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The lipoprotein lipase (Asn291 → Ser) mutation is associated with elevated lipid levels in families with familial combined hyperlipidaemia

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Abstract

Familial combined hyperlipidaemia (FCHL) is one of the major genetic causes of coronary heart disease (CHD) and is characterised by elevated levels of plasma cholesterol and/or triglycerides in individuals within a single family. Decreased lipoprotein lipase (LPL) activity has been found in some cases of FCHL. A recent study revealed a common mutation in the LPL gene, LPL(Asn291 → Ser), with a frequency of 9.3% in Dutch FCHL patients (Reymer et al., Circulation, 90 (1994) I-998). This mutation was found in 3 out of 17 FCHL families. Extensive family studies were subsequently performed to determine the effect of this mutation on the phenotypic expression of FCHL. Using a pedigree-based maximum likelihood estimate, we demonstrated that the LPL(Asn291 → Ser) mutation significantly affects the levels of plasma and very low density lipoprotein (VLDL) triglycerides (2.03 ± 0.21 vs. 1.14 ± 0.13 and 1.21 ± 0.16 vs. 0.62 ± 0.09 mmol/l, carriers and non-carriers, respectively) and VLDL- and high density lipoprotein (HDL) cholesterol (0.83 ± 0.10 vs. 0.38 ± 0.06 and 1.02 ± 0.08 vs. 1.29 ± 0.05 mmol/l, carriers and non-carriers, respectively), but not those of plasma and low density lipoprotein (LDL) cholesterol. These findings indicate that the LPL(Asn291 → Ser) mutation is associated with elevated lipid levels, indicating it may be one of the genetic factors predisposing to FCHL in the families studied.

Keywords: Lipoprotein lipase; Familial combined hyperlipidaemia; LPL(Asn291 → Ser) mutation; Triglycerides; Cholesterol

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1. Introduction

Familial combined hyperlipidaemia (FCHL) is commonly found among survivors of premature myocardial infarction (MI). Goldstein et al. [1] were the first to show that the FCHL syndrome was distinct from familial hypercholesterolaemia and familial hypertriglyceridaemia. FCHL is one of the major genetic causes of coronary heart disease (CHD), with an estimated frequency of 0.3%–2% in the general population [1].

FCHL probands show elevated plasma levels of cholesterol, triglyceride or both. The FCHL phenotype may vary from time to time in a given patient or among affected relatives within a single family [2]. In addition, characteristics such as increased very low density lipoprotein (VLDL) production, predominance of small dense low density lipoprotein (LDL), hypertension and insulin resistance have been associated with FCHL [3–5]. Considering elevated levels of either VLDL, LDL or both as affected phenotype in family studies, FCHL was initially suggested to be an autosomal dominant disorder [1,6]. Recently, using complex segregation analysis, Cullen et al. [7] found evidence for a major gene acting on triglycerides in families with FCHL.

Several genes, including the apolipoprotein B (APOB) gene the APOA1-C3-A4 gene cluster, the LDL receptor and the lipoprotein lipase (LPL) genes [8–13], have been suggested to be associated with the appearance of FCHL. However, despite extensive studies in FCHL families, a major genetic defect underlying this heterogeneous and possibly polygenetic disorder has not been reported.

Babirak et al. [12] showed in a study among relatives from homozygous LPL-deficient probands that the heterozygous state for LPL deficiency, determined by measurement of post-heparin LPL activity and mass, often segregates with hyperlipidaemia and decreased levels of high density lipoprotein (HDL)-cholesterol. In addition, decreased LPL activity has been found in one third of the cases with FCHL [13]. These results suggest that heterozygosity for LPL mutations may be one of the factors influencing the lipid phenotype of FCHL.

Recently, the LPL(Asn291 → Ser) mutation was identified in 9.3% of Dutch patients with FCHL [14]. The identification of this mutation in three unrelated FCHL probands enabled us to study the inheritance of this mutation within FCHL families and estimate its effect on the lipid levels. Statistical analysis showed that the LPL(Asn291 → Ser) mutation significantly affects lipid parameters, implying that this LPL mutation may be one of the genetic factors contributing to the FCHL phenotype in these families.

