A PCSK9 variant and familial combined hyperlipidaemia

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ABSTRACT

Background: Our discovery in 2003 of the first mutations of PCSK9 gene causing autosomal dominant hypercholesterolaemia (ADH) shed light on an unknown factor that strongly influences the level of circulating low density lipoprotein cholesterol (LDL-C). PCSK9 gain of function mutations cause hypercholesterolaemia by a reduction of LDL receptor levels, while PCSK9 loss of function variants are associated with a reduction of LDL-C values and a decreased risk of coronary heart disease.

Methods and results: We report an insertion of two leucines (p.L21tri also designated p.L15_L16ins2L) in the leucine stretch of the signal peptide of PCSK9 that is found in two of 25 families with familial combined hyperlipidaemia (FCHL). This mutant is associated with high total cholesterol and LDL-C values in these families and is found also in a patient with familial hypercholesterolaemia and her father.

Conclusion: PCSK9 variants might contribute to FCHL phenotype and are to be taken into consideration in the study of this complex and multigenic disease with other genes implicated in dyslipidaemia.

Our discovery in 2003 of the first mutations of PCSK9 in cholesterol metabolism, by identifying mutations of this gene causing autosomal dominant hypercholesterolaemia, revealed a previously unknown mechanism that strongly influences the concentration of circulating low density lipoprotein cholesterol (LDL-C). Since then it has been widely ascertained that PCSK9 is the third major gene implicated in autosomal dominant hypercholesterolaemia (ADH) and is a main determinant contributing to the variability of circulating cholesterol concentrations in several populations. ADH (OMIM 144400) is characterised by a selective increase of LDL cholesterol concentrations in plasma giving rise to tendon and skin xanthomas, arcus corneae and cardiovascular deposits, leading to premature and premature atherosclerosis, coronary heart disease (CHD) and mortality. The frequency of this genetic disorder is 1 in 500 heterozygotes and 1 per million for homozygotes. It is principally associated with mutations in the genes LDLR (encoding the LDL receptor) or APOB encoding apolipoprotein B, the LDL receptor ligand.

Seven PCSK9 gain of function mutations have been reported in autosomal dominant hypercholesterolaemia (p.S127R, p.D129G, p.F216L, p.R218S, p.R357H, p.D574Y, p.D574H) and have been well investigated. Other PCSK9 mutations, especially nonsense mutations (p.Y142X, p.C679X), are associated with hypercholesterolaemia due to a loss of function mechanism. These mutations are much more frequent in individuals of African descent. In the USA, 1 of every 50 African Americans has a nonsense mutation in PCSK9 and these mutations are associated with a 28% lower plasma concentration of LDL-C.

Several PCSK9 variants are associated with an increase or a decrease in total serum cholesterol (TC) and LDL-C values and in CHD. These PCSK9 single nucleotide polymorphisms (SNPs) are unequally distributed in different ethnic groups contributing to the variability of cholesterol values and to CHD incidence in these populations. PCSK9 is a member of the mammalian serine proprotein convertase (PC) family that is responsible for the proteolytic maturation of secretory proteins including neuropeptides, pro-hormones, cytokines, growth factors, receptors, serum and cell surface proteins. It is synthesised as an inactive proenzyme and contains a triad of residues (D186, H226, and S386) that are required for catalytic activity. The ~74 kDa precursor form of PCSK9 undergoes intramolecular autocatalytic cleavage in the endoplasmic reticulum (ER), which produces a 60 kDa catalytic fragment. Autocatalytic cleavage of thezymogen in the ER is essential for transport from this compartment and secretion. PCSK9 hypercholesterolaemic mutations (p.S127R, p.D574Y) are associated with an increased apob100 secretion but they cause hypercholesterolaemia mainly by reducing the number of cell surface LDL receptors. Thus, as for overexpression of wild type PCSK9, gain of function mutants of PCSK9 overexpressed in the liver of mice lead to hypercholesterolaemia due to a dramatic decrease of hepatic LDLR protein values through a post-transcriptional mechanism. PCSK9 might work in a post-ER compartment where it targets the LDL receptor for degradation in lysosome. But secreted PCSK9 might also bind to the first epidermal growth factor-like repeat (EGF-A) of the LDL receptor at the cell surface and the PCSK9/LDLR complex could be internalised into endosomal/lysosomal compartments. PCSK9-LDLR affinity is increased in the endosome due to higher acidity. Failure to release PCSK9 might hinder receptor recycling and reduce the cell surface abundance of LDL receptor.

