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REVIEW

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Establishing the relationship between familial dysbetalipoproteinemia and genetic variants in the *APOE* gene

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Abstract

Familial Dysbetalipoproteinemia (FD) is the second most common monogenic dyslipidemia and is associated with a very high cardiovascular risk due to cholesterol-enriched remnant lipoproteins. FD is usually caused by a recessively inherited variant in the *APOE* gene (ε2ε2), but variants with dominant inheritance have also been described. The typical dysbetalipoproteinemia phenotype has a delayed onset and requires a metabolic hit. Therefore, the diagnosis of FD should be made by demonstrating both the genotype and dysbetalipoproteinemia phenotype. Next Generation Sequencing is becoming more widely available and can reveal variants in the *APOE* gene for which the relation with FD is unknown or uncertain. In this article, two approaches are presented to ascertain the relationship of a new variant in the *APOE* gene with FD. The comprehensive approach consists of determining the pathogenicity of the variant and its causal relationship with FD by confirming a dysbetalipoproteinemia phenotype, and performing *in vitro* functional tests and, optionally, *in vivo* postprandial clearance studies. When this is not feasible, a second, pragmatic approach within reach of clinical practice can be followed for individual patients to make decisions on treatment, follow-up, and family counseling.

KEYWORDS

Apolipoprotein E, *APOE* gene, next generation sequencing, familial dysbetalipoproteinemia, dyslipidemia, pathogenicity, genetics, SNP, type III hyperlipoproteinemia

1 | INTRODUCTION

Familial Dysbetalipoproteinemia (FD) is the second most common monogenic dyslipidemia, with an estimated prevalence of 1 in 1000 to 1 in 2500 individuals.[1] It is characterized by a mixed hyperlipidemia (i.e., increased plasma cholesterol and triglycerides (TG)), although it can also present as predominant hypertriglyceridemia or hypercholesterolemia. The lipid abnormalities in FD are caused by cholesterol-enriched remnant lipoprotein accumulation; and associated with an increased risk of premature atherosclerotic cardiovascular disease (ASCVD). The classical diagnosis of FD requires the presence of a specific lipoprotein phenotype obtained by ultracentrifugation,[2] as well as pathogenic variants in the *APOE* gene that predispose to FD. Because ultracentrifugation is often not available in clinical practice, approaches using apolipoprotein B (apoB) can be used to establish a dysbetalipoproteinemia phenotype. In most cases (90%) the genetic basis of FD is homozygosity for the ε2 allele (ε2ε2 genotype). The other 10% of cases consist of other variants, of which 23 have been described (Supplementary Table 1). Rarely, hepatic lipase deficiency is responsible for a similar dysbetalipoproteinemia phenotype.[6] Generally, only 10%–15% of people with an ε2ε2 genotype develop the specific dysbetalipoproteinemia phenotype later in life, involving additional metabolic stress, usually obesity, insulin resistance or diabetes mellitus.[7,8] FD has a genetic background and is therefore hereditary, but in most cases it is a recessive disorder, with a low penetrance. So although FD is a genetic disease, the disorder does not usually run in the family and is therefore not "familial." When FD is suspected, genetic testing should be performed to confirm the diagnosis. Many laboratories can perform *APOE* genotyping for the common isoforms in the *APOE* gene (ε2, ε3, or ε4). When ε2 homozygosity is ruled out, the next step is Next Generation Sequencing (NGS) to identify other variants in the *APOE* gene.

It can however, be difficult to translate the results of NGS to clinical practice, for example when NGS reveals a variant in the *APOE* gene that has not been described before in a patient with a dysbetalipoproteinemia phenotype. The question arises: is the variant causally related to the observed lipid abnormalities? Furthermore, it is not uncommon that a new variant in the *APOE* gene is detected without an initial clinical suspicion of FD. In this case the question is whether there is a dysbetalipoproteinemia phenotype in the patient, and if so, if the variant is causally related to the observed lipid abnormalities. In this article, we discuss two approaches to establish whether a new *APOE* variant is causally related to FD. The first is a comprehensive approach that consists of determining the pathogenicity of the variant and its causal relationship with FD by confirming the dysbetalipoproteinemia phenotype; and by performing *in vitro* functional tests and, optionally, *in vivo* postprandial clearance studies. When this approach is not feasible, a second, pragmatic approach within reach of clinical practice is suggested, that can be followed for individual patients to make decisions on treatment, follow-up, and family counseling.

