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Predicted pathogenic mutations in *STAP1* are not associated with clinically defined familial hypercholesterolemia

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HIGHLIGHTS

- *STAP1* has been proposed as a candidate gene for FH with controversial results.
- Predicted pathogenic mutations in *STAP1* in genetic negative FH were studied.
- These mutations in *STAP1* did not cosegregate with hypercholesterolemia in families.
- *STAP1* does not seem to play a major role in the etiology of FH.

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ABSTRACT

Background and aims: Autosomal dominant familial hypercholesterolemia (FH) is caused by mutations in *LDLR*, *APOB* and *PCSK9*. Two new putative *loci* causing FH have been identified recently, the p.(Leu167del) mutation in *APOE* and new mutations in the signal transducing adaptor family member *STAP1*. We aimed at investigating the role of *STAP1* mutations in the etiology of FH.

Methods: We sequenced *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *APOE*, *LIPA* and *STAP1* with the LipidInCode platform in 400 unrelated subjects from Spain with a clinical diagnosis of FH. All subjects carrying rare predicted pathogenic variants in *STAP1* gene, described as pathogenic by at least three bioinformatic analysis and having an allelic frequency lower than 1% in general population, were selected for family study. Available relatives were recruited, including both hypercholesterolemic and non-hypercholesterolemic family members.

Results: Sequencing analysis of *STAP1* gene revealed seventeen rare variants, four of them being described as pathogenic by bioinformatic analysis. We studied the cosegregation with hypercholesterolemia of four rare predicted pathogenic variants, c.-60A > G, p.(Arg12His), p.(Glu97Asp), p.(Pro176Ser) in seven families. We did not observe any cosegregation between genotype and phenotype, even carriers of rare variants in *STAP1* had lower LDL cholesterol levels than non-carriers.

Conclusions: This study analyzes the family cosegregation of four rare predicted pathogenic variants of STAP1, p.(Arg12His), p.(Glu97Asp), p.(Pro176Ser) and c.-60A > G, in seven families, showing absence of cosegregation in all of them. These results would suggest that *STAP1* gene is not involved in hypercholesterolemia of these families.

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1. Introduction

Familial hypercholesterolemia (FH) is a genetic disorder characterized by very high plasma total cholesterol concentrations, due to increased low-density lipoprotein cholesterol (LDL cholesterol), with a high risk of premature coronary heart disease (CHD) [1]. Traditionally, FH has been described as a monogenic disease, with autosomal codominant transmission and an estimated prevalence of around 1:500 in the general population [2]. Recent studies have revealed that clinically defined FH is probably more common than previously reported, with a prevalence of 1:217 in the Copenhagen General Population study [3]. This prevalence is as high as 1:70 in some populations with a founder gene effect, such as Afrikaners from South Africa [4].

The clinical diagnosis of FH relies on a high level of LDL cholesterol in plasma, a family history of hypercholesterolemia, a personal and family history of premature CHD and signs of cholesterol deposition such as tendon xanthomas and premature arcus cornealis. These variables are often clinically scored 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 [5].

FH is caused by mutations in *LDLR*, the gene coding for the LDL receptor; *APOB*, encoding apolipoprotein B [6] and *PCSK9*, which encodes the enzyme proprotein convertase subtilisin/kexin type 9 [7]. One new putative *locus* causing FH has been identified: the p.(Leu167del) mutation in *APOE* [8]. Besides, several mutations in the signal transducing adaptor family member *STAP1* have been associated recently with FH etiopathology [9]. However, 20–40% of patients with possible familial hypercholesterolemia have not mutation in candidate genes [10], which could be due to the presence of new genes or mutations, previously not described, or the overlapping between FH with polygenic hypercholesterolemia. In this line, Talmud et al. [11] described 12 single nucleotide polymorphisms (SNPs), which could help discriminated FH from polygenic hypercholesterolemia.

STAP1 gene was associated with FH by Fouchier et al. [9] in 2014 using the exome sequencing and linkage analysis in one family with a clear FH phenotype. Besides, these authors sequenced the *STAP1* gene in normolipemic patients and patients with FH without mutation in *LDLR*, *PCSK9* and *APOB* genes, and identified four new missense variants (p.(Glu97Asp), p.(Leu69Ser), p.(Ile71Thr) and p.(Asp207Asn) in *STAP1*, described as pathogenic by bioinformatic analysis, only present in patients clinically defined as FH. Fouchier et al. reported that patients carrying mutations in *STAP1* have significantly lower values in their lipid profile than patients carrying mutations in *LDLR*, presenting a lipid phenotype very similar to carriers of *APOB* mutations.

