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

BY

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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 delesie 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.

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CHAPTER THREE

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
α	alpha
α^{32}P-dCTP	dCTP labelled in the α position with ^{32}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_3H_5NO
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: $(NH_4)S_2O_8$
AP-SA	alkaline phosphatase-labelled streptavidin
AR	autosomal recessive
ATP	adenosine triphosphate
β	beta
Bam HI	restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Bam* HI gene from Bacillus amyloliquefaciens H, with recognition site 5'-G↓GATCC-3'
Bgl II	restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Bgl* II gene from *Bacillus globigii*, with recognition site 5'-A↓GATCT-3'
bisacrylamide	N,N'-methylene-bis-acrylamide:$C_7H_{10}O_2N_2$
Bln I	restriction endonuclease isolated from from an *E. coli* strain that carries the cloned *Bln* I gene from *Brevibacterium linens*, with recognition site 5'-C↓CTAGG-3'
BMD	Becker muscular dystrophy
boric acid	boracic acid: H_3BO_3
bp	base pair
BPB	bromophenol blue (3',3",5',5"-tetrabromophenolsulfonephthalein): $C_{14}H_{10}BrO_5S$
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 dÉtude 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 3.7×10^{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®[1]	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
δ	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_20	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	dithiothreithol: 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
ε	epsilon
Eco RI	restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Eco* RI gene from *Escherichia coli* RY 13, with recognition site 5'-G↓AATTC-3'
EDMD	Emery-Dreifuss muscular dystrophy
EDMD-AD	autosomal dominant Emery-Dreifuss muscular dystrophy

[1] CPD-Star® 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 *in situ* hybridisation
formamide	carbamaldehyde: CH_3NO
FRG1	FSHD region gene 1
FRG1P	FRG1 protein
FRG2	FSHD region gene 2
FSHD	facioscapulohumeral muscular dystrophy
γ	gamma
γ^{32}P-dATP	dATP labelled in the γ position with ^{32}P isotope
G	gram
G or g	guanine (in DNA sequence)
GDB	Genome database
gDNA	genomic DNA
Genbank	Genbank® [1] : 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
hhsmp3	human DNA insert showing sperm-specific hypomethylation
HIBM	hereditary inclusion body myopathy
HLA	human leukocyte antigens
Hmix	Xenopus mesoderm induced homeobox
HmprD	*Drosophila* paired
HSPG	heparan sulfate proteoglycan
IAA	isoamyl alcohol
IgG	immunoglobulin G
IQ	intelligence quotient
ITGA7	integrin α7
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
Kpn I	restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Kpn* I gene from *Klebsiella pneumoniae*, 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]

[1] Genbank® is a registered trademark of the National Institutes of Health, Bethesda, MD, U.S.A.

LAMA2	laminin α2 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/Isl-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
Lsau	long *Sau* 3A DNA repeats
µ	micro: 10^{-6}
µCi	micro Curie
µg	microgram
µl	microlitre
µM	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 $MgCl_2$, 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^{2+}	magnesium ion
Mg-acetate	magnesium acetate: $C_4H_6O_4Mg.4H_2O$
$MgCl_2$	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_2EDTA	disodium EDTA: $C_{10}H_{14}N_2Na_2O_8.2H_20$
NaH_2PO_4	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: $C_{16}H_{10}N_2O_7S2Na_2$
ORF	open reading frame
Otx	orthodenticle homeobox gene
%	percent
p	pico: 10^{-12}
^{32}P	phosphorus isotope: maximum β 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-Rα	platelet-derived growth factor receptor α
PDGF-Rβ	platelet-derived growth factor receptor β
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+ 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
Sac I	restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Sac* I gene from *Streptomyces achromogenes*, with recognition site 5'-GAGCT↓C-3'
Sau 3AI	restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Sau* 3AI gene from *Staphylococcus aureus 3A*, with recognition site 5'-↓GATC-3'
SCK	serum creatine kinase
SDS	sodium dodecyl sulphate: $C_{12}H_{25}NaSO_4$
sec	seconds
Sequenase	Sequenase®[1] Version 2.0 T7 DNA Polymerase
Sequenase buffer	200 mM Tris-HCl (pH7.5); 100 mM $MgCl_2$; 250 mM NaCl
SERCA1	sarcoplasmic reticulum Ca^{2+} 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: $C_7H_{19}N_3$
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_2PO_4, 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_a	annealing temperature
T_m	melting temperature
Taq polymerase	deoxynucleosidetriphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7, from *Thermus aquaticus* BM, recombinant (*E. coli*)
TBE buffer	Tris borate-EDTA buffer: 89.15mM Tris®[2] (pH 8.0), 88.95 mM boric acid, 2.498 mM Na_2EDTA
TCAP	telethonin
TE	10 mM Tris-HCl (pH 7.5); 1 mM EDTA
TEL	telomere
TEMED	N,N,N,N'-tetramethylethylenediamine: $C_6H_{16}N_2$
temp	temperature
TetNR	tetranucleotide repeat
TMD	tibial muscular dystrophy
TPM3	α tropomyosin
TriNR	trinucleotide repeat
Tris	Tris®: tris(hydroxymethyl)-amino-methane: 2-amino-2-(hydroxymethyl)-1,3-propanediol: $C_4H_{11}NO_3$
Tris-HCl	2-amino-2-2(hydroxymethyl)-1,3-propanediol hydrochloride: $C_4H_{11}NO_3.H_2O$
Triton X-100	triton X-100®[3]: octylphenolpoly(ethylene-glycolether)$_n$: $C_{34}H_{62}O_{11}$, for n = 10
TUBB4Q	human beta-tubulin gene
U	units

[1] Sequenase® is a registered trademark of United States Biochemical Corporation, Cleveland, OH, U.S.A.
[2] Tris ® is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.
[3] Triton X-100® is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.

UK	United Kingdom
Urea	CH_4N_2O
USA	United States of America
UV	ultraviolet
V	volt
VNTR	variable number of tandem repeats
VPDMD	vocal cord and pharyngeal weakness with ausotomal dominant distal myopathy
W	watt
Xap I (isoschizomer *Apo* I)	restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Apo* I gene from *Arthrobacter protophormiae* with recognition site 5'-Pu↓AATTPy-3'
XC	xylene cyanole FF: $C_{25}H_{27}N_2O_6S_2Na$
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

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