Novel Loss-of-Function PCSK9 Variant Is Associated with Low Plasma LDL Cholesterol in a French-Canadian Family and with Impaired Processing and Secretion in Cell Culture

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BACKGROUND: PCSK9 (proprotein convertase subtilisin/kexin type 9) is a polymorphic gene whose protein product regulates plasma LDL cholesterol (LDLC) concentrations by shuttling liver LDL receptors (LDLRs) for degradation. PCSK9 variants that cause a gain or loss of PCSK9 function are associated with hyper- or hypocholesterolemia, which increases or reduces the risk of cardiovascular disease, respectively. We studied the clinical and molecular characteristics of a novel PCSK9 loss-of-function sequence variant in a white French-Canadian family.

METHODS: In vivo plasma and ex vivo secreted PCSK9 concentrations were measured with a commercial ELISA. We sequenced the PCSK9 exons for 15 members of a family, the proband of which exhibited very low plasma PCSK9 and LDLC concentrations. We then conducted a structure/function analysis of the novel PCSK9 variant in cell culture to identify its phenotypic basis.

RESULTS: We identified a PCSK9 sequence variant in the French-Canadian family that produced the PCSK9 Q152H substitution. Family members carrying this variant had mean decreases in circulating PCSK9 and LDLC concentrations of 79% and 48%, respectively, compared with unrelated noncarriers (n = 210). In cell culture, the proPCSK9-Q152H variant did not undergo efficient autocatalytic cleavage and was not secreted. Cells transiently transfected with PCSK9-Q152H cDNA had LDLR concentrations that were significantly higher than those of cells overproducing wild-type PCSK9 (PCSK9-WT). Cotransfection of PCSK9-Q152H and PCSK9-WT cDNAs produced a 78% decrease in the secreted PCSK9-WT protein compared with control cells.

CONCLUSIONS: Collectively, our results demonstrate that the PCSK9-Q152H variant markedly lowers plasma PCSK9 and LDLC concentrations in heterozygous carriers via decreased autocatalytic processing and secretion, and hence, inactivity on the LDLR.

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Familial hypercholesterolemia (FH)6 is characterized by high circulating concentrations of LDL particles and increases the risk of cardiovascular disease (1). Mutations in the LDLR7 (low density lipoprotein receptor), APOB [apolipoprotein B (including Ag(x) antigen)], and PCSK9 (proprotein convertase subtilisin/kexin 9) genes are linked with FH1, FH2, and FH3, respectively (1, 2). Recently, 2 other loci for hypercholesterolemia have been identified on chromosome 16q22.1 (FH4) and 8q24.22, although the cognate genes have not yet been defined (3, 4). The LDL receptor (LDLR) clears LDL particles from the circulation, whereas apolipoprotein B100 is the protein component of the LDL particle that interacts with the LDLR (5). PCSK9 is a secreted glycoprotein (6) that interacts with the LDLR and mediates its lysosome-dependent degradation (7–10). Largely produced in the liver and the intestine (6), PCSK9 is synthesized in the endoplasmic reticulum.
respectively) 47% for PCSK9-C679X and -R46L heterozygotes, carriers of loss-of-function PCSK9 variants (88% and 47%) to demonstrate reductions in the risk of coronary artery disease in individuals with PCSK9 loss-of-function activities at the protein level (10, 12). Longitudinal population studies have shown significant reductions in the risk of coronary artery disease in carriers of loss-of-function PCSK9 variants (88% and 47% for PCSK9-C679X and -R46L heterozygotes, respectively) (21).

The characterization of naturally occurring gain-of-function and loss-of-function human PCSK9 variants has increased our understanding of the cell biology and function of this secreted glycoprotein. This work has included identification of the amino acid residues important for PCSK9 autocatalytic processing, secretion, and biological activity—information that provides insight into the mechanism by which PCSK9 mediates LDLR degradation. The mechanisms of many PCSK9 variants remain unknown, however. In this study, we identified a novel PCSK9 sequence variant in a white French-Canadian family that is associated with low circulating LDL cholesterol concentrations. We carried out cell culture studies to characterize the molecular mechanism behind this loss-of-function PCSK9 phenotype.

