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Adiposity and the development of dyslipidemia in APOE ε2 homozygous subjects: A longitudinal analysis in two population-based cohorts

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ABSTRACT

Background and aims: Familial dysbetalipoproteinemia (FD), characterized by remnant lipoprotein accumulation and premature cardiovascular disease, occurs in homozygous carriers of the *APOE* ε2 allele, but genetic predisposition alone does not suffice for the clinical phenotype. Cross-sectional studies suggest that a second metabolic hit – notably adiposity or insulin resistance – is required, but the association between these risk factors and development of FD has not been studied prospectively.

Methods: For this study, we evaluated 18,987 subjects from two large prospective Dutch population-based cohorts (PREVEND and Rotterdam Study) of whom 118 were homozygous *APOE* ε2 carriers. Of these, 69 subjects were available for prospective analyses. Dyslipidemia – likely to be FD – was defined as fasting triglyceride (TG) levels >3 mmol/L in untreated subjects or use of lipid lowering medication. The effect of weight, body mass index (BMI), waist circumference, type 2 diabetes mellitus and non-TG metabolic syndrome on development of dyslipidemia was investigated.

Results: Eleven of the 69 ε2ε2 subjects (16%) developed dyslipidemia – likely FD – during follow-up. Age-, sex- and cohort-adjusted risk factors for the development of FD were BMI (OR 1.19; 95%CI 1.04–1.39), waist circumference (OR 1.26 95%CI 1.01–1.61) and presence of non-TG metabolic syndrome (OR 4.39; 95%CI 1.04–18.4) at baseline. Change in adiposity during follow-up was not associated with development of dyslipidemia.

Conclusions: Adiposity increases the risk of developing an FD-like lipid phenotype in homozygous *APOE* ε2 subjects. These results stress the importance of healthy body weight in subjects at risk of developing FD.

1. Introduction

The apolipoprotein E gene *(APOE)* codes for the ApoE protein, which plays a crucial role in lipoprotein metabolism by effecting hepatic clearance of triglyceride rich lipoproteins (TRLs) comprising chylomicrons, very low density lipoprotein (VLDL) and their remnants [1,2]. There are three *APOE* variants designated *APOE*-ε3, -ε4, and -ε2, with corresponding allele frequencies of approximately 78%, 14% and 8%, respectively [3]. Subjects with an *APOE* ε2ε2 genotype generally have lower plasma total cholesterol, lower low-density lipoprotein cholesterol (LDL-C) and lower apolipoprotein B (ApoB) plasma levels [3,4] and are therefore, on average, at lower risk of cardiovascular disease (CVD) compared to subjects with other *APOE* genotypes [3,5,6]. However, approximately 15% of ε2 homozygotes develop familial dysbetalipoproteinemia (FD), which is characterized by increased remnant lipoprotein plasma concentrations [7]. These cholesterol-enriched remnant lipoproteins cause foam cell accumulation and low-grade inflammation in the vascular wall of arteries, contributing to the process of atherosclerosis. Hence, in FD, the protective ε2 lipid profile transforms to a highly atherogenic lipoprotein phenotype. This 'switch' from the favorable hypolipidemic to dysbetalipoproteinemic state is most likely caused by secondary metabolic abnormalities, in addition to the genetic

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predisposition. Several additional risk factors, including adiposity and insulin resistance, have been postulated to be associated with FD lipid phenotype in ε2ε2 subjects. However, the direction of this association between adiposity and insulin resistance and the development of FD is unclear, considering the majority of the studies were limited to a cross-sectional study design [8–13]. The exact underlying pathophysiological mechanism is uncertain, but might relate to hepatic overproduction of VLDL particles and impaired triglyceride (TG) lipolysis due to insulin resistance [14]. In ε2 homozygotes, the altered conformation in the ApoE2 protein decreases the affinity for the low-density lipoprotein receptor (LDL-R) compared to ApoE3 by >98% [14]. In ε2 homozygotes, remnant lipoproteins cannot be cleared efficiently from the circulation by the LDL-R, but in most subjects this is of little consequence because the second remnant clearing receptor, the heparan sulphate proteoglycan receptor (HSPG-R), functions normally. However, studies in mice have shown that, in an insulin resistant state, the HSPG-R is degraded by upregulation of sulfatase 2 (Sulf2) [15]. This mechanism could be causally implicated in the extensive remnant accumulation seen in FD [16–19]. Furthermore, it has been shown that ε2 heterozygotes could also develop a typical FD lipoprotein phenotype, demonstrated with ultracentrifugation [20]. The aim of this study was to prospectively evaluate the association between adiposity, type 2 diabetes mellitus (T2DM), non-TG metabolic syndrome (MetS) and the development of dyslipidemia – likely FD – in ε2ε2 subjects from the general population.