2 | DIAGNOSING FD

Before the two approaches will be outlined, a brief introduction to FD and the *APOE* gene will be provided in this section. The dysbetalipoproteinemia phenotype of FD, also known as hyperlipoproteinemia type III or remnant removal disease, is characterized by the accumulation of cholesterol-enriched remnant lipoproteins, usually reflected in a mixed hyperlipidemia. In general, men develop FD in young adulthood and women after menopause.[9] Although very rare, finding an orange palmar crease xanthoma on physical examination of the patient, is considered pathognomonic.[10] FD confers a very high risk of premature ASCVD, and timely and adequate lipid-lowering treatment is important to lower ASCVD risk.[11,12] Furthermore, when TGs are >10 mmol/L, these patients are also at risk for pancreatitis. Diagnosis of FD results in a clear treatment strategy of dietary lipid restriction along with prescription of statins and fibrates. Non-high density lipoprotein cholesterol (non-HDL-C) rather than low-density lipoprotein cholesterol (LDL-C) is used as treatment goal to ensure best control of atherogenic lipoproteins.[13] In addition, risk calculators to estimate 10-year ASCVD risk are not applicable in genetic lipid disorders, including FD, as they underestimate the true ASCVD risk.

A formal diagnosis of FD requires the demonstration of the dysbetalipoproteinemia phenotype *and* an *APOE* genotype that is shown to be causally related to FD (i.e., the ε2ε2 genotype or any of the rare variants described in Supplementary Table 1). Making a formal diagnosis of FD is important for several reasons. First, not all pathogenic variants in *APOE* are causally related to FD, even when patients present with hyperlipidemia. Variants in *APOE* have been associated with LDL hypercholesterolemia resembling Familial Hypercholesterolemia (FH),[14,15] hypertriglyceridemia[3] or lipoprotein glomerulopathy.[16] Other pathogenic variants in *APOE* are linked to neurological dysfunction or Alzheimer's disease, age-related macular degeneration[17] or sea blue histiocytosis.[18] Second, not all patients with a pathogenic variant for FD develop the dysbetalipoproteinemia phenotype (incomplete penetrance). This is best illustrated by the ε2ε2 genotype. Only 10-15% of subjects with this genotype develop the dysbetalipoproteinemia phenotype although functional tests have demonstrated that all apoE2 protein binds with less than 2% to the LDL-receptor (LDL-R) compared to the apoE3 protein.[9,19] Thus, despite apoE2 being pathogenic, not all patients carrying it will have (or get) the disease.[1,7] Third, it was demonstrated that only a minority (38%) of patients with an ultracentrifugally proven dysbetalipoproteinemia phenotype, has the ε2ε2 genotype and the remainder are presumed to have a multifactorial dysbetalipoproteinemia phenotype.[20] This is relevant because, in that study, patients that had a dysbetalipoproteinemia phenotype and an ε2ε2 genotype had an 11-fold increased risk of peripheral artery disease compared to those with the dysbetalipoproteinemia phenotype without the ε2ε2 genotype.[20] For these three reasons it is important to determine the presence of a specific dysbetalipoproteinemia phenotype and genotype, when making a FD diagnosis.

TABLE 1 Cut-offs and diagnostic properties of laboratory tests to establish an FD lipoprotein phenotype

