Genetic variation in the arachidonate 5-lipoxygenase-activating protein (ALOX5AP) is associated with myocardial infarction in the German population

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ABSTRACT

Genetic variation in the genes ALOX5AP (arachidonate 5-lipoxygenase-activating protein) and LTA4H (leukotriene A4 hydrolase) has previously been shown to contribute to the risk of MI (myocardial infarction) and stroke in Icelandic and Scottish populations. Both genes encode proteins playing a role in the synthesis of the pro-inflammatory leukotriene B mediators, possibly providing a link between MI and inflammation. The aim of the present study was to investigate whether these associations could be confirmed in a large study of German MI patients. Two previously described four SNP (single nucleotide polymorphism) haplotypes of the ALOX5AP gene (termed haplotype A and B) and one SNP (rs2660899) of the LTA4H gene conferring the greatest risk of MI in previous studies were genotyped in 1211 unrelated MI cases from the German MI Family Study and in 1015 healthy married-in spouses serving as controls. Haplotype B of the ALOX5AP gene was associated with an increased risk of MI in the German population, confirming previously reported associations of this haplotype with CAD (coronary artery disease) in populations from Scotland and Italy. No association with the risk of MI was detected for haplotype A of the ALOX5AP gene or for SNP rs2660899 representing the LTA4H gene. In conclusion, haplotype B of the ALOX5AP gene is associated with an increased risk of MI in a large German study. The present study is the third independent report from a European population describing an increased risk of CAD for carriers of haplotype B of the ALOX5AP gene, which substantiates further a role of this gene in the pathogenesis of CAD in Europeans.

Key words: arachidonate 5-lipoxygenase-activating protein (ALOX5AP), coronary artery disease (CAD), leukotriene A4 hydrolase (LTA4H), MAF, minor allele frequency; myocardial infarction, single nucleotide polymorphism (SNP).

Abbreviations: ALOX5AP, arachidonate 5-lipoxygenase-activating protein; CAD, coronary artery disease; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; LTA4H, leukotriene A4 hydrolase; MAF, minor allele frequency; MI, myocardial infarction; OR, odds ratio; SNP, single nucleotide polymorphism.

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INTRODUCTION

Despite numerous advances in treatment, CAD (coronary artery disease) and its major complication MI (myocardial infarction) still represent the leading causes of mortality in the Western world [1]. Both environmental and genetic factors contribute to the pathogenesis of CAD and MI. Genetic epidemiological approaches, including genome-wide linkage and association studies, have identified several chromosomal regions and polymorphisms related to MI and/or CAD [2–4]. However, independent validations as well as mechanistic explanations of the underlying functional basis for many of these findings are still under investigation.

In two studies, Helgadottir and co-workers reported a strong genetic association between the ALOX5AP gene and the risk of MI and stroke in an Icelandic population [5,6]. ALOX5AP encodes arachidonate 5-lipoxygenase-activating protein, which plays a key role in the biosynthesis of pro-inflammatory leukotriene B mediators, providing a potential link between inflammation and cardiovascular disease. In a genome-wide scan of 296 multiplex Icelandic families including 713 individuals with MI, suggestive linkage led to the identification of the ALOX5AP gene as a strong candidate for MI. Within the ALOX5AP gene the authors identified a four SNP (single nucleotide polymorphism) haplotype (haplotype A) conferring a 2-fold risk for MI [5]. In functional studies using isolated neutrophils from carriers of haplotype A, an increased production of the pro-inflammatory molecule leukotriene B was demonstrated [5]. However, the association of haplotypes A and B of the ALOX5AP gene with leukotriene B production was not confirmed in a recent study [7].

In a subsequent study, the same authors who first reported the association of haplotype A with MI in an Icelandic population were unable to confirm this association in a cohort of British patients [8]. However, the authors identified another four SNP haplotypes of the ALOX5AP gene, termed haplotype B, which conferred a significant risk of both MI and stroke [8]. Similarly, a recent angiography-based study from Italy has detected an increased risk of angiographically proven CAD for carriers of haplotype B but not for carriers of haplotype A [9]. In contrast with the aforementioned European studies, no association of either haplotype A or B of the ALOX5AP gene with MI was detected in further studies from the US [10] and Japan [11].