2. Patients and methods

2.1. Study population

Subjects from two large Dutch population-based, prospective cohorts were included: the Prevention of Renal and Vascular End-Stage Disease cohort and the Rotterdam Study. Details of the study design and recruitment have been described in previous reports [21–23]. In brief, the PREVEND cohort investigates renal and vascular damage in the general population. In 1997–1998, all inhabitants of the city of Groningen, aged 28–75 years (n = 85,421), were asked to complete a short questionnaire for collection of demographics and cardiovascular morbidity and to provide a sample of early morning urine. Of the responders, all subjects with a urinary albumin concentration ≥10 mg/L were invited for a baseline visit and 6000 were enrolled. Additionally, a randomly selected group with a urinary albumin concentration of <10 mg/L was invited for a baseline visit and 2592 were enrolled. In total, 8592 subjects completed the baseline visit. The Rotterdam Study aims to unravel the etiology and natural history of chronic diseases in mid-life and late-life, including cardiovascular, endocrine, hepatic and neurological diseases, among inhabitants of the Ommoord district in the city of Rotterdam. This ongoing prospective cohort started in 1990, and initially all inhabitants above 55 years were invited for participation. The cohort was subsequently expanded in 2000 and again in 2005, with inclusion of subjects above 45 years. Subjects are invited for an interview and an extensive set of examinations every 3–4 years. From the Rotterdam Study, we included all subjects who attended the research center between 1997 and 2006 for the third examination cycle of the first cohort, and the baseline examination of both expansion cohorts (n = 10,395).

For the present study, we combined both studies resulting in 18,987 subjects. Thereafter, we excluded subjects without an *APOE* ε2 genotype (n = 17.924) or subjects without *APOE* genotype measurement (n = 945), resulting in 118 homozygous subjects (0.6%) with the *APOE* ε2 genotype. There were no important differences in participant (age, sex, body mass index (BMI), waist and blood pressure) and clinical (CVD, T2DM, total cholesterol (TC) and TG) characteristics between subjects with and without *APOE* genotyping. The median time interval between baseline and follow-up in the PREVEND cohort was 4.2 (IQR 4.0–4.3) years and in the Rotterdam Study 10.4 (IQR 5.6–10.7) years. For the prospective analyses in this study, ε2ε2 subjects with FD-like lipid phenotype at baseline (n = 23) were excluded. Of the remaining 95 subjects, 69 were re-examined during follow-up. See Supplementary Fig. 1 for a flowchart of subjects included in or excluded from this study. All subjects gave written informed consent and the Ethics Committee of the institutions approved the studies.

2.2. Baseline and follow-up measurements in PREVEND and Rotterdam Study

In both cohorts, examinations were performed as part of a standardized screening protocol as previously described [24,25]. BMI was calculated as weight in kilograms (kg) divided by height in meters (m) squared. Alcohol consumption in PREVEND was defined as self-reported current alcohol consumption (≥10 g every month) and no alcohol use was defined as rare (<10 g/every month) or no alcohol consumption. In the Rotterdam Study, alcohol consumption was defined as minimum alcohol intake of 1 g/day and no alcohol use was defined as <1 g/day. Smoking was defined as current smoking. In PREVEND, information on medication use was based on questionnaires and combined with information from a pharmacy-dispensing registry, which has complete information on drug usage for >95% of subjects. In the Rotterdam Study, medication use was assessed by interview at every visit. T2DM was defined as a fasting blood glucose concentration ≥7.0 mmol/L, a non-fasting blood glucose concentration ≥11.1 mmol/L (when fasting samples were unavailable), or the use of blood glucose-lowering drugs. MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III criteria [26]. For non-TG metabolic syndrome (non-TG MetS), the criterion for MetS was used by replacing the criterion of elevated TG with elevated high sensitivity C-reactive protein (hsCRP) (≥2 mg/L), because TG was used to define dyslipidemia – likely FD. This was based on previous works in which waist circumference was replaced by hsCRP in the definition of MetS [27,28]. This implies that subjects must fulfill ≥3 individual criteria of non-TG MetS, which is not necessarily the hsCRP criterion, just like in the original MetS criterion. In PREVEND, previous coronary artery disease (CAD) and stroke were based on interview at baseline. CAD was defined as myocardial infarction or coronary revascularization and stroke was defined as previous ischemic or hemorrhagic stroke. In the Rotterdam Study, history of myocardial infarction and stroke was assessed by interview and confirmed by medical records (from general practitioner and/or hospital). CAD was defined as previous myocardial infarction and stroke was defined as previous ischemic or hemorrhagic stroke. Both studies instructed subjects to have their blood samples taken in a fasting state and lipids were determined by standard analytical methods [24,25].

2.3. Outcome

In this study, dyslipidemia – likely FD – or FD-like lipid phenotype was defined as fasting plasma TG levels >3 mmoL/l or use of lipid lowering medication. This definition was used as the reference standard for the diagnosis of FD (ultracentrifugation [29]) is not part of standard laboratory analyses. To overcome this, measurement of apolipoprotein B (ApoB) can distinguish between other causes of mixed hyperlipidemia or hypertriglyceridemia and FD [30]. However, currently there are no prospectively validated algorithms to screen for FD. In addition, previously developed ApoB algorithms were all validated in cohorts with dyslipidemic patients, while the current study consists of subjects from the general population. Furthermore, ApoB levels were only measured in half of the study population.

2.4. Analyses

Baseline characteristics are presented for the total study population. Baseline data are presented as number and percentage for categorical

variables, mean and standard deviation (SD) for normally distributed variables or median with interquartile range (IQR) in case of unevenly distributed variables. For the cross-sectional analyses, we included 118 subjects with a homozygous *APOE* ε2 genotype and evaluated the association with risk factors and the presence of FD-like lipid phenotype at baseline. For the prospective analyses, 69 ε2ε2 subjects without FD-like lipid phenotype at baseline and with a follow-up visit were included. Lipid measures were only evaluated during the first and last follow-up visit of the Rotterdam Study. Baseline characteristics and difference in change of these characteristics in subjects who did and did not develop dyslipidemia – likely FD – during follow-up were evaluated. Thereafter, the effect of baseline characteristics and change in clinical characteristics between baseline and follow-up was assessed with logistic regression models adjusted for age, sex and cohort. The models assessing change between baseline and follow-up were additionally adjusted for baseline values. HsCRP at the follow-up measurement, and therefore change in non-TG MetS status, was not available in half of the cohort (Rotterdam Study). Missing data (with a maximum of 18% for use of lipid-lowering- and antihypertensive mediation in PREVEND and with a maximum of 17% for alcohol use in the Rotterdam Study) were imputed by single imputation using predictive mean matching. All analyses were conducted in R statistical software, version 3.5.1. For all analyses, a *p*-value <0.05 was considered statistically significant.

3. Results

3.1. Study population

Baseline characteristics of 118 subjects with an *APOE* ε2 genotype are presented in Table 1. In total, 46% were male, age 58 ± 14 years. Their mean BMI was 26.7 ± 4.7 kg/m^2 and waist circumference was 92 ± 14 cm. CAD was present in 5% of the subjects and 3% had a previous stroke. Furthermore, 10% had T2DM and 37% non-TG MetS at baseline. To compare the clinical variables of these ε2 homozygotes with the general population (including carriers of an ε3 and ε4 allele), an overview of both cohorts is given in Supplementary Table 1. This table shows that clinical variables at baseline of ε2 homozygotes are very similar compared to subjects with other *APOE* genotypes.

Table 1
Baseline characteristics of 118 subjects with an *APOE* ε2ε2 genotype.

Male sex (n)	54 (46%)
Age (years)	58 ± 14
Weight (kg)	78 ± 16
Body mass index (kg/m^2)	26.7 ± 4.7
Systolic blood pressure (mmHg)	136 ± 21
Diastolic blood pressure (mmHg)	77 ± 11
Waist circumference (cm)	92 ± 14
Current smoking (n)	34 (29%)
Alcohol consumption (n)	80 (68%)
Coronary heart disease (n)	6 (5%)
Stroke (n)	3 (3%)
Diabetes mellitus type 2 (n)	12 (10%)
Metabolic syndrome (n)	42 (36%)
Non-TG metabolic syndrome (n)[a]	44 (37%)
Lipid lowering medication (n)	10 (8%)
Antihypertensives (n)	26 (22%)
Total cholesterol (mmol/L)	5.23 ± 1.69
HDL-cholesterol (mmol/L)	1.39 ± 0.39
Non-HDL-cholesterol (mmol/L)	3.84 ± 1.78
Triglycerides (mmol/L)[b]	1.57 (1.07–2.29)
hsCRP (mg/L)[b]	1.5 (0.7–2.7)
Creatinine (umol/L)[b]	79 (70–89)

