*The Journal of Clinical Endocrinology & Metabolism*, 2022, Vol. 107, No. 2, 538–548
https://doi.org/10.1210/clinem/dgab648
Clinical Research Article

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# Dysbetalipoproteinemia: Differentiating Multifactorial Remnant Cholesterol Disease From Genetic ApoE Deficiency

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**Abbreviations:** Apo, apolipoprotein; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CAD, coronary artery disease; CeVD, cerebrovascular disease; DBL, dysbetalipoproteinemia; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HLP, hyperlipoproteinemia; IRCM, Montreal Clinical Research Institute; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; PVD, peripheral vascular disease; TG, triglyceride; TGRL, triglyceride rich lipoproteins; VLDL, very-low-density lipoprotein.

Received: 15 June 2021; Editorial Decision: 26 August 2021; First Published Online: 1 September 2021; Corrected and Typeset: 25 September 2021.

## Abstract

**Context:** Dysbetalipoproteinemia (DBL) is characterized by the accumulation of remnant lipoprotein particles and associated with an increased risk of cardiovascular and peripheral vascular disease (PVD). DBL is thought to be mainly caused by the presence of an *E2/E2* genotype of the apolipoprotein E (*APOE*) gene, in addition to environmental factors. However, there exists considerable phenotypic variability among DBL patients.
**Objective:** The objectives were to verify the proportion of DBL subjects, diagnosed using the gold standard Fredrickson criteria, who did not carry E2/E2 and to compare the clinical characteristics of DBL patients with and without E2/E2.
**Methods:** A total of 12 432 patients with lipoprotein ultracentrifugation as well as *APOE* genotype or apoE phenotype data were included in this retrospective study.
**Results:** Among the 12 432 patients, 4% (n = 524) were positive for Fredrickson criteria (F+), and only 38% (n = 197) of the F+ individuals were E2/E2. The F+ E2/E2 group had significantly higher remnant cholesterol concentration (3.44 vs 1.89 mmol/L) and had higher frequency of DBL-related xanthomas (24% vs 2%) and floating beta (95% vs 11%) than the F+ non-E2/E2 group ($P < 0.0001$). The F+ E2/E2 group had an independent higher risk of PVD (OR 11.12 [95% CI 1.87-66.05]; $P = 0.008$) events compared with the F+ non-E2/E2 group.



ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

**Conclusion:** In the largest cohort of DBL worldwide, we demonstrated that the presence of E2/E2 was associated with a more severe DBL phenotype. We suggest that 2 DBL phenotypes should be distinguished: the multifactorial remnant cholesterol disease and the genetic apoE deficiency disease.

**Key Words:** dysbetalipoproteinemia, Fredrickson, diagnosis, *APOE*, remnant lipoprotein, lipoprotein ultracentrifugation

Dysbetalipoproteinemia (DBL), also known as type III hyperlipoproteinemia (HLP) according to the Fredrickson classification (OMIM# 617347), is a disorder characterized by pathologic accumulation of remnant lipoprotein particles (intermediate-density lipoprotein and chylomicron remnants) in circulation caused by a reduced clearance, often associated with increased triglyceride (TG)-rich lipoproteins (TGRL) production (1-3). This excess in circulating cholesterol-enriched remnant lipoproteins results in a mixed dyslipidemia, where both total cholesterol and TG are elevated, but low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (apo) B concentrations are generally reduced (4, 5). Clinical manifestations and complications of this disease include the presence of xanthomas (tuberous, tuberoeruptive, striated palmar, and eruptive) as well as accelerated atherosclerosis that predispose to premature coronary artery disease (CAD) and peripheral vascular disease (PVD) (4, 6-8).

In 1967, Fredrickson et al described the type III HLP phenotype as an unusual form of mixed dyslipidemia defined by the presence of "floating beta lipoproteins" in plasma following electrophoresis of the d < 1.006 g/mL fraction (1). Several years later, in 1975, they established the well-known gold standard criteria for the diagnosis of type III HLP using lipoprotein ultracentrifugation. In their retrospective analysis, they analyzed a cohort of 182 adults with primary familial hypertriglyceridemia for whom 3100 complete lipoprotein analyses were available. These patients came from the Clinical Center of the National Institutes of Health in the United States and were recruited from 1965 to 1973. Among the 182 patients, 59 presented floating beta bands on lipoprotein electrophoresis. The authors concluded that both triglycerides between 150 and 1000 mg/dL (1.7-11.29 mmol/L) and a very-low-density lipoprotein cholesterol (VLDL-C)/TG ratio ≥ 0.30 (in mg/dL, or 0.69 in mmol/L) represented more specific criteria for the diagnosis of type III HLP than the presence of floating beta lipoproteins since the floating beta also appeared in other types of dyslipidemias (9). The same year, *APOE* was identified as the genetic basis of DBL by Utermann et al (10) and it was later shown that the presence of secondary metabolic factors, such as hyperinsulinemia or increased body mass index (BMI), are necessary to precipitate the disease (11). Indeed, it is now well established that DBL is caused by genetic variants in the *APOE* gene that produce dysfunctional apoE proteins that interact poorly with the LDL receptor (2, 12). It was estimated that E2 homozygosity accounts for 90% of cases (recessive form) whereas 10% of these patients carry a single deleterious rare variant (dominant form) (2, 13-15). However, the few original reports on which this affirmation is based included a limited number of subjects (13) or did not use the gold standard criteria (15).

In order to simplify the diagnosis of DBL in the context of clinical practice, several criteria have been proposed such as the apoB algorithm, the non-HDL-C/apoB ratio or the apoB/total cholesterol ratio in order to circumvent the use of lipoprotein ultracentrifugation (3, 5, 16-21). Although these criteria could be useful for screening in the clinical context, they do not replace the gold standard diagnostic criteria of DBL that are based on lipoprotein ultracentrifugation.

The objectives of the present study were twofold. Our first aim was to verify the proportion of DBL subjects diagnosed using the gold standard Frederickson criteria who did not carry E2/E2. Our second objective was to compare the clinical characteristics of DBL patients with vs without the E2/E2.

