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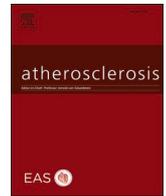
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APOE gene variants in primary dyslipidemia

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ABSTRACT

Apolipoprotein E (apoE) is a major apolipoprotein involved in lipoprotein metabolism. It is a polymorphic protein and different isoforms are associated with variations in lipid and lipoprotein levels and thus cardiovascular risk. The isoform apoE4 is associated with an increase in LDL-cholesterol levels and thus a higher cardiovascular risk compared to apoE3. Whereas, apoE2 is associated with a mild decrease in LDL-cholesterol levels. In the presence of other risk factors, apoE2 homozygotes could develop type III hyperlipoproteinemia (familial dysbetalipoproteinemia or FD), an atherogenic disorder characterized by an accumulation of remnants of triglyceride-rich lipoproteins. Several rare APOE gene variants were reported in different types of dyslipidemias including FD, familial combined hyperlipidemia (FCH), lipoprotein glomerulopathy and *bona fide* autosomal dominant hypercholesterolemia (ADH). ADH is characterized by elevated LDL-cholesterol levels leading to coronary heart disease, and due to molecular alterations in three main genes: *LDLR*, *APOB* and *PCSK9*. The identification of the APOE-p.Leu167del variant as the causative molecular element in two different ADH families, paved the way to considering APOE as a candidate gene for ADH. Due to non mendelian interacting factors, common genetic and environmental factors and perhaps epigenetics, clinical presentation of lipid disorders associated with APOE variants often strongly overlap. More studies are needed to determine the spectrum of APOE implication in each of the diseases, notably ADH, in order to improve clinical and genetic diagnosis, prognosis and patient management. The purpose of this review is to comment on these APOE variants and on the molecular and clinical overlaps between dyslipidemias.

1. Introduction

ApoE is a major apolipoprotein that controls lipoprotein metabolism. ApoE is expressed in many cells, primarily in the liver, and also in the brain, spleen, kidneys, gonads, adrenals, and macrophages [1]. The widespread production of apoE indicates its importance in various pathways such as lipoprotein, fat-soluble vitamins and glucose/energy metabolisms, signal transduction, metastasis, angiogenesis or neurosciences.

ApoE is a component of chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and lipoprotein (a) (Lp(a)). Classical knowledge is that LDL does not have apoE, nevertheless, since apoE binds to lipids through its C-terminal domain it seems difficult that

it binds to HDL, VLDL, IDL and Lp(a) but not LDL at all. Indeed, lipoproteins isolated by flotation sequential ultracentrifugation showed that apoE represents 0.40%, 0.10% and 0.38% of the total mass of VLDL, LDL and HDL, respectively [2], and is found in HDL (61 ± 27%), VLDL (35 ± 25%), Lp(a) (4 ± 9%) and LDL (1 ± 1%) in fasting plasma [3]. Lipoproteins isolated by anti-apoE immunoaffinity chromatography showed the presence of 21, 19, and 5 molecules of apoE in a portion of VLDL, HDL, and LDL respectively [4]. ApoE plays a key role in regulating the clearance of these lipoproteins from the plasma and controls plasma lipid levels as well as homeostasis of tissue lipid content as the ligand for cell-surface lipoprotein receptors. ApoE mediates the interaction between apoE-carrying lipoproteins and the LDL receptor, the LDL receptor-related protein (LRP), the VLDL receptor, the apoE receptor-2 and heparan sulfate proteoglycans (HSPG) [5] (Fig. 1). As a ligand for

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heparin and HSPG in extracellular matrices or on cell membranes, apoE may also influence cellular responses to signals [1]. ApoE also contributes to the catabolism of VLDL particles. Once secreted, triglyceride-rich VLDL can be hydrolyzed by lipoprotein lipase (LPL). An excessive hepatic production of VLDL can cause a saturation of this pathway and an increase in plasma levels of triglyceride-rich lipoproteins [6]. It is now well established that apoE, as an apo CIII co-factor, inhibits LPL and the triglyceride-rich lipoprotein lipolysis [7,8]. Also, apoE could increase the hepatic production of VLDL by activating the particle assembly cascade [9]. These data underline the potential impact of a dysfunctional apoE on the metabolism of triglyceride-rich lipoproteins and the etiology of dyslipidemia. Furthermore, apoE plasma levels are associated with different causes of mortality: high apoE levels are associated with cardiovascular and cancer mortality while low apoE levels were causally associated with dementia-associated mortality [10].

The *APOE* gene is located at chromosome 19q13.2 and encodes a 317 amino acid apolipoprotein E precursor (NM_000041.4). After cleavage of the 18-amino acid signal peptide and glycosylation, mature apoE is secreted as a 299 amino acid protein with a relative molecular mass of 34 200 kDa.

The variants that give rise to the apoE isoforms are rs429358, p.Cys130Arg (E4), and rs7412, p.Arg176Cys (E2). According to the frequencies given by the Genome Aggregation Database (gnomAd), with the sequencing of about 100 000 subjects from various disease-specific and population genetic studies, the rs429358 allele frequency is 14.25% and the rs7412 allele frequency is 6.542% in the total gnomAd population. Thus, the approximate prevalences for E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4 are 0.4, 6.5, 0.9, 75.9, 14.3 and 2.0%, respectively.

Which is overall in agreement with previous reports in healthy subjects [11–13], but different from what is observed in hyperlipidaemic patients for whom the E4 isoform is more frequent and the E2 less frequent than in a control group [13].

ApoE accounts for 1%–8.3% of the total variance of LDL cholesterol [12]. Because the different isoforms have different affinity for lipoproteins and receptors, apoE2 and apoE4 have a significant impact on interindividual variation of lipid and lipoprotein levels in normal subjects. The residues that determine apoE isoforms are in the receptor-binding domain (154–168 and Arginine 190), separated from the lipid-binding domain (262–290) by a hinge region (218–233) (Fig. 2) [5,14]. The structure and electric charge of apoE are crucial for the optimal function of the protein and the binding to lipids is necessary for receptor affinity. Once binding to lipoproteins, apoE undergoes conformational changes and adopt a circular horseshoe shape in which the lipid-binding and the N-terminal domains join to wrap around the lipoprotein and the critical residues for binding, 154–168 and Arg-190, meet nearby [14]. In apoE4, the substitution Cys130Arg lead to a more compact structure, due to N- and C- terminal ends interaction, which changes its affinity preference from HDL to VLDL but have less impact on the receptor binding properties [14]. ApoE4 is associated with higher apo B, total-, LDL-, and remnant-cholesterol levels [15] due to its preference for VLDL, and its higher production rate [16], and to its higher VLDL-lipolysis activity, or less inhibitory effect, relative to apoE3 [8] (Fig. 1). The other classical hypothesis, according to which the increased level of LDL in apoE4 carriers would be due to the downregulation of LDL receptor expression consequent to the accelerated hepatic uptake of apoE4-rich VLDL, could complete the pathophysiology of the apoE4

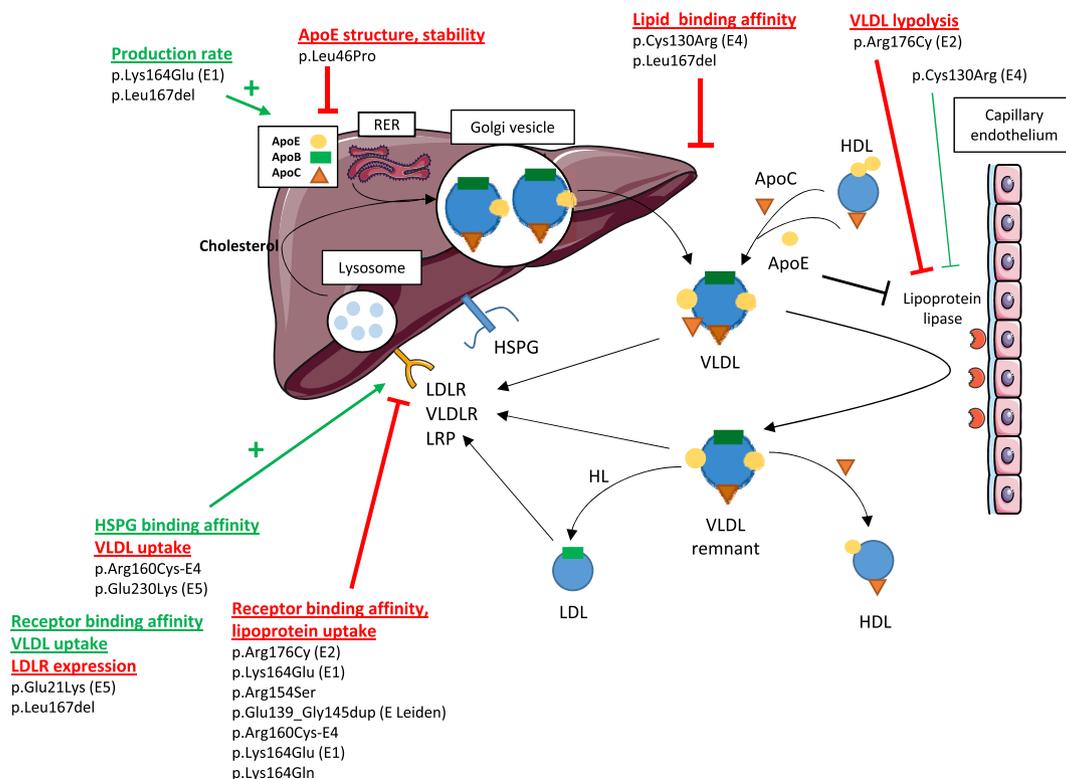


Fig. 1. Effect of *APOE* variants on the metabolic pathways of triglyceride rich lipoproteins.

Several apoproteins including apoE are produced by the liver in the rough endoplasmic reticulum. ApoE, apoB100, apoC are then associated with triglycerides and cholesteryl esters to form VLDL particles. Nascent VLDL particles are secreted by the liver via golgi vesicles. Once in the circulation, VLDL particles can acquire more apoE and apoC molecules form HDL particles. In the vessels of peripheral tissues, VLDLs are hydrolyzed and converted to VLDL remnants by Lipoprotein lipase (LPL) located on capillary endothelium. ApoE, as a co-factor for apoCIII, inhibits LPL. VLDL particles are also converted to LDL by loss of triglycerides and apoE. ApoE binds with high affinity to cell-surface lipoprotein receptors including LDL receptor, the LDL receptor-related protein (LRP), the VLDL receptor, the apoE receptor-2. It also binds to cell surface HSPGs which facilitates the interaction with the LRP and possibly other receptors. Common and rare *APOE* variants are associated with dyslipidemia by affecting triglyceride rich lipoproteins metabolic pathways. Apo: apoprotein; VLDL: very low-density lipoprotein; HDL: high-density lipoprotein; LDL: low-density Lipoprotein; RER: rough endoplasmic reticulum; LPL: lipoprotein lipase; HL: hepatic lipase.

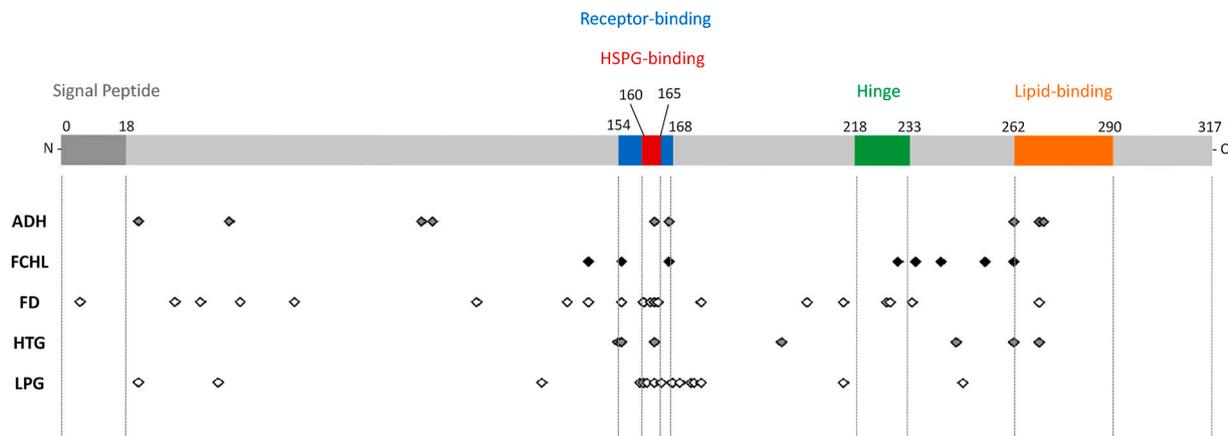


Fig. 2. Distribution of the *APOE* gene variants in the apoE protein.

