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Case report

A rare *STAP1* mutation incompletely associated with familial hypercholesterolemia

Francisco Blanco-Vaca[a,c,e,*], Jesús M. Martín-Campos[b,e], Antonio Pérez[a,d,e], Pablo Fuentes-Prior[b,**]

[a] Hospital de la Santa Creu i Sant Pau (HSCSP), Serveis de Bioquímica i d'Endocrinologia i Nutrició - Institut d'Investigacions Biomèdiques (IIB) Sant Pau, Barcelona, Spain
[b] Institut de Recerca de l'HSCSP – IIB Sant Pau, Spain
[c] Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain
[d] Departament de Medicina, Universitat Autònoma de Barcelona, Barcelona, Spain
[e] CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain

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ABSTRACT

Autosomal dominant hypercholesterolemia, being referred to as familial hypercholesterolemia (FH), is mainly due to defective LDL receptor (LDLR) function, but is also associated with variants in genes encoding APOB (LDLR ligand) and PCSK9, the catabolic regulator of LDLR. The *signal-transducing adaptor family member 1* (*STAP1*) gene has been recently linked to FH. We describe the case of a 56-year-old male patient found to have hypercholesterolemia at age 34, but who did not continue follow-up nor received treatment with lipid-lowering drugs. At age 55 he suffered a myocardial infarction. A systematic NGS analysis did not show point mutations in the *LDLR*, *APOB*, *LDLRAP1*, or *PCSK9* genes, nor large rearrangements of the *LDLR* gene, but revealed the heterozygous missense variant rs199787258 of *STAP1* (c.526C > T; p.Pro176Ser). This variant was also found in heterozygosis in the two siblings of the index case, who also had hypercholesterolemia, but did not cosegregate in his progeny. A bioinformatics analysis and available structural information predicts p.Pro176Ser as the most damaging of all *STAP1* missense variants associated with familial hypercholesterolemia. Our findings confirm and extend the linkage between *STAP1* variants and FH, and point to an important role of this adaptor protein within a signaling pathway that affects cholesterol homeostasis.

1. Introduction

The clinical diagnosis of autosomal dominant hypercholesterolemia (ADH) relies on a high plasma LDL-cholesterol (LDL-c) level (> 190 mg/dl), a family history of hypercholesterolemia, a personal and/or first-degree family history of premature coronary heart disease (CHD), and signs of cholesterol deposition such as tendinous xanthomata and/or premature arcus cornealis. These variables are often scored clinically by applying the Make Early Diagnosis to Prevent Early Death (MEDPED) criteria, the Dutch Lipid Clinic Network (DLCN) MEDPED modification, or the Simon Broome Register Group (SBRG) criteria [1].

ADH, commonly referred to as familial hypercholesterolemia (FH, OMIM #143890), is mainly due to defective cellular LDL receptor (LDLR) function. FH is also associated with variants in other genes encoding proteins that interact with the LDLR, such as: (i) its ligand, apolipoprotein B-100 (APOB) [2], referred to as familial ligand-defective hypercholesterolemia (OMIM #144010), (ii) the LDLR catabolic regulator, the proprotein convertase subtilisin/kexin type 9 (PCSK9) [3], referred to as FH3 (OMIM #603776) and, more recently, (iii) the *signal-transducing adaptor family member 1* (*STAP1*) gene, which has also been postulated as a FH4 locus [4].

Heterozygous ADH is relatively common, and recent data suggests that the disorder affects approximately 1 in 250 individuals world-wide [5–7]. Cholesterol-lowering treatment with statins has been shown to dramatically reduce CHD risk in patients with ADH [8]. Therefore, the early detection of subjects carrying pathogenic variants in *LDLR*, *APOB* and/or *PCSK9*, and eventually *STAP1*, combined with a cascade

* Corresponding author at: Hospital de la Santa Creu i Sant Pau (HSCSP), Serveis de Bioquímica i d'Endocrinologia i Nutrició - Institut d'Investigacions Biomèdiques (IIB) Sant Pau, C/ Sant Antoni Maria Claret, 167, Barcelona 08025, Spain.
** Corresponding author at: Institut de Recerca de l'HSCSP – IIB Sant Pau, C/ Sant Antoni Maria Claret, 167, Barcelona 08025, Spain.
E-mail addresses: fblancova@santpau.cat (F. Blanco-Vaca), pfuentes@santpau.cat (P. Fuentes-Prior).

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detection and a cholesterol-lowering therapy should be used to decrease CHD mortality [2].

2. Material and methods

2.1. Case description

A 56-year-old male patient was referred to the Endocrinology Department of our hospital for dyslipidemia management. At age 34 he was found to have hypercholesterolemia, but he neither continued follow-up nor received treatment with lipid-lowering drugs. At age 55 he suffered a myocardial infarction. Angiography showed widespread coronary artery disease that required the placement of two stents. The patient was subsequently treated with atorvastatin (80 mg/day) and ezetimibe (10 mg/day), which reduced his LDL-c level to 65 mg/dl. Of note, there was no history of premature coronary artery disease or cardiovascular death in his family. Siblings and offspring of the patient were later included in the study.

2.2. DNA extraction and sequencing

Genomic DNA was extracted from leukocytes of peripheral whole blood samples, obtained after 12 h of fasting, or saliva collected in Oragene®. DNA was then analyzed using the next-generation sequencing (NGS) kit SEQPRO LIPO RS® (Progenika Grifols Biopharma, ref. [9]). This kit detects mutations in *LDLR*, *APOB*, *PCSK9*, LDLR adapter protein 1 (*LDLRAP1*) and *STAP1* genes, and copy number variation (CNV) in *LDLR*. The identified variant was analyzed in the family by PCR amplification and Sanger sequencing.