The LTA4H gene encodes leukotriene A4 hydrolase, a protein in the same biochemical pathway as 5-lipoxygenase-activating protein, the gene product of ALOX5AP. A subsequent study reported a moderately increased risk of MI for carriers of a haplotype in the LTA4H gene (haplotype K) [6] in an Icelandic population. In a similar fashion as previously demonstrated for ALOX5AP, the authors [6] were again able to show an increased production of pro-inflammatory leukotriene B associated with haplotype K of the LTA4H gene. In the same study, the authors replicated the association of haplotype K with MI and stroke in pooled populations from three North American cities comprising 1591 MI cases with European ancestry and 197 MI cases with African-American ancestry. The increased chance conferred by haplotype K was markedly higher in African-Americans and for subjects with MI and concomitant cerebrovascular disease [6].

Since the published data on the association of ALOX5AP with CAD is conflicting and no further studies on the association of genetic variation in LTA4H with MI have been reported so far, in the present study we investigated the association of genetic variation in both genes with MI in the German MI Family Study.

MATERIALS AND METHODS

Study population

MI families were identified through patients at 17 cardiac rehabilitation centres distributed throughout Germany. All patients had suffered from an MI by 60 years of age [12]. If at least one sibling presented with MI or severe CAD (defined as percutaneous coronary intervention or coronary artery bypass grafting) by 70 years of age, the nuclear family (patient, available parents, and all affected and unaffected siblings) was contacted and invited to participate in the study. All study participants answered a standardized questionnaire about medical history, presence of coronary risk factors, clinical events, medication, anthropometric data and socioeconomic background. This information was validated by retrospective analyses of medical records. Additionally, all patients underwent a medical examination during a visit scheduled at their primary care physician’s office. For the present study, we examined 1211 unrelated MI patients. Healthy married-in spouses (1015 individuals) served as a control group.

The study protocol was approved by the local institutional review committee and all subjects gave written informed consent.

Genotyping

SNPs comprising haplotype A (consisting of the common allele of rs17222814 (G), the common allele of rs10507391 (T), the common allele of rs4769874 (G) and the rare allele of rs9551963 (A)) and haplotype B (rs17216473, rs10507391, rs9315050 and rs17222842) in the ALOX5AP gene and one SNP in the LTA4H gene (rs2660899) were genotyped using 5′-exonuclease activity (TaqMan) on a ABI Prism 7900HT (Applied Biosystems). TaqMan genotyping assays with probes labelled with the fluorophores FAM (6-carboxyfluorescein) and VIC respectively, were purchased from Applied Biosystems. Genotyping was performed on 384-well plates prepared...
with the GENESIS Freedom pipetting robot from TECAN. The universal PCR master mix from Applied Biosystems was used in a 5 μl total reaction volume with 10 ng of DNA per reaction. Allelic discrimination was measured automatically on the ABI Prism 7900HT (Applied Biosystems) using the Sequence Detection Systems 2.1 software (autocaller confidence level 95 %).

In order to check for consistency and to ensure intra- and inter-plate genotype quality control 10 % of all genotypes were repeated in independent PCR reactions. No genotyping discrepancies were detected between the repeated samples.

**Statistical analysis**

We performed power analyses based on the OR (odds ratio) provided from the literature for haplotypes A and B [5]. For this, we applied a two-sided Fisher’s exact test at a global test level of 5 %.

Deviation of genotype distribution from and compatibility with HWE (Hardy–Weinberg equilibrium) were analysed using a Monte Carlo χ² goodness-of-fit test (threshold of significance every P > 0.05, HWEχ²) and the exact uniformly most powerful equivalence test with δi = 2 and δi = 2/3 respectively, for all patient groups and all control groups for all markers separately [13].

The primary aim of the present study was to show the association between the ALOX5AP and LTA4H genes and MI. Therefore for the ALOX5AP gene we examined the two haplotypes, A and B, that are sufficient to cover genetic variation in this gene as described by Helgadottir et al. [5]. Given that a previous study has demonstrated that SNP rs2660899 in the LTA4H gene provides the best single surrogate marker of haplotype K, which is associated with the greatest risk of MI [6], we have only genotyped rs2660899 for the LTA4H gene. P values of the haplotype analyses were adjusted for multiple testing according to Holm [14] and were considered as significant if they were less than 5 %.

