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Plasma proprotein convertase subtilisin kexin type 9 levels are related to markers of cholesterol synthesis in familial combined hyperlipidemia

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Abstract *Background and aims:* Two recent independent studies showed that patients with familial combined hyperlipidemia (FCHL) have elevated plasma levels of proprotein convertase subtilisin kexin type 9 (PCSK9) and markers of cholesterol synthesis. Both PCSK9 expression and cholesterol synthesis are downstream effects of hepatic activation of sterol regulatory element binding protein 2 (SREBP2). The present study was conducted to study the relationship between plasma PCSK9 and markers of cholesterol synthesis in FCHL.

Abbreviations: FCHL, familial combined hyperlipidemia; LDLR, LDL receptor; PCSK9, proprotein convertase subtilisin kexin type 9; SREBP2, sterol regulatory element binding protein 2; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; VLDL-C, VLDL cholesterol; VLDL-TG, VLDL triglycerides; RLPC, remnant lipoprotein cholesterol; sdLDL-C, small-dense LDL cholesterol; BMI, body mass index; SOLAR, Sequential Oligogenic Linkage Analysis Routines; SPSS, Statistical Package of Social Sciences; CAD, coronary artery disease.

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Methods and results: Markers of cholesterol synthesis (squalene, desmosterol, lathosterol), cholesterol absorption (campesterol, sitosterol, cholestanol) and PCSK9 were measured in plasma of FCHL patients ($n = 103$) and their normolipidemic relatives (NLR; $n = 240$). Plasma PCSK9, lathosterol and desmosterol levels were higher in FCHL patients than their NLR ($p < 0.001$, age and sex adjusted). Heritability calculations demonstrated that 35% of the variance in PCSK9 levels could be explained by additive genetic effects ($p < 0.001$). Significant age- and sex-adjusted correlations were observed for the relationship between PCSK9 and lathosterol, both unadjusted and adjusted for cholesterol, in the overall FCHL population (both $p < 0.001$). Multivariate regression analyses, with PCSK9 as the dependent variable, showed that the regression coefficient for FCHL status decreased by 25% (from 0.8 to 0.6) when lathosterol was included. Nevertheless, FCHL status remained an independent contributor to plasma PCSK9 ($p < 0.001$).

Conclusions: The present study confirms the previously reported high and heritable PCSK9 levels in FCHL patients. Furthermore, we now show that high PCSK9 levels are, in part, explained by plasma lathosterol, suggesting that SREBP2 activation partly accounts for elevated PCSK9 levels in FCHL.

Introduction

Familial combined hyperlipidemia (FCHL) is the most common genetic dyslipidemia in western society and responsible for a substantial proportion of premature coronary events [1]. To date, the exact pathogenesis of FCHL has remained unknown, due to its complex nature. We recently integrated the currently known metabolic and genetic defects into a biological model that accounts for the characteristic dyslipidemia in FCHL: elevated apolipoprotein B and triglycerides levels [2]. Hepatic overproduction of triglyceride-rich VLDL particles is one of the pathophysiological hallmarks of FCHL that develops in a background of obesity with multi-organ insulin resistance and hepatic steatosis. Several genetic defects in the clearance pathway of apolipoprotein B containing particles explain the pronounced dyslipidemia. Examples of genes that have already been identified are apolipoprotein CIII, apolipoprotein AV, lipoprotein lipase and hepatic lipase [2].

The role of another important player in particle clearance, the LDL receptor (LDLR), is now being further elucidated in the pathogenesis of FCHL. Hepatic LDLR expression is regulated by, amongst others, proprotein convertase subtilisin kexin type 9 (PCSK9), which does not only act locally on LDLR expression, but is also secreted in plasma from where it can degrade the LDLR [3]. The recently reported phase II intervention study with PCSK9 blocking antibodies underlines the relevance of circulating PCSK9 as a determinant of plasma LDL cholesterol levels [4].

Our laboratory has previously shown that circulating PCSK9 levels are elevated, heritable and related to the FCHL phenotype in the Maastricht FCHL cohort [5]. These observations could be the consequence of genetic defects in *PCSK9* itself, or, alternatively, result from defects in proteins that regulate the expression of *PCSK9*, such as hepatocyte nuclear factor 1 and sterol regulatory element binding protein 2 (SREBP2) [6—8]. It has been suggested that plasma PCSK9 levels reflect hepatic SREBP2 activity, since fasting induces changes in circulating PCSK9 and markers of cholesterol synthesis in humans, which are both downstream effects of SREBP2 [9,10]. Of interest, markers of cholesterol synthesis, not of cholesterol absorption, have also been shown to be increased in the Nijmegen FCHL cohort [11].

In the present collaboration study, we have determined plasma PCSK9 levels in the Nijmegen FCHL cohort to examine whether the previously reported observations in the Maastricht FCHL cohort can be confirmed in an independent sample. Moreover, the relationship between PCSK9 levels and markers of cholesterol synthesis and absorption was studied to gain more insight into the processes contributing to the elevated plasma PSCK9 levels in FCHL. We hypothesized that, if elevated plasma PCSK9 and cholesterol synthesis markers are also reflective of SREBP2 activation in FCHL, an association should be observed between plasma PCSK9 and markers of cholesterol synthesis, but not between plasma PCSK9 and markers of cholesterol absorption.

Methods

Study population

This study was conducted in the Nijmegen FCHL cohort that has been described in detail before [11]. In short, the study population consisted of 343 subjects derived from 32 well-defined pedigrees. FCHL affection status was assessed by the previously established nomogram, which includes absolute apolipoprotein B levels and age- and sex-adjusted plasma triglyceride and total cholesterol levels [12]. Subjects visited the lipid clinic after an overnight fast. Any lipid lowering medication was stopped four weeks prior to their visit [11]. Patients with acute coronary syndrome within the 3 months prior to their visit were not asked to stop their lipid lowering medication. Of note, these patients were not present in this cohort. Furthermore, the patient's primary physician (cardiologist or internist) was consulted whether it was medically justifiable to temporarily withdraw any lipid lowering medication. The study was approved by the ethics committee of the Radboud University Nijmegen Medical Center. All subjects gave written informed consent.

Laboratory measurements

Plasma total cholesterol, triglycerides, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), apolipoprotein B,