

Expression of Type III Hyperlipoproteinemia in Apolipoprotein E2 (Arg158→Cys) Homozygotes Is Associated With Hyperinsulinemia

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Abstract—Type III hyperlipoproteinemia (HLP) is mainly found in homozygous carriers of apolipoprotein E2 (apoE2, Arg158→Cys). Only a small percentage (<5%) of these apoE2 homozygotes develops hyperlipidemia, indicating that additional environmental and genetic factors contribute to the expression of type III HLP. In the present study, first, the prevalence of type III HLP among apoE2 homozygotes was estimated in a Dutch population sample of 8888 participants. Second, 68 normocholesterolemic and 162 hypercholesterolemic apoE2 homozygotes (type III HLP patients) were collected to investigate additional factors influencing type III HLP expression. In the Dutch population sample, apoE2 homozygosity occurred with a frequency of 0.6% (57 of 8888 individuals). Among the 57 E2/2 subjects, 10 type III HLP patients were identified (prevalence 18%). Comparison of normocholesterolemic E2/2 subjects and type III HLP patients showed that the latter had a significantly increased body mass index (25.6 ± 4.0 versus 26.9 ± 3.8 kg/m², respectively; $P=0.03$) and prevalence of hyperinsulinemia (26% versus 63%, respectively; $P<0.001$). Multiple linear regression analysis demonstrated that most of the variability in type III HLP expression can be explained by fasting insulin levels (partial correlation coefficient ≈ 0.50 , $P<0.001$). In contrast to men, apoE2 homozygous women aged ≥ 50 years had significantly higher plasma lipid levels than their counterparts aged <50 years. These data demonstrate that the expression of type III HLP in E2/2 subjects is elicited to a large extent by hyperinsulinemia. In addition, in female apoE2 homozygotes, the expression increases with age; this increase is most likely due to the loss of estrogen production. (*Arterioscler Thromb Vasc Biol.* 2002;22:294-299.)

Key Words: type III hyperlipoproteinemia ■ apolipoprotein E ■ hyperinsulinemia

Familial dysbetalipoproteinemia (FD) is an inborn error of metabolism characterized by defective apoE, leading to the impaired clearance of remnant lipoproteins by the liver.^{1,2} FD subjects are characterized by an accumulation of chylomicron and VLDL remnants in the circulation. These so-called β -VLDL particles are enriched in cholesterol esters and apoE.^{3,4}

ApoE is a ligand for the receptor-mediated uptake of chylomicron and VLDL remnants by the liver.⁵⁻⁷ Three alleles encode for 3 apoE isoforms: apoE2 (Arg158→Cys), apoE3 (Cys112, Arg158), and apoE4 (Cys112→Arg).⁸ In contrast to the other 2 common apoE isoforms, apoE2 displays <1% binding affinity for the hepatic LDL receptor (LDLR).^{9,10} More than 90% of all FD subjects are homozygous carriers of apoE2 (Arg158→Cys).^{1,11} In white populations, apoE2 homozygosity occurs with a frequency of $\approx 1\%$.²

Despite the accumulation of remnants in the circulation, most (>95%) apoE2 homozygous subjects are normolipi-

dem or even hypolipidemic because of low LDL cholesterol levels.¹¹⁻¹³ However, apoE2 homozygosity together with the presence of additional environmental and/or genetic factors that interfere with normal lipoprotein metabolism will often result in a hyperlipidemic phenotype known as type III hyperlipoproteinemia (HLP).¹³

It has been suggested that the expression of type III HLP is influenced by factors that stimulate the production of lipoproteins (eg, obesity, diabetes mellitus, and excessive caloric intake), by impaired clearance (eg, decreased LDLR activity secondary to increasing age, hypothyroidism, or low estrogen levels during menopause), or by impaired lipolysis.^{2,3,14-17}