Analyses of haplotypes A and B were performed using the EM-algorithm. Here, score statistics based on a χ² distribution with one degree of freedom and corresponding P values were calculated as proposed by Schaid et al. [15]. We determined Monte Carlo P values from 1000000 replications. Score statistics were re-calculated from a permuted re-ordering of the affection status and the original ordering of the genotype matrix. The simulated P value is the number of times the simulated score statistic exceeds the observed score statistic, divided by the total number of simulations. For the simulated P values presented in Tables 3(a) and (b), 1000000 replications were performed. Interestingly, even with such a high number of permutations nominal and simulated P values are approximately the same.

In addition, for every SNP of haplotype A and B separately, as well as for rs2660899, differences in genotype frequencies between MI cases and controls were tested using the two-sided Cochran–Armitage trend test; ORs and corresponding 95 % CIs (confidence intervals) were calculated.

For haplotypes A and B and rs2660899, we furthermore performed additional subgroup analyses investigating patients who were either suffering from MI by 45 or 50 years of age, or those patients with MI who had a positive history of stroke or other cerebrovascular disease (history of transient ischaemic attack or known supra-aortic stenosis), because previous studies [5,8,9] have indicated that the association of polymorphisms in both genes with the risk of cardiovascular disease is markedly increased in individuals with early-onset MI or in individuals affected by both MI and concomitant cerebrovascular disease.

### RESULTS

Baseline characteristics of the study population are shown in Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MI cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1211</td>
<td>1015</td>
</tr>
<tr>
<td>Men (n)</td>
<td>782 (64.6)</td>
<td>377 (37.1)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.2 ± 8.6</td>
<td>56.8 ± 9.9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.5 ± 3.7</td>
<td>26.7 ± 4.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>138.0 ± 19.7</td>
<td>134.0 ± 17.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82.4 ± 10.3</td>
<td>82.1 ± 9.8</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>227.7 ± 46.0</td>
<td>238.3 ± 42.3</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>50.6 ± 13.3</td>
<td>60.5 ± 15.3</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>151.1 ± 42.5</td>
<td>147.5 ± 34.5</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>822 (67.9)</td>
<td>509 (50.1)</td>
</tr>
<tr>
<td>Diabetics (n)</td>
<td>197 (16.3)</td>
<td>56 (5.5)</td>
</tr>
<tr>
<td>Taking antihypertensive medication (n)</td>
<td>1066 (88.0)</td>
<td>382 (7.6)</td>
</tr>
<tr>
<td>Taking lipid-lowering medication (n)</td>
<td>801 (66.1)</td>
<td>94 (9.3)</td>
</tr>
</tbody>
</table>

For the power analysis, we assumed a haplotype frequency of 15.8 % for haplotype A and an OR of 1.8, as reported in the initial study [5]. This resulted in sufficient power to detect differences (99.9 %), even a more moderate effect of 1.4 is detectable with the present study (power=85.6 %). The power to detect an OR of 1.95, as reported in the initial study [5] for haplotype B with a frequency of 7.5 %, was 99.7 %.

To evaluate genotyped SNPs for HWE, we checked the patient and control groups for all markers separately to determine whether there were deviations in genotype distribution with a Monte Carlo χ² goodness-of-fit test (Table 2). No deviation from HWE was detected this way (every P > 0.05, HWEχ²). Secondly, we analysed...
### Table 2  Overview of single SNP analyses in the ALOX5AP and LTA4H genes