The function of *STAP1*, which encodes a signal-transducing adaptor protein, is still unknown. STAP1 contains a domain with several tyrosine phosphorylation sites, which allows it to interact with the membrane or with membrane proteins [9]. Therefore, it seems that the involvement of STAP1 in cholesterol metabolism could be through interaction with membrane proteins. Subsequent studies have reported a subject clinically defined as FH, with premature CHD, carrying the p.(Glu97Asp) mutation in *STAP1* gene [12]. Another study with 255 patients with a personal and family history of premature CHD reported one patient carrier of a new mutation in *STAP1* [13]. Consequently, the *STAP1* gene has been included in many sequencing platforms for the genetic diagnosis of FH, as a new candidate gene causing FH.

We have analysed the *STAP1* mutations found in FH subjects, to select those predicted to be pathogenic and to research the family cosegregation of four variants in *STAP1,* two of them not previously described, with the aim to investigate if these mutations in *STAP1* could explain the phenotype of FH.

2. Materials and methods

All genetic studies of FH performed with the LipidInCode platform (GenInCode, Terrassa-Barcelona, Spain), including *STAP1,* were reviewed until December 2017 and those variants in *STAP1* described as pathogenic by bioinformatic tools were selected. These predicted pathogenic variants were identified in FH index cases from the Lipid Clinics at Hospital Universitario Miguel Servet in Zaragoza, Hospital Universitario Puerta de Hierro in Madrid and Hospital Universitario Insular in Gran Canaria, all in Spain.

2.1. Subjects

Unrelated subjects above the age of 18, with a clinical diagnosis of FH according of the DLCN criteria, untreated LDLc concentrations above 95th percentile of the Spanish population [14], triglycerides (TG) below 200 mg/dL, and familial presentation (at least 1 first-degree relative with the same phenotype) were selected for this study. Exclusion criteria were secondary causes of hypercholesterolemia including: obesity (body mass index (BMI) $>$ 30 kg/m^2), poorly controlled type 2 diabetes (HbA1c $>$ 8%), renal disease with glomerular filtration rate $<$ 30 mL/min and/or macroalbuminuria, liver disease (alanine transaminase $>$ 3 times upper normal limit), hypothyroidism (thyroid stimulating hormone $>$ 6 mIU/L), pregnancy, autoimmune diseases, and treatment with protease inhibitors. The assessment of cardiovascular risk factors, a personal and family history of cardiovascular disease, drugs intake affecting intestinal or lipid metabolism, and anthropometric measurements were performed in all participants.

Of all subjects carrying rare predicted pathogenic variants in *STAP1* gene, reported pathogenic by at least three bioinformatic analysis and with an allelic frequency lower than 1% in general population, the available relatives were recruited, including both hypercholesterolemic and non-hypercholesterolemic family members.

2.2. Lipid analysis

Lipid and lipoprotein analyses were performed on EDTA plasma samples collected after at least 10 h overnight fast and without lipid lowering drugs in the previous 6 weeks. Total cholesterol and triglyceride (TG) levels were determined by standard enzymatic methods. HDL cholesterol was measured directly by an enzymatic reaction using cholesterol oxidase (UniCel DxC 800; Beckman Coulter, Inc, Brea, CA). LDL cholesterol was calculated by Friedewald's formula. All subjects signed an informed consent to a protocol previously approved by our local ethics committees. Lipoprotein(a) protein was determined by IMMAGE kinetic nephelometry (Beckman-Coulter, Inc) in all subjects from Hospital Universitario Miguel Servet in Zaragoza and Hospital Universitario Insular in Gran Canaria.

2.3. Genetic analysis

Whole blood genomic DNA was isolated using standard methods. Promoters, coding regions, and intron-exon boundaries of *LDLR* (NM_000527.4), *APOB* (NM_000384.2), *PCSK9* (NM_174936.3), *APOE* (NM_000041.3), *STAP1* (NM_0121108.3), *LDLRAP1* (NM_015627.2) and *LIPA* (NM_000235.3) genes were sequenced in the LipidInCode platform (GenInCode, Terrassa-Barcelona, Spain).

Data were analysed by the bioinformatic tool Gendicall (Gendiag.exe/FiC) using the BWA-mem alignment software with the gene reference grch37/hg19. The detection of variants was done with the SAMtools and Gendicall programs. The variants that presented between 15% and 85% of alternative or reference sequences were considering heterozygous. To evaluate the variants pathogenicity we used PolyPhen-2 [15], Mutation Taster [15] and PredictSNP [16]. The effect of variants in potential splicing sites was predicted with FruitFly [17]. To compare the frequency of identified variants in the general population, we have compiled the allele frequencies of the identified variants from the 1000 Genomes Project [18], ExAc Browser Data [19] and genome aggregation data base (gnomAD) [20].

