

# **Genetic heterogeneity in South African facioscapulohumeral muscular dystrophy (FSHD) families**

BY

**ANNELIZE VAN DER MERWE**

Thesis submitted in partial fulfilment of the requirements for  
the Magister Scientia (M.Sc.) degree in Human Genetics

Department of Human Genetics and Developmental Biology  
Faculty of Medicine, University of Pretoria,  
South Africa

**SUPERVISOR : Prof. ANTONEL OLCKERS**  
Centre for Genome Research,  
Potchefstroom University for Christian Higher Education, South Africa  
(Formerly from the Department of Human Genetics and Developmental Biology,  
Faculty of Medicine, University of Pretoria, South Africa)

**CO-SUPERVISOR : Dr. CLARA SCHUTTE`**  
Department of Neurology, Faculty of Medicine,  
University of Pretoria, South Africa

February 2002



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

TO MY PARENTS

# ABSTRACT

---

FSHD is the third most common inherited disorder of muscle after Duchenne and Myotonic dystrophy. On a clinical level FSHD is characterised by progressive weakening and muscle atrophy. Initially the face, shoulder-girdle and upper arm muscles are affected, and other skeletal muscles may also become involved over time. Clinical diagnosis of the FSHD phenotype is complex due to the extreme variability and penetrance. The expression varies in the severity, rate of progression and the age of onset.

The FSHD phenotype segregates as an autosomal dominant trait. Linkage was established in 1990 to the sub-telomeric region of chromosome 4q35. This sub-telomeric region was observed to contain 3.3 kb tandem repeats. In 1993 it was concluded that FSHD is caused by a deletion of an integral number of these 3.3 kb repeats. Probe p13E-11 is utilised to detect the FSHD associated DNA rearrangements, but was observed to cross-hybridise to chromosome 10q26 as well as the Y-chromosome. Restriction mapping of chromosome 10q26 indicated that this region contains similar 3.3 kb repeat units to those on chromosome 4q35. Comparison of sequences between 4q35 and 10q26 fragments indicated the presence of an unique chromosome 10 specific *Bln* I site, allowing discrimination between chromosome 4 and 10 alleles. Translocation events between the repeats on chromosomes 4 and 10 were demonstrated via the presence of *Bln* I sites within the 3.3 kb repeats on chromosome 10q26. The interchromosomal exchanges have implications for the specificity and sensitivity of diagnostic DNA testing of FSHD.

This was the first study to investigate the molecular aetiology of FSHD in the South African population. Five extended FSHD families (F10, F20, F30, F40 and F60), consisting of 100 individuals, from the South African population were selected for this study. Haplotype analyses were performed to study the segregation of nine short tandem repeat polymorphism (STRP) markers, mapped for the first time here, in the 4q35 region with the FSHD phenotype. The FSHD associated DNA rearrangements at the D4Z4 locus were detected via Southern blot analyses utilising probe p13E-11.

Results of this study confirmed the presence of DNA rearrangements in the five FSHD families investigated. No evidence for genetic heterogeneity was therefore observed in the selected population group. Identical FSHD-associated haplotypes were observed in three

families (F10, F30 and F40), co-segregating with a *Bln* I resistant deletion fragment of 24 kb. However, an FSHD-associated haplotype, different than the one observed in F10, F30 and F40, was observed in the two other families (F20 and F60). A *Bln* I resistant deletion fragment of 34 kb co-segregated with this FSHD-associated haplotype in these families. Individual 15-31 was found to be a compound heterozygote with regard to the two FSHD-associated genotypes that segregated in the South African families investigated. This individual from family F10 therefore represents the link between the two groups of South African families.

Two distinct haplotypes were observed, each co-segregating with a specific *Bln* I resistant deletion fragment. A dual Founder Effect was therefore observed in this unique population and excludes the possibility of a single ancestral mutation in the South African FSHD population.

# OPSOMMING

---

Fasioskapulohumerale spierdistrofie (FSHD) is die derde algemeenste oorerflike spiertoestand na Duchenne en Miotoniese distrofie. FSHD word geklassifiseer op 'n kliniese vlak deur progressiewe verswakking en spieratrofie. Die gesig, skouergordel en bo-arm spiere word gewoonlik eerste aangetas, maar ander skeletale spiere kan ook mettertyd aangetas word. Die kliniese diagnose van die FSHD fenotipe is kompleks as gevolg van ekstreme variasie en penetrasie. Die ekspressie varieer in die graad van aantasting, tempo van progressie en ouderdom van presentering.

Die FSHD fenotipe segregeer as 'n outosomale dominante toestand. Koppeling is gevind in die sub-telomeriese gebied van chromosoom 4q35 in 1990 en bevat 3.3 kb direk-herhalende volgordes. Dit is in 1993 gepostuleer dat FSHD veroorsaak word deur 'n deleisie van 'n aantal van hierdie 3.3 kb herhalings. Peiler p13E-11 is gebruik om die FSHD-geassosieerde DNA herrangskikkings te herken, maar is waargeneem om ook te hibridiseer met chromosoom 10q26 so wel as met die Y-chromosoom. Restriksiekartering van chromosoom 10q26 het aangedui dat hierdie gebied dieselfde 3.3 kb herhalingseenhede bevat as chromosoom 4q35. Vergelyking tussen die DNA volgorde van 4q35 en 10q26 fragmente het die teenwoordigheid van 'n unieke chromosoom 10 spesifieke *Bln* I setel aangedui wat dit moontlik maak om tussen die allele van chromosoom 4 en 10 te kan onderskei. Die *Bln* I setels in elke 3.3 kb herhaling van 10q26 het die teenwoordigheid van translokasiegebeurtenisse tussen die herhalings op chromosoom 4 en 10 aangedui. Die interchromosomale uitruilings het implikasies vir die spesifisiteit en sensitiwiteit van diagnostiese DNA toetsing vir FSHD.

Hierdie was die eerste studie om die molekulêre etiologie van FSHD in die Suid-Afrikaanse populasie te ondersoek. Vyf uitgebreide FSHD families (F10, F20, F30, F40 en F60), bestaande uit 100 individue, van die Suid-Afrikaanse populasie is geselekteer. Haplotipe analise is onderneem om die segregasie van nege direk-herhalende polimorfiese merkers (STRP), wat ook gekarteer is in hierdie studie, in die 4q35 gebied met die FSHD fenotipe te bestudeer. Die FSHD-geassosieerde DNA herrangskikkings by die D4Z4 lokus is waargeneem met Southern klad analise deur gebruik te maak van peiler p13E-11.

Resultate van hierdie studie het die teenwoordigheid van DNA herrangskikkings in die vyf FSHD families bevestig. Geen bewys vir genetiese heterogeniteit is waargeneem in hierdie geselekteerde populasie nie. Identiese FSHD-geassosieerde haplotipes is waargeneem in drie families (F10, F20 en F30) wat saam met 'n 24 kb *Bln I* bestande delesiefragment segregeer. 'n Verskillende FSHD-geassosieerde haplotipe as die wat waargeneem is in F10, F30 en F40 is waargeneem in die ander twee families (F20 en F60). 'n *Bln I* bestande delesiefragment van 34 kb het saam met dié FSHD-geassosieerde haplotipe in hierdie twee families gesegregeer. Individu 15-31 is gevind om 'n saamgestelde heterosigoot ten opsigte van die twee FSHD-geassosieerde genotipes wat in die Suid-Afrikaanse bevolking waargeneem is, te wees. Hierdie individu van familie F10 is dus die skakel tussen die twee groepe van Suid-Afrikaanse families.

Twee kenmerkende haplotipes, wat elkeen met 'n spesifieke *Bln I* bestande delesiefragment segregeer, is waargeneem. 'n Dubbele stigterseffek is waargeneem in hierdie unieke populasie en dit sluit dus enige moontlikheid van 'n enkele voorouerlike mutasie vir die Suid-Afrikaanse FSHD populasie uit.

# TABLE OF CONTENTS

---

LIST OF ABBREVIATIONS AND SYMBOLS .....	i
LIST OF FIGURES AND GRAPHS .....	viii
LIST OF TABLES .....	x
ACKNOWLEDGEMENTS .....	xi

## CHAPTER ONE

INTRODUCTION .....	1
--------------------	---

## CHAPTER TWO

THE AETIOLOGY AND PATHOGENESIS OF FACIOSCAPULO- HUMERAL MUSCULAR DYSTROPHY .....	3
---	---

2.0 THE MUSCULAR DYSTROPHIES .....	3
2.1 FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY .....	6
2.1.1 CLINICAL ASPECTS OF FSHD .....	7
2.1.1.1 Presenting symptoms .....	7
2.1.1.1.1 The facial muscles .....	8
2.1.1.1.2 The upper extremities, shoulder girdle and neck muscles .....	9
2.1.1.1.3 The truncal muscles .....	11
2.1.1.1.4 The lower extremities and the pelvic girdle muscles .....	11
2.1.1.1.5 Asymmetry of muscle involvement .....	11
2.1.1.1.6 Extramuscular involvement .....	11
2.1.1.2 Clinical heterogeneity in the FSHD phenotype .....	13
2.1.1.2.1 Infantile FSHD .....	16
2.1.1.3 Current treatments for FSHD .....	17
2.1.2 GENETIC ASPECTS OF FSHD .....	20
2.1.2.1 Linkage of FSHD to chromosome 4q35 .....	20
2.1.2.2 The FSHD locus on chromosome 4q35 .....	22
2.1.2.2.1 Translocation events between chromosomes 4q and 10q .....	26
2.1.2.2.2 Hybrid repeat arrays and deletion of p13E-11 hybridisation site ...	28
2.1.2.2.3 The <i>Bgl</i> II – <i>Bln</i> I dosage test .....	30
2.1.2.3 Somatic and germline mosaicism .....	31
2.1.2.4 Anticipation .....	33
2.1.2.5 Female and male transmission effects .....	34
2.1.2.6 Sporadic FSHD .....	34
2.1.2.7 Phenotypic-Genotypic correlation .....	35
2.1.2.8 Prenatal diagnosis .....	37
2.1.2.9 Genetic heterogeneity .....	38
2.1.2.10 Candidate genes .....	39
2.1.2.10.1 Actinin-associated LIM protein gene (ALP) .....	39
2.1.2.10.2 Adenine nucleotide translocator gene (ANT) .....	39

2.1.2.10.3	Double homeobox gene 4 (DUX4) .....	40
2.1.2.10.4	Fibroblast growth factor (FGF) and FGF receptors .....	41
2.1.2.10.5	The FSHD region gene 1 (FGR1) .....	41
2.1.2.10.6	The FSHD region gene 2 (FRG2) .....	42
2.1.2.10.7	Human beta-tubulin gene (TUBB4Q) .....	43
2.1.2.11	Molecular models proposed for the aetiology of FSHD .....	43
2.1.2.11.1	Homeodomain .....	43
2.1.2.11.2	Position effect variegation .....	44
2.1.2.12	Objectives of this study .....	46