In order to assess the clinical and biological spectrum of dyslipidaemias associated with mutations in the PCSK9 gene, we studied probands with autosomal dominant hypercholesterolaemia as...
well as probands with familial combined hyperlipidaemia (FCHL), the most common hereditary dyslipidaemia (OMIM 144250). The classic FCHL proband has elevated TC, triglycerides (TG) and also increased apolipoprotein B (apoB) concentrations and low high density lipoprotein cholesterol (HDL-C). FCHL family members exhibit varying degrees of dyslipidaemia with either isolated high TG, TC, or both in the combined phenotype. The genetic basis of FCHL is apparently complex and poorly understood.14

We sequenced PCSK9 in probands with ADH and FCHL and identified an insertion of two leucines that occurs in the stretch of leucine of the signal peptide in two FCHL and one ADH families.

METHODS

Family recruitment

Probands were selected among consecutive French Canadian patients seen at the Clinique de Nutrition, Métabolisme et Athérosclérose of the Clinical Research Institute of Montreal. Twenty-five French Canadian probands with FCHL have been included in this study. The diagnosis of FCHL relies on population age and sex specific cut-offs for the lipid measurements. The French Canadian FCHL probands recruited had the combined phenotype with LDL-C and TG values >90th centile, and at least a first degree relative with dyslipidaemia.14 Probands with a diagnosis of familial hypercholesterolaemia, a body mass index (BMI) >35, diabetes or secondary hyperlipidaemia were excluded from FCHL recruitment. The affected relatives included were those: (1) with LDL-C and TG >90th centile for age and sex; or (2) with LDL-C >95th centile for age and sex; or (3) with TG >95th centile for age and sex.

Furthermore, 25 French Canadian probands with familial hypercholesterolaemia have been included in this study. Familial hypercholesterolaemia was diagnosed on the basis of high LDL-C values, the presence of tendinous xanthomas, and a family history of hypercholesterolaemia and premature CHD according to MedPed recommendations.15 The lipid values used for inclusion were: TC >7.49 mmol/l and LDL-C >5.68 mmol/l for adults over 20 years; TC >6.97 mmol/l and LDL-C >5.17 mmol/l for youths.15 All French Canadian subjects with familial hypercholesterolaemia were tested for five common mutations of the LDLR gene. Because of a founder effect, these mutations (a 15 kb deletion, a 5 kb deletion, exon 3, 4 and 10 mutations) represent more than 85% of the LDLR mutations found in the French Canadian population.16 APOB was only tested for the p.R3500Q mutation. Thus, none of these mutations in the LDLR or APOB genes were found in the hypercholesterolaemic probands included in the study.

The control population comprised 100 French Canadian normocholesterolaemic and normotriglyceridaemic individuals who originated from the same geographic regions as the probands and 100 French normocholesterolaemic and normotriglyceridaemic individuals. This approach was approved by the institutional ethics committee and all patients signed an informed consent form.

Laboratory and biochemical test

Blood was obtained from subjects who had fasted for 12 h overnight. It was drawn under vacuum from an arm vein into tubes containing EDTA (final concentration 1.5 mg/ml). Plasma was separated from blood cells by centrifugation (15 min, 3000 rpm, 4°C). Cholesterol and TG concentrations were determined by enzymatic colorimetric test from Roche according to manufacturer’s instructions, on an autoanalyzer (Cobas Mira, Roche). HDL-C concentration was determined by assaying cholesterol in the supernatant, after precipitation of apoB containing lipoproteins from the d >1.006 g/ml fraction (1.0 ml) with heparin–manganese.17 LDL-C values were calculated as the difference between cholesterol in the d >1.006 g/ml fraction and HDL-C in the same fraction.17 Plasma apoB concentrations were determined by nephelometry (Behring Nephelometer 100 Analyser).

