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Association of variants in the *BAT1-NFKBIL1-LTA* genomic region with protection against myocardial infarction in Europeans

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Single-nucleotide polymorphisms within the *BAT1-NFKBIL1-LTA* genomic region (6p21.3) and the *LGALS2* gene (22q13.1), encoding a regulator for lymphotoxin-α, the product of the *LTA* gene, have been reported to be linked with the risk of myocardial infarction in Japanese. We employed nine polymorphisms from the *BAT1-NFKBIL1-LTA* region and one polymorphism from the *LGALS2* gene, and investigated whether such associations were also present in Europeans. The study included 3657 patients with myocardial infarction and 1211 control individuals with angiographically normal coronary arteries. Minor homozygous genotypes of polymorphisms in *BAT1* (rs2239527, −23C/G), *NFKBIL1* (rs2071592, −63T/A) and *LTA* (rs1800683, −162G/A; rs909253, 252G/A; rs1041981, Thr26Asn) were associated with moderately protective effects against myocardial infarction ($P \leq 0.045$). The most abundant 9-marker haplotype of the *BAT1-NFKBIL1-LTA* region, named haplotype 1 (28% frequency in the study population), included the alleles of the five protective genotypes and was related with a significantly lower risk of myocardial infarction (OR 0.88, 95% CI 0.80–0.98; $P = 0.015$). Moreover, homozygosity for haplotype 1 was associated with an OR 0.72 (95% CI 0.57–0.90; $P = 0.0047$). Multiple logistic regression analysis revealed an independent protective effect against myocardial infarction in the homozygous carriers of haplotype 1 (adjusted OR 0.78, 95% CI 0.62–0.99; $P = 0.043$). A putative risk genotype of the polymorphism in the *LGALS2* gene (rs7291467; 3279T/C) was not associated with myocardial infarction (OR 0.98, 95% CI 0.83–1.16; $P = 0.84$). Our finding that protective effects are linked with minor homozygous genotypes and haplotype 1 of the *BAT1-NFKBIL1-LTA* region in Europeans is opposite to the observation of associated risks in Japanese.

INTRODUCTION

The pathogenesis of coronary atherosclerosis involves inherited, behavioural and environmental factors (1). Acute thrombosis at the site of a ruptured lipid-rich atherosclerotic plaque is understood as a crucial event in the transition from stable or subclinical atherosclerotic disease to myocardial infarction (MI) (2,3). Inflammatory processes are thought to promote atherogenesis from early lesion formation to unstable plaque rupture (1–3).

With the use of single-nucleotide polymorphisms (SNPs) as markers, candidate gene analyses and genome-wide approaches have revealed multiple susceptibility loci for MI, including a 50 kb portion in the class III region of the major

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histocompatibility complex located on the short arm of chromosome 6 (4–6). Risk effects were found to be linked with minor alleles and a major haplotype of this region in Japanese (4). High linkage disequilibrium across this section (4,7,8) disperses association findings, and the genetic elements functionally responsible for the modulation of MI risk have not been established definitely. The MI-related locus includes the genes *BAT1* (encoding HLA-B-associated transcript 1), *NFKBIL1* (encoding nuclear factor of κ light chain gene enhancer in B cells inhibitor-like 1) and *LTA* (encoding lymphotoxin-α) (4,9). However, other studies from Japan have not replicated the original association results (5,7).

Secretion of lymphotoxin-α is regulated by the cellular factor galectin-2, the product of *LGALS2* (gene map locus: 22q13.1), another gene that has been implicated in the susceptibility to MI (10). Lymphotoxin-α and galectin-2 are present in intimal cells of atherosclerotic plaques, mostly in smooth muscle cells or smooth muscle-cell-derived foam cells and occasionally in macrophages, but absent from quiescent or normal medial smooth muscle cells (10). Within the cells, lymphotoxin-α and galectin-2 are co-localized in the microtubule cytoskeleton network (10).

