

ARTICLE

# Genetic influences contributing to LDL particle size in familial combined hyperlipidaemia

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The nature of the genetic and environmental factors influencing low density lipoprotein (LDL) particle size in patients with familial combined hyperlipidaemia (FCHL) is under debate. We measured LDL peak particle size in 553 subjects belonging to 48 Finnish FCHL families. Individuals with high triglyceride (TG) concentrations (phenotype IV) or combined hyperlipidaemia (phenotype IIB) had significantly smaller LDL particles than those with hypercholesterolaemia (phenotype IIA) or unaffected subjects ( $P < 0.001$ ). In stepwise regression analyses, serum TGs ( $r^2=43\%$ ,  $P < 0.001$ ) and high density lipoprotein cholesterol (HDL-C) ( $r^2=4.5\%$ ,  $P < 0.001$ ) were the only significant predictors of LDL peak particle size. Familial correlations support the conclusion that LDL peak particle size is familial, and most probably influenced by genes in these families. Segregation analysis of LDL peak particle size, a quantitative trait, was performed to model this genetic influence. Our results suggest a polygenic background for LDL size with a recessive major gene that may contribute to large LDL peak particle size in women. Serum TG and HDL-C concentrations predict the majority of variations in LDL particle size.

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## Introduction

Familial combined hyperlipidaemia (FCHL) is a common hereditary dyslipidemia with a prevalence of 1–2%. It is characterised by elevated levels of serum total cholesterol (TC), triglycerides (TGs), or both.<sup>1,2</sup> Decreased high density lipoprotein cholesterol (HDL-C) levels are also frequently found in FCHL, especially in association with high TG values.<sup>3</sup> Possible defects in FCHL are proposed to be the

overproduction and impaired clearance of apolipoprotein B (apoB) containing lipoproteins.<sup>4</sup> Both environmental and genetic factors are suggested to affect the complex FCHL phenotype,<sup>5</sup> although the precise metabolic and genetic defects responsible for FCHL are still not known.

Low density lipoprotein (LDL) particles are heterogeneous. LDL subclasses can be differentiated according to the density or the electrophoretical movement translated into the terms of the particle size.<sup>6,7</sup> LDL particle size classification has been of interest because of identified associations between small, dense LDL particles and coronary heart disease (CHD).<sup>8</sup> In addition, the LDL subclass distribution has been associated with serum TG and HDL-C concentrations,<sup>9</sup> TC to apoB ratio,<sup>9</sup> hepatic lipase (HL) activity,<sup>10</sup> and insulin resistance.<sup>11</sup>

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Previous studies implicated genetic susceptibility and environmental factors in the expression of LDL particle size and density in FCHL families.<sup>12,13</sup> A recent study supported the strong association between small LDL particles, high serum TGs, and low HDL-C in FCHL and familial hypertriglyceridaemia families, and it was inferred that the same gene(s) affect all three traits.<sup>14</sup> Metabolic associations between high serum TG concentrations, low HDL-C concentrations, and small dense LDL are well established.<sup>9,15</sup> The purpose of the current study was to add to our understanding of the regulation of LDL peak particle size in FCHL.

## Materials and methods

### Subjects

The study sample consisted of 48 Finnish FCHL families ( $n=720$ ). The study protocol has been described in detail elsewhere.<sup>3</sup> Affection status for family members was based on the age and sex specific 90th percentile levels of serum TC and TGs, derived in the general Finnish population, as described earlier.<sup>16,17</sup> Subjects using lipid-lowering medication were asked to discontinue the medication for 30 days before collection of blood samples, which were taken after a 10–12 h overnight fast. All traits were measured on 553 individuals: 441 family members and 112 spouses. The study was approved by the ethics committees of the participating centres, and all participants gave their informed consent.

### LDL peak particle size and other measurements

LDL peak particle diameters, serum TC, TGs, HDL-C, and apoB were determined as described earlier.<sup>18,19</sup> TC values were transformed to g/l values in the TC/apoB ratios by dividing the TC values by 2.6. The lipid values of each subject were determined in one to three visits within short intervals. The maximum of all available values was used except for HDL-C and TC/apoB ratios, for which the minimum was used. Body mass index (BMI) was calculated as  $\text{weight(kg)/height(m)}^2$ .