isoform. The substitution Arg176Cys in apoE2 causes the disruption of a salt bridge, lowers the positive potential, and alters the receptor-binding domain which affects drastically apoE2 affinity for its receptors (<2%) but not its affinity for HSPG [5]. Thus, apoE2 is associated with higher remnant-cholesterol levels [15], as observed for 1–10% of the apoE2 homozygotes, under the influence of other genetic or environmental factors, who develop type III hyperlipoproteinemia (OMIM #617347). These factors (oestrogen deficiency, hypothyroidism, obesity and diabetes) lead to a saturated or impaired lipoprotein clearance. ApoE2 also inhibits the VLDL lipolysis significantly more than apoE3 and apoE4 [8] probably by a reduction in the apoCII content of VLDL [17] (Fig. 1). Thus, explaining why apoE2 is also associated with lower apo B, total-, and LDL-cholesterol levels, relative to apoE3 [15]. The other classical hypothesis, according to which the decreased level of LDL in apoE2 carriers would be due to an opposite mechanism to that of apoE4 (the relative impoverishment of apoE2 in VLDL would slow down hepatic uptake and consequently upregulate the LDL receptor), could complete the pathophysiology of the apoE2 isoform.

On average, apoE2 lowers total cholesterol levels by approximately 14 mg/dL and apoE4 raises them by approximately 8 mg/dL [12]. Thus, although LDL contains only few apoE, its isoforms influence LDL concentration and size along with many other factors including sex, age and triglyceride levels. The recent analysis of 228 serum metabolites in a cohort of 2234 young Finns showed that the apoE4 isoform influences LDL and VLDL particle sizes as well as their composition increasing their concentrations of free/esterified cholesterol, triglycerides, phospholipids and total lipid, thus increasing their size [18]. The opposite being observed for the apoE2 isoform [19]. Furthermore, apoE isoforms influence the Lp(a) mass that is 65% higher for apoE4 homozygous carriers relative to apoE2 homozygous carriers [20].

In addition to the three major isoforms apoE2, E3 and E4, three minor apoE isoforms have been observed by isoelectric focusing, E1, E5, and E7, each one presenting several nucleotide sequence variants with different amino acid substitutions [21]. The minor E1 isoform has an isoelectric point more acid, by two units of charge, than apoE3 due to either p.Lys164Glu, p.Arg168Gly-E2, p.Gln174_Gly190del variants (Table 1). The apoE1 p.Lys164Glu variant, within the receptor binding domain of apoE, is produced at a higher rate and catabolized significantly slower than apoE3, due to its reduced affinity for the LDL receptor and for heparin [22,23] (Fig. 1). The minor E5 isoform has an isoelectric point more basic by two units of charge than apoE3, due to either p. Glu21Lys, p.Gln99Lys, p.Pro102Arg, p.Val153_Arg160dup, or p. Glu230Lys variants (Table 1). The apoE5 p.Glu21Lys variant, within the N-terminal domain of apoE, presents a twofold increased receptor-binding activity while the activity is unaffected by the apoE5 p. Pro102Arg variant also localized in the N-terminal domain [24] (Figs. 1 and 2). This indicates that the enhanced receptor-binding activity of

basic apoE isoforms depends on the position at which additional positively charged amino acids are incorporated. The apoE5 p.Glu230Lys variant, in the heparin binding domain, displays enhanced receptor- and heparin-binding activity, but decreased catabolism in cultured fibroblasts [25] (Figs. 1 and 2). Since receptor-mediated endocytosis of apoE lipoproteins is facilitated by proteoglycan ligands transfer, the stronger binding of apoE5 p.Glu230Lys variant to proteoglycans could reduce the rate at which it is finally delivered to the endocytosis pathways. The minor E7 isoform, also named apoE-Suita, has an isoelectric point more basic by four units of charge than apoE3, due to the replacement of two glutamine residues by two lysine residues at positions 262 and 263 in the lipid-binding domain [26] (Fig. 2). Each of the two nucleotide variants contributing to the apoE7 isoform, rs140808909 and rs190853081, are only found in the Japanese population with a relative frequency of 0.7% for the apoE7 allele in a cohort of 1269 Japanese subjects [27]. According to the frequencies given in the Genome Aggregation Database, with the sequencing of about 8000 Est Asian subjects from various disease-specific studies, the rs140808909 and rs190853081 frequency is 0.27% for both, giving a frequency of 0.073% for the apoE7 allele.

Moreover, rare apoE mutants have been associated with different dyslipidemias such as familial dysbetalipoproteinemia (FD, type III) (OMIM # 617347), familial combined hyperlipidaemia (FCHL, type IIb) (OMIM # 144250), ADH (type IIa), hypertriglyceridemia (HTG, type IV and V), lipoprotein glomerulopathy (LPG) (OMIM #611771) [21,28], sea-blue histiocytosis (OMIM #269600) [29] or late-onset Alzheimer disease (OMIM # 104310) [30].

2. ApoE and autosomal dominant hypercholesterolemia (ADH)

Familial Hypercholesterolemia (FH) is an autosomal codominant genetic lipoprotein disorder, type IIa hyperlipoproteinemia according to Fredrickson's classification [31,32], initially identified through mutations into the low density lipoprotein receptor encoded by the *LDLR* gene at 19p13.2 (OMIM #143890, #606945). FH is a codominant disease since each allele contributes to the phenotype and heterozygotes present with an intermediate phenotype of that of homozygotes. The same phenotype is also observed with mutations in the apolipoprotein B [*APOB* gene at 2p24.1 (familial defective apolipoprotein B (OMIM #107730, #144010)), the proprotein convertase subtilisin/kexin type 9 [*PCSK9* gene at 1p32.3 (OMIM # 607786)] - and the apolipoprotein E [*APOE* gene at 19q13.32 (OMIM #107741)]. While homozygous carriers of an *APOB* mutation are very rare, Familial defective apolipoprotein B appears to be a dominant disease with homozygotes reported to have cholesterol concentrations in the range for heterozygotes carriers [33]. To our knowledge, no homozygous carrier of a *PCSK9* or *APOE* hypercholesterolemic mutation has been reported yet, thus the status of the transmission mode for these diseases cannot be defined as

Table 1
APOE gene mutations reported with dyslipoproteinemias.

rs number	cDNA position*	Protein position*	Segregation	Common name	Transmission	Phenotype	Ref.
-	c.15G > A	p.Trp5*	2 families			FD (RP)	[74]
rs121918392	c.61G > A	p.Glu21Lys	2 cases	ApoE5		HC	[24,87]
rs121918392	c.61G > A-E2	p.Glu21Lys-E2	1 case	ApoE5-ApoE2		LPG	[88]
rs201672011/rs769455	c.91G > A/c.487C > T	p.Glu31Lys/p.Arg163Cys	1 family, 6 htz, 1 hmz	Philadelphia		FD	[89]
CM980097	c.114G > A-E2	p.Trp38*-E2	1 case			FD	[90]
rs121918399	c.127C > T	p.Arg43Cys	6 individual cases, 2 families	Kyoto		LPG	[28,79,91]
rs769452	c.137T > C	p.Leu46Pro	1 case			HC	[48]
-	c.146delG	p.Gly49Valfs*29	2 family members			FD	[92]
-/rs267606664	c.146delG/c.434G > A-E2	p.Gly49Valfs*29/p.Gly145Asp-E2	1 case			FD	[92]
rs370594287	c.192G > C	p.Gln64His	1 case			FD	[79]
rs1180612218	c.295G > A-E4	p.Gln99Lys-E4	1 case	Frankfurt, ApoE5		HC	[54]
rs11083750	c.305C > G	p.Pro102Arg	1 case	ApoE5		HC	[24]
-	c.339dupG	p.Glu114Glyfs*50	1 family	Groningen	dominant	FD	[93]
rs11542041	c.394C > T	p.Arg132Cys	1 case	Tsukuba		LPG	[77]
rs397514254	c.415_435dup21-E4	p.Glu139_Gly145dup-E4	1 family	Leiden	dominant	FD	[67,94]
rs267606664	c.434G > A-E2	p.Gly145Asp-E2	1 family, 3 cases			Mixed (RP), FD	[48,69]
rs267606664	c.434G > A	p.Gly145Asp	9 family members		dominant	FD	[94]
rs267606664/rs267606661	c.434G > A/c.805C > G	p.Gly145Asp/p.Arg269Gly	1 case			FD	[94]
-	c.457_480dup24	p.Val153_Arg160dup	1 case	ApoE5ss		HTG	[95]
rs121918393	c.460C > T	p.Arg154Cys	1 family		dominant	FD	[96]
rs121918393	c.460C > A	p.Arg154Ser	15 cases, 3 families	Christchurch	dominant	FD (RP), FCHL, HTG	[59,63,68–70]
CM950078	c.461G > A	p.Arg154His	9 family members		recessive?	HTG	[97]
rs121918393	c.460C > T-E2	p.Arg154Cys-E2	3 families, 1 case			FD	[84,98,99]
-		p.Leu159_Lys161del	1 case	Tokyo		LPG	[100]
CM043808	c.478C > A	p.Arg160Ser	1 case			FD	[101]
CM950079	c.479G > T	p.Arg160Leu	2 family members			FD	[102]
CM890009	c.478C > T-E4	p.Arg160Cys-E4	6 family members		dominant	FD	[71]
-		p.Arg160_Leu162del	1 case	Maebashi		LPG	[103]
-	c.477_491del15	p.Lys161_Arg165 del	5 family members			LPG (RP)	[80]
-	c.484_492del9	p.Leu162_Lys164del	1 case			FD	[100]
rs769455	c.487C > T	p.Arg163Cys	1 family, 1 htz and 1 hmz			ADH	[48]
rs769455	c.487C > T-E2	p.Arg163Cys-E2	43 cases, 39 htz, 4 hmz, 1 family		dominant	FD (RP)	[75,84]
rs121918397	c.488G > A	p.Arg163His	1 family, 1 case	Kochi		HTG, FD	[101,104]
CM972792	c.488G > C	p.Arg163Pro	3 cases	Sendai		LPG	[105]
CM972792/rs121918392	c.488G > C/c.61G > A	p.Arg163Pro/p.Glu21Lys	1 case	Sendai/ApoE5		LPG	[106]
CM890010	c.490A > G	p.Lys164Glu	2 families, 1 case	ApoE1	dominant	FD (RP)	[22,23,107]
rs121918394	c.490A > G-E2	p.Lys164Gln-E2	3 families		dominant	FD	[66,108]
rs121918394	c.490A > C-E2	p.Lys164Gln-E2	5 family members	Harrisburg	dominant	FD	[73]
-/-	c.492G > C/c.493C > T	p.Lys164Asn/p.Arg165Trp	3 family members	Hammersmith	dominant	FD	[109]
CM064979	c.494G > C	p.Arg165Pro	2 cases	Chicago		LPG	[28,110]
CM064979/rs121918392	c.494G > C/c.61G > A	p.Arg165Pro/p.Glu21Lys	1 case	Chicago/ApoE5		LPG	[111]
rs746494694	c.500_502delTCC	p.Leu167del	2 ADH families, 11 cases, 3 FCHL families		dominant	ADH, HC, FCHL	[13,46–48,58,59]
CM087959	c.502C > T	p.Arg168Cys	1 case, 1 family	Modena, Shenzhen		LPG (RP)	[81,112]
CM075988	c.503G > C	p.Arg168Pro	1 family	Guangzhou		LPG (RP)	[82]
CM104291	c.509C > A	p.Ala170Asp	1 case	Las Vegas		LPG	[113]
CM088091	c.502C > G-E2/E2	p.Arg168Gly-E2/E2	1 case	Okayama, ApoE1		LPG	[114]
-	c.518T > C	p.Leu173Pro	2 family members	Chengdu		LPG	[115]
-	c.520-573del54	p.Gln174_Gly190del	2 family members	ApoE1		LPG	[116]
rs7412	c.526C > T	p.Arg176Cys	hmz	ApoE2	recessive	FD	[117]
CM111115	c.527G > C	p.Arg176Pro	7 cases	Osaka/Kurashiki		LPG	[28,118,119]
CM111115	c.527G > C-E2	p.Arg176Pro-E2	2 cases	Osaka/Kurashiki		LPG	[120,121]
rs1426426514	c.592C > T	p.Arg198Cys	1 case	Baden		HTG	[122]
CM980098	c.613C > G	p.Gln205Glu	1 case	Toranomon		FD	[123]
-	c.644C > G-E2/E2	p.Ser215Cys-E2/E2	3 case	Toyonaka		LPG, FD	[78,124,125]
CD961785	c.679_688del10	p.Ala227Glyfs*20	1 family, 10 htz and 1 hmz		dominant	FD	[76]

(continued on next page)

Table 1 (continued)

rs number	cDNA position*	Protein position*	Segregation	Common name	Transmission	Phenotype	Ref.
rs121918396	c.683G > A	p.Trp228*	1 family, 2 htz and 1 hnz	Washington	dominant	FD	[126]
rs567353589	c.688G > A	p.Glu230Lys	1 family (6 htz, 2 hnz)	ApoE5	dominant	FCHL	[25]
CD961785	c.701C > T	p.Ala234Val	1 case	Nananuma		FD	[101]
rs530010303	c.703C > T	p.Arg235Trp	1 case			mixed	[46]
rs267606663	c.725G > A	p.Arg242Gln	1 case	Fukuoka		mixed	[65]
rs267606664	c.434G > A-E2	p.Gly145Asp-E2	3 family members			FD	[92]
rs121918395	c.736C > T-E2	p.Arg246Cys-E2	twins	Dumedin		HTG	[57]
-/rs121918399	c.742G > T/c.127C > T	p.Asp248Tyr/p.Arg43Cys	1 case	Hong Kong/Kyoto		LPG	[127]
rs199768005	c.761T > A-E2	p.Val254Glu-E2	1 case			mixed	[57]
rs140808909/rs190853081	c.784G > A/c.787G > A	p.Glu262Lys/p.Glu263Lys	14 cases	Suita/ApoE7		HC, mixed, HTG	[26,27,52,53,55,56]
rs267606661	c.805C > G	p.Arg269Gly	1 case, 1 family			HTG, FD	[57,94]
rs267606661	c.805C > G	p.Arg269Gly	1 case			type IIa	[46]
-	c.808C > G-c.809T > A-E2	p.Leu270Glu-E2	1 case			type IIa	[57]

* Transcript ENST00000252486.9 (Ensembl Reference Sequence), NM_000041.4 (NCBI Reference Sequence).

Htz: heterozygote. Hnz: homozygote.

ADH: autosomal dominant hypercholesterolemia (Type IIa). FCHL: familial combined hyperlipidemia (type IIb). FD: familial dysbetalipoproteinemia (type III). LPG: lipoprotein glomerulopathy. HTG: hypertriglyceridemia (Type IV and V). HC: hypercholesterolemia. RP: reduced penetrance.

dominant or codominant. Nowadays, frequently the denomination FH is used for all these forms of type IIa hyperlipoproteinemia while the most suitable denomination is Autosomal Dominant Hypercholesterolemia (ADH) as FH refers to *LDLR* mutation carriers only. There is the need of an international consensus on precise terminology to avoid misunderstanding.

ADH is one of the most frequent genetic diseases with a prevalence of 1 in 313 or 311 according to two recent meta-analyses of 11 [34] and 7.3 [35] million subjects. ADH is characterized by a selective increase of circulating Low Density Lipoproteins (LDL) in the plasma since birth giving rise to premature mortality from cardiovascular disease (CVD), with a 13-fold higher coronaropathy risk [36]. ADH has proven to be genetically heterogeneous and associated with defects in at least four different genes. In 1973, Goldstein et al. showed that ADH may result from defects in the LDL receptor that removes LDL from plasma [37]. Subsequently, Innerarity et al. revealed the genetic heterogeneity of ADH by reporting hypercholesterolemic patients with normal LDL receptor activity [38]. Their work on these patients led to the detection of the first hypercholesterolemic mutation in the *APOB* gene, which encodes the main ligand for the LDL receptor: apolipoprotein B-100 (apo B-100) implicated in the uptake of LDL particles from blood [39]. Fourteen years later, gain of function mutations in *PCSK9* (proprotein convertase subtilisin kexin 9) were reported in ADH families unlinked to either the *LDLR* or the *APOB* gene [40]. *PCSK9*, the ninth member of the proprotein convertase (PC) family, has been identified as a major regulator of cholesterol homeostasis [41]. *PCSK9* binds the extracellular epidermal growth factor-like repeat A (EGF-A) domain of the LDL receptor and thus disrupts its recycling to the cell surface by targeting it to the lysosomal pathway for degradation [42]. Further genetic heterogeneity of ADH has been established more recently, with the demonstration that 19% of ADH cases are not caused by a defect in either the *LDLR*, *APOB* or *PCSK9* gene [43]. Two other ADH loci have been localized on chromosomes 16q22.1 [44] and 8q24.22 [45], as well as mutations that segregate with the ADH disease in the apolipoprotein E (apoE) gene (*APOE*) [46–48] and the signal transducing adaptor family member 1 gene (*STAP1*) [49]. However recent functional studies showed that *STAP1* does not alter plasma LDL-cholesterol in mice and humans [50], and familial analyses showed no cosegregation with the disease for four predicted pathogenic variants [51], delisting *STAP1* as an authentic ADH gene.

Nine apoE variants (p.Glu21Lys, p.Leu46Pro, p.Gln99Lys-E4, p.Pro102Arg, p.Arg163Cys, p.Leu167del, apoE7-Suita, p.Arg269Gly, p.Leu270Glu) have been observed in single cases with hyperLDLemia (type IIa hyperlipoproteinemia), but the possible co-segregation with hypercholesterolemia in the families was not always analyzed [24,27, 46–48,52–58] (Table 1).

Two variants in the *APOE* gene, p.Arg163Cys [48] and p.Leu167del [46,47], were observed with a co-segregation with hyperLDLemia in the families. The p.Arg163Cys is carried by the mother, who is suffering from hypercholesterolemia with a LDL cholesterol of 270 mg/dL, and her son (LDL cholesterol 415 mg/dL) who is homozygote carrier [48]. The p.Leu167del variation was reported in two different families [46, 47]. A large French ADH family, with 14 affected members, was analyzed through whole genome linkage and whole exome sequencing and revealed that the p.Leu167del variation in the *APOE* gene is responsible of the ADH phenotype [46]. Shortly after, an ADH family from Italian origin with two affected members, one with tendinous xanthomas, was analyzed through whole exome sequencing and also revealed the co-segregation of the p.Leu167del variant [47]. Kinetic studies of apo B-100-containing lipoproteins was performed in one p. Leu167del carrier of the French ADH family [46]. LDL kinetic parameters were similar to those from FH patients (mutation in the *LDLR* gene) with an increased LDL pool, which was the consequence of both an increase in VLDL production rate and a decrease in LDL catabolism, and thus explaining the hyperLDLemia observed in the family [46]. An explanation of the decreased LDL catabolism was given by the study of

VLDL from p.Leu167del carriers showing a reduced expression of the *LDLR* gene when compared to VLDL from E3/E3 carriers [13] (Fig. 1).