## CHAPTER THREE

<b>MATERIALS AND METHODS .....</b>	<b>47</b>
<b>3.1 FSHD FAMILIES .....</b>	<b>47</b>
3.1.1 Family F10 .....	48
3.1.2 Family F20 .....	49
3.1.3 Family F30 .....	50
3.1.4 Family F40 .....	51
3.1.5 Family F60 .....	51
<b>3.2 DNA ISOLATION .....</b>	<b>52</b>
3.2.1 Isolation of genomic DNA using sodium perchlorate and chloroform ....	52
3.2.2 Isolation of genomic DNA utilising the Wizard® Genomic kit .....	53
<b>3.3 HAPLOTYPE ANALYSIS .....</b>	<b>55</b>
3.3.1 STRP marker information .....	56
3.3.1.1 Marker UT1366 at locus D4S1523 .....	57
3.3.1.2 Marker GATA5B02 at locus D4S1652 .....	57
3.3.1.3 Marker AFMa224xh1 at locus D4S2930 .....	58
3.3.1.4 Marker ATA22F02 at locus D4S2390 .....	59
3.3.1.5 Marker UT5785 at locus D4S2299 .....	60
3.3.1.6 Marker UT2219 at locus D4S2283 .....	60
3.3.1.7 Marker UT7694 at locus D4S2688 .....	61
3.3.1.8 Marker AFMa190zf5 at locus D4S2921 .....	62
3.3.1.9 Marker AFM238ve3 at locus D4S426 .....	63
3.3.2 5' - End labelling of PCR primers .....	64
3.3.3 The polymerase chain reaction (PCR) .....	64
3.3.4 Multiplex PCR .....	65
3.3.5 Single stranded DNA sequencing .....	65
3.3.6 Denaturing gel electrophoresis and autoradiography .....	66
<b>3.4 SOUTHERN BLOT ANALYSIS .....</b>	<b>67</b>
3.4.1 Restriction fragment length polymorphism (RFLP) analysis .....	67
3.4.2 Agarose gel electrophoresis .....	67
3.4.3 Genomic DNA Transfer .....	68
3.4.4 Isolation of p13E-11 .....	68
3.4.5 Radio-active detection .....	70
3.4.5.1 Labelling of probe p13E-11 and molecular weight markers .....	71
3.4.5.2 Hybridisation conditions .....	71
3.4.5.3 Wash conditions .....	71
3.4.5.4 Autoradiography .....	72
3.4.6 Non radio-active detection .....	72



3.4.6.1	Labelling of probe p13E-11 .....	72
3.4.6.2	Quantification of labelled probe .....	72
3.4.6.3	Hybridisation conditions .....	74
3.4.6.4	Wash conditions .....	74
3.4.6.5	Detection .....	74

## CHAPTER FOUR

### RESULTS AND DISCUSSION ..... 75

<b>4.1</b>	<b>SHORT TANDEM REPEAT POLYMORPHISM (STRP) ANALYSIS .....</b>	<b>75</b>
4.1.1	Short tandem repeat marker UT1366 at locus D4S1523 .....	76
4.1.2	Short tandem repeat marker GATA5B02 at locus D4S1652 .....	77
4.1.3	Short tandem repeat marker AFMa224xh1 at locus D4S2930 .....	78
4.1.4	Short tandem repeat marker ATA22F02 at locus D4S2390 .....	79
4.1.5	Short tandem repeat marker UT5785 at locus D4S2299 .....	80
4.1.6	Short tandem repeat marker UT2219 at locus D4S2283 .....	81
4.1.7	Short tandem repeat marker UT7694 at locus D4S2688 .....	82
4.1.8	Short tandem repeat marker AFMa190zf5 at locus D4S2921 .....	83
4.1.9	Short tandem repeat marker AFM238ve3 at locus D4S426 .....	85
<b>4.2</b>	<b>SOUTHERN BLOT ANALYSIS .....</b>	<b>86</b>
<b>4.3</b>	<b>RADIO-ACTIVE VERSUS NON RADIO-ACTIVE DETECTION .....</b>	<b>89</b>
<b>4.4</b>	<b>MOLECULAR ANALYSIS OF SPECIFIC FSHD FAMILIES .....</b>	<b>91</b>
4.4.1	FSHD family F10 .....	91
4.4.2	FSHD family F20 .....	95
4.4.3	FSHD family F30 .....	99
4.4.4	FSHD family F40 .....	102
4.4.5	FSHD family F60 .....	103

## CHAPTER FIVE

### CONCLUSION ..... 107

5.1	SOUTH AFRICAN FSHD FAMILIES INVESTIGATED .....	107
5.2	GENETIC HETEROGENEITY .....	110
5.3	DUAL FOUNDER EFFECT .....	110
5.4	FUTURE DIRECTIONS IN FSHD RESEARCH .....	111

### REFERENCES ..... 113

## APPENDIX A

### CONFERENCES AND MEETINGS AT WHICH RESEARCH CONTAINED IN THIS THESIS WERE PRESENTED ..... 122

A.1	RESEARCH PRESENTED AT INTERNATIONAL CONFERENCES .....	122
A.2	RESEARCH PRESENTED AT NATIONAL CONFERENCES .....	123

A.3	RESEARCH PRESENTED AT THE FACULTY OF MEDICINE, UNIVERSITY OF PRETORIA .....	124
A.4	PUBLISHED ABSTRACTS IN INTERNATIONAL PEER-REVIEWED JOURNALS .....	124

## **APPENDIX B**

MODE OF INHERITANCE OF DIFFERENT TYPES OF MUSCULAR DYSTROPHIES .....	125
---	-----

## **APPENDIX C**

DIAGNOSTIC CRITERIA FOR FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY .....	128
---	-----

## **APPENDIX D**

NUCLEOTIDE SEQUENCE OF REPEAT UNITS AND FLANKING REGIONS AT THE D4Z4 LOCUS .....	132
---	-----

## **APPENDIX E**

COMPARISON OF NUCLEOTIDE SEQUENCE FROM ONE <i>Kpn</i> I REPEAT UNIT DERIVED FROM CHROMOSOMES 4q35 AND 10q26 .....	139
---	-----

## **APPENDIX F**

EXTENDED FAMILY PEDIGREES .....	144
---------------------------------	-----

# LIST OF ABBREVIATIONS AND SYMBOLS

---

Abbreviations are listed in alphabetical order.