## Methods

### Study Population and Data Collection

Subjects included in this retrospective study came from the lipid clinic research database of the Montreal Clinical Research Institute (IRCM). A total of 12 432 patients who had undergone quantification of lipoproteins by ultracentrifugation were included in the present study after excluding those with missing data for ultracentrifugation (n = 2376) as well as those with missing data for *APOE* genotype or apoE phenotype, patients < 18 years of age, and patients with invalid or incomplete lipid profile (n = 2301). The data collection corresponds to the first visit at the lipid clinic, where each patient underwent a 4-week washout of fibrate before the baseline blood sampling. Patients without any prior history of cardiovascular disease also underwent a 4-week washout of statin therapy. Data from the study subjects were included in the

database over a 35-year period (from 1969 to 2004). The patients included in this cohort were of French Canadian origin. The gold standard Fredrickson criteria used to diagnose DBL were TG between 1.7 and 11.29 mmol/L and a VLDL-C/TG ratio > 0.69 (in mmol/L) as determined by lipoprotein ultracentrifugation (9). DBL-related xanthomas included tuberous xanthoma, tuberoeruptive xanthoma, striated palmar xanthoma, and eruptive xanthoma (see Fig. 1 for illustrations of these clinical manifestations). Atherosclerotic cardiovascular disease (ASCVD) events included CAD (angina, myocardial infarction, coronary angioplasty, and coronary bypass surgery), PVD (claudication [confirmed by peripheral arterial Doppler or ankle brachial index], peripheral angioplasty, and peripheral arterial surgery), and cerebrovascular disease (CeVD) events (transient ischemic attack, stroke, and carotid endarterectomy). All patients included in the research database provided a written informed consent, approved by the IRCM ethics institutional review board on research on humans. The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

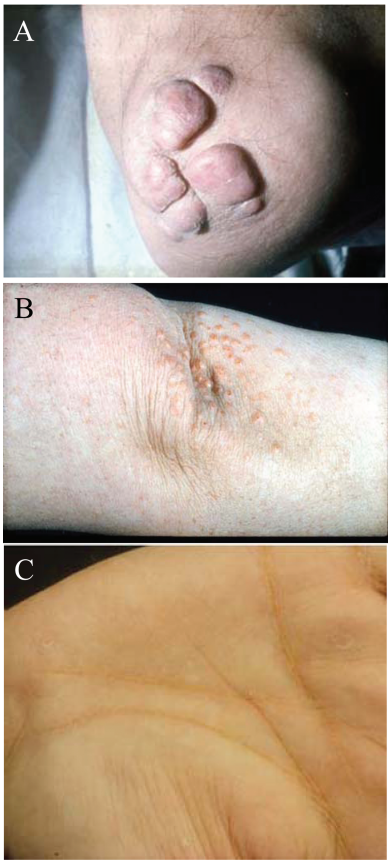


**Figure 1.** Clinical manifestations of dysbetalipoproteinemia. (a) Picture of tuberous/tuberoeruptive xanthomas. (b) Picture of eruptive xanthomas. (c) Picture of striated palmar xanthoma.

## Biochemical Analysis

Blood samples were obtained after a 12-hour overnight fast. All lipoprotein ultracentrifugation and lipoprotein electrophoresis procedures were done by qualified technicians at the IRCM lipid clinic according to standardized protocols. Plasma lipoproteins were separated by ultracentrifugation according to the protocol of the Lipid Research Clinics (22). Plasma samples (5 mL) were spun at d = 1.006 g/mL and HDLs were separated from the d > 1.006 g/mL fraction by precipitation of apoB-containing lipoproteins using heparin-manganese. Cholesterol and TG concentrations were measured enzymatically with an automated analyzer (ABA-100 Bichromatic Analyzer, Abbott Laboratories; a Cobas Mira S chemistry system, Roche Diagnostic Systems; or a Hitachi 717). All assays were calibrated to the Centers for Disease Control and Prevention reference standard. Cholesterol contents in the LDL and HDL fractions were measured by assaying lipid concentrations in the d > 1.006 g/mL fraction via beta-quantification, although this method could lead to an overestimation of LDL-C in subjects with a significant concentration of remnant lipoproteins. Cholesterol contents in the VLDL fraction (VLDL-C or remnant-C) was measured by assaying lipid concentrations in the d < 1.006 g/mL fraction. Agarose gel electrophoresis (23) of total plasma, d < 1.006 g/mL (VLDL), and d > 1.006 g/mL (LDL + HDL) fractions was carried out in order to identify "floating beta bands" using a Beckman Paragon Electrophoresis System (Beckman Instruments) according to the manufacturer's instructions.

ApoE phenotypes were determined after isoelectric focusing of delipidated VLDL (24) or by immunoblotting of plasma separated by minigel electrophoresis (25). *APOE* genotype was obtained by restriction isotyping (restriction enzyme isoform genotyping) as described elsewhere (26). E2 was used throughout the manuscript to designate both the ε2 allele as determined by genotyping and E2 isoform as determined by isoelectric focusing.

A commercial enzyme-linked immunosorbent assay (ELISA) kit (Macra EIA Kit; Strategic Diagnostics Industries, Inc, Newark) was used to measure lipoprotein (a) concentration. The measurement of apolipoprotein B was done by electroimmunoassay (Behringwerke, Marburg, Germany).

## Statistical Analyses

Statistical analyses have been performed using SPSS statistical software version 26 (IBM Corp, Armonk, NY). Statistical significance level was set to $P < 0.05$. Continuous normally distributed variables are expressed as mean ± SD, whereas skewed distributed variables are presented as median (Q1-Q3). These abnormally distributed variables were

log-transformed prior to analysis. The Student *t* test, the ANOVA, and the chi-square test were used to compare variables between groups. The Tukey test was used for multiple comparison testing. When the normality of the distribution was not reestablished by the logarithm transformation, the nonparametric U Mann-Whitney test or the nonparametric Kruskall-Wallis test were used. Odds ratio (OR) were obtained using either uncorrected or corrected models of logistic regression. Missing data were not imputed.

