Detection of Familial Hypercholesterolemia in South Africa via Cascade Screening: The Wits Find-FH Program

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BACKGROUND

Familial hypercholesterolemia (FH) is an autosomal co-dominant disorder usually resulting from mutations in the LDL receptor (LDLR) gene and less commonly from mutations in apoB100, PCSK9 or LDLRAP1. This condition is characterized by elevated levels of LDL-cholesterol (LDL-C) and premature cardiovascular disease, particularly coronary artery disease (CAD)[1,2].

The heterozygous phenotype (HeFH) is characterized by elevated LDL-C levels approximately twice the normal levels (190 – 400mg/dL), tendon xanthoma and premature CAD[1,2]. If untreated, the cumulative risk of a coronary event by the age of 60 years is at >50% in men and 30% in women[3]. The homozygous phenotype (HoFH) is characterized by LDL-C levels >500 mg/dL, skin and tendon xanthoma beginning soon after birth and if untreated CAD prior to age 20 years of age. There is some overlap in the clinical phenotype between HeFH and HoFH when assessed by genotype.

FH is one of the commonest inherited diseases in the world with an estimated frequency of 1:200 to 1:250 for Caucasian populations[4]. However in some countries such as South Africa, the prevalence in certain population groups such as the Afrikaner, Jewish and south-Asian Indians it is as high as 1:80[5,6], probably due to a founder effect.

In South Africa 70 to 80% of subjects of Afrikaner, Jewish or Indian origin with clinical heterozygous FH identified to date have one of 5 founder mutations – table 1. However the vast majority of FH patients remain undiagnosed and untreated and have not been screened for other mutations in the LDLR, apoB or PCSK9 genes.

TABLE 1: Founder FH LDLR mutations common in South Africa

LOCATION ON LDLR GENE	CODON CHANGE	AMINO ACID CHANGE	COMMON NAME	POPULATION GROUP
Exon 4	c.681C>G	D206E	FH Afrikaner 1	Afrikaner*
Exon 9	c.1285G>A	V408M	FH Afrikaner 2	Afrikaner*
Exon 4	c.523G>A	D154N	FH Afrikaner 3	Afrikaner*
Exon 14	c.2054C>T	P664L	FH Gujarat	Indian
Exon 4	654_656del	Gly219del	FH Lithuania	Jewish

** Includes both Caucasian and SA 'coloured' populations*

Until recently no systematic program existed to detect subjects with FH or to test their family members. Furthermore, information regarding prevalence of FH in black South Africans is sparse. The Wits FIND-FH program was initiated in late 2016 with the goal of addressing both these problems

RESULTS

One full-time and 1 part-time research nurse were hired and trained in late 2016 and beginning in January 2017 follow up of family members commenced based on index patients identified from the Wits Lipid Clinic. In the 1st 7 months, 310 family members were screened and 236 suspected FH cases identified, including black and Indian families. Demographic and LDL-C levels are shown in table 2. Genetic analysis was performed in 236 subjects suspected of FH; 130/236 (55.1%) were confirmed with a mutation consistent with FH including 16/236 (6.8%) with homozygous, compound heterozygous or double heterozygous FH.

TABLE 2:

	VARIANT IDENTIFIED						
	TOTAL (130)	MALE (59)	FEMALE (71)	< 18 YEARS (20)	≥ 18 YEARS (110)	TREATED (82)	UNTREATED (48)
LDL-C (mg/dL)	188.9 ± 86.4	197.5 ± 95.7	181.7 ± 77.5	256.0 ± 121.8	176.7 ± 72.4	167.7 ± 80.3	224.8 ± 85.2
Apolipoprotein B (mg/dL)	133.4 ± 42.7	137.1 ± 45.1	130.2 ± 40.7	153.2 ± 57.9	129.8 ± 38.6	124.8 ± 41.5	147.9 ± 41.3
Lipoprotein (a) (nmol/L)	54 (17 – 129)	50 (17 – 116)	59.5 (16.5 – 145)	47.5 (22.25 – 92.5)	54 (14 – 139)	83 (13 – 136)	41.5 (19.75 – 125.5)