9B6A	probe complimentary to the homeobox sequences within each 3.3 kb repeat unit
10qter	telomeric region of the long arm of chromosome 10
$\alpha$	alpha
$\alpha$ - <sup>32</sup> P-dCTP	dCTP labelled in the $\alpha$ position with <sup>32</sup> P isotope
A or a	adenine (in DNA sequence)
$A_{260}/A_{280}$	ratio of absorbency measured at 260 nm and 280 nm
ACD	acid citrate dextrose
acrylamide	C <sub>3</sub> H <sub>5</sub> NO
ACTA	actin alpha skeletal muscle
AD	autosomal dominant
ADP	adenosine diphosphate
ALP	actinin-associated LIM protein
ANT	adenine nucleotide translocator
ANT1	adenine nucleotide translocator isoform 1
ANT2	adenine nucleotide translocator isoform 2
ANT3	adenine nucleotide translocator isoform 3
APS	ammonium persulfate: (NH <sub>4</sub> )S <sub>2</sub> O <sub>8</sub>
AP-SA	alkaline phosphatase-labelled streptavidin
AR	autosomal recessive
ATP	adenosine triphosphate
$\beta$	beta
<i>Bam</i> HI	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Bam</i> HI gene from <i>Bacillus amyloliquefaciens</i> H, with recognition site 5'-G↓GATCC-3'
<i>Bgl</i> II	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Bgl</i> II gene from <i>Bacillus globigii</i> , with recognition site 5'-A↓GATCT-3'
bisacrylamide	N,N'-methylene-bis-acrylamide: C <sub>7</sub> H <sub>10</sub> O <sub>2</sub> N <sub>2</sub>
<i>Bln</i> I	restriction endonuclease isolated from from an <i>E. coli</i> strain that carries the cloned <i>Bln</i> I gene from <i>Brevibacterium linens</i> , with recognition site 5'-C↓CTAGG-3'
BMD	Becker muscular dystrophy
boric acid	boracic acid: H <sub>3</sub> BO <sub>3</sub>
bp	base pair
BPB	bromophenol blue (3',3'',5',5''-tetrabromophenolsulfonephthalein): C <sub>14</sub> H <sub>10</sub> BrO <sub>5</sub> S
BSA	bovine serum albumin
C or c	cytosine (in DNA sequence)
°C	degrees centigrade
%C	percentage crosslinking monomer
ca.	circa: approximately
CAPN3	calpain-3
CAV3	caveolin-3
CCD	central core disease
cDNA	complementary DNA
CEB8	probe complementary to locus D4F35S1
CEN	centromere



CEPH	Centre d'Étude du Polymorphisme Humain (Centre for the Study of Human Polymorphisms)
CHLC	Co-operative Human Linkage Centre
chr	chromosome
CI	cardiac involvement
Ci	curie: quantity of any radioactive nuclide in which there are $3.7 \times 10^{10}$ disintegrations per second
CK	creatine kinase
cm	centimeter: $10^{-2}$ meter
cM	centimorgan
CMD1B	congenital muscular dystrophy with secondary merosin deficiency
CNS	central nervous system
COL6A1	collagen type VI subunit $\alpha 1$
COL6A2	collagen type VI subunit $\alpha 2$
COL6A3	collagen type VI subunit $\alpha 3$
CPD-Star <sup>®1</sup>	disodium 4-chloro-3-(methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro) tricyclodecan}-4-yl)phenyl phosphate: $C_{18}H_{19}Cl_2O_7PNa_2$
CS	clinical severity
CT-scan	computed tomography scan
$\delta$	delta
dATP	2'-deoxyadenosine-5'-triphosphate
DBP	detector block powder
dCTP	2'-deoxycytidine-5'-triphosphate
ddATP	2',3'-dideoxyadenosine-5'-triphosphate
ddCTP	2',3'-dideoxycytidine-5'-triphosphate
ddGTP	2',3'-dideoxyguanosine-5'-triphosphate
ddH <sub>2</sub> O	double distilled water
ddNTP	2',3'-dideoxynucleotide
ddTTP	2',3'-dideoxythymidine-5'-triphosphate
DEPC	diethyl pyrocarbonate
DES	desmin
DGC	dystrophin-glycoprotein complex
dGTP	2'-deoxyguanosine-5'-triphosphate
DMD	Duchenne muscular dystrophy
DMPK	Myotonin-protein kinase gene
DMRV	distal myopathy with rimmed vacuoles
DNA	deoxyribonucleic acid
DNR	dinucleotide repeat
dNTP	2'-deoxynucleotide triphosphate
DRM	desmin related myopathy
dsDNA	double stranded DNA
DTT	dithiothreitol: threo-1,4-dimercapto-2,3-butanediol: $C_4H_{10}O_2S_2$
dTTP	2'-deoxythymidine-5'-triphosphate
DUX1	double homeobox gene 1
DUX2	double homeobox gene 2
DUX3	double homeobox gene 3
DUX4	double homeobox gene 4
$\epsilon$	epsilon
<i>Eco</i> RI	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Eco</i> RI gene from <i>Escherichia coli</i> RY 13, with recognition site 5'-G↓AATTC-3'
EDMD	Emery-Dreifuss muscular dystrophy
EDMD-AD	autosomal dominant Emery-Dreifuss muscular dystrophy

<sup>1</sup> CPD-Star<sup>®</sup> is a registered trademark of Tropix Inc., Bedford, MA, U.S.A.



