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Case Report Open Access

A Case Report of a South Asian Family with Homozygous and Heterozygous Familial Defective APOB-100 Caused by p.(Arg3527Trp)

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Abstract

Familial hypercholesterolemia (FH) is an autosomal dominant disorder most commonly caused by mutations in the gene for the Low-Density Lipoprotein (LDL) receptor (*LDLR*), but about 5% of patients in the UK with a clinical diagnosis of FH have a mutation in the gene for apolipoprotein B (*APOB*). This disorder is called Familial Defective *APOB-100* (FDB), and while plasma total- and LDL-cholesterol levels overlap between patients with FDB and those with *LDLR* mutations, usually those with FDB present with a milder form of the disease, especially in homozygous FDB compared to LDLR mutation-caused FH. The most common mutation in *APOB* is p.(Arg3527Gln), but another *APOB* mutation p.(Arg3527Trp) has previously been identified in a family of South Asian origin. Here we describe a consanguineous marriage of parents of South Asian origin with both homozygous and heterozygous offspring with the *APOB* p.(Arg3527Trp) mutation. The mean untreated levels of LDL-cholesterol in the three heterozygous, Father and Mother (age 45 years) and a girl (age 9 years) were 5.5 mmol/l, 4.5 mmol/l, and 4.2 mmol/l respectively, while the mean untreated levels of LDL-cholesterol in the two homozygous boys (age 15 years and 11 years) were 6.2 mmol/l and 7.0 mmol/l respectively and this was reduced by ~30% on statin treatment. This confirms the milder phenotype and good response to statin therapy even for homozygous FDB.

Keywords: Familial Hypercholesterolemia (FH); Familial Defective *APOB* (FDB); South Asia

Introduction

Familial hypercholesterolemia (FH) (OMIM 143890) is an autosomal dominant disorder resulting in significantly elevated total- and Low-Density Lipoprotein (LDL)-cholesterol (LDL-C) and premature coronary heart disease (CHD) [1]. The diagnostic criteria for FH include LDL-C over 4.9 mmol/l in an adult and over 4.0 mmol/l in a child, plus the presence of a family history of elevated cholesterol and or a family history of premature CHD. A clinical diagnosis of definite FH is given if the patient also has stigmata of elevated cholesterol of tendon xanthomas [2], but these are rarely present in a child. In FH patients in the UK around 93% have a mutation in the gene for the LDL-receptor (*LDLR*), ~5% a mutation in the gene for apolipoprotein B (APOB) and ~2% in the gene coding for Proprotein convertase subtilisin/kexin Type 9 (*PCSK9*) [3].

Where a patient has a mutation in the *APOB* gene, strictly speaking the disorder is called familial defective *APOB-100* (OMIM 107730) or FDB. The clinical and lipid phenotype of FDB patients overlap with those carrying an LDLR mutation, but on average they have a milder presentation than FH due to LDLR mutations [4,5] and in heterozygous FDB, serum cholesterol varies between 7.5 – 9.0 mmol/l. Similarly, in the few cases reported of homozygous FDB, serum cholesterol level seen are between 10-16 mmol/l [6,7] and the severity of the disease is more comparable to heterozygous familial hypercholesterolemia caused by LDLR mutations [8-10].

The most common mutation causing FDB alters the Arginine at position 3527 to Glutamine (p.(Arg3527Gln)) [11-16], with the LDL containing *APOB*-Gln showing very low affinity for the LDL-receptor in *in vitro* assays [16,17] and reduced clearance from the blood in turnover studies (18). The frequency of this mutation in (non-Finish) European populations reported in the ExAC database is 0.034% (http://exac.broadinstitute.org/gene/ENSG00000084674). A second mutation at this same codon has also been reported p.(Arg3537Trp) [12] in a subject of Pakistani origin. The mutation co-segregated with hyperlipidaemia in the family, and the LDL-Trp showed poor binding to the LDL-receptor. In three adults carrying the mutation mean total- and LDL-C was 7.18 mmol/l and 5.38 mmol/l respectively, and in three children carrying the mutation levels were 5.65 mmol/l and 4.05 mmol/l respectively. The ExAC database shows that this mutation is essentially only found in subjects of South and East Asian origin, where the frequency reported is 0.064% (http://exac.broadinstitute.org/variant/2-21229161-G-A). To date no individuals homozygous for this mutation have been reported.

