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ORIGINAL ARTICLE

Plasma Proprotein Convertase Subtilisin Kexin Type 9 as a Predictor of Carotid Atherosclerosis in Asymptomatic Adults

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Background Atherosclerosis is a lipid-driven inflammatory disease of the arterial wall involving complex and multifactorial processes. Proprotein convertase subtilisin kexin type 9 (PCSK9) may play a role in the development of atherosclerosis.

Methods We investigated the associations between serum PCSK9 and carotid intima-medial wall thickness (IMT), a measure of subclinical atherosclerosis that predicts cardiovascular events, in 295 asymptomatic subjects from community. Carotid IMT was determined by high-resolution B-mode carotid ultrasonography and serum PCSK9 was measured by immunoassay.

Results In univariate analysis, serum PCSK9 concentration was positively ($P<0.05$ in all) associated with age ($r=0.204$), BMI ($r=0.149$), waist circumference ($r=0.139$), systolic blood pressures ($r=0.116$), glucose ($r=0.211$), insulin ($r=0.178$), HOMA score ($r=0.195$), plasma triglyceride ($r=0.285$), total cholesterol ($r=0.241$) and LDL-cholesterol concentrations ($r=0.172$). In multivariate regression including male gender, hypertension, smoking status, HOMA score, obesity, LDL-cholesterol, lipoprotein (a) or markers of inflammation, serum PCSK9 remained an independent predictor of mean carotid IMT ($P<0.001$).

Conclusions These data suggest that serum levels of PCSK9 may contribute to increased risk of subclinical carotid atherosclerosis independent of conventional risk factors. Whether PCSK9 inhibition improves cardiovascular outcomes remains to be demonstrated in large, ongoing clinical trials.

Keywords PCSK9 • Carotid IMT and Cardiovascular disease

Introduction

Atherosclerosis is a chronic, lipid-driven inflammatory disease of the arterial wall involving complex and multifactorial processes, such as endothelial dysfunction, influx and modification of low-density lipoprotein (LDL), leukocyte recruitment, foam cell formation and plaque development [1]. Individuals with subclinical atherosclerosis are at increased risk for future cardiovascular disease (CVD) [2,3]. Although traditional plasma lipid and inflammatory

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factors are important for the development of atherosclerosis, they do not fully account for the variation in risk of CVD. Hence, identifying new plasma markers associated with subclinical atherosclerosis can have important application for clinical practice.

Proprotein convertase subtilisin kexin type 9 (PCSK9), a secretory protease produced by the liver and detectable in human plasma, has recently been suggested to play a role in the development of atherosclerosis [4]. Proprotein convertase subtilisin kexin type 9 is a key regulator of the LDL receptor and hence the metabolism of LDL [5]. In vitro and animal studies demonstrate that secreted PCSK9 binds to and redirects the LDL receptor to lysosomes for degradation; this inhibits the intracellular recycling of LDL receptors and the subsequent removal of LDL particles from plasma. Gain-of-function mutations of PCSK9 can express as familial hypercholesterolaemia (FH) with high risk of coronary artery disease, whereas PCSK9 deficiency results in low LDL-cholesterol and protection against coronary artery disease [6,7]. More recently, experimental studies also indicate that PCSK9 could accelerate atherosclerosis by promoting vascular inflammation [8].

High-resolution B-mode carotid ultrasonography has been used for non-invasive detection of subclinical atherosclerosis in large community-based cohorts [9]. We and others have demonstrated that carotid intima-medial wall thickness (IMT), a measure of subclinical atherosclerosis that predicts incident coronary heart disease, is correlated with standard cardiovascular risk factors [10–12]. A report by Cohen et al. also found that genetic variations in PCSK9 were associated with changes in carotid IMT in a large population study [7]. In the present study, we investigated the association between serum PCSK9 concentration and carotid IMT in a cross sectional, community-based sample of asymptomatic subjects in Western Australia.

Methods

Study Population

The data presented here were derived from a sample of participants in the Perth Carotid Ultrasound Disease Assessment Study (CUDAS) [12,13]. The selection criteria and study design of this community-based study have been detailed previously. The participants were randomly selected from the Perth Community population and assessed for cardiovascular risk factors and had carotid B-mode ultrasound performed. This present study sample was confined to the 295 asymptomatic subjects (151 men and 144 women; age 53 ± 13 years [mean ± SD], range 28 to 77) in whom we had available serum (stored at -70 °C) to measure PCSK9. A self-administered questionnaire similar to that used by the 1994 Australian National Heart Foundation Perth Risk Factor Prevalence Survey was used to record a history of smoking, hypertension, hyperlipidaemia, diabetes, angina pectoris, myocardial infarction, stroke or a family of premature-onset myocardial infarction (MI) or stroke by age 55 years in first degree relatives. Anthropomorphic measurements and the lower of two resting blood pressures were recorded. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. The study protocol was approved by Sir Charles Gairdner Group Human Research Ethics Committee (Reference No: 2014-086). Written informed consent was obtained from all study participants.

Biochemical Analysis

A fasting blood sample was obtained from each subject. The methods for measurements of biochemical analyses were previously described [12,13]. Total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride and glucose levels were determined enzymatically on a Hitachi 747 autoanalyser (Tokyo, Japan). The LDL-cholesterol was calculated by Friedewald's method; all individuals had fasting triglyceride levels <4.5 mmol/L. Insulin was measured using a two-site immunoenzymometric assay (Tosoh AIA-600 immunoassay analyser, San Francisco). Lipoprotein(a) (Lp(a)) concentration was determined by rate nephelometry (Beckman-Coulter Inc., Fullerton, CA, USA). Insulin resistance was estimated using the HOMA formula: fasting insulin (mU/L) X fasting plasma glucose (mmol/L)/22.5. Serum PCSK9 and plasma interleukin-6 (IL-6) were measured by immunoassay (R & D Systems); the inter-assay coefficients of variation for these methods were <5%. Serum hs-CRP was measured by a microparticle turbidity assay (Hitachi 917, Roche). Monocyte and white cell counts were measured by a Coulter counter.

Carotid Ultrasound

Bilateral carotid B-mode ultrasound was performed by two trained sonographers using a 7.5-MHz annular phased-array transducer on an Interspec (Apogee) CX 200 ultrasound machine as previously described [12,13]. The IMT was defined as the distance between the characteristic echoes from the lumen–intima and media–adventitia interfaces on the far wall of the distal common carotid artery measured over a 1 cm segment length. A thorough search of the distal common carotid, carotid bulb, and internal and external carotid arteries was also made to determine the presence of focal plaque. Plaque was defined as a clearly identified area of focal increased thickness (≥1 mm) of the intima–media layer. Three end-diastolic images were analysed from the right and left distal common arteries at a site free of any discrete plaque and measurements averaged to give the mean IMT. Repeat measurements of randomly selected scans revealed no significant variation in the IMT measurements. The intraobserver coefficient of variability for image acquisition and analysis in our laboratory was 2.9% for sonographer no. 1 and 4.8% for sonographer no. 2. The interobserver coefficient of variability was 5.9%. The mean (±SD) difference in carotid artery IMT between repeat measurements varied from 0.03 ± 0.02 mm for intraobserver variability to 0.05 ± 0.04 mm for interobserver variability.

Statistical Analysis

All analyses were performed using SPSS 21 (SPSS, Inc., Chicago). Associations were examined by simple and