

DYSBETALIPOPROTEINEMIA (TYPE III HYPERLIPOPROTEINEMIA)

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ABSTRACT

Dysbetalipoproteinemia is an underrecognized and underdiagnosed genetic lipid disorder characterized by pathogenic variants in the *APOE* gene, which encodes apolipoprotein (apo) E. It leads to the abnormal accumulation of triglyceride-rich remnant lipoproteins, elevated levels of both cholesterol and triglycerides, and an increased risk of cardiovascular disease. Typically, patients with autosomal recessive form of dysbetalipoproteinemia are homozygous for the $\epsilon 2$ allele, which is associated with decreased binding of apo E to the LDL receptor and/or heparan sulfate proteoglycans, resulting in impaired remnant clearance. However, only a minority of apo $\epsilon 2$ homozygotes become hyperlipidemic, often due to metabolic conditions that either increase lipoprotein production or decrease remnant clearance. Rarer variants in the *APOE* gene are linked to autosomal dominant dysbetalipoproteinemia. Palmar xanthoma is considered a characteristic feature of dysbetalipoproteinemia, although it is observed in fewer than half of affected individuals. Both total cholesterol and triglyceride levels are typically elevated and may be of similar magnitude. A low apo B level relative to a high total cholesterol level or a discrepancy between calculated LDL-cholesterol (LDL-C) and direct LDL-C levels can raise suspicion of this condition. There is no simple diagnostic test for dysbetalipoproteinemia, and diagnosis traditionally requires the detection of β -VLDL (remnant lipoproteins) and pathogenic variants in the *APOE* gene, both of which are not routinely available in

clinical laboratories. Several algorithms using various lipid and apo B parameters have been proposed for screening and selecting candidates for genetic testing. Recent data suggest that the phenotype of dysbetalipoproteinemia is heterogeneous. The term multifactorial remnant cholesterol disease has been proposed to describe a milder form of dysbetalipoproteinemia in individuals without the apo $\epsilon 2/\epsilon 2$ genotype, differentiating them from the more severe form associated with apo $\epsilon 2/\epsilon 2$ genotype. Patients with dysbetalipoproteinemia are at an increased risk of cardiovascular diseases, particularly coronary artery disease and peripheral arterial disease. However, they generally respond well to lifestyle modifications and conventional lipid-lowering therapies, including statins and fibrates.

INTRODUCTION

Dysbetalipoproteinemia has been recognized since the 1950s and several names have been used in the literature, including xanthoma tuberosum, familial dysbetalipoproteinemia, broad beta disease, type III hyperlipoproteinemia, and remnant removal disease. Originally described by Gofman and colleagues, affected patients developed tuberous xanthoma of the extensor tendons and palmar xanthoma of the skin creases (1). An abnormal lipoprotein profile determined by analytical ultracentrifugation showed an increase in lipoproteins corresponding to small very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL). Using a combination of ultracentrifugation and paper electrophoresis, these

cholesterol-enriched lipoprotein fractions displayed abnormal flotation and β electrophoretic mobility, instead of the normal pre- β mobility (2), and were referred to as floating beta lipoproteins or β -VLDL (3,4). The term “dysbetalipoproteinemia” was used to describe the presence of these β -VLDL in the circulation although the amount may not be high enough to cause elevated lipid levels. Overt hyperlipidemia observed in certain patients with dysbetalipoproteinemia was identified as being identical to type III hyperlipidemia, as classified by Fredrickson et al. in 1967 (4). The term “broad beta disease” represented the peculiar migration pattern of these abnormal β -VLDL on paper electrophoresis (4). These β -VLDL were shown to be remnants of apolipoprotein (apo) B-containing lipoproteins of both hepatic and intestinal origin (5), which accumulated in the plasma due to defective clearance (6). Havel and Kane later demonstrated that subjects with type III hyperlipidemia exhibited elevated levels of apo E, originally referred to as arginine-rich protein (7). Using isoelectric focusing, Utermann et al. found that apo E3 was absent in these subjects (8) and homozygosity for the pathogenic variant of apo E, referred to as apo E2 as opposed to the normal apo E3, was later found to be the underlying genetic defect (9,10). The complete amino acid sequences of different isoforms of apo E were determined by Mahley et al., which helped define the molecular abnormality of apo E in the pathogenesis of dysbetalipoproteinemia (11). Apo E was subsequently shown to be a major ligand for the LDL receptor and heparan sulfate proteoglycans (HSPGs), establishing apo E as the main apolipoprotein responsible for the uptake of remnant particles into hepatocytes (12). Defective binding of apo E to the receptors and impaired hepatic uptake could therefore explain the accumulation of remnant lipoproteins in these patients (13), although other environmental factors could modulate the expression of the abnormal lipid profile.

It is important to note that different terminology is often used in the literature to describe dysbetalipoproteinemia. In general, the term

dysbetalipoproteinemia is used to indicate the presence of β -VLDL remnant particles in the circulation, whereas type III hyperlipidemia or type III hyperlipoproteinemia refers to the hyperlipidemic phenotype resulting from the accumulation of these remnant lipoproteins. In this article, we use the term dysbetalipoproteinemia to refer to the lipoprotein disorder characterized by the presence of β -VLDL in the circulation, which is often associated with hyperlipidemic phenotype.

