Association of AHSG Gene Polymorphisms With Fetuin-A Plasma Levels and Cardiovascular Diseases in the EPIC-Potsdam Study

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Background—Elevated circulating levels of fetuin-A in blood have been associated with increased risk of cardiovascular disease. The goal of our study was to prospectively investigate the potential causal nature of the association between fetuin-A levels and myocardial infarction (MI) and ischemic stroke by applying a Mendelian randomization approach.

Methods and Results—Five tagging single-nucleotide polymorphisms (rs2248690, rs2070633, rs2070635, rs4917, and rs6787344) capturing the common genetic variation of the fetuin-A coding gene α2-Heremans-Schmid glycoprotein (AHSG) were genotyped in a case-cohort comprising 214 MI cases, 154 ischemic stroke cases, and 2152 persons who remained free of cardiovascular disease events in the European Prospective Investigation into Cancer and Nutrition-Potsdam study. One single-nucleotide polymorphism (rs6787344) was discarded because of Hardy-Weinberg disequilibrium. All AHSG tagging single-nucleotide polymorphisms were associated with fetuin-A plasma levels (P<0.0001). AHSG rs4917 C>T showed the strongest association, explaining 21.2% of the phenotypic variance independent of potential confounding factors (+35.5 μg/mL increase per C-allele, P=2×10^-121). Furthermore, the rs4917 C-allele showed a significant association with MI (adjusted hazard rate ratio [RR] 1.34, 95% CI 1.05 to 1.70, P=0.02). Based on this association, the expected RR for MI corresponding to 1 SD in fetuin-A was 1.54 and, thus, strikingly matches the previously observed association between fetuin-A plasma levels and MI risk (RR 1.59).

Conclusions—These data provide evidence for the causal nature of the recently reported association between fetuin-A plasma levels and MI risk, thereby suggesting an involvement of fetuin-A in the pathogenesis of cardiovascular disease. (Circ Cardiovasc Genet. 2009;2:607-613.)

Key Words: fetuin-A ■ α2-HS glycoprotein ■ tagging single-nucleotide polymorphism ■ myocardial infarction ■ ischemic stroke ■ prospective cohort study ■ Mendelian randomization

Fetuin-A, also referred to as α2-Heremans-Schmid glycoprotein (AHSG), is synthesized and secreted by the liver almost exclusively,1 particularly in hepatic steatosis.2 The negative acute-phase 62-kDa glycoprotein fetuin-A is an abundant serum protein of the cystatin superfamily of cysteine proteases and is found throughout the body in the extracellular space. Fetuin-A is an endogenous inhibitor of the insulin-stimulated insulin receptor tyrosine kinase3–5 and an important circulating inhibitor of ectopic mineral calcification.6 Fetuin-A deficiency in chronic kidney disease is known to contribute to vascular complications.7

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Recent data indicate an important role of circulating fetuin-A in the pathophysiology of insulin resistance2,8 and type 2 diabetes.9–11 Furthermore, fetuin-A was associated with signs of the metabolic syndrome.11–13 Our novel finding in the European Prospective Investigation into Cancer and
Nutrition (EPIC)-Potsdam study suggests a strong link between high circulating fetuin-A levels and risk of cardiovascular disease (CVD); fetuin-A plasma levels were associated with incident myocardial infarction (MI) and ischemic stroke (IS) independent of established risk factors for CVD. In support of this finding, fetuin-A levels correlated positively with intima media thickness of the common carotid artery, an early marker of atherosclerosis, in a clinical study. Furthermore, findings from animal in vivo and human adipocyte in vitro studies suggest a direct effect of fetuin-A on the induction of subclinical inflammation and cytokine expression. Thus, fetuin-A is believed to be directly involved in the pathogenesis of CVD.