**Study Participants and Methods**

**POPULATION STUDY INDIVIDUALS**

After obtaining written informed consent, we collected blood samples from all study participants after a 12-h fast and made clinical measurements according to study protocols approved by the ethics committees of the Ottawa Hospital Research Institute and Clinical Research Institute of Montreal. We obtained blood samples from 15 participants recruited to the Clinical Research Institute of Montreal. The comparison group consisted of 210 individuals recruited to the Ottawa Hospital Lipid Clinic. Participants underwent anthropometric measurements, including height and weight measurements. Body mass index was calculated as the weight in kilograms divided by the square of the height in meters.

**MEASUREMENT OF PLASMA LIPIDS AND LIPOPROTEINS**

Blood was collected into EDTA-containing Vacutainer tubes (BD), and plasma and blood leukocytes were obtained by centrifuging blood samples at 1560g for 10 min at 22 °C. Serum for lipid measurements was obtained by collecting blood into BD SST™ Vacutainer tubes, allowing the blood sample to clot at room temperature for 20 min, and centrifuging the sample at 1560g for 10 min at 22 °C. Total cholesterol and triglycerides were measured with enzymatic methods on an Ortho Clinical Diagnostics Vitros 250 analyzer. HDL cholesterol was measured by a direct enzymatic method (Beckman Coulter) on the Synchron LX20 Pro analyzer (Beckman Coulter); the LDLC concentration was calculated with the Friedewald equation.

**GENOTYPING**

Genomic DNA was isolated from blood leukocytes with the QIAamp DNA Blood Kit (Qiagen). The primers and PCR conditions used for amplifying individual PCSK9 exons were as described by Abifadel et al. (2). Standard DNA-sequencing services were carried out by Bio Basic.

**MEASUREMENT OF PLASMA PCSK9**

The plasma PCSK9 concentration was quantified with a human PCSK9 ELISA from CycLex. This assay has an intraassay CV of 1.5%–2.6% and an interassay CV of 2.9%–7.1%. All samples were quantified 4 times.

**CONSTRUCTS AND ANTIBODIES**

The cDNA of human PCSK9 was cloned into the pIREs2-EGFP vector with a C-terminal V5 tag, as previously described (6). Mutations were introduced by site-directed mutagenesis, also as described (22). The mouse anti-V5 IgG used for immunoprecipitation and immunoblotting of V5-tagged recombinant PCSK9 was obtained from Invitrogen. The anti-PCSK9 antibody used for immunoblotting (anti–IB PCSK9 Ab) was produced by recombinant PCSK9 vaccination (7). The rabbit anti–human LDLR IgG and the mouse anti–transferrin receptor IgG were from Cedarlane. Secondary antimouse and antirabbit IgGs were from Amersham/GE Healthcare Life Sciences.
standard protocol for Effectene® (Qiagen) (6). Media from cultures of HuH7 cells transiently transfected with cDNA encoding human PCSK9 or a nonrelevant DNA control were collected 48 h later in the presence of a Complete Mini Protease Inhibitor Cocktail (Roche) and a phosphatase inhibitor (200 μmol/L sodium orthovanadate) and centrifuged at 13 000g for 3 min. Cell lysis was carried out in 1X RIPA buffer (50 mmol/L Tris, pH 7.6, 150 mmol/L NaCl, 10 mL/L NP-40, 5 g/L deoxycholate, 1 g/L SDS) in the presence of the inhibitors mentioned above. The protein concentrations of total cell lysates were measured with the Bradford dye-binding method (Bio-Rad Protein Assay Kit; Bio-Rad Laboratories).

IMMUNOBLOTTING
Proteins were electrophoresed through a 7% NuPAGE Tris-acetate gel (Invitrogen), electroblotted onto nitrocellulose, and immunoblotted according to a standard protocol. The primary anti-PCSK9 antibody used for immunoblotting (anti–IB PCSK9 Ab-03) (7) was raised in rabbits against recombinant PCSK9 amino acid residues 31–454 and used at a dilution of 1 part in 500. Immunoblots were revealed by chemiluminescence with Western Lightning Plus (PerkinElmer) on X-Omat film (Kodak). The Chemigenius 2XE imager and GeneTools software (Syngene) were used for densitometric quantification of signals.

STATISTICAL ANALYSES
The results of quantification of secreted PCSK9 by ELISA were expressed as the mean and SE (n = 4, human plasma; n = 3, spent media). LDLR was quantified via immunoblotting followed by densitometry analysis (n = 3). Representative immunoblots are provided (see below). The unpaired Student t-test was used for statistical analyses of differences. P values <0.05 were considered statistically significant.

Results

IDENTIFICATION OF THE NOVEL PCSK9-Q152H LOSS-OF-FUNCTION VARIANT IN A FRENCH-CANADIAN FAMILY
We identified an individual in a French-Canadian family with a very low circulating PCSK9 concentration (Fig. 1; see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue10), compared with concentrations in a general white Canadian population (n = 210; 68.5 μg/L vs 326.9 μg/L), as well as low LDLC concentrations (14th percentile, adjusted for age and sex). We sequenced the 12 PCSK9 exons and the exon–intron boundaries in this individual. She carried a missense mutation at base pair 456 (G→C) in exon 3 that yielded a proPCSK9 amino acid substitution (Q152H) at the P1 site of autocatalytic cleavage (Fig. 2A). We recruited members of the family and sequenced their PCSK9 exons and exon–intron boundaries (Fig. 1; see Table 1 in the online Data Supplement). These individuals' circulating PCSK9 concentrations were 84.3% (II.2), 87.3% (III.3), 79.0% (II.4), and 64.7% (II.9) lower than those of unrelated individuals (see Table 1 in the online Data Supplement). These individuals' circulating PCSK9 concentrations were 84.3% (II.2), 87.3% (III.3), 79.0% (II.4), and 64.7% (II.9) lower than those of the general white Canadian population (see Table 1 in the online Data Supplement). Although the Q152H carriers and the general population had mean plasma triglyceride and HDL cholesterol concentrations that did not differ significantly [triglycerides, 135.8 mg/dL and 138.2 mg/dL (1.53 mmol/L vs 1.56 mmol/L), respectively (P = 0.94); HDL cholesterol, 54.7 mg/dL and 46.4 mg/dL (1.42 mmol/L vs 1.20 mmol/L), respectively (P = 0.14), Q152H carriers had significantly lower plasma concentrations of total cholesterol and LDL cholesterol than the general population [154.6 mg/dL vs 213.6 mg/dL (4.00 mmol/L vs 5.53 mmol/L) (P = 0.0069) and 72.8 mg/dL vs 140.1 mg/dL (1.91 mmol/L vs 3.63 mmol/L) (P = 0.0031), respectively]. After adjustment for age and sex, the plasma LDL cholesterol concentrations for 3 of the 4 Q152H carriers were below the fifth percentile, and the proband was at the 14th percentile (Fig. 1; see Table 1 in the online Data Supplement). Noncarriers of the Q152H mutation within the family had LDL cholesterol concentrations that ranged from the fifth percentile to the 90th percentile, after adjustment for age and sex (Fig. 1; see Table 1 in the online Data Supplement).

Sequencing of the 12 PCSK9 exons in this family also revealed that 6 members (II.2, II.5, II.7, II.8, III.5, III.6; Fig. 1) carried a Leu insertion (c.43_44insCTG and denoted L10ins) within a stretch of 9 Leu residues in the signal peptide for PCSK9. This insertion was associated with lower LDL cholesterol concentrations in a white population (17). Two other members (II.6, III.4) carried both the L10ins and the R46L sequence variants within the PCSK9 propeptide, which are also associated with PCSK9 loss of function (21) (Fig. 1; see Table 1 in the online Data Supplement). In fact, several pop-
Population studies have found that the risk of cardiovascular disease is decreased by approximately 50% for PCSK9-R46L heterozygotes, the LDL-C concentrations of which are reduced by approximately 14% on average, compared with age- and sex-matched controls (21, 23, 24). The PCSK9-L10ins variant is associated with an approximately 14% reduction in LDL-C concentrations in white populations (17), but it is not associated with a significant lowering in the LDL-C concentration in individuals of African descent (25).

Three individuals (II.2, III.2, III.3) also carried the PCSK9-I474V variant, which has been found in several other populations. The I474V variant is not associated with any changes in the LDL-C concentration (25, 26).

The fact that several members of this family carried multiple loss-of-function PCSK9 variants may indicate that these variants may be more frequent in some French-Canadian cohorts than in the general population.

