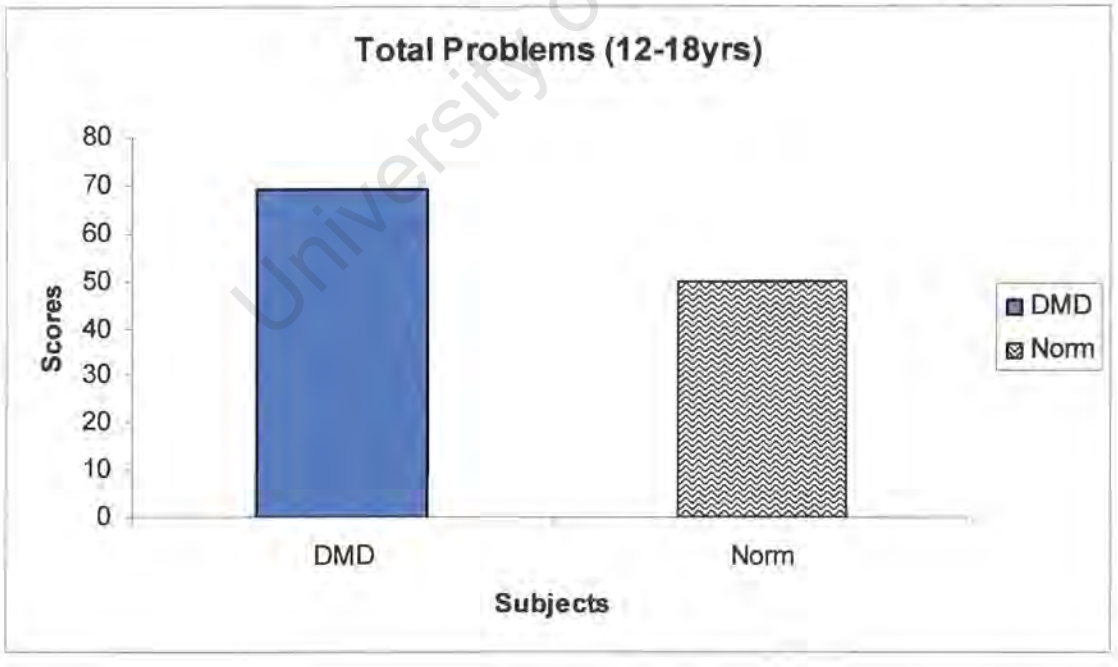
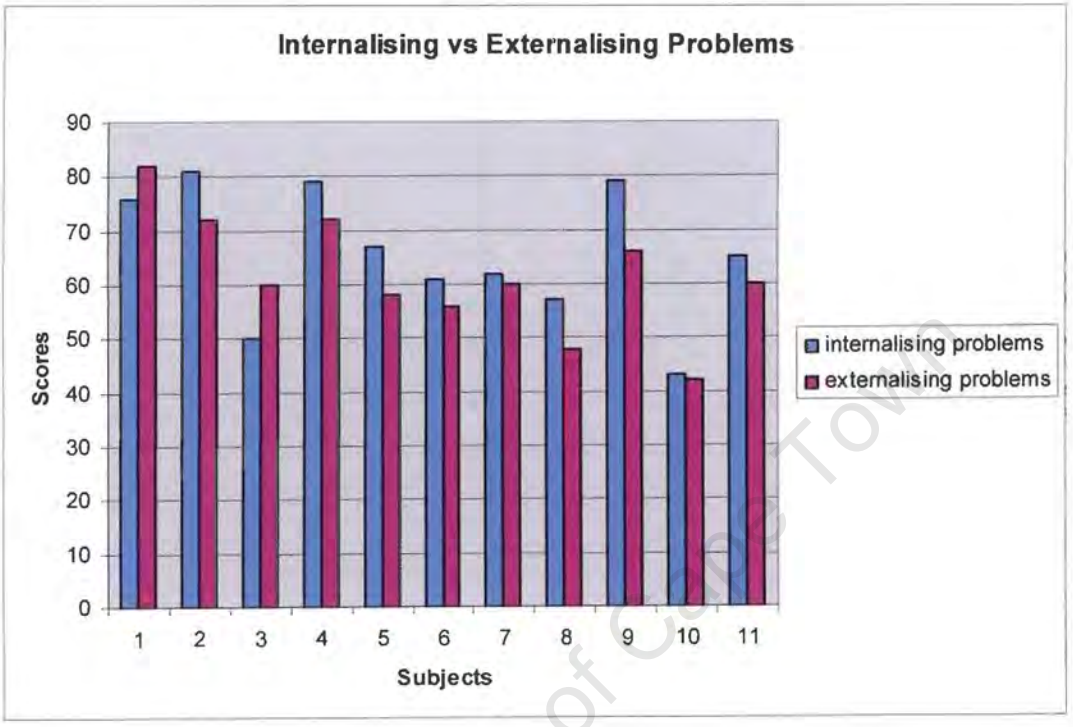