APOE = apolipoprotein E TG = triglycerides; HDL = high-density lipoprotein; non-HDL = non-high-density lipoprotein; HsCRP = high sensitivity C-reactive protein.
[a] Adaptation of original criterion for MetS by replacing the criterion of elevated TG for elevated high sensitivity C-reactive protein (hsCRP) (≥2 mg/L).
[b] Median with interquartile range.

3.2. Association between baseline characteristics and presence of FD-like lipid phenotype

At baseline, 19% (n = 23) of the subjects had dyslipidemia – likely FD – and 81% (n = 95) did not (Supplementary Table 2; cross-sectional analyses are presented in the Supplementary Materials because the focus of this study is the prospective analyses). In general, subjects with dyslipidemia at baseline were more often male and had an older age. Subjects with dyslipidemia at baseline had higher body weight (OR 1.24 95%CI 1.05–1.47), BMI (OR 1.14 95% CI 1.03–1.28), waist circumference (OR 1.35 95%CI 1.11–1.69) and more often non-TG MetS (OR 14.90 (95% CI 4.64–57.5) (Supplementary Fig. 2). The latter association with non-TG MetS was driven by glucose (≥5.6 mmol/L), systolic blood pressure (≥130 mmHg), waist circumference (>102 cm for men and >88 cm for women) and HDL-C (≤1.01 for men and ≤1.10 for women) components from the non-TG MetS definition (Supplementary Table 3A).

3.3. Association between baseline characteristics and development of FD-like lipid phenotype

Of the 95 homozygous *APOE* ε2 subjects without dyslipidemia – likely FD – at baseline, 69 (73%) were re-examined during follow-up. Eleven of the 69 ε2ε2 subjects (16%) developed dyslipidemia between baseline and follow-up while 58 (84%) subjects did not (Table 2). Homozygous *APOE* ε2 subjects who developed dyslipidemia between baseline and follow-up had a higher weight, BMI and waist circumference at baseline compared to subjects without development of dyslipidemia. Subjects that developed dyslipidemia between baseline and follow-up had 15% more T2DM, and 29% more non-TG MetS at baseline, compared to subjects who did not develop dyslipidemia. In subjects who developed dyslipidemia, lipids at baseline, including total cholesterol, non-HDL-C and TGs, were higher compared to subjects without dyslipidemia during follow-up. Fig. 1 shows the association between baseline characteristics and development of dyslipidemia – likely FD – between baseline and follow-up in ε2ε2 carriers adjusted for age, sex and cohort. BMI (OR 1.19, 95%CI 1.04–1.39), waist circumference (OR 1.26 95% CI 1.01–1.61) and non-TG MetS (OR 4.39 95%CI 1.04–18.4) at baseline were associated with the development of dyslipidemia during follow-up. Non-TG MetS was mainly driven by glucose and HDL-C components from the non-TG MetS definition (Supplementary

Table 2
Baseline characteristics of ε2ε2 subjects who did and did not develop FD-like lipid phenotype between baseline and follow-up.

	No FD-like lipid phenotype during follow-up (n = 58)	FD-like lipid phenotype during follow-up (n=11)
Male sex (n)	23 (40%)	4 (36%)
Age (years)	54 ± 13	56 ± 14
Weight (kg)	74 ± 15	83 ± 21
BMI (kg/m^2)	25.2 ± 3.7	29.5 ± 6.7
Waist circumference (cm)	87 ± 14	96 ± 17
Diabetes mellitus type 2 (n)	2 (3%)	2 (18%)
Metabolic syndrome (n)	6 (10%)	8 (72%)
Non-TG metabolic syndrome (n)[a]	9 (16%)	5 (45%)
Use of lipid lowering medication (n)	0 (0%)	0 (0%)
Total cholesterol (mmol/L)	4.63 ± 1.11	6.65 ± 1.94
Non-HDL-cholesterol (mmol/L)	3.06 ± 1.13	5.48 ± 1.85
Triglycerides (mmol/L)[b]	1.20 (0.87–1.59)	2.48 (1.98–2.65)

BMI = Body Mass Index; TG = triglycerides; non-HDL = non-high-density lipoprotein.
[a] Adaptation of original criterion for MetS by replacing the criterion of elevated TG for elevated high sensitivity C-reactive protein (hsCRP) (≥2 mg/L).
[b] Median with interquartile range.