<table>
<thead>
<tr>
<th>Allele</th>
<th>rs17222814</th>
<th>rs10507391</th>
<th>rs4769874</th>
<th>rs9551963</th>
<th>rs17216473</th>
<th>rs9315050</th>
<th>rs17222842</th>
<th>rs2660899</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
</tr>
<tr>
<td>1</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
</tr>
<tr>
<td>2</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
<td>MI</td>
<td>Cont.</td>
</tr>
<tr>
<td>Frequency of rare allele</td>
<td>10.5</td>
<td>8.7</td>
<td>33.0</td>
<td>30.9</td>
<td>4.8</td>
<td>4.4</td>
<td>49.5</td>
<td>46.9</td>
</tr>
<tr>
<td>HWE $\chi^2$ (196, 220)</td>
<td>0.5232</td>
<td>0.5731</td>
<td>0.7849</td>
<td>0.1741</td>
<td>0.5064</td>
<td>0.4154</td>
<td>0.0538</td>
<td>0.4164</td>
</tr>
<tr>
<td>HWE aequi (384, 562)</td>
<td>0.7849</td>
<td>0.1741</td>
<td>0.5064</td>
<td>0.4154</td>
<td>0.0538</td>
<td>0.4164</td>
<td>0.2787</td>
<td>0.2592</td>
</tr>
<tr>
<td>$P$ trend</td>
<td>0.05675</td>
<td>0.1656</td>
<td>0.5027</td>
<td>0.1118</td>
<td>0.09297</td>
<td>0.8659</td>
<td>0.0175</td>
<td>0.6534</td>
</tr>
<tr>
<td>OR (95% CI)*</td>
<td>1.22</td>
<td>(0.99, 1.51)</td>
<td>1.10</td>
<td>(0.96, 1.25)</td>
<td>1.11</td>
<td>(0.82, 1.49)</td>
<td>1.11</td>
<td>(0.98, 1.25)</td>
</tr>
<tr>
<td>OR (95% CI)†</td>
<td>1.50</td>
<td>(0.99, 2.27)</td>
<td>1.20</td>
<td>(0.93, 1.56)</td>
<td>1.23</td>
<td>(0.68, 2.22)</td>
<td>1.22</td>
<td>(0.95, 1.56)</td>
</tr>
</tbody>
</table>

*OR for one allele
†OR for two alleles

ALOX5AP haplotype A, SNP rs17222814 (position 30197553 and major/minor allele G/A); ALOX5AP haplotype A, haplotype B, SNP rs10507391 (position 30210096 and major/minor allele T/A); ALOX5AP haplotype A, SNP rs4769874 (position 30197553 and major/minor allele G/A); ALOX5AP haplotype A, SNP rs9551963 (position 30230547 and major/minor allele G/A); ALOX5AP haplotype B, SNP rs17216473 (position 30201965 and major/minor allele G/A); ALOX5AP haplotype B, SNP rs9315050 (position 30234045 and major/minor allele T/A); ALOX5AP haplotype B, SNP rs17222842 (position 30238117 and major/minor allele G/T); LTA4H, SNP rs2660899 (position 94932888 and major/minor allele G/T). Genotype abbreviations: 0 = homozygous for common allele, 1 = heterozygous and 2 = homozygous for rare allele; HWE $\chi^2 = P$ value of Monte Carlo $\chi^2$ goodness-of-fit test for deviation from HWE; HWE aequi = CI for heterozygotes for compatibility with HWE using the exact uniformly most powerful equivalence test with $\delta_2 = 2$ and $\delta_1 = 2/3$ respectively. P trend, unadjusted two-sided $P$ values of the Cochrane–Armitage trend test. Cont., controls.
the compatibility with HWE using the exact uniformly
most powerful equivalence test with \( \delta_2 = 2 \) and \( \delta_1 = 2/3 \)
respectively (HWE aequi). For SNPs rs4769874 and
rs9315050 we detected slightly more heterozygotes in
the MI cases than expected. However, such findings are
not unusual in MI cases in regions linked to a disease.

Frequencies and MAFs (minor allele frequencies) of
the single SNPs comprising haplotypes A and B of the
ALOX5AP gene and rs2660899 of the LTA4H gene
are shown in Table 2. In addition, \( P \) values from the
Cochrane–Armitage trend test and corresponding ORs
(95% CIs) are shown. No association of rs2660899 with
MI was detected.

The frequency of haplotype A in the present study
population was comparable with that reported previously
in the literature [5,8,9]. However, we detected no
association between MI and haplotype A (Monte Carlo
\( P = 0.2807 \); Table 3a) despite sufficient power. Accord-
ingly, neither the other haplotypes of the four SNPs
comprising haplotype A nor the global association score
test had a significant association with MI (each Monte
Carlo \( P = 0.0112 \)). This was in contrast with the haplotype
AAAG that was associated with an increased risk of MI (haplotype score = 5.0), the haplotype
AAAA had a protective effect (haplotype score = −2.5).

