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Analysis of the frequency and spectrum of mutations recognised to cause familial hypercholesterolaemia in routine clinical practice in a UK specialist hospital lipid clinic

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ABSTRACT

Aim: To determine the frequency and spectrum of mutations causing Familial Hypercholesterolaemia (FH) in patients attending a single UK specialist hospital lipid clinic in Oxford and to identify characteristics contributing to a high mutation detection rate.
Methods: 289 patients (272 probands) were screened sequentially over a 2-year period for mutations in *LDLR*, *APOB* and *PCSK9* using standard molecular genetic techniques. The Simon Broome (SB) clinical diagnostic criteria were used to classify patients and a separate cohort of 409 FH patients was used for replication.
Results: An FH-causing mutation was found in 101 unrelated patients (*LDLR* = 54 different mutations, *APOB* p.(Arg3527Gln) = 10, *PCSK9* p.(Asp374Tyr) = 0). In the 60 SB Definite FH patients the mutation detection rate was 73% while in the 142 with Possible FH the rate was significantly lower (27%, $p < 0.0001$), but similar (14%, $p = 0.06$) to the 70 in whom there was insufficient data to make a clinical diagnosis. The mutation detection rate varied significantly ($p = 9.83 \times 10^{-5}$) by untreated total cholesterol (TC) levels (25% in those <8.1 mmol/l and 74% in those >10.0 mmol/l), and by triglyceride levels (20% in those >2.16 mmol/l and 60% in those <1.0 mmol/l ($p = 0.0005$)), with both effects confirmed in the replication sample (p for trend = 0.0001 and $p = 1.8 \times 10^{-6}$ respectively). There was no difference in the specificity or sensitivity of the SB criteria versus the Dutch Lipid Clinic Network score in identifying mutation carriers (A_{ROC} respectively 0.73 and 0.72, $p = 0.68$).
Conclusions: In this genetically heterogeneous cohort of FH patients the mutation detection rate was significantly dependent on pre-treatment TC and triglyceride levels.

1. Introduction[1]

Familial Hypercholesterolaemia (FH) is a common autosomal dominant disease caused by mutations affecting the plasma clearance of LDL-cholesterol (LDL-C) [1]. FH patients have elevated levels of total cholesterol (TC) and LDL-C from birth, and if untreated, develop coronary heart disease (CHD) by the age of 55 in 50% of men and 30% of women [2]. The clinical phenotype of FH is known

Abbreviations: FH, familial hypercholesterolaemia; ARMS, amplification refractory mutation system; HRM, high resolution melting; MLPA, multiplex ligation probe-dependent amplification; TC, total cholesterol; TG, triglycerides; CHD, coronary heart disease; DLCN, Dutch Lipid Clinic Network; NGS, next generation sequencing; NICE, National Institute for Health and Clinical Excellence; DFH, definite FH; PFH, possible FH; UH, unclassified hypercholesterolaemia.

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[1] Human Genes: LDLR—low density lipoprotein receptor, APOB—apolipoprotein B (including Ag(x) antigen), PCSK9—proprotein convertase subtilisin/kexin type 9.

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to be due to mutations in three genes encoding proteins involved in the uptake of LDL-C from the plasma, *LDLR*, *APOB* and *PCSK9*. In the UK, the Simon Broome Register criteria are used for the clinical diagnosis of FH, whereas other European countries may use a score developed by the Dutch Lipid Clinic Network (DLCN) [1,3]. The estimated frequency of heterozygous FH in the UK is 1 in 500 to 1 in 600 [4], and about 120,000 individuals would therefore be predicted to be affected by FH, although only about 15% of them are currently being treated at lipid clinics [5].

To date, there are over 1200 different *LDLR* mutations reported [6] but only one common *APOB* (c.10580G > A, p.(Arg3527Gln)) and one *PCSK9* (c.1120G > T, p.(Asp374Tyr)) [7]. The spectrum of FH mutations in Europe varies between countries, from Greece with only six mutations, which account for 60% of FH in the country, to the Netherlands with one of the most heterogeneous spectrum [8,9]. In the UK there are over 200 different mutations reported [10], which is similar to other western countries. *LDLR* mutations include mainly single nucleotide changes, which alter the amino acid composition of the mature protein, affect the correct splicing of the transcript, or binding of key transcription factors, if located in the promoter region (publication in revision, Khamis A et al.). Large deletions and insertions account for approximately 5–6% of all FH genetic defects [10,11]. The high number of different FH mutations makes genetic testing labour-intensive and costly, which has encouraged the development of novel assays and techniques such as next-generation sequencing (NGS) for diseases like FH [12].