EPIDEMIOLOGY

The prevalence of dysbetalipoproteinemia varies depending upon the definition used for diagnosis and the study population. Using the original gold standard diagnostic criteria by Fredrickson et al. (14) (a VLDL-cholesterol/plasma triglyceride (VLDL-C/TG) ratio >0.30 and the presence of β -VLDL on gel electrophoresis without requiring apo E genotype), the population-based prevalence of dysbetalipoproteinemia in the Northern American population was reported around 0.4% (1 in 250) in men aged 20 years or older and 0.2% (1 in 500) in similarly aged women (15,16). The prevalence is higher in men than in women and it tends to occur earlier in men (17,18). A similar prevalence of 0.2-0.4% was reported from a free-living population in California and in Vermont using lipoprotein electrophoresis as a diagnostic tool (19,20). In the 2011-2014 National Health and Nutrition Examination Survey (NHANES) participants in the U.S., the prevalence of 0.2-0.8% was reported using the lipoprotein levels from ultracentrifugation, but it increased to 1.97% when using only lipid and apo B levels (21). In studies using both lipid levels and the apo E genotype, a prevalence of 0.1% (1 in 889) was reported among 8,888 Dutch population (22), and a prevalence of 0.2% (1 in 469) was reported from 452,469 UK Biobank participants (23). The prevalence among different genetic ancestries was relatively similar and did not exceed 0.2% in any ancestry (23). Another study in Russia showed a prevalence of 0.67% (1 in 150) using the apo E genotype and

triglyceride level ≥ 130 mg/dL or 1.5 mmol/L (24). Collectively, the overall prevalence of dysbetalipoproteinemia, based on gold standard criteria and genetic testing, is estimated to be around 0.1–0.8%. Interestingly, this estimate is comparable to that of familial hypercholesterolemia (25).

GENETICS

Dysbetalipoproteinemia is caused by a genetic defect in the *APOE* gene, which encodes apo E. Apo E is a polymorphic glycoprotein found in various lipoprotein particles, including chylomicrons, chylomicron remnants, VLDL, VLDL remnants, and HDL. The main function of apo E is to mediate the interaction between apo E-containing lipoproteins and lipoprotein receptors. The N-terminal domain of apo E is involved in the interaction with the LDL receptor, the LDL receptor-related protein (LRP), and HSPGs, whereas the C-terminal domain is responsible for lipid binding. Amino acid residues 154-168 in the N-terminus contained several critical basic amino acids (i.e., arginine and lysine) that interact with acidic amino acid residues of the lipoprotein receptors and HSPGs.

The *APOE* gene is in the apolipoprotein gene cluster on the long arm of chromosome 19. It has 4 exons and 3 introns. Apo E is primarily synthesized in the liver, but other tissues can also produce apo E, including the brain, spleen, lung, kidneys, adrenals, ovaries, macrophages, and smooth muscle cells (26). After cleavage of the 18-amino acid signal peptide, the mature apo E protein has 299 amino acids. Three major isoforms of apo E (E3, E2, and E4) exist, which are caused by a single amino acid substitution at two different sites of the protein (27) as shown in Table 1. The differences among these isoforms result from different apo E alleles. The alleles are given designations using the Greek letter epsilon, i.e., $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. Although the $\epsilon 3$ is suggested to be a normal or wild-type allele, evidence exists that $\epsilon 4$ allele may be the ancestral allele (28). The 3 apo E alleles yield 6 possible phenotypes, i.e., E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4. The classical molecular abnormality causing dysbetalipoproteinemia is the homozygous variant known as the E2/E2 phenotype, which leads to a substitution of arginine for cysteine at position 176 (p.Arg176Cys). This variant is associated with an autosomal recessive inheritance of dysbetalipoproteinemia.

Table 1. Major Isoforms of Apo E Due to Different Amino Acids and Charges			
Isoform	E2	E3	E4
Apo E allele	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$
rs number	rs7412	-	rs429358
HGVSc	c.526C>T	-	c.388T>C
HGVSp	p.Arg176Cys	-	p.Cys130Arg
Residue 130 (112*)	Cys	Cys	Arg
Residue 176 (158*)	Cys	Arg	Arg
Charge	0	+1	+2
Lipoprotein preference	HDL	HDL	VLDL

* Used in the old literature, which does not include the 18-amino acid signal peptide.

In almost all populations, the $\epsilon 3$ allele makes up a majority of the apo E gene pool (70-80%), followed by $\epsilon 4$ (10-15%) and $\epsilon 2$ (5-10%). Therefore, the most common phenotype is the apo E3/E3 phenotype, which is found in 50-70% of the population, whereas the apo E2/E2 phenotype is relatively rare (24). Data

from the UK Biobank indicate that apo E2 homozygosity is present in 0.2–1.3% of individuals, depending on genetic ancestry (23), and less than 20% of those with the apo E2/E2 phenotype develop overt hyperlipidemia (22), despite having

demonstrable β -VLDL in the plasma, characteristic of dysbetalipoproteinemia.

Rarer variants in the *APOE* gene cause an autosomal dominant form of dysbetalipoproteinemia. Except for *APOE* Leiden, which has a tandem duplication of 21 nucleotides coding for 7 amino acids, most of these rare variants involve substitutions of neutral or acidic amino acids for basic ones in the critical amino acid residues 154-168 that interact with lipoprotein receptors. The p.Arg163Cys variant is particularly common in subjects of African descents with the prevalence of 5-12% (29). Another rare cause of autosomal dominant dysbetalipoproteinemia is due to apo E deficiency (30,31).

PATHOPHYSIOLOGY

Chylomicrons produced by the small intestine and VLDL produced by the liver are both processed by lipoprotein lipase in the lipolytic cascade, resulting in triglyceride hydrolysis and the formation of chylomicron remnants and VLDL remnants, respectively. Normally, these remnant lipoproteins are cleared by receptors in the liver, including the LDL receptor and LDL receptor-related protein (LRP). Apo E plays a critical role in the binding, uptake, and

hepatic clearance of remnant lipoprotein particles. Synthesized primarily by hepatocytes, apo E is secreted into the space of Disse, where it associates with remnant lipoproteins. Two major pathways mediate the clearance of remnant lipoprotein particles (32,33). First, apo E-containing remnant lipoproteins directly interact with the LDL receptor and are internalized into hepatocytes via the classical LDL receptor-mediated pathway. Second, apo E-enriched remnant lipoproteins interact with the cell-surface HSPGs before being transferred to LRP and internalized into hepatocytes through LRP. In addition, HSPGs alone can directly mediate lipoprotein uptake. HSPGs are transmembrane core proteins with attached heparan sulfate chains. These heparan sulfate chains are highly negatively charged sugar polymers capable of capturing lipoproteins and other ligands (33). Thus, the ligand-binding domains of HSPGs are carbohydrates, not proteins. Syndecan-1, an abundant HSPG on the hepatic surface in the space of Disse, is particularly important for remnant clearance (34,35). Furthermore, there is evidence suggesting that scavenger receptor class B type I (SR-BI) may also serve as an additional hepatic remnant receptor (36). Figure 1 illustrates the pathways involved in the clearance of remnant lipoproteins.

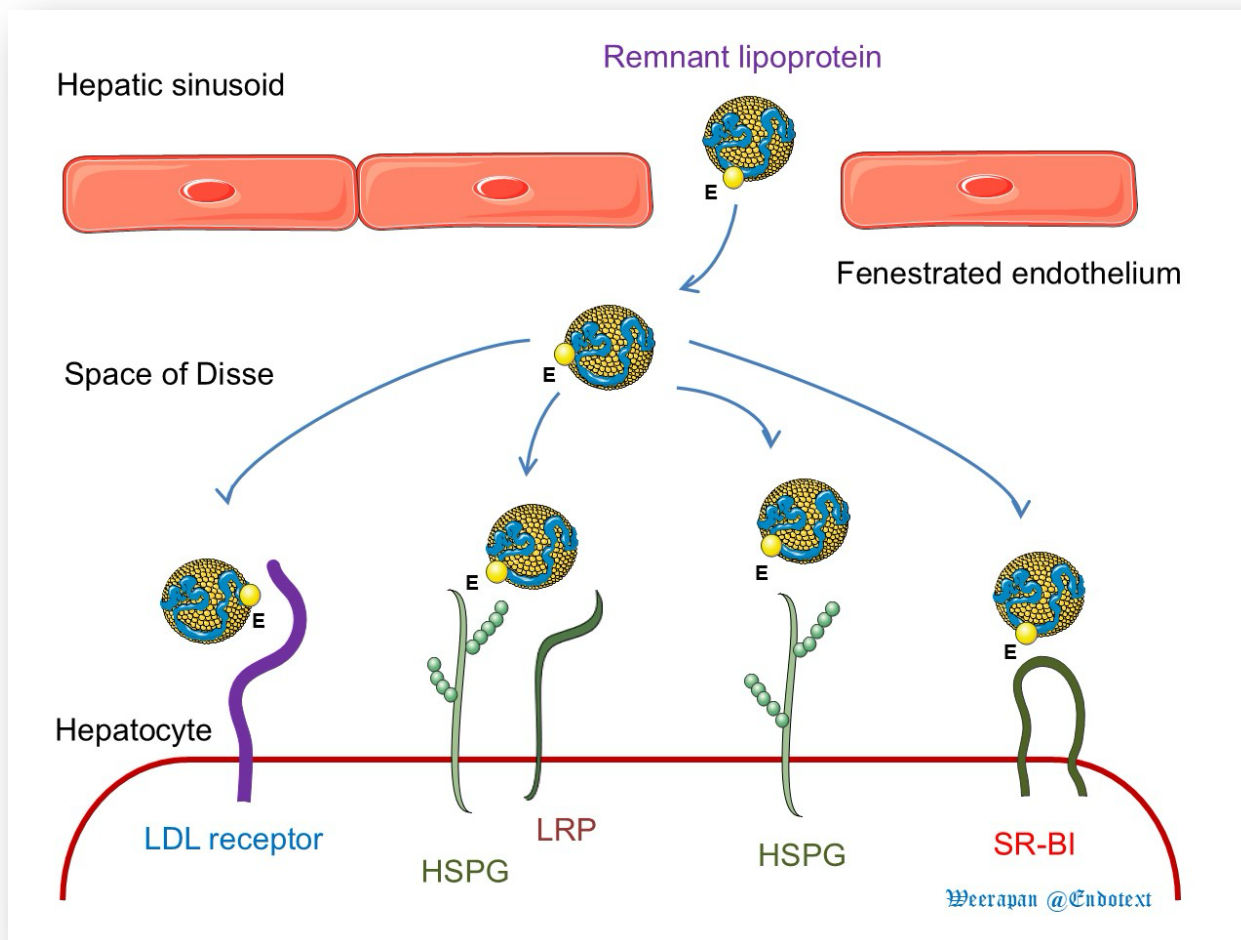


Figure 1. Clearance pathways of remnant lipoproteins.

The presence of abnormal apo E, which is defective in binding to HSPGs or hepatic receptors, or the absence of apo E could lead to impaired clearance of these remnant particles and the accumulation in the circulation, resulting in elevated levels of cholesterol and triglyceride. Different apo E variants likely affect different pathways of remnant lipoprotein particles, resulting in different patterns of hyperlipidemia and inheritance (37). Patients with homozygous familial hypercholesterolemia (FH) who lack LDL receptors do not have remnant accumulation (38). However, mice lacking syndecan-1, a core protein of HSPG, develop remnant accumulation despite intact LDL receptors (35). These studies suggest that HSPGs play a more

important role in remnant clearance than the LDL receptor.

Accumulation of cholesterol-rich remnant particles leads to their uptake by macrophages, resulting in foam cells found in atherosclerotic lesions and xanthoma. Elevated levels of remnant cholesterol have been associated with an increased risk of cardiovascular disease, with a hazard ratio similar to that of elevated LDL-C (39,40).

Besides its role in the uptake and clearance of remnant particles, apo E also modulates lipolytic activity. Elevated levels of apo E can impair triglyceride hydrolysis by displacing or masking apo C-

II, a cofactor for lipoprotein lipase, resulting in hypertriglyceridemia (41). In addition, apo E has been shown to stimulate hepatic VLDL production in animals, further increasing circulating triglyceride levels (42). However, evidence in humans is rather limited. In individuals with complete apo E deficiency, hypertriglyceridemia is usually not observed since there is no excess apo E and triglyceride lipolysis is not impaired.