	NO VARIANT						
	TOTAL (106)	MALE (49)	FEMALE (57)	< 18 YEARS (7)	≥ 18 YEARS (99)	TREATED (48)	UNTREATED (57)
LDL-C (mg/dL)	136.4 ± 41.3	134.0 ± 43.4	138.3 ± 39.8	151.1 ± 34.0	135.3 ± 41.7	117.3 ± 44.1	150.8 ± 32.1
Apolipoprotein B (mg/dL)	108.5 ± 24.6	109.6 ± 23.9	107.5 ± 25.4	107.1 ± 22.3	108.6 ± 24.9	102.3 ± 25.5	113.0 ± 22.7
Lipoprotein (a) (nmol/L)	34 (9.75 – 129)	24.5 (7.75 – 69.5)	48 (20 – 148)	81 (26 – 128.5)	33 (8.25 – 128.75)	46.5 (11.25 – 154.75)	32 (8.75 – 107)

LDL-C and Apolipoprotein B presented as mean ± standard deviation. Lipoprotein (a) presented as median (IQR).

METHODS

After obtaining an IRB approved written informed consent from a known FH index case, 1st degree relatives were contacted and a home or clinic visit arranged where after individual informed consent was obtained a targeted medical, cardiovascular, family and medication history, physical (including for skin and tendon xanthoma and corneal arcus) and blood sample were obtained. Fasting blood samples obtained from subjects were analysed at Medpace Reference Laboratories (MRL), Leuven, Belgium for lipids and apolipoproteins and select chemistries to exclude underlying metabolic conditions known to cause secondary elevations of LDL-C. Whole blood samples were drawn into a K2EDTA tube and initially processed at MRL Belgium and genomic DNA isolated using the QIAamp DNA Blood kit (Germantown, MD). The genomic DNA (gDNA) was sequenced at MRL in Cincinnati, Ohio. Capture-based target enrichment library preparation was performed using the Roche SeqCap EZ HyperCap workflow, after which the libraries were sequenced on an Illumina MiSeq Dx sequencing platform (San Diego, CA) using 2x 150 base pair (bp) paired-end chemistry. Sequencing was performed for the coding regions of four genes (LDLR, APOB, PCSK9, LDLRAP1) known to account for the majority of cases of FH[7].

The .FASTQ files generated by the MiSeq were processed using a custom workflow (Developed by Dr. Robert Hegele, Robarts Research Institute, London, ON) for the QIAGEN Biomedical Genomics Workbench version 4.1.1 (Redwood City, CA). This workflow aligned the sequence to the Genome Reference Consortium Human Build 37 (GRCh37/hg19) along with producing variant call files (.vcf) as well as coverage statistics for the targeted regions (.bam). The .vcf and .bam files were then processed with the Golden Helix VarSeq (Bozeman, MT) version 1.4.6 for variant annotation.

TABLE 3:

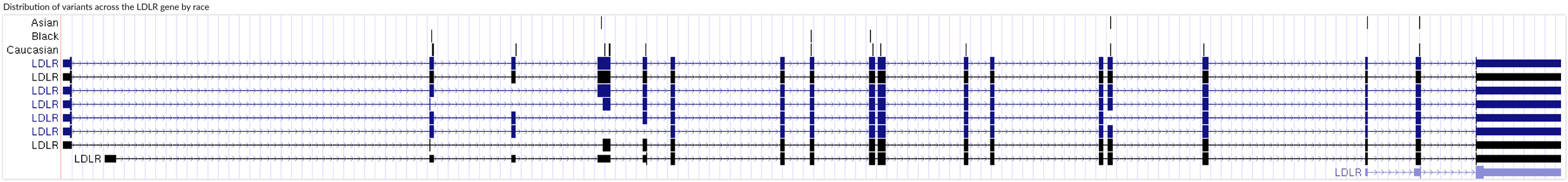
RACE	MUTATION +VE	MUTATION -VE	SPECTRUM OF FH CAUSING MUTATIONS
Caucasian (141)*	87 (62%)	54 (38%)	**HoFH/compound HeFH/double heterozygous FH** LDLR FH Afrikaner-1 (c.681C>G)/LDLR FH Afrikaner-2 (c.1285G>A) = 1 LDLR FH Afrikaner-2 (c.1285G>A)/LDLR Paris 9(c.2177C>T) = 1 LDLR FH Afrikaner-1 (c.681C>G)/ApoB (c.152A>G) =1 LDLR FH Afrikaner-2 (c.1285G>A)/ApoB (c.3383G>A) =3 LDLR FH Lebanese/ApoBx2 (c.3927T>A)/(c.5254G>A) = 1 LDLR FH Padova 1(c.662A>G)/LDLR (c.2359G>A) = 1 PCSK9 (c.267G>A)GOF/LDLR (c.1104C>T)# = 1 **HeFH** **LDLR mutations** FH Afrikaner-1 (c.681C>G) = 28] FH Afrikaner-2 (c.1285G>A) = 21] 54 (62%) FH Afrikaner-3 (c.523G>A) = 5] FH Genoa (c.1646G>A) = 3 FH Padova 1 (c.662A>G) = 1 FH Lancashire (c.301G>A) = 1 FH Reggio (c.761A>C) = 1 FH Irish (c.1444G>A) = 1 (c.651_653delTGG) = 3 (c.165C>T) = 2# =2 (c.2478C>G)# =1 (c.148G>A) =2 **ApoB mutations** (c.10580G>A) = 2 (c.3383G>A) = 2 (c.5474G>A) = 1 **PCSK9 GOF mutation** (c.267G>A) = 1 (c.137G>T) = 2
Asian (90)	39 (43%)	51 (57%)	**HoFH/compound HeFH/double heterozygous FH** LDLR FH Gujarat (c.2054C>T)/LDLR Alexandra (c.401G>A) = 1 LDLR FH Gujarat (c.2054C>T)/LDLR (c.2478C>G)# = 1 LDLR FH Gujarat (c.2054C>T)/apoB (c.8227C>A) = 2 ApoB/ApoB (c.7619G>T)/(c.1794C>T) = 1 **HeFH** **LDLR mutations** FH Gujarat (c.2054C>T) = 26 (66%) c.2356A.T = 1 c.2478C.G# = 1 **ApoB mutations** (c.6929T>C) = 3 (c.6639_6641delTGA) = 1 (c.8227C>A) = 1 (c.10657G>A) = 1
Black (5)	4 (80%)	1 (20%)	**HoFH/compound HeFH/double heterozygous FH** LDLR Cape Town 1(c.137>142del)/LDLR Cape Town 1(c.137>142del) = 1 LDLR Algeria (c.1222G>A)/LDLR (c.1104C>T)# = 1 **HeFH** **LDLR mutations** LDLR Cape Town 1 (c.137-142 del) = 2

** includes subjects of mixed ancestry (coloured)*

 # = not previously reported (n=7)

FIGURE 1

Distribution of variants across the LDLR gene by race



Distribution of variants across the APOB gene by race

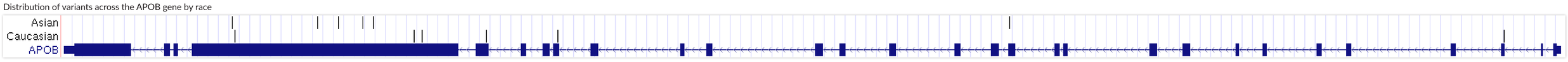


Figure 1. Representation of variant locations for Asian, Black, and Caucasian (including mixed race) populations mapped to the University of California Santa Cruz (UCSC) Genes track and visualized with the UCSC Genome Browser (https://genome.ucsc.edu). Exons are represented as blocks whereas the thin horizontal line connecting the exons represent introns. Variants for Asian, Black, and Caucasian/Mixed Race populations are represented by vertical lines above the gene track corresponding to the position where the variant was identified.

NOVEL MUTATIONS

Mutations either not previously described or listed in the literature as 'of uncertain significance' were evaluated against clinical findings using the Simon Broome criteria for FH to determine if the associated mutations were pathogenic or not. Using this method 7 novel FH associated mutations and 15 mutations of previously uncertain clinical significance were assessed as being FH pathogenic mutations.

DISCUSSION

The Wits FIND-FH program in its 1st year of operation has screened a monthly average of 40 subjects with a clinical FH diagnosis in 75% of subjects and genetic confirmation in 55% with 1/3rd untreated. The ability to collect and utilize clinical data to assess FH phenotype for evaluation against new mutations or those of uncertain significance has resulted in ascertainment of a number of new FH pathogenic mutations. In addition, a number of genetic HoFH or compound HeFH patients who do not meet the traditional clinical criteria, especially LDL-C, for HoFH have been found. The South African multi-ethnic society and well described founder effects emphasize the need for differential approaches to diagnosis and management of FH. Cascade testing of index cases based on phenotype is an important start and has identified many family members who were previously unaware that they had FH. Studies involving larger cohorts and inclusive of different ethnicities are paramount to establishing an accurate prevalence of FH in black South Africans. While the prevalence of FH in virtually all populations in the world demonstrates a gene frequency of 1 per 200 to 500 there are no similar studies in black Africans where even case reports of FH in the literature are few.

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