Table 4: Internalising vs. Externalising problems (N=11)

	Mean	SD	%t-score>63	t-score range
Internalising problems	65.45	12.52	45	43-81
Externalising problems	61.45	11.28	36	42-82
Total problems	65.18		55	41-81

Figure 21: DMD boys' performance on *Internalising vs. Externalising Problems* subscale: (a) individual scores (b) group performance

21(a)



21(b)

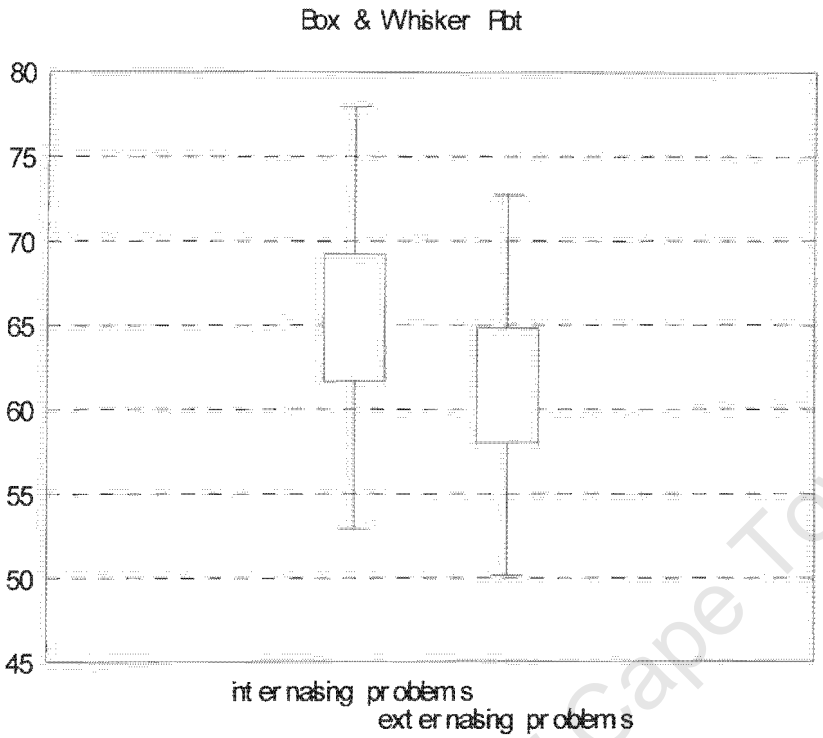


Table 5: Occurrence of *Total Behaviour Problems* (clinically significant) in boys receiving steroids and those not receiving steroids.

Treatment	Clinically significant behavioural problems	Non-clinically significant behavioural problems
Steroid group	2	3
Non-steroid group	4	2

There was, in fact, a greater proportion of the non-steroid group with a clinically significant overall behaviour score than the steroid group. This did not achieve statistical significance (Chi-square = 0.78). The average behaviour scores for the steroid and non-steroid group revealed a higher average for the non-steroid group **(Table 6)**, but the t-test analysis did not achieve statistical significance (t=0.76).

Table 6: Means for "total" behavioural problems

	Means	SD
Steroid Group (N=5)	62.02	14.62
Non-Steroid Group (N=6)	67.67	8.98

DISCUSSION

The main purpose of this study was to describe the cognitive profile of the DMD boys in the <7 year age bracket and the behavioural profile of the whole cohort. There are many factors which may contribute to the performance of children in these areas.

Environmental risk factors particularly prevalent in South African society include maternal depression, violence, social isolation and poverty. These frequently coexist with biological risks, and emotional problems are common causes and consequences of cognitive and language disorders (Dooling, 1993; Richter & Grieve, 1991). In this small pilot study it was not possible to obtain in-depth background on all such possible risk factors. To provide information about economic background the focus was on family income. As parental education can particularly impact on the child's development, this was also assessed for the cognitive group and their controls.

The demographic data for this group was based on family income as measured by the hospital fee categorisation structure. This measure does have disadvantages as families may under-report their income in order to avoid paying higher fees. Of the 5 patients who participated in the cognitive evaluation, 2 (40%) fell into the very lowest income category. Of the 11 boys who participated in the behaviour evaluation, 8 (63%) fell into the lowest income category (family income <R4800 per month).

What is not known is the effect of low socio-economic status and environment on the cognition of this study population. Violence and poverty affect both the psychological and intellectual competence of young children (Donald & Dawes, 1994). Educational factors may also contribute, as often schools in low socio-economic areas are overcrowded, with a high pupil to teacher ratio. Standards in these areas may not be optimal, and learning difficulties and behavioural

problems are less likely to be picked up and/or acted upon. Transport/access to schools may also be a problem, especially for children living outside the larger metropolitan areas. Recurrent illness and poor nutrition may further exacerbate developmental and behavioural problems. Children who live in poverty are particularly susceptible to the cumulative burdens of both social stress and the greater biological vulnerability related to a higher prevalence of nutritional deficiencies. Boys with DMD in South African society may be exposed to these environmental risk factors as well as the additional serious biological risk factor of their underlying condition.

COGNITION

Prior to enrolment, none of the participants were considered to have severe developmental or cognitive problems. However, the results indicate that most of the DMD children fell at the lower spectrum of expected neuro-cognitive function for age and 2 boys fell into the mild intellectual disability category (GQ<70). In fact, the boy who had the weakest performance in the Griffith's assessment attends a mainstream school and currently receives no additional academic support. This is a possible indication of the inadequacy of current methods of assessment and support for children with this condition.

The Griffiths Scales correlate with verbal and non-verbal sub quotients of the Junior South African Intelligence Scales (JSAIS) IQ scales (unpublished, Lewis *et al*). As the intelligent quotient (IQ) is a well-known measure, it can be used to help with interpretation of results comparing it with guidelines set out for interpretation of IQ: Charts are available to relate percentile scores to IQ scores.

Box1:IQInterpretation

N >70
Mild intellectual disability = 50-70
Moderate intellectual disability = 35-50
Severe intellectual disability <35

Even with the removal of the motor scale from the scores of the Duchenne boys, the GQ scores were lower than their normally developing counterparts. Differences between the DMD and control group for general intellectual ability (GQ (no MS)) was statistically significant, where M_{DMD} = 78.90; M_{CONT} = 106.20; p = 0.027 with effect size = 1.89. Assigning an average motor score to the DMD boys and their controls still resulted in a statistically significant difference between the groups for general intellectual ability (GQ), where M_{DMD} = 82.48, M_{CONT} = 105.15, p = 0.027 with effect size = 1.88.

The scores achieved by the DMD group are consistent with the large meta-analysis of all studies undertaken to look at intellectual functioning in boys with this condition up to that time (1224 boys) which was published in 2001 (Cotton *et al* 2001). Mean full-scale IQ scores were approximately one standard deviation below the normal population (80.2%; SD19.3). Looking at the individuals in our group, 2 had GQ <70 and 1 boy approaching the lower cut-off score with a GQ of 72 (using the calculation excluding the motor scale). Therefore, 40% of the Red Cross group falls into the formal category of intellectual disability, a percentage which is comparable to the 34% of boys in Cotton *et al's* meta-analysis. Both boys with GQs <70 in our group, fell into the mild intellectual disability range, while in Cotton *et al's* analysis 79.3% displayed mild learning difficulty (FIQ 50-70), but 19.3% fell into the moderate range(FIQ 35-50) and 1.1% fell into the severe range (FIQ 20-35).

Effect sizes were reported for analysis of the Griffiths Developmental scale which gives an indication of the likelihood that the difference found in the sample will be found in the general population. Effect sizes were substantially significant which, despite a small sample size, suggests that the differences found in this study may be generalised to the wider population.

The majority of papers published on intellectual functioning in DMD have been assessments of boys of school-going age. Limited data are available on the cognitive profile of preschool boys with DMD. Smith *et al* (1990) reported global developmental delay in a group of 33 boys <6 years old with DMD (mean GQ= 79 (14.3)). The authors reported that the areas most severely affected amongst their boys were the locomotor and language domains, but there was no calculated statistical significance for the differences between subscale quotients. With the exception of the locomotor scores, the boys' performance in all the subscales remained largely static over serial testing (Smith *et al*, 1990). The boys in our small group displayed similar findings to those of this study with a pattern of global developmental delay. We excluded the locomotor subscale from analysis because the boys performed so poorly in comparison to their controls on this subscale and we felt taking it out of the calculation for the GQ would be a fairer reflection of their cognitive abilities. In addition to their significantly lower mean GQ, the group only displayed significantly poorer performances on 1 out of the 5 remaining subscales. However 3 out of the remaining subscales showed strong trends towards significance. The *Hand-eye Co-ordination* subscale was the area in which the DMD boys performed worst in relation to their controls ($p=0.02$), *Hearing & Speech* as well as *Personal & Social* subscales both approached significance with $p=0.05$ as did the *Performance* subscale, with $p=0.08$. The numbers in our group were too small to comment on the possible statistical significance of the differences in performance between subscales.

