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Case report

A rare *STAP1* mutation incompletely associated with familial hypercholesterolemia

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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

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Fig. 1. Inheritance, impact on cholesterol levels and structural analysis of the putatively pathogenic variant of human *STAP1*, p.Pro176Ser. (A) Values under each symbol indicate, from top to bottom, age (in years), body mass index (in kg/m^2), total triglycerides (TG), total cholesterol (TC), and VLDL, LDL and HDL cholesterol (c), respectively, in milligrams per deciliter, without lipid-lowering therapies, and finally the LDL-c genetic score. Shaded symbols indicate carriers of the *STAP1* p.Pro176Ser variant in heterozygosis. A question mark indicates subjects not studied. HC, hypercholesterolemic. (B) LDL-c weighted score of the members of the family studied, as compared to the 503 European subjects included in the 1000 Genomes study. (C) Schematic domain organization of human STAP1 protein. The adaptor is essentially comprised of tandem PH (Leu24-Thr152) and SH2 domains (Asn173-Cys269). Point mutants identified to date in FH4 patients are indicated with stars. Mutations linked to liver carcinomas are marked with black ovals, those reported in all other cancer types with gray ovals. Ser/Thr or Tyr residues reported to be phosphorylated are highlighted with black and gray arrowheads, respectively. (D) Partial sequence alignment of N-terminal residues from STAP1 SH2 domain. (E) Overall structure of the STAP1 SH2 domain bound to a Tyr-phosphorylated peptide. The globular domain is shown as an orange cartoon, and the peptide as Van-der-Waals spheres, color-coded (carbon, green; oxygen, red; nitrogen, blue; and phosphor, orange). The side chain atoms of Pro176 are shown as spheres. Notice that the residue is embedded and excluded from bulk solvent by an aliphatic/aromatic cage formed by several residues (shown as sticks). The side chains of a few STAP1 residues directly involved in the recognition of the phosphorylated Tyr residue are also given with all their non hydrogen atoms. Putative pathways along which structural perturbations might propagate from the Pro176-binding pocket to the phosphotyrosine binding site are indicated with red arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Predicted impact of missense variants of the human *STAP1* gene associated with familial hypercholesterolemia (FH4).

Variant	SNP?	PROVEAN[b]	PolyPhen-2 (HumDiv)[c]	PolyPhen-2 (HumVar)	SIFT[d]	CUPSAT (ΔΔG, kcal/mol)
c.139A > G (p.Thr47Ala)	rs793888522	Neutral (−0.135)	Benign (0.001)	Benign (0.005)	Tolerated (0.38)	Destabilizing (−3.15)
c.206 T > C (p.Leu69Ser)	rs938523789	Deleterious (−3.643)	Probably damaging (0.994)	Possibly damaging (0.873)	Deleterious (0.00)	Destabilizing (−0.55)
c.212 T > C (p.Ile71Thr)	rs141647940[a]	Neutral (−2.038)	Possibly damaging (0.915)	Possibly damaging (0.870)	Deleterious (0.03)	Stabilizing (3.30)
c.291G > C (p.Glu97Asp)	rs779392825	Neutral (−1.649)	Probably damaging (0.979)	Probably damaging (0.956)	Tolerated (0.41)	Destabilizing (−0.43)
c.526C > T (p.Pro176Ser)	rs199787258	Deleterious (−5.254)	Probably damaging (1.000)	Probably damaging (0.997)	Deleterious (0.00)	Destabilizing (−3.36)
c.619G > A (p.Asp207Asn)	rs146545610	Neutral (−1.308)	Benign (0.056)	Benign (0.046)	Tolerated (0.07)	Stabilizing (0.46)

[a] 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).
[b] The PROVEAN threshold between neutral and deleterious replacements is −2.5.
[c] PolyPhen scores vary between 0.0 (benign) and 1.0 (probably damaging), both for HumVar and HumDiv.
[d] Amino acids with SIFT scores < 0.05 are considered deleterious.

four different *in silico* predictive software packages (Table 1). Most notably, PROVEAN classified the p.Pro176Ser missense variant as deleterious, with a score of −5.254 (threshold between neutral and deleterious replacements: −2.50). Similarly, PolyPhen-2 classified the Pro176 → Ser exchange as 'probably damaging' with the maximum score of 1.0. Finally, CUPSAT identifies the p.Pro176Ser variant as strongly destabilizing against thermal denaturation of the SH2 domain (Table 1).