Pathogenic variants in the *APOE* gene play a key role in the pathophysiology of dysbetalipoproteinemia. Most cases of dysbetalipoproteinemia are autosomal recessive, with the majority of affected individuals harboring two $\epsilon 2$ alleles. Apo E2 has a binding capacity for the LDL receptor that is only 1–2% of that of apo E3 (43). Notably, the amino acid residue 176 lies outside the critical binding region involved in ionic interaction with lipoprotein receptors. This amino acid change appears to reorganize the salt bridges and alter the conformation of the amino acid residues 154–168, thereby indirectly impairing the receptor binding (44,45). In contrast, apo E2 retains significant binding affinity for HSPGs and the HSPG/LRP (37,46). Therefore, relatively normal binding of apo E2 to HSPG may compensate for defective binding to the

LDL receptor, thereby protecting against the development of hyperlipidemia. In fact, most subjects with the E2/E2 phenotype are either normolipidemic or even hypolipidemic (9) and have a reduced risk for coronary artery disease (CAD) (47). Overt hyperlipidemia, also known as type III hyperlipidemia or type III hyperlipoproteinemia, develops only in the presence of additional environmental or genetic factors. These secondary factors may involve conditions associated with overproduction of VLDL or impaired clearance via the LDL receptor or the HSPG/LRP pathways as shown in Table 2. Insulin resistance, for example, is associated with the activation of the *SULF2* gene, which encodes sulfatase 2 and causes degradation of HSPGs in mice (48). Thus, the presence of apoE2/E2 is necessary but not sufficient to cause an abnormal lipid profile. In the recessive form of dysbetalipoproteinemia, elevated lipid levels rarely appear before adulthood. Estrogen is known to enhance both LDL receptor expression and the lipolytic process. Therefore, women who are $\epsilon 2/\epsilon 2$ homozygotes are protected against the development of overt hyperlipidemia until after menopause. Additionally, common gene polymorphisms involved in triglyceride metabolism influence susceptibility to overt hyperlipidemia (49).

Table 2. Metabolic Conditions Known to Precipitate Hyperlipidemia in Dysbetalipoproteinemia	
Lipoprotein overproduction	Impaired clearance
<ul style="list-style-type: none"> - Insulin resistance - Type 2 diabetes - Nephrotic syndrome - Excess alcohol intake - Estrogen treatment - Pregnancy - High fat diets - Medications: corticosteroids, retinoids, atypical antipsychotics, antiretrovirals, immunosuppressive drugs 	<ul style="list-style-type: none"> - Increased age - Menopause - Hypothyroidism - Insulin resistance

In approximately 10% of patients with dysbetalipoproteinemia, the disease is caused by autosomal dominant pathogenic variants in the *APOE* gene. These rare variants typically involve single

amino acid substitutions within the critical binding region of apo E (residues 154–168) that interacts with the LDL receptor, thereby directly impairing receptor binding (50). Other variants disrupt the receptor

binding of apo E or result in apo E deficiency. Furthermore, these dominant variants exhibit severely impaired binding to HSPGs. This defective HSPG binding in the dominant form of dysbetalipoproteinemia suggests that normal LDL receptor binding alone is not sufficient to ensure proper clearance of remnant lipoproteins. As a result, the HSPG binding affinity of apo E variants is considered a key determinant of the inheritance pattern of dysbetalipoproteinemia. In the autosomal dominant form, a single allele carrying these variants

is sufficient to cause overt hyperlipidemia without the need for secondary factors and lipid abnormalities in these cases presumably begin at birth. To date, approximately 30 *APOE* variants associated with autosomal dominant dysbetalipoproteinemia have been reported (50-52). Autosomal dominant dysbetalipoproteinemia can occasionally be misdiagnosed as FH (50). The key differences between the autosomal recessive and autosomal dominant forms of dysbetalipoproteinemia are shown in Table 3.

Table 3. Characteristics of Autosomal Recessive and Autosomal Dominant Dysbetalipoproteinemia		
	Recessive	Dominant
Presence of β -VLDL	Yes	Yes
Lipoprotein preference of apo E	HDL	Triglyceride-rich lipoproteins
LDL receptor binding	Defective	Defective
HSPG binding	Normal	Defective
Defect in receptor binding	Indirect	Direct
Secondary factors	Required	Not required
Occurrence of hyperlipidemia	Adulthood	From birth

Hypercholesterolemia in dysbetalipoproteinemia arises from impaired receptor-mediated clearance of cholesterol-rich remnant lipoproteins, while hypertriglyceridemia results from both impaired lipolytic processing of remnant particles and increased hepatic VLDL production driven by elevated levels of apo E (41,42). Low LDL-cholesterol levels in individuals with dysbetalipoproteinemia are primarily due to impaired conversion of VLDL to IDL, caused by elevated levels of apo E, and reduced hepatic lipase-mediated conversion of IDL to LDL by apo E2. Apo E plays a crucial role in hepatic lipase activity, with apo E3 and apo E4 being more effective than apo E2 in activating hepatic lipase-mediated lipolysis (53,54). Animal studies also suggest that low LDL-cholesterol levels are not due to upregulation of LDL receptors or enhanced hepatic clearance of LDL (41).

CLINICAL FEATURES

Patients with dysbetalipoproteinemia exhibit variable clinical features. Cutaneous xanthomas, especially

palmar xanthoma and tuberous or tuberoeruptive xanthoma, are commonly observed. Palmar xanthoma (or xanthoma striata palmaris) is characterized by yellowish lipid deposits in the palmar creases and is found in 18–72% of patients (figure 2) (17,18,55-57). Although once considered specific to dysbetalipoproteinemia, it is now recognized in other conditions (57). Tuberous xanthoma, frequently found on the knuckles, elbows, knees, and buttocks, may be more common than palmar xanthoma (17,18). Tendon xanthoma is also present in some cases. Neither tuberous xanthoma nor tendon xanthoma is unique to dysbetalipoproteinemia; they can occur in other types of dyslipidemia. These xanthomas typically disappear once lipid levels are brought under control. Several metabolic conditions, including type 2 diabetes, hyperinsulinemia, obesity, hyperuricemia, and hypothyroidism, are associated with dysbetalipoproteinemia, as outlined in Table 2.



