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The contribution of PCSK9 levels to the phenotypic severity of familial hypercholesterolemia is independent of LDL receptor genotype

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ABSTRACT

Autosomal dominant familial hypercholesterolemia (FH) is caused by genetic mutations in the LDL receptor (LDLR), its ligand apolipoprotein (apo) B, or proprotein convertase subtilisin/kexin type 9 (PCSK9). Although PCSK9 levels have been shown to correlate with LDL-cholesterol (LDL-C) levels in FH, the extent to which PCSK9 levels modulate the phenotypic severity of this disease independent of LDLR genotype remains to be clarified.

Objective. To assess the relationship between LDLR genotype and the plasma levels of PCSK9, LDL-C, and lipoprotein (a) (Lp(a)) in a large cohort of genetically defined FH heterozygotes (HeFH).

Methods. A total of 292 HeFH carrying one of the nine French-Canadian mutations in the LDLR gene were recruited. The cohort included 226 carriers of a negative-receptor (NR) mutation and 66 carriers of a defective-receptor (DR) LDLR gene mutation. Fifty-six control subjects, who were matched with the HeFH subjects based on gender and body mass index, were also recruited.

Results. PCSK9 levels were higher in the HeFH group than in the control group (317.9 ± 107.1 ng/mL vs. 203.3 ± 59.8 ng/mL; $P < 0.0001$). The strength of the association between PCSK9 and LDL-C levels was similar among controls ($r = 0.37$; $P = 0.005$) and HeFH ($r = 0.31$; $P < 0.0001$). Furthermore, a multiple linear regression analysis revealed that the positive correlation between PCSK9 and LDL-C levels remained significant after adjusting for LDLR genotype in the HeFH group.

Conclusion. These results suggested that the contribution of PCSK9 levels to the phenotypic severity in FH heterozygotes is independent of LDLR genotype.

Abbreviations: ApoB, Apolipoprotein B; BMI, Body mass index; CAD, Coronary artery disease; DR, Defective-receptor; EGF(A), Epidermal growth factor precursor homology domain A; FH, Familial hypercholesterolemia; HDL-C, HDL-cholesterol; HeFH, FH heterozygotes; LDL-C, LDL-cholesterol; LDLR, LDL receptor; Lp(a), Lipoprotein (a); NR, Negative-receptor; PCR, Polymerase-chain reaction; PCSK9, Proprotein convertase subtilisin/kexin type 9; TG, Triglyceride.

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1. Introduction

Autosomal dominant familial hypercholesterolemia (FH) is caused by genetic mutations in the LDL receptor (LDLR), its ligand apolipoprotein (apo) B, or proprotein convertase subtilisin/kexin type 9 (PCSK9) [1,2]. Mutations in the LDLR gene disrupt the normal clearance of LDL particles from the plasma, causing a marked increase in LDL-cholesterol (LDL-C) levels [1]. Atherosclerotic coronary artery disease (CAD) usually occurs between the age of 35 and 55 years in untreated FH heterozygotes (HeFH) [3]. The LDLR mutations have been classified based on biosynthetic and functional studies in fibroblasts derived from skin biopsy specimens [4]. Negative-receptor (NR) mutations are associated with <2% of the normal LDLR allele activity, whereas defective-receptor (DR) mutations exhibit between 2% and 30% of the normal allele activity [4]. In HeFH subjects who possess a single functional LDLR allele, the ability of LDLR to bind LDL particles is reduced by 20% to 50% compared with non-FH subjects. Previous studies have shown that CAD occurs earlier in HeFH carrying NR mutations compared with HeFH carrying DR variants [5]. In the province of Quebec (Canada), nine mutations are responsible for 90% of the HeFH cases, as defined based on clinical and biochemical criteria [6,7].