### Statistical analyses of traits

Lipid characteristics of different FCHL phenotypes were compared by analysis of variance (ANOVA). Scheffe's (equal variances) and Tamhane's (non-equal variances) *post hoc* tests were conducted when appropriate. The differences between groups with different lipid phenotypes were tested with a two-way ANOVA using the phenotype as a fixed factor and family membership as a random factor.<sup>20</sup> Family number was also incorporated in regression analyses as a factor to adjust for the non-independence of family members. Stepwise regression was performed to study the relationships among traits correlated with LDL particle size. Spouses were excluded from these analyses. Statistical tests were performed with SPSS 8.0 (SPSS Inc., Chicago, IL, USA). A  $P$ -value  $<0.05$  was considered significant. The data are shown as mean  $\pm$ SD. Natural logarithmic transforma-

tions were applied to reduce skewness when appropriate. No transformation of the LDL size trait was made.

### Analyses of family data

Familial correlations were estimated using the FCOR2 program of SAGE.<sup>21</sup> The uniform weight to pedigrees option was chosen. This programme estimates correlations among relatives taking into account the lack of independence among family members in assessing the statistical significance. Prior to analysis, adjustments for age and gender on LDL size were done by regression analysis, and correlations were conducted on the residuals.

To extract the maximum amount of information, all traits were treated as quantitative. Segregation analyses of LDL peak particle size were performed using the REGC programme of SAGE.<sup>22</sup> This programme follows the approach described by Bonney *et al.*<sup>23</sup> Trait distributions are generated by conditioning each individual's value on that of his antecedents. The class A models, used here, assume that the dependency of the trait among siblings can be explained solely by their common parentage. The models do not contain a separate component for shared environment. This can be considered reasonably appropriate for these Finnish families which are culturally more homogeneous than families not originating from isolated populations. Multivariate normality of the trait in the relatives is assumed, and the correlation structure among the relatives is explained by models which include parameters representing the effects of a major gene, polygenes, gender and other covariates. Parameters for the mean and standard deviation of each major genotype, as well as the residual correlations among relatives after the major gene is modelled are included. The major gene is also parametrised by its frequency and the mode of transmission. The polygenic model assumes mendelian inheritance and that the major gene has an allele frequency of zero. Table 3 shows the estimated parameters of the segregation analyses. Analyses were conducted by conditioning on the original FCHL proband in each family. We have not conditioned on the family members contributing to the diagnosis of FCHL. Many of them do not exhibit a particular LDL size profile, making this reasonable. Age and BMI were treated as covariates. When evaluating a particular segregation model, a statistic of negative two times the log likelihood ( $-2\ln\text{lik}$ ) of the data under that model with the 'best' parameter estimates is obtained. This statistic is used to compare the different segregation models in two ways. When two models are not nested, the likelihoods of these models are compared using Akaike's information criterion (AIC).<sup>24</sup> The AIC is the  $-2\ln\text{lik}$  value with twice the number of independent estimated parameters added to it. When models are nested, the relative goodness of fit of the models is tested by calculating negative twice the difference in the log likelihoods which is distributed as  $\chi^2$  with the difference in the degrees of freedom (equal to the number of independent parameters estimated by the two models).

## Results

Subject characteristics are shown in Table 1. The proportions of men and women were similar in all study groups. Fifty-one per cent of FCHL family members were unaffected, and they were younger and leaner than their affected relatives and spouses. LDL peak particle sizes were especially small in groups with high TGs (IIB and IV) ( $P < 0.001$  vs unaffected family members, IIA, and spouses). The prevalence of small LDL particles (LDL peak particle diameter  $< 25.5$  nm) was greatest in those with phenotype IIB or IV (Table 1). When LDL peak particle size was adjusted for age and BMI all three phenotypes had significantly smaller LDL particles than unaffected family members ( $25.9 \pm 0.5$ ,  $25.8 \pm 0.4$ ,  $25.8 \pm 0.5$ , and  $26.4 \pm 0.5$  nm for IIA, IIB, IV, and unaffected groups, respectively,  $P < 0.001$  for each).

In FCHL family members, serum TGs ( $r = -0.66$ ), TC/apoB ratio ( $r = 0.59$ ), HDL-C ( $r = 0.53$ ), apoB ( $r = -0.51$ ), TC ( $r = -0.40$ ), age ( $r = -0.36$ ), and BMI ( $r = -0.35$ ) were significantly correlated ( $P < 0.001$  for all) with LDL particle size. In regression analyses using all 441 FCHL family members and adjusting for their non-independence, serum TGs and HDL-C were retained in a predictive model and explained 41.0 and 4.5% of LDL peak particle size variation, respectively. Other traits were not retained in this model due to their high correlations with these traits.