Figure 2. Palmer xanthoma

In a large cohort collected over a 35-year period in Canada, 524 patients met the gold standard Fredrickson criteria for dysbetalipoproteinemia (plasma triglyceride between 150-1,000 mg/dL and VLDL-cholesterol/triglyceride mass ratio >0.30) (58). However, only 197 subjects (38%) had the apo $\epsilon 2/\epsilon 2$ genotype. This finding contrasts with earlier reports based on a smaller number of subjects, which indicated that 90% of patients with dysbetalipoproteinemia carried the apo $\epsilon 2/\epsilon 2$ genotype. Clinically, patients who met the Fredrickson criteria and had the apo $\epsilon 2/\epsilon 2$ genotype exhibited more severe phenotypes than those without it. These individuals had significantly higher levels of remnant cholesterol, a greater frequency of xanthomas, and a higher prevalence of atherosclerotic cardiovascular disease (ASCVD) (58). Additionally, those with the apo $\epsilon 2/\epsilon 2$ genotype demonstrated an 11-fold increased risk of peripheral artery disease (PAD) compared to those without it. This study suggests that dysbetalipoproteinemia may manifest as a less severe multifactorial remnant cholesterol disease in individuals without the apo $\epsilon 2/\epsilon 2$ genotype and as a more severe form associated with the apo $\epsilon 2/\epsilon 2$ genotype (58).

Premature ASCVD, particularly CAD and PAD of the lower extremities, is more common in patients with dysbetalipoproteinemia and elevated lipid levels (17,55,59). The risk of CAD is reported to increase by approximately 5- to 10-fold (59). For PAD, the risk is

elevated 13-fold compared to normolipidemic controls and 3-fold compared to patients with FH (60). Factors such as age, hypertension, smoking, waist circumference, triglyceride levels, and a polygenic risk score are significant predictors of cardiovascular disease in these individuals (61,62).

A contemporary cross-sectional study of 305 patients with dysbetalipoproteinemia in Europe found CAD in 19% of participants, while PAD was present in 11% (63). Similarly, among 964 patients in the UK Biobank, CAD was identified in 12% and PAD in 3% (23). Notably, as with other genetic lipid disorders, standard risk calculators for estimating the 10-year risk of ASCVD are not applicable, as they tend to underestimate the actual risk.

Rare mutations in the *APOE* gene are associated with lipoprotein glomerulopathy, a condition most commonly reported in Japan. The most frequent mutation identified is APOEc.488G>C (p.Arg163Pro), also known as apoE Sendai (64,65). This disorder is characterized by progressive proteinuria. Histologically, lipoprotein thrombi are observed in markedly dilated glomeruli. Approximately half of the reported cases progress to renal failure.

BIOCHEMICAL FEATURES

The lipid profile of subjects with dysbetalipoproteinemia is highly variable and sensitive

to day-to-day changes in diet (66). Typically, there is an increase in both total cholesterol and triglyceride levels. Plasma triglyceride levels may be within the same range with the total cholesterol levels (cholesterol to triglyceride molar ratio around 2:1) or sometimes higher than total cholesterol levels. Severe hypertriglyceridemia resulting in acute pancreatitis has been reported in some cases of dysbetalipoproteinemia. Although total cholesterol levels are usually elevated, LDL-C levels are almost always reduced (17). The cause of low LDL-C levels in dysbetalipoproteinemia is due to an impairment in the apo E-mediated conversion of remnant lipoproteins to LDL (67). Normally, once apo E on remnant lipoproteins binds to HSPGs on hepatocytes, HSPG-bound hepatic lipase removes triglyceride from these remnants and converts them into LDL. The presence of abnormal apo E2 in dysbetalipoproteinemia appears to impair this process, leading to low levels of LDL-C.

Since remnant lipoproteins are enriched in cholesterol with a higher VLDL-cholesterol/triglyceride (VLDL-C/TG) ratio, a fixed ratio of VLDL-C/TG used in the Friedewald formula is invalid. In fact, dysbetalipoproteinemia is listed as one of the exceptions in the original report that the Friedewald formula should not be used (68). VLDL-C levels, calculated by triglyceride/5, are therefore underestimated, leading to overestimation of calculated LDL-C. Calculated LDL-C levels derived from the Friedewald formula or the NIH equation, as well as measured LDL-C levels from a homogeneous direct LDL-C assay, have been shown to overestimate plasma LDL-C levels in patients with dysbetalipoproteinemia (69,70). HDL cholesterol levels are also modestly reduced in subjects with dysbetalipoproteinemia. Apo B levels are typically not markedly elevated. Although Apo E levels are higher in individuals with dysbetalipoproteinemia, there is an overlap with those without the condition (71).

Based on lipid phenotypes, dysbetalipoproteinemia should be suspected in the following situations (72).

- (1) dyslipidemia patients whose total cholesterol and triglyceride levels are both elevated and approximately equal
- (2) mixed hyperlipidemia in which apo B level is relatively low in relation to total cholesterol level
- (3) Significant disparity between calculated LDL-C and directly measured LDL-C levels

DIAGNOSIS

Dysbetalipoproteinemia cannot be diagnosed with a single straightforward test, nor can it be identified solely through conventional lipid values. Historically, diagnosis usually requires a biochemical approach to demonstrate the presence of remnant accumulation in the circulation and a genetic approach to characterize the apo E genotype. The presence of β -VLDL indicates dysbetalipoproteinemia regardless of whether hyperlipidemia is present or not.

Lipoprotein electrophoresis is a classical method originally used to characterize different lipoproteins and to classify various types of dyslipidemia. Different lipoprotein families display distinct bands on serum electrophoresis. Using paper, agarose, or cellulose acetate as the media, chylomicron stays at the origin whereas HDL migrates to the most advanced position, which is called an α band. Between the origin and the α band, a β band indicates LDL, whereas a pre- β band represents VLDL. On polyacrylamide gel, however, the migration pattern is slightly different in that VLDL (pre- β lipoproteins) migrate behind instead of in front of the LDL (β -lipoproteins) (73).