As the number of boys in this study is so very small, only tentative conclusions can be drawn from our findings on their neuro-cognitive profile. However, it is encouraging to see that our results have a similar profile to those achieved by larger groups elsewhere.

Of the 5 boys in the preschool group, two tested positive for a deletion (both in the 3' hotspot or distal). The first boy with this distal deletion was 1 of 2 boys who fell into the mild intellectual disability range. However, the other boy who was

deletion positive scored the highest GQ in the group. The remaining three boys were mutation negative. The numbers in this group are unfortunately too small and the variance in performance between individuals too great to make any meaningful associations between gene deletion status and degree of intellectual disability.

BEHAVIOUR

The results of parental reports on the behaviour of the DMD boys in our group reveal higher rates of general behavioural problems (54.5%) than normative data. Previously conducted reports on general behavioural problems in boys with DMD have likewise described an increased rate. Darke and colleagues (2006) reported behavioural problems in 41.5% of their children with neuromuscular disorders and Leibowitz & Dubowitz reported behavioural problems in 33% of their cohort. The higher rates in our group may be due to the exposure of the majority of our children to significant socio-economic stressors. This discrepancy may, however, also be an erroneous finding as a result of our small sample size.

Hinton and colleagues (2006) reported a specific behavioural profile in their sample of DMD boys. Their findings included particularly high ratings in the area of *Social Problems* (34%). They did also describe higher rates of behavioural problems in the areas of *Attention* (24%), *Withdrawn/Depressed* (22%) and *Thought Problems* (22%). Our cohort scored higher than normative data on all scales of behaviour. However, they scored particularly highly in the syndromes of *Somatic Complaints* (45%), *Thought Problems* (44%), *Anxious/Depressed behaviours* (36%) and *Withdrawn/Depressed* (27%). Our group did not score particularly highly on the questions related to *Social Problems* (18%). This may, in part, be due to different ways that societies treat children with disabilities.

Three of the 4 top-scoring syndromes (as listed above) in our cohort fall into the category of "*Internalising Behaviours*". This is further reflected in the mean score

for the whole group for *Internalising Behaviours* falling into the clinically significant category. Other studies which have looked at behaviours in Duchenne boys using different tools have also found results weighted towards anxiety and depression (Leibowitz & Dubowitz, 1981 and Fitzpatrick *et al* 1986).This may well be explained in terms of an adjustment disorder to their condition. Internalising behavioural problems are well described as having an association with chronic illness. Hinton *et al* (2006) reported that the boys in their cohort did not reflect anxiety and depressive behaviours as the predominant characterisation of their profile. They argued that the emphasis on social problems in their group possibly represented a particular behaviour profile associated with DMD and its direct central nervous system effects rather than purely a reactive phenomenon. Our results do not agree with this hypothesis. However, the size of our sample makes our findings significantly less robust.

It is well described that oral corticosteroids may cause behavioural problems (Stuart, Segal &. Keady, 2005). Hinton et al (2006) in their large study of 181 DMD boys using the same behavioural scale analysed their group split into those on oral steroids (34%) and those not on steroid medication. They found no significant differences between the two groups across all 8 of the syndrome scales. In our group of 11 boys, 5 were on a regimen of intermittent oral steroids at the time of assessment (45%). In a Chi-square analysis of our group a greater proportion of the non-steroid group achieved a clinically significant overall behaviour score than the steroid group. There was also a higher mean total behavioural problems score in the non-steroid group. This did not achieve statistical significance. These results may have been due to the fact that the by definition the non-steroid group was made up of older boys already in wheelchairs (our hospital treatment protocol involves interrnittent steroid use until ambulation is lost, then steroids are withdrawn). This finding does not rule out that steroids may be a contributing factor to behavioural problems in individual cases and should be screened for in the clinical setting.

STUDY LIMITATIONS

Due to the small sample sizes in both the neuro-cognitive group (N = 5) and the behavioural group (N = 11), the analysis lacks power. Stated otherwise, there is less statistical sensitivity to differences that may exist in the sample. This situation can be remedied by increasing the sample size in the future.

The lack of South African paediatricians expert in dealing with boys who have complex neuromuscular conditions such as DMD is a serious concern. The result is a system where boys with the condition may have to travel over 500km to attend the Red Cross muscle clinic every 3 months. The serious logistical factors involved in transporting a wheelchair-bound child this distance means a visit to the hospital requires at least 1 and sometimes both parents taking unpaid time off work. Quite understandably it was these parents who generally refused permission for their boys to be involved in the study. Special educational facilities or even support is frequently lacking in these peripheral areas and hence parents of boys living in such areas are not likely to have any hope of a positive result for their sons should a cognitive or behavioural problem be identified.