2. Subjects, materials and methods

2.1. Subjects

FCHL probands were selected from patients attending the lipid clinics in Nijmegen and Amsterdam for analysis of a lipoprotein disorder when they fulfilled the following criteria: (i) elevated levels of both total cholesterol and triglycerides (at first measurement), (ii) a personal or family history of premature cardiovascular disease, and (iii) at least one first degree relative with elevated total cholesterol and/or triglyceride levels. None of the FCHL probands had specific clinical signs, like tendon xanthomata, and none were homozygous for the APOE*2 allele. For all probands, a secondary cause of hyperlipidaemia was excluded by standard laboratory tests. Using these criteria, 17 probands were diagnosed to have FCHL. The study protocol was approved by the ethical committee of the universities of Amsterdam and Nijmegen. Family members with total cholesterol and/or triglyceride levels above the 90th percentile using the age- and sex-related percentile levels of the PROCAM study are indicated in Fig. 1.

2.2. Lipid and lipoprotein analysis

Ethylenediamine tetraacetic acid (EDTA) blood samples were obtained from the three probands and family members after an overnight fasting. No lipid lowering drugs were administered to the subjects for 6 weeks at the onset of the study, except for individual II-5 of family A. This individual was still on medication when the blood samples were collected. Plasma was separated from cells by centrifugation at 500 × *g* for 10