For each proband, we calculated LDL score using the weighted sum of the risk allele of these SNPs: rs11220462 in *ST3GAL4*; rs1367117 in APOB; rs1564348 in *SCL22A1*; rs1800562 in HFE; rs2479409 in PCSK9; rs3757354 in *MYLIP*; rs429358 in APOE; rs4299376 in ABCG5/G8; rs629301 in CELSR2; rs6511720 in *LDLR*; rs7412 in *APOE* and rs8017377 in *NYNRIN* sequenced in the LipidInCode platform (GenInCode, Terrassa-Barcelona, Spain). The weights used were the corresponding per-allele (risk) beta coefficients reported by the Global Lipid Genetic Consortium (Supplemental Table 1). Calculated score above 0.73 indicated high probability of polygenic hypercholesterolemia according to the research carried out by Talmud et al. [11]. Besides, all proband families had determined two SNPs (rs10455872A > G and rs3798220T > C), which are closely related with the Lpa levels [21].

To define the pathogenicity classification of genomic variants according to the American College of Medical Genetics and Genomics (ACMG), we calculate the probability of observed cosegregation if not pathogenic variant in each family. We calculated a simple probability that the observed variant-affected status data occur by chance, rather than due to cosegregation. We assume that the proband(s) have that variant and full penetrance and that the allele is rare enough that all occurrences in the observed pedigrees are identical by descent, rather than the same variant entering the pedigree from more than one ancestor. Under a dominant model, this probability is $N = (1/2)^m$, where m is the number of meiosis of the variant of interest that are informative for cosegregation [22]. Proposed Cosegregation Evidence to Support Each ACMG-AMP Pathogenicity Evidence Level is described in the Supplemental Table 2.

2.4. Statistical analysis

Analyses were performed using statistical computing software R [23] version 3.5.0. The level of significance was set at $p < 0.05$. The distribution of the variables was analysed by the Shapiro test. Quantitative variables with a normal distribution were expressed as mean ± standard deviation and were analysed by the Student *t*-test. Variables with a skewed distribution were expressed as medians and interquartile ranges and were analysed with the Mann-Whitney *U* test. Qualitative variables were expressed as percentages and were analysed by the Chi-squared test.

3. Results

Supplemental Table 3 shows all variants identified by the Systematic NGS study of *STAP1* until December 2017 with the LipidInCode platform. We selected four rare variants predicted as pathogenic, and they were studied in seven index cases and their relatives. One of the selected variants was located in the promoter region and three of them were located in the coding region of *STAP1* gene, giving rise to missense variants.

Rare variants reported as pathogenic with bioinformatic analysis are shown in Table 1. The rare variant c.-60 A > G is located in the promoter region, its frequency in the general population is 0.17% and it has not been previously associated with FH. We found three patients who carried this rare variant. In Fig. 1, the available family members are shown. Interestingly, proband of family 2 presented higher levels of total and LDL cholesterol than the others probands who carried the same rare variant (c.-60A > G). These higher levels could be explained because this proband is a carrier of the variant rs10455872A > G in heterozygosity in *LPA* gene and presents higher level of LDL score. In Family 2, the proband brother is carrier of the c.-60 A > G variant, but he presents normal levels of total and LDL cholesterol. Same absence of cosegregation between phenotype and genotype is observed in Family 7, as a proband brother, who is not carrier of the variant, shows high levels of total and LDL cholesterol. The proband daughter has normal levels in her lipid profile, although she is carrier of the rare variant.

Table 1
Frequency, bioinformatic and clinical significance of rare variants reported in *STAP1* gene.

Variant	Nucleotide change	Amino acid change	Bioinformatic analysis				Frequency in general population		Previously associated with FH
			Mutation Taster	Proven	Polyphen-2	Predict SNP	1000G	gnomAD[a] (Allele count/Allele number)	
rs201996284	**c.-60A > G**[d]	–	Disease causing	Deleterious	–	Deleterious	0.0000	27/15426	Not
rs141647540	**c.35G > A**	**p.(Arg12His)**	Disease causing	Neutral	Probably damaging	Deleterious	0.0000	41/128972	Not
rs779392825	**c.291G > C**	**p.(Glu97Asp)**	Disease causing	Neutral	Probably damaging	Deleterious	0.0000	3/112720	Yes[b]
rs199787258	**c.526C > T**	**p.(Pro176Ser)**	Disease causing	Deleterious	Probably damaging	Deleterious	0.0000	76/125912	Yes[c]

[a] GnomAD has been used, using as reference the European population (non-Finnish).
[b] Fouchier et al. The first mutation discovered in *STAP1* gene cosegregating with FH phenotype.
[c] Blanco-Vaca et al. This mutation did not cosegregate with the FH phenotype.
[d] This variant showed that the score in the promoter region decreases from 0.89 with the wild type allele (A) to 0.35 with the mutation allele (G) (https://www.fruitfly.org/seq_tools/splice.html).