## Results

As presented in Table 1, of the 12 432 subjects included in the study, 524 (4%) were positive for the Fredrickson criteria (F+), whereas 11 908 were negative (F–). There were significantly more men (59% vs 54%), the subjects were on average 2 years older, and the BMI was 1.1 kg/m$^2$ higher in the F+ group compared with the F– group ($P = 0.04$, $P = 0.02$, and $P \leq 0.0001$, respectively). All lipids, lipoproteins, and their ratio (total cholesterol, triglycerides, measured LDL-C, non-HDL-C, VLDL-C, VLDL-TG, VLDL-C/TG ratio, apolipoprotein B, lipoprotein (a), total cholesterol/apoB ratio, and non-HDL-C/apoB ratio) were significantly higher in the F+ group compared with the F– group ($P \leq 0.01$), except for HDL-C which was significantly lower. The prevalence of DBL xanthoma (10% vs 1%), floating beta (47% vs 4%), E2/E2 (38% vs 1%), ASCVD event (17% vs 11%), CAD event (13% vs 9%),

**Table 1.** Baseline characteristics of patients who are negative vs positive for the classical Fredrickson criteria

| Variables | Reference | Fredrickson negative | Fredrickson positive | *P* value |
|---|---|---|---|---|
| | | N = 11908 | N = 524 | |
| Sex | Male (%) | 4698/8774 (54%) | 252/429 (59%) | **0.04** |
| Age | (year) | 47 ± 14 | 49 ± 13 | **0.02** |
| Systolic blood pressure | mmHg | 120 (110-132) | 127 (112-132) | 0.48 |
| Diastolic blood pressure | mmHg | 74 (70-80) | 76 (70-84) | 0.20 |
| Smoking | ever (%) | 9443 (79%) | 422 (81%) | 0.49 |
| Diabetes | n (%) | 526 (4%) | 18 (3%) | 0.28 |
| BMI | (kg/m$^2$) | 26.1 (23.4-29.1) | 27.2 (24.8-29.7) | **≤0.0001** |
| Menopause | n (%) | 1497/2749 (54%) | 91/126 (72%) | **<0.0001** |
| Total cholesterol | (mmol/L) | 6.06 (5.04-7.26) | 7.88 (6.45-9.59) | **≤0.0001** |
| Triglycerides | (mmol/L) | 1.81 (1.14-3.23) | 2.80 (2.05-4.22) | **≤0.0001** |
| Measured LDL-C | (mmol/L) | 3.68 (2.82-4.70) | 3.88 (2.66-5.40) | **0.002** |
| HDL-C | (mmol/L) | 0.96 (0.78-1.20) | 0.85 (0.75-1.02) | **≤0.0001** |
| Non-HDL-C | (mmol/L) | 5.03 (4.00-6.26) | 6.92 (5.55-8.81) | **≤0.0001** |
| VLDL-C (remnant-C) | (mmol/L) | 0.93 (0.60-1.52) | 2.32 (1.65-3.59) | **≤0.0001** |
| VLDL-TG | (mmol/L) | 1.36 (0.77-2.64) | 2.33 (1.61-3.80) | **≤0.0001** |
| VLDL-C/TG ratio | | 0.49 (0.40-0.59) | 0.79 (0.73-0.91) | **≤0.0001**[a] |
| Apolipoprotein B | (g/L) | 1.48 (1.16-1.87) | 1.72 (1.24-2.18) | **≤0.0001** |
| Lipoprotein (a) | (mg/dL) | 11.0 (3.0-31.0) | 16.0 (5.0-35.0) | **0.0003** |
| Total cholesterol/apoB ratio | mmol/g | 4.14 (3.69-4.63) | 4.52 (3.96-5.49) | **≤0.0001**[a] |
| Non-HDL-C/apoB ratio | mmol/g | 3.36 (3.06-3.74) | 4.03 (3.44-4.84) | **≤0.0001**[a] |
| DBL xanthoma | n (%) | 126 (1%) | 55 (10%) | **≤0.0001** |
| Tuberous or tuberoeruptive xanthoma | n (%) | 50 (0.4%) | 15 (3%) | **≤0.0001** |
| Eruptive xanthomas | n (%) | 28 (0.2%) | 6 (1%) | **≤0.0001** |
| Striated palmar xanthoma | n (%) | 59 (0.5%) | 48 (9%) | **≤0.0001** |
| Floating beta | n (%) | 241/5702 (4%) | 108/232 (47%) | **≤0.0001** |
| E2/E2 | n (%) | 103 (1%) | 197 (38%) | **≤0.0001** |
| ASCVD | n (%) | 1352 (11%) | 91 (17%) | **≤0.0001** |
| CAD | n (%) | 1072 (9%) | 69 (13%) | **0.001** |
| PVD | n (%) | 377 (3%) | 29 (6%) | **0.003** |
| CeVD | n (%) | 305 (3%) | 17 (3%) | 0.34 |

Data for continuous normally distributed variables are expressed as mean ± SD. Continuous logarithmic variables are expressed as median (Q1-Q3). All ratios are expressed in SI units.

Fredrickson criteria are the following: TG between 1.7 and 11.29 mmol/L + VLDL-C/TG > 0.69 (in mmol/L). Bold type indicates *P* values < 0.05.

Abbreviations: Apo, apolipoprotein; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CAD, coronary artery disease; CeVD, cerebrovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PVD, peripheral vascular disease; TG, triglycerides; VLDL, very-low-density lipoprotein.

[a]Nonparametric U Mann-Whitney test

and PVD event (6% vs 3%) was significantly higher in the F+ group compared with the F– group ($P \leq 0.001$).

Among subjects in the F+ group, only 197 were E2/E2 (38%) and 327 (62%) were non-E2/E2 (Table 2). The diabetes prevalence was higher (8% vs 1%) and the median of BMI was 1.4 kg/m$^2$ higher in the F+ E2/E2 than in the F+ non-E2/E2 group ($P \leq 0.0001$ and $P = 0.003$, respectively). LDL-C, apolipoprotein B, and lipoprotein (a) were significantly lower in the F+ E2/E2 than in the F+ non-E2/E2 group ($P < 0.01$), whereas TG, VLDL-C, VLDL-TG, VLDL-C/TG ratio, total cholesterol/apoB ratio, and non-HDL-C/apoB ratio were significantly higher ($P \leq 0.0001$). There was no difference of total cholesterol, HDL-C, and non-HDL-C between groups. The prevalence of DBL xanthoma (24% vs 2%), floating beta (95% vs 11%), ASCVD event (23% vs 14%), and PVD event (12% vs 2%) was significantly higher in the F+ E2/E2 group compared with the F+ non-E2/E2 group ($P \leq 0.01$). When men and women were analyzed separately, similar results were observed (Table 3).

