APOE and familial hypercholesterolemia

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Purpose of review
Autosomal dominant hypercholesterolemia is a common cause of cardiovascular disease. In addition to the classic genes that cause hypercholesterolemia, *LDLR, APOB and PCSK9*, a new locus has emerged as a candidate to be the cause of this hyperlipidemia, the p.(Leu167del) mutation in the *APOE* gene.

Recent findings
Various studies have demonstrated the involvement of the p.(Leu167del) mutation in the *APOE* gene in hypercholesterolemia: Studies of family segregation, lipoprotein composition by ultracentrifugation and proteomic techniques, and functional studies of VLDL-carrying p.(Leu167del) internalization with cell cultures have demonstrated the role of this mutation in the cause of hypercholesterolemia. The phenotype of individuals carrying the p.(Leu167del) in *APOE* is indistinguishable from familial hypercholesterolemia individuals with mutations in the classic genes. However, a better response to lipid-lowering treatment has been demonstrated in these *APOE* mutation carrier individuals.

Summary
Therefore, *APOE* gene should be considered a candidate *locus* along with *LDLR, APOB*, and *PCSK9* to be investigated in the genetic diagnosis of familial hypercholesterolemia.

Keywords
apolipoprotein E, familial hypercholesterolemia, p.(Leu167del) mutation

INTRODUCTION

Mature apolipoprotein (apo) E is a glycoprotein of 299 amino acids, synthetized in many tissues, including liver, brain, and tissue macrophages with a crucial role in lipid metabolism [1]. Apo E is an important protein in the lipoprotein metabolism, being necessary for lipoprotein remnant clearance. Apo E interacts with several members of the LDLR family on the surface of cells [2]. The LDLR family includes more than 10 different receptors participating in receptor-mediated endocytosis and cellular signaling. In addition to the LDLR, the family includes LRP/LRP1, megalin/LRP2, VLDLR, ApoER2/LRP8, SORLA-1/LR11, LRP4, LRP5, LRP6, and LRP1B [3]. Apo E is a component of chylomicrons, VLDL and HDL, and through interaction with these different receptors and heparan sulphate proteoglycans promotes the clearance of remnants of chylomicrons and VLDL by the liver [4]; facilitates cholesterol-efflux to HDL from macrophages incorporating free cholesterol and phospholipid from ABCA1 [5]; and may stimulate adipogenesis from triglyceride-rich lipoproteins [6].

The *APOE* gene is mapped to chromosome 19 in a cluster with apolipoprotein C1 (*APOC1*) and apolipoprotein C2 (*APOC2*) genes. The *APOE* gene consists of four exons and three introns, totaling 3597 base pairs, with more than 85% of the mature protein coded by exon 4. *APOE* is transcriptionally activated by the liver X receptor (an important regulator of cholesterol, fatty acid, and glucose homeostasis) and peroxisome proliferator-activated receptor γ, nuclear receptors that form heterodimers with retinoid X receptors [7].

In humans, there are three common genetically determined isoforms of apo E, named apoE2, apoE3, and apoE4, that are under the control of 3 *APOE* alleles APOE2, APOE3, and APOE4. The isoforms differ in primary structure at two sites: residues 130 (single nucleotide variation [SNV] rs429358)

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KEY POINTS

- *APOE* p.(Leu167del) mutation causes autosomal dominant hypercholesterolemia with a lipid profile similar to classical familial hypercholesterolemia.
- The mechanism by which *APOE* p.(Leu167del) is associated with familial hypercholesterolemia appears to be a downregulation of the LDL receptor.
- *APOE* gene should be considered a candidate *locus* to be investigated in the genetic diagnosis of familial hypercholesterolemia.

and 176 (SNV rs7412). In concordance with its critical role in lipid metabolism, the genetic variation in *APOE* gene, mostly in exon 4 of *APOE* gene, where the vast majority of mutations are located [8▪▪,9▪], is associated with monogenic disorders, including familial dysbetalipoproteinemia [10] and familial hypercholesterolemia [11], but also contributes to polygenic hypercholesterolemia [12] and to the interindividual variability in blood cholesterol of normolipidemic individuals in the general population [13].