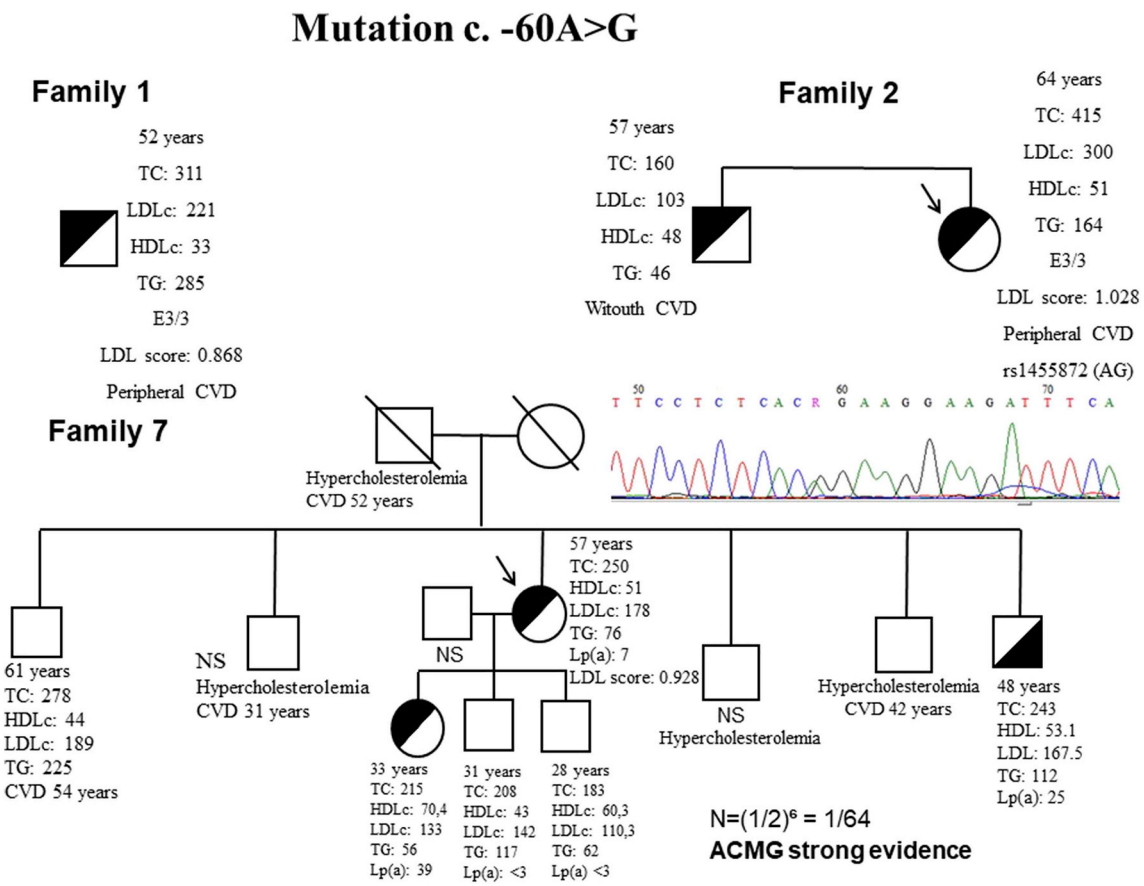

Fig. 1. Families carrying the c.-60A > G mutation in *STAP1* gene.
NS: Not studied; TC: Total cholesterol; LDLc: Low Density Lipoprotein cholesterol; HDLc: High Density Lipoprotein cholesterol; TG: Triglyceride; CVD: Cardiovascular Disease; Lp(a): Lipoprotein a; ACMG: American College of Medical Genetics and Genomics.

Besides, according to the ACMG guideline, Family 7 showed strong evidence of lack of segregation phenotype-genotype. All family members in Family 7 showed normal levels of Lp(a).

The rare variant c.35G > A is located in exon 1 and is predicted to cause a substitution of arginine by histidine at amino acid 12 of STAP1, p.(Arg12His). This amino acid change has not been previously described in FH patients, although it has been described as deleterious by bioinformatic analysis. Besides, its frequency in the general population is extremely low, less than 0.1% (Table 1). We found one patient with this rare variant, who presented high levels of total and LDL cholesterol. However, his daughter presented normal levels in her lipid profile, although she was also carrier of the same rare variant (Fig. 2).

The rare variant c.291G > C is located in exon 3 and is predicted to cause a substitution of glutamic acid by aspartic acid at amino acid 97 in STAP1 protein, p.(Glu97Asp). This rare variant has been previously described in FH patients by Fouchier et al. [9] and it has been described as pathogenic by bioinformatic analysis. The proband and his daughter in Family 3 are carriers of the variant; the daughter presents normal levels in her lipid profile (Fig. 3).

