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# Are AHSG polymorphisms directly associated with coronary atherosclerosis?

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ABSTRACT

*Background:* Fetuin-A (AHSG) has been proposed as a new cardiovascular risk factor. Fetuin-A levels as well as AHSG single nucleotide polymorphisms (SNPs) have been associated with intima media thickness and incident vascular events, respectively. However, the association between AHSG variants and angiographically determined coronary artery disease (CAD) has not been reported yet. Therefore, we aimed at investigating the association of AHSG SNPs with angiographically characterized coronary atherosclerosis.
*Methods:* We genotyped AHSG variants rs4917, rs2248690, rs2518136, and rs2077119 in a cross-sectional study including 1,649 patients undergoing coronary angiography. Significant CAD was diagnosed in the presence of coronary stenoses ≥50%.
*Results:* Variant rs2077119 deviates significantly from Hardy–Weinberg equilibrium and was excluded from further analysis. Neither under an additive, nor under a recessive or dominant model of inheritance, the association between investigated AHSG variants and angiographically determined CAD reached statistical significance. Haplotypes derived from these AHSG variants also were not significantly associated with coronary lesions. Further, no significant associations between investigated SNPs and the extent or severity of CAD could be observed (all p-values > 0.05).
*Conclusion:* Our data do not support a significant direct association between AHSG variants rs4917, rs2248690, and rs2518136 and clinical atherosclerosis as exemplified by angiographically characterized coronary atherosclerosis.



## 1. Introduction

Coronary artery disease (CAD) is the leading cause of mortality in industrialized nations [1]. Recently, the secreted liver protein fetuin-A, which is encoded by the α2-Heremans-Schmid glycoprotein (AHSG) gene, has been proposed as a new cardiovascular risk factor: Fetuin-A levels have been positively correlated with surrogate parameters of atherosclerosis such as intima media thickness (IMT) [2,3] and arterial stiffness [4]. Most importantly, the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study showed that fetuin-A serum levels proved associated with incident myocardial infarction (MI) and ischemic stroke [5].

Fetuin-A levels are partly genetically determined; among several single nucleotide polymorphisms (SNPs), which influence fetuin-A plasma levels, variant rs4917 (Thr248Met) has been reported to show strongest association with fetuin-A plasma levels in the EPIC study [6]. Indeed, a strong link between rs4917 (and other AHSG SNPs) and risk of MI has been shown by the same study. Of note, MI is precipitated by plaque rupture and thrombus formation and thus may not specifically reflect the atherogenicity of a certain risk factor [7,8]. Coronary angiography, to the opposite, preferentially assesses atherosclerosis. Therefore, it is important to also study the association of novel risk factors with atherosclerosis per se as e.g. angiographically characterized CAD. However, the association between AHSG polymorphisms and angiographically determined coronary atherosclerosis has not been investigated yet.

Therefore, we aimed at investigating the association of AHSG SNPs with putative functional effects including variant rs4917 [6,9], rs2248690 (−799A>T) [6,10], rs2518136 (IVS6+98C>T) [9], as well as rs2077119 (−469G>T) [9,10], with angiographically characterized coronary atherosclerosis in a large cohort of consecutive angiographied coronary patients.

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## 2. Methods

### 2.1. Study subjects

The present study included a total of 1,649 white patients, who were referred to coronary angiography for the evaluation of suspected or established stable CAD on the basis of current guidelines [11] at the Department of Medicine and Cardiology of the Academic Teaching Hospital Feldkirch, Austria. Coronary angiography was performed with the Judkins technique [12]; significant CAD was diagnosed in the presence of coronary artery stenoses with lumen narrowing of 50% or more; the severity of CAD was calculated as the sum of all stenoses' percentages of a given patient divided by the number of coronary stenoses in this patient, and the extent of CAD as the number of significant coronary stenoses in a given patient, as described previously [13,14]. The study has been carried out in accordance with the principles of the Declaration of Helsinki and the Ethics Committee of the Medical University of Innsbruck approved the present study; all participants gave written informed consent.

### 2.2. Genotyping

Genomic DNA was extracted from EDTA blood samples using the peqGOLD® Blood DNA Mini kit (PEQLAB Biotechnologie Ltd., Erlangen, Germany). Genotyping of AHSG variants rs4917, rs2248690, rs2518136, and rs2077119 were carried out by the 5′ nuclease assay using TaqMan® MGB probes on a LightCycler® 480 Real-Time PCR System (F. Hoffmann-La Roche Ltd, Basel, Switzerland). TaqMan® MGB probes were provided together with corresponding PCR primers by the Assay-on-demand™ service (Applied Biosystems, Forster City, CA). Reference controls as well as non template controls were included into each run. Genotypes were automatically determined by LightCycler® software 1.5 followed by a visual control of accurate genotype classification.

### 2.3. Tagging analysis

To investigate how well included DNA polymorphisms are tagging the AHSG locus, tagging analysis was conducted using Tagger software [15] implemented in Haploview program [16]. Identification of tagging SNPs was based on HapMap SNP database [17], release #27; analysis panel: CEU + TSI (Utah residents with ancestry from Northern and Western Europe as well as Tuscan residents in Italy), using as criteria a minor allele frequency $\geq 0.1$ and pairwise $r2 \geq 0.8$. Included region ranged from position 187,811,000 to 187,823,000 on chromosome 3, completely covering AHSG gene.

### 2.4. Statistical analysis

Univariate logistic regression analyses and Chi-squared tests were performed for evaluating the association of the AHSG variants and haplotypes, respectively, with angiographically characterized CAD. To evaluate the association of the AHSG SNPs with the severity or extent of coronary lesions the Kruskal–Wallis-Test was applied. Observed numbers of each genotype were compared with those expected when the sample was in Hardy–Weinberg equilibrium using the Chi-Square test with one degree of freedom. Statistical analyses were performed with the software package SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL). Statistical significance was defined as a two-tailed p-value $< 0.05$.

To measure linkage disequilibrium, the squared correlation coefficient r2 was calculated for each pair of SNPs using CubeX software (http://www.oege.org/software/cubex [18]). Haplotype frequencies were evaluated by the Estimate Haplotype (EH) program (ftp://linkage.rockefeller.edu/software/eh [19]). For power calculations, the software package Quanto 1.2.3 was used [20]. Power analysis was calculated for an additive model of inheritance. On the basis of a minor allele frequency between 20% and 45% depending of included variant and assuming a prevalence of significant coronary stenoses of 60%, a priori power analysis indicated that 1,600 patients would be sufficient to demonstrate an OR between 1.22 and 1.27 at an alpha fault of 0.05 with a power of 80%.

## 3. Results

Demographic data of our patients were characteristic for patients undergoing coronary angiography for the evaluation of CAD, with a preponderance of male gender (66.6%) and a high prevalence of T2DM (23.8%), hypertension (53.2%), and smoking (59.4%). Coronary angiography revealed significant coronary stenoses in 952 patients (57.7%). Clinical and biochemical characteristics of the study population with respect to the presence of significant CAD have been reported previously [21].

Genotypes were successfully called in 1,649 patients for SNP rs4917 and rs2248690 (100%), in 1,634 patients for SNP rs2518136 (99.1%), and in 1,611 patients for SNP rs2077119 (97.7%). The genotype distribution of variant rs2077119 deviates significantly from Hardy–Weinberg equilibrium and, therefore, this SNP was excluded from further analysis. All other genotype frequencies followed Hardy–Weinberg law. Results from tagging analysis showed that under predefined selection criteria the AHSG gene is completely tagged by SNPs rs4917, rs2248690, and rs2518136 (mean max $r2 = 0.92$). The analysed SNPs were in linkage disequilibrium: pairwise squared correlation coefficients were $r2 = 0.516$ between rs4917 and rs2248690, $r2 = 0.489$ between rs4917 and rs2518136, and $r2 = 0.242$ between rs2248690 and rs2518136.

Table 1 shows the associations between significant coronary stenoses and AHSG polymorphisms rs4917, rs2248690, and rs2518136. Neither under an additive, nor under a recessive or dominant model of inheritance, the association between these variants and angiographically determined CAD reached statistical significance. Haplotype analysis was conducted to further evaluate the association between the genetic diversity of the AHSG gene and significant CAD. Estimated frequencies of the five most common haplotypes derived from included AHSG variants and their associations with CAD are shown in Table 2. Neither haplotype was significantly associated with significant coronary stenoses.

To evaluate the quantitative contribution of SNPs rs4917, rs2248690, and rs2518136 to angiographically characterized coronary atherosclerosis, we investigated the association of these genetic variants with the extent and severity of coronary lesions. Results are shown in Table 3. No significant associations between investigated SNPs and the extent or severity of coronary lesions were observed.

## 4. Discussion

In the present study, we found no association of the AHSG polymorphisms rs4917, rs2248690, and rs2518136 with angiographically characterized coronary atherosclerosis. To the best of our knowledge, our study is the first study addressing the association between AHSG polymorphisms and angiographically characterized CAD. Genetic association of included AHSG SNPs have been reported with varying fetuin-A levels (rs4917 [6,22], rs2248690 [6,10]), plasma cholesterol (rs4917, rs2248690) [23], T2DM (rs2518136) [9], the extent of coronary artery calcified plaque (rs2248690) [24], and, the incidence of MI (rs4917, rs2248690) [6]. All together, these findings point at a putative genetic role of AHSG in the pathophysiology of cardiovascular diseases. However, our data do not support an impact of these AHSG polymorphisms in coronary atherogenesis.

In our investigation, diagnosis of CAD was based on coronary angiography, an objective and quantitative measure of coronary atherosclerotic burden. In contrast, the incidence of cardiovascular events is related to plaque instability, plaque rupture, and thrombogenesis, often without significant narrowing plaques. Our data, therefore, do not indicate an association of selected AHSG polymorphisms with