2.3. Plasma lipid, lipoprotein and apolipoprotein analyses

Blood samples were collected after an overnight fast. Standard commercially-available assays adapted to an Architect C4000 (Abbott Diagnostics, USA) were used to determine plasma total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c) and apoB. LDL-c was calculated by the Friedewald-formula [10], since all TG were < 300 mg/dl.

2.4. Genotyping and calculation of weighted LDL-c score

Briefly, eleven SNPs[1] were genotyped using TaqMan® probes (Thermo Fisher Scientific, USA): *PCSK9* (rs2479409), *CELSR2-SORT1* (rs629301), *APOB* (rs1367117), *ABCG8* (rs4299376), *SLC22A1-LPA* (rs1564348), *HFE* (rs1800562), *MYLIP/IDOL* (rs3757354), *NYNRIN* (rs8017377), *LDLR* (rs6511720), and *APOE* (rs429358 and rs7412). A LDL-c-specific gene score was calculated using the per-allele beta coefficients reported by the GLGC [11].

2.5. Bioinformatics analysis

STAP1 missense mutations identified in various cancer types were taken from the COSMIC resource (http://cancer.sanger.ac.uk/cosmic). Phosphorylation sites on STAP1 protein were extracted from the PhosphoSitePlus (www.phosphosite.org), PRIDE (https://www.ebi.ac.uk/pride) or MaxQB (http://maxqb.biochem.mpg.de) databases. The three-dimensional structures of STAP1 domains were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) and inspected with MIFit (https://github.com/mifit/). The impact of point variants on protein structure and function was assessed with PROVEAN[2] (http://provean.jcvi.org/, ref. [12]), PolyPhen-2[3] (http://genetics.bwh.harvard.edu/pph2/index.shtml, ref. [13]), SIFT[4] (http://sift.jcvi.org/www/SIFT_seq_submit2.html, ref. [14]), and CUPSAT (http://cupsat.tu-bs.de/; ref. [15]). Structure figures were prepared with PyMol (www.pymol.org).

3. Results

3.1. A rare STAP1 variant identified in a hypercholesterolemic patient and his relatives

To understand the molecular pathology of a 56-year-old hypercholesterolemic male patient referred to the Endocrinology Department of our hospital (see 2.1.), we conducted a systematic NGS study of *LDLR*, *APOB*, *PCSK9*, *LDLRAP1* and *STAP1* genes. This analysis did not show point mutations in the *LDLR*, *APOB*, *PCSK9* or *LDLRAP1* genes, nor large rearrangements of *LDLR*. Instead, it revealed the heterozygous missense variant rs199787258 of *STAP1* (c.526C > T; p.Pro176Ser).

These findings prompted us to extend our genetic and biochemical studies to other family members. The putative pathogenic variant was also found in heterozygosis in the two siblings of the index case, who also had hypercholesterolemia (Fig. 1A). The daughter of the patient, currently aged 26, has also inherited the Ser176 allele, but so far has not shown hypercholesterolemia, while the 21-years-old son has hypercholesterolemia but is not a carrier of the *STAP1* variant (Fig. 1A).

3.2. Polygenic contribution to hypercholesterolemia in the studied family

The results presented above, in particular those referred to the siblings of the index case, suggested a polygenic contribution to hypercholesterolemia in the family. It has been recently reported that raised LDL-c concentrations might have a polygenic cause in a significant proportion of patients with FH but without a known mutation [11]. This polygenicity can be assessed by genotyping patients for 12 common LDL-c SNPs allowing the construction of a weighted LDL-c gene score. Individuals with polygenic hypercholesterolemia had a significantly higher mean weighted LDL-c score than controls. In line with these findings, the mean weighted LDL-c score of all members of the family studied here (1.0525 ± 0.0739) was significantly higher than that of 503 Europeans analyzed in the 1000 Genomes study (0.8866 ± 0.214; http://www.internationalgenome.org) ($p = 0.03$) (Fig. 1B). Further, the score was higher in the four members of the family with hypercholesterolemia than in the sole normocholesterolemic member (in all cases over the 75th percentile). However, no phenotypic data is publicly available for each of the individual samples in the 1000 Genomes project and, therefore, a plasma LDL-c concentration could not be assigned to each quartile of the weighted LDL-c gene score.

3.3. STAP1 variant p.Pro176Ser is predicted to be deleterious

Analysis of the p.Pro176Ser variant following the recommendations of the American College of Medical Genetics [16] strongly suggests pathogenicity. First, it is noteworthy that it affects a residue that appears to be strictly conserved from fishes to humans, pointing to an important structural role (see Fig. 1C for the domain organization of the protein and Fig. 1D for a sequence alignment around position 176).

[1] The reported SNP (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=141647940) refer to both A and C alleles, which would result in replacements of residue Thr71 by a lysine or a threonine, respectively. However, only the C allele is reported with a relevant minor allele frequency (1.65×10^{-5} in the ExAc_Aggregated_Populations; https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ss.cgi?ss=ss1687508046).

[2] The PROVEAN threshold between neutral and deleterious replacements is -2.5.

[3] PolyPhen scores vary between 0.0 (benign) and 1.0 (probably damaging), both for HumVar and HumDiv.

[4] Amino acids with SIFT scores < 0.05 are considered deleterious.