FIRST DESCRIPTIONS OF THE P.(LEU167DEL) MUTATION IN THE *APOE* GENE

The first description of the p.(Leu167del) mutation in the *APOE* gene is due to Kenneth J. Livak and James W. Hainer, from Du Pont Merck Pharmaceutical Company, who in 1994 analyzed the *APOE* genotype by solid-phase minisequencing in a group of 75 participants in a clinical trial of a lipid-lowering drug with a personal history of hypercholesterolemia. One of the participants carried in heterozygosity a deletion of codon 149 (with the old nomenclature) in the *APOE* gene. He was a healthy 22-year-old man with normal total cholesterol levels [14]. No further investigation of the pathogenicity of the variant was reported at that time.

The first association of the p.(Leu167del) mutation in the *APOE* gene with disease was due to Nguyen *et al.* [15] in 2000 in a cooperative study that included researchers from the Mayo Clinic and the University of California. In this important work, authors described two unrelated individuals of French-Canadian ancestry with severe hyperlipidemia after splenectomy because hepatosplenomegaly of unknown cause. The *APOE* sequencing was performed due to suspicion of dysbetalipoproteinemia, as the presence of β-VLDL and an elevated VLDL-C/triglyceride ratio in plasma were found in both cases. The liver biopsy showed significant macrovesicular steatosis and the splenic biopsy showed a high content of foamy histiocytes in both individuals. The functional analysis of the deletion showed an increase in the uptake of the VLDL fraction of these individuals by mouse macrophages, inducing a significant accumulation of cholesterol esters that, in the opinion of the researchers, could explain the presence of hepatosplenomegaly and the increase in dyslipidemia after splenectomy.

A third proband with the association of splenomegaly with sea-blue histiocytes, hypertriglyceridemia and thrombocytopenia was described a few years later [16]. The proband, also of French origin, was a carrier of the p.(Leu167del) and an E2 allele. The family study revealed hyperlipidemia in relatives carrying the mutation, been limited to isolated hypercholesterolemia in the case of the proband's mother and brother without signs of dysbetalipoproteinemia, and hypertriglyceridemia in the father without the mutation. The authors drew attention to the variability of the phenotype associated with the deletion in *APOE*, and the possibility that the presence of APOE2 in the other allele and genetic load of paternal hypertriglyceridemia could be responsible for the patient's phenotype [16].

A third report of the association of p.(Leu167del) with combined hyperlipidemia was reported by Rahalkar *et al.* [17]. In this case, a 49-year-old man developed combined hyperlipemia with severe hypertriglyceridemia after splenectomy for spontaneous splenic rupture. The spleen was enlarged with infiltration of lipid-laden histiocytes. This case highlights the association of p.(Leu167del) to dyslipidemia with splenomegaly and infiltrates of foamy histiocytosis, and severe dyslipidemia after splenectomy. It also reveals the variability of the phenotype associated with the p.(Leu167del) *APOE* mutation and the need for other unknown associated factors for the presence of splenomegaly and hypertriglyceridemia.

ASSOCIATION OF P.(LEU167DEL) MUTATION WITH HYPERCHOLESTEROLEMIA

Due to the association of the genetic variation in *APOE* with dysbetalipoproteinemia and the presence of rare variants in *APOE* with a dominant form of this disease [18], Solanas-Barca *et al.* [19] studied the *APOE* gene in a large group of unrelated individuals with combined hyperlipidemia of suspected genetic origin, but without mutation in the *LDLR* gene, from a lipid unit in Spain. The hypothesis was that some rare *APOE* variants could be responsible for combined hyperlipidemia, probably dysbetalipoproteinemia, but mimicking familial combined

hyperlipidemia due to the dominant transmission of dysbetalipoproteinemia of some rare variants [20]. In 279 unrelated individuals with the sequencing of the *APOE* gene, four individuals (1.4%) with the p.(Leu167del) mutation, and two individuals in an independent group of 160 individuals from other locations in Spain were detected. VLDL and IDL fractions were isolated by sequential ultracentrifugation and, in contrast to other *APOE* mutations, the deletion did not show accumulation of cholesterol-rich VLDL, with ratios (in mg/dl) VLDL-C/triglycerides of 0.18 ± 0.06, without differences compared to individuals with APOE3/3 genotype and much lower than homozygous APOE2/2 individuals, who had a ratio of 0.44 ± 0.14, that is characteristic of dysbetalipoproteinemia. Furthermore, carriers of the p.(Leu167del) allele developed hyperlipidemia with high concentration of apolipoprotein B and without dysbetalipoproteinemia, suggesting that both VLDL and LDL particles were increased but without significant accumulation of remnant lipoproteins. This study also included the analysis of the families of three probands, demonstrating cosegregation of p.(Leu167del) with hyperlipoproteinemia, although, surprisingly, the lipid phenotype of family members carriers of the p.(Leu167del) was isolated high LDL-C with normal triglycerides in five out of eight individuals, and with a lipid phenotype undistinguishable from familial hypercholesterolemia [19].