Although our sample sizes are extremely small compared to studies published elsewhere in the world, it is of great importance to document the results. Duchenne muscular dystrophy is an inherited condition which we have demonstrated has a similar genetic profile in this country to elsewhere in the world. However the conditions in which boys with the condition must live and go to school in South Africa are very different from the majority of countries from which previous research has been published. As detailed above, many of these boys have the double disadvantage of their underlying condition as well as their poor social context. Both cognitive and behavioural problems are likely to be under-recognised and avenues for management remain limited.

Further limitations to the study included the use of a single tool to evaluate both cognition and behaviour. The CBCL is also a parent-report questionnaire and this was not further validated by direct interviews with the boys or teacher questionnaires. Although widely used in this context, the CBCL has also been shown to have some limitations in assessing the behavioural adjustment of children with chronic illness (including increased overall scores due to high scores in the somatic complaints subscale). This may well be a valid observation in our group.

Despite these limitations, it was important to document our findings. Young children with DMD may have serious problems (both cognitive and behavioural) well before muscle weakness is a significant issue. Help can be motivated for in both these spheres.

RECOMMENDATIONS

Although numbers are small in this pilot study, the results do echo findings of international groups. It would be valuable to continue assessing boys with DMD formally for intellectual disability or more specific learning difficulties. A regular screen for behavioural problems and prompt referral to appropriate help may be of great benefit in alleviating family distress and allowing the boys to achieve their optimal development.

We feel the information obtained will be valuable in counselling the parents of children with this disorder. In addition we have identified the need for screening for both cognitive and behavioural problems from diagnosis. The service should aim to target these children for more intensive developmental follow up and educational support from as early as possible.

Darke et al (2006) in their survey of behavioural problems in a cohort of children with neuromuscular conditions in the United Kingdom reported that families

tended to under-report behavioural problems under normal circumstances and largely failed to seek professional help. This problem is likely to be more marked in South African society where significant socio-economic stressors may overshadow concerns about perceived minor behavioural problems. We will need to be active both in screening for issues as well as setting up good links with child developmental and psychiatric services in order for our boys with DMD to reach their maximum potential.

REFERENCES

Achenbach TM & Edelbrock C. (1983) Manual for Child Behaviour Checklist and revised Child Behaviour Profile. Burlington, VC: University of Vermont, Department of Psychiatry.

Achenbach, TM, Rescorla, LA (2001) Achenbach System of Empirically Based Assessment, An Integreated System of Multi-informant Assessment. ASEBA, 1 South Prospect, Burlington, Vermont.

Ahn AH, Kunkel LM. The structural and functional diversity of dystrophin. *Nat Genet* 1993; 3:283-291

Allan MM, Luiz DM, Foxcroft CD. The performance of South African Normal Preschool Children on the Griffiths Scales of mental development: A comparative study, University of Port Elizabeth. Presented at the 10th National Congress of the Psychological Association of South Africa, October 1992

Anderson JL, Head SI, Rae C et al. Brain function in Duchenne Muscular Dystrophy. *Brain* 2002; 125:4-13

Ballo R, Viljoen D & Beighton P. Duchenne and Becker muscular dystrophy prevalence in South Africa and molecular findings in 128 persons affected. *SAMJ* 1994 Aug; 84 (8): 497-497

Bardoni A, Felisari G, Sironi M Comi G, Lai M, Robotti M, Bresolin N. Loss of Dp140 regulatory sequences is associated with cognitive impairment in dystrophinopathies. *Neuromuscul Disord* 2000; 10(3): 194-9

Billard C, Gillet P, Barthez M, Hommet C & Bertrand P. Reading ability and processing in Duchenne muscular dystrophy and spinal atrophy. *Dev Med Child Neurol* 1998; 40: 12-20

Billard C, Gillet P, Signoret JL, Uicaut E, Bertrand P, Fardeau M, Barthez-Carpentier MA, Santini JJ. Cognitive functions in Duchenne muscular dystrophy a reappraisal and comparison with Spinal muscular atrophy. *Neuromusc disord* 1992; 2 (5-6): 371-8

Blake DJ & Kroger S: The neurobiology of DMD: learning lessons from muscle? *Trends Neurosci* 2000; 23: 92-99

Bresolin N, Castelli E, Comi GP, Felisari G, Bardoni A, Perani D, Grass F, Turconi A, Mazzucchelli F, Galloti D et al. Cognitive impairment in Duchenne muscular dystrophy. *Neuromuscul Disord* 1994; 4(4): 359-69

Bushby KM, Appleton R, Anderson LV, Welch JL, Kelly P Gardner-Medwin D. Deletion status and intellectual impairment in Duchenne muscular dystrophy. *Dev Med Child Neurol* 1995 Mar; 37(3):260-9

Cotton S, Voudaris NJ, Greenwood KM. Intelligence and Duchenne muscular dystrophy: Full-Scale, Verbal and Performance intelligence quotients. *Dev Med Child Neurol* 2001; 43:497-501

Cotton SM, Voudaris NJ, Greenwood KM. Association between intellectual functioning and age in children and young adults with Duchenne muscular dystrophy: further results from a meta-analysis. *Dev Med Child Neurol* 2005 Apr; 47(4):257-65

D'Angelo MG, Civati F, Lorusso M, Marini A, Comi GP, Turconi AC, Fabbro F & Bresolin N. Neurpsychological assessment in Duchenne muscular dystrophy. Poster at congress of European Paediatric Neurology Society, 2007.

Darke J, Bushby K, Le Couteur A, et al. Survey of behaviour problems in children with neuromuscular diseases. *Eur J Paed Neurol* 2006; 10: 129-134

Dellafave L & McNally EM. Cardiomyopathy in neuromuscular disorders. *Progress in Paediatric Cardiology* 2007; 24: 35-46

Donald DR & Dawes A (eds). 1994. Childhood and adversity: Psychological perspectives from South African Research. David Phillip, Cape Town. p136-153

Dooling EC. Cognitive disorders in children. *Curr Opin Pediatr* 1993;5: 675-9

Dorman C, Hurley AD & D'Avignon J. Language and learning disorders of older boys with Duchenne muscular dystrophy. *Dev Med Child Neurol* 1988; 30: 316-327

Fabro,F, Felisari,G, Bresolin et al. Language disturbances in a group of participants suffering from Duchenne Muscular Dystrophy: A Pilot study. *Perceptual and Motor Skills* 2007; 104: 663-676

Felisari G, Martinelli Boneschi F, Bardoni A, Sironi M, Comi G, Lai M, Robotti M, Turconi AC, Corrao G, Bresolin N. Loss of Dp140 dystrophin isoform and intellectual impairment in Duchenne dystrophy. *Neurology* 2000 Aug; 55(4):559-64

Fitzpatrick C, Barry C, Garvey C. Psychiatric disorders among boys with Duchenne muscular dystrophy. *Dev Med Child Neurol* 1986;28: 589-595

Hallworth-Pillay K, Bill P, Maduri S et al. Molecular deletion patterns in Duchenne and Becker muscular dystrophy patients from Kwazulu Natal. *Journal of the Neurological Sciences* 2007; 252: 1-3

Hendricksen JGM, Vles JSH. Neuropsychiatric Disorders in Males With Duchenne Muscular Dystrophy: Frequency Rate of Attention-Deficit Hyperactivity Disorder (ADHD), Autism Spectrum Disorder and Obsessive Compulsive Disorder. *J Child Neurol* 2008; 23:477-481

Hinton VJ, De Vivo DC, Nereo NE, Goldstein E, Stern Y. Poor verbal working memory across intellectual level in boys with Duchenne dystrophy. *Neurology* 2000 Jun; 54(11):2127-32

Hinton VJ, De Vivo DC, Nereo NE, Goldstein E, Stern Y. Selective deficits in verbal working memory associated with known genetic etiology. *J Int Neuropsychol Soc* 2001; 7(1): 45-54

Hinton VJ, Nereo NE, Fee RJ, Cyrulnik SE. Social Behaviour problems in boys with Duchenne muscular dystrophy. *Developmental and Behavioral Pediatrics* 2006; 27(6): 470-476

Hinton VJ, De Vivo DC, Fee R, Goldstein E & Stern Y. Investigation of poor academic performance in children with Duchenne muscular dystrophy. *Learn Disabil Res Pract* 2004; 19(3): 146-154

Holt, K.S. Developmental Paediatric Perspectives and Practices; Butterworth 1977

Ibraghimov-Beskronaya O,Ervati JM, Leveille CJ et al. Primary structure of dystrophin-associated glycoproteins linking dystrophin to extracellular matrix. *Nature* 1992; 335: 696-702

Ivanova M, Achenbach TM, Dumenci L et al. Testing the 8-syndrome structure of the child behaviour checklist over 30 societies. *Journal of Clinical Child and Adolescent Psychology* 2007; 36 (3): 405-417

Karagan NJ, Zellweger HU. Early verbal disability in children with Duchenne muscular dystrophy. *Dev Med Child Neurol* 1978 Aug; 20(4):435-41.