Several MI-related SNPs in the *BAT1-NFKBIL1-LTA* region (rs2239527, rs2071592 and rs909253; named here as BAT1-C SNP, NFKBIL1-A SNP and LTA-B SNP, respectively) and in *LGALS2* (rs7291467; LGALS2-A SNP, known also as the 3279T/C SNP in intron 1 of *LGALS2*) are located in regulatory elements of the particular genes (4,10–14). These SNPs were reported to affect nuclear factor binding and/or transcriptional activity and thus may influence the levels of gene expression and, as a consequence, cell physiology (4,10–14). Another MI-related SNP in *LTA* (rs1041981; LTA-C SNP), located in exon 3, affects amino acid position 26 (threonine or asparagine residue) of the mature lymphotoxin-α protein and has been found to differentially influence mRNA levels specific for the synthesis of the vascular cell adhesion molecule-1 (VCAM-1) and selectin E in cultured human coronary vascular smooth muscle cells (4). VCAM-1 and selectin E have been implicated in inflammatory processes underlying atherosclerosis (15). In addition to their relationship with MI, associations with autoimmune diseases have been reported of the NFKBIL1-A, LTA-B and LTA-C SNPs and SNP-based haplotypes of the *BAT1-NFKBIL1-LTA* region, including rheumatoid arthritis, allergen-induced asthma and type 1 diabetes (16–20).

Because associations of the *BAT1-NFKBIL1-LTA* region and *LGALS2* with MI were originally observed in case–control studies that included participants from Japan (4,10), we investigated whether such associations exist also in Europe. A group of patients with MI ($n = 3657$) and two control groups were included in the analysis. One control group ($n = 1211$) consisted of individuals with angiographically normal coronary arteries and the other control group ($n = 4115$) comprised representative subjects from a local population in southern Germany (KORA S4 sample) (21–24).

RESULTS

Main baseline characteristics of the control group with coronary angiography, the population-based control group and the group of patients with MI are shown in Table 1. The overall genotype distributions of nine SNPs located in the *BAT1-NFKBIL1-LTA* genomic region and one SNP of *LGALS2* were not significantly different between the control group with coronary angiography and the MI group (Table 2). Genotype proportions among control individuals and patients were in agreement with those expected for a sample in Hardy–Weinberg equilibrium. We observed a high degree of allelic association among the SNPs in the *BAT1-NFKBIL1-LTA* region. For example, $D' = 0.993$ between the BAT1-A and LTA-C SNPs. Allelic associations among SNPs, as inferred from the present genotype analysis, corresponded well with HapMap data.

Frequencies of the minor alleles and rare homozygous genotypes of the SNPs in the *BAT1-NFKBIL1-LTA* region and *LGALS2* are presented in Table 3. The minor allele of the LTA-B SNP and the rare homozygous genotypes of the BAT1-C, NFKBIL1-A, LTA-A, LTA-B and LTA-C SNPs were significantly more frequent in the control group with coronary angiography than in the MI group (Table 3). These MI-related SNPs were highly intercorrelated ($r^2 \geq 0.86$) (Table 3). We considered the non-synonymous LTA-C SNP, a representative of the five tightly linked SNPs, and determined the genotype distribution of this SNP in the population-based KORA S4 sample, which served as an independent control group. In the KORA S4 cohort, genotype data of the LTA-C SNP were obtained from 4030 individuals, and the CC, CA and AA genotypes were present at 47.7, 42.1 and 10.2%, respectively, a distribution not significantly different from that in the group of controls with coronary angiography ($P = 0.60$) and the MI group ($P = 0.093$), which are shown in Table 2. Importantly, like in the control group with coronary angiography (Table 3), the AA genotype of the LTA-C SNP was significantly more prevalent in the KORA S4 group than in the MI group (10.2% versus 8.8%; OR 0.85, 95% CI 0.73–0.99; $P = 0.032$).

Genotype distributions of the LGALS2-A SNP were not significantly different between the control group with coronary angiography and the MI group (Table 2) and between the KORA S4 sample (3537 genotypes; TT = 33.3%, TC = 48.3%, CC = 18.4%) and the MI group ($P = 0.77$). The minor allele or rare homozygous genotype of the LGALS2-A SNP was not significantly associated with MI in comparisons including the control group with coronary angiography (Table 3) or the KORA S4 sample ($P \geq 0.55$).