Whilst both of these *APOB* mutations are relatively uncommon, their incidence is likely to be increased in situations where patients share common gene pools. Consanguineous marriage remains popular in many parts of Asia and Africa and it is estimated that currently 10.4% of the global population are either married to a partner related as second cousin or closer ($F \geq 0.0156$) or are the progeny of such a union [18,19]. Although a decline in first-cousin marriage has been observed in some communities, no similar trend seems to have occurred in the United Kingdom's Pakistani population [20] with the highest prevalence of 69% of parental consanguinity being observed in the Pakistani Muslim

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community [21], where 17-38% are marriages of first cousins and closer [22]. We report here a consanguineous family of Pakistani origin where both parents are carriers of the *APOB* p.(Arg3527Try) and where two children are homozygous and one heterozygous for this mutation.

Case Presentation

A 42-year-old Pakistani female was referred to the lipid clinic by her general practitioner, having total cholesterol of 8.3 mmol/l. She was at the same time diagnosed with type 2 diabetes but was not prescribed any medication, managing it by diet only. She has never smoked or taken alcohol, but prior to the diagnosis of hypercholesterolemia had a diet rich in fat and sugar and did not do regular exercise. She is married to her first-degree cousin, with their mothers being sisters. Her two sisters and her husband's two sisters have high cholesterol, two of three uncles have high cholesterol but there is no family history of cardiovascular disease. The couple have five children between the ages of 5 and 18 years old. On examination of the children there were no xantomata, xanthelasma or corneal arcus and no indicators of developing cardiovascular disease. Family screening was advised, with the pedigree and lipid levels shown in Figure 1 and Table 1.

Sample collection

The Oragene•DNA (OG-500) kit (http://www.dnagenotek.com/ROW/pdf/PD-BR-017.pdf) was used to collect saliva samples as recommended by the manufacturer, and prepIT®•L2P reagent was used for the purification of genomic DNA.

Molecular study

A custom designed targeted-next generation sequencing (NGS) method was used [23]. Primers to amplify coding regions (± 25 base pairs (bp)) of the three autosomal dominant FH genes (*LDLR, APOB, PCSK9*), and the autosomal recessive FH gene (*LDLRAP1*) for targeted sequencing were designed using the Illumina Design Studio. Amplicon length was set at 250 bp. The library preparation was performed using the TruSeq Custom Amplicon (TSCA) v1.5 kit (Illumina, San Diego, CA) and sequencing (in both directions) was done using the Illumina MiSeq platform. The raw data acquired were aligned to human reference genome (Hg19). The criteria for the standard variant calling pipeline were: coverage ≥30×, a minimum of five reads for an altered allele, a Phred quality ≥20, and a strand bias filter. A sensitive pipeline was used to ensure that variants were not missed (a coverage ≥15x, a minimum of two reads for an altered allele, a Phred quality ≥ zero, and no strand bias filter). Reported variants were filtered based on their frequency and functional affects. Variants with minor allele frequency >1% according to the 1000 Genomes genotype data [24] and the ExAC database (http://www.nature.com/nature/journal/v536/n7616/full/nature19057.html?foxtrotcallback=true) were considered non-pathogenic and excluded from further analysis. The remaining variants were flagged as rare (frequency= 0.005) or novel (frequency=0). Also, variants were flagged as functional when they were most likely to affect a protein's function.

Sanger sequencing was used to confirm all called mutations by NGS. PCR was carried out in the Rotor-Gene6000 (Qiagen Ltd, Crawley, West Sussex, UK). *APOB*- Exon 26 PCR primers were forward primer (TGTCAAGGGTTCGGTTCTTT) reverse primer (GGGTGGCTTTGCTTGTATGT). The PCR conditions were as follows; started at 95°C for 5 minutes, followed by 40-45 cycles of denaturing at 950˚C for 5 seconds, annealing at 600°C for 10 seconds, and extending at 70°C for 20 seconds. The amplified fragment was sequenced using Sanger sequencing. The DNA sequence was assessed manually.

Results

All family members were offered cascade screening. Both parents had high LDL-C levels (>4.9 mmol/l) (Table 1). Of the five children, two (F-6 and F-7) had normal cholesterol levels, while the other three had high levels of LDL–C (>4.0 mmol/l). All of the affected children were offered appointments in the lipid clinic and agreed to be registered on the Paediatric FH register. As part of this project the children were offered genetic testing.

To assess the consistency of the targeted sequencing assay a threshold of 30x read depth was used. The percentage of bases covered above the 30x threshold were as follows: *APOB* 98.8%, *LDLR* 97.0%, *PCSK9* 93.0%. The least covered gene was the LDLRAP1, which is involved in the very rare autosomal recessive form of hypercholesterolaemia, with 85.3% bases with read depth above 30x.

The genetic analysis showed that both parents are heterozygous for the *APOB* p.(Arg3527Trp) mutation, and of their five children, the mutation was identified in three children, where one is heterozygous for the mutation and the other two are homozygous. The family pedigree and mutation analysis are shown in Figure 1. The *APOB* p.(Arg3527Trp) mutation was identified by the standard variant calling pipeline. The sensitive variant calling pipeline did not reveal any further mutation candidates, which would have been missed by the standard pipeline.

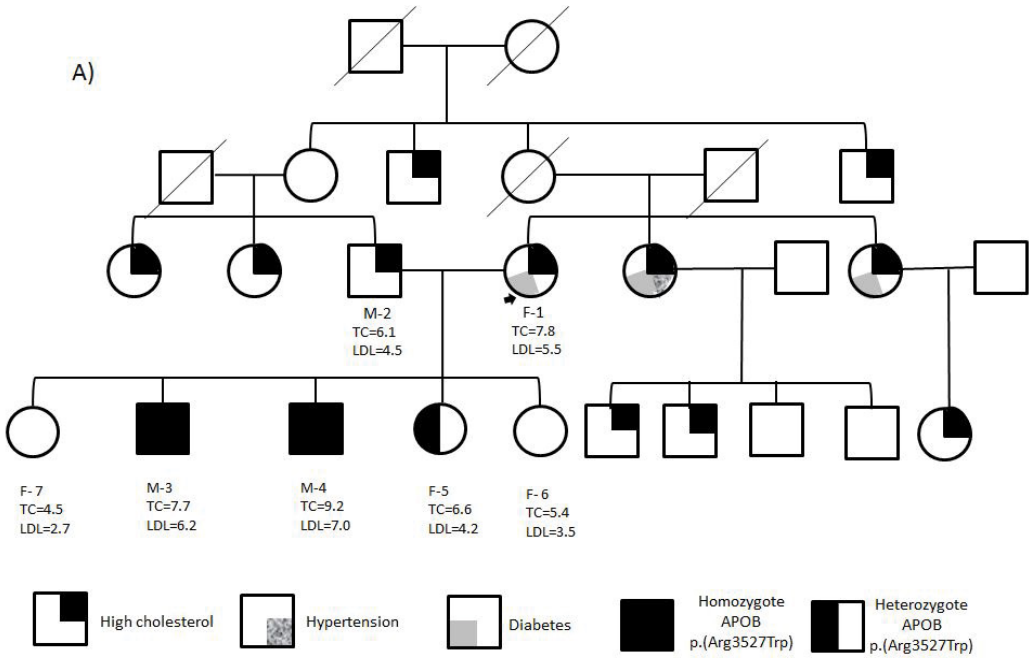
Discussion

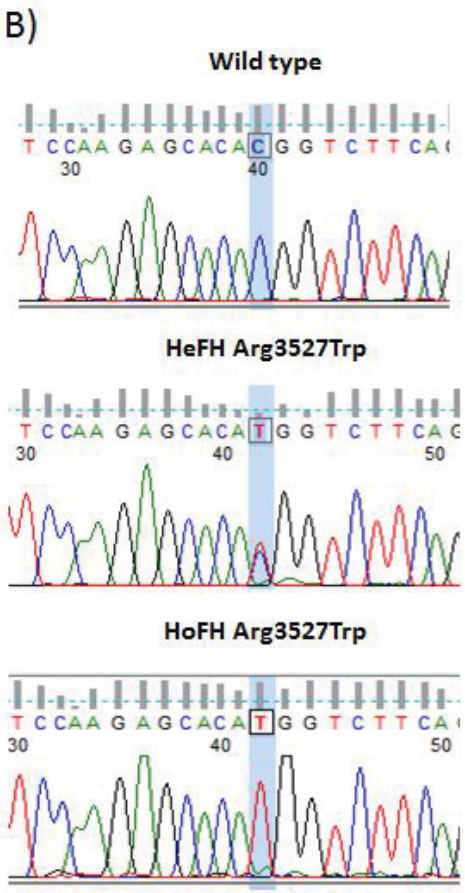
FDB is caused by defective binding of *APOB-100* to the LDL Receptor, leading to failure of hepatic LDL-C clearance and accumulation of LDL-C particles in the circulation [25], and the phenotype therefore overlaps with FH caused by mutations in LDLR. There are a number of mutations in the *APOB* gene reported as causing FH. The most common mutation is p.(Arg3527Gln) in subjects of European origin [11-16,26]. A second mutation has been reported at the same codon (p.(Arg3527Trp)) resulting in a substitution of tryptophan in place of arginine, which causes FDB in South and East Asians [12,27-29]. An additional mutation at this same codon p.(Arg3527Leu), has also been reported in a single case from the Netherlands [30].