The rare variant c.526C > T is located in exon 5 and is predicted to cause a substitution of proline by arginine at amino acid 176, p.(Pro176Ser). This variant has been previously described by Blanco-Vaca et al. [24], being incompletely associated with FH phenotype. We have found two unrelated probands carriers of this rare variant. Both probands presented high levels of LDL score, higher than 0.73, calculated from 12 SNPs indicated by Talmud et al. [11]. Proband's Family 6, who had higher LDL score and E4/4 *APOE* genotype, showed higher levels of total and LDL cholesterol than proband Family 5, although both were carriers of the same rare variant in *STAP1*. As it is shown in Fig. 4, all available family members in Family 5 are carriers of this variant, but only the proband presented high levels of total and LDL cholesterol. Interestingly, proband Family 5 and her sons had high levels of Lipoprotein (a), although only the proband presented hypercholesterolemia. The same absence of cosegregation between phenotype and genotype was found in Family 6, in which the proband and her sons are carriers of this rare variant, although the daughter showed normal levels of total and LDL cholesterol. All family members in Family 6 showed normal levels of Lp(a). Besides, both families showed strong evidence of lack of cosegregation phenotype-genotype, according to the ACMG guidelines.

Table 2 shows biochemical characteristics of carriers and non-carriers of rare variants identified in *STAP1* gene. Carriers of rare variants presented significantly higher levels of HDL cholesterol than non-carriers ($p = 0.019$), with no further differences in their lipid profile, even the carriers present lower levels of LDL cholesterol than non-carriers. Due to the allele frequency of the variant in the promoter region (c.-60A > G) in the general population of 0.17%, which would be above the cut-off recommended for filtering of variants causing Mendelian diseases [25], we reanalyzed the biochemical characteristics of carriers and non-carriers of rare variants identified in *STAP1* gene without carriers of promoter variant. Carriers of missense rare variants (p.(Arg12His), p.(Glu97Asp) and p.(Pro176Ser)) in *STAP1* presented significantly lower age and higher levels of HDL cholesterol than non-carriers of missense rare variants ($p = 0.041$ and $p = 0.007$, respectively), with no further differences in their lipid profile, showing that even the carriers present lower levels of LDL cholesterol than non-carriers (Supplemental Table 4).

4. Discussion

Our results do not confirm a major role of *STAP1* in the etiology of

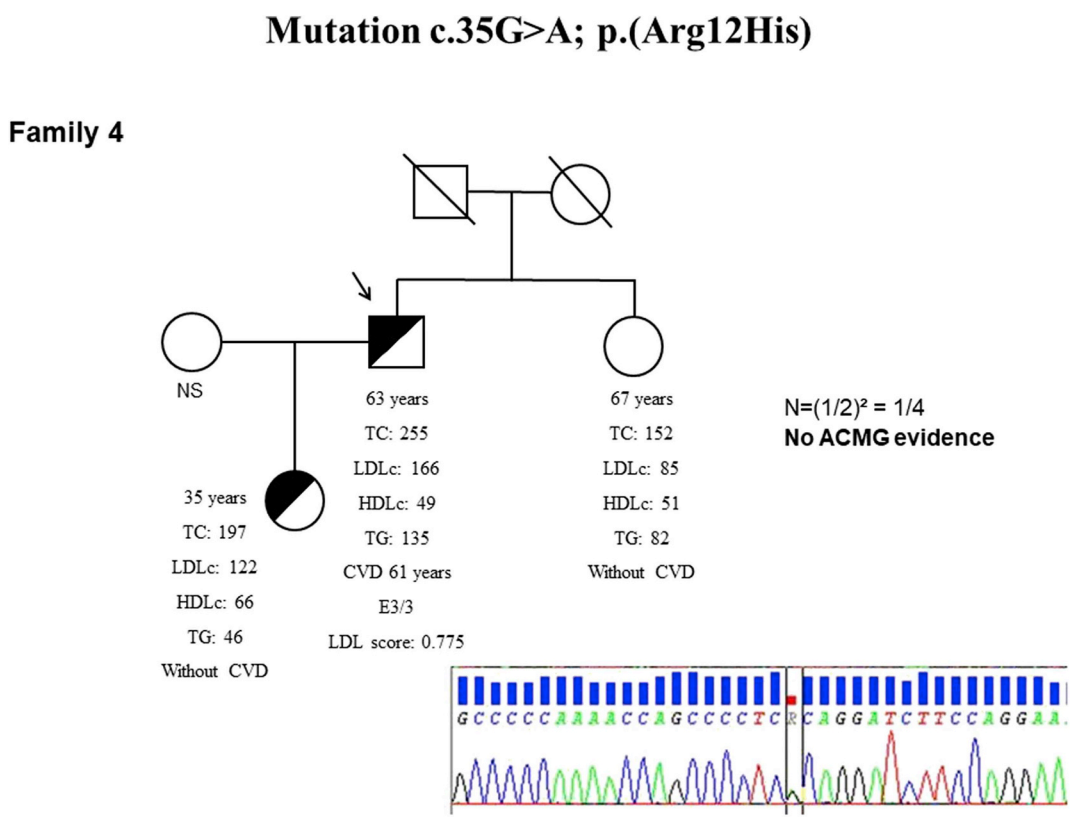

Fig. 2. Family carrying the c. 35G > A, p.(Arg12His) mutation in *STAP1* gene.
NS: Not studied; TC: Total cholesterol; LDLc: Low Density Lipoprotein cholesterol; HDLc: High Density Lipoprotein cholesterol; TG: Triglyceride; CVD: Cardiovascular Disease; Lp(a): Lipoprotein a; ACMG: American College of Medical Genetics and Genomics.