APOE P.(LEU167DEL), A FOURTH CAUSATIVE GENE FOR FAMILIAL HYPERCHOLESTEROLEMIA

Several works have been published in recent years to establish the potential role of p.(Leu167del) in the etiology of familial hypercholesterolemia. Cenarro *et al.* [11] studied the presence of the mutation in 288 unrelated individuals with the clinical diagnosis of familial hypercholesterolemia and in whom the presence of pathogenic variants in *LDLR, APOB,* and *PCSK9* genes had been ruled out. As a control group, 220 unrelated individuals with normal LDL-C levels were studied. In the group with hypercholesterolemia, nine individuals (3.1%) were carriers of p.(Leu167del), and no carrier was found in the control group [11]. This study included the analysis of eight families of the probands, demonstrating a clear cosegregation of hypercholesterolemia with the deletion, since of the 10 carriers, six of them presented isolated hypercholesterolemia, three combined hyperlipidemia, and only one young woman, with BMI 19.5 kg/m^2, had total cholesterol below the 90th percentile. None of the p.(Leu167del) carriers showed splenomegaly [11].

Confirmation of the association between p.(Leu167del) and familial hypercholesterolemia comes from the study of Marduel *et al.* [21]. They identified the mutation in a proband with suspected familial hypercholesterolemia, demonstrated cosegregation with isolated hypercholesterolemia in the family, and decreased catabolism of the LDL particle associated with this variant of apo E by kinetic studies [21]. A similar study with clear co-segregation of the deletion with high LDL-C in individuals from Canada [22] and from an Iranian family from three generations with the clinical diagnosis of familial hypercholesterolemia has been recently reported by Norian *et al.* [23].

FREQUENCY OF P.(LEU167DEL) AS CAUSE OF FAMILIAL HYPERCHOLESTEROLEMIA

In the French National Research Network on Hypercholesterolemia, which includes 38 clinicians from all over France and 5743 probands diagnosed with primary dyslipidemia, 58% of them with autosomal dominant hypercholesterolemia, the p.(Leu167del) was carried by 14 individuals (0.42%), a similar frequency of the *PCSK9* gain-of-function mutations and 1% of the clinical suspected familial hypercholesterolemia individuals in whom pathogenic mutation in the well established genes, *LDLR, APOB,* and *PCSK9,* were absent [24▪▪].

LIPIGEN, an Italian network aimed at the early identification of patients with genetic dyslipidemias, analyzed 1592 unrelated patients from 20 outpatient clinics with clinical diagnosis of familial hypercholesterolemia and a Dutch Lipid Clinic Network (DLCN) score of 6 points. They were genetically tested for the presence of variants in familial hypercholesterolemia causing genes, and one patient was found to be heterozygous for p.(Leu167del) (0.06%) [25].

In a multicenter study, including 41 families of European origin with extreme concentration of LDL-C or HDL-C, with an autosomal dominant transmission pattern and without mutations in the familial hypercholesterolemia candidate genes, three unrelated carriers of p.(Leu167del) in *APOE* were detected, with familial segregation also demonstrated [26]. This represents 7.3% of these families.

In the study of 478 consecutive unrelated individuals with a clinical diagnosis of familial hypercholesterolemia from the UK, the presence of the *APOE* deletion was detected in four patients, which represents approximately 1% [27].

The analysis of the different studies reveals that consistently, at least in European populations, the deletion in *APOE* is found between 0.1 and 1% of

individuals with clinical suspicion of familial hypercholesterolemia, and that percentage can reach 3–7% if we consider only those individuals in whom pathogenic variants in *LDLR, APOB,* and *PCSK9* have been ruled out. For this reason, different authors recommend the inclusion of *APOE* in the genetic study of familial hypercholesterolemia [24▪▪,25–27].

MECHANISM OF HIGH LDL-C PRODUCED BY P.(LEU167DEL)

The lipoprotein composition analysis showed that apo E content is reduced in the VLDL fraction in individuals carrying the p.(Leu167del) mutation, but with normal VLDL particle number as determined by the content of apo B in VLDL. Hence, the apo E-VLDL/apo B-VLDL ratio was significantly lower in individuals carrying the p.(Leu167del) mutation than in normolipemic controls [11]. This would suggest that each VLDL particle from mutation carriers would have a lower content of apo E molecules per VLDL particle.