Serum agarose gel electrophoresis has been traditionally used to detect the remnant lipoproteins and to diagnose dysbetalipoproteinemia. On paper, agarose, or cellulose acetate electrophoresis, the demonstration of a broad β band, extending from the β position into the pre- β position, indicates the presence of remnant lipoproteins (74) as shown in Figure 3. However, a broad β band is found in less than half of patients (75) and can be found in other conditions (76), suggesting that the presence of a

broad β -band in lipoprotein electrophoresis is neither sensitive nor specific for the diagnosis of dysbetalipoproteinemia. On polyacrylamide gel electrophoresis, the presence of small VLDL and IDL

along with the absence of a β -migrating lipoprotein band have also been used to indicate dysbetalipoproteinemia (73,75).

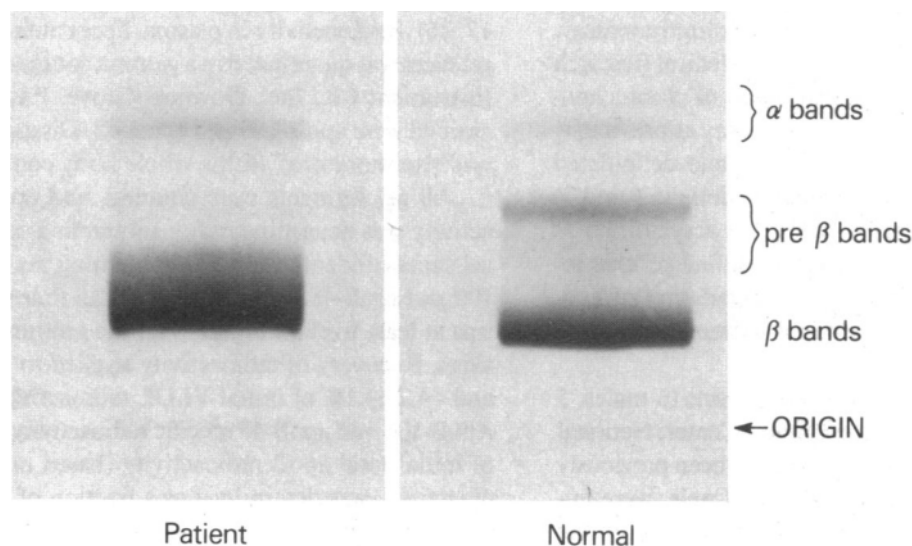


Figure 3. Plasma lipoprotein electrophoresis in 0.5% agarose gel demonstrated a broad β band in a patient with dysbetalipoproteinemia (left) and a normal pattern in a normal subject (right) (from (31)).

Another method used to characterize different lipoproteins is ultracentrifugation. Using preparative ultracentrifugation to isolate various lipoprotein families, lipid composition of different lipoprotein fractions can then be determined. Compared to the normal pre β -VLDL, β -VLDL remnant particles are more cholesterol-enriched and triglyceride-depleted. Normally, the cholesterol/triglyceride mass ratio in VLDL is 0.2 or less and the cholesterol/triglyceride ratio in β -VLDL is higher. A VLDL-C/VLDL TG mass ratio (both in mg/dL) >0.42 or VLDL-C/VLDL TG molar ratio (both in mmol/L) >0.97 is considered diagnostic of dysbetalipoproteinemia (77). Several studies have also tried to identify cut points for detection of β -VLDL using a VLDL-C/plasma or total TG ratio. The most frequently used cutoff for diagnosis of dysbetalipoproteinemia is the Fredrickson criteria, which is VLDL-C/TG >0.30 (mass ratio) or >0.69 (molar ratio) and plasma triglyceride level between 150-1,000 mg/dL (14). A mass ratio between 0.25-

0.30 or a molar ratio between 0.57-0.69 is considered suggestive of dysbetalipoproteinemia (14,78).

Both lipoprotein electrophoresis and preparative ultracentrifugation described above are, however, not readily available in routine clinical laboratories. Therefore, other diagnostic methods using common lipid and apolipoprotein levels have been explored.

Two methods for estimating the VLDL-C level without a need for ultracentrifugation have recently been described (79). The first method used results obtained from the standard lipid panel and the Sampson-NIH equation. At a cut point of 0.194, a sensitivity of 74% and a specificity of 83% were reported (79). The second method modified the Sampson-NIH equation by including apo B level for predicting VLDL-C. At a cut-point of 0.209, a better sensitivity of 97% and a better specificity of 95% were demonstrated (79).

Remnant lipoprotein cholesterol (RLP-C) can be measured in serum and serum RLP-C/triglyceride

ratio has been shown to be an effective alternative to VLDL-C/triglyceride ratio (80,81). Serum RLP-C/triglyceride ratio >0.23 is highly correlated with the presence of β -VLDL and has been demonstrated to be useful for screening for dysbetalipoproteinemia (81,82)

Since plasma apo B levels are not typically elevated in subjects with dysbetalipoproteinemia, Blom et al. showed that an apo B (in g/L)/total cholesterol (in mmol/L) ratio of <0.15 could identify patients with dysbetalipoproteinemia with a sensitivity of 89% (95% confidence interval [CI] 78-96%) and a specificity of 97% (95% CI 94-98%) among 333 patients with mixed hyperlipidemia with 57 having confirmed dysbetalipoproteinemia (83). In another study of 1,771 patients with various types of dyslipidemia along with 38 confirmed cases of dysbetalipoproteinemia, Sniderman et al. reported that a total cholesterol (in mmol/L)/apo B (in g/L) ratio of >6.2 and a triglyceride/apo B ratio of <10.0 have been shown to discriminate among other types of dyslipidemia based on the Fredrickson classification (84). However, when this method was compared to the ultracentrifugation-based definition of dysbetalipoproteinemia among 3,695 individuals (16 with dysbetalipoproteinemia), a higher prevalence was found (1.43% vs. 0.43%), suggesting that the method of Sniderman et al. using lipids and apo B levels might not be specific (16). When the triglyceride cutoff was raised from 160 mg/dL to 200 mg/dL, the specificity is significantly improved, indicating that triglyceride level is also important in this screening algorithm (16). With increasing levels of triglyceride, more severe cases of dysbetalipoproteinemia may be identified using the apo B algorithm but the sensitivity to detect milder cases drops significantly (85). Similarly, a recent study from Germany using the apo B/total cholesterol ratio as diagnostic criteria proposed by Sniderman et al. (84) or Blom et al. (83), showed that although subjects with the apo $\epsilon 2/\epsilon 2$ genotype were more likely to develop dysbetalipoproteinemia, most subjects with dysbetalipoproteinemia did not have the apo $\epsilon 2/\epsilon 2$ genotype (86). Resequencing of *APOE* gene further