Familial correlations were estimated to assess the potential genetic contribution to LDL peak particle size (Table 2). The parent-offspring and sibling-sibling correlations exhibited values of 0.17 and 0.16 ( $P \leq 0.01$ ), whereas spousal correlations were non-significant, suggesting that LDL peak particle size in Finnish FCHL families is familial, and probably genetic, although the sample size may not be sufficient to detect a significant spousal correlation. Table 2 also shows the correlations of TC, TGs, apoB and HDL-C with LDL peak particle size. These data indicate that LDL peak particle size is familial, and appears to be correlated with TGs and HDL-C in family members, suggesting possible common genetic contributions to these traits.

To further assess the genetic contribution to LDL peak particle size, we conducted segregation analysis (Table 3). We began by testing a general transmission model with 12 free parameters, but unfortunately the model did not converge, although multiple sets of initial values were tried. However, we were able to fit more limited models. First, the frequency of a major gene allele in a mendelian model was set to 0, resulting in one normal distribution capturing polygenic and random environmental effects (column 1 of Table 3). The addition of parameters for residual familial correlations (column 2) resulted in a significant improvement in the fit ( $P < 0.05$ ), indicating that family members

**Table 1** Subject characteristics

	FCHL family members				P value <sup>3</sup>	Spouse n=112 (55 m/57 f)
	Unaffected n=224 (121 m/103 f)	IIA n=62 (32 m/30 f)	IIB n=78 (42 m/36 f)	IV n=77 (34 m/43 f)		
Age (years)	32.1 ± 12.8	42.2 ± 13.1*	43.7 ± 13.9*	43.0 ± 15.5*	< 0.001	50.9 ± 8.4
BMI (kg/m <sup>2</sup> )	24.1 ± 3.9	26.7 ± 3.7*	27.8 ± 3.4*	28.0 ± 4.2*	< 0.001	26.5 ± 4.4
LDL size (nm)	26.7 ± 1.2	26.2 ± 1.3‡¶	25.3 ± 1.5*†	25.1 ± 1.6*†	< 0.001	26.7 ± 1.3
Prevalence of small LDL <sup>1</sup> (%)	16.1‡¶	27.4‡¶	57.7*†	55.8*†	< 0.001	17.9
Total cholesterol (mmol/l)	5.1 ± 0.9	7.3 ± 0.8	7.8 ± 1.0	6.0 ± 0.8		5.9 ± 1.0
HDL cholesterol (mmol/l)	1.25 ± 0.32‡¶	1.40 ± 0.43‡¶	1.08 ± 0.38*†	1.03 ± 0.33*†	< 0.001	1.32 ± 0.41
Triglycerides (mmol/l)	1.26 ± 0.54	1.72 ± 0.58	4.43 ± 2.90	3.33 ± 1.76		1.40 ± 0.62
Apolipoprotein B (apoB) (g/l)	0.89 ± 0.24‡¶	1.28 ± 0.24*†	1.59 ± 0.42*†¶	1.23 ± 0.25*†	< 0.001	1.03 ± 0.24
Total cholesterol/apoB ratio <sup>2</sup>	2.23 ± 0.37‡¶	2.19 ± 0.32‡¶	1.87 ± 0.39*†	1.88 ± 0.34*†	< 0.001	2.18 ± 0.32

Data are mean ± standard deviation. <sup>1</sup>LDL peak particle diameter  $< 25.5$  nm. <sup>2</sup>Total cholesterol values transformed to g/l. <sup>3</sup>One-way ANOVA for members in the FCHL family blood lines. Analyses adjusted for non-independence of family members. \* $P < 0.05$  vs Unaffected; † $P < 0.05$  vs IIA; ‡ $P < 0.05$  vs IIB; ¶ $P < 0.05$  vs IV.

**Table 2** Age-sex adjusted familial correlations among LDL peak particle size and TC, TGs, apoB, HDL-C

Relative pairs		LDL size	TC	Trait TGs	apoB	HDL-C
Spousal (n=96)	LDL size of mother†	-0.17	0.35 (**)	0.28 (**)	0.31 (**)	0.01
	LDL size of father††	-0.17	0.19	0.17	0.23 (*)	-0.01
Parent/Offspring (n=437)	LDL size of parent‡	0.17 (**)	0.00	-0.13 (**)	-0.05	0.14 (**)
	LDL size of offspring‡‡	0.17 (**)	-0.04	-0.17 (**)	-0.10	0.11 (*)
Sibling (n=497)	LDL size	0.16 (**)	0.01	-0.18 (**)	-0.05	0.14 (*)