Serum aspects were noted after 12 h refrigeration. Lipoprotein electrophoretic patterns were determined using a Paragon electrophoresis kit (Beckman-Coulter, Fullerton, California, USA); apoE phenotype was determined by isoelectric focusing gel electrophoresis followed by immunoblotting of whole plasma.18

DNA sequencing

DNA was extracted from white blood cells using an automated 340A DNA extractor (Applied Biosystems, Foster City, California, USA). The 12 exons of PCSK9 were amplified with thermostable DNA polymerase from Integen on GeneAmp PCR system 9700 (Perkin Elmer) as described previously.1 DNA sequencing was carried out with Big Dye Terminator version 1.4 on GeneAmp PCR system 9700 (Applied Biosystems, Applera France SA, Courtaboeuf, France) apparatus, under conditions supplied by the manufacturer. Electrophoregrams were analysed using Sequencing Analysis 3.4 and SeqED.

Statistical analyses

For basic statistical analysis we used a statistical non-parametric test (Mann–Whitney test) to compare lipid values between carriers and non-carriers of the L11 allele. Furthermore, in order to study the association between the L11 allele and TC, LDL-C, HDL-C, TG and apoB values within the two families, we used a classical Family Based Association Testing (FBAT) framework.19 As these values are expected to be correlated to covariates such as age, sex or apoE, we first used a linear model separately on each trait in order to take into account these covariates (as age, sex or apoE, we first used a linear model separately on each trait in order to take into account these covariates (as age, sex or apoE; or LDL-C, sex; or HDL-C, sex; or apoB, sex). The resulting residuals were normalised and taken as phenotypic traits in FBAT. Since only two pedigrees constitute our data, and the default minimum number of pedigrees in FBAT is 10, we hence tried the EM-FBAT, an Expectation-Maximization implementation of FBAT, which is more robust both for missing data and genotypic errors.20

RESULTS

In two probands with FCHL an alteration was found in the first exon of PCSK9: an insertion of two leucines in the leucine stretch leading to 11 leucine repeats (p.L21tri also designated p.L15_L16ins2L or L11 and at nucleic level c.61_63triCTG) instead of nine leucine repeats (L9) for the normal allele (fig 1). The two probands’ families were recruited. In family A (fig 2A), only the proband’s parents had a history of CHD. The L11 allele was found in six of 12 family members. The mean (SD) TC and LDL-C values were 6.28 (0.99) and 4.08 (0.87) mmol/l, respectively, in L11 heterozygous carriers versus 5.24 (0.97) and 3.21 (0.76) mmol/l, respectively, in non-carriers. All family A members that have high LDL-C values were carriers of the L11 allele except A.II.9 patient (fig 2A). The L11 allele was not found
in 100 French Canadian and 100 French normocholesterolaemic and normotriglyceridaemic controls. The only carrier of the L11 allele with a normal cholesterol value was individual A.II.5, the spouse of one of the affected family members carrying the L11 allele. She and her husband both originated from the Lac St-Jean region, known for high frequency of founder effects in various inherited diseases. To the best of their knowledge, the patients are not first degree relatives.

The second family is represented in fig 2B. Patient B.II.10 presents xanthomas, but no CHD was noted. All three brothers and three sisters of the first generation of this kindred have combined hyperlipidaemia, but a second dyslipidaemic trait also segregated that associated with the presence of the apoE epsilon-2 allele. Patients B.1.7, B.1.9 and B.1.10 have floating betalipoproteinaemia on lipoprotein electrophoresis (presence of beta-VLDL (very low density lipoprotein), with an elevated VLDL/C/TG ratio) with B.1.2, B.1.9 and B.1.10 being apoE2/E2 carriers, which explains their familial dysbetalipoproteinaemia phenotype (HLP type III according to Fredrickson’s classification; OMIM 107741). The B.1.5 and B.1.6 patients have elevated cholesterol and TG values without floating betalipoprotein or apoE2/E2 variant. These two patients are carriers of the L11 allele. However, moderate dyslipidaemia in those of their children who carry the L11 allele has been noted. The lipid profile, BMI and age of the members of the two kindreds are given in fig 2.

We compared (by a statistical non-parametric test) lipid values between carriers (n = 10) and non-carriers (n = 11) of the L11 allele in the two families after the exclusion of the four members with type III dysbetalipoproteinaemia and their children (encased within dashed lines in fig 2). Mean (SD) TC values and LDL-C values are significantly higher (p = 0.0067 and p = 0.0167) in the L11 carriers (6.59 (0.97) and 4.06 (0.82) mmol/l, respectively) than in the non-carriers (5.18 (0.81) and 3.13 (0.70) mmol/l, respectively). A significant difference was also observed between the two groups for apoB (139 (28) vs 111 (26) mg/dl; p = 0.020) but not for HDL-C and TG, although this was slightly higher in the L11 carrier group (table 1). The association between the L11 allele and lipid parameters within our family data, after taking into account covariates (age, sex, and apoE), was studied by a classical FBAT test and by the alternative method EM-FBAT. Using the classical FBAT on the families’ data, no significant results were observed, whereas statistically significant results were observed with EM-FBAT for TC, LDL-C, HDL-C and apoB (table 1). Furthermore the comparison of the frequencies of the occurrence of the L11 variant between 25 French Canadian FCHL patients and 100 French Canadian controls by Fisher’s exact test is significant (p = 0.059).