**BIOSYNTHESIS AND FUNCTIONAL ANALYSIS OF THE LOSS-OF-FUNCTION PCSK9-Q152H VARIANT**

Fig. 2A depicts the domain structure of preproPCSK9, its sites of posttranslational modifications (sulfation at Y38, phosphorylation at S47 and S688, and glycosylation at N533), the residues surrounding the site of prosegment cleavage, the catalytic residues (D186, H226, S386), and the oxyanion hole residue (N317). Secreted PCSK9 can interact with the LDLR and enter with it into the endocytic recycling pathway, thereby decreasing the rate of LDLR recycling and increasing lysosome-dependent LDLR degradation (7–9, 27).

To compare the clinical lipoprotein profiles and plasma PCSK9 measurements of individuals carrying the novel PCSK9-Q152H variant, we investigated the biosynthesis and secretion of PCSK9 and its effect on LDLR degradation. Western blotting results for V5-tagged PCSK9 (Fig. 2B) show the distribution of proPCSK9 and intracellular PCSK9 (PCSK9 in panel B) in total cell lysates from liver HuH7 cells transiently transfected with wild-type (WT) PCSK9 or PCSK9-Q152H (lanes 1 and 2, respectively). The Q152H amino acid substitution at position 152 greatly reduced the ability of proPCSK9 to undergo autocatalytic cleavage, compared with WT (lane 2). Immunoprecipitation and subsequent immunoblotting procedures revealed secretion of PCSK9-WT into the medium but detected no PCSK9-Q152H secretion into the medium (Fig. 2B, lanes 3 and 4, respectively). This reduced proprotein processing and loss of secretion produced LDLR concentrations (Fig. 2C) that were not sig-

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Fig. 1. Pedigree of proband with missense mutation G→C at base pair 456 in PCSK9.

Age- and sex-matched adjusted LDL-C percentiles and plasma PCSK9 concentrations are shown. Family members younger than 16 years (III.1 and III.7) were not tested. Single line through symbol represents deceased individual. ND, not determined; NM, no exonic amino acid–changing mutation (for LDL-C conversion of mg/dL to mmol/L multiply by 0.0259).
significantly higher (relative concentration, approximately 1.4) than those in mock-transfected control cells (relative concentration, 1; \( P/H11005 \geq 0.25 \)); however, these LDLR concentrations were significantly higher (relative concentration, 1.4) than those in cells transfected with PCSK9-WT (relative concentration, approximately 0.4; \( P/H11005 \leq 0.03 \)).

The inset in Fig. 2C shows representative immunoblots for the LDLR and the transferrin receptor control.

**THE PCSK9-Q152H VARIANT DECREASES WT SECRETION IN CELL CULTURE**

In addition to blocking cleavage of the proPCSK9 zymogen, the Q152H variant also affected PCSK9-WT processing and secretion (Fig. 3, A and B, respectively), and this effect protected LDLR from degradation in cell culture (Fig. 3C). Fig. 3A shows equal amounts of PCSK9 secreted from HuH7 cells transfected with 250 ng of either PCSK9-WT (untagged) or PCSK9-WT-V5 (V5-tagged) cDNA (lanes 1 and 2, respectively). Lanes 1 and 2 of Fig. 3B show the intracellular processing of proPCSK9 to PCSK9. PCSK9-Q152H-V5 was not secreted (Fig. 3A, lane 3). Cotransfection of PCSK9-WT with increasing amounts of PCSK9-WT-V5 was recompensed with increased PCSK9 secretion (Fig. 3A, lanes 4–6), and both proforms were processed as in single transfections (compare lanes 1 and 2

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**Fig. 2. Effect of the Q152H mutation on the synthesis and secretion of PCSK9.**

(A), Schematic of proPCSK9 domain structure. SP, signal peptide; PD, propeptide domain; CHRD, C-terminal Cys/His-rich domain.

(B), PCSK9 immunoblotting of cells and immunoprecipitated media from overproduction of C-terminally V5-tagged PCSK9-WT and PCSK9-Q152H in HuH7 cells. PCSK9-\( \Delta N_{218} \), PCSK9 cleaved by furin following R218; WB, Western blotting; Ab, antibody.