PCSK9 is the ninth member of the proprotein convertase family [8] and is predominantly expressed in the liver, small intestine and kidneys [9]. Mechanistic studies have shown that PCSK9 interacts with the epidermal growth factor precursor homology domain A (EGF(A)) of LDLR at the cell surface and promotes its intracellular lysosomal degradation [8,10]. High levels of PCSK9 decrease the LDLR density on the cell surface and therefore reduce LDL-C clearance, leading to an accumulation of LDL particles in the plasma [11]. LDL-C levels and PCSK9 levels are positively correlated in non-FH and untreated FH subjects [12–15], and previous studies have shown that PCSK9 levels are higher in HeFH than in non-FH controls [13–16]. Although a recent report from a South African group suggested that the detrimental effect of PCSK9 levels on the FH phenotype is independent of LDLR genotype [15], it is unclear whether this observation also applies to other FH groups. Therefore, the primary objective of this study was to examine the extent to which LDLR genotype modulates the relationship between LDL-C and PCSK9 levels in a large French-Canadian cohort of genetically defined HeFH subjects. We also examined the relationship between PCSK9 levels and lipoprotein (a) (Lp(a)) levels in FH because hepatic apoB synthesis and secretion have been shown to be modulated by PCSK9 [17]. We hypothesized that the relationship between PCSK9 and LDL-C levels in FH heterozygotes would be dependent on LDLR genotype.

2. Methods

2.1. Population

A total of 292 HeFH (133 men and 159 women) and 56 control subjects, matched as a group for gender and body mass index (BMI), were recruited in the Quebec City area to participate in the study. All eligible subjects were required to discontinue their use of lipid-lowering medications for at least 6 weeks before blood sample collection. Subjects with a previous history of cardiovascular disease, acute liver disease, hepatic dysfunction, persistent elevations of serum transaminases, secondary hyperlipidemia due to any cause, a recent history of alcohol or drug abuse, diabetes mellitus, a history of cancer or any other conditions that may interfere with optimal participation in the study were ineligible.

All the HeFH subjects were carriers of one of the nine previously identified French-Canadian mutations in the LDLR gene [6]. Of the 292 HeFH subjects that were included in the study, 183 had the >15 kb deletion at the 5′ end of the gene [3], 57 had the W66G mutation in exon 3 [18], 24 had the C646Y mutation in exon 14 [4], 17 had the Y468X mutation in exon 10 [19], 4 had the C347R mutation in exon 8 [6], 3 had the E207K mutation in exon 4 [4], 1 had the C152W mutation in exon 4 [6], 1 had the R329X mutation in exon 7 [6], and 2 had the 5 kb deletion in exons 2 and 3 [4]. The >15 kb deletion and the R329X, Y468X and C646Y point mutations were considered to be NR mutations because the receptor encoded by these alleles exhibits <2% of the normal activity. The 5 kb deletion and the C152W, C347R, E207K and W66G point mutations were considered to be DR mutations because the receptor encoded by these alleles exhibits 2% to 30% of the normal activity.

The study was approved by the Laval University Medical Center ethical review committee, and informed consent was obtained from each patient. This trial was registered at clinicaltrials.gov as NCT02225340.

2.2. DNA Analysis

All the FH patients were screened for nine mutations in the LDLR gene using genomic DNA; the two deletions were analyzed by Southern blotting [20], and seven point mutations were analyzed by restriction enzyme fragment analysis [6,19]. Genotyping of apoE was performed by PCR amplification of a 244 bp fragment of exon 4 of the apoE gene with oligonucleotides F4 and F6 and digestion of the resulting PCR fragments with the restriction enzyme Hhal [21].

2.3. Plasma Lipids, Lipoproteins and Apoproteins

Blood samples were collected after a 12-hour fast in tubes containing disodium EDTA (Na_2EDTA) and benzamidine (0.03%) [22]. The samples were then immediately centrifuged at 4 °C for 10 min at 3000 rpm to obtain plasma, which was stored at 4 °C and processed within 5 days. Cholesterol and triglyceride (TG) levels were determined in the plasma and lipoprotein fractions by enzymatic methods (Randox, Crumlin, UK) using an RA-500 analyzer (Bayer Corporation, Tarrytown, NY) as previously described [23]. Plasma VLDL (d < 1.006/mL) was isolated by preparative ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranatant (d > 1.006 g/mL) using heparin and MnCl. The cholesterol and TG content of the infranatant fraction was measured before and after the precipitation step to obtain the LDL-C level. Plasma apoB levels were measured using a Behring Nephelometer BN-100 (Behring Diagnostic,