The odds ratios for ASCVD, CAD, PVD, and CeVD events according to the presence of E2/E2 in F+ individuals are presented in Table 4. In the model corrected for traditional ASCVD risk factors, the presence of E2/E2 was

**Table 2.** Baseline characteristics of patients who are positive for the Fredrickson criteria, according to the absence or presence of E2/E2

| Variables | Reference | Fredrickson positive | Fredrickson positive | *P* value |
|---|---|---|---|---|
| | | Non-E2/E2 | E2/E2 | |
| | | N = 327 | N = 197 | |
| Sex | Male (%) | 145/246 (59%) | 107/183 (58%) | 0.92 |
| Age | (year) | 48 ± 12 | 50 ± 13 | 0.14 |
| Systolic blood pressure | mmHg | 125 ± 17 | 125 ± 14 | 0.97 |
| Diastolic blood pressure | mmHg | 76 ± 10 | 77 ± 12 | 0.51 |
| Smoking | ever (%) | 261 (80%) | 161 (82%) | 0.59 |
| Diabetes | n (%) | 3 (1%) | 15 (8%) | **≤0.0001** |
| BMI | (kg/m$^2$) | 26.9 ± 4.0 | 28.3 ± 3.5 | **0.003** |
| Total cholesterol | (mmol/L) | 7.96 (6.83-9.58) | 7.55 (5.86-9.68) | 0.08 |
| Triglycerides | (mmol/L) | 2.36 (1.97-3.24) | 3.79 (2.75-5.67) | **≤0.0001** |
| Measured LDL-C | (mmol/L) | 4.76 (3.62-6.12) | 2.67 (2.11-3.49) | **≤0.0001** |
| HDL-C | (mmol/L) | 0.85 (0.75-1.02) | 0.85 (0.72-1.03) | 0.79[a] |
| Non-HDL-C | (mmol/L) | 7.08 (5.89-8.82) | 6.70 (5.02-8.72) | 0.07 |
| VLDL-C (remnant-C) | (mmol/L) | 1.89 (1.53-2.67) | 3.44 (2.42-5.78) | **≤0.0001** |
| VLDL-TG | (mmol/L) | 1.84 (1.46-2.78) | 3.23 (2.23-4.99) | **≤0.0001** |
| VLDL-C/TG ratio | | 0.75 (0.71-0.83) | 0.90 (0.79-1.06) | **≤0.0001**[a] |
| Apolipoprotein B | (g/L) | 2.00 ± 0.59 | 1.39 ± 0.59 | **≤0.0001** |
| Lipoprotein (a) | (mg/dL) | 19.0 (7.0-43.8) | 10.0 (4.0-27.0) | **0.002** |
| Total cholesterol/apoB ratio | mmol/g | 4.12 (3.75-4.55) | 5.89 (5.00-7.09) | **≤0.0001** |
| Non-HDL-C/apoB ratio | mmol/g | 3.64 (3.28-4.09) | 5.13 (4.40-6.21) | **≤0.0001** |
| DBL xanthoma | n (%) | 8 (2%) | 47 (24%) | **≤0.0001** |
| Tuberous or tuberoeruptive xanthoma | n (%) | 5 (2%) | 10 (5%) | **0.03** |
| Eruptive xanthomas | n (%) | 1 (0.3%) | 5 (3%) | **0.03** |
| Striated palmar xanthoma | n (%) | 4 (1%) | 44 (22%) | **≤0.0001** |
| Floating beta | n (%) | 15/134 (11%) | 93/98 (95%) | **≤0.0001** |
| ASCVD | n (%) | 46 (14%) | 45 (23%) | **0.01** |
| CAD | n (%) | 42 (13%) | 27 (14%) | 0.78 |
| PVD | n (%) | 6 (2%) | 23 (12%) | **≤0.0001** |
| CeVD | n (%) | 8 (2%) | 9 (5%) | 0.18 |

Data for continuous normally distributed variables are expressed as mean ± SD. Continuous logarithmic variables are expressed as median (Q1-Q3). All ratios are expressed in SI units.

Fredrickson criteria are the following: TG between 1.7 and 11.29 mmol/L + VLDL-C/TG > 0.69 (in mmol/L). Bold type indicates *P* values < 0.05.

Abbreviations: Apo, apolipoprotein; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CAD, coronary artery disease; CeVD, cerebrovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PVD, peripheral vascular disease; TG, triglycerides; VLDL, very-low-density lipoprotein.

[a]Nonparametric U Mann-Whitney test

**Table 3.** Baseline characteristics of men vs women who are positive for the Fredrickson criteria, according to the absence or presence of E2/E2

| Variables | Reference | Fredrickson positive Non-E2/E2 Men N = 145 | Fredrickson positive E2/E2 Men N = 107 | *P* value for comparison between men | Fredrickson positive Non-E2/E2 Women N = 101 | Fredrickson positive E2/E2 Women N = 76 | *P* value for comparison between women |
|---|---|---|---|---|---|---|---|
| Age | (year) | 45 ± 10 | 45 ± 11 | 0.68 | 51 ± 14 | 56 ± 11 | **0.04** |
| Systolic blood pressure | mmHg | 126 ± 17 | 124 ± 14 | 0.69 | 120 (110-141) | 130 (110-135) | 0.92 |
| Diastolic blood pressure | mmHg | 77 ± 10 | 79 ± 13 | 0.59 | 80 ± 9 | 71 ± 7 | **0.04** |
| Smoking | ever (%) | 127 (88%) | 93 (87%) | 0.87 | 68 (67%) | 54 (71%) | 0.60 |
| Diabetes | n (%) | 1 (1%) | 9 (8%) | **0.002** | 2 (2%) | 6 (8%) | 0.08 |
| BMI | ($kg/m^2$) | 26.4 ± 2.9 | 27.5 ± 2.9 | **0.04** | 27.8 ± 5.2 | 29.3 ± 3.9 | 0.12 |
| Total cholesterol | (mmol/L) | 8.20 ± 2.10 | 7.71 ± 2.68 | 0.12 | 7.96 (6.92-9.79) | 7.84 (6.57-10.02) | 0.91 |
| Triglycerides | (mmol/L) | 2.54 (2.00-3.49) | 3.72 (2.61-5.93) | **≤0.0001** | 2.58 (1.97-3.47) | 3.89 (2.85-5.41) | **≤0.0001** |
| Measured LDL-C | (mmol/L) | 4.92 ± 2.03 | 2.67 ± 1.09 | **≤0.0001** | 4.80 (3.63-6.43) | 3.12 (2.31-4.14) | **≤0.0001** |
| HDL-C | (mmol/L) | 0.83 (0.72-0.91) | 0.80 (0.68-0.95) | 0.49 | 0.88 (0.80-1.10) | 0.95 (0.80-1.11) | 0.63[a] |
| Non-HDL-C | (mmol/L) | 7.38 ± 2.14 | 6.86 ± 2.73 | 0.11 | 7.05 (6.02-8.94) | 6.87 (5.69-9.06) | 0.93 |
| VLDL-C (remnant-C) | (mmol/L) | 2.07 (1.61-2.73) | 3.43 (2.34-5.61) | **≤0.0001** | 1.91 (1.48-2.87) | 3.46 (2.60-6.03) | **≤0.0001** |
| VLDL-TG | (mmol/L) | 2.06 (1.54-3.16) | 3.30 (2.21-5.29) | **≤0.0001** | 1.91 (1.40-2.65) | 3.26 (2.31-4.28) | **≤0.0001** |
| VLDL-C/TG ratio | | 0.75 (0.72-0.83) | 0.90 (0.76-1.02) | **≤0.0001**[a] | 0.74 (0.71-0.81) | 0.90 (0.80-1.12) | **≤0.0001**[a] |
| Apolipoprotein B | (g/L) | 1.97 ± 0.50 | 1.37 ± 0.63 | **≤0.0001** | 2.15 (1.68-2.51) | 1.25 (1.04-1.56) | **≤0.0001** |
| Lipoprotein (a) | (mg/dL) | 20.0 (6.0-45.0) | 14.5 (4.0-27.0) | 0.08 | 18.0 (5.0-58.5) | 7.0 (3.0-25.5) | **0.01** |
| Total cholesterol/apoB ratio | mmol/g | 4.12 (3.73-4.54) | 5.75 (4.92-7.17) | **≤0.0001**[a] | 4.02 (3.76-4.55) | 6.13 (5.24-7.07) | **≤0.0001**[a] |
| Non-HDL-C/apoB ratio | mmol/g | 3.67 (3.284.11) | 5.06 (4.25-6.27) | **≤0.0001**[a] | 3.56 (3.30-3.92) | 5.33 (4.52-6.25) | **≤0.0001**[a] |
| DBL xanthoma | n (%) | 3 (2%) | 28 (26%) | **≤0.0001** | 4 (4%) | 19 (25%) | **≤0.0001** |
| Tuberous or tuberoeruptive xanthoma | n (%) | 2 (1%) | 7 (7%) | **0.04** | 2 (2%) | 3 (4%) | 0.65 |
| Eruptive xanthomas | n (%) | 1 (1%) | 4 (4%) | 0.17 | 0 (0%) | 1 (1%) | 0.43 |
| Striated palmar xanthoma | n (%) | 2 (1%) | 25 (23%) | **≤0.0001** | 2 (2%) | 19 (25%) | **≤0.0001** |
| Floating beta | n (%) | 9/69 (9%) | 45/47 (96%) | **≤0.0001** | 5/47 (11%) | 39/41 (95%) | **≤0.0001** |
| ASCVD | n (%) | 24 (17%) | 30 (28%) | **0.03** | 11 (11%) | 13 (8%) | 0.23 |
| CAD | n (%) | 22 (15%) | 17 (16%) | 0.88 | 11 (11%) | 8 (11%) | 0.94 |
| PVD | n (%) | 4 (3%) | 16 (15%) | **0.001** | 1 (1%) | 6 (8%) | **0.04** |
| CeVD | n (%) | 3 (2%) | 6 (6%) | 0.18 | 1 (1%) | 3 (4%) | 0.32 |

Data for continuous normally distributed variables are expressed as mean ± SD. Continuous logarithmic variables are expressed as median (Q1-Q3). All ratios are expressed in SI units.

Fredrickson criteria are the following: TG between 1.7 and 11.29 mmol/L + VLDL-C/TG > 0.69 (in mmol/L). Bold type indicates *P* values < 0.05.

Abbreviations: Apo, apolipoprotein; BMI, body mass index; CAD, coronary artery disease; CeVD, cerebrovascular disease; ASCVD, atherosclerotic cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; VLDL, very-low-density lipoprotein.

[a]Nonparametric U Mann-Whitney test

a significant predictor of both ASCVD events (OR 2.99 [95% CI, 1.21-7.37]; *P* = 0.02) and PVD events (OR 11.12 [95% CI, 1.87-66.05]; *P* = 0.008). The association was not significant for CAD and CeVD.

The prevalence of individuals with 0 vs 1 vs ≥ 2 features of DBL is presented in Fig. 2A and 2B. The features include DBL-related xanthoma (tuberous, tuberoeruptive, striated palmar, and eruptive), floating beta on electrophoresis, and

**Table 4.** Odds ratio (OR) of ASCVD, CAD, PVD and CeVD events according to the presence of E2/E2 in F+ individuals according to different regression models

| Model | OR (95% CI) | *P* value |
|---|---|---|
| ASCVD uncorrected | 1.81 (1.15-2.85) | **0.01** |
| ASCVD corrected | 2.99 (1.21-7.37) | **0.02** |
| CAD uncorrected | 1.08 (0.64-1.81) | 0.78 |
| CAD corrected | 1.33 (0.49-3.59) | 0.58 |
| PVD uncorrected | 7.07 (2.83-17.70) | **≤0.0001** |
| PVD corrected | 11.12 (1.87-66.05) | **0.008** |
| CeVD uncorrected | 1.91 (0.72-5.03) | 0.19 |
| CeVD corrected | 3.04 (0.36-25.62) | 0.31 |

