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ORIGINAL ARTICLE

Lymphotoxin-α and galectin-2 SNPs are not associated with myocardial infarction in two different German populations

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Abstract Recent data provided strong evidence for the association of single nucleotide polymorphisms (SNPs) in the lymphotoxin-α (LTA) and galectin-2 (LGALS2) genes with myocardial infarction (MI) in a Japanese population. For populations of other genetic background, the relevance of these polymorphisms in the pathogenesis of MI remains controversial. We aimed to define the role of LTA and LGALS2 SNPs in two German MI populations with markedly

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different ascertainment strategies. Two different MI populations were studied. In the first population, MI patients were ascertained by a strong family history of MI (n=1214). Controls were unrelated disease-free participants of the study (n=1080). The second population included patients suffering from sporadic (nonfamilial) MI from the German KORA register (n=607). The control group consisted of participants of the WHO MONICA survey in Germany (n=1492). TaqMan assays were used to determine the genotypes of 4 SNPs in the LTA genomic region and 1 SNP in the LGALS2 gene. Single SNPs in both genomic regions as well as haplotypes in the LTA genomic region were tested for association in various models of inheritance. No association with MI could be found for any of the examined SNPs in the LTA genomic region and LGALS2 gene, or for haplotypes spanning the LTA genomic region. In two MI populations of European descent with markedly different ascertainment strategies, we were not able to identify a significant association of SNPs in the LTA genomic region or the LGALS2 gene with MI. These variants are unlikely to play a significant role in populations of European origin.

Keywords Genetics · Lymphotoxin-alpha · Galectin-2 · Myocardial infarction · Coronary artery disease

Introduction

According to global WHO statistics, among noninfectious diseases, cardiovascular diseases remain the leading cause of mortality, accounting worldwide for 30% of all deaths—or 17.5 million people—in 2005. In the Western industrialized countries, cardiovascular diseases are responsible for up 50% of all deaths [1]. In most developed countries, coronary artery disease (CAD) and MI mortality rates steadily decreased over the past 40 years [2]. However, in other regions, morbidity and mortality rises (e.g., Russia, India, and Latin America) or is expected to rise dramatically in the near future (e.g., China, South America, and Africa) [3].

MI is a complex genetic phenotype resulting from interplay of established environmental and individual risk factors. Importance of family history as an independent risk factor resulting from complex interactions of independent heritable genetic components has been well documented [4, 5].

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Much interest has been paid in the recent years to inflammatory pathways in the pathogenesis of atherosclerosis [6]. Genes involved in these pathways have become ideal candidate genes for genