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Association between the surfactant protein D (*SFTPD*) gene and subclinical carotid artery atherosclerosis

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ABSTRACT

Objective: Surfactant protein D (SP-D) is a defense collectin with inflammation-modulating properties. SP-D deficiency inhibits atherosclerosis *in vivo*, and the circulatory SP-D levels have been previously associated with cardiovascular disease mortality. We hypothesized that plasma SP-D (pSP-D) and SP-D gene (*SFTPD*) single nucleotide polymorphisms (SNPs) are risk factors for atherosclerosis.
Methods: We evaluated individuals who were all 60 years old and participated in *The Glostrup Population* Study. Subclinical atherosclerosis was diagnosed based on the ultrasonographic measurement of intima-media thickness (IMT) and protruding plaques in the right carotid artery. Associations between cardiovascular traits and the levels of pSP-D (n = 687) or two coding *SFTPD* SNPs rs3088308 and rs721917 (n = 396) were investigated using multiple linear regressions and logistic regressions.
Results: There was no significant association between pSP-D and the presence of plaques or IMT. The *SFTPD* SNP rs3088308 was nominally associated with the presence of plaques, and rs721917 was nominally associated with IMT. The directions of effects of associations were markedly dependent on current smoking status.
Conclusions: The results do not support that pSP-D levels influence the development of subclinical atherosclerosis. However, the *SFTPD* SNP data support previous observations from animal studies that SP-D plays a role in the etiology of atherosclerotic disease development. The nominal significant effects are likely to be mediated by structural variant SP-D modulation of effects of tobacco smoking and are independent of pSP-D levels. The data warrant confirmation in larger cohorts.

1. Introduction

Cardiovascular disease is the most frequent cause of death globally and atherosclerosis dominates the death and disability statistics and is caused by interactions between genetic and environmental factors. Both the innate as well as the adaptive immune systems are implicated in disease development [1], and genetic differences in the molecules related to innate immunity have been linked to disease susceptibility [2].

Surfactant protein D (SP-D) is a multimeric collectin that is involved in innate immune defense and is synthesized by type 2 pneumocytes in the lungs, in additional epithelia [3] and in the endothelium throughout the vasculature [4,5]. Circulatory SP-D has

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been found to be a promising blood biomarker of lung inflammation [6], injury related to cigarette smoking and chronic obstructive pulmonary disease [7–9]. However, the sources of circulating SP-D are not fully clarified and may involve multiple sites of SP-D synthesis in addition to the lung [10]. In the lungs, and in additional mucosal sites, SP-D exerts major anti-microbial and anti-inflammatory effects [11]. However, in the systemic circulation, SP-D was shown to influence lipid metabolism and enhance the risk of atherosclerosis *in vivo*; in addition, SP-D expression was evident in atherosclerotic plaques [4,12]. Circulatory SP-D has been positively associated with mortality in general as well as mortality due to cardiovascular disease [13–15]. SP-D was further linked to metabolic and cardiovascular disease based on the following observations: immune-dampening effects in human coronary artery smooth muscle cells [16], direct binding to lipoprotein particles [17], the development of obesity in SP-D deficient mice [18], decreased SP-D expression in human adipose tissue with increasing body mass index [19], pancreatic islet SP-D gene (*SFTPD*) expression [20,21], and depression of circulating SP-D in obesity and type 2 diabetes [22–24]. A common *SFTPD* single nucleotide polymorphism (SNP), rs721917, in codon 11 in the mature protein without a leader sequence (Met/Thr [11]) affects the oligomeric form of the SP-D molecule [25] and was found to be associated with type 2 diabetes and insulin resistance, independent of circulating SP-D levels [26]. Moreover, there was a tendency for an association between this *SFTPD* SNP and coronary stenosis in a study of 236 adults admitted for elective first-time coronary angiography [27].

In the present study, we sought to extend the above observations and test the hypotheses that circulatory SP-D could serve as a proxy for subclinical atherosclerosis and that *SFTPD* variants contribute to the genetic variations in subclinical atherosclerosis in a cohort of 60-year old Danes.

2. Materials and methods

2.1. Study population

A cohort of all men and women born in 1936 who were living in one of four municipalities served by Glostrup Hospital in Copenhagen (n = 1198) has been followed since 1976 [28]. In 1996, when the subjects in the cohort were 60 years of age, the cohort was re-examined with a participation rate of 65% (n = 695) of the living cohort members. The present association study was performed using this cohort of 60-year old individuals because we were specifically interested in studying the relationship in a fairly elderly but still relatively healthy, active population. The local Ethical Committee approved the study (KA96008), and informed consent was obtained from all study participants.

2.2. Intima media thickness (IMT) and plaque measurements

Ultrasonographic measurements of carotid artery IMT (mean of three measurements) and protruding plaques was performed using B-mode ultrasound with a VingMed CFM 800 scanner with an annular transducer (7.5 MHz) as previously described [29].

2.3. Assessment of covariates

A standardized questionnaire was used to collect information about tobacco smoking status, physical activity, inhalation glucocorticoids, diabetes, and use of cardiovascular medication. Bodyweight was estimated to the nearest 0.1 kg, and height was estimated to the nearest centimeter. Systolic blood pressure (mmHg) was measured by a single observer in a standardized way according to WHO recommendations using a London School of Hygiene and Tropical Medicine Sphygmomanometer. Enzymatic methods were used to assess the serum concentration of HDL cholesterol and total cholesterol (Roche, Mannheim, Germany) [29].

2.4. Blood sampling for SP-D analysis

Venous blood samples were drawn in the fasting state and 4.5 mL of blood was collected in 0.5 mL 0.129 mol L^{-1} trisodium citrate. The plasma samples were stored at −80 °C in small aliquots until analysis. Blood samples were available from 687 individuals.

2.5. Plasma SP-D (pSP-D) analysis

The concentration of pSP-D was measured by an ELISA as described previously [30] for serum SP-D. The only exception to this protocol was that SP-D was measured in citrated plasma. The repeatability and agreement tests of SP-D measurements in citrated plasma and in serum were previously performed and showed the following: intraclass correlation coefficient (ICC), 0.9973, $P < 0.00005$; 95% limits of agreement (LOA) [($e^{0.179}$; $e^{0.304}$)] 1.20–1.36, range 0.16, precision ± 3%, demonstrating that serum measurements were relatively higher than citrate-plasma but that the measurements were parallel [31].

2.6. Genotyping

DNA was isolated by ammonium acetate precipitation from white blood cells in citrated blood. DNA samples were available from 406 individuals, but samples from only 396 individuals were analyzed for both pSP-D and *SFTPD* genotypes and were therefore used in the subsequent genotype analysis. Genotyping was performed using TaqMan Genotyping Assays according to the manufacturer's recommendations (Applied Biosystems, Foster City, California) and essentially as previously described [8,32]. *SFTPD* genotyping was performed for 2 SNPs that alter amino acids in the mature protein (without the leader region): rs721917 in codon 11 (Met/Thr [11]) and rs3088308 in codon 270 (Ser/Thr^{270}). Genotyped SNPs had a call rate of 0.95.

2.7. Statistical analysis

Variations in pSP-D between smokers and non-smokers were compared by Mann–Whitney test, and correlations between pSP-D, presence of plaques or IMT and smoking pack years were assessed using Spearman rank-order correlation coefficients. The associations between pSP-D and other variables were analyzed using multiple linear regressions. Ln(SP-D) was used to achieve a Gaussian distribution. Association analyses between pSP-D and potential confounders included the following covariates; gender/smoking status/BMI, or a predefined set of covariates, which are used to generate Framingham risk scores; gender/smoking status/HDL cholesterol/total cholesterol/systolic blood pressure in addition to BMI. The same predefined sets of covariates were also used in association analyses between pSP-D and use of inhalation glucocorticoids, use of cardiovascular medication or diabetes. Furthermore, analysis of associations between presence of plaques or IMT as dependent variables and pSP-D were performed using logistic regression and multiple linear regression, respectively. Gender/smoking status/HDL cholesterol/total cholesterol/systolic blood pressure/BMI and pSP-D-smoking interaction term were used as covariates.

The Hardy–Weinberg equilibrium of the bi-allelic polymorphism was assessed using the χ^2-test. The SNPs were in Hardy–Weinberg equilibrium (rs721917; p = 0.53, rs3088308;