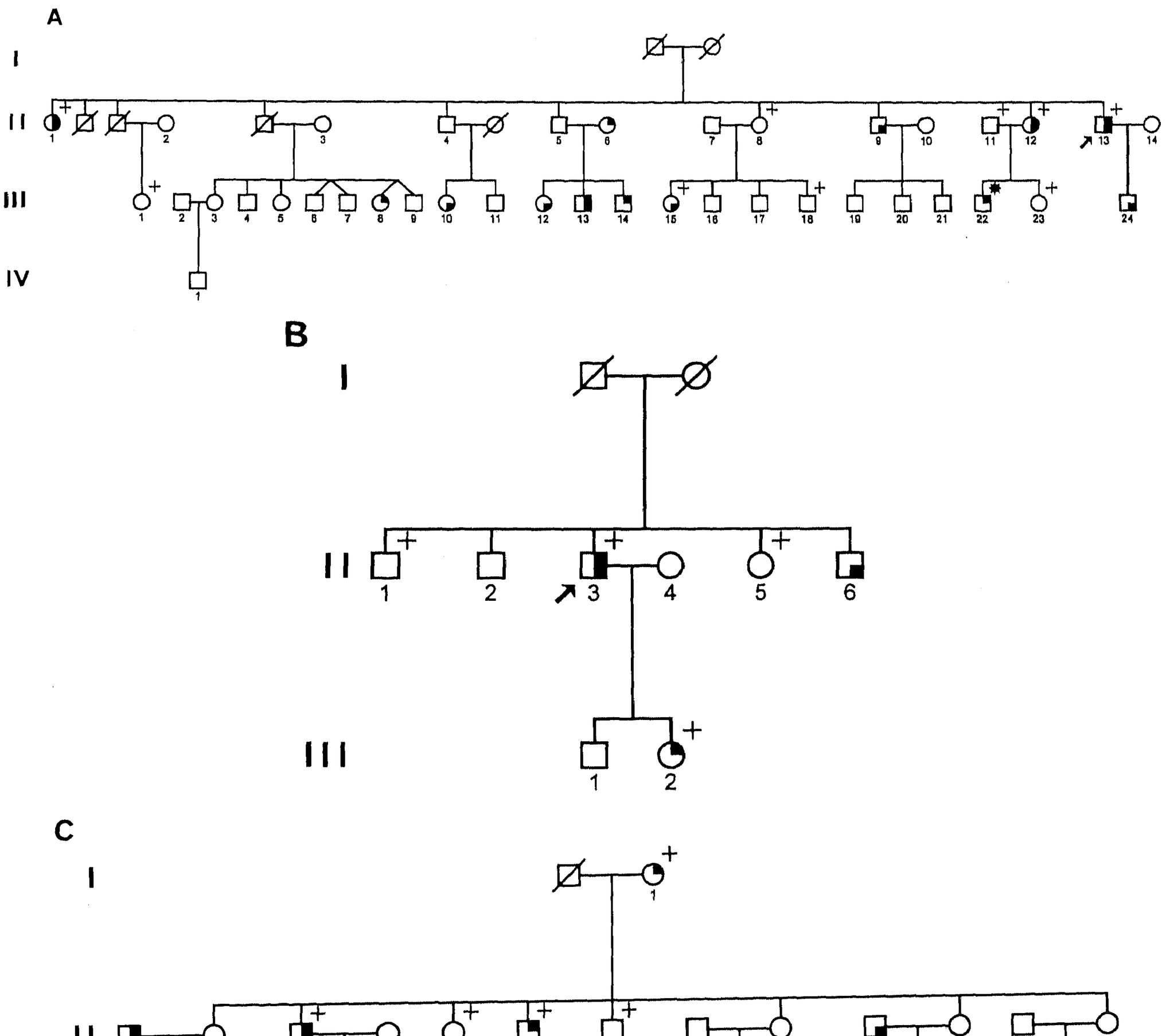

Fig. 1. Pedigrees of the families of the three probands. Probands are indicated with an arrow. The symbol definitions are indicated in the figure. The homozygous carrier of the LPL(Asn291 → Ser) mutation is marked with an asterisk. Individual II-5 from family A was using lipid-lowering drugs.

min at room temperature, and was used for lipid and lipoprotein analysis.

VLDL ($d < 1.006$ g/ml) was isolated by ultracentrifugation for 16 h at 36 000 rev./min in an fixed-angle TFT 45.6 rotor (Kontron, Zurich) [16]. Plasma and lipoprotein cholesterol and triglyceride concentrations were determined by enzymatic, commercially available reagents (No. 237574; Boehringer-Mannheim, FRG; Sera-pak, No. 6639; Tournai Belgium). HDL-cholesterol was determined in whole plasma using the polyethylene glycol 6000 method [17]. LDL cholesterol was subsequently calculated using the formula of Friedewald et al. [18].

2.3. Detection of the LPL(Asn291→Ser) mutation

Genomic DNA was isolated from leukocytes according to Miller et al. [15]. Identification of the LPL(Asn291→Ser) allele carriers in probands and their families was performed by polymerase chain reaction (PCR) using a mutagenic amplification primer approach. Primer LPL291L: 5′-*ATA ATA TAA AAT ATA AAT A*CT GCT TCT TTT GGC TCT GAC-TG TA-3′ was designed with a nucleotide mismatch (underlined) as compared to the wild type LPL sequence [19]. In the case of the mutant allele, an *Rsa*I restriction site is introduced due to the germline missense mutation and the nucleotide mismatch in the primer. The primer was elongated with a TA-rich stretch (italics) at the 5′ end to facilitate the subsequent electrophoretic screening (see below). PCR was performed using primer LPL291R 5′-GCC GAG ATA CAA TCT TGG TA-3′ and primer LPL291L. The reaction mixture included 15 pmol of each primer, 0.5 μg genomic DNA, 0.2 mM of each dNTP, 10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCL, 0.01% (w/v) gelatin, 0.1% Triton X-100, 0.1 unit *Taq* polymerase (Super Taq HT biotechnology Ltd, UK), and 10% dimethylsulphoxide (v/v) in a total volume of 50 μl. Amplification was performed for 32 cycles of 30 s at 94°C, 30 s at 53°C and 1 min at 72°C, with an initial denaturation period of 3 min. Some 20 μl of PCR products were digested with the restriction enzyme *Rsa*I according to recommendations of the supplier (Pharmacia). Thereafter, fragments were separated on a 4% MP agarose gel (Boehringer Mannheim, FRG) and stained with ethidium bromide. Digestion of PCR products revealed two fragments for the mutant allele of approximately 240 bp and 40 bp, and one fragment of 280 bp for the normal allele.

2.4. Statistics

Studying three FCHL families implies, in a strict sense, that there were only three independent observations which can be used for statistical analysis. To test for the statistical significance of the effect of the mutant LPL(Asn291→Ser) allele on the lipoprotein traits in these three families, we used a pedigree-based maximum likelihood method developed by Lange et al. [20]. Using standard statistical analyses would give similar results concerning the univariate statistical calculations, but inappropriate standard errors due to the fact that individuals are related.

For a given pedigree of n individuals, a vector of observations (x) is defined and a vector of expected values (E(x)), that can depend on measured variables such as gender or measured genotype. The covariance between the residual part of the observations, i.e., the part that is not accounted for by the measured genotype or other variables, depends on the relationship between the pedigree members and on the genetic model assumed for the observations. Throughout, we have modeled the variances not accounted for by the measured genotype as consisting of additive genetic and random environmental variance, recognizing that the genetic part may also reflect environmental influences shared by family members. However, our main interest is to test for the influence of the measured genotype. For a given E(x) and expected covariance matrix Σ the log likelihood of obtaining the observation vector x is:

$L = -\frac{1}{2}\ln|\Sigma| - 1/2[x - E(x)]'\Sigma^{-1}[x - E(x)] +$ constant, where $'$ denotes matrix transpose.

