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 LDL-C levels, premature coronary artery disease, as well as atherosclerosis beginning as early as the teen years and rarely before the age of 40 years. LDL-C levels are influenced by the loss of function of the LDL receptor, resulting in increased plasma LDL-C levels. FH is an autosomal dominant, a heterogeneous disorder affecting about 1 in 500 individuals worldwide. Some FH individuals may present with early-onset CAD before the age of 20 years.

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 FH Clinic. In the 18th month, 331 family members were screened and 236 suspected FH cases were identified (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 mutations.

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>
<thead>
<tr>
<th>POPULATION</th>
<th>TOTAL (106)</th>
<th>MALE (59)</th>
<th>FEMALE (46)</th>
<th>8 YEARS (25)</th>
<th>8 YEARS (30)</th>
<th>TREATED (46)</th>
<th>8 YEARS (37)</th>
<th>UNTREATED (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR-Cig/1</td>
<td>118.8 ± 56.4</td>
<td>117.1 ± 54.7</td>
<td>116.7 ± 53.5</td>
<td>120.6 ± 121.8</td>
<td>176.7 ± 72.6</td>
<td>167.7 ± 60.5</td>
<td>219.4 ± 62.9</td>
<td>119.6 ± 41.2</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>112.8 ± 5.2</td>
<td>117.1 ± 5.7</td>
<td>116.2 ± 5.7</td>
<td>132.2 ± 37.9</td>
<td>129.1 ± 36.6</td>
<td>124.8 ± 41.5</td>
<td>167.9 ± 61.2</td>
<td>124.8 ± 32.1</td>
</tr>
<tr>
<td>Lipoprotein (a)</td>
<td>54 (17 - 116)</td>
<td>60 (17 - 114)</td>
<td>56 (14.5 - 145)</td>
<td>61 (22.8 - 29.3)</td>
<td>54 (13 - 183)</td>
<td>53 (13 - 136)</td>
<td>45.6 (18.7 - 125.3)</td>
<td></td>
</tr>
</tbody>
</table>

**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 xanthomas and cornal arcus) and blood sample were obtained. Fasting blood samples obtained from subjects were analysed at Medpace Reference Laboratories (MRL) Laboratories, Auckland, New Zealand for blood lipids 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 was isolated using the QIAamp DNA Blood kit (QiaGen, Hilden, Germany). The genomic DNA (gDNA) was sequenced at MRL in Cincinnati, Ohio. Capture-based target enrichment library preparation was performed using the Roche's NimbleGen EZ Hybrid Capture system, 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 exome regions of up to four genes per sample (LDLR, APoB, PCSK9, LDLRAP1) known to account for the majority of cases of FH. The FASTQ files generated by the MiSeq were processed using a custom workflow (Developed by Dr. Robert Halegou, Roberts Research Institute, London, ON) for the OAGEN Biomedical Genomics Workbench version 4.1.1 (Bioedit Corp, 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 2.4.4 for variant annotation.

Table 3: Spectrum of FH-causing mutations

<table>
<thead>
<tr>
<th>MUTATION</th>
<th>RACE</th>
<th>MUTATION</th>
<th>VARIANTS IDENTIFIED</th>
</tr>
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**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 is 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 1/3 untreated. The ability to collect and utilize clinical data to assess FH genotypes 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 FHHT or compound heterozygous mutations were identified in patients who do not meet the traditional clinical criteria, especially LDL-C, for FH 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 starting point and has identified many family members who were previously unaware that they had FH. Studies involving larger cohorts and inclusive of different ethnic groups are paramount to establishing an accurate prevalence of FH in black South Africans. While the prevalence of FH is virtually equal in populations in the world it demonstrates a gene frequency of 1 per 2000 in the general population.

REFERENCES


The Authors have no relevant financial or nonfinancial relationships to disclose.