We asked whether associations existed between SNP-based haplotypes and MI. The structures and frequencies of the five most abundant 9-marker haplotypes of the *BAT1-NFKBIL1-LTA* region are shown in Table 4. Together, these five haplotypes represented 90.5% of the *BAT1-NFKBIL1-LTA* haplotypes in the study sample (Table 4). The most frequent haplotype, named haplotype 1, but none of the other four abundant haplotypes, included the alleles of the five rare homozygous genotypes that were more prevalent in the control group with coronary angiography than in the MI group (Table 4). Like each of these genotypes, haplotype 1 was significantly more frequent in the control group than in the group with MI (29.6% versus 27.1%; OR 0.88, 95% CI 0.80–0.98; $P = 0.015$) (Table 4). This result indicated that haplotype 1 was associated with protection against MI. There was no

Table 1. Baseline clinical characteristics of the control group with coronary angiography, the population-based control group (KORA S4 sample) and the MI group

	Control group with angiography ($n = 1211$)	Population-based control group ($n = 4115$)	MI group ($n = 3657$)
Age (years)	60.3 ± 11.9	49.3 ± 13.9	64.0 ± 12.0
Women	598 (49.4)	2079 (50.5)	885 (24.2)
Arterial hypertension	589 (48.6)	751 (18.3)	2246 (61.4)
Hypercholesterolaemia	602 (49.7)	1122 (27.3)	2067 (56.5)
Current cigarette smoking	184 (15.2)	1067 (25.9)	1849 (50.6)
Diabetes mellitus	65 (5.4)	165 (4.0)	754 (20.6)

Age is mean ± SD; other variables are presented as number (%) of individuals.

Table 2. Genotype distributions of SNPs in the *BAT1-NFKBIL1-LTA* region and the LGALS2-A SNP in the control group with coronary angiography and the MI group

Polymorphism	Genotype	Control group ($n = 1211$)	MI group ($n = 3657$)	*P*
BAT1-A (rs2075582)	TT	687 (56.7)	2044 (55.9)	0.25
	TC	433 (35.8)	1379 (37.7)	
	CC	91 (7.5)	234 (6.4)	
BAT1-B (rs2075580)	CC	686 (56.6)	2041 (55.8)	0.28
	CG	434 (35.8)	1380 (37.7)	
	GG	91 (7.5)	236 (6.5)	
BAT1-C (rs2239527)	CC	568 (46.9)	1787 (48.9)	0.068
	CG	514 (42.4)	1559 (42.6)	
	GG	129 (10.7)	311 (8.5)	
NFKBIL1-A (rs2071592)	TT	590 (48.7)	1850 (50.6)	0.077
	TA	501 (41.4)	1519 (41.5)	
	AA	120 (9.9)	288 (7.9)	
NFKBIL1-B (rs2239707)	TT	600 (49.5)	1772 (48.5)	0.55
	TC	485 (40.0)	1527 (41.8)	
	CC	126 (10.4)	358 (9.8)	
NFKBIL1-C (rs2516479)	CC	381 (31.5)	1169 (32.0)	0.89
	CG	607 (50.1)	1804 (49.3)	
	GG	223 (18.4)	684 (18.7)	
LTA-A (rs1800683)	GG	560 (46.2)	1757 (48.0)	0.11
	GA	521 (43.0)	1580 (43.2)	
	AA	130 (10.7)	320 (8.8)	
LTA-B (rs909253)	AA	558 (46.1)	1760 (48.1)	0.057
	AG	521 (43.0)	1580 (43.2)	
	GG	132 (10.9)	317 (8.7)	
LTA-C (rs1041981)	CC	558 (46.1)	1758 (48.1)	0.11
	CA	523 (43.2)	1577 (43.1)	
	AA	130 (10.7)	322 (8.8)	
LGALS2-A (rs7291467)	TT	418 (34.5)	1210 (33.1)	0.57
	TC	574 (47.4)	1795 (49.1)	
	CC	219 (18.1)	652 (17.8)	

Variables are presented as number (%) of control individuals and patients.

sex-specific connection between haplotype 1 and MI (women: OR 0.87, 95% CI 0.74–1.02; men: OR 0.88, 95% CI 0.77–1.01).

The proportion of homozygous carriers of haplotype 1 was higher in the control group than in the MI group (9.3% versus 6.9%; OR 0.72, 95% CI 0.57–0.90; $P = 0.0047$). After adjustments were made for potentially confounding covariates (age, gender, history of arterial hypertension, history of hypercholesterolaemia, current cigarette smoking and diabetes mellitus), multiple logistic regression analysis revealed an independent, moderately protective effect against MI in the homozygous carriers of haplotype 1 (adjusted OR 0.78, 95% CI 0.62–0.99; $P = 0.043$).