4. Discussion

We present a FH family in which we found a heterozygous p.Pro176Ser variant in the *STAP1* gene. Our findings confirm and extend the recently proposed linkage between *STAP1* variants and FH [4,17]. However, functional analysis of the mutant is not feasible at this point as there is no known, measurable STAP1 function. Increased LDL-c and mutation cosegregated in the index case and in his two siblings, but not in his offspring. Incomplete penetrance was found in one of the families reported by Fouchier and coworkers [4], and may reflect the need for other concomitant factors, such as a more advanced age or a polygenic susceptibility, to reach phenotypic expression. Indeed, both a polygenic susceptibility and the *STAP1* mutation may have contributed to hypercholesterolemia in the proband as well as in his siblings. The fact that the 21-years-old son of the index case present hypercholesterolemia without being a carrier of the *STAP1* variant also points to a polygenic source of hypercholesterolemia in the family [11]. On the other hand, reduced polygenic predisposition could contribute to the normocholesterolemia present in the young *STAP1* mutation-positive individual since age, increased body mass index (BMI), as well as other genetic, epigenetic and environmental factors, could also influence the differential expression of hypercholesterolemia within the family. This is also consistent with the finding that both grandparents on his father's side were also hypercholesterolemic (Fig. 1A).

Because the role of the docking protein, STAP1, in cholesterol metabolism remains largely unknown [4,17], we were set to a bioinformatics analysis of pathogenicity. Interestingly, the consensus result of different algorithms strongly predicts variant p.Pro176Ser to be more deleterious than any other previously reported FH4-linked allele (Table 1). For instance, p.Pro176Ser had the lowest PROVEAN score of all variants (−5.254); the only other mutation that affects the SH2 domain of STAP1 previously identified in a FH4 family, p.Asp207Asn [4], has a PROVEAN score of −1.308, and is therefore considered as neutral. Further, it is one of the two variants classified as "probably damaging" by both HumDiv and HumVar PolyPhen-2 prediction models, and CUPSAT also identifies the Pro176 → Ser replacement as the most destabilizing to the protein structure of all six FH4 variants.

The NMR solution structure of the N-terminal PH domain of STAP1 (residues Leu24 to Thr152) has been deposited in the Protein Data Bank (PDB), and the crystal structure of the following SH2 domain (Asn173-Cys269) bound to a tyrosine-phosphorylated peptide has also been reported ([18]; Fig. 1E). This 3D structural information allows for a thorough analysis of the impact of point mutations on STAP1 structure, as is the case of the current p.Pro176Ser variant. This replacement is interpreted as deleterious for several, not mutually exclusive reasons. Pro176 is fully embedded within an aromatic/aliphatic cage formed by the side chains of residues Phe179, Leu202, Leu249, Phe250, and Ile253 (Fig. 1E). Therefore, insertion of a polar hydroxyl group in this environment would be extremely disfavored. The structural impact of this exchange would be even larger if it generates a neophosphorylation site, because a charged, phosphorylated Ser176 would be definitely excluded from the apolar Pro176-binding pocket. This, in turn, would force the whole N-terminal stretch of the SH2 domain to move away from the core of the module. Along these lines, a number of phosphosites have been reported in the human STAP1 protein (Fig. 1C).

In addition to an important effect on the stability of the mutant protein, functional implications of the p.Pro176Ser variant are also conceivable. Indeed, protein rearrangements caused by the introduction of a polar serine at position 176 might propagate to the nearby

phosphotyrosine binding cleft of the SH2 domain (Fig. 1E). In this regard, it is noteworthy that Pro176 is located close to the two arginine residues that coordinate the phosphate group of the bound phosphotyrosine, Arg184 and Arg203. Phe179 may act as link between the two sites, as it contacts both Pro176 and residues at the start and end of a loop that forms a 'latch' over the bound phosphotyrosine peptide, Pro204 and Tyr211. Further, another residue that belongs to the Pro176-binding pocket, Leu202, immediately precedes one of the phosphoTyr ligands, and also contacts Tyr211. Finally, an increased average distance between PH and SH2 modules might interfere with physiological activities that require a precise minimum distance and/or orientation between the ligand binding sites on the respective domains.

Although all the evidence strongly suggests that the p.Pro176Ser variant would affect STAP1 structure and/or function, it is unknown whether this is the direct cause of FH, as the molecular mechanism(s) by which the adaptor protein could affect cholesterol homeostasis remain unclear. STAP1 appears to be expressed at most at low levels in the liver, and it can be speculated that its expression is either temporarily or spatially limited in this organ. In this context, it is noteworthy that several missense mutations of the *STAP1* gene have been reported in patients with liver carcinomas (highlighted with black ovals in Fig. 1C), supporting a critical (patho)physiological role for this docking protein. On the other hand, STAP1 functions downstream of the receptor tyrosine kinase c-*kit* [19], and c-*kit*-deficient mice showed hyperlipidemia [20,21]. Consistently, receptor tyrosine kinase inhibitors administered to patients with chronic myeloid leukemia were associated with increased plasma cholesterol levels [22,23]. Altogether, these observations suggest that STAP1 is part of a signaling pathway that regulates cholesterol homeostasis. Regardless of the actual mechanism, association of multiple STAP1 variants with FH points to an important role of the adaptor protein in lipid metabolism.

Conflicts of interest

No authors declared potential conflicts of interest.

Author contributions

All authors confirm that they have contributed to the intellectual content of this paper and have met the following three requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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