Hyperinsulinemia is known to stimulate VLDL production (see review¹⁸). Resistance to insulin-stimulated glucose uptake results in compensatory hyperinsulinemia and, subsequently, a decreased uptake and/or an increased release of free fatty acids (FFAs) by adipose tissue. An increased FFA flux stimulates the secretion of VLDL in humans (see

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review¹⁹). In a previous study, we analyzed a group of 49 apoE2 homozygotes, including normolipidemic E2/2 subjects and type III HLP patients. An association of high insulin levels with type III HLP expression was observed, whereas an interaction between hyperinsulinemia and the *SsII* polymorphism in apoC3 resulted in severe hyperlipidemia.²⁰

The low frequency of overt type III HLP (≈ 1 to 5 per 5000 in white populations^{13,21}) makes it difficult to collect a sample large enough to identify the additional factors necessary for the expression of the disorder. Furthermore, most reported studies included only type III HLP patients, whereas no comparison with normolipidemic E2/2 subjects was made. In the present study, a multicenter study was used to include normocholesterolemic and hypercholesterolemic E2/2 subjects. This approach enabled us to determine any additional factors contributing to the expression of type III HLP in a population of 230 apoE2 homozygotes. In addition, we have estimated the prevalence of type III HLP among apoE2 homozygotes in a Dutch population sample.

Methods

The Methods section can be found online at <http://www.atvb.ahajournals.org>.

Results

Prevalence of Type III HLP Among E2/2 Subjects in the Dutch Population Sample

Data from the Rotterdam Study ($n=6870$) and the Dutch population study among 2018 randomly selected 35-year-old men were combined to determine the prevalence of apoE2 homozygosity in the Netherlands.

Of 8888 participants, 57 (0.6%) were homozygous carriers of apoE2 (Arg158→Cys). Among the 57 E2/2 subjects, 10 type III HLP patients (7 in the Rotterdam Study and 3 in the population study among 35-year-old men) were identified. Thus, the prevalence of type III HLP among E2/2 subjects in the Dutch population sample was 18%.

Influence of Additional Factors on the Expression of Type III HLP

Characteristics of Normocholesterolemic E2/2 Subjects and Type III HLP Patients

To determine additional factors that contribute to the expression of type III HLP in apoE2 homozygotes, clinical and biochemical characteristics of 162 type III HLP patients and 68 normocholesterolemic E2/2 subjects were compared.

The mean age of the type III HLP patients was significantly lower and their body mass index (BMI) was higher compared with corresponding values in the normocholesterolemic E2/2 subjects (Table 1). Coronary artery disease and peripheral vascular disease were more frequently present in type III HLP patients, but there were no differences found in the occurrence of diabetes mellitus or hypertension or in the number of smokers and alcohol consumers between the groups. The use of antihypertensive drugs was significantly higher among type III HLP patients.

Total cholesterol, triglyceride, VLDL cholesterol (VLDL-C), and VLDL triglyceride (VLDL-TG) levels and the VLDL-C/VLDL-TG ratio were increased in type III HLP patients, whereas HDL cholesterol (HDL-C) was decreased (Table 2). It has been demonstrated that type III HLP patients

TABLE 1. Clinical Characteristics of Normocholesterolemic E2/2 Subjects and Type III HLP Patients

Parameter	Normocholesterolemic E2/2		Type III HLP	
	n (%)	N	n (%)	N
Male	39 (57)	68	104 (64)	162
Female	29 (43)	68	58 (36)	162
Age (range), y	60±17 (25–88)	68	51±12 (22–80)†	162
BMI, kg/m ²	25.6±4.0	63	26.9±3.8†	162
Xanthomas*	ND	...	67 (54)	123
CAD	6 (10)	58	58 (36)‡	162
PVD	1 (2)	58	31 (19)‡	162
CVD	1 (2)	58	11 (7)	162
Hypertension	14 (22)	65	47 (29)	162
Antihypertensive drugs	3 (5)	62	26 (20)‡	132
Diabetes mellitus	5 (8)	62	16 (10)	162
Smokers	23 (39)	59	64 (40)	161
Alcohol consumers	34 (72)	47	90 (71)	126

n indicates subgroup of patients with given parameter; N, total number of subjects with available data about the respective parameter; CAD, coronary artery disease; PVD, peripheral vascular disease; CVD, cerebrovascular disease; and ND, not determined. Age and BMI are presented as mean±SD.