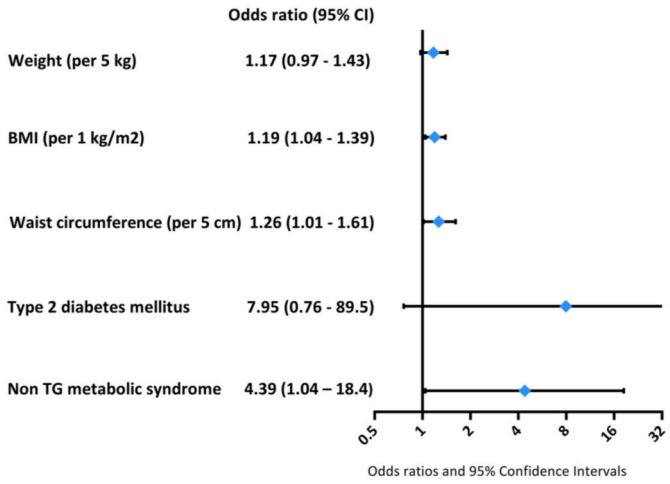

Fig. 1. Logistic regression analyses showing association between baseline characteristics and development of FD-like lipid phenotype between baseline and follow-up in ε2ε2 subjects.
Models adjusted for age + sex + cohort.

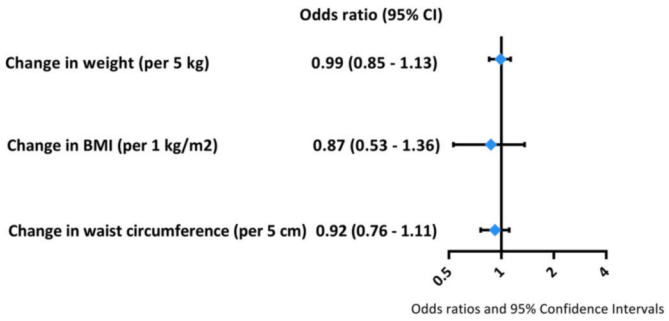

Fig. 2. Logistic regression analyses showing association between change in baseline characteristics and development of FD-like lipid phenotype between baseline and follow-up in ε2ε2 subjects.
Models adjusted for age + sex + cohort + baseline value.

Table 3B). Weight (OR 1.17 95%CI 0.97–1.43) and presence of T2DM at baseline (OR 7.95 95%CI 0.76–89.5) did not show statistically significant associations with development of dyslipidemia – likely FD – between baseline and follow-up.

3.4. Association between change in baseline characteristics during follow-up and development of FD-like lipid phenotype

During follow-up, subjects gained 1.7 kg in weight on average. Weight gain was less pronounced in subjects who developed dyslipidemia – likely FD – than in those who did not (1.1 kg *versus* 1.8 kg Table 3). In subjects who developed dyslipidemia between baseline and follow-up, total cholesterol and non-HDL-C levels decreased during this time interval, and the use of lipid lowering medication increased by 73%. Lipid levels in subjects without development of dyslipidemia did not change substantially. Furthermore, 2 subjects developed T2DM during follow-up but did not switch to an FD-like lipid phenotype, while development of an FD-like lipid phenotype was not accompanied by the development of T2DM. Fig. 2 shows the odds ratios for the association between change in baseline characteristics and development of dyslipidemia – likely FD – between baseline and follow-up. No statistically significant or clinically relevant associations were seen. Furthermore, additional analyses to evaluate the development of dyslipidemia – likely FD – in ε2ε3 subjects were performed, of the 1329 subjects with an ε2ε3 genotype in this cohort, 146 (11%) developed dyslipidemia. These analyses show that differences in baseline characteristics in ε2ε3 subjects with and without development of dyslipidemia are less prominent compared to subjects with an ε2ε2 genotype (Supplementary Tables 5–7 and Supplementary Fig. 3 and 4).

4. Discussion

In this prospective study, baseline adiposity increased the risk of developing dyslipidemia – likely FD – in ε2ε2 subjects from the general population. BMI, waist circumference and non-TG MetS at baseline were associated with development of dyslipidemia during follow-up, but *change* in these clinical variables did not significantly influence the risk for development of dyslipidemia – likely FD – in ε2ε2 subjects.