In addition, we performed subgroup analyses for
haplotypes A and B of the ALOX5AP gene and
rs2660899 of the LTA4H gene (Table 4). Although we
did not detect a significant association of either haplotype
A or rs2660899 in any subgroup, the effect of haplo-
type B was found in every subgroup investigated.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Association of haplotypes A (a) and B (b) in ALOX5AP with MI</th>
</tr>
</thead>
</table>
| Analyses were performed in 1211 MI cases and 1015 controls. (a) SNPs were: rs17222814 (G/A), rs10507391 (T/A), rs4769874 (G/A) and rs9551963 (C/A). The global scores were \( P = 0.2067 \) (nominal) and \( P = 0.2058 \) (stimulated). The specific haplotype scores are displayed. Haplotype A is shown in bold. (b) SNPs were:
| haplotype \( A \) or \( rs2660899 \) in any subgroup, the effect of haplo-
type B in our population proved to be
very similar to the previous reports from the U.K. [8] and
Italy [9], but different to a North American study [10]
in which no difference in frequency of haplotype B was
detected between MI cases and controls. The fact that the
association of haplotype B with the risk of CAD has now
been replicated three times in European populations
but proved negative in a North American study might suggest
differences in genetic structure, namely differences in
SNP linkage disequilibrium and haplotype structure,
between European and North American populations.

For the LTA4H gene the present study did not find any
association of SNP rs2660899 representing haplotype
K with the risk of MI in a German population. In the

DISCUSSION

Consistent with two previous reports from the U.K. [8]
and Italy [9], we detected an increased risk of MI for carri-
ers of haplotype B of the ALOX5AP gene in our German
study, whereas no association of haplotype A with MI risk
was observed. Haplotype frequency, OR and direction
effect for haplotype B in our population proved to be
Table 4  Subgroup analyses of haplotypes A and B in ALOX5AP and rs2660899 in LTA4H
The global and specific haplotype scores for the haplotypes are displayed. For rs2660899 MAF, two-sided P values of the Cochrane–Armitage trend test; ORs and 95 % CI are presented. Each subgroup was analysed separately compared with the 1015 controls.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>MI</th>
<th>MI plus cerebrovascular disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 45 years</td>
<td>≤ 50 years</td>
</tr>
<tr>
<td>Number of affected individuals</td>
<td>1211</td>
<td>279</td>
</tr>
<tr>
<td>Haplotype A ALOX5AP</td>
<td>304</td>
<td>509</td>
</tr>
<tr>
<td>Haplotype frequency</td>
<td>15.4</td>
<td>15.9</td>
</tr>
<tr>
<td>Haplotype score</td>
<td>−1.1</td>
<td>−0.5</td>
</tr>
<tr>
<td>Nominal P</td>
<td>0.2804</td>
<td>0.5859</td>
</tr>
<tr>
<td>Simulated P</td>
<td>0.2807</td>
<td>0.587</td>
</tr>
<tr>
<td>Haplotype B ALOX5AP</td>
<td>4.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Haplotype frequency</td>
<td>5.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Haplotype score</td>
<td>5.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Nominal P</td>
<td>&lt; 0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Simulated P</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>rs2660899</td>
<td>18.0</td>
<td>18.3</td>
</tr>
<tr>
<td>MAF</td>
<td>18.7</td>
<td>18.7</td>
</tr>
<tr>
<td>P trend</td>
<td>0.6534</td>
<td>0.8895</td>
</tr>
<tr>
<td>OR per one rare allele (95 % CI)</td>
<td>0.96 (0.83, 1.13)</td>
<td>0.98 (0.77, 1.26)</td>
</tr>
<tr>
<td>OR per two rare alleles (95 % CI)</td>
<td>0.93 (0.68, 1.27)</td>
<td>0.97 (0.59, 1.58)</td>
</tr>
</tbody>
</table>

In original study the risk associated with haplotype K was particularly pronounced in individuals suffering from MI with concomitant cerebrovascular disease and in African-American individuals [6]. However, a subgroup analysis of individuals with MI and concomitant stroke in the present German study did not yield any additional information. The lack of association between rs2660899, representing haplotype K, with MI in the German MI study consisting entirely of Caucasians might again be due to population-specific effects. Further studies in populations of different ethnic origins might be needed in order to better understand the association of genetic variation in LTA4H with MI and stroke.

In summary, the present study was able to confirm the association between a common haplotype of ALOX5AP and CAD in a European population. There is now increasing evidence from several studies for a role of ALOX5AP in the pathogenesis of CAD, particularly in subjects of European descent.

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