The p.Leu167del variant has been previously shown to segregate with familial combined hyperlipidemia (FCHL) in three families with a dominant transmission pattern [59]. The p.Leu167del variant has also been previously associated with familial splenomegaly and thrombocytopenia in two unrelated probands with mild hypertriglyceridemia that worsened after splenectomy [29] and in one member from a family in which dyslipidemia segregated with the apoE p.Leu167del variant [58]. Splenomegaly has been explained by an increased uptake of mutant p.Leu167del apoE-containing lipoproteins by macrophages [29], but probably requires some other unknown defect to develop.

Modelling analysis based on the NMR structure of the full-length apoE3 isoform was used for interpreting the effect of three variations identified in patients with type IIa hyperlipoproteinemia: p.Leu46Pro, p.Arg163Cys and p.Leu167del [48]. The p.Leu46Pro variant, located in the first of the four α -helix in the N-terminal domain, inserts a proline residue and thus was predicted to be destabilizing the α -helix. A previous analysis of the E4-p.Leu46Pro variant - which is associated with an increased risk of Alzheimer's disease (AD) (odds ratio (OR) 13.2) increasing the risk of the apoE4 alone by five times [60] - showed an affected stability and structure of the protein [61]. The p.Arg163Cys and p.Leu167del variants, in the receptor-binding domain (region 154–168) (Fig. 2), are thought to impair receptor-binding properties of the apoE protein (Fig. 1). Homology modeling of the human wild type apoE protein and of the deleted mutant was used for interpreting the effect of the p.Leu167del mutant [46,47]. The p.Leu167del variation interrupts one α -helix in a group of four helices stabilized by a leucine zipper within the receptor-binding domain. Disruption of one helix in the group very probably alters the interaction with the three others. The electrostatic surface charges are altered in apoE p.Leu167del, that also likely influences interaction with lipids and affinity of apoE to its receptors [46].

Finally, among the 41 carriers of the apoE7-Suita reported, 27 presented normal lipid values, 6 were with hypertriglyceridemia, 5 were with isolated LDL increase, 2 presented mixed hyperlipidemia, and 1 was diagnosed with type III hyperlipoproteinemia (familial dysbetalipoproteinemia (FD)) (Table 2).

3. ApoE and familial combined hyperlipidemia (FCHL)

Familial combined hyperlipidemia (FCHL) is a common disorder of lipid metabolism which leads to elevated levels of VLDL, LDL, or both in plasma, leading to a mixed hyperlipidemia with both high total-cholesterol and triglycerides levels. FCHL occurs in up to 1–3% of the general population and may account for one third to one half of familial causes of early coronary heart disease [62]. The phenotype of FCHL is highly variable among family members, depending on genetic and environmental factors, and may present as mixed hyperlipidemia, isolated hypercholesterolemia, hypertriglyceridemia, or as a normal serum lipid profile in combination with abnormally elevated levels of apolipoprotein B. The presence of small dense LDL particles has also been associated with FCHL. Small dense LDL particles are generated when triglyceride-rich VLDL particles are abundant. VLDL particles can exchange their triglyceride molecules for cholesteryl esters from LDL particles which results in cholesterol-depleted LDL particles [6]. In the circulation, LDL particles are substrate for endothelial-bound lipases which leads to the formation of smaller and more dense particles [7]. Affected individuals may have variable degrees of elevated total cholesterol, triglycerides, or LDL cholesterol and are at high risk for premature atherosclerotic cardiovascular disease.

FCHL is a genetically complex disorder with reduced penetrance. Most cases of FCHL are considered polygenic with the interaction of multiple susceptibility factors. Many of the genes contributing to FCHL are unknown: susceptibility loci have been reported at 1q21-23, 11p14.1 and 16q22–24.1, and a consistent association with the

chromosome 11 loci has been shown [62]. This locus contains several candidate genes such as the upstream transcription factor 1 gene (*USF1*) which encodes a transcription factor that regulates numerous genes of the lipoprotein metabolism including *APOE*. However, several genes have already been described in FCHL and have been associated with three major metabolic pathways: adipose tissue dysfunction, hepatic fat accumulation and overproduction, disturbed metabolism and delayed clearance of apolipoprotein B-containing particles [6]. ApoE serves as the ligand for the clearance of triglyceride-rich lipoproteins and as a co-factor for lipoprotein lipase (LPL) responsible for the hydrolysis of triglycerides in VLDL driving the formation of IDL and LDL. Thus, a variation of either the structure or the function of apoE could have an impact on the metabolism and clearance of triglyceride-rich lipoproteins and the development of FCHL [6]. Eight variants in the *APOE* gene have been reported with either FCHL or mixed hyperlipidemia (Table 1). Three variants - p.Arg154Ser, p.Leu167del, p.Glu230Lys - were reported in families with a dominant transmission of a mixed hyperlipidaemia phenotype defining FCHL [25,59]. The two first variants are in the receptor-binding domain, while the p.Glu230Lys is in the hinge domain (Fig. 2). Receptor-binding studies have shown that p.Arg154Ser apoE mutant had only 41% of the apoE3 receptor binding capacity. Moreover, lipoprotein turnover studies showed a significantly reduced catabolic rate of VLDL particles from patient carrying the p.Arg154Ser variant [63] (Fig. 1). When associated with apoE2, p.Glu230Lys modifies the binding preference of apoE-containing particles from lipoprotein receptors to proteoglycans reducing the uptake and degradation of these particles. This could explain the increased of triglycerides in p.Glu230Lys carriers [64] (Fig. 1). Five variants - p.Gly145Asp-E2, p.Arg235Trp, p.Arg242Gln, p.Val254Glu-E2, apoE7 - were reported in single cases presenting with a mixed hyperlipidaemia phenotype [27,46,48,56,57,65]. However, without notion of familial segregation, it is not possible to confirm the involvement of these variants in the pathogenesis of FCHL. Furthermore, without information on the level of VLDL-remnants (IDL), it is also not possible to validate the participation of these variants in the genetic heterogeneity of type III hyperlipidaemia (familial dysbetalipoproteinemia (FD)).