Previously, several cross-sectional studies were performed that evaluated the association between adiposity and presence of FD. In line with our finding that adiposity increases the risk of presence of dyslipidemia – likely FD – in ε2ε2 subjects, it was previously observed that high BMI and hyperinsulinemia were more prevalent in hyperlipidemic ε2ε2 subjects compared to normolipidemic ε2ε2 subjects form the general population [10,31]. Furthermore, recent Bayesian network analysis confirmed that insulin resistance (indirectly) increases the prevalence of FD in ε2ε2 subjects from the general population [11]. Another study in patients with an ε2ε2 genotype and vascular disease showed that adiposity measures and MetS were associated with the presence of FD [9]. In the present study, presence of T2DM appears to be associated with development of dyslipidemia – likely FD, with an OR of 7.95 (95% CI 0.76–89.5), but its wide confidence interval resulted in non-significant associations, probably due to insufficient power.

The only other prospective study was performed in 1999 in 10 men with an ε2ε2 genotype evaluated total cholesterol, TG and BMI values at baseline and after 10 years and found no significant changes [31]. However, this study did not report the presence or development of FD lipid phenotype.

The potential mechanism behind the relation between adiposity and development of FD may be degradation of the HSPG-R, an important hepatic remnant clearance receptor, because the affinity of the ApoE2 ligand is very low for the other remnant-clearing receptor (LDL-R) in

Table 3
Change in baseline characteristics in ε2ε2 subjects and development of FD-like lipid phenotype between baseline and follow-up.

	No FD-like lipid phenotype during follow-up (n = 58)	FD-like lipid phenotype during follow-up (n = 11)
Change in weight (kg)	1.8 ± 3.8	1.1 ± 3.3
Change in BMI (kg/m^2)	0.6 ± 1.3	0.8 ± 2.4
Change in waist circumference (cm)	2.6 ± 4.9	2.6 ± 4.8
Change in diabetes mellitus type 2 status (n)	2 (3%)	0 (0%)
Change in metabolic syndrome status (n)	2 (3%)	−2 (−18%)
Change in use of lipid lowering medication (n)	0 (0%)	8 (73%)
Change in total cholesterol (mmol/L)	0.09 ± 0.69	−1.43 ± 2.55
Change in non-HDL-cholesterol (mmol/L)	0.14 ± 0.74	−1.48 ± 2.58
Change in triglycerides (mmol/L)[a]	0.09 (−0.25–0.36)	0.20 (−0.54–0.84)

BMI = Body Mass Index; TG = triglycerides; non-HDL = non-high-density lipoprotein.
[a] Median with interquartile range.

ε2ε2 subjects, thereby severely limiting remnant lipoprotein clearance [15]. In obese and diabetic mice, it was shown that lower HSPG-R status in an insulin resistant state is caused by Sulf2, an extracellular sulphatase and heparin sulphate remodeling enzyme that disrupts the structure of HSPG-R by removing 6-O sulphate groups [16,18]. In the present study, it was observed that obesity at baseline was associated with development of dyslipidemia – likely FD – between baseline and follow-up, but *change* in obesity during follow-up was not. This suggests that the 'switch' to an FD-like lipid phenotype is preceded by a slow and gradual process of increasing adiposity, insulin resistance and remnant accumulation, which probably takes longer than the time between baseline and follow-up in this study (median follow-up 4.2 (IQR 4.0–4.3) years in PREVEND and 10.4 (IQR 5.6–10.7) years in Rotterdam Study). Mean age of ε2ε2 subjects at baseline in the present study was 59 years, and the metabolic changes that lead to the development of dyslipidemia – likely FD – probably start already at younger age. In line with this, it could be hypothesized that the HSPG-R remnant clearance system functions normally for a long time, even when part of the HSPG-receptors are damaged by Sulf2 upregulation due to adiposity or insulin resistance. In that case, the 'switch' to FD will only take place when a certain threshold of damage to the number of HSPG-R occurs (in combination with a certain threshold of remnant accumulation by VLDL overproduction).