identified only a few cases of rare *APOE* variants (86). These studies suggest that using only lipid phenotypes and apo B alone without the apo $\epsilon 2$ genotype tends to either include more false positive cases or capture milder cases of true dysbetalipoproteinemia (16,21).

In addition to using the total cholesterol/apo B ratio as a screening criterion, the non-HDL-cholesterol (non-HDL-C)/apo B ratio has also been examined. A small study in 9 Japanese patients with dysbetalipoproteinemia proposed a non HDL-C/apo B ratio (both in mg/dL) of >2.6 to differentiate from those with combined hyperlipidemia (87), whereas a subsequent larger study in England ($n = 1,637$) with 63 cases of dysbetalipoproteinemia showed that a non HDL-C (in mmol/L)/apo B (in g/L) ratio of >4.91 had better diagnostic performance than a total cholesterol/apo B ratio (88).

A study from Canada has also evaluated different lipid values among 4,891 patients with mixed hyperlipidemia (total cholesterol ≥ 5.2 mmol/L [200 mg/dL] and triglyceride ≥ 2.0 mmol/L [175 mg/dL]), 188 of whom had dysbetalipoproteinemia defined from elevated VLDL-C/plasma TG ratio and the presence of apo $\epsilon 2/\epsilon 2$ genotype (56). In this cohort, Paquette et al. showed that the non-HDL-C/apo B ratio was the best predictor of dysbetalipoproteinemia, which was better than the total cholesterol/apo B ratio (56). The non HDL-C/apo B ratio cut point of 3.69 mmol/g or 1.43 in conventional units (both in mg/dL) followed by the identification of apo $\epsilon 2/\epsilon 2$ genotype provided a good sensitivity (94.8%) and specificity (99%) with 99.4% accuracy (56). A review of previous diagnostic strategies proposed for dysbetalipoproteinemia further demonstrated that all other criteria (16,82-84,87-89) exhibited either low sensitivity or low specificity when validated using this cohort.

A combination of non HDL-C/apo B ratio of ≥ 1.7 and TG/apo B ratio of ≥ 1.35 , all in mg/dL (non HDL-C in mmol/L/apo B in mg/dL ≥ 4.4 and TG in mmol/L/apo B in mg/dL ≥ 3.5) has recently been proposed as a

screening tool for further *APOE* genotyping in subjects with TG >150 mg/dL, LDL-C >160 mg/dL or non HDL-C >190 mg/dL (90). This algorithm has been shown to give excellent sensitivity and high specificity compared with other algorithms. Although apo B levels are affected by lipid-lowering therapy, this algorithm has been proposed to be used in those with and without lipid-lowering medications. In the population with lower levels of apo B, however, the algorithm that used non HDL-C/apo B ratio has been shown to give excellent sensitivity but very low specificity for detecting apo $\epsilon 2/\epsilon 2$ genotype (24).

More recently, a large study of dysbetalipoproteinemia patients (n=964) from the UK Biobank has been reported (23). Dysbetalipoproteinemia was diagnosed using the apo $\epsilon 2/\epsilon 2$ genotype and mixed hyperlipidemia (total cholesterol ≥ 200 mg/dL [5.2 mmol/L] and triglyceride ≥ 175 mg/dL [2.0 mmol/L]). The performances of 6 different criteria (56,79,83,84,88,90) were evaluated and 3 criteria by Boot et al.(88), Blom et al. (83), and Sniderman et al. (84) showed sensitivity, specificity, and accuracy

>90% with the area under the curve (AUC) of ≥ 0.95 and the negative predictive value of 100% (23). The number of those who met the criteria and should be assessed for *APOE* genotype in these 3 criteria ranged from 1-6%. When the non HDL-C/apo B cutoff ratio originally proposed by Paquette et al. (56) was raised from ≥ 1.43 (in mg/dL) [3.69 (in mmol/g)] to ≥ 1.78 (in mg/dL) [4.61 (in mmol/g)], the sensitivity, specificity, accuracy and the AUC were all improved similar to the 3 criteria, and the number of individuals that should undergo *APOE* genetic testing was lower from 23% to 3% (23). It is important to note that all of these criteria should be used for screening for further genetic testing and should not be used solely for diagnosis of dysbetalipoproteinemia. All of these screening criteria have very low positive predictive value, meaning that only a few of those who meet the criteria actually have dysbetalipoproteinemia when tested for *APOE* genotype (23).

The description of various criteria proposed for further evaluation for dysbetalipoproteinemia is shown in Table 4.

Table 4. Criteria Proposed for Further Evaluation for Dysbetalipoproteinemia	
References	Proposed criteria
Apo B assay	
Blom et al., 2005 (83)	- apo B (in g/L)/total cholesterol (in mmol/L) <0.15
Sniderman et al., 2007 (84)	- total cholesterol (in mmol/L)/apo B (in g/L) >6.2 - triglyceride (in mmol/L)/apo B (in g/L) <10.0 - triglyceride >75 th percentile for age and gender
Murase et al., 2010 (87)	- non-HDL-C/apo B ratio (both in mg/dL) >2.6
Hopkins et al., 2014 (16)	- total cholesterol (in mmol/L)/apo B (in g/L) >6.2 - triglyceride (in mmol/L)/apo B (in g/L) <10.0 - triglyceride >200 mg/dL (>2.3 mmol/L)
Boot et al., 2019 (88)	- total cholesterol >5.0 mmol/L (>193 mg/dL) and triglyceride >1.5 mmol/L (>133 mg/dL) - non-HDL-C (in mmol/L)/apo B (in g/L) >4.91
Paquette et al., 2020 (56)	- total cholesterol ≥ 5.2 mmol/L (≥ 200 mg/dL) and triglyceride ≥ 2.0 mmol/L (≥ 175 mg/dL) - non-HDL-C/apo B >3.69 mmol/g or 1.43 (both in mg/dL)

Bea et al, 2023 (90)	<ul style="list-style-type: none"> - triglyceride >150 mg/dL and LDL-C >160 mg/dL or non-HDL-C >190 mg/dL - non-HDL-C/apo B ≥ 1.7 (both in mg/dL) or non-HDL-C (in mmol/L) /apo B (in mg/dL) ≥ 4.4 - triglyceride/apo B ≥ 1.35 (both in mg/dL) or triglyceride (in mmol/L) /apo B (in mg/dL) ≥ 3.5
Remnant lipoprotein cholesterol assay	
Nakajima et al., 2007 (82)	- RLP-C/triglyceride >0.23

Identification of apo E phenotype and/or genotype can help establish the diagnosis of dysbetalipoproteinemia. Nowadays, conventional apo E phenotyping by isoelectric focusing is replaced by a number of simple and more accurate *APOE* genotyping methods. When apo $\epsilon 2/\epsilon 2$ homozygosity is discovered in subjects with dysbetalipoproteinemia, immediate family members should be screened for the presence of hyperlipidemia. The presence of apo $\epsilon 2/\epsilon 2$ by itself without overt hyperlipidemia is not a critical risk factor for premature ASCVD. Therefore, counseling should be focused on eliminating secondary factors known to cause hyperlipidemia, such as obesity, diabetes, or alcohol consumption.

In patients with suspected dysbetalipoproteinemia, if the apo $\epsilon 2/\epsilon 2$ homozygosity is excluded, next generation sequencing can be performed to identify rare *APOE* variants associated with the autosomal dominant form. Since not all identified variants in the *APOE* gene are causally related to dysbetalipoproteinemia, a comprehensive approach is advised to determine the pathogenicity of the variants detected using both *in vitro* and *in vivo* functional studies (52). In this condition, 50% of first-degree relatives are affected. Therefore, cascade screening should be performed in a manner similar to that for FH. Once the diagnosis is confirmed, appropriate treatment should be initiated.

TREATMENT

Dysbetalipoproteinemia responds well to therapy (17). However, data from the UK Biobank and the US

NHANES cohorts show that the majority of subjects with dysbetalipoproteinemia remain untreated despite their high atherogenic risk (21,23). Dietary modifications and addressing secondary metabolic factors form the cornerstone of therapy. Restriction of caloric intake in those who are overweight and reduction of saturated fat and cholesterol in the diet help normalize lipid levels (18). There are no specific dietary guidelines for patients with dysbetalipoproteinemia (91); however, reducing dietary cholesterol and saturated fat while increasing polyunsaturated fat intake is recommended (18). Weight reduction, glycemic control of diabetes, cessation of alcohol intake, and treatment of hypothyroidism can also lower lipid levels.

LDL-C levels cannot be accurately measured or calculated in patients with dysbetalipoproteinemia (70). In addition, LDL-C levels are typically not elevated and do not reflect high cardiovascular risk in these patients. Therefore, LDL-C levels should not be used as a treatment target in dysbetalipoproteinemia. It is recommended that the primary target of treatment is non-HDL-C level (50,73), which can be reliably measured using standard assays of total cholesterol and HDL-C (70). The secondary target of treatment is triglyceride level. In some cases, medications are required to lower cholesterol and triglyceride levels, and statins and fibrates are the medications of choice, respectively. Evolocumab, a PCSK9 inhibitor, has also been shown to reduce total cholesterol, remnant cholesterol, and triglyceride levels in patients with dysbetalipoproteinemia (92,93). Resolution in xanthomas and regression of atherosclerotic lesions

have been observed after normalization of lipid levels (94).

CONCLUSION

Dysbetalipoproteinemia remains an underrecognized genetic lipid disorder. Pathogenic variants in the *APOE* gene lead to defective apo E-mediated remnant clearance and accumulation of remnant lipoproteins characterized by elevation of both total cholesterol and triglyceride levels, palmar and tuberous xanthomas, and an increased risk of CAD and PAD. The HSPG-binding affinity of the apo E variants appears to be an important determinant of the different modes of inheritance. Historically, diagnosis requires sophisticated methods to demonstrate the presence of remnant lipoprotein particles (β -VLDL) and the pathogenic variants in the *APOE* gene. Currently, a simple diagnostic test for dysbetalipoproteinemia does not exist and several algorithms using various lipid and apo B parameters have been proposed for screening for this condition and further genetic testing. Recent data suggest that the phenotype of

dysbetalipoproteinemia may be more heterogeneous and a milder form of dysbetalipoproteinemia without the apo $\epsilon 2/\epsilon 2$ genotype is called multifactorial remnant cholesterol disease to differentiate it from the more severe form in those with apo $\epsilon 2/\epsilon 2$ genotype. Nevertheless, subjects with dysbetalipoproteinemia are usually responsive to lifestyle modifications and conventional lipid-modifying therapy, including statins and fibrates. Despite renewed interest and recent advances in understanding this condition, several knowledge gaps remain. These include the precise mechanisms involved in the clearance of remnant lipoproteins, the true prevalence within the general population, the roles of genetic and environmental factors in modifying disease expression, the underlying mechanisms of PAD involvement, the development of a simplified diagnostic test for clinical use, the establishment of standard guidelines for screening, and the creation of evidence-based guidelines for optimal treatment and cardiovascular risk reduction.

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