However, it cannot be excluded that the observed relationship between fetuin-A levels and CVD risk is due to reverse causation or confounding, i.e., that fetuin-A constitutes a marker of yet unknown metabolic processes. Evidence for a causal role of circulating fetuin-A in the pathogenesis of CVD can be generated by applying the concept of Mendelian randomization. The Mendelian randomization approach assumes that any factor that possibly confounds a true association between an intermediate phenotype (e.g., fetuin-A blood levels) and a disease (e.g., CVD) is distributed evenly among those who carry and those who do not carry a predisposing genotype for the intermediate phenotype (high fetuin-A levels predicting alleles). Therefore, carriage of an allele or haplotype that exposes individuals to long-term elevated circulating fetuin-A should be associated with disease risk proportionally to the difference in circulating fetuin-A, attributable to the genetic variant.

In this study, we determined 4 tagging (tag) single-nucleotide polymorphisms (SNPs) of the fetuin-A gene (AHSG) and examined the hypothesis that AHSG heterogeneity is directly related to CVD risk due to changes in fetuin-A blood levels.

Methods

Study Population

The EPIC-Potsdam study is part of the multicenter prospective cohort study EPIC that involves over half a million people from 10 European countries. In Potsdam, Germany, 27,548 subjects, mainly aged 35 to 65 years, from the general population were recruited between 1994 and 1998. The baseline examination included anthropometric and blood pressure measurements, blood sampling, a self-administered validated food-frequency questionnaire, and a personal interview on lifestyle habits and medical history. Follow-up questionnaires have been administered every 2 to 3 years to obtain information on current medication and newly developed diseases, including CVDs. Informed consent was obtained from all study participants, and approval was given by the Ethical Committee of the State of Brandenburg, Germany.

The association of AHSG tagSNPs with risk of MI and IS was analyzed using a case-cohort design using EPIC-Potsdam data from the study population previously described. The study population included a random sample of 2500 subjects (subcohort) and all newly arisen MI and IS cases from the EPIC-Potsdam cohort verified in each follow-up round until November 30, 2006. Two hundred sixty-nine incident MI and 199 incident IS cases were confirmed during a mean follow-up time of 8.2 ± 2.2 years in the EPIC-Potsdam cohort. If both events occurred in an individual, only the first event was considered. After exclusion of participants with a history of MI or IS at baseline, with missing covariate assessment, fetuin-A blood concentration, AHSG genotypes, and fully obtained follow-up data, the case cohort comprised 2520 individuals (214 MI cases, 154 IS cases, and 2152 noncases). In agreement with the case-cohort design, 45 incident cases (22 MI cases and 23 IS cases) belonged to the subcohort (n=2197). The subcohort was used to investigate the association between AHSG alleles/haplotypes and fetuin-A plasma levels.

Ascertaining of Incident MI and Stroke

Potential cases of incident MI or stroke were identified by self-report in 1 of the 4 follow-up questionnaires, or by death certificate. To increase sensitivity, the questionnaire included additional questions about typical stroke symptoms. All self-reports for CVD incidences were verified by contacting the patients’ attending physicians or by review of death certificates according to World Health Organization Monitoring Trends Determinants in Cardiovascular Disease criteria. According to ICD-10, cases were classified as incident MI (ICD-10 I21), IS (ICD-10 I63.0-I63.9), intracerebral hemorrhage (ICD-10 I60.0-I60.9), or undetermined stroke (ICD-10 I64.0-I64.9) by 2 physicians in the study center.

Biochemical and Genetic Analyses

Plasma levels of fetuin-A, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, high-sensitivity C-reactive protein, creatinine, and γ-glutamyltransferase were measured with the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany). Glomerular filtration rate was estimated from plasma creatinine levels by using the prediction equation described by Levey et al. For determination of fetuin-A, an immunoturbidimetric method was used with specific polyclonal goat anti-human fetuin-A antibodies to human fetuin-A (BioVendor Laboratory Medicine, Modreci, Czech Republic). This method was evaluated in a side-by-side comparison with an ELISA (intra-assay CV 3.5% and interassay CV 5.4%, BioVendor) showing a correlation of r=0.93.