EDTA	ethylenediamine tetraacetic acid: $C_{10}H_{16}N_2O_8$
EMD	X-linked recessive Emery-Dreifuss muscular dystrophy
EMG	electromyography
EST	expressed sequence tag
EtBr	ethidium bromide (2,7-Diamino-10-ethyl-9-phenyl-phenanthridinium bromide): $C_{21}H_{20}BrN_3$
EtOH	ethanol: $CH_3CH_2OH$
FCMD	Fukuyama congenital muscular dystrophy
FER-1	dysferlin
FGF	fibroblast growth factor
FGF-R1	fibroblast growth factor receptor 1
FGF-R3	fibroblast growth factor receptor 3
FISH	fluorescence <i>in situ</i> hybridisation
formamide	carbamide: $CH_3NO$
FRG1	FSHD region gene 1
FRG1P	FRG1 protein
FRG2	FSHD region gene 2
FSHD	facioscapulohumeral muscular dystrophy
$\gamma$	gamma
$\gamma^{32}P$ -dATP	dATP labelled in the $\gamma$ position with $^{32}P$ isotope
G	gram
G or g	guanine (in DNA sequence)
GDB	Genome database
gDNA	genomic DNA
Genbank	Genbank <sup>® 1</sup> : United States repository of DNA sequence information
Gm	allotype associated with IgG heavy chains
H buffer	high salt buffer [10X buffer contains: 100 mM Tris-HCl (pH 7.5), 100 mM $MgCl_2$ , 10 mM Dithiothreitol, 1000 mM NaCl]
$H_2O$	water
HCl	hydrochloric acid
HET	heterozygosity
Hz	hertz
<i>hsm3</i>	human DNA insert showing sperm-specific hypomethylation
HIBM	hereditary inclusion body myopathy
HLA	human leukocyte antigens
Hmix	Xenopus mesoderm induced homeobox
HmprD	<i>Drosophila</i> paired
HSPG	heparan sulfate proteoglycan
IAA	isoamyl alcohol
IgG	immunoglobulin G
IQ	intelligence quotient
ITGA7	integrin $\alpha 7$
K buffer	potassium buffer [10X buffer contains: 200 mM Tris-HCl (pH 8.5), 100 mM $MgCl_2$ , 10 mM Dithiothreitol, 1000 mM KCl]
K-acetate	potassium acetate: $CH_3COOK$
kb	kilo ( $10^3$ ) base pair
KCl	potassium chloride
KPL	Kirkegaard & Perry Laboratories
<i>Kpn</i> I	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Kpn</i> I gene from <i>Klebsiella pneumoniae</i> , with recognition site 5'-GGTAC↓C-3'
L buffer	low salt buffer [10X buffer contains: 100 mM Tris-HCl (pH 7.5), 100 mM $MgCl_2$ , 10 mM Dithiothreitol]

<sup>1</sup> Genbank<sup>®</sup> is a registered trademark of the National Institutes of Health, Bethesda, MD, U.S.A.



LAMA2	laminin $\alpha$ 2 chain of merosin
LGMD	limb-girdle muscular dystrophy
LGMD1A	limb-girdle muscular dystrophy type 1A
LGMD1B	limb-girdle muscular dystrophy type 1B
LGMD1C	limb-girdle muscular dystrophy type 1C
LGMD1D	limb-girdle muscular dystrophy type 1D
LGMD1E	limb-girdle muscular dystrophy type 1E
LGMD2A	limb-girdle muscular dystrophy type 2A
LGMD2B	limb-girdle muscular dystrophy type 2B
LGMD2C	limb-girdle muscular dystrophy type 2C
LGMD2D	limb-girdle muscular dystrophy type 2D
LGMD2E	limb-girdle muscular dystrophy type 2E
LGMD2F	limb-girdle muscular dystrophy type 2F
LGMD2G	limb-girdle muscular dystrophy type 2G
LGMD2H	limb-girdle muscular dystrophy type 2H
LGMD2I	limb-girdle muscular dystrophy type 2I
LIM	Lin-11/Is1-1/Mec-3
LINE	long interspersed nuclear elements
LMNA	lamin A/C (gene encoding two components of the nuclear lamina, lamins A and C)
LOD	logarithm of the odds
<i>Lsau</i>	long <i>Sau</i> 3A DNA repeats
$\mu$	micro: $10^{-6}$
$\mu$ Ci	micro Curie
$\mu$ g	microgram
$\mu$ l	microlitre
$\mu$ M	micromolar
m	milli: $10^{-3}$
M	molar: moles per litre
M buffer	medium salt buffer [10X buffer contains: 100 mM Tris-HCl (pH 7.5), 100 mM MgCl <sub>2</sub> , 10 mM Dithiothreitol, 500 mM NaCl]
M13mp18	vector number 18 of the mp series of bacteriophage M13
MD	muscular dystrophy
MD-EBS	epidermolysis bullosa simplex associated with late-onset muscular dystrophy
MEAX	myopathy with excessive autophagy
MFD	Marshfield Research Foundation
mg	milligram
mg	magnesium
Mg <sup>2+</sup>	magnesium ion
Mg-acetate	magnesium acetate: C <sub>4</sub> H <sub>6</sub> O <sub>4</sub> Mg.4H <sub>2</sub> O
MgCl <sub>2</sub>	magnesium chloride
Mhox	muscle specific homeodomain protein
MIM	mendelian inheritance in man
min	minutes
ml	millilitres
mm	millimetre
mM	millimolar
MM	Miyoshi myopathy
MPD1	autosomal dominant distal myopathy
MPRM1	autosomal dominant myopathy with proximal weakness and early respiratory muscle involvement
mRNA	messenger RNA
MTMX	myotubular myopathy
n	nano: $10^{-9}$