DISCUSSION

A haplotype analysis in the *BAT1-NFKBIL1-LTA* region on chromosome 6 revealed the presence of a 9-marker haplotype, named haplotype 1, with a protective effect against MI. Among the five most prevalent haplotypes in the study sample, the minor alleles of five SNPs, the BAT1-C, NFKBIL1-A, LTA-A, LTA-B and LTA-C SNPs, were unique to haplotype 1. Based on high allelic association across this genomic region, homozygosity of each of these minor alleles was associated with protection against MI. The LGALS2-A SNP in the gene for galectin-2 was not detectably associated with MI, a result different from an observation in a Japanese sample (10). Although adjustments are not necessarily required for replication studies examining an existing hypothesis, a possible influence of multiple hypothesis testing should be considered before accepting the main findings of our study.

The control group in which all subjects had some indication for coronary angiography did not represent a typical sample of healthy individuals. However, we consider this group especially suitable as a control group in the setting of this study, because the absence of MI was rigorously established on the basis of history, electrocardiography, left ventricular angiography and coronary angiography. Evidence for the adequacy of this group as a control group was provided by highly conform genotype distributions of the LTA-C and LGALS2-A SNPs in the control group with coronary angiography and the KORA S4 sample that served as an independent control group. The KORA S4 sample consists of representative subjects from a local population in southern Germany and was previously used in genetic association studies (21–23). Additional evidence for the usefulness of the group with coronary angiography as a control group is provided by the high degree of similarity of the genotype distributions of the LTA-B and LTA-C SNPs between this group and other control groups that consisted of Caucasians (25,26).

Prior case–control studies have addressed the relationships of SNPs in the *BAT1-NFKBIL1-LTA* region with MI (4,5,7,26) or the acute coronary syndrome (MI or unstable angina pectoris) (27). Table 5 shows the risks associated with homozygosity of the minor allele (A allele) of the LTA-C SNP

Table 3. Frequencies of the minor alleles and rare homozygous genotypes of SNPs in the *BAT1-NFKBIL1-LTA* region and the LGALS2-A SNP in the control group with coronary angiography and the MI group

Polymorphism	Minor allele	Control group (2422 alleles)	MI group (7314 alleles)	OR (95% CI)	*P*	Rare homozygous genotype	Control group ($n = 1211$)	MI group ($n = 3657$)	OR (95% CI)	*P*	r^2 bins[a]
BAT1-A	C	615 (25.4)	1847 (25.3)	0.99 (0.89–1.10)	0.89	CC	91 (7.5)	234 (6.4)	0.84 (0.65–1.18)	0.18	1
BAT1-B	G	616 (25.4)	1852 (25.3)	0.99 (0.89–1.10)	0.91	GG	91 (7.5)	236 (6.5)	0.85 (0.66–1.09)	0.20	1
BAT1-C	G	772 (31.9)	2181 (29.8)	0.91 (0.82–1.00)	0.057	GG	129 (10.7)	311 (8.5)	0.78 (0.63–0.97)	0.024	2
NFKBIL1-A	A	741 (30.6)	2095 (28.6)	0.91 (0.82–1.01)	0.067	AA	120 (9.9)	288 (7.9)	0.78 (0.62–0.97)	0.027	2
NFKBIL1-B	C	737 (30.4)	2243 (30.7)	1.01 (0.92–1.12)	0.83	CC	126 (10.4)	358 (9.8)	0.93 (0.75–1.16)	0.54	3
NFKBIL1-C	G	1053 (43.5)	3172 (43.4)	1.00 (0.91–1.09)	0.93	GG	223 (18.4)	684 (18.7)	1.02 (0.86–1.21)	0.82	4
LTA-A	A	781 (32.2)	2220 (30.4)	0.92 (0.83–1.01)	0.080	AA	130 (10.7)	320 (8.8)	0.80 (0.64–0.99)	0.039	2
LTA-B	G	785 (32.4)	2214 (30.3)	0.91 (0.82–1.00)	0.048	GG	132 (10.9)	317 (8.7)	0.78 (0.63–0.96)	0.020	2
LTA-C	A	783 (32.3)	2221 (30.4)	0.91 (0.83–1.01)	0.070	AA	130 (10.7)	322 (8.8)	0.80 (0.65–1.00)	0.045	2
LGALS2-A	C	1012 (41.8)	3099 (42.4)	1.02 (0.93–1.12)	0.61	CC	219 (18.1)	652 (17.8)	0.98 (0.83-1.16)	0.84	–