Statin drug therapy significantly reduces the morbidity and mortality from premature coronary disease in FH, particularly if affected individuals are identified and treated in childhood or early adulthood [13–15]. The UK National Institute for Health and Clinical Excellence (NICE) guidelines published in 2008 recommended that all FH patients be offered a DNA test to confirm the diagnosis and that identified mutations should be used as the basis for cascade testing of first-degree relatives of index cases. Patients newly identified by such screening can then be offered treatment to reduce the risk of premature cardiac events [16]. DNA testing for FH has also been shown to complement cholesterol measurement in the management of affected individuals [17].

This study is aimed to assess the frequency and spectrum of mutations recognised to cause FH among patients attending the Oxford Lipid Clinic. The frequency of specific mutations in the UK differs between areas, with p.(Glu101Lys) being the most common in Manchester [18], p.(Arg350*) in South of England [19], and p.(Cys184Tyr) in Glasgow [20]. This study examined whether there are any specific mutations that occur with an unexpected frequency among patient attending the Oxford lipid clinic, which is a specialist clinic with a catchment population of over 620,000 people [4]. The correlation between the measured pre-treated cholesterol, pre-treated triglycerides and the mutation detection rate was also assessed to test the hypothesis that the individuals carrying a FH mutation have higher pre-treatment cholesterol levels and lower triglyceride level compared to those with no mutation. The likelihood of identifying mutation carriers was compared using two different clinical diagnostic criteria: the Simon Broome criteria and the DLCN score. In addition, the study examined whether the effectiveness of lipid-lowering therapy varied between patients with different genetic causes of FH.

2. Materials and methods

2.1. Patient selection criteria

The Oxford FH cohort comprised individuals who attended sequentially the Oxford Lipid Clinic, in England over the period 2009–2011. All participants were Caucasian, aged 18 or over, and were diagnosed with either definite FH (DFH) or possible FH (PFH) using the Simon Broome clinical diagnostic criteria [3,21], or as having unclassified hypercholesterolaemia (UH) which was defined as a total cholesterol and/or LDL-C concentration above the Simon Broome criteria cut off (respectively >7.5 mmol/l and/or >4.9 mmol/l) but with no family history of early CHD or with no such family history that could be elicited. The Simon Broome diagnostic criteria for FH exclude subjects with a triglyceride level of >4.5 mmol/l and none of the patients exceeded this level. There were a total of 289 patients in the cohort, of which 272 probands were apparently unrelated. The Simon Broome British Heart Foundation study (SBBHF) of 409 individuals was used for the replication of the FH clinical diagnosis methods comparison between the Simon Broome FH criteria and the DLCN score, and for the testing of the mutation detection association with TC and TG quartiles. This was a cross-sectional comparison of white patients aged 18 years or more with treated DFH with and without clinically documented CHD recruited from clinics in London, Oxford and Manchester. Recruitment methods, inclusion and exclusion and diagnostic criteria have been described previously [21]. The cohort consisted of 328 FH-mutation positive (FH/M+) and 81 FH-mutation negative (FH/M-) patients and the baseline characteristics of the cohort are shown in Supplemental Data Table 1 (pre-treatment TG levels were not available for the analysis).

2.2. Molecular genetic analysis

Genomic DNA was isolated from whole blood using standard methods [22]. Samples were first screened for the 20 most common UK mutations, including p.(Arg3527Gln) in *APOB* and p.(Asp374Tyr) in *PCSK9*, with a commercially available Elucigene™ FH20 (Gen-Probe Life Sciences, UK) Amplification Refractory Mutation System (ARMS) kit [11]. Next the promoter, intron–exon junctions and the coding sequence of the *LDLR* gene (NM_000527.2) were screened by High Resolution Melting (HRM) method using the Rotor-Gene 6000 [23]. The *LDLR* gene was then screened for gross deletions and insertions using Multiplex Ligation-dependent Probe Amplification (MLPA) assay, SALSA P062 from MRC-Holland (Amsterdam), on the 96-capilary ABI 3730 XL and GeneMarker software. Mutations were designated according to the Human Genome Variation Society guidelines (http://www.hgvs.org/mutnomen/).

2.3. Mutation prediction

Novel *LDLR* variants were assessed by *in silico* mutation prediction tools, including PolyPhen2, SIFT, and Mutation Taster. Analysis of conservation and structure, as previously described [6], were additionally used for variants with an ambiguous effect. Mutation nucleotide numbers were designated using the *LDLR* sequence reported (https://grenada.lumc.nl/LOVD2/UCL-Heart/home.php?select_db=LDLR) with the cDNA numbering beginning with A (A = 1) of the initiating ATG codon.

2.4. Statistical analysis

All statistical analyses were carried out using R (R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0). Matched pre- and post-treatment LDL-C values were available for 104 patients (69 mutation negative, 35 mutation positive). Concentrations of serum cholesterol, LDL-C, HDL-C and triglyceride were not normally distributed, and were presented as geometric means with an approximate standard deviation. Matched pre-treatment TC and TG values were available for 159 patients (62 mutation positive). Dutch scores were calculated using the weights for diagnostic traits as described [1]. SBBHF study subjects were