Five tagSNPs (rs2248690, rs2070633, rs2070635, rs4917, and rs4831) were identified in the HapMap 22phaseI CEPH (Utah residents with ancestry from northern and western Europe) population data using stringent criteria (minor allele frequency >0.05 and pairwise r²≥0.8) according to Tagger software implemented in Haploview program. The tagged gene region comprised 10.6 kb of chromosome 3, position 187811624 to 187822224, including 2000 bp 5'-flanking and 345 bp 3'-flanking sequences of the AHSG gene. The positions of the selected tagSNPs are illustrated in Figure 1A. SNP rs4831 has been reported previously to have failed TaqMan analysis. Therefore, the 3'-flanking SNP rs6787344 was chosen as a surrogate for rs4831 because both SNPs showed strong linkage disequilibrium (r²=0.84). Genotyping of whole-genome-amplified DNA samples was performed with the TaqMan System (Applied Biosystems, Foster City, Calif) using 5 ng of DNA. The average genotyping success rate in the 5 SNPs was >98%.

Statistical Analysis

Linkage disequilibrium between SNPs was calculated by determination of the squared correlation coefficient r² and Lewontin’s D². Haplotype frequencies were estimated by the expectation-maximization algorithm. Based on haplotype trend regression analysis described by Zaykin et al, individual haplotypes were estimated, including all AHSG haplotypes with frequencies >2%. Analysis of covariance considering an additive genetic model was used to assess the association between genotypes or haplotypes and fetuin-A levels. SNPs were coded according to the direction of association with fetuin-A: plasma level: rs2248690 (TT=0, AT=1, and AA=2), rs2070633 (TT=0, CT=1, and CC=2), rs2070635 (AA=0, AG=1, and GG=2), rs4917 (TT=0, CT=1, and CC=2). Haplotype analysis was restricted to individuals with a probability of 100% for either no copy or 2 copies present or a probability of 49% to 50% for the presence of 1 copy of the respected haplotype. The haplotype-specific effect was estimated as the difference of the means in fetuin-A between carriers of 1 or 2 copies of a haplotype compared with those without the haplotype. Candidate covariates
were selected by stepwise logistic regression with age and sex forced into the multivariable model.

Association of single AHSG SNPs with risk of MI and IS and combined CVD endpoints were calculated as hazard rate ratios (RR) with 95% CI using Cox proportional-hazard regression, modified according to the Prentice method to account for the case-cohort design and using a robust estimator for CI. Age was the underlying time variable in the counting processes, with entry defined as the subjects’ age at the time of recruitment and exit defined as age at the diagnosis of MI and IS, respectively, or censoring. The statistical models were adjusted for sex and potential confounding and effect modifying factors according to previous analyses and candidate covariate selection (cigarette smoking, history of diabetes, history of hypertension, total cholesterol, high-density lipoprotein-cholesterol, C-reactive protein level, physical activity, body mass index, waist circumference, alcohol intake, and educational attainment). Smoking status was defined as “never smoker,” “former smoker,” “<20 cigarettes per day,” or “≥20 cigarettes per day.” Physical activity was calculated from mean time spent on sporting activities during summer and winter seasons (h/wk) and categorized as “<2 h/wk,” and “≥2 h/wk.” Alcohol intake comprised 3 categories specified for each gender (men: “<2 g/d,” “2 to 15 g/d,” “>15 g/d”; women: “<1 g/d,” “1 to 7.5 g/d,” “>7.5 g/d”). Educational attainment was classified as “vocational school or less,” “technical school,” and “university degree.” Prevalent hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or self-reporting of a diagnosis or of use of antihypertensive medication. The prevalence of diabetes at baseline was evaluated by a physician using information on self-reported medical indications (order of SNPs: rs2248690 A>C, rs4917 C>T, rs2070633 C>T, rs2070635 A>G, and rs4917 C>T). The most prevalent haplotype A-C-G-C (frequency, 44%), harboring the major alleles of SNPs rs2248690, rs2070633, and rs4917, was associated with increased fetuin-A levels (+27.5 μg/mL per copy, P<0.0001). Haplotype T-T-A-T (frequency, 24%) characterized by 3 minor SNP alleles showed an inverse association with fetuin-A levels (−35.2 μg/mL per C-allele, P=2×10−12). The polymorphism explained as much as 21.2% of total plasma fetuin-A variation (Table 2).