The L11 variant was also found in one French Canadian proband with autosomal dominant hypercholesterolaemia and in this proband’s father. The French Canadian ADH proband is a 44-year-old woman who was diagnosed on the basis of high LDL-C values, the presence of tendinous xanthomas, and a family history of hyperlipidaemia and cardiovascular disease. Her father is also hypercholesterolaemic. The mean lipid values for this French Canadian patient and her father were: TC 7.72 mmol/l; LDL-C 5.57 mmol/l (table 2).

DISCUSSION

The aim of our study was to identify an association between PCSK9 variants and FCHL. We showed that an insertion of two leucines in the leucine stretch of exon 1 of PCSK9 leading to 11 leucine repeats (p.L21tri also designated p.L15_L16ins2L or L11 and at nucleic level c.61_63triCTG) is associated with FCHL in two French Canadian families. This variant has already been reported by Chen et al21 but it is the first time that a variant of PCSK9 is associated with FCHL.

We previously reported another variant in exon 1 of PCSK9 occurring also in the stretch of nine leucines.1 This polymorphism c.61_63dupCTG (formerly designated c.43_44insCTG) was an in-frame insertion of one leucine (p.L21dup, also designated p. L15_L16NL and denoted as L10) that is common both in African American (allele frequency 0.27) and Caucasian populations (allele frequency 0.143).22 This variant was found with a higher frequency in a low LDL-C population than in a normal population and segregated with the hypercholesterolaemia phenotype in three families displaying hypobetalipoproteinemia. It showed complete linkage disequilibrium in these families with an SNP located 108 bp upstream in exon 1: the c.-64C>T SNP in the 5’UTR region of PCSK9. The L10 variant is associated with lower LDL-C in the Caucasian population tested, the L9/L10 (nine repeats/10 repeats) heterozygous carriers having 10–15 mg/dl lower LDL-C than normal.22 Finally, these results showed that the L10 variation is an independent predictor of LDL-C, accounting for about 5.0% of LDL-C variation.22 This leucine stretch is more heterogeneous and an insertion of two leucines (L11) instead of one (L10) seems associated with a different phenotype in the French Canadian families we report here. The allele with 11 leucine repeats (L11) was reported by Chen et al21 with a very low frequency in their population. They found the L11 allele in

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**Figure 1** PCSK9 variants with an addition of two leucines (L11) to the stretch of nine leucines in the signal peptide (PCSK9 exon 1 forward sequence).
Figure 2  The p.L15_L16ins2L variant study in familial combined hyperlipidaemia (FCHL) families. (A) Segregation of the L11 variant in French Canadian family A. (B) Segregation of the L11 variant in French Canadian family B. This family has a second dyslipidaemic trait, type III dysbetalipoproteinaemia (encased within dashed lines). Half-blackened symbols indicate affected members, clear symbols indicate unaffected members. Age (in years), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) (all mmol/l) and apoB (mg/dl) are given. Body mass index (BMI) and apoE phenotype are given too. B float indicates the presence (+) or traces (t) of B floating lipoprotein. L11 indicates the presence (also noted +2L) or absence (2L) of the L11 allele. The probands of each family are indicated by an arrow. NA, not available.
seven out of 572 (1.3%) individuals of the LCAS population study (Lipoprotein Coronary Atherosclerosis Study), which comprised American subjects who had plasma LDL-C values of 115–190 mg/dl (2.96–4.90 mmol/l), despite diet and one or more coronary lesions causing 30–75% diameter stenosis.