(C), Relative LDLR concentrations in these cells. Inset shows representative immunoblotting of the LDLR and the transferrin receptor (TfR) (loading control). Data are presented as the mean (SE) (n = 3) and compared with the Student t-test. C, mock-transfected controls.
with lanes 3–5 in Fig. 3B). In contrast, cotransfection of PCSK9-WT with increasing amounts of PCSK9-Q152H-V5 significantly decreased PCSK9-WT secretion (Fig. 3A, lanes 7–9), even at the highest ratio of WT cDNA to Q152H cDNA (4:1) (Fig. 3A; compare lanes 1 and 7; \( P = 0.002 \)). Cotransfection of equal amounts of PCSK9-Q152H-V5 with PCSK9-WT significantly decreased PCSK9 secretion by 78% and 90% (Fig. 3A; compare lane 1 or 6 with lane 9; \( P = 0.0002 \) and \( P = 0.0004 \), respectively), whereas immunoblotting showed that the intracellularly processed form of PCSK9-WT was reduced (Fig. 3B; compare iPCSK9-no tag lane 1 or 6 with lane 9). Lanes 1–9 of Fig. 3C show a representative immunoblot of LDLR in HuH7 cells transiently transfected with PCSK9-WT, PCSK9-WT-V5, or PCSK9-Q152H-V5. As expected, the LDLR concentration decreased with cotransfection of increasing amounts of PCSK9-WT-V5 with PCSK9-WT (Fig. 3C, lanes 4–6); however, lanes 7–9 of Fig. 3C show that cotransfection of increasing amounts of PCSK9-Q152H-V5 protected the LDLR from degradation by PCSK9-WT. Transfections with 250 ng of PCSK9-WT significantly decreased LDLR concentrations relative to those for control cells (Fig. 3C, lane 1 vs lane 3; \( P = 0.05 \)); however, cotransfection in the presence of either 125 ng or 250 ng of Q152H (Fig. 3C, lanes 8 and 9) significantly increased the relative LDLR concentration to 0.94 (\( P = 0.03 \)) and 1.1 (\( P = 0.02 \)), respectively, compared with
PCSK9-WT (0.79; lane 1). This approximately 40%–50% increase in relative LDLR concentration from that expected for WT can be attributed at least partially to the decrease in secreted PCSK9-WT in the presence of the Q152H variant. In Fig. 1 in the online Data Supplement, we demonstrate the linearity of PCSK9 secretion for the amounts of PCSK9-WT cDNA (untagged) and PCSK9-WT-V5 cDNA used (see above). Fig. 2 in the online Data Supplement shows that endogenous concentrations of intracellular PCSK9 were decreased upon overproduction of PCSK9-Q152H, compared with mock-transfected cells, and there was a corresponding significant upregulation of LDLR in these cells ($P = 0.02$). Fig. 3 in the online Data Supplement illustrates the cotransfection of our plasmids by immunocytochemistry.

**Discussion**

The loss-of-function PCSK9-Q152H variant is the first described in a white Canadian population to have such a profound effect on plasma cholesterol concentrations. Several other loss-of-function PCSK9 variants with strong phenotypic effects on the LDLC concentration have been described for other populations. Two such variants occur in carriers of African descent, and both are nonsense variants. One of the variants occurs in the prodomain of PCS9. No PCS9 is produced, owing to an early truncation (PCSK9-Y142X). The other variant is a C-terminal nonsense mutation (PCSK9-C679X) that causes the retention of autocatalytically cleaved PCS9 in the ER (16, 21). The LDLC concentrations in carriers of these variants range from the first percentile to the 50th percentile, with a mean lowering in the LDLC concentration of 40%, after adjustment for age- and sex-matched controls (16). The plasma PCS9 concentrations in carriers of the C679X and Y142X variants are approximately 60% lower than those in their control population (28). The third variant, a compound mutation (R104C/V114A) found in a French family and associated with familial hypobetalipoproteinemia, exhibits a dominant negative effect on PCSK9 secretion (29). In Fig. 3A, we show that cotransfection of equal amounts of PCS9-Q152H-V5 and nontagged PCS9-WT decreased WT secretion by approximately 80% (from 175 ng/L to 35 ng/L). This decrease was not a general effect of cotransfection, because secretion of equal amounts of PCS9-WT-V5 and nontagged PCS9-WT (Fig. 3A, lane 6) increased the concentration of secreted PCS9 to 275 ng/L. Therefore, in the ex vivo cell culture conditions of cotransfection, our PCS9-Q152H variant does have a dominant negative effect on PCS9-WT secretion. Whether this effect also occurs in vivo is not known; however, persons carrying this variant have 79% less circulating PCS9 than unrelated noncarriers. If this effect does occur in vivo, we expect that such an effect would amplify the loss-of-function phenotype of the Q152H variant.