Mean plasma fetuin-A levels differed also across 5 common haplotypes of AHSG (order of SNPs: rs2248690 A>T, rs2070633 C>T, rs2070635 A>G, rs4917 C>T). The most prevalent haplotype A-C-G-C (frequency, 44%), harboring the major alleles of SNPs rs2248690, rs2070633, and rs4917, was associated with increased fetuin-A levels (+27.5 μg/mL per copy, P<0.0001). Haplotype T-T-A-T (frequency, 24%) characterized by 3 minor SNP alleles showed an inverse association with fetuin-A levels (−35.2 μg/mL per copy, P=6.7×10−98). Three other, low-frequency (8%, 9%, and 13%) haplotypes (A-C-A-C, A-T-A-T, and A-T-A-C) were also associated with fetuin-A (P=0.02 to P<0.0001 per copy). All analyses were multivariable adjusted according to the single SNP analyses.

We then determined the association of AHSG tagSNPs with risk of CVD endpoints (MI and IS). The characteristics of the case-cohort study subjects are described in Table 1. Disease risks were evaluated in 214 incident MI and 154 incident IS cases and 2152 persons who have remained free of
Table 1. Baseline Characteristics in EPIC-Potsdam Subcohort Subjects, Incident MI, and IS Cases

<table>
<thead>
<tr>
<th></th>
<th>Subcohort, N=2197</th>
<th>MI Cases, N=214</th>
<th>IS Cases, N=154</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>50.0±8.9</td>
<td>55.8±7.0</td>
<td>57.0±7.5</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current, &gt;20 cigarettes/d</td>
<td>6</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>Current, &lt;20 cigarettes/d</td>
<td>15</td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td>Former</td>
<td>32</td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td>Never</td>
<td>47</td>
<td>27</td>
<td>42</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Hypertension</td>
<td>47</td>
<td>70</td>
<td>74</td>
</tr>
</tbody>
</table>

**Educational attainment**

Vocational school or less: 37 39 44
Technical school: 25 22 30
University degree: 38 39 26

**Physical activity**

<2 h/wk: 76 82 84
≥2 h/wk: 24 18 16

**Alcohol intake**

Men, <2 g/d: 7 6 5
Men, 2–15 g/d: 46 56 48
Men, >17.5 g/d: 47 38 47

**BMI, kg/m²**

26.0±4.2 27.7±4.1 27.6±4.2

**Waist circumference, cm**

85.5±12.7 93.9±11.9 92.1±12.9

**Total cholesterol, mg/dL**

173.6±37.6 188.9±38.2 177.5±41.6

**Triglycerides, mg/dL**

113.1±80.2 158.3±122.6 144.5±148.0

**C-reactive protein, mg/L**

1.7±2.2 2.2±2.8 3.3±9.9

**Creatinine, mg/dL**

0.72±0.15 0.80±0.16 0.75±0.17

**Fetuin-A, μg/mL**

228.0±52.1 247.0±47.1 242.7±47.3

Mean±SD or %.

MI indicates myocardial infarction; IS, ischemic stroke; GFR, estimated glomerular filtration rate.

*Because of missing data creatinine values and GFR estimates are based on additional adjustment for creatinine.