Na-citrate	citric acid trisodium salt: $C_6H_5Na_3O_7$
NaCl	sodium chloride
Na <sub>2</sub> EDTA	disodium EDTA: $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$
NaH <sub>2</sub> PO <sub>4</sub>	sodium phosphate monobasic
NaOH	sodium hydroxide
ng	nanogram
NCBI	National Center for Biotechnology Information, U.S.A.
NEM	nemaline myopathy
NIH	National Institutes of Health, U.S.A.
nm	nanometer: $10^{-9}$ meter
nM	nanomolar
NMR	nuclear magnetic resonance
No	number
OD	optical density
OMIM	online mendelian inheritance in man
OPMD	oculopharyngeal muscular dystrophy
orange G	7-hydroxy-8-phenylazo-1,3-naphthalenedisulfonic acid: $C_{16}H_{10}N_2O_7S_2Na_2$
ORF	open reading frame
Otx	orthodenticle homeobox gene
%	percent
p	pico: $10^{-12}$
<sup>32</sup> P	phosphorus isotope: maximum $\beta$ emission energy 1.71 MeV: half-life 14.3 days
p13E-11	probe complimentary to 3.3 kb repeat units at locus D4Z4
PAB	phosphatase assay buffer
PABP2	poly(A) binding protein 2
PAGE	polyacrylamide gel electrophoresis
Pax	paired box gene
Pax 3	paired box gene 3
Pax 6	paired box gene 6
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PDGF-R $\alpha$	platelet-derived growth factor receptor $\alpha$
PDGF-R $\beta$	platelet-derived growth factor receptor $\beta$
PDZ	proteins comprising the Postsynaptic density protein, Disc-large tumor suppressor and the Zonula occludens protein
PEG	polyethylene glycol: $HO(C_2H_4O)_nH$
PEV	position effect variegation
PFGE	pulsed field gel electrophoresis
pH	indicates acidity: numerically equal to the negative logarithm of H <sup>+</sup> concentration expressed in molarity
pH30	probe complimentary to locus D4S139
pmol	pico mole
prd	paired gene
P/S	ratio of probe to standard
Pu	purine
Py	pyrimidine
q	long arm of a chromosome
qter	telomeric region of the long arm of a chromosome
RFLP	restriction fragment length polymorphism
RMD	rippling muscle disease
RNA	ribonucleic acid
rpm	revolutions per minute
RSA	relative specific activity



RSMD-1	congenital muscular dystrophy with rigid spine
RT-PCR	reverse transcriptase PCR
RYR1	ryanodine receptor
<i>Sac</i> I	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Sac</i> I gene from <i>Streptomyces achromogenes</i> , with recognition site 5'-GAGCT↓C-3'
<i>Sau</i> 3AI	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Sau</i> 3AI gene from <i>Staphylococcus aureus</i> 3A, with recognition site 5'-↓GATC-3'
SCK	serum creatine kinase
SDS	sodium dodecyl sulphate: C <sub>12</sub> H <sub>25</sub> NaSO <sub>4</sub>
sec	seconds
Sequenase	Sequenase <sup>®1</sup> Version 2.0 T7 DNA Polymerase
Sequenase buffer	200 mM Tris-HCl (pH7.5); 100 mM MgCl <sub>2</sub> ; 250 mM NaCl
SERCA1	sarcoplasmic reticulum Ca <sup>2+</sup> ATPase
SGC	sarcoglycan complex
SGCA	α-sarcoglycan
SGCB	β-sarcoglycan
SGCD	δ-sarcoglycan
SGCG	γ-sarcoglycan
SJS	Schwartz-Jampel syndrome
spermidine	N-[3-Aminopropyl]-1,4-butanediamine: C <sub>7</sub> H <sub>19</sub> N <sub>3</sub>
SSC	saline-sodium-citrate buffer: 0.15 M NaCl, 15mM Na-citrate, pH 7.0
ssDNA	single stranded DNA
SSPE	saline-sodium-phosphate-EDTA buffer: 0.15 M NaCl, 10 mM NaH <sub>2</sub> PO <sub>4</sub> , 1 mM EDTA, pH 7.4
stop buffer	95% formamide; 0.05% xylene cyanol FF; 0.05% bromophenol blue; 20mM EDTA
STRP	short tandem repeat polymorphism
STS	sequence tagged site
T or t	thymine (in DNA sequence)
T buffer	Tris acetate buffer [10X contains: 330 mM Tris-acetate (pH 7.9), 100 mM Mg-acetate, 5 mM Dithiothreitol, 660 mM K-acetate]
T <sub>a</sub>	annealing temperature
T <sub>m</sub>	melting temperature
Taq polymerase	deoxynucleosidetriphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7, from <i>Thermus aquaticus</i> BM, recombinant ( <i>E. coli</i> )
TBE buffer	Tris borate-EDTA buffer: 89.15mM Tris <sup>®2</sup> (pH 8.0), 88.95 mM boric acid, 2.498 mM Na <sub>2</sub> EDTA
TCAP	telethonin
TE	10 mM Tris-HCl (pH 7.5); 1 mM EDTA
TEL	telomere
TEMED	N,N,N,N'-tetramethylethylenediamine: C <sub>6</sub> H <sub>16</sub> N <sub>2</sub>
temp	temperature
TetNR	tetranucleotide repeat
TMD	tibial muscular dystrophy
TPM3	α tropomyosin
TriNR	trinucleotide repeat
Tris	Tris <sup>®</sup> : tris(hydroxymethyl)-amino-methane: 2-amino-2-(hydroxymethyl)-1,3-propanediol: C <sub>4</sub> H <sub>11</sub> NO <sub>3</sub>
Tris-HCl	2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride: C <sub>4</sub> H <sub>11</sub> NO <sub>3</sub> .H <sub>2</sub> O
Triton X-100	triton X-100 <sup>®3</sup> : octylphenolpoly(ethylene-glycolether) <sub>n</sub> : C <sub>34</sub> H <sub>62</sub> O <sub>11</sub> , for n = 10
TUBB4Q	human beta-tubulin gene
U	units

<sup>1</sup> Sequenase<sup>®</sup> is a registered trademark of United States Biochemical Corporation, Cleveland, OH, U.S.A.