This increase in remnant accumulation due to insulin resistance may also be relevant for patients that have a high cardiovascular risk despite low levels of LDL-C, as remnant cholesterol is an important CVD risk factor [32]. In patients without ε2ε2 genotype, obesity may lead to insulin resistance and remnant lipoprotein accumulation by similar mechanisms as in ε2ε2 and FD patients, although the remnant accumulation will be less severe because the LDL-R clearing system functions normally in non-ε2ε2 subjects. Previously, it was shown that in healthy individuals, and patients with obesity and T2DM, genetic variants in HSPG and Sulf2 influenced postprandial remnant clearance [17,33]. Therefore, the Sulf2 and HSPG pathway may be an attractive target for future pharmacological interventions.

The findings in the present study emphasize the importance of a healthy lifestyle in ε2ε2 subjects. This has clinical implications for healthy people with an *APOE* ε2ε2 genotype, in particular relatives of FD patients identified with cascade screening. For these subjects, maintaining a healthy weight may contribute to the prevention of FD.

Strengths of the study are the combination of two large well-defined population-based cohorts from different areas in the Netherlands and the prospective cohort design with a long follow-up period, although a longer follow-up period would be ideal but such studies are not yet available.

Some limitations should also be considered. First, due to the lack of ApoB measurement in the total study population, the definition of FD-like lipid phenotype in this study could only be based on fasting TG > 3.0 mmoL/l or use of lipid lowering medication. The cut-off of triglycerides >3.0 mmoL/l is assumed to be acceptable as TG levels >3.0 mmoL/l are high enough not be a random finding and low enough to diagnose potential primary disorders in triglyceride metabolism. However, this could have resulted in misclassification of the diagnosis of FD-like lipid phenotype, especially in subjects with TG levels around 3.0 mmoL/l due to natural variations of TG levels, which is partly based on dietary influences. Also, subjects with an ordinary hypertriglyceridemia and subjects with the presence of a cholesterol-enriched triglyceride rich lipoprotein fraction, characteristic of FD, could not be distinguished. Although the use of lipid-lowering medication in ε2ε2 subjects is very likely to be influenced by FD, as ε2ε2 genotype is usually associated with hypocholesterolemia. Furthermore, more detailed information about (changes in) alcohol consumption or diet was not available to evaluate more precisely the influence of diet and alcohol on the development of dyslipidemia during follow-up. It is uncertain whether individual dietary patterns remain stable over prolonged periods. Furthermore, there was no information about the type of lipid lowering medication use, however, a considerable part of the population that is defined as having dyslipidemia – likely FD – was not aware of the diagnosis, as *APOE* genotype was performed in a research setting years after inclusion of the subjects. Therefore, treatment decisions for these patients in clinical practice were not based or influenced by *APOE* genotype. It is also important to emphasize that lipid levels in subjects allocated as having dyslipidemia – likely FD – are on average reduced due to the use of lipid-lowering medication. Second, aggregating cohorts with over 18,000 subjects still yielded no more than 118 ε2ε2 subjects, emphasizing the challenge to obtain sufficient statistical power to investigate the preclinical disease course of FD in the population. This also leads to small numbers of subjects with, for example, T2DM, which is also indicated by the large confidence intervals of the odds ratios, leading to less precision of the estimate, making firm conclusions based on these numbers difficult. Third, by design it was unknown if the change in risk factors occurred before or after the onset of FD-like lipid phenotype, which might have resulted in an underestimation of the true effect of the change in risk factors over time. Fourth, 32 of the 118 subjects did not have a follow-up visit, however, as shown in Supplementary Table 4 there were, except for age, no important differences in baseline characteristics of subjects with or without follow-up, thereby confirming a limited effect of potential selection bias. Fifth, in this study dominant variants in the *APOE* gene causing 10% of the FD cases were not taken into account [15].

In conclusion, in this prospective study, baseline adiposity increases the risk of developing FD-like lipid phenotype in ε2ε2 subjects from the general population. BMI, waist circumference and presence of non-TG MetS at baseline were associated with development of FD-like lipid phenotype during follow-up. These results stress the importance of a healthy body weight to lower the risk of development of dyslipidemia – likely FD – in these subjects.

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CRediT authorship contribution statement

Britt E. Heidemann: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Frank J. Wolters:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Maryam Kavousi:** Writing – review & editing. **Eke G. Gruppen:** Methodology, Formal analysis, Writing – review & editing. **Robin PF. Dullaart:** Conceptualization, Investigation, Writing – review & editing. **A. David Marais:** Investigation, Writing – review & editing. **Frank LJ. Visseren:** Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **Charlotte Koopal:** Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2021.04.001.

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