We have identified this L11 allele in two French Canadian families with FCHL and in one French Canadian woman and her father with hypercholesterolaemia. The L11 allele was not found in 100 French Canadian and 100 French normocholesterolaemic controls and segregates with hypercholesterolaemia in the families tested. In family A all the carriers of this mutation have TC values >6 mmol/l (>2.3 g/l), except the spouse of one of the affected family members carrying the L11 allele. She comes from the same region of Quebec as her husband, the Lac St-Jean, a region known for its high frequency of founder effects. She shared partially a common haplotype (the intragenic SNF and the centromeric microsatellite marker tested were conserved) (data not shown). Her family was not available for genotyping to understand better if she constitutes a case of incomplete penetrance or has a modifier gene that protects against dyslipidaemia, or variants in genes that lower cholesterol, or HDL-C values. It is the ability for EM-FBAT to infer precisely the missing parental genotypes that fits the most with the two studied pedigrees. As a result, the conditional distributions of the offspring are expected to be far more realistic with EM-FBAT than when using the basic conditional rule introduced by Rabinowitz and Laird.

The association between the L11 allele and high TC or LDL-C values has already been reported for all the other gain of function variants of PCSK9. Other genetic or environmental factors contribute to the FCHL phenotype, especially to the high TG values observed in these families. Thus their FCHL might be explained partially by the PCSK9 variant we have identified, acting concomitantly with other genetic, nutritional and environmental causes.

Western blot analysis of PCSK9 in cells transfected with PCSK9 carrying two additional Leu to the Leu stretch (L11 allele) of the signal peptide, done in order to study its impact on the enzyme maturation and processing, showed a profile similar to the one observed with the p.S127R hypercholesterolaemic mutant. In vivo kinetics of apoB100 containing lipoproteins showed that this mutant dramatically increased the production rate of apoB100 (threefold) compared with controls or LDLR mutated patients and led to a higher direct over-production of very low density lipoprotein (VLDL) (threefold), intermediate density lipoprotein (IDL) (threefold) and LDL (fivefold). A lower conversion rate of VLDL and IDL compared with controls and heterozygous familial hypercholesterolaemia (FH) patients was also observed. Finally, LDL fractional catabolic rate was slightly decreased (by 30%) compared with controls but still higher than LDLR mutated subjects.

No notable hypertriglyceridaemia was observed in the carriers of PCSK9 mutations in human studies. But massive hypertriglyceridaemia in mice overexpressing PCSK9 was shown only in 24 h fasted mice with a striking increase in VLDL, TG and apoB100 hepatic output because of the lack of modulation of hepatic VLDL mostly by the LDLR. This is in agreement with the elevated VLDL-apoB100 production seen under fasting conditions in the lipoprotein kinetics of patients carrying the higher cholesterol values than their brothers or sisters not carrying the L11 allele.

Familial segregation and non-parametric statistical analysis showed that the L11 allele is associated with higher TC, LDL-C, and apoB values, but not TG values.

No significant results were obtained using the classical FBAT, and this was expected since we worked on two pedigrees only, whereas the minimum number of pedigrees in FBAT is 10. Nevertheless, a statistically significant association was observed using EM-FBAT between the L11 allele and TC, LDL-C, apoB and HDL-C values. It is the ability for EM-FBAT to infer exactly the missing parental genotypes that fits the most with the two studied pedigrees. As a result, the conditional distributions of the offspring are expected to be far more realistic with EM-FBAT than when using the basic conditional rule introduced by Rabinowitz and Laird.

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Table 1

<table>
<thead>
<tr>
<th>L11 presence</th>
<th>n (10)</th>
<th>n (11)</th>
<th>Mann-Whitney</th>
<th>EM-FBAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/l)</td>
<td>6.39</td>
<td>5.18</td>
<td>0.0667</td>
<td>0.0014</td>
</tr>
<tr>
<td>(g/l)</td>
<td>2.47</td>
<td>2.00</td>
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</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>4.06</td>
<td>3.13</td>
<td>0.0167</td>
<td>0.000051</td>
</tr>
<tr>
<td>(g/l)</td>
<td>1.57</td>
<td>1.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.413</td>
<td>1.154</td>
<td>0.860</td>
<td>0.002</td>
</tr>
<tr>
<td>(g/l)</td>
<td>0.54</td>
<td>0.44</td>
<td></td>
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</tr>
<tr>
<td>TG (mmol/l)</td>
<td>2.23</td>
<td>1.85</td>
<td>0.307</td>
<td>0.69</td>
</tr>
<tr>
<td>(g/l)</td>
<td>1.95</td>
<td>1.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>139.5</td>
<td>111.4</td>
<td>0.0201</td>
<td>0.00086</td>
</tr>
</tbody>
</table>