4. ApoE and familial dysbetalipoproteinemia (FD)

Familial dysbetalipoproteinemia (FD), also called type III hyperlipoproteinemia, is an atherogenic disorder characterized by a mixed hyperlipidaemia, due to accumulation of VLDL remnants and IDL, similar to the FCHL phenotype. As for FCHL, the FD phenotype is highly variable. The diagnosis of FD can easily be performed by lipoprotein electrophoresis using either agarose or polyacrylamide gel disc electrophoresis or by gel-permeation high-performance liquid chromatography (GP-HPLC). FD is diagnosed based upon the presence of broad beta pattern and increase of chylomicron and VLDL remnants. FD is due to dysfunctional genetic variants of apolipoprotein E with the homozygous E2/E2 in most of the cases. However, only 1–4% of the E2/E2 carriers will develop FD [66] indicating that additional factors are necessary for manifestation of FD. In contrast to lipoprotein analysis, the identification of the apoE isoform is easily obtained by isoelectric focusing, PCR-mediated site-directed mutagenesis, or direct gene sequencing.

Thirty-one variants in the *APOE* gene have been reported in hyperlipidemic patients presenting FD diagnosed by the presence of a broad β migrating band on the electrophoresis separation of lipoproteins (Table 1). The first apoE mutant in type III hyperlipoproteinemia (FD) reported is the ApoE3-Leiden variant characterized by an in-frame repeat of 21 nucleotides in exon 4 leading to a 7 amino-acid duplication, p.Glu139_Gly145dup, localized 9 residues before the receptor-binding domain (154–168) [67]. The seven-amino acid insert introduces one extra negatively charged glutamyl residue when compared with the common apoE4 variant and thus leads to a focusing on the apoE3 position on isoelectric focusing gels [67]. ApoE3-Leiden is

Table 2
Characteristics of ApoE7-Suita variant carriers.

ApoE7 diagnosis	Age (y.o.)	Sex	Family	ApoE isoforms	Total-cholesterol	Triglycerides	LDL-cholesterol	HDL-cholesterol	Hyperlipidemia	Reference
IEF	37	M	Proband	E4/E7	316	48	298	15	hLDL	[52]
IEF	32	M	Brother	E4/E7	350	47	296	38	hLDL	[52]
IEF	57	M	Proband	E3/E7	311	121	262	38	hLDL	[52]
IEF	23	F	Daughter	E2/E7	206	82	158	38	N	[52]
IEF	74	F	Proband	E3/E7	188	146	136	31	N	[52]
IEF	40	M	Proband	E4/E7	271	586	80	26	HTG	[52]
IEF	54	-	Proband	E3/E7	235	144	-	-	N	[53]
IEF + SEQ	55	M	Proband	E3/E7	205	483	-	49	HTG	[26]
IEF	-	-	Proband	E3/E7	-	-	-	-	Type IIa	[55]
IEF	-	-	Proband	E4/E7	-	-	-	-	Type III	[55]
IEF	-	-	Proband	E4/E7	-	-	-	-	Type IV	[55]
IEF + PCR	85	F	Proband	E3/E7	184	87	86	80	N	[27]
IEF + PCR	67	M	Proband	E3/E7	177	99	122	35	N	[27]
IEF + PCR	60	M	Proband	E4/E7	265	180	191	37	N	[27]
IEF + PCR	55	M	Proband	E3/E7	201	164	137	32	N	[27]
IEF + PCR	55	M	Proband	E3/E7	259	195	188	32	N	[27]
IEF + PCR	54	F	Proband	E3/E7	202	73	122	66	N	[27]
IEF + PCR	52	F	Proband	E3/E7	203	92	114	70	N	[27]
IEF + PCR	51	M	Proband	E3/E7	183	78	124	43	N	[27]
IEF + PCR	50	M	Proband	E3/E7	304	160	217	55	hLDL	[27]
IEF + PCR	46	F	Proband	E3/E7	189	116	132	33	N	[27]
IEF + PCR	45	M	Proband	E3/E7	209	206	109	59	Mixed	[27]
IEF + PCR	32	M	Proband	E3/E7	252	199	177	35	N	[27]
IEF + PCR	26	M	Proband	E3/E7	207	183	124	46	N	[27]
IEF + PCR	22	F	Proband	E3/E7	186	49	103	73	N	[27]
IEF + PCR	21	F	Proband	E3/E7	257	84	168	72	N	[27]
IEF + PCR	17	M	Proband	E3/E7	205	90	135	52	N	[27]
IEF + PCR	17	F	Proband	E4/E7	204	78	129	60	N	[27]
IEF + PCR	17	F	Proband	E3/E7	125	182	47	61	N	[27]
IEF + PCR	66	M	Proband	E3/E7	268	157	182	36	N	[56]
IEF + PCR	60	M	Proband	E3/E7	310	167	228	46	N	[56]
IEF + PCR	44	M	Proband	E3/E7	263	507	114	50	HTG	[56]
IEF + PCR	48	M	Proband	E3/E7	127	338	39	33	HTG	[56]
IEF + PCR	55	M	Proband	E3/E7	193	326	66	41	HTG	[56]
IEF + PCR	47	M	Proband	E3/E7	281	376	-	72	Mixed	[56]
IEF + PCR	48	F	Proband	E3/E7	165	179	56	46	N	[56]
IEF + PCR	25	M	Proband	E3/E7	192	99	126	40	N	[56]
IEF + PCR	22	F	Proband	E3/E7	176	93	87	56	N	[56]
IEF + PCR	22	F	Proband	E3/E7	157	65	60	75	N	[56]
IEF + PCR	10	M	Proband	E3/E7	152	99	62	64	N	[56]
IEF + PCR	11	M	Proband	E4/E7	131	66	63	30	N	[56]

Lipid parameters are given in mg/dL. Type IIa, IIa, III, IV clinical phenotypes were distinguished according to WHO criteria. Type III hyperlipidemia was recognized by the presence of a mid-band larger than the LDL band on electrophoresis [55].

IEF: isoelectric focusing. SEQ: Sequencing of the p.Glu262Lys/p.Glu263Lys variant. PCR: PCR-mediated site-directed mutagenesis of the p.Glu262Lys/p.Glu263Lys variant. hLDL: hyperLDLemia. HC: primary hypercholesterolaemia. HTG: hypertriglyceridemia. N: normolipidemic.

defective in binding to the LDL receptor. The p.Arg154Ser variant, *APOE*-Christchurch, has been identified in hyperlipidemic patients presenting FCHL, FD or primary hypertriglyceridemia (HTG) illustrating the variability of the phenotype and the difficulty of clinical diagnosis [59,63,68–70]. The p.Arg160Cys-E4 variant has been shown to segregate with the FD phenotype in a 6-member family on the dominant mode [71]. Functional analysis of this variant either on the E3 or E4 isoform showed a significantly reduced receptor- and heparin-binding activity on human fibroblasts indicating that the cysteine at residue 160, and not the E4 arginine at residue 130, is responsible for the decreased receptor-binding activity of the variant [72]. The apoE1 p.Lys164Glu variant has also been shown to segregate with the FD phenotype in a 12-member family on the dominant mode [73] and characterized with a higher production rate, and a reduced catabolism due to its reduced affinity for the LDL receptor and for heparin [22,23]. The p.Lys164Gln, associated with the E2 isoform, has defective binding activity for the lipoprotein receptors [66]. Moreover, 4 variants - p.Trp5*, p.Arg145Cys, p.Arg154Ser, p.Ala227Glyfs*20 [68,74–76] - were shown to be inherited on the autosomal dominant mode with reduced penetrance, adding complexity to the molecular diagnosis.