*Including xanthochromia striata palmaris.

† $P<0.05$ vs normocholesterolemic E2/2 subjects (by unpaired Student *t* test); ‡ $P<0.05$ vs normocholesterolemic E2/2 subjects (by χ^2 test).

have low LDL cholesterol (LDL-C) levels and increased IDL cholesterol (IDL-C) levels.^{2,4,11–13} Thus, the significantly increased levels of LDL-C+IDL-C in type III HLP patients (Table 2) can be attributed to the high IDL levels present in these patients. Furthermore, compared with normocholesterolemic E2/2 subjects, type III HLP patients had significantly higher fasting insulin levels. In fact, hyperinsulinemia was observed in 51 (63%) of 81 type III HLP patients, whereas 7 (26%) of 27 normocholesterolemic E2/2 subjects were hyperinsulinemic ($P<0.001$).

TABLE 2. Biochemical Characteristics of Normocholesterolemic E2/2 Subjects and Type III HLP Patients

Parameter	Normocholesterolemic E2/2		Type III HLP	
	Mean±SD	n	Mean±SD	n
TC, mmol/L	5.59±0.92	68	10.90±3.43*	162
TG, mmol/L	2.11±0.88	63	6.57±4.72†	162
HDL-C, mmol/L	1.36±0.34	61	1.03±0.28†	161
VLDL-C, mmol/L	1.47±0.42	12	6.19±3.82†	134
LDL-C+IDL-C, mmol/L	2.28±0.83	12	3.58±1.12†	134
VLDL-TG, mmol/L	1.66±0.63	12	5.22±4.53†	125
VLDL-C/VLDL-TG	0.93±0.21	12	1.30±0.41†	125
Glucose, mmol/L	5.2±0.8	28	5.4±1.2	122
Insulin, pmol/L	68±41	27	154±133†	81

n indicates total number of subjects with available data about the respective parameter; TC, total cholesterol; and TG, total triglycerides. TC, TG, VLDL-C, VLDL-TG, and insulin levels were logarithmically transformed before statistical analysis.

*Selection criterion.

† $P<0.05$ vs normocholesterolemic E2/2 subjects (by unpaired Student *t* test).

TABLE 3. Multiple Linear Regression Analysis for Logarithmically Transformed Plasma Lipid and Lipoprotein Levels in E2/2 Subjects

Dependent Variable	Independent Variable	Partial <i>r</i>	Multiple <i>r</i>	<i>P</i> *	<i>r</i> ²
Ln(TC)	Ln(insulin)	0.46		<0.001	
	BMI	-0.02		0.85	
	Age	-0.01		0.90	
	Sex†	-0.13		0.18	
	Alcohol†	0.07		0.48	
	Full model			0.51	<0.001
Ln(TG)	Ln(insulin)	0.56		<0.001	
	BMI	-0.02		0.83	
	Age	-0.12		0.24	
	Sex†	0.09		0.39	
	Alcohol†	0.05		0.64	
	Full model			0.58	<0.001
Ln(VLDL-C)	Ln(insulin)	0.47		<0.001	
	BMI	-0.08		0.50	
	Age	-0.02		0.87	
	Sex†	-0.05		0.64	
	Alcohol†	-0.07		0.56	
	Full model			0.49	<0.001
Ln(VLDL-TG)	Ln(insulin)	0.51		<0.001	
	BMI	-0.08		0.47	
	Age	-0.15		0.20	
	Sex†	0.01		0.96	
	Alcohol†	-0.05		0.69	
	Full model			0.52	<0.001

Partial *r* indicates partial correlation coefficient; multiple *r*, multiple correlation coefficient; and *r*², determination coefficient. Normocholesterolemic E2/2 subjects and type III HLP patients were included in the statistical analyses.