The joint log-likelihood of obtaining all pedigrees is the sum of the log-likelihood of the separate pedigrees. Estimation involves selection of parameter values under a specific model that maximizes the joint likelihood of all pedigrees. The likelihood obtained for different models can

be compared with chi-squared difference tests where $X^2 = 2(L_1 - L_0)$ and L_1 and L_0 denote the log likelihood for the general (H_1) and the constrained (H_0) hypothesis. The degrees of freedom (df) for this test are equal to the number of independent parameters between H_1 and H_0 [21]. The Fisher package [19] was used for genetic modelling. Ascertainment correction was carried out by conditioning on the probands. Extensive description of model definition: A: most general model allowing for: (i) age regression, (ii) gender-difference, and (iii) difference between carriers and non-carriers; B: Equal to model A but no age differences; C: Equal to model A but no gender difference except for the trait 'HDL-chol' where model C is equal to model B but no gender difference; D: Equal to model C but no difference between carriers and non-carriers, except for the trait 'VLDL-Tg' where model D is equal to model A but no difference between carriers and non-carriers and the trait 'HDL-chol' where model D is equal to model B but no difference between carriers and non-carriers. Testing procedure: (1) Model B is tested against model A. The data from the probands were omitted from statistical calculations in order to avoid possible ascertainment bias.

3. Results

3.1. Probands and families

As a part of an ongoing study aimed at the identification of genetic risk factors underlying FCHL, we screened 17 FCHL probands for the presence of a common LPL mutation LPL(Asn291 → Ser). Screening revealed three apparently unrelated carriers. The lipid parameters of these three probands, at their first visit to a lipid clinic, are shown in Table 1.

Family studies were performed for all three probands including 67 relatives. During screening of these 3 families, 19 carriers for the LPL(Asn291 → Ser) mutation were found including an individual related by marriage. One of the offsprings of this marriage proved to be homozygous for the mutation (Fig. 1). This homozygous carrier of the LPL(Asn291 → Ser) mutation appeared to have hypertriglyceridaemia (total cholesterol: 4.8 mmol/l and total triglycerides: 3.1 mmol/l) at the age of 22 despite very lean body constitution (body mass index (BMI) 21 kg/m^2). Although total cholesterol levels were not elevated, the VLDL cholesterol was rather high, 1.44 mmol/l, while HDL levels are decreased, 0.66 mmol/l. Screening 114 random individuals of a general Dutch population revealed no carriers, suggesting that this mutation occurs with a low frequency in the general population.

3.2. Lipid and lipoprotein levels in carriers vs. non-carriers

Plasma samples of the three probands and 67 additional family members were studied for lipid and lipoprotein parameters. The clinical details of allele carrying ($n = 19$) family members, including the homozygous carrier, and non-carrying ($n = 48$) family members are presented in Table 2. The statistical analyses of these data are presented in Table 3. Compared to non-carriers, the LPL(Asn291 → Ser) allele carriers exhibited markedly increased levels of plasma and VLDL-triglycerides (2.03 ± 0.21 vs. 1.14 ± 0.13 and 1.21 ± 0.16 vs. 0.62 ± 0.09 mmol/l, respectively) and VLDL-cholesterol (0.83 ± 0.10 vs. 0.38 ± 0.06) (Table 2). HDL-cholesterol was slightly decreased in carriers (1.02 ± 0.08 vs. 1.29 ± 0.05 mmol/l).

As indicated in the Materials and methods section, we considered allele carriers and non-carriers of three different families, implying that, in a strict sense, there were only three independent observations. Therefore, to test for the effect of the mutant LPL(Asn291 → Ser) allele in

Table 1
Clinical characteristics of three LPL(Asn291 → Ser) probands

	A:II-13	B:II-3	C:II-3
Age (yrs)	45	56	52
Gender	M	M	M
BMI (kg/m^2)	22.5	26.9	28.5
Plasma cholesterol (mmol/l)	7.6	8.40	9.50
Plasma triglycerides (mmol/l)	4.22	3.71	6.49
VLDL-cholesterol (mmol/l)	ND	1.98	ND
LDL-cholesterol (mmol/l)	ND	5.61	ND
HDL-cholesterol (mmol/l)	0.87	0.97	0.63

Plasma samples were collected after an overnight fasting.