5. ApoE and lipoprotein glomerulopathy (LPG)

Lipoprotein glomerulopathy (LPG) is a renal disease in which lipid deposition is limited to the kidney and is believed to cause glomerulosclerosis. This disease is characterized by glomerular lipoprotein thrombi and clinically by proteinuria and type III hyperlipoproteinemia with apoE abnormality [21,28]. The elevated plasma apoE concentration caused by reduced receptor-binding activity is an important determinant for the development of LPG. However, hyperlipidaemia in LPG is often milder than in type III hyperlipoproteinemia, or not even recognized in some cases of LPG. Seventeen variants in the *APOE* gene have been reported in lipoprotein glomerulopathy (LPG) (Table 1). Several variants were reported in single cases with various disease expression from the p.Arg132Cys in a normolipidemic patient with normal levels of apoE [77] to the p.Ser215Cys-E2/E2 in a type III hyperlipidaemic patient [78]. Four variants - p.Arg43Cys, p.Lys161_Arg165del, p.Arg168Cys, p.Arg168Pro - were reported in families with a dominant transmission and a reduced penetrance [79–82].

6. Overlaps between ADH, FCHL, FD and LPG

Mutations in the *LDLR* gene were reported in patients with a clinical diagnosis of FCHL [83]. A study conducted on 143 unrelated FCHL patients showed that 19.6% were carriers of *LDLR* mutations. Some of these mutations have been previously identified as the cause of ADH indicating that patients with ADH presenting with hypertriglyceridemia may be misdiagnosed with FCHL [83]. Interestingly, *APOE* variants were also identified in patients diagnosed with FCHL [83] and the same *APOE* variant can be reported with both FCHL and ADH. Rare *APOE* mutations are responsible for 3.5% of FCHL cases in a Spanish population [59] out of which 1.4% were carriers of the p.Leu167del variant [59] already identified as the causative mutations of ADH in two different families [46,47]. The *APOE* p.Arg163Cys variant was found, in association with the E2 isoform, in a single case with FD [84], and in a family with ADH [48]. The association of the p.Arg163Cys variant with the E2 common polymorphism may amplify effects contributing to the triglyceride elevation that differentiate FD from ADH. Moreover, the overlapping clinical presentation of FCHL and ADH exists [83], hypertriglyceridemia can sometimes be observed in ADH subjects, mainly because of the many common genetic, metabolic and environmental factors contributing to triglyceride elevation, or perhaps epigenetic and other non-mendelian interacting effects. Variants in the *LDLR* or *APOE* gene may amplify the effect of these factors, and thus, according to the number or the nature of these factors, could be associated with an overlapping phenotype between FCHL, ADH and sometimes FD when the subject is

E2/E2. The genotyping of *APOE* and the exclusion of *LDLR* and *APOE* variants in patients with combined dyslipidemia could be very informative for the differential diagnosis of lipid disorders. The current diagnostic criteria for ADH, excluding all patients presenting high triglyceride levels, could be responsible for an underdiagnosis and hence an undertreatment of the disease. Furthermore, many glomerular disorders caused by abnormalities in apoE-containing lipoproteins have been identified. Of these disorders, lipoprotein glomerulopathy and apoE2 homozygote glomerulopathy have been characterized and differentiated histologically [21]. However, both disorders present with a lipid profile similar to FD [21]. In fact, apoE homozygosity is considered responsible for FD, but only 10 cases of apoE2 homozygote glomerulopathy were reported worldwide. The presence of other factors seems necessary to trigger the disease. LPG was first identified as a glomerular disease associated with type III hypercholesterolemia. Genetic studies identified numerous heterozygotes *APOE* variants associated with LPG, most of them are located within the receptor-binding domain. Whilst, it was previously perceived that FD observed in the patients is responsible for the LPG phenotype, current studies claim that the abnormal lipid-free apoE structure and aggregation caused by *APOE* variants are the cause behind the development of LPG. Pathological studies have shown that, in contrast with homozygote E2 glomerulopathy, treatment with fibrates is effective for the management of hypertriglyceridemia in LPG, but not homozygote E2 glomerulopathy, and could prevent the glomerular damage [85,86]. Thus, both genotyping and sequencing of *APOE* could be very useful in patients with a FD-like phenotype in order to set the diagnosis, the prevention and the treatment of the glomerular diseases and their consequences on renal dysfunction.

7. Conclusion

Common *APOE* variants are associated with variations in lipid and lipoprotein levels alongside with other common genetic and environmental factors influencing the clinical presentation of lipid disorders associated with rare *APOE* variants. The identification of the *APOE* p.Leu167del variant as the causative molecular element in two different ADH families [46,47], paved the way to consider *APOE* as a candidate gene for ADH. Recent works showed that causal variants in *APOE* are not an insignificant cause of ADH with a frequency of 1.3% of affected patients in a French cohort [48] and 3.1% in a Spanish cohort [13]. On the contrary, *APOE* causative variants are not a common cause of hypercholesterolemia in a Norwegian population with a frequency of 0.2% [74]. Altogether, these results show that screening of the *APOE* gene is warranted in the setting of molecular diagnosis of ADH along with the *LDLR*, *APOB*, and *PCSK9* genes. Which will be routinely possible with the progressive use of next generation sequencing (NGS). The molecular diagnostics is warranted to allow the constitution of homogeneous cohorts of patients, and to understand the natural history and the evolution with age of lipid diseases associated with the different variants of apoE. This will also make it possible, as has been widely reported for FH, to set up cascade screening allowing the diagnosis of relatives at risk and their early treatment. Finally, for the patient itself, it has been shown that knowledge of the mutation leads to better adherence to treatment. More studies are needed to determine the spectrum of implication of *APOE* in various lipid disorders, notably ADH, in order to improve clinical and genetic diagnosis, prognosis and patient care management.

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All authors contributed to the writing of this review.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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