Variables are presented as number (%) of control individuals and patients. *P*-values <0.050 are underlined.
[a]SNPs in the *BAT1-NFKBIL1-LTA* region that are intercorrelated with r^2 values >0.8 are indicated by the same numbers.

Table 4. Haplotype frequencies in the control group with coronary angiography and the MI group

Haplotype No	Allele combination	Control group (2422 alleles)	MI group (7314 alleles)	*P*
1	TCGATCAGA	718 (29.6)	1981 (27.1)	0.015
2	TCCTTGGAC	509 (21.0)	1544 (21.1)	0.92
3	CGCTCGGAC	489 (20.2)	1437 (19.6)	0.56
4	TCCTTCGAC	264 (10.9)	875 (11.9)	0.16
5	TCCTCCGAC	236 (9.7)	761 (10.4)	0.35
	Other	206 (8.5)	716 (9.8)	0.061

Data are numbers (%) of haplotypes in the control and MI groups. Each haplotype is defined as a specific chromosome-based allele combination estimated from the genotypes of nine SNPs in the *BAT1-NFKBIL1-LTA* region. The order of the alleles in the haplotypes is in accordance with the chromosomal positions of the SNPs (from left to right): BAT1-A, BAT1-B, BAT1-C, NFKBILl-A, NFKBILl-B, NFKBILl-C, LTA-A, LTA-B and LTA-C (8). The alleles were selected from the Watson strand of chromosome 6 ($5'p \rightarrow 3'q$). Alleles present in haplotype 1, but not in haplotypes 2, 3, 4, and 5, are underlined in haplotype 1.

(recessive model) that were determined in the present study or calculated from data shown in reports from the prior studies (4,5,7,26,27). A moderately protective effect of the AA genotype was detected in the present study which included two independent control groups (Table 5). Different from this finding, the AA genotype was not associated with MI in a study that was conducted by Tobin *et al*. (26) in the UK (Table 5). In a study that included a group of patients with MI and two control groups from Japan, Ozaki *et al*. (4) observed a highly significant risk effect associated with the AA genotype (Table 5). Unlike this result, the AA genotype was not related with MI risk in Japanese study samples examined by Iwanaga *et al*. (5) and Yamada *et al*. (7) (Table 5). A non-significant trend towards a protective effect of the AA genotype was present in Americans of European origin examined by Morgan *et al*. (27) (Table 5). Their case group was composed of patients with MI (73%) or unstable angina pectoris (27%) (27).

Investigation of family trios recruited from different European countries suggested a relationship of the AA genotype or A allele of the LTA-C SNP with an increased risk of MI, as indicated by their excess transmission to affected individuals (6). Results of the present study rather provided evidence for an association of the AA genotype with a reduced risk of MI. In summary, examinations in samples of Europeans showed different effects of the AA genotype of the LTA-C SNP: the AA genotype was linked to a reduced risk (present study) or increased risk (6) of MI or was not associated with the risk of MI (26). Inconsistencies among data from the studies may be related to different recruitment strategies (family-based versus population-based) or the use or not of coronary angiography for the establishment of case or control status.

Results obtained by Ozaki *et al*. (4) in a Japanese sample suggested that homozygosity of the minor alleles of the BAT1-C, NFKBIL1-A, LTA-A, LTA-B and LTA-C SNPs conferred increased risks of MI (recessive models), instead of exhibiting rather protective effects, as found in the present population. A specific SNP-based haplotype, named

Table 5. Estimated risks of MI or acute coronary syndrome (with 95% CIs) associated with the AA genotype of the LTA-C SNP (rs1041981; Thr26Asn) in case–control studies[a]