*Probability for *t* statistics (partial correlation) or F statistics.

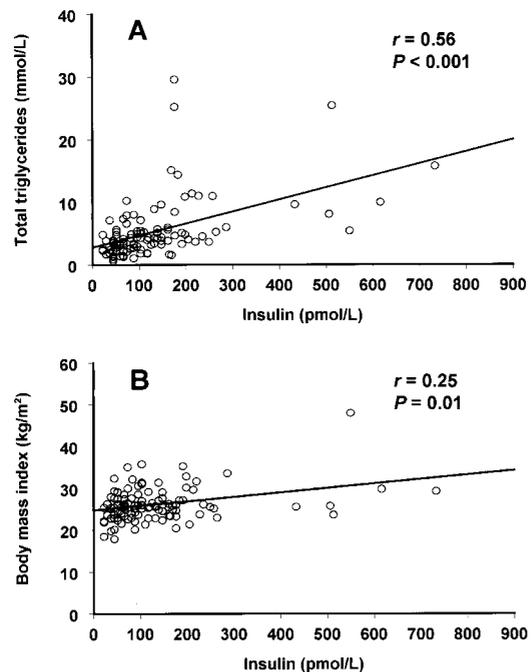
†For sex and alcohol, a numerical code was entered in this analysis.

Multiple Linear Regression

Multiple linear regression analysis was used to determine the effect of age, sex, BMI, hyperinsulinemia, and alcohol consumption on the total cholesterol, total triglyceride, VLDL-C, and VLDL-TG levels of the E2/2 population (normocholesterolemic E2/2 subjects plus type III HLP patients). Inclusion of all factors in the model explained ≈30% of the variability in the expression of type III HLP (Table 3). Plasma insulin levels had a strong independent effect on total cholesterol, total triglyceride, VLDL-C, and VLDL-TG levels. Panel A of the Figure shows that the strongest relationship was observed between plasma insulin and total triglyceride levels (Spearman correlation coefficient 0.56, $P < 0.001$). This relationship remained significant after exclusion of 6 apoE2 homozygotes with insulin levels >400 pmol/L (Spearman correlation coefficient 0.50, $P < 0.001$). Furthermore, a significant linear correlation between plasma insulin and BMI was found (Spearman correlation coefficient 0.25, $P = 0.01$; Figure, panel B).

Differences Between Males and Females in the Onset of Expression of Type III HLP

Type III HLP was more prevalent in men than in women (Table 1). In men, type III HLP is usually expressed



Scatterplots showing the associations between total triglycerides and plasma insulin (A) and BMI and plasma insulin (B) in the E2/2 population (normocholesterolemic E2/2 subjects plus type III HLP patients, $n = 108$). The associations are expressed as Spearman correlation coefficients.

between 30 and 40 years of age, whereas in women type III HLP is expressed after menopause.^{2,3} In accordance, the prevalence of postmenopausal women was increased in type III HLP patients compared with normocholesterolemic E2/2 subjects (91% versus 69%, respectively; $P < 0.05$; results not shown).

To investigate the effect of sex on the age of onset of type III HLP, plasma lipid levels were compared between males and females subdivided in 2 age classes. Table 4 shows that women aged <50 years had significantly lower levels of total cholesterol and triglycerides than did the men, whereas HDL-C levels were increased. Compared with males, females aged >50 years had significantly increased levels of total cholesterol and similar levels of total triglycerides and HDL-C. These data indicate that in E2/2 subjects there is indeed a significant influence of sex on the age of manifestation of type III HLP. Furthermore, total cholesterol and triglyceride levels decreased in males as age increased. In contrast, females aged >50 years had significantly higher levels of total cholesterol and triglycerides than did their counterparts aged <50 years, indicating that in females the expression of type III HLP increases with age, which is most likely due to the loss of estrogen production.