Knuesel I, Mastrocola M, Zuellig RA et al. Altered synaptic clustering of GABA receptors in mice lacking dystrophin (mdx mice). *European Journal of Neuroscience* 1999; 11:4457-4462

Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet* 1998; 45:498-506

Komoto J, Usui S, Otsuki S et al. Infantile Autism and DMD. *J Autism Dev Disord* 1984; 14: 191-195

Leibowitz D & Dubowitz V. Intellect and behaviour in Duchenne muscular dystrophy. *Dev Med Child Neurol* 1981 Oct; 23(5):577-90

Lidov HGW, Selig S, Kunkel LM. Dp 140: A novel 140kDa CNS transcript from the dystrophin locus. *Hum Mol Genet* 1995; 4: 329-335

Luiz D. Griffiths Scales of Mental Development: South African Studies (Research paper noC25) 1988

Marini A, Lorusso M, D'Angelo MG et al. Evaluation of narrative abilities in patients suffering from Duchenne Muscular Dystrophy. *Brain and Language* 2007; 102: 1-12

McIntosh N, Helms P & Smyth R. (eds) 2003 Forfar and Arneil's Textbook of Paediatrics. 6th ed. Churchill Livingstone, London.

Menkes JH, Sarnat, Maria (eds) 2006 Child Neurology. 7th ed. Lippincot Williams & Wilkins, Philadelphia.

Moizard MP, Toutain A, Berret F, Billard C, Marmin N, Moraine C. Are Dp71 and Dp 140 brain dystrophin isoforms related to cognitive impairment in Duchenne muscular dystrophy? *Am J Med Genet* 1998 Oct; 80(1):32-41

Moizard MP, Toutain A, Fournier D, Berret F, Raynaud M, Billard C, Andres C, Moraine C. Severe cognitive impairment in DMD: obvious clinical indication for Dp71 isoform point mutation screening. *Eur J Hum Genet* 2000 Jul; 8(7):552-6

Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol* 2003; 2: 731-748

Niepieklo, A. 2008, email, 15 July

Nudel U, Zuk D, Einat P et al. Duchenne muscular dystrophy gene product is not identical in muscle and brain. *Nature* 1989; 337: 76-78

Richter L & Grieve KW. Home Environment and Cognitive Development of Black Infants in impoverished South African Families. *Infant Mental Health Journal* 1991; 12 (2): 88-102

Rishel CW, Greeno C, Marcus SC et al. Use of the Child Behaviour Checklist as a Diagnostic Screening Tool in Community Mental Health. *Research on Social Work Practice* 2005: 15: 195-203

Rosman NP. The cerebral defect and myopathy in Duchenne Muscular Dystrophy. A comparative clinopathologic study. *Neurology* 1970; 20: 329-335

Rutter M. A children's behaviour questionnaire for completion by teachers: preliminary findings. *Journal of Child Psychology and Psychiatry* 1967; 8: 1-11

Smith RA, Sibert JR & Harper PS. Early development of boys with Duchenne muscular dystrophy. *Dev Med Child Neurol* 1990 Jun; 32(6):519-27

Stuart FA, Segal TY & Keady S. Adverse psychological effects of corticosteroids in children and adolescents. *Archives of Disease in Childhood* 2005; 90: 500-506

Tremblay RE, Vitaro F, Gagnon C et al. A prosocial Scale for the Preschool Behaviour Questionaire: Concurrent and Predictive Correlates. *International Journal of Behavioural Development* 1990: 15; 227-245

Wicksell RK, Kihlgren M, Melin L Eeg-Olofsson O. Specific cognitive deficits are common in children with Duchenne muscular dystrophy. *Dev Med Child Neurol* 2004 Mar; 46(3):154-9

Wu JY, Kukan KC, Epi SM et al. Association of Duchenne Muscular Dystrophy with Autistic Spectrum Disorder. *J Child Neurol* 2005; 20: 790-796

APPENDIX A

INFORMATION LEAFLET AND CONSENT FORM FOR DUCHENNE MUSCULAR DYSTROPHY PROJECT

Principal investigator: Dr K Donald

School of Adolescent and Child Health

Red Cross Children's Hospital

Rondebosch, Cape Town

Contact number: (083) 4194188

Your child is being invited to take part in a research project and we would like to ask for your consent. Please take some time to read the information presented here which will explain the details of the project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you understand what the research entails and how you and your child may be involved. Your child's participation is entirely voluntary. If you or your child is at all uncomfortable with the process you may contact myself (Dr K Donald) or Dr M Blockman. You are also free to withdraw at any stage, even if you do agree to take part.

This study has been approved by the Committee for Human Research at the University of Cape Town and will be conducted according to the ethical guidelines and principles of the International Declaration of Helsinki, South African Guidelines for Good Clinical Practice and Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

Some research overseas has shown that children with Duchenne Muscular Dystrophy have an increased risk of learning difficulties. A few studies have

shown that a deletion on a specific part of the dystrophin gene is associated with an even higher risk of having learning problems.

We wish to look at whether this is true for South African boys with Duchenne muscular dystrophy. If we are able to identify a subgroup of boys who are at higher risk of having intellectual difficulties, we will be able to target these children for more intensive developmental follow up and educational support from an early age.