Study (country)	AA carriers (cases/total)	AC and CC carriers (cases/total)	OR (95% CI)	*P*
Present (Germany)	322/452[b]	3335/4416	0.80 (0.65–1.00)	0.045
	322/735[c]	3335/6952	0.85 (0.73–0.99)	0.032
Tobin *et al*. (26) (United Kingdom)	74/136	473/916	1.12 (0.78–1.60)	0.55
Ozaki *et al*. (4) (Japan)	213/329[d]	920/1810	1.78 (1.39–2.27)	0.0000033
	213/313[e]	920/1692	1.79 (1.38–2.31)	0.0000073
Iwanaga *et al*. (5) (Japan)	81/130	397/714	1.32 (0.90–1.94)	0.16
Yamada *et al*. (7) (Japan)	334/635	1557/3054	1.07 (0.90–1.27)	0.46
Morgan *et al*. (27) (United States)	80/158	720/1297	0.82 (0.59–1.14)	0.24

[a]The case groups consisted entirely of patients with MI, with the exception of the case group in the study of Morgan *et al*. (27), which included patients with MI or unstable angina pectoris (combining term: acute coronary syndrome).
[b]Analysis included the control group with coronary angiography.
[c]Analysis included the KORA S4 sample as a control group.
[d]Analysis included control group 1.
[e]Analysis included control group 2.

haplotype A, was associated with an increased risk of MI in the Japanese population (4). Haplotype A is structurally related to haplotype 1 (8) that showed a protective effect in the present European sample. Subsequently, Ozaki *et al*. reported that the CC genotype of the LGALS2-A SNP conferred a higher risk of MI than the other genotypes of this SNP in a Japanese population (OR 1.21, 95% CI 1.08–1.37; $P = 0.0016$) (10). In contrast to this finding, we did not detect a relationship between the CC genotype and MI in the present sample (OR 0.98, 95% CI 0.83–1.16; $P = 0.84$). Thus, the LGALS2-A SNP appears to be a marker of MI risk in Japanese (10) but not in Europeans. Ethnic factors, including different genetic background and genetic heterogeneity of the phenotype, may explain, at least in part, the contradictory findings between the European and Japanese populations. Substantially different allele frequencies and genotype distributions exist for the SNPs in the *BAT1-NFKBIL1-LTA* region and the LGALS2-A SNP between the present European and the Japanese samples (4,10). Interaction with specific behavioural factors and environmental exposures may further complicate comparisons of the genetic association results between these populations.

Iwanaga *et al*. (5) found significant associations of the NFKBIL-A, LTA-A, LTA-B and LTA-C SNPs with MI in Japanese. The total of minor allele carriers (combined homozygous and heterozygous carriers), but not the homozygous carriers alone, were more frequent in the patient group than in the control group (5). These results suggested dominant effects of the minor alleles on MI risk and were different from the finding of recessive risk effects of the minor alleles in the sample examined by Ozaki *et al*. (4,5). In disagreement with these positive association results (4,5), Yamada *et al*. (7) reported that the LTA-B and LTA-C SNPs were not associated with MI in another sample from Japan. The divergent findings among the Japanese samples may be related to heterogeneities in the compositions of the control groups which exhibited strikingly different genotype distributions (4,5,7). Different recruitment criteria and age ranges are further possible explanations for the conflicting results among the Japanese studies (4,5,7).

Despite the fact that findings were apparently inconsistent among studies, evidence has been accumulated to suggest at least a moderate genetic effect of the *BAT1-NFKBIL1-LTA* region in the modulation of MI risk in Europeans and Japanese. These genes probably play only minor roles in atherosclerosis and MI, a possibility that calls for further investigation of other important genetic and environmental factors that may interact with them. On the other side, inconsistencies across studies may be conceived as an indication that the functions of the genes or the polymorphisms are irrelevant for disease risk. It remains to be clarified whether causal links exist between differential *in vitro* and *in vivo* effects in gene regulation, as observed with the BAT1-C, NFKBIL1-A, LTA-B and LTA-C SNPs (4,11–14), and MI.

MATERIALS AND METHODS

Patients and controls

Participants were examined at Deutsches Herzzentrum München or 1. Medizinische Klinik rechts der Isar der Technischen Universität München. They were recruited from southern Germany from 1993 to 2002. After catheterization, 5264 individuals were deemed eligible for inclusion into the MI or control group. Written informed consent for genetic analysis was obtained from 97.1% ($n = 5111$) of these individuals. Blood samples assigned for DNA preparation had been collected from 95.2% of the individuals who agreed to participate in the study ($n = 4868$; 3657 patients with MI and 1211 controls).