†Mother's LDL peak particle size correlation with father's other trait (LDL peak particle size, TC, TGs, apoB, or HDL-C). ††Father's LDL peak particle size correlation with mother's other trait (LDL peak particle size, TC, TGs, apoB, or HDL-C). ‡Parent's LDL peak particle size correlation with offspring's other trait (LDL peak particle size, TC, TGs, apoB, or HDL-C). ‡‡Offspring's LDL peak particle size correlation with parent's other trait (LDL peak particle size, TC, TGs, apoB, or HDL-C). \* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table 3** Segregation analysis of LDL peak particle size

Parameter	Model*					
	1 Sex-dependent no major gene no familial correlations	2 Sex-dependent no major gene with familial correlations	3 Dominant	4 Sex-dependent dominant	5 Recessive	6 Sex-dependent recessive
1. Frequency of major gene allele A	0	0	$0.4 \times 10^{-6}$	$0.8 \times 10^{-6}$	0.05 (0.03)	0.06 (0.03)
2. Frequency of AA	–	–	$0.1 \times 10^{-12}$	$0.6 \times 10^{-12}$	0.002 (0.003)	0.004 (0.004)
3. Frequency of AB	–	–	$0.7 \times 10^{-6}$	$0.2 \times 10^{-5}$	0.09 (0.05)	0.12 (0.06)
4. Frequency of BB	–	–	1.0	1.0	0.90 (0.05)	0.88 (0.06)
	Female/Male	Female/Male		Female/Male		Female/Male
5. Mean AA	–	–	3.5 (1.4)	23.0/3.2	3.5 (1.4)	31.2/3.4
6. Mean AB	–	–	3.5 (1.4)	23.0/3.2	28.9 (0.4)	29.3/28.5
7. Mean BB	–	–	28.9 (0.4)	29.3/28.5	28.9 (0.4)	29.3/28.5
8. Mean of Polygenic Distribution	29.4/28.4	29.4/28.4	–	–	–	–
9. Variance for each major gene distribution	3.05 (0.2)	3.04 (0.2)	2.0 (0.1)	1.8 (0.1)	1.99 (0.1)	1.82 (0.1)
10. Spouse Correlation	–	–0.04 (0.06)	–0.20 (0.08)	–0.14 (0.08)	–0.20 (0.08)	–0.14 (0.08)
11. Parent-Offspring Correlation	–	0.10 (0.04)	0.18 (0.04)	0.20 (0.04)	0.18 (0.04)	0.20 (0.04)
12. Regression Coefficient of Age	–0.015	–0.015	–0.009	–0.01	–0.009	–0.01
13. Regression Coefficient of BMI	–0.08	–0.08	–0.09	–0.08	–0.09	–0.08
14. Number of independent parameters	5	7	8	10	8	10
15. $-2\ln\text{lik}$	2052	2045	1842	1796	1823	1774
16. AIC**	2062	2059	1858	1816	1839	1794

\*A general model with all parameters free to be estimated did not converge. \*\*AIC indicates Akaike Information Criterion. Standard error (SE) values are shown in the parentheses. Model 2 vs model 1,  $P < 0.05$ ; Model 4 vs model 3,  $P < 0.0001$ ; Model 6 vs model 5,  $P < 0.0001$

are correlated for the trait beyond that accounted for by polygenes. Further, the inclusion of a major gene resulted in a marked improvement in the fit. Modeling this gene was first done without considering the effects of gender (columns 3 and 5). However, impossible parameter estimates associated with the means and major gene allele frequencies of these models led us to continue by testing models that include gender effects. Models with gender effects (columns 4 and 6) added two extra parameters for female genotypic means (lines 5–7). Their inclusion improved the fit of both models significantly ( $P < 0.0001$ ). However, only the estimated means of the female major genotypes were realistic. An additional model fixing the parameters for the residual spousal and parent-offspring correlations to zero for this recessive model with gender differences gave an AIC of 1800 (data not shown), indicating that these residual correlations contribute to the explanation of these data.

In conclusion, the estimated means and frequencies for the major genotypes of AA and AB for dominant and recessive models are unrealistic for all models except for the recessive one identified in women. This result suggests that the LDL size trait has a polygenic background with a recessive major gene that may contribute to large LDL peak particle size in women.

## Discussion

We investigated the genetic contributions to LDL peak particle size in 48 FCHL families originating from the genetically isolated Finnish population,<sup>25</sup> which offers advantageous study material for complex disorders such as

FCHL, as genetic and environmental homogeneity beyond that observed in more mixed populations are expected. Even in this homogeneous population, however, a polygenic model for LDL peak particle size is likely. A recessive, low frequency major gene may also contribute to LDL peak particle size in females.