Variables included in the corrected models: age, sex, diabetes, body mass index, smoking, LDL-C, and HDL-C.
Bold type indicates *P* values < 0.05.
Abbreviations: ASCVD, atherosclerotic cardiovascular disease; CAD, coronary artery disease; CeVD, cerebrovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PVD, peripheral vascular disease.

atherosclerotic cardiovascular disease event. The prevalence of subjects without any feature was higher in the F– compared to the F+ groups (79% vs 44%, respectively) and the prevalence of subjects presenting ≥ 2 features of DBL was lower in the F– group compared with the F+ group (1% vs 23%, respectively) $P \leq 0.0001$. When the F+ non-E2/E2 and the F+ E2/E2 groups were compared, the majority of the F+ non-E2/E2 group had 0 features (73%), whereas 24% had 1 feature, and 3% had 2 or more features. In the F+ E2/E2, 4% had 0 features, 45% had 1 feature, and 51% had 2 or more features ($P \leq 0.0001$).

Characteristics of F+ patients carrying a single copy of the ε2 allele/E2 isoform (E4/E2 or E3/E2) are presented in Table 5 and compared with F+ non-E2 and F+ E2/E2 groups. The highest prevalence of diabetes (8%), DBL-related xanthoma (24%), floating beta (95%), ASCVD (23%), and PVD (12%) was observed in the F+ E2/E2 group, whereas the lowest prevalence was found in the F+ non-E2 group for diabetes (<1%), floating beta (7%), ASCVD (13%), and PVD (1%) and in the F+ E4/E2 or E3/E2 group for DBL-related xanthomas (2%). BMI was significantly higher in the F+ E2/E2 group than in the F+ non-E2 group ($P = 0.009$), whereas total cholesterol was significantly lower ($P = 0.04$). Triglycerides, VLDL-C, and VLDL-TG were significantly higher in the single E2 group compared with the F+ non-E2 and significantly lower than in the F+ E2/E2 group ($P < 0.0001$). LDL-C and apoB were both significantly lower in the single E2 group than in the F+ non-E2 and significantly higher than in the F+ E2/E2 group ($P < 0.0001$). VLDL-C/TG, total cholesterol/apoB ratio, and non-HDL-C/apoB ratio were significantly higher in the F+ E2/E2 group than in the other groups ($P < 0.0001$). Lipoprotein (a) was significantly lower in the in the F+ E2/E2 group than in the other groups ($P = 0.008$).

## Discussion

This study describes one of the largest cohorts of DBL patients worldwide diagnosed using the gold standard criteria. Among our cohort of 12 432 dyslipidemic patients, 4% were positive for the Fredrickson criteria and 2% were F+ E2/E2. It was previously reported that 90% of DBL patients carry the E2/E2 genotype (2, 13-15), but our data do not support this observation. In this study, we demonstrated that only 38% of individuals positive for the gold standard Fredrickson criteria for DBL also carry apoE2/E2, and that only 47% had floating beta on lipoprotein electrophoresis. Importantly, individuals who were Fredrickson positive (F+) with E2/E2 had a more severe phenotype than those who were F+ without E2/E2. Indeed, the F+ E2/E2 group had significantly higher remnant cholesterol concentration and had higher frequency of DBL-related xanthomas (tuberous, tuberoeruptive, striated palmar, or eruptive) than the F+ non-E2/E2 group.

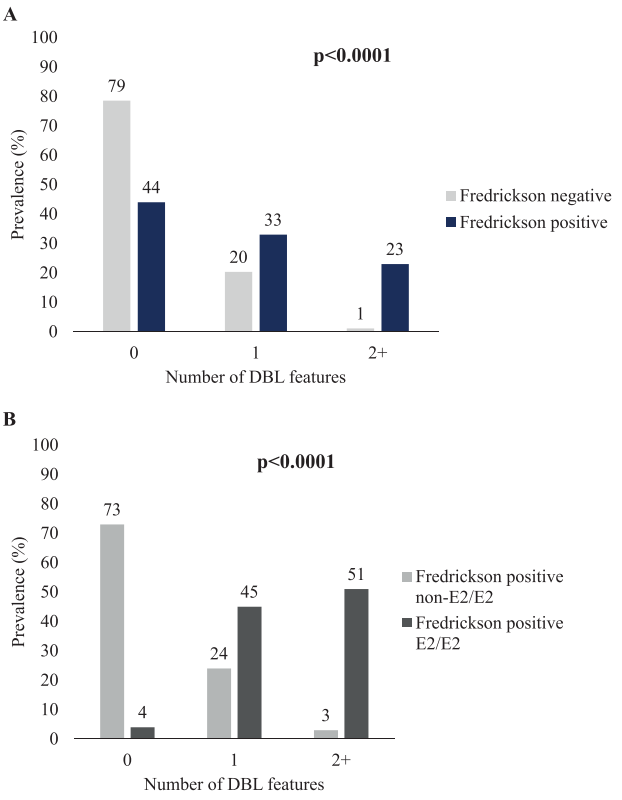


**Figure 2.** Prevalence of individuals with 0 vs 1 vs ≥ 2 features of DBL. A, In Fredrickson negative vs Fredrickson positive patients. B, In Fredrickson positive non-E2/E2 vs Fredrickson positive E2/E2 patients. The features include DBL-related xanthoma (tuberous, tuberoeruptive, striated palmar, and eruptive), floating beta on electrophoresis and atherosclerotic cardiovascular disease event. Abbreviation: DBL, dysbetalipoproteinemia.