CVD during follow-up. In multivariable adjusted models, similar hazard rate ratios were found for all AHSG tagSNPs (Table 3). The AHSG SNP rs4917 C-allele was associated with a 1.34-fold increase in future MI risk (95% CI 1.05 to 1.70, P=0.02) and a 1.07-fold increase in future IS risk (95% CI 1.03 to 1.10, P=0.6). Exclusion of those participants displaying <60 mL/min/1.73 m² glomerular filtration rate (n=22) or additional adjustment for creatinine did not influence the association between AHSG SNP rs4917 and MI (RRmodel 1 [excluding subjects with glomerular filtration rate <60 mL/min/1.73 m²] =1.37 [95% CI 1.06 to 1.75, P=0.015]; RRmodel 1 [with additional adjustment for creatinine] =1.36 [95% CI 1.06 to 1.74, P=0.02]). In combined MI and IS cases, the risk estimates were RR=1.20 (95% CI 0.99 to 1.44, P=0.06). After further adjustment for fetuin-A, all associations were completely abolished and comparison between the respective risk estimates of model 1 (without adjustment for fetuin-A) and model 2 (with adjustment for fetuin-A) clearly showed significant inequality (all P<0.0001).

On the basis of the assumption underlying Mendelian randomization, we calculated the size of the effect of 1 SD (52.1 μg/mL) change in fetuin-A levels on MI by combining the estimates obtained from the association of the AHSG SNP rs4917 C-allele with risk of MI (RR, 1.34 [95% CI 1.05 to 1.70]) and the association of the C-allele with fetuin-A levels (35.5 μg/mL difference per allele), that resulted in a theoretically predicted MI risk of 1.54-fold for 1 SD change in fetuin-A (Figure 2).

**Discussion**

This report is the first to provide evidence for a putative causal nature of the association between elevated fetuin-A blood levels and MI. We used an instrumental variable method to evaluate the potential causal relationship between elevated circulating fetuin-A and future risk of MI or IS implied from Mendelian randomization.7 Mendelian randomization involves comparison of phenotype and genotype effects in observational studies. If the association between a modifiable risk factor and disease is causal, the genetic variant associated with this risk factor should be related to the disease outcome to the extent predicted by the magnitude of its association with the risk factor. The imputed estimate is likely to be equivalent with the direct estimate from an observational study provided that a very high degree of precision can be assumed for all 3 estimates.35 Our imputed estimate suggested that the effect of 1 SD change in circulating fetuin-A (fetuin-A levels in healthy individuals averaged at about 228 μg/mL with 1 SD=52 μg/mL) on disease risk leads to an increase of 54% in the risk of MI, which is comparable with the observed association between circulating fetuin-A and MI in our previous study (risk increase 59%).14 This study provided a high accuracy of the association between genotype (AHSG rs4917 C-allele) and phenotype (fetuin-A levels), because the association was established in a large population-based, ethnically homogenous sample and was controlled for other confounding factors.

Compared with other studies on genotype-phenotype relationships, eg, for blood proteins including plasminogen activator inhibitor 1,37 monocyte chemoattractant protein 1,38 and C-reactive protein,39 in which SNPs in the gene encoding the respective blood protein explained, on average, 1% to 2.5% of the phenotypic variation, the contribution of AHSG SNPs on circulating fetuin-A found in our study (≈21%) indicates a very strong relationship.

Our findings confirm previous reports that the common alleles of rs4917 and rs2248690 are associated with increased plasma fetuin-A levels.40–43 The AHSG rs2248690 (g.-799 A/T) polymorphism has been shown to change a transcription factor binding site in the 5′-flanking gene region and to alter fetuin-A expression levels in HepG2 cells.42 Transcription factor AP-1, a corepressor, showed higher affinity to the
rs2248690 T-coding sequence. Thus, a greater transcriptional repression of the gene might occur in hepatocytes of minor T-allele carriers, which would lead to lower circulating fetuin-A in T-allele carriers compared with A-allele carriers. The minor T-allele was associated with significantly lower fetuin-A in T-allele carriers compared with A-allele carriers. T-allele carriers, which would show similar linkage with all tagSNPs. As a matter of fact, this variant would exert even a higher impact on fetuin-A plasma levels than those 4 tagSNPs investigated.