Discussion

The present study has demonstrated that the prevalence of type III HLP among middle-aged Dutch apoE2 homozygotes is ≈18%. Furthermore, we found that hyperinsulinemia is an important contributor to the expression of type III HLP, whereas age has an effect only in women with type III HLP.

TABLE 4. Differences Between Male and Female E2/2 Subjects in Plasma Lipid Levels After Subdivision Into 2 Age Classes

	Age <50 y				Age ≥50 y			
	Males		Females		Males		Females	
	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n
TC, mmol/L	10.08±4.08	81	6.29±3.22*	11	8.19±2.99*	62	9.89±3.78†‡	76
TG, mmol/L	6.33±4.98	81	2.88±2.36*	11	4.55±3.14*	60	5.21±4.92‡	73
HDL-C, mmol/L	1.01±0.33	78	1.52±0.43*	9	1.10±0.27	60	1.20±0.32‡	75

n indicates total number of subjects with available data about the respective parameter. Normocholesterolemic E2/2 subjects and type III HLP patients were included in the statistical analyses. TC and TG levels were logarithmically transformed before statistical analysis.

* $P < 0.05$ vs males aged <50 y; † $P < 0.05$ vs males aged ≥50 y; and ‡ $P < 0.05$ vs females aged <50 y.

The prevalence of type III HLP among apoE2 homozygotes has been estimated to be <5%.^{11,13,21} The higher prevalence of type III HLP among apoE2 homozygotes in the present study could be the result of an increased frequency of apoE2 homozygosity in the Dutch population. However, the gene frequency in the present study (0.6%) is very similar to that in other white populations (see review²). Alternatively, the prevalence of overt type III HLP might be higher in the Netherlands than in other white populations. Indeed, the results of the Dutch population sample revealed a prevalence of ≈1:900 (10 of 8888), which is comparable to the highest prevalence documented in the literature.¹³ There are several possible explanations for this finding. First, the high age of the participants of the Rotterdam study might contribute to the higher prevalence. However, when only the data from the population study among 35-year-old men are used, the prevalence of type III HLP is estimated to be ≈1:700 (3 of 2018). Second, we have estimated the prevalence in a population-based study containing middle-aged Dutch individuals, whereas the estimations reported by most other investigators have been determined in kindreds of type III HLP patients.^{11,21} Finally, most estimates have been reported in the 1970s and 1980s (see review¹³). Improved methodology for the diagnosis of type III HLP since then could have resulted in an increased frequency. More recently, a study by Feussner et al²² has shown that the prevalence of type III HLP among apoE2 homozygous relatives of German type III HLP patients is 30%, which is even higher than the prevalence estimated in the present population-based study.

It has been suggested that the expression of type III HLP is influenced by factors that either stimulate production or impair the clearance or lipolysis of lipoproteins.² To investigate these additional factors, we have compared a large group of type III HLP patients and normocholesterolemic E2/2 subjects. The increased levels of plasma insulin in type III HLP patients together with the results of the multiple linear regression analysis clearly demonstrate that hyperinsulinemia is an important additional factor in the expression of type III HLP. This is in line with our previous findings in a smaller group of apoE2 homozygotes.²⁰ Recently, Brümmer et al²³ have compared 8 type III HLP patients and 3 normolipidemic E2/2 subjects. Their study demonstrated increased postprandial insulin levels but normal fasting insulin levels in type III HLP patients, which were most likely due to the small sample size. Hyperinsulinemia is known to stimulate VLDL produc-

tion through an increased FFA flux into the liver.^{18,19} Hepatic VLDL overproduction in combination with a defective LDLR binding could lead to a severe accumulation of lipoproteins in the circulation and overt HLP in apoE2 homozygotes. On the other hand, hyperinsulinemia could affect lipoprotein lipase-mediated lipolysis, inasmuch as lipoprotein lipase may be downregulated by insulin.²⁴