ApoB, apolipoprotein B; EM-FBAT, Expectation-Maximization-Family Based Association Testing; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Table 2

<table>
<thead>
<tr>
<th>Lipid values</th>
<th>Proband</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/l)</td>
<td>7.66</td>
<td>7.78</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>5.73</td>
<td>5.41</td>
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<tr>
<td>HDL-C (mmol/l)</td>
<td>1.37</td>
<td>1.02</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.40</td>
<td>1.74</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>227</td>
<td>175</td>
</tr>
<tr>
<td>ApoE phenotype</td>
<td>3/4</td>
<td>3/4</td>
</tr>
</tbody>
</table>

ApoB, apolipoprotein B; ApoE, apolipoprotein E; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

PCSK9, Proprotein Convertase Subtilisin/Kexin type 9; FBAT, Family Based Association Testing; LDL, low density lipoprotein; TC, total cholesterol.
p.S127R mutation in PCSK9 and with the fact that the p.D374Y gain of function mutation was associated with an increased apoB100 secretion.\textsuperscript{9}

Like other PCSK9 variants that are associated with cholesterol variability, the L11 allele is associated with hypercholesterolemia in the FCHL families studied according to family segregation and statistical analysis. Furthermore it is found in two of 25 French Canadian FCHL families, and in one French Canadian patient and her father with ADH, while it is absent from controls. Thus, L11 is a very rare PCSK9 allele that seems to increase total and LDL-C values in a moderate way, and might also be observed in FCHL where it contributes with other genetic and environmental factors to the appearance of the phenotype.

FCHL is a common disorder characterised by elevated values of either plasma cholesterol or TG or both in members of the same family.\textsuperscript{4} Apolipoprotein B values are also elevated in these individuals.\textsuperscript{5} Some candidate genes might be involved in the disease process of FCHL: lipoprotein lipase (LPL) and two members of the APOA1/C3/A4/A5 gene cluster, APOC3 and APOA5, that are linked to hypertriglyceridaemia in several studies.\textsuperscript{6, 7} These genes may be characterised as FCHL modifier genes, since none of them has provided evidence for a major effect.\textsuperscript{7} The main FCHL gene identified until now is USF1, located in the 1q21–23 region.\textsuperscript{27} It encodes a transcription factor that is a key regulator of lipid and glucose metabolism with SNP associated to FCHL.\textsuperscript{28} These loci (LPL, APOA1/C3/A4/A5 and USF1) linked to dyslipidaemia in genome-wide studies\textsuperscript{29} do not segregate with FCHL in the French Canadian families reported here (data not shown).

The genetic basis of FCHL is apparently complex and more than one genetic factor account for this phenotype. Some PCSK9 variants might contribute to the increased apoB rich lipoprotein production seen in FCHL, and constitute one of the genetic factors underlying FCHL. The association found between FCHL and PCSK9 suggests that in some cases of FCHL part of the phenotype may be driven by variations at the PCSK9 locus. Thus, PCSK9 might be considered as modifier gene or one of the genes contributing to the FCHL phenotype. Studies of PCSK9 variants in a larger number of FCHL families are needed to confirm these data and to identify precisely the role of PCSK9 in the FCHL phenotype. It is noteworthy that the p.R496Q variant in PCSK9 had been identified by Cameron et al\textsuperscript{9} in a subject homozygous for apolipoprotein E-2 who presented with type III hyperlipoproteinaemia, but the effect on cholesterol values was not investigated.

The complex genetic background of FCHL and the lack of unified diagnostic criteria for FCHL made difficult the collective interpretation of the findings from several genomes scans done in order to identify genes implicated in this disease.\textsuperscript{9, 10} Thus, it is very important to carefully phenotype population cohorts, especially FCHL families used in linkage analysis study, and genotype the candidate genes already known to be implicated in FCHL to better dissect the FCHL phenotype in these families, the contribution of each gene that can be implicated in the phenotype in a moderate way, and the selection of the families that can facilitate the identification of a new gene in FCHL.

Thus, PCSK9 variants might contribute to FCHL in a moderate way, and are to be taken into consideration in the study of this disease with other known factors (LPL, APOE, etc) in order to help in the detection of unknown factors that are required to understand this multigenic and complex atherogenic disease.

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