<sup>2</sup> Tris<sup>®</sup> is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.

<sup>3</sup> Triton X-100<sup>®</sup> is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.





UK	United Kingdom
Urea	CH <sub>4</sub> N <sub>2</sub> O
USA	United States of America
UV	ultraviolet
V	volt
VNTR	variable number of tandem repeats
VPDMD	vocal cord and pharyngeal weakness with autosomal dominant distal myopathy
W	watt
Xap I (isoschizomer Apo I)	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Apo I</i> gene from <i>Arthrobacter protophormiae</i> with recognition site 5'-Pu↓AATTPy-3'
XC	xylene cyanole FF: C <sub>25</sub> H <sub>27</sub> N <sub>2</sub> O <sub>6</sub> S <sub>2</sub> Na
xg	gravitational acceleration
XR	X-linked recessive
YAC	yeast artificial chromosome
■ / ●	male/female: tested FSHD normal
■ / ●	male/female: FSHD equivocal
□ / ○	male/female: never tested for FSHD (phenotypical status unknown)
■ / ●	male/female: tested FSHD positive
■ / ●	obligate gene carrier
♁ / ♀	male/female: deceased
◇	sex unknown
◇	multiple individuals, exact number of individuals unknown
—#—	divorced
↖	proband
∧	dizygotic twins
∧	monozygotic twins
▶	recombination event

# LIST OF FIGURES AND GRAPHS

---

Figure 2.1:	Distribution of muscle groups predominantly affected in various muscular dystrophies .....	5
Figure 2.2:	Muscle membrane proteins .....	6
Figure 2.3:	Facial muscles affected in FSHD .....	8
Figure 2.4:	Upper extremities, lower extremities, shoulder girdle and truncal muscles affected in FSHD .....	10
Figure 2.5:	Scapular fixation in FSHD .....	18
Figure 2.6:	Partial map indicating the relative position and loci and restriction endonuclease recognition sites within the 4q35 region .....	23
Figure 2.7:	Restriction endonuclease map of the 4q35 and 10q26 regions.....	25
Figure 2.8:	Schematic representation of hybrid chromosomes .....	29
Figure 2.9:	Schematic representation of the <i>Bgl</i> II – <i>Bln</i> I dosage test .....	31
Figure 3.1:	Excerpt from a pedigree of family F10 .....	49
Figure 3.2:	Excerpt from a pedigree of family F21 .....	50
Figure 3.3:	Excerpt from a pedigree of family F30 .....	50
Figure 3.4:	Excerpt from a pedigree of family F40 .....	51
Figure 3.5:	Excerpt from a pedigree of family F60 .....	52
Figure 4.1:	Representative autoradiograph of marker UT1366 .....	77
Figure 4.2:	Representative autoradiograph of marker GATA5B02 .....	78
Figure 4.3:	Representative autoradiograph of marker AFMa224xh1 .....	79
Figure 4.4:	Representative autoradiograph of marker ATA22F02 .....	80
Figure 4.5:	Representative autoradiograph of marker UT5785 .....	81
Figure 4.6:	Representative autoradiograph of marker UT2219 .....	82
Figure 4.7:	Representative autoradiograph of marker UT7694 .....	83
Figure 4.8:	Representative autoradiograph of marker AFMa190zf5 .....	84
Figure 4.9:	Representative autoradiograph of marker AFM238ve3 .....	85
Figure 4.10:	Representative autoradiograph of the optimised Southern blot analysis .....	89
Figure 4.11:	Comparison between radio-active and non radio-active detection .....	90
Figure 4.12:	Haplotypes of selected individuals from family F10 .....	94
Figure 4.13:	Southern blot analysis of family F15 .....	95
Figure 4.14:	Haplotypes of selected individuals from family F20 .....	98
Figure 4.15:	Haplotypes of selected individuals from family F30 .....	101
Figure 4.16:	Haplotypes of selected individuals from family F40 .....	103
Figure 4.17:	Haplotypes of selected individuals from family F60 .....	104
Figure 4.18:	Southern blot analysis of family F60 .....	106
Figure 5.1:	Relationship between five FSHD families investigated in this study .....	111
Figure F.1:	Full pedigree of South African FSHD family F10 .....	145
Figure F.2:	Full pedigree of South African FSHD family F11 .....	148
Figure F.3:	Full pedigree of South African FSHD family F12 .....	151
Figure F.4:	Full pedigree of South African FSHD family F13 .....	152
Figure F.5:	Full pedigree of South African FSHD family F14 .....	153
Figure F.6:	Full pedigree of South African FSHD family F15 .....	156

Figure F.7:	Full pedigree of South African FSHD family F21 .....	157
Figure F.8:	Full pedigree of South African FSHD family F30 .....	159
Figure F.9:	Full pedigree of South African FSHD family F40 .....	163
Figure F.10:	Full pedigree of South African FSHD family F60 .....	164
Graph 2.1:	Proportion of patients with different clinical severity scores and <i>Eco</i> RI deletion fragment sizes .....	37