FH. This study analyzes the cosegregation of four predicted pathogenic rare variants in seven families, showing the absence of correlation between genotype and phenotype. The four rare variants reported in this study have been predicted as pathogenic by at least three bioinformatic analysis; three of them produce an amino acid change, p.(Arg12His), p.(Glu97Asp), p.(Pro176Ser) and one of them is located in the promoter region, c.-60A > G. Only two of these four predicted pathogenic rare variants had been previously described, one of them associated with FH [9] and the other one has been described as incompletely associated with FH [24].

The rare variant p.(Glu97Asp) in *STAP1* had been described associated with FH by Fouchier et al. [9] in 2014 from the linkage analysis combined with exome sequencing in an FH family. After studying a large family with 27 family members, the authors demonstrated a good correlation between genotype and phenotype, but there were some inconsistencies in the family cosegregation: they reported four affected family members with LDL cholesterol above 95th percentile, who did not carry this rare variant and one non-affected family member, who was carrier of this variant. Despite several studies have included the sequencing of *STAP1* in FH patients or patients with CVD in the last years, the results have not been conclusive. Corral et al. [26] sequenced 69 subjects with clinical diagnosis of FH, with more than 6 points DLCN and they did not identify any mutation in *STAP1*. In the same line, Pirillo et al. [27] sequenced the candidate FH genes in 1592 unrelated patients with clinical diagnosis of FH, finding 1076 carriers of 216 mutations in *LDLR, APOB, PCSK9, LDLRAP1* and *APOE*, but no *STAP1* carriers. Amor-Salamanca et al. [12] sequenced *LDLR*, *APOB*, *PCSK9*, *APOE*, *STAP1*, *LDLRAP1* and *LIPA* genes in 821 patients with premature CVD, reporting only one patient, who was carrier of the same mutation described by Fouchier et al. [9]. Although they did not report the cholesterol levels of the *STAP1* carrier, they showed that the proband daughter, who was carrier of the same mutation, presented normal levels of total and LDL cholesterol (166 mg/dL and 99 mg/dL, respectively). Blanco-Vaca et al. [24] reported one predicted pathogenic rare variant with incomplete association with FH. They studied one family with three hypercholesterolemic siblings, who were carriers of the rare variant p.(Pro176Ser) in *STAP1* gene. However, not all siblings showed high cholesterol levels and none of them presented LDL cholesterol concentrations compatible with the classical FH phenotype. Besides, the proband son, who was carrier of the same variant, presented normal total and LDL cholesterol levels (212 mg/dL and 121 mg/dL, respectively). Finally, Brænne et al. [13] sequenced the candidate FH genes in 255 subjects with premature CVD with at least one sibling with CVD before 70 years, reporting only one variant in *STAP1* gene, p.(Thr47Ala). This rare variant had not been previously described and the authors have classified it as pathogenic, although both carrier siblings presented different concentrations of LDL cholesterol, one of them below the 95th percentile.

Several genome wide association studies (GWAS) have been performed with the aim of searching for new genes involved in cholesterol metabolism [28–30]. However, none of these studies has demonstrated an association between genetic variants in *STAP1* and cholesterol levels. To our knowledge, the unique association with *STAP1* variants located has been described with Parkinson disease by Fung et al. [31]. The authors genotyped 276 unrelated patients with Parkinson disease and 276 unrelated controls, reporting the variant rs2242330, located in an intronic region, which was significantly associated with the development of Parkinson disease.

Several exome sequencing studies had the goal of searching for new genes that could explain the etiology of FH without mutations in candidate genes. However, except that of Fouchier et a.l [9], there are no other studies reporting mutations in *STAP1* as cause of FH. Exome sequencing studies allowed to discover new mutations in *LDLR*, *APOB* and *PCSK9*. For example, Futema et al. [32] sequenced the exome of 125