Laboratory test	Cut-off	Sensitivity (compared to ultracentrifugation)	Specificity (compared to ultracentrifugation)	References
Ultracentrifugation (reference standard)	*VLDL-C/VLDL-TG molar ratio*: >0.97 (or mass ratio >0.42 mass) *VLDL-C/total TG molar ratio*: >0.69 (or mass ratio >0.30) Suggestive: molar ratio >0.57 (or mass ratio >0.25)	–	–	2
PGGE (qualitative)	Increased IDL and/or VLDL and no detectable LDL	–	–	22
PGGE (quantitative)	Videodensitometric analysis of the ratio of area under the curve > 0.5 for IDL-LDL	89%	100%	
Non-HDL-C/apoB ratio	>4.91 mmol/g	96.8% (95%CI 89.0–99.6)	95.0% (95%CI 93.8–96.0)	25
Non-HDL-C/apoB ratio	>3.69 mmol/g	94.8% (95%CI 90.0–97.7)	66.1% (95%CI 64.7–67.6)	26
ApoB/TC ratio	<0.15 g/mmol	89% (95%CI 78–96)	97% (95%CI 94–98)	24
ApoB, TC, and TG levels	3-step-algorithm. (1) TG >75th percentile (2) TC/apoB ratio ≥6.2 mmol/g (3) TG/apoB ratio <10.0 mmol/g	AUC-ROC of combination 0.988		27

3 | THE DYSBETALIPOPROTEINEMIA PHENOTYPE

The dysbetalipoproteinemia phenotype cannot be detected with the standard investigations for dyslipidemia alone. Standard investigations comprise total cholesterol (TC), HDL-C, TG, and LDL-C. In FD standard investigations will often result in a non-specific mixed hyperlipidemia. The reference standards for determining the dysbetalipoproteinemia phenotype are ultracentrifugation and polyacrylamide gradient gel electrophoresis (PGGE), although the specific dysbetalipoproteinemia pattern is also recognized by paper-, cellulose acetate- or agarose electrophoresis.[21] In addition, although the broad beta band on agarose gel electrophoresis was found to be highly specific for dysbetalipoproteinemia it had low sensitivity compared with PGGE.[22] The dysbetalipoproteinemia phenotype is defined by ultracentrifugation as an increased ratio of cholesterol to TG within very-low density lipoprotein (VLDL) (>0.42 by mass or >0.97 by molar measurements) or increased VLDL-C/total plasma TG ratio (>0.30 or >0.69 by mass or molar measurements, respectively; and respectively, >0.25 and >0.57 ratios are suggestive/borderline).[2,23] With PGGE a dysbetalipoproteinemia phenotype displays lipid staining in the intermediate-density lipoprotein (IDL) and/or smaller VLDL range, with little or no LDL.[22] When these methods are not available, the measurement of apoB is recommended to distinguish FD from other causes of mixed dyslipidemia such as familial combined hyperlipidemia (FCHL).[24–27] Several approaches to establish a dysbetalipoproteinemia phenotype based on apoB have been developed. Compared to ultracentrifugation, the sensitivity of these approaches ranges from 89% to 97% and the specificity ranges from 95% to 97%. The diagnostic approach with the best diagnostic properties is the non-HDL-C/apoB ratio, with a cut-off of >4.91 mmol/g (sensitivity 96.8% (95% CI 89.0–99.6) and specificity 95.0% (95% CI 93.8–96.0)). All diagnostic methods for the dysbetalipoproteinemia phenotype are summarized in Table 1.

4 | ANALYSIS OF GENETIC VARIANTS IN THE *APOE* GENE

Pathogenic variants in the *APOE* gene that have been shown to have a causal relationship with the dysbetalipoproteinemia phenotype are listed in Supplementary Table 1. Pathogenicity in general is the process in which a genetic variant leads to translation of a dysfunctional protein with pathogenic mechanistic properties.

As mentioned before approximately 10% of FD patients have other variants than ε2ε2 in *APOE*, and those variants are often inherited in a dominant mode.[4] Some variants inherit in a co-dominant fashion, meaning that the isoform of the other allele determines the outcome: if the other allele is ε2, the condition will resemble ε2 homozygosity. When a new variant is detected by NGS, the variant is classified on general genetic principles rather than specific mechanistic studies that would determine a causal relationship between gene and disease. Classification is based on the guidelines by the American College of Medical genetics and genomics (ACMG).[28] These are general guidelines, and therefore not specific for the *APOE* gene and not aimed at identifying FD. In brief, variants are placed in 5 classes: "benign" (class 1), "likely benign" (class 2), "uncertain significance" (class 3), "likely pathogenic," (class 4) or "pathogenic" (class 5). The classification of pathogenicity is based on several levels of evidence ranging from very strong to supportive. There are many types of evidence that can be used to determine pathogenicity, the details of

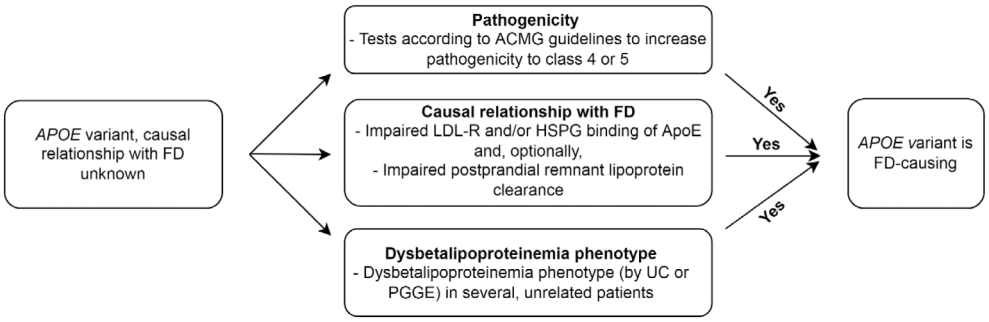

FIGURE 1 Comprehensive evaluation of an *APOE* variant for causal relationship for FD. When the causal relationship with FD of a variant in the *APOE* gene is unknown, attempts should be made to evaluate this. The assessment should following 3 steps. The first step is determining pathogenicity of this variant according to the ACMG guidelines; the second step is determining a causal relation with FD by *in vitro* functional studies (impaired LDL-R and/or HSPG binding of apoE) and, optionally, *in vivo* functional studies (impaired postprandial lipoprotein clearance). The third step is demonstration of a dysbetalipoproteinemia phenotype in several, unrelated patients with the same variant. Class 4 variant, likely pathogenic variant, class 5 variant, pathogenic variant. ACMG, American College of Medical Genetics and Genomics; ApoE, Apolipoprotein E; FD, Familial Dysbetalipoproteinemia; HSPG, heparan sulphate proteoglycan; LDL-R, low-density lipoprotein receptor; PGGE, polyacrylamide gradient gel electrophoresis; UC, ultracentrifugation

which are outside the scope of this article. Examples of strong evidence are *in vitro* and *in vivo* functional studies or an increased prevalence of the variant in affected subjects, compared to controls. An example of moderate evidence is that the variant is located in a functional domain of a protein. Examples of supporting evidence are the presence of a highly specific phenotype and *in silico* predictions. *In silico* predictions are based on the probable impact of amino acid substitutions on the structure and function of a protein (based on the degree of evolutionary conservation of the wild type amino acid and the 3D structure of the new protein).[28,29]

5 | APPROACHES TO ESTABLISH A CAUSAL RELATION BETWEEN A NEW *APOE* VARIANT AND FD

When NGS reveals a variant in *APOE* of which the causal relationship with FD is unknown we suggest two approaches. The comprehensive approach consists of determining the pathogenicity of the variant and its causal relationship with FD by confirming a dysbetalipoproteinemia phenotype using reference methods, and performing *in vitro* functional tests and, optionally, *in vivo* postprandial clearance studies. We strongly recommend that when the comprehensive method is used for a new variant to establish or exclude a causal relationship with FD, the results of this research should be published in peer-reviewed journals for use in clinical practice. However, this approach requires resources, infrastructure, specific expertise and time. Therefore, a pragmatic approach is suggested which describes how to make clinical decisions by combining presence of the dysbetalipoproteinemia phenotype with the (preliminary) degree of pathogenicity of the variant.

5.1 | Comprehensive approach

The comprehensive approach consists of three parts: 1. determining pathogenicity; 2. determining a causal relation with FD; and 3. determining a dysbetalipoproteinemia phenotype in several, unrelated patients with the same variant (Figure 1). All three steps are necessary to make a definite FD diagnosis, although point 2 can be part of point 1, as will be explained later.

The first step is to determine the pathogenicity of the variant, using the ACMG guidelines as was described in the previous paragraph.

Step two of the comprehensive approach is determining the causal relationship of the variant with FD. This should be done by establishing impaired LDL-R and/or heparan sulphate proteoglycan (HSPG) binding of remnant lipoproteins by *in vitro* functional hepatic receptor binding studies. Delayed postprandial remnant clearance with *in vivo* functional tests can be used to confirm the causal relationship with FD. An example of a postprandial remnant clearance study can be to evaluate the effect of an oral fat load (e.g., with fresh cream) and to assess retinyl palmitate levels up to 12 or even 24 h after ingestion of the oral fat load, and to compare the response with healthy subjects. Inclusion of retinyl palmitate to the oral fat load enables tracking chylomicrons and their remnants.[30] *In vitro and in vivo* functional tests can, but do not have to be part of the determination of pathogenicity in step one. Geneticists are free to decide which levels of evidence from the ACMG guidelines they use to determine the pathogenicity of a variant. Although in most cases functional tests are likely to be part of the pathogenicity assessment, this is not essential if other criteria provide sufficient evidence for the pathogenicity of the variant. The third step in the comprehensive approach is to determine whether the variant is associated with the dysbetalipoproteinemia phenotype in several, unrelated patients with the same variant in *APOE* using the reference standards. It should be noted here that, at least theoretically, subjects carrying an *APOE* variant that is causally related to FD may not (yet) have developed the specific dysbetalipoproteinemia phenotype. That is the reason we recommend using several patients for establishing the dysbetalipoproteinemia phenotype. When a variant has been shown to be pathogenic and to lead to impaired receptor binding of the ApoE protein, it can still be classified as FD-causing, even when not all patients carrying the variant express the dysbetalipoproteinemia phenotype. However, when

TABLE 2 ApoE-Leiden (p.Glu165-Gly171dup) variant in the *APOE* gene

Phenotype assessment		Comment and explanation
Ultracentrifugation		Several studies showed presence of beta-VLDL and VLDL-C/plasma TG >0.69 mmol/L in several unrelated heterozygotes
Pathogenicity assessment according to ACMG guidelines		
Criterion	Weight	
Functional tests	Strong	*In vitro*: LDL-R binding is 11%–25%, HSPG binding is 5% compared to apoE3 protein
Location in gene	Moderate	Location 165–171 is not in functional domain (but variant influences the functional domain)
Protein length changes as a result of inframe insertions	Moderate	ApoE-Leiden consists of tandem repeat.
Patients phenotype (highly) specific for a disease	Supporting	FD lipoprotein phenotype confirmed in subjects evaluated in several studies
Cosegregation with disease in multiple affected family members	Supporting	In one kindred 100% segregation of genotype and phenotype
Conclusion	(1) FD lipoprotein phenotype? Yes (2) (Likely) pathogenic? Yes 1. Strong criterion, 2. Moderate criteria and 2 supporting criteria for pathogenicity met, resulting in class 5 (pathogenic)	Variant is FD-causing

Note: Based on previous publications.[19,31,32]

the patients are under sufficient metabolic stress (e.g., metabolic syndrome, diabetes mellitus, or post-menopausal state in women), and still lack the specific phenotype, a definite relationship with FD cannot be determined and careful monitoring of the lipoprotein profiles is warranted.

Two examples of how to use the comprehensive approach are provided in Tables 2[31,32] and 3.[33,34] The first example describes the apoE-Leiden (p.Glu165-Gly171dup (NM_001302688.1, Supplementary Material)[28] variant in the *APOE* gene. In this example there are five arguments for pathogenicity (according to the ACMG guidelines): one strong, two moderate and two supporting. These criteria are sufficient to classify the variant as pathogenic (class 5). Furthermore, the causal relationship with FD was established with functional *in vitro* tests showing decreased LDL-R binding of the apoE-Leiden protein. In addition, the specific dysbetalipoproteinemia phenotype was demonstrated in several unrelated patients that carried this variant, using ultracentrifugation (the reference standard). A causal relationship between this *APOE* variant and FD is thus verified.