Normal VLDL contains multiple copies of apo E, which bind to LDL receptors with up to 20-fold higher affinity than LDL, which contains only one copy of apo B-100 [28]. The in-vitro cultured cell studies carried out demonstrated that VLDL carrying apo E with the p.(Leu167del) mutation, and carrying also wild-type apo E (they were isolated from heterozygous individuals), have a higher uptake by HepG2 and by THP-1 cells and, subsequently, LDL receptor expression is repressed. The decrease of LDL receptor expression in surface membrane of hepatocytes would result in a decrease of LDL internalization, and therefore in an increase of LDL particles circulating in plasma and an increase in LDL cholesterol levels. It has also been demonstrated that in VLDL isolated from mutant heterozygous individuals, wild-type apo E3 is almost a five-fold increase compared to mutant p.(Leu167del) apo E, by quantitative proteomic techniques [11]. These results are consistent with a higher uptake of the lipoproteins carrying the mutant apo E by HepG2 cells, and also with the lower total apo E content observed in VLDL and LDL from carriers. These results suggest that p.(Leu167del) mutation is a gain-of-function mutation for the lipoprotein uptake by the LDL receptor or other members of the LDL receptor family involved in VLDL catabolism.

P.(LEU167DEL) PHENOTYPE AND TREATMENT RESPONSE

Twenty-two patients with the p.(Leu167del) mutation attending a Lipid Unit in Spain and 44 age and sex-matched individuals with genetically defined heterozygous familial hypercholesterolemia from the same Unit were randomly selected as control group to compare lipid phenotype and lipid-lowering response. No differences were found on total cholesterol, LDL-C, HDL-C, and non-HDL-C. Triglycerides were slightly higher and lipoprotein (a) lower in p.(Leu167del) carriers than in familial hypercholesterolemia (Table 1) [28]. The mean percentage reduction in non-HDL-C with the same statin dose was significantly higher in the p.(Leu167del) carriers (−52.7%) than in the *LDLR* familial hypercholesterolemia (−34.1%) ($P=0.048$). Similar differences were observed in triglycerides: −43.2 and −6.63%, respectively ($P=0.016$). Lower doses of lipid-lowering drugs were needed to reach lipid-lowering goals in p.(Leu167del) carriers. Authors concluded that p.(Leu167del) mutation carriers have a higher lipid-lowering response to statins with or without ezetimibe than *LDLR* familial hypercholesterolemia [28].

Table 1. Biochemical characteristics of *APOE* p.(Leu167del) mutation carriers and matched familial hypercholesterolemia individuals with *LDLR* functional mutation[a]

Variable	p.(Leu167del) carriers $n=22$	*LDLR* mutation carriers $N=44$	*P*
Total cholesterol (mg/dl)	345 ± 85.0	346 ± 70.1	0.959
LDL cholesterol (mg/dl)	255 ± 75.4	267 ± 65.6	0.539
HDL cholesterol (mg/dl)	62.0 ± 22.4	58.0 ± 12.8	0.422
Non-HDL cholesterol (mg/dl)	283 ± 72.4	287 ± 73.7	0.875
Triglycerides (mg/dl)	146 (119–282)	101 (73.3–126)	<0.001
Apolipoprotein A1 (mg/dl)	284 ± 72.4	287 ± 74.0	0.074
Apolipoprotein B (mg/dl)	169 ± 31.4	154 ± 28.3	0.506
Lipoprotein (a) (mg/dl)	7.41 (1.50–29.0)	38.0 (11.0–69.2)	0.002

[a]Modified from [28].

CONCLUSION

Different studies support the involvement of *APOE* p.(Leu167del) in autosomal dominant hypercholesterolemia, even with some variability of LDL-C or non-HDL-C levels in carriers; the presence of hypertriglyceridemia in some carriers could be explained by a concomitant polygenic defect or environmental influences. The mechanism by which this mutation is associated with familial hypercholesterolemia appears to be a downregulation of the LDL receptor by VLDL carrying the variant apo E, resulting in higher plasma LDL-C levels. Therefore, *APOE* gene should be considered a candidate *locus* along with *LDLR, APOB*, and *PCSK9* to be investigated in the genetic diagnosis of familial hypercholesterolemia.

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Conflicts of interest

There are no conflicts of interest.

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