What would participation in this study involve?

All children who we think are likely to have Duchenne muscular dystrophy are offered gene testing (a simple blood test) as a routine part of their care. This is so that we can confirm the diagnosis and counsel you as parents about the likelihood of the problem occurring in children you may have in the future.

Although developmental assessment is offered to children under school age we have not to date offered formal psychometric testing of your child's intellectual development. With your permission we would like your child to undertake these tests. They are tests of intelligence and learning. In addition we would like you to complete a questionniare about aspects of your child's behaviour. The tests will take approximately 2 hours and will be arranged at your convenience at Red Cross Hospital. If your child is of school age, the testing will be performed by a postgraduate psychology student (supervised by Dr Kevin Thomas, senior lecturer in the department of Psychology, University of Cape Town.) If your child is not yet at school Dr Kirsty Donald will perform the tests.

Participation is entirely voluntary. If you decide that your child should not participate in this study, it will not affect the way we treat your child. He will continue to receive the same standard of care that he presently experiences.

We will also be testing children from similar socio-economic backgrounds without Duchenne Muscular Dystrophy to make a comparison. These children will be chosen from your neighbourhood or from your child's school. We may ask for your help in recruiting these children.

Will you benefit from taking part in this research?

The results of the tests will be available to yourselves in full and should we discover any particular problems, we hope to be able to offer more informed advice about choice of schooling in pre-school children and the need for support for specific learning difficulties in children of school-going age.

Who will have access to your child's records?

All information collected will be treated as confidential and protected. If it is used in a publication or thesis, the identity of the participant will remain anonymous. The only people who will have access to the information collected will be Dr Kirsty Donald and Dr Kevin Thomas. As part of the study the research records may need to be reviewed by auditors or the Research Ethics Committee.

Will you be paid to take part in the study and are there any costs involved?

No, you will not be paid to take part in the study, but transport for yourself and your child will be paid for visits during the study.

Is there anything else you should know or do?

Please don't hesitate to contact Dr Kirsty Donald at telephone (083) 4194188 should you have any further queries or encounter any problems

shown that a deletion on a specific part of the dystrophin gene is associated with an even higher risk of having learning problems.

We wish to look at whether this is true for South African boys with Duchenne muscular dystrophy. If we are able to identify a subgroup of boys who are at higher risk of having intellectual difficulties, we will be able to target these children for more intensive developmental follow up and educational support from an early age. In order for us to do this we need to test boys who do not have Duchenne Muscular Dystrophy and compare them with the boys in our clinic who do.

What would participation involve?

With your permission we would like your child to undertake these tests. They are tests of intelligence and learning. In addition we would like you to complete a questionnaire about aspects of your child's behaviour. The tests will take approximately 2 hours and will be arranged at your convenience at Red Cross Hospital. Your transport money will be reimbursed. If your child is of school age, the testing will be performed by a postgraduate psychology student (supervised by Dr Kevin Thomas, senior lecturer in the department of Psychology, University of Cape Town). If your child is not yet at school Dr Kirsty Donald will perform the tests.

Will you benefit from taking part in this research?

The results of the tests will be available to yourselves in full and should we discover any particular problems, we hope to be able to offer more informed advice about choice of schooling in pre-school children and the need for support for specific learning difficulties in children of school-going age.

Who will have access to your child's records?

All information collected will be treated as confidential and protected. If it is used in a publication or thesis, the identity of the participant will remain anonymous. The only people who will have access to the information collected will be Dr Kirsty Donald and Mr Kevin Thomas. As part of the study the research records may need to be reviewed by auditors or the research ethical committee.

Will you be paid to take part in the study and are there any costs involved?

No, you will not be paid to take part in the study, but transport for yourself and your child will be paid for visits during the study.

Is there anything else you should know or do?

Please don't hesitate to contact Dr Kirsty Donald at telephone (083) 4194188 on above number should you have any further queries or encounter any problems

You can contact the Committee for Human Research at 021-4066338 (Health sciences faculty, Research Ethics Committee, Room E52-24 Groote Schuur Hospital, Old Main Building, Observatory, 7925) if you have any concerns or complaints that have not been adequately addressed by your study doctor

CONSENT

By signing below, I.....give consent for my child .....to take part in the research study entitled: Intellectual functioning and Duchenne Muscular Dystrophy: Intellegence testing and correlation with genetic diagnosis

I declare that:

I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.

I have had a chance to ask questions and all my questions have been adequately answered

I understand that taking part in this study is voluntary and I have not been pressurised to take part

I may choose to leave the study at any time

Signed at (place).....on (date).....

.....

.....

Signature of guardian/parent Signature of witness

RELATION TO CHILD: .....