Table 2
Descriptive statistics (means ± S.E.) of the LPL(Asn291 → Ser) allele carriers and their non-carrier relatives

	LPL(Asn291 → Ser) allele carrier relatives ($n = 19$)	Non-carrier relatives ($n = 48$)
BMI(kg/m^2)	24.91 ± 0.83	23.76 ± 0.52
Plasma TG[a]	2.03 ± 0.21	1.14 ± 0.13
VLDL-TG	1.21 ± 0.16	0.62 ± 0.09
Plasma chol	5.85 ± 0.29	5.34 ± 0.18
VLDL-chol	0.83 ± 0.10	0.38 ± 0.06
LDL-chol	3.98 ± 0.26	3.57 ± 0.16
HDL-chol	1.02 ± 0.08	1.29 ± 0.05

Means and asymptotic standard errors were estimated from the most general model using the maximum likelihood estimate procedures implemented in the Fisher program. Probands were excluded for these quantitative analyses. [a]All levels are expressed in mmol/l.

these families, we used a pedigree-based maximum likelihood method [20]. The principle of this statistical method is briefly described in the Materials and methods section. The legend of Table 3 indicates the four different models that were considered for the quantitative variables. Model A is the most general model, estimating the effects of age, gender and carrier status. Models B, C and D subsequently leave out each of these effects and are tested for a significant decrease in likelihood. In this table we present, when significant, the percentage of the total variance that can be explained by age, gender or carrier status respectively. BMI did not differ between the two groups. Therefore, it cannot explain the differences found between carriers and non-carriers and is not used for one of the models. Table 3 shows that age influences BMI and all lipid parameters measured except HDL-cholesterol. In addition, gender influences levels of VLDL-triglycerides and HDL-cholesterol. No significant effects of additional genetic or environmental variances could be detected on the residual variance (results not shown).

Independently from the effects of age and gender, it is apparent that carrier status of the LPL(Asn291 → Ser) mutation significantly affects the levels of plasma and VLDL-triglycerides, VLDL- and HDL-cholesterol, but not of plasma and LDL-cholesterol. In general, carrier status appears to explain approximately 14% of the total variance of these traits.

4. Discussion

FCHL is a frequently occurring lipid disorder in which multiple lipoprotein phenotypes occur within one family [1,6,7]. Although no major underlying defect has been found so far, some studies suggested a link between heterozygosity for LPL deficiency and FCHL [12,13]. Therefore, we investigated the LPL gene as a candidate gene in Dutch FCHL families.

We screened FCHL families for a common mutation LPL(Asn291 → Ser), which occurs with a frequency of 9.3% among Dutch FCHL patients [14]. Moreover, in vitro site-directed mutagenesis revealed that the LPL(Asn291 → Ser) mutation affects the catalytic function of LPL by causing a 50% reduction of LPL activity and mass [22], making it a feasible candidate gene for FCHL.

Three probands from our study carried the LPL(Asn291 → Ser) mutation. This allowed us to determine the effect of this mutation on lipid and lipoprotein parameters within FCHL families. We have used a pedigree-based maximum likelihood method described by Lange et al. [20] which allowed us to study the influence of measured alleles on quantitative traits under different models, since the subjects included are not unrelated and derive from only three families.

Since age and gender effects have an considerable influence on interindividual variability [23], we have estimated the effects of age and gender on the quantitative lipid traits and BMI for carri-

Table 3
Log-likelihood for four different models testing the effects of age, gender, and carrier status for the quantitative traits and BMI in the LPL(Asn291 → Ser) pedigrees

	A	B	C	D	Percent variance explained by		
					Age	Gender	Carrier status
BMI	−104.19	−119.51***	−104.21	−104.32	35.9	—	—
Plasma TG	−21.41	−25.92**	−22.44	−26.09**	9.9	—	12.9
VLDL-TG	−1.83	−5.17*	−4.04*	−5.43*	7.9	5.6	13.9
Plasma chol	−33.39	−47.16***	−34.57	−34.73	29.7	—	—
VLDL-chol	26.89	21.67***	25.99	22.24**	11.5	—	13.5
LDL-chol	−27.25	−38.37***	−27.49	−27.60	24.6	—	—
HDL-chol	46.58	46.55	35.81***	40.90***	—	23.6	16.2