A second control group consisted of 4115 probands from the population-based KORA S4 epidemiological survey conducted between 1999 and 2001 in unrelated residents of German nationality aged 25–74 years (response 67%) and living in or near Augsburg, Germany (24).

The study protocol was approved by the institutional ethics committee and the reported investigations were in accordance

with the principles of the current version of the Declaration of Helsinki and Somerset West.

Definitions

Individuals recruited at the clinics in München were considered disease-free and, therefore, eligible as controls when their coronary arteries were angiographically normal and when they had no history of MI, no symptoms suggestive of MI, no electrocardiographic signs of MI, no regional wall motion abnormalities and no relevant valvular abnormalities in echocardiograms. Coronary angiography in the control individuals was performed for the evaluation of chest pain. The diagnosis of MI was established in the presence of chest pain lasting >20 min combined with ST-segment elevation or pathologic Q waves on a surface electrocardiogram. Patients with MI had to show either an angiographically occluded infarct-related artery or regional wall motion abnormalities corresponding to the electrocardiographic infarct localization, or both. Systemic arterial hypertension was defined as a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg (28), at least on two separate occasions, or antihypertensive treatment. Hypercholesterolemia was defined as a documented total cholesterol value ≥240 mg/dL (≥6.2 mmol/L) or current treatment with cholesterol-lowering medication. Persons reporting regular smoking in the previous 6 months were considered as current smokers. Diabetes mellitus was defined as the presence of an active treatment with insulin or an oral antidiabetic agent; for patients on dietary treatment, documentation of an abnormal fasting blood glucose or glucose tolerance test based on the World Health Organisation criteria (29) was required for establishing this diagnosis.

Genetic analysis

TaqMan reactions were used for genotyping in the control individuals and MI patients from the clinics in München. The assay systems employed here for the analysis of SNPs in the *BAT1-NFKBIL1-LTA* region [BAT1-A (rs2075582), BAT1-B (rs2075580), BAT1-C (rs2239527), NFKBIL1-A (rs2071592), NFKBIL1-B (rs2239707), NFKBIL1-C (rs2516479), LTA-A (rs1800683), LTA-B (rs909253) and LTA-C (rs1041981)] were described previously (8). The TaqMan assay used for genotyping of the LGALS2-A SNP (rs7291467) included the oligonucleotide primers 5′-CGCCACACAGACACTCACAGA-3′ and 5′-AGGAGGCAGGGAGCCATCT-3′ and the minor groove binder group-containing probes FAM—5′-ACACACAC GTCTAACA-3′ and VIC—5′-ACACACACATCTAAC-3′. With each of the TaqMan systems, 20% of the DNA samples were re-typed to control for correct sample handling and data acquisition. Genetic analyses were done without knowledge of the case–control status of the DNA samples.

Genotyping of the LTA-C and LGALS2-A SNPs in the KORA S4 sample was performed by MALDI-TOF mass spectroscopy (Sequenom, San Diego, CA), as previously described (21). SNP genotypes exceeded a call rate of 0.8 and a Hardy–Weinberg *P*-value of 0.05.

Haplotypes and measures of linkage disequilibrium (D' and r^2) between SNPs were calculated from primary genotype data with the use of the software package Haploview (30). A partition–ligation–expectation–maximization algorithm was used to infer individual haplotypes (31).

Statistical analysis

The analysis consisted of comparisons of genotype distributions and haplotype frequencies between the control group and the MI group. Discrete variables are expressed as counts and compared by the χ^2-test. Continuous variables were compared by means of the unpaired, two-sided *t*-test and are expressed as mean ± SD. Independence of associations between SNPs and MI were assessed after adjustment for other potentially confounding factors (age, gender, history of arterial hypertension, history of hypercholesterolemia, current cigarette smoking and diabetes mellitus) using multiple logistic regression analysis and calculating the adjusted odds ratios and their 95% confidence intervals. Statistical significance was accepted for *P*-values <0.05.

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Conflict of Interest statement. None declared.

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