# LIST OF TABLES

---

Table 2.1:	Penetrance of FSHD .....	13
Table 2.2:	Composition of translocated arrays .....	27
Table 2.3:	Clinical Severity Scale for FSHD .....	36
Table 3.1:	Average DNA yield from various amounts of starting material .....	53
Table 3.2:	Genetic map of human chromosome 4q35 .....	55
Table 3.3:	Primers for short tandem repeat polymorphism markers located on chromosome 4q35 .....	56
Table 3.4:	Allele frequencies for marker UT1366 at locus D4S1523 .....	57
Table 3.5:	Partial gDNA sequence at marker UT1366 at locus D4S1523 .....	57
Table 3.6:	Allele frequency for marker GATA5B02 at locus D4S1652 .....	57
Table 3.7:	Partial gDNA sequence at marker GATA5B02 at locus D4S1652 ...	58
Table 3.8:	Allele frequency for marker AFMa224xh1 at locus D4S2930 .....	58
Table 3.9:	Partial gDNA sequence at marker AFMa224xh1 at locus D4S2930	59
Table 3.10:	Allele frequency for marker ATA22F02 at locus D4S2390 .....	59
Table 3.11:	Partial gDNA sequence at marker ATA22F02 at locus D4S2390 ....	59
Table 3.12:	Allele frequency for marker UT5785 at locus D4S2299 .....	60
Table 3.13:	Partial gDNA sequence at marker UT5785 at locus D4S2299 .....	60
Table 3.14:	Allele frequency for marker UT2219 at locus D4S2283 .....	61
Table 3.15:	Partial gDNA sequence at marker UT2219 at locus D4S2283 .....	61
Table 3.16:	Allele frequency for marker UT7694 at locus D4S2688 .....	61
Table 3.17:	Partial gDNA sequence at marker UT7694 at locus D4S2688 .....	62
Table 3.18:	Allele frequency for marker AFMa190zf5 at locus D4S2921 .....	62
Table 3.19:	Partial gDNA sequence at marker AFMa190zf5 at locus D4S2921	63
Table 3.20:	Allele frequency for marker AFM238ve3 at locus D4S426 .....	63
Table 3.21:	Partial gDNA sequence at marker AFM238ve3 at locus D4S426 ....	63
Table 4.1:	Optimised conditions for nine short tandem repeat polymorphism markers located on chromosome 4q35 .....	75
Table 4.2:	Relative enzyme activity in Amersham™ buffers .....	86
Table B.1:	Mode of inheritance of different types of muscular dystrophies .....	125
Table C.1:	Diagnostic criteria for FSHD .....	129
Table D.1:	Nucleotide sequence of repeat units at D4Z4 locus .....	132
Table E.1:	Comparison of one <i>KpnI</i> repeat unit nucleotide sequence derived from 4q35 and 10q26 .....	139

# ACKNOWLEDGEMENTS

---

I would like to express my appreciation towards the following individuals and institutions:

The **FSHD families** for their participation in this project. Their interest and contribution to this project was invaluable and without their involvement this study would have been impossible.

My supervisor, **Prof. Antonel Olckers**, for her never-ending support and for sharing her wisdom, experience and insight with me to enable me to fulfil my dreams. I am especially grateful to her for providing me with opportunities of a lifetime, and for making a tremendous impact on my scientific career. She is truly committed to the education of her students, and not just involved. **Dr. Clara Schutte**, my co-supervisor, for the clinical examinations of the patients and for partial funding to attend the 6<sup>th</sup> World Muscle Society meeting and the 51<sup>st</sup> American Society of Human Genetics meeting. The **Department of Neurology** for hosting of the weekly FSHD clinics and for assisting with compilation of the initial family pedigrees. **Dr. Engela Honey** for the genetic counseling and blood collection at the FSHD clinics. **Mr. Francois Honiball** for running the FSHD support group. **Debbie Prosser**, a colleague and true friend, for her encouragement and support throughout the years. **Marco Alessandrini** for joining the FSHD team and for sharing his ideas and enthusiasm with us. I am also grateful for the chromosome 10 haplotype analysis he performed which allowed us to determine the chromosomal origin of some deletion fragments. To the other members of the team: **Bronwyn Wedge**, **Coenie Goosen**, **Dr. Francois Maree** and **Wayne Towers**, for their encouragement and support. The sense of humour and laughter in the lab ensured an uplifting environment every day. **Debbie**, **Marco** and **Bronwyn** for proof reading parts of my thesis. The **MDF National Office** and **Gauteng Branch** for financial assistance to attend both national and international conferences and for the grants awarded to Dr. Antonel Olckers to fund the FSHD project and to enable completion of this study. **Intergrated DNA Technologiec Inc.** (especially Ric Devor, Mark Behlke and Vicky Struzynski-Olson) for accepting me onto the J1-Gene Quantification programme and allowing me to gain experience and expertise to broaden my scientific knowledge. **Prof. Doug Wallace**, for inviting me to his lab to gain experience in pulse field gel electrophoresis. **Prof. Rune Frants** and **Prof.**

**Silvére van der Maarel** for the kind donation of the p13E-11 probe and the original Southern blot protocol. **Martie van der Walt** and other librarians of the Faculty of Medicine for assistance. The former **Department of Human Genetics and Developmental Biology**, the **Faculty of Medicine** and the **University of Pretoria** for providing the infrastructure to complete this study. The research committee of the University of Pretoria (NAVKOM) for the grants awarded to me in 2000 and 2001. A former colleague, **Yolande Havenga**, for the DNA isolation of selected patients. **Vincent Procaccio** for the French translation of the title of the first FSHD article.

My **parents** and immediate family (**Ronel, Wilna, Marlize, Kayla, Riaan and Chris**) for loving and believing in me and for their patience, genuine encouragement and support. I am especially grateful for the ability and endurance I received from **God** to complete my study.