It is commonly assumed that obesity has an impact on the expression of type III HLP,² because obesity is known to stimulate the production and secretion of VLDL.^{25,26} Although we found a significantly increased BMI in type III HLP patients compared with normocholesterolemic E2/2 subjects, BMI had no significant independent effect on the expression of type III HLP in apoE2 homozygotes. However, in our apoE2 homozygous population, a significant relationship was present between plasma insulin levels and BMI. Because of this correlation, part of the effect of hyperinsulinemia on the expression of type III HLP could be derived from the effect of BMI. Nevertheless, the use of BMI as a measure for obesity has some limitations: (1) subjects with a low BMI could have the same amount of fat as those with a high BMI, and (2) at different ages, subjects with the same BMI could have different amounts and distribution of body fat. The waist-to-hip ratio (WHR) might be a better measure for obesity and body fat distribution than BMI,²⁷ because it has been found that women with a high WHR (ie, abdominal obesity) are more insulin resistant than are women with a low WHR and the same BMI.²⁸ Unfortunately, we were unable to collect data about the WHR in our study population. Therefore, we can only speculate about the importance of a high WHR for the expression of type III HLP.

Although in apoE2 homozygotes an effect of diabetes mellitus on the expression of type III HLP has been suggested,^{2,3} we found no differences in the frequency of diabetes mellitus between type III HLP patients and normocholesterolemic E2/2 subjects. The prevalence of diabetes mellitus observed in both groups is similar to the prevalence observed in the Dutch population (8.3%),²⁹ indicating that diabetes mellitus is no major determinant of type III HLP. In addition, alcohol consumption had no significant effect on the expression of type III HLP. This observation is in agreement with recent data from our group demonstrating that in type III HLP patients normal alcohol consumption has no effect on plasma lipid levels.³⁰

A factor that could have a profound effect on lipoprotein clearance is the number of LDLRs on the liver cell. Although it has been reported that LDLR number decreases with age,³¹ our data demonstrate that age is no important independent contributor to the expression of type III HLP. However, the observation that women develop type III HLP mostly during menopause³ and our findings that plasma cholesterol and triglyceride levels were increased in female apoE2 homozygotes aged >50 years of age strongly suggest that in women type III HLP expression is influenced by estrogen balance rather than age itself. In contrast, a small decrease in plasma lipids was observed in men aged ≥ 50 years. The latter is in agreement with the results from a study among male apoE2 homozygotes aged 35 years showing that their lipid levels remained essentially unchanged after 10 years of follow-up.²⁰ However, it is important to note that the apparent leveling off in expression of type III HLP in men with advancing age might be confounded by ascertainment bias in the overrepresentation of younger men with more severe forms of the disorder in vascular or dermatology clinics and the later appearance of men with probably milder forms of type III HLP who have escaped complications until an older age. Furthermore, in men, the manifestation of type III HLP might also increase with age, but this could remain undetected because of the earlier cardiovascular deaths of those affected with type III HLP.

The present data show that $\approx 25\%$ of the variability in expression of type III HLP in apoE2 homozygotes can be explained by hyperinsulinemia and that an additional 3% can be explained by sex, age, BMI, and alcohol consumption. Other additional risk factors remain to be determined. Family studies have provided evidence that the simultaneous occurrence of apoE2 homozygosity and genes causing familial combined hyperlipidemia or hypertriglyceridemia can result in the clinical expression of type III HLP.^{11,22,32} These observations in combination with our data suggest that possible candidates could be mutations in genes regulating VLDL metabolism and in genes associated with insulin resistance. The insulin resistance syndrome is characterized by dyslipidemia, hypofibrinolysis, hypertension, and insulin resistance^{18,33,34}. Although euglycemic clamp studies have not been performed in type III HLP patients to determine their insulin sensitivity, the increased BMI and the high prevalence of hypertension and hyperinsulinemia found in the present study suggest that the insulin resistance syndrome plays a pivotal role in the etiology of type III HLP.

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