**Table 5.** Baseline characteristics of patients who are positive for the Fredrickson criteria, according to the number of ε2 allele/E2 isoform

| Variables | Reference | Non-E2 | E4/E2 or E3/E2 | E2/E2 | *P* value |
|---|---|---|---|---|---|
| | | F+ | F+ | F+ | |
| | | N = 217 | N = 110 | N = 197 | |
| Sex | Male (%) | 86/150 (57%) | 59/96 (61%) | 107/183 (58%) | 0.81 |
| Age | (year) | 47 ± 13 | 48 ± 12 | 50 ± 13 | 0.30 |
| SBP | mmHg | 126 ± 20 | 123 ± 13 | 125 ± 14 | 0.75 |
| DBP | mmHg | 76 ± 10 | 75 ± 9 | 77 ± 12 | 0.75 |
| Smoking | ever (%) | 173 (80%) | 88 (80%) | 161 (82%) | 0.87 |
| Diabetes | n (%) | 1 (<1%) | 2 (2%) | 15 (8%) | **0.0002** |
| BMI | ($kg/m^2$) | 26.8 ± 4.3 [a] | 27.3 ± 3.4 [ab] | 28.4 ± 3.5 [b] | **0.009** |
| Total cholesterol | (mmol/L) | 8.12 (6.88-9.79)[a] | 7.58 (6.52-9.11) [ab] | 7.55 (5.86-9.68) [b] | **0.04**[a] |
| Triglycerides | (mmol/L) | 2.23 (1.95-2.93) [a] | 2.76 (2.00-4.54) [b] | 3.79 (2.75-5.67) [c] | **<0.0001**[a] |
| Measured LDL-C | (mmol/L) | 4.99 (3.96-6.67) [a] | 4.07 (2.91-5.31) [b] | 2.67 (2.11-3.49) [c] | **<0.0001** |
| HDL-C | (mmol/L) | 0.85 (0.75-1.03) | 0.85 (0.75-0.98) | 0.85 (0.72-1.03) | 0.90 |
| Non-HDL-C | (mmol/L) | 7.19 (5.99-8.92) | 6.81 (5.55-8.23) | 6.70 (5.02-8.72) | 0.052[a] |
| VLDL-C (remnant-C) | (mmol/L) | 1.78 (1.50-2.39) [a] | 2.17 (1.63-3.52) [b] | 3.44 (2.42-5.78) [c] | **<0.0001**[a] |
| VLDL-TG | (mmol/L) | 1.69 (1.42-2.33) [a] | 2.32 (1.63-3.97) [b] | 3.23 (2.23-4.99) [c] | **<0.0001**[a] |
| VLDL-C/TG ratio | | 0.75 (0.71-0.83) [a] | 0.75 (0.71-0.83) [a] | 0.90 (0.79-1.06) [b] | **<0.0001**[a] |
| ApoB | | 2.07 ± 0.56 [a] | 1.89 ± 0.64 [b] | 1.39 ± 0.59 [c] | **<0.0001** |
| Lipoprotein (a) | (mg/dL) | 17.0 (7.0-41.0) [a] | 22.0 (6.8-47.5) [a] | 10.0 (4.0-27.0) [b] | **0.008** |
| Total cholesterol/apoB ratio | | 4.10 (3.76-4.42) [a] | 4.28 (3.74-4.94) [a] | 5.89 (5.00-7.09) [b] | **<0.0001**[a] |
| Non-HDL-C/apoB ratio | (mmol/g) | 3.62 (3.28-3.99) [a] | 3.67 (3.28-4.27) [a] | 5.13 (4.40-6.21) [b] | **<0.0001**[a] |
| DBL xanthoma | n (%) | 6 (3%) | 2 (2%) | 47 (24%) | **<0.0001** |
| Floating beta | n (%) | 6/88 (7%) | 9/46 (20%) | 93/98 (95%) | **<0.0001** |
| ASCVD | n (%) | 29 (13%) | 17 (15%) | 45 (23%) | **0.03** |
| PVD | n (%) | 2 (1%) | 4 (4%) | 23 (12%) | **<0.0001** |

Bold type indicates *P* values < 0.05.

The Tukey test was used for multiple comparison testing, where a is significantly different from b, ab is not different from neither a nor b, and c is different from a and b.

Abbreviations: ApoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PVD, peripheral vascular disease; SBP, systolic blood pressure; TG, triglycerides; VLDL, very-low-density lipoprotein.

[a]Nonparametric Kruskall-Wallis test.

Furthermore, subjects in the F+ E2/E2 group had significantly lower LDL-C than the F+ non-E2/E2 group and this could be due to the lower conversion of VLDL to LDL in more severely affected individuals, although LDL quantification by beta-quantification may be inexact when significant remnant lipoproteins are present. A similar effect could be seen for apoB. The lower lipoprotein (a) concentration observed in F+ E2/E2 compared with the F+ non E2/E2 may be due to the decreased covalent binding of apo(a) to the apoB of the larger triglyceride rich lipoproteins observed in E2/E2 individuals.

More importantly, the F+ E2/E2 group had a significantly higher prevalence of floating beta (95% vs 11%), as well as ASCVD (23% vs 14%) and PVD (12% vs 2%) events, compared with the F+ non-E2/E2 group. A main clinical finding of this study is the independent 3-fold increased risk of ASCVD and 11-fold increased risk of PVD in F+ E2/E2 individuals compared with the F+ non-E2/E2. Furthermore, when the numbers of DBL features were evaluated (DBL-related xanthomas, floating beta, and ASCVD), the majority of F+ individuals had 0 features (44%), but when the E2/E2 criterion was added, the majority had ≥ 2 features (51%).

We therefore conclude that combining the Fredrickson criteria for DBL with the presence of E2/E2 is associated with a more severe dyslipidemia and a higher frequency of DBL-related xanthomas, ASCVD, and PVD. These results strongly suggest that there exist 2 distinct forms of DBL: a multifactorial form and a form associated with genetic apoE dysfunction (Fig. 3). The 11-fold increased risk of PVD in subjects with genetic apoE dysfunction compared with those with multifactorial DBL suggests that the former