The second example describes the p.Leu72Pro variant in the *APOE* gene. This variant does not affect the part of the ApoE protein that is critical for the clearance of remnant lipoproteins, but does typically disrupt protein structure. The likely pathogenic (class 4) status of the variant was established with one strong and two supporting arguments according to the ACMG guidelines. Binding of this apoE protein to the LDL-R was, however, normal and postprandial remnant clearance was not impaired. None of the patients had a specific dysbetalipoproteinemia phenotype determined by ultracentrifugation. A causal relationship of this variant of apoE with FD was thus excluded. This example shows that a putative pathogenic variant in *APOE* is not always causally related to FD, although the variant may still be related to dyslipidemia or other disorders.

5.2 | Pragmatic approach

Healthcare providers could be faced with a situation in which an *APOE* variant is found in a patient, but definitive information on the relationship between this variant and FD is not (yet) available. To provide some guidance in these situations, the following pragmatic approach is suggested for individual patients (Table 4).

When a patient presents with hyperlipidemia and FD is suspected, apoB-based diagnostic methods should be used to establish a dysbetalipoproteinemia phenotype (or, if available, one of the reference standards) (Table 1). Second, the preliminary classification of the pathogenicity of the variant should be taken into account. This classification should be provided by the genetic laboratory that performed the NGS.

When a patient has a variant that is classified as (likely) pathogenic (class 4/5) and the patient has a dysbetalipoproteinemia phenotype according to an apoB-based diagnostic strategy such as the non-HDL-C/apoB ratio, the patient can be classified as having presumptive FD. In this case the patient can be treated as FD, but a definite diagnosis can only be made by following the comprehensive approach. When a patient has a class 3 (unknown significance) variant and the dysbetalipoproteinemia phenotype is present, the patient can be diagnosed as having probable FD and can be treated accordingly.

When a variant is (likely) pathogenic (class 4/5) and the dysbetalipoproteinemia phenotype is *not* present, there are three possibilities to consider. First, the variant may not be causal for FD (e.g., the p.Leu72Pro variant). Second, the variant causes FD, but due to delayed penetrance, has not come to expression yet. This can be the case when a variant is found in cascade screening. A third reason for the

TABLE 3 p.Leu72Pro variant in the *APOE* gene

Phenotype assessment		Comment and explanation
Ultracentrifugation		In homozygotes: None VLDL-C/VLDL-TG molar ratio >0.97 or VLDL-C/plasma TG molar ratio >0.69 In 60 heterozygotes: No specific hyperlipoproteinemia phenotype
Pathogenicity assessment according to ACMG guidelines		
Criterion	Weight	
Functional tests	Strong	*In vitro*: Excluded a binding defect to LDL-R. *In vivo*: Excluded accumulation of remnants
Compare prevalence variant in controls/cases (OR ≥ 5.0) or CI of OR does not include 1.0.	Strong	OR for CAD 3.1 (95% CI 1.20–8.0) in carriers relative to non-carriers.
Location in gene	Moderate	Location 72 is not in functional domain.
Absent from controls	Moderate	Prevalence of p.Leu72Pro in European (non-Finnish) population: 0.34%
Patients phenotype (highly) specific for a disease	Supporting	All four homozygotes suffered from various forms of hyperlipoproteinemia and had three different types of hypertriglyceridemia
Cosegregation with disease in multiple affected family members	Supporting	Heritability and cosegregation of genotype and phenotype were studied in 7 study participants and 56 of their relatives. Genotype and phenotype were congruent in all families
Multiple lines of computational evidence of a deleterious effect	Supporting	In silico predictions on Gnomad. Polyphen: possibly damaging, SIFT: tolerated
Conclusion	1) FD lipoprotein phenotype? No 2) (Likely) pathogenic? Yes, 1 strong, 2 supporting	Variant is likely pathogenic according to ACMG guidelines, but does not cause FD. However, this variant can increase risk for atherosclerosis by other (dyslipidemia) mechanisms

Note: Based on previous publication about the p.Leu72Pro variant and website of Gnomad.[33,34]

TABLE 4 Pragmatic approach to diagnose FD in an individual patient

Phenotype		Non-HDL-C/apoB ratio >4.91 mmol/g (or if available: ultracentrifugation or PGGE)	
Pathogenicity		**Yes**	**No**
(Likely) pathogenic (class 4/5)	Yes	Presumptive FD (treat as FD)	Unknown • Variant is not causally associated with FD • Variant may eventually lead to FD under sufficient metabolic stress
	No	Possibly FD (treat as FD)	Exclude FD • Monitor updates on pathogenicity classification and lipoprotein phenotype of patient

absence of the dysbetalipoproteinemia phenotype, could (theoretically) be the limited specificity of the apoB algorithm.