Significant familial correlations of LDL peak particle size with TGs and HDL-C may indicate a common genetic background contributing to these traits. Correlations among LDL peak particle size, TGs, and HDL-C have also been identified in epidemiological studies conducted in general populations that have not been ascertained for disease<sup>9</sup> as well as in FCHL families.<sup>13</sup> A recent study inferred that in FCHL and familial hypertriglyceridaemia a large proportion of these correlations can probably be explained by the influence of at least one gene affecting all of these traits.<sup>14</sup> However, non-shared environmental and possibly genetic factors also had a strong effect on them. These results could also be interpreted to imply that one or more shared genes has an effect on one of the traits, which in turn has secondary effects on the others. Accordingly, the primary dyslipidemic feature could be a high TG concentration leading to secondary changes in LDL size and HDL-C concentration.<sup>26</sup>

Our quantitative segregation analysis of LDL peak particle size in 48 Finnish FCHL families suggests that this trait is the result of the additive effects of multiple genes where a recessive major gene of low frequency (0.06%) may contribute to large LDL peak particle size in women. It is possible that this major gene is underrepresented among the hyperlipidaemic female FCHL family members

when compared to the general female population, since hyperlipidaemic individuals tend to have small LDL sizes. The equal frequencies of men and women in these segregation analyses indicate that the same opportunity to identify a major gene for LDL size was available for both sexes. However, this sample may only have provided the information to identify the presence of this gene in females by chance alone. On the other hand, premenopausal women tend to have larger and less dense LDL particles than males of the same age, likely due to their lower hepatic lipase activity and lower serum TG concentration.<sup>9,10</sup> These traits are regulated by genetic as well as other factors, such as obesity and sex hormone levels, suggesting that our finding reflects the genetic background and/or gene-environment interactions in these women.

There are limitations to interpreting the results of the segregation analyses, such as their dependence on the parameters used, models, method of analysis, and ascertainment.<sup>27</sup> The relatively small study sample (553 subjects) indicates that inferences drawn from this study need to be conservative. However, our results may contribute to refining the complex FCHL phenotype for further genetic analyses. This is of especial interest, since it has recently been suggested that small dense LDL and apoB may be better candidates for defining the FCHL diagnosis<sup>28</sup> when compared to the currently used serum TC and/or TG traits.<sup>1,2</sup> The current study suggests that LDL size may not be such an attractive candidate for genetic studies, whereas several earlier studies suggested major gene effects for serum TG and apoB levels.<sup>29,30</sup>

Candidate genes for small, dense LDL have been examined intensively. Results of these studies have often been contradictory, and some of the positive results have not been confirmed. A recent study showed evidence indicating that several genes influence LDL size in FCHL, including lecithin:cholesterol acyltransferase, manganese superoxide dismutase, and the apolipoprotein AI-CIII-AIV gene cluster.<sup>31</sup> These data also lend support for a polygenic model for LDL particle size.

In a previous study, seven FCHL families consisting of 234 members showed that pattern B was inherited as an autosomal trait, with either a dominant or an additive mode of inheritance, and a small, but significant additional multifactorial component.<sup>12</sup> In comparison to the current analyses, LDL particle size was treated as qualitative. This result was consistent with those of an earlier study of primarily healthy families,<sup>32</sup> in which LDL particle size defined as a dichotomous trait fit a single dominant gene model of inheritance with reduced penetrance. However, when the same study sample was analysed using the LDL peak particle diameter as a continuous variable that was adjusted for age and gender, the models of inheritance could not be distinguished.<sup>33</sup> Evidence for a recessive model of inheritance with an additional polygenic component was implicated for the small dense LDL trait in 40 Dutch FCHL

families with 623 individuals,<sup>13</sup> although in this study, unlike in the present one, the LDL subfractions were determined by LDL density, and not size. More recently, using those same families, a model containing a major gene with codominant alleles for both the LDL density and apoB traits, and distinct polygenes for each trait, was identified.<sup>34</sup> Interestingly, in a study by Hokanson *et al*, lowering of serum TGs with gemfibrozil in FCHL patients resulted in a marked increase of LDL buoyancy, while LDL peak particle size remained small,<sup>35</sup> suggesting that LDL particle size and buoyancy are regulated, at least in part, via different pathways. Analytic, methodological and population based-differences may account for these somewhat contradictory results.

In conclusion, small LDL particles are a typical feature of Finnish FCHL patients, found especially in individuals with phenotype IIB or IV. While we have not established that LDL peak particle size for men follows a strictly polygenic model, these analyses indicate that LDL size in men is unlikely to be influenced by a major gene in this population. In women, a low frequency recessive major gene allele may be contributing to larger LDL peak particle sizes. The observed correlations between LDL peak particle size, TGs, and HDL-C may be due to a common genetic background and/or metabolic interplay.

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