It is important to consider the potential limitations of our study. Our case-cohort study sample consisted of a modest number of incident CVD patients, and thus, the lack of association between others, with the exception of the AHSG rs4917 polymorphism and MI or IS or both CVD events, may be due to insufficient statistical power. Our estimates suggested that we had >80% power to detect an odds ratio of ≥1.25 per risk allele if we assumed an allele frequency of 45% or more. Studies with larger numbers of affected individuals would be more suitable to precisely assess the

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**Table 2. Association Between AHSG Polymorphisms and Fetuin-A Concentration in the Subcohort**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>MAF</th>
<th>rHWE</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>P0.05</th>
<th>P0.01</th>
<th>Adj R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2248690</td>
<td>T</td>
<td>A</td>
<td>0.257</td>
<td>0.20</td>
<td>178.9 (171.6, 186.1)</td>
<td>211.5 (208.3, 214.7)</td>
<td>245.3 (242.7, 247.9)</td>
<td>6.5×10⁻²²</td>
<td>33.5 (1.6)</td>
<td>33.4 (1.6)</td>
</tr>
<tr>
<td>rs2070633</td>
<td>T</td>
<td>C</td>
<td>0.451</td>
<td>0.09</td>
<td>192.6 (188.5, 196.7)</td>
<td>225.3 (222.6, 228.1)</td>
<td>256.5 (253.1, 259.9)</td>
<td>6.2×10⁻¹⁵</td>
<td>31.9 (1.3)</td>
<td>32.0 (1.4)</td>
</tr>
<tr>
<td>rs2070635</td>
<td>A</td>
<td>G</td>
<td>0.463</td>
<td>0.21</td>
<td>202.5 (198.9, 206.1)</td>
<td>236.8 (227.9, 233.6)</td>
<td>256.3 (252.1, 260.5)</td>
<td>4.7×10⁻⁷⁷</td>
<td>27.0 (1.4)</td>
<td>27.0 (1.4)</td>
</tr>
<tr>
<td>rs4917</td>
<td>T</td>
<td>C</td>
<td>0.336</td>
<td>0.79</td>
<td>250 (249.0, 251.2)</td>
<td>256 (254.6)</td>
<td>256 (254.6)</td>
<td>2.0×10⁻¹²</td>
<td>35.5 (1.4)</td>
<td>35.6 (1.5)</td>
</tr>
</tbody>
</table>

Means (95% CI) of fetuin-A (g/mL) adjusted for age, sex, C-reactive protein, triglycerides, total cholesterol, γ-glutamyltransferase, high alcohol intake, and waist circumference; SNPs were coded according to the direction of their association with fetuin-A (minor alleles are in bold letters).

**Table 3. Association Between AHSG tagSNPs and CVD End Points**

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Hazard Ratio (95% CI)</th>
<th>P</th>
<th>Model 1</th>
<th>Hazard Ratio (95% CI)</th>
<th>P</th>
<th>Model 2</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2248690</td>
<td>A/T</td>
<td>1.22 (0.95 to 1.57)</td>
<td>0.12</td>
<td>0.78 (0.59 to 1.04)</td>
<td>0.09</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>rs2070633</td>
<td>C/T</td>
<td>1.23 (0.98 to 1.53)</td>
<td>0.07</td>
<td>0.79 (0.61 to 1.04)</td>
<td>0.09</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>rs2070635</td>
<td>A/G</td>
<td>1.22 (0.98 to 1.52)</td>
<td>0.08</td>
<td>0.87 (0.68 to 1.10)</td>
<td>0.25</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>rs4917</td>
<td>C/T</td>
<td>1.34 (1.05 to 1.70)</td>
<td>0.02</td>
<td>0.84 (0.64 to 1.12)</td>
<td>0.23</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2248690</td>
<td>A/T</td>
<td>1.21 (0.91 to 1.60)</td>
<td>0.19</td>
<td>0.88 (0.66 to 1.19)</td>
<td>0.41</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>rs2070633</td>
<td>C/T</td>
<td>1.04 (0.83 to 1.31)</td>
<td>0.73</td>
<td>0.72 (0.54 to 0.95)</td>
<td>0.02</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>rs2070635</td>
<td>A/G</td>
<td>1.00 (0.77 to 1.29)</td>
<td>0.99</td>
<td>0.74 (0.55 to 0.99)</td>
<td>0.04</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>rs4917</td>
<td>C/T</td>
<td>1.07 (0.83 to 1.34)</td>
<td>0.59</td>
<td>0.72 (0.52 to 0.98)</td>
<td>0.04</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>MI + IS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2248690</td>
<td>A/T</td>
<td>1.18 (0.97 to 1.43)</td>
<td>0.09</td>
<td>0.81 (0.65 to 1.01)</td>
<td>0.07</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>rs2070633</td>
<td>C/T</td>
<td>1.14 (0.96 to 1.35)</td>
<td>0.14</td>
<td>0.77 (0.62 to 0.94)</td>
<td>0.01</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>rs2070635</td>
<td>A/G</td>
<td>1.12 (0.94 to 1.33)</td>
<td>0.20</td>
<td>0.83 (0.68 to 1.01)</td>
<td>0.06</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>rs4917</td>
<td>C/T</td>
<td>1.20 (0.99 to 1.44)</td>
<td>0.06</td>
<td>0.79 (0.63 to 0.99)</td>
<td>0.0385</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Hazard rate ratios are given for the allele associated with a higher fetuin-A level → bold letters.