Log-likelihoods for four models. Model definition: A: most general model allowing for: (i) age regression, (ii) gender-difference, and (iii) difference between carriers and non-carriers; B: Equal to model A but no age differences; C: Equal to model A but no gender difference; D: Equal to model C but no difference between carriers and non-carriers. Testing procedure: (1), Model B is tested against model A. When twice the difference in log-likelihoods of these models is higher than the χ^2 corresponding to df = 1, this indicates a significant age difference as indicated by $^*P < 0.05$ ($\chi^2 > 3.84$), $^{**}P < 0.01$ ($\chi^2 > 7.88$) or $^{***}P < 0.001$ ($\chi^2 > 10.83$); (2) Model C (with df = 1) is tested against model B as described above. When model B was significantly different from model A, then model C was tested against model A. (3) Model D (with df = 1) is tested against model C or, in case of a significant effect of gender in model C, model B, or in case of a significant effect of age in model B, model A.

ers and non-carriers. In addition to the influences of age and gender, the LPL(Asn291 → Ser) allele appeared to contribute considerably (14%) to the total variance in VLDL and HDL levels. Recently, a segregation analysis of 55 FCHL families, predicted a model for FCHL in which a major gene predominantly acting on triglycerides, would explain 20% of the phenotypic variance in triglyceride levels [7]. The influence found for the LPL(Asn291 → Ser) mutation on the variance in triglyceride levels and the absence of associations with total cholesterol levels are in agreement with this predicted effect.

Hamsten and co-workers [24] estimated for serum triglyceride concentration in families that the genetic (0.33) and cultural i.e., smoking, alcohol intake and obesity (0.23), inheritance was of similar significance. In our study, carrier status explains approximately 13% of the total variance in plasma triglycerides, indicating that almost half of the genetic heritability is due to this mutation within the three families studied. For HDL-cholesterol, carrier status explains a similar effect of the genetic heritability. This reduction of levels of HDL cholesterol was also observed in patients with premature atherosclerosis carrying the LPL(Asn291 → Ser) mutation [25].

So far, only a few studies were performed on heterozygosity for LPL mutations and the effect of carrier status on the lipid parameters. Wilson et al. [26] found that heterozygotes for the LPL(Gly188 → Glu) mutation showed moderate fasting hypertriglyceridaemia only after an age of 40. Secondary factors such as obesity, hyperinsulinaemia and lipid-raising drug use were aggravating factors on the genetic defect. Similar results were found with two Austrian families carrying the LPL(Gly188 → Glu) mutation, in which pronounced postprandial lipidaemia was found [27]. In contrast with the LPL(Gly188 → Glu) mutation, homozygous carriers of the LPL(Asn291 → Ser) mutation do not develop severe hyperchylomicronaemia [this study, 28]. Although the heterozygous carriers of both mutations seem to develop hyperlipidaemia in the presence of other predisposing factors, the homozygous carriers do differ in their phenotype. These differences in phenotypic effects of mutations in the LPL gene suggest different underlying mechanisms.

Two studies, in which FCHL patients with impaired LPL activity were screened for DNA changes in the LPL gene, showed that only poly-

morphisms but no mutations causing defective catabolic activity could be found in the LPL gene, implying that LPL is not a significant primary factor leading to FCHL [29,30]. Recently, Mailly and co-workers [31] showed, among 773 healthy men, that carriers of the LPL(Asp9 → Asn) substitution have a significantly higher triglyceride concentration compared with non-carriers. This study suggests that the common LPL(Asp9 → Asn) variant is a mutation that has insufficient impact on its own to cause hyperlipidaemia. In combination with other predisposing factors, LPL(Asp9 → Asn) is associated with the development of hyperlipidaemia. As a consequence, this mutation is also found among healthy individuals, but its frequency is lower than in patients with hyperlipidaemia.

Our study showed similar results for the LPL(Asn291 → Ser) mutation, associated with a significant increase of triglycerides and VLDL-cholesterol and a decrease of HDL-cholesterol in carriers. In addition, this mutation is found with an increased frequency in patients with premature atherosclerosis and FCHL [14]. Taken together, these findings suggest that, within the families described in this study, the LPL(Asn291 → Ser) mutation is one of the predisposing genetic factors to FCHL but does not cause the disease on its own. Additional genetic and environmental factors are needed for complete expression of the FCHL phenotype.

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