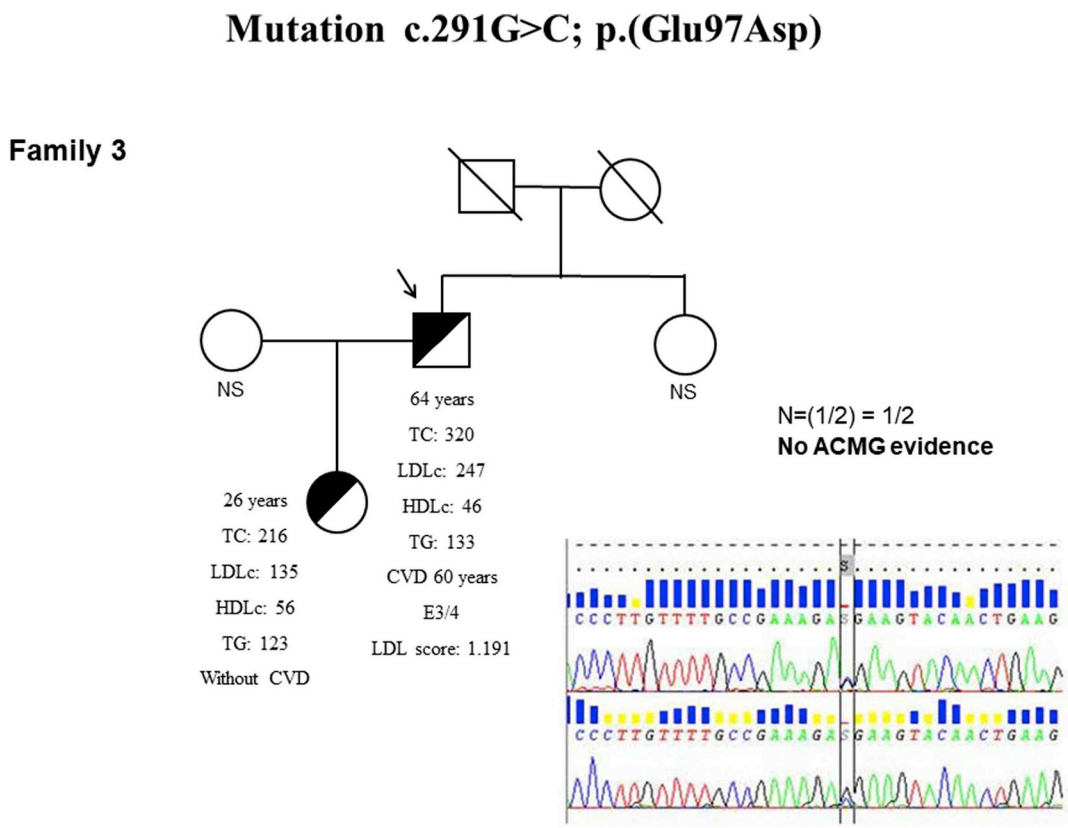

Fig. 3. Family carrying the c.291G > C, p.(Glu97Asp) mutation in *STAP1* gene.
NS: Not studied; TC: Total cholesterol; LDLc: Low Density Lipoprotein cholesterol; HDLc: High Density Lipoprotein cholesterol; TG: Triglyceride; CVD: Cardiovascular Disease; Lp(a): Lipoprotein a; ACMG: American College of Medical Genetics and Genomics.

definite FH patients, without mutation in *LDLR*, *APOB* neither *PCSK9* genes. They reported 23 new mutations in *LDLR* and two new mutations in *APOB*, but no mutation in *STAP1*. Brænne et al. [33], using the exome sequencing analysis in three FH affected family members, discovered one new mutation in the *LDLR* gene. In the same line, Han et al. [34] used exome sequencing to look for new mutations in *LDLR*, *APOB*