Model 1, adjusted for age, sex, BMI, waist circumference, history of diabetes, history of hypertension, cigarette smoking, educational attainment, physical activity, total cholesterol (mg/dL), HDL-cholesterol (mg/dL), and C-reactive protein (mg/L); Model 2, adjusted as model 1 plus additional adjustment for fetuin-A (μg/mL).

*Testing the hypothesis that hazard rate ratios in models 1 and 2 are equal.
modest effect sizes of fetuin-A genetic variation on CVD endpoints. We have not accounted for multiple testing in this analysis. The Bonferroni correction would probably have been too conservative owing to the high degree of correlation among the tests performed. However, the associations of AHSG tagSNPs and haplotypes with fetuin-A levels would have survived even a Bonferroni correction.

In summary, our application of Mendelian randomization plausibly suggested a functional link between elevated fetuin-A plasma levels and MI risk. Thus, interventions to modify fetuin-A levels might be expected to influence disease risk.

Acknowledgments

We thank Bianca Krüger-Carstensen, Anna Bury, Sabina Herbert, and Albrecht Pfa¨fflin who were involved in the biochemical and genetic analyses. We are indebted to Manuela Bergmann and Wolfgang Fleischhauer for case ascertainment and to Ellen Kohlsdorf for data management.

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Disclosures

None.

References


2. Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Krober SM, Machicao F, Fritsche A, Haring HU. Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation among the tests performed. However, the associations of AHSG tagSNPs and haplotypes with fetuin-A levels would have survived even a Bonferroni correction.


Findings from animal in-vivo and human adipocyte in-vitro studies suggest an effect of fetuin-A on the induction of subclinical inflammation and cytokine expression, thereby supporting the assumption that fetuin-A is directly involved in the pathogenesis of cardiovascular disease (CVD). In humans, elevated circulating levels of fetuin-A in blood are associated with incident myocardial infarction (MI) and ischemic stroke, independently of established risk factors for CVD. By using a Mendelian randomization approach, we investigated whether the observed association reflects a causal relationship or whether elevated circulating fetuin-A is merely a marker of CVD. We observed that the expected risk for MI associated with 1 standard deviation increase in circulating fetuin-A due to genetic variants in the fetuin-A gene (AHSG) was very similar to the observed association between fetuin-A plasma levels and MI risk. These data provide strong support for a functional link between elevated circulating fetuin-A and MI risk.
Association of AHSG Gene Polymorphisms With Fetuin-A Plasma Levels and Cardiovascular Diseases in the EPIC-Potsdam Study