When the variant is classified as class 3 and the dysbetalipoproteinemia phenotype is *not* present, the diagnostic label of FD should not be used until the pathogenicity of the variant is clear from (functional) studies or the dysbetalipoproteinemia phenotype supervenes.

Although the causal relationship with FD can only be determined by specialized laboratories using data of several, unrelated patients, as described in the comprehensive approach, it is possible for individual health care providers to shed some light on the potential relation between the *APOE* variant and FD in the individual patient. This can for example be useful when a variant is classified as class 3 (unknown significance). First, *in silico* predictions can be used. Several in silico prediction software programs can be found on www.gnomad.broadinstitue.org. However, multiple *in silico* prediction tools sometimes provide inconsistent results for the same variants, so results should be interpreted with caution. Second, the location of the variant on the gene can be considered. The LDL-R binding domain of apoE is the most vulnerable region and is located in the fourth helix, at position 180-194 (NM_001302688.1; Supplementary Material),[28,35-70] so when a variant is located there, the variant is more likely to be pathogenic. When using these methods it is important to note that they can never by themselves provide definite information on the causal

relationship between a genetic variant and FD. Furthermore, treatment decisions are made based on the presence of a dysbetalipoproteinemia phenotype, and these strategies (*in silico* predictions or gene location) can only be supportive in this regard.

6 | DISCUSSION AND CONCLUSION

FD is a complex disorder with a very specific dysbetalipoproteinemia phenotype, a delayed penetrance, and a heterogeneous genetic basis. Not all pathogenic variants in the *APOE* gene are causally related to FD, and not all patients with a genetic predisposition to FD develop the dysbetalipoproteinemia phenotype (incomplete penetrance). The diagnosis of FD can therefore only be made by demonstration of both the specific dysbetalipoproteinemia phenotype and a specific causal *APOE* genotype.

In this article, two strategies are proposed to establish whether a variant in *APOE* causes FD. The first approach requires comprehensive investigation which is only feasible at specialized laboratories which should collect information in several unrelated patients with the same variant. The second, pragmatic approach is aimed at clinical practice. This approach requires the addition of apoB to demonstrate the dysbetalipoproteinemia phenotype (although with less confidence).

Currently, the ACMG guidelines standardize the classification and reporting of the pathogenicity of all new genetic variants, irrespective of the gene or the disease. When a (likely) pathogenic variant in *APOE* is automatically classified as FD causing, without determining a causal relationship, this might lead to misdiagnosis of patients.

Cooperation between physicians and laboratories is encouraged to investigate clusters of patients with the same variant. A registry of new variants in the *APOE* gene, that includes lipid profiles of patients, will enhance linking novel genetic variants to FD. Such information should be published according to ClinVar (a public database for clinical laboratories, researchers, expert panels, and others to share their interpretations of variants along with their evidence) and ClinGen regulations.

The main limitation of this article is that the recommendations are based on expert opinion. This article was written to address a current need for guidance in the interpretation of the relationship between new variants in the *APOE* gene and FD in clinical practice, but further studies to substantiate these approaches are warranted.

To conclude, FD is an important cause of mixed hyperlipidemia that is highly atherogenic and whose diagnosis consists of a specific phenotype and genotype. To evaluate whether a new *APOE* variant is causally related to FD is challenging. In this article, we present two approaches that can be followed. The comprehensive approach consists of determining the pathogenicity of the variant and establishing a causal relation with FD in several unrelated patients with the same variant with more detailed lipoprotein characterization and functional studies. The pragmatic strategy was developed for clinical practice and can be followed for individual patients to make decisions on treatment, follow-up, and family counseling.

AUTHOR CONTRIBUTIONS

All authors contributed to either the acquisition, analysis, or interpretation of the data for the work. All authors have given final approval of the manuscript, and agree to be accountable for the work.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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