Conversely, there are some gain-of-function PCS9 variants—S127R and D127G—that display decreased autocatalytic cleavage and secretion but are associated with hypercholesterolemic phenotypes (10, 30). In vitro binding assays have shown 5-fold increased binding of the PCS9-S127R variant to the LDLR compared with WT, a result that could partly account for its gain of function (31). Alternatively, these variants may mediate intracellular LDLR degradation more efficiently. This pathway has been described by Poirier et al. (32), although the relative contributions of the intracellular and extracellular degradation routes with respect to PCS9-mediated LDLR degradation are not fully understood.

Several studies have reported a positive correlation between the plasma PCS9 concentration and the LDLC concentration in general populations (28, 33–36). Other studies have documented variable changes in plasma PCS9 for carriers of PCS9 loss-of-function and gain-of-function variants compared with control individuals (noncarriers of a particular PCS9 variant) (34, 36, 37). In fact, circulating PCS9 may differentially affect plasma LDL concentrations, depending on whether an individual carries a PCS9 variant that alters its LDLR-degrading activity and depending on the mode of action of that particular PCS9 mutation and/or variant. Overall, this variability means that the plasma PCS9 concentration as measured by ELISA does not necessarily predict the LDL concentration, because the LDLC concentration can, in some instances, be strongly influenced by the mode of action of a PCS9 variant (36). For instance, carriers of the gain-of-function PCS9-D374Y variant, which is associated with autosomal dominant hypercholesterolemia and very high LDL concentrations, have lower plasma PCS9 concentrations than the general population (37), a finding that conflicts with the reports of a positive correlation between the plasma PCS9 concentration and the LDLC concentration (28, 33–36). The mode of action of the PCS9-D374Y variant has been well studied, however. It binds 10 times better to the liver LDLR than PCS9-WT, thereby decreasing the plasma PCS9 concentrations in D374Y carriers compared with noncarriers and augmenting LDLR degradation (38). On the other hand, plasma PCS9 concentrations are also reduced in carriers of the loss-of-function PCS9-R46L variant, which is associated with reduced LDL concentrations (24, 36, 37), although the reason for this observation is less clear. Our studies with HEK293 cell cultures showed no change in the rate of PCS9-R46L secretion.
route for PCSK9-mediated LDLR degradation affects the downregulation of cell surface LDLR. His for Glu prevents autocatalytic cleavage by PCSK9 that the naturally occurring amino acid substitution of R46L variants are approximately 80% of non-secreted, and therefore plasma concentrations are low, protecting the liver LDLRs from PCSK9-mediated LDLR degradation (Figs. 1 and 2).

In a previous report, we described our investigation of the effect of amino acid substitutions around the autotranscriptase cleavage site of PCSK9 (VFAQ152 ▼ SIP) (10). We showed that PCSK9 tolerates an Ala substitution for Glu at P1 and that its relative processing and secretion are approximately 80% of PCSK9-WT (10). In the present study, we have shown that the naturally occurring amino acid substitution of His for Glu prevents autocatalytic cleavage by PCSK9 in the ER, thereby precluding PCSK9 secretion (Figs. 2 and 3). Consequently, this PCSK9 variant no longer affects the downregulation of cell surface LDLR through the endosomal/lysosomal pathway, a major route for PCSK9-mediated LDLR degradation (40).

Our cell biology findings are consistent with the findings of our human studies, in which individuals carrying the PCSK9-Q152H mutation showed very low plasma PCSK9 concentrations (79% reduction compared with the general Canadian population) and consequently low concentrations of circulating LDLC owing to the upregulation of liver LDLR. This report is the first of a loss-of-function mutation in PCSK9 within the white Canadian population that displays such a profound effect on cholesterolemia. It also reinforces the suggestion that lowering the PCSK9 concentration by blocking PCSK9 synthesis, processing, or secretion could be an effective therapeutic strategy to complement current lipid-lowering drugs.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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PCSK9 Variant Causes Low Plasma PCSK9 and LDL Cholesterol