Eva Fisher, Norbert Stefan, Kathrin Saar, Dagmar Drogan, Matthias B. Schulze, Andreas Fritsche, Hans-Georg Joost, Hans-Ulrich Häring, Norbert Hubner, Heiner Boeing and Cornelia Weikert

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**SUPPLEMENTAL MATERIAL**

**Supplement Table 1**  
Statistical power of the MI and IS case-cohort studies

<table>
<thead>
<tr>
<th>allele frequency</th>
<th>MI</th>
<th>IS</th>
<th>MI+IS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>214 cases / 2,152 non-cases</td>
<td>154 cases / 2,152 non-cases</td>
<td>368 cases / 2,152 non-cases</td>
</tr>
<tr>
<td>0.45</td>
<td>1.35</td>
<td>1.40</td>
<td>1.25</td>
</tr>
<tr>
<td>0.50</td>
<td>1.35</td>
<td>1.40</td>
<td>1.25</td>
</tr>
<tr>
<td>0.55</td>
<td>1.35</td>
<td>1.40</td>
<td>1.25</td>
</tr>
<tr>
<td>0.60</td>
<td>1.35</td>
<td>1.45</td>
<td>1.30</td>
</tr>
<tr>
<td>0.65</td>
<td>1.40</td>
<td>1.45</td>
<td>1.30</td>
</tr>
<tr>
<td>0.70</td>
<td>1.40</td>
<td>1.50</td>
<td>1.30</td>
</tr>
<tr>
<td>0.75</td>
<td>1.45</td>
<td>1.55</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Values of the with >80% power detectable odds ratios for risk alleles (high fetuin-A level associated alleles) of varying frequencies calculated with Quanto (http://hydra.usc.edu/GxE/) assuming a disease frequency = 2%, a two-sided type 1 error rate of 5%.
### Supplement Table 2  Correlates of fetuin-A level: Stepwise Linear Regression

Model $R^2=0.0632$ in the subcohort

<table>
<thead>
<tr>
<th></th>
<th>β-estimate (SE)</th>
<th>partial $R^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>6.88 (2.70)</td>
<td>0.0234</td>
<td>0.0109</td>
</tr>
<tr>
<td>age (per Y)</td>
<td>-1.17 (0.13)</td>
<td>0.0234</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>total cholesterol (per 1 mg/dL)</td>
<td>0.22 (0.03)</td>
<td>0.0199</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>high alcohol intake</td>
<td>-8.62 (2.23)</td>
<td>0.0064</td>
<td>0.0001</td>
</tr>
<tr>
<td>hs-CRP (per 1 mg/L)</td>
<td>0.92 (0.35)</td>
<td>0.0050</td>
<td>0.0098</td>
</tr>
<tr>
<td>triglycerides (per 1 mg/dL)</td>
<td>-0.05 (0.02)</td>
<td>0.0022</td>
<td>0.0014</td>
</tr>
<tr>
<td>waist circumference</td>
<td>0.32 (0.11)</td>
<td>0.0036</td>
<td>0.0046</td>
</tr>
<tr>
<td>GGT (per 1 U/L)</td>
<td>0.04 (0.02)</td>
<td>0.0018</td>
<td>0.0389</td>
</tr>
</tbody>
</table>

Age and sex were forced into the multivariable model; significance level for other variables entering the model was set at 0.05; Partial $R^2$ indicates the increment to $R^2$ as each variable was added in the order listed.

Candidate covariates in stepwise selection included body mass index, waist circumference, current cigarette smoking, former cigarette smoking, never cigarette smoking, total cholesterol, HDL-cholesterol, triglycerides, γ-glutamyltransferase (GGT), high-sensitivity C-reactive protein (hs-CRP), lipid-lowering therapy, diabetes, hypertension, no sports activity, <2h/week sports activity, >2h/week sports activity, no or low alcohol intake (<2 g/d for men, <1 g/d for women), medium alcohol intake (2-15 g/d for men, 1-7.5 g/d for women), high alcohol intake (>15 g/d for men, >7.5 g/d for women).