The p.(Arg3527Trp) mutation appears to have arisen independently in East Asian ethnic groups [12,27-29]. In this study we report a South Asian family with p.(Arg3527Trp) mutation, where the mutation was identified in two patients homozygous for the mutation, with the disease expression milder than in comparative LDLR homozygous patients, but with characteristics in heterozygous subjects, which is essentially similar to p.(Arg3527Gln) patients.

There are several contributing factors to explain the lower plasma lipid levels found in FDB patients. Firstly, studies in FDB homozygotes showed that the binding affinity of LDL-C to receptors in homozygous FDB was 10% to 20% of normal affinity [6], which thus will still allow clearance of some LDL-C via the receptor. Secondly, the poor *APOB*-mediated clearance of lipoproteins can partially be compensated by an increased uptake of VLDL remnants via apoE-mediated clearance [4]. Thirdly, the increased uptake of apoE containing particles from the plasma results in a decrease of hepatic *APOB-100* production rate as well as that of LDL-C particles [4].

In the studied family the parents, having the same age and mutation, display different levels of total- and LDL-C, and this is in line with previously reported differences in cholesterol levels of age matched males and females [31]. Whereas in our case the 45-year-old





(A) A family pedigree of the proband patient (F1) with the mutation indicated by arrow. Six members of the family (M2, F7, M3, M4, F5, and F6) were screened and sequenced for the mutation. Four members of the family were found to carry the variant. Circle represents female and square represents male, also age (years), TC level (mmol/L) and LDL-C level (mmol/L) included. (B) *APOB* exon 26 sequencing for wild type and base change in patients (appropriate base highlighted).

Figure 1: Family pedigree and segregation of the c.10579 C>T, p. (Arg3527Trp) *APOB* mutation.

Subject	Age, yrs	Gender	Before tretment		After tretment		LDL reduction, %	Treatment
			Total C mmol/l	LDL-C mmol/l	Total C mmol/l	LDL-C mmol/l		
F-1	45	F	8.3	5.5	5.4	3.8	35 %	Atorvastatin 20 mg Ezetimibe 10 mg
M-2	45	M	6.1	4.5	na	na	na	none
M-3	15	M	7.7	6.2	5.4	3.7	30 %	Atorvastatin 40 mg
M-4	11	M	9.2	7.0	6.6	4.9	28 %	Simvastatin 40 mg
F-5	9	F	6.6	4.2	4.6	2.3	30 %	Simvastatin 20 mg
F-6	19	F	5.4	3.5	-	-	-	-
F-7	8	F	4.5	2.7	-	-	-	-

Table 1: Lipid levels at screening and on treatment.

female would be suspected to have a genetic disorder, the 45-year-old male would have not risen as much suspicion and a genetic diagnosis would be unlikely to be made. Similar differences have been reported in children [32]. Homozygous FDB children display lower levels than homozygous FH children, which is illustrated well in this family. Although the differences between *APOB*-FDB and *LDLR*-FH in homozygotes are quite obvious, there is more considerable overlap in lipid levels in the heterozygotes. In this family both heterozygous FDB females display sufficiently raised serum cholesterol to be clinically diagnosed FH, whereas in the heterozygous male this might not be obvious.

Previously a pronounced decrease of total- and LDL-C by 26% and 31% respectively was reported in FDB patients when treated with HMG-CoA reductase inhibitors [33]. In the family examined here, with both homozygous and heterozygous FDB children we observed a reduction in LDL-C level of between 28% – 35%. However, in all these subjects the cholesterol reduction was lower than predicted for the corresponding dose of the statin from published data [34]. Hence a higher dose of statins may be necessary to achieve a satisfactory level of cholesterol.

Conclusion

In summary we report a Pakistani family carrying the common Asian *APOB* mutation in which the heterozygote adult mother demonstrated a serum cholesterol level sufficient to indicate a diagnosis of FH. The heterozygote male however had serum cholesterol that did not reach the diagnostic cut-off and it is important to consider such a diagnosis in individuals with mildly raised cholesterol and family history of hypercholesterolaemia.

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Author Contributions

IT, RHF, EAH and SEH equally contributed to write the manuscript. RHF, MF produced and interpreted the data. SEH and EAH jointly supervised the work.

Conflict of Interest

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. This work is not considered for publication elsewhere and has not been published in the past in any form.

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