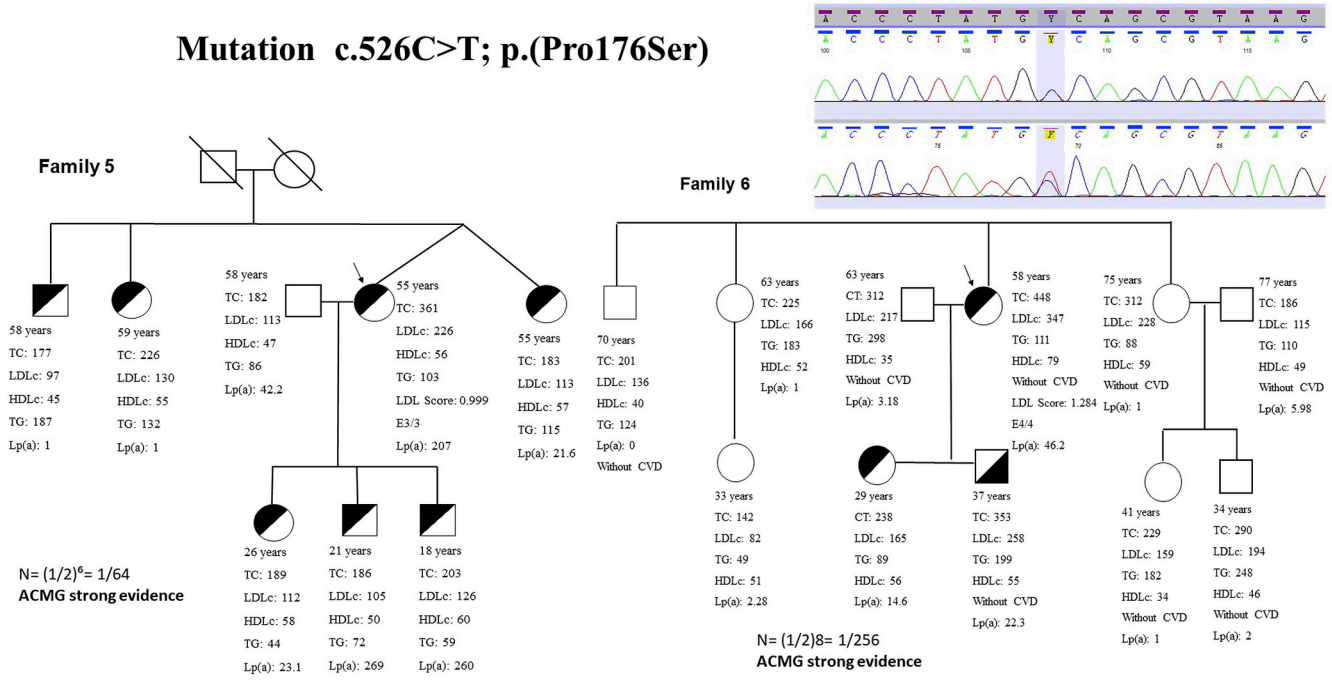

Fig. 4. Family carrying the c.526C > T, p.(Pro176Ser) mutation in *STAP1* gene.
NS: Not studied; TC: Total cholesterol; LDLc: Low Density Lipoprotein cholesterol; HDLc: High Density Lipoprotein cholesterol; TG: Triglyceride; CVD: Cardiovascular Disease; Lp(a): Lipoprotein a; ACMG: American College of Medical Genetics and Genomics.

Table 2
Biochemical characteristics of carriers and non-carriers of pathogenic rare variants identified in *STAP1* gene.

	Carriers of rare variants in *STAP1* (N = 20)	Non carries of rare variants in *STAP1* (N = 14)	*p*
Age, years	45.6 ± 16.0	53.9 ± 17.9	0.189
Men, n (%)	7 (35.0%)	9 (64.3%)	0.061
Total cholesterol, mg/dL	226 (193–280)	208 (183–278)	0.443
LDL cholesterol, mg/dL	135 (118–200)	142 (113–189)	0.716
HDL cholesterol, mg/dL	56.0 (49.5–67.5)	49.0 (43.0–52.0)	0.019
Triglycerides, mg/dL	111 (65.5–132)	117 (86.0–183)	0.357

Quantitative variables are expressed as mean ± standard deviation, except for variables not following normal distribution, expressed as median (interquartile range). Qualitative variables are expressed as n (%). The *p* value was calculated by Student's *t*-test, Mann-Whitney U and Chi - square as appropriate.

and *PCSK9* genes, not reporting any variant in *STAP1* gene. Jiang et al. [35] used targeted exome sequencing in young patients with severe hypercholesterolemia and they discover 27 mutations in *LDLR*, including 3 novel mutations in *LDLR* gene. Iacocca et al. [36] used targeted next-generation sequencing to discover copy number variations in *APOB, PCSK9, LDLRAP1, APOE, STAP1*, *LIPA*, and *ABCG5/8* genes. The authors sequenced 704 patients with FH and they did not find any copy number variation in these candidate genes.

Our study has some limitations: some families had few available members, especially in Family 3, that carried the mutation p.(Glu97Asp), which could be an important limitation. However, despite that, the absence of relation between phenotype and genotype was clear in this family. Theses pathogenic mutations need a functional analysis to be considered totally pathogenic. Another possible limitation could be the limited number of rare variants studied for the family analysis. Nevertheless, we concentrated on those variants predicted to be pathogenic. If these variants have not reported any relationship with the FH phenotype, then those variables with dubious pathogenicity and more frequently found in the population, probably would have not explained the pathogenesis of FH. Finally, familial cosegregation analysis provides initial evidence of a major effect on a particular phenotypic trait with a certain *locus* when the trait is under the control of a single gene. It is well established that some clinically defined FH are in fact polygenic diseases [11,37]. If *STAP1* genetic variation contributes to these polygenic FH cannot be excluded from our study.

4.1. Conclusions

Our study including the predicted pathogenic mutations in *STAP1* does not confirm the role of *STAP1* in FH. This study analyzes the cosegregation of four predicted pathogenic rare variants, three of them producing an amino acid change, p.(Arg12His), p.(Glu97Asp), p.(Pro176Ser) and one of them, located in the promoter region, c.-60A > G, in seven families, showing an absence of correlation between genotype and phenotype.

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Declaration of competing interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.11.025.

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