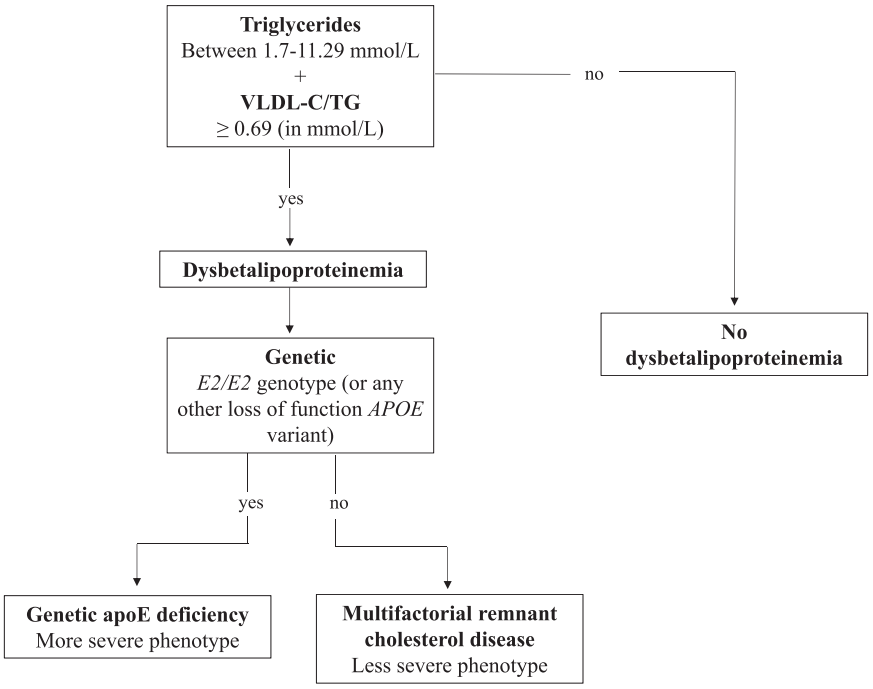


**Figure 3.** Proposed algorithm for dysbetalipoproteinemia phenotypes.

group should be treated earlier and more aggressively. Furthermore, information concerning the *APOE* genotype in DBL subjects could be useful to initiate discussion with the patient concerning the importance of maintaining a cardioprotective lifestyle and to promote compliance to long term pharmacological.

Interestingly, individuals who are F+ and carrying a single copy of the ε2 allele/E2 isoform present an intermediate phenotype that is closer to the non-E2 group than the E2/E2 group. Indeed, the values for the VLDL-C/TG ratio as well as non-HDL-C/apoB ratio are very similar between this group and the non-E2 group. The proportion of individuals with ASCVD and PVD are slightly higher in the single E2 group than in the non-E2 group, but markedly lower than in the E2/E2 group. Therefore, we suggest that the F+ individuals carrying a single copy of the ε2 allele/E2 isoform should still be classified as having multifactorial remnant cholesterol disease.

Our study also supports the fact that genetic testing such as *APOE* genotyping or *APOE* sequencing is an important component of the diagnosis algorithm of DBL since the presence of a defective apoE is associated with a more severe DBL phenotype. Patients with a VLDL-C/TG ratio ≥ 90$^{th}$ percentile (0.93 in the F+ non-E2/E2 group) or those with DBL-related features should be screened in priority.

Our study comprises several strengths and limitations. The major strengths include the large and well-characterized sample size of dyslipidemic patients that includes 524 F+ patients. Furthermore, the number of subjects with the DBL criteria was much higher than in the initial publication by Frederickson et al (9) and our study represents one of the largest published cohorts of DBL patients diagnosed using the gold standard criteria.

Limitations include the fact that the *APOE* gene was not sequenced. Therefore, it is probable that a few individuals with a rare dominant variant in *APOE* gene were included in the F+ non-E2/E2 group. This could contribute to attenuate the differences between groups. It is therefore likely that, with a proper reclassification, our results would be further strengthened. However, according to findings in other cohorts, rare variants are not significant contributory factors in the development of DBL and the prevalence among DBL patients could be less than 10% (15). Another limitation is the fact that the patients included in the present cohort have been recruited in a specialized lipid clinic and not in the general population. Therefore, a referral bias is present and all prevalence data presented in this study should be interpreted with caution. Indeed, since patients were referred for dyslipidemia, there was an enrichment in E2/E2 individuals (2.4% vs < 1% in Caucasian populations) (27). Furthermore, we observed an enrichment of the prevalence of F+ individuals (4.2% vs 0.2%-0.68%) (28-30) and in the proportion of those with E2/E2 who developed DBL (65.7% vs < 20%) (11). The prevalence of xanthomas and other clinical manifestations of dyslipidemia should also be interpreted with caution since their presence may not have

been deeply investigated equally for each patient. Finally, electrophoresis results were only present for a subgroup of patients, which may have introduced a bias.

## Conclusion

In this very large cohort of well-characterized dyslipidemic patients, we observed that the prevalence of DBL according to the gold standard Fredrickson criteria was 4% and that only 38% of those positive individuals were carriers of E2/E2. Among the F+ patients, the presence of E2/E2 was associated with a more severe DBL phenotype, including higher remnant cholesterol concentration and a higher frequency of DBL-related xanthomas, floating beta, ASCVD, and PVD. These results suggest that the DBL phenotype is heterogeneous and that genetic testing of the *APOE* gene is a crucial part of the screening in order to identify more severe cases of the disease and the individuals who would benefit from more aggressive lifestyle changes and treatment.

## Acknowledgments

The authors want to thank the Montreal Clinical Research Institute (IRCM) research team and the nursing staff for their everyday help, support, and involvement.

***Financial Support:*** None.

***Author Contributions:*** All authors contributed to the discussion, analysis, and interpretation of data and have reviewed the article for the intellectual content. M.P. performed statistical analysis and has drafted the manuscript. All authors have approved the final article. A.B. had primary responsibility for final content.

## Additional Information

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***Disclosures:*** A.B. received research grants from Akcea, Amgen, Astra Zeneca, the Fondation Leducq, Merck Frosst, and Sanofi. He has participated in clinical research protocols from Acasti Pharma Inc., Akcea, Amgen, Astra Zeneca, Ionis Pharmaceuticals, Inc., The Medicines Company, Merck Frosst, Novartis, Pfizer, Regeneron Pharmaceuticals Inc., and Sanofi. He has served on advisory boards and received honoraria for symposia from Akcea, Amgen, and Sanofi.

S.B. has participated in clinical research protocols from Akcea, Amgen, The Medicines Company, and Sanofi. She has served on advisory boards for Akcea, Amgen, HLS Therapeutics, Novartis, Novo Nordisk, and Sanofi, and received honoraria for symposia from Akcea, Amgen, Novo Nordisk, and Sanofi-Aventis.

G.P. has served on advisory boards and received honoraria for symposia from Akcea, Amgen, Bayer, and Sanofi.

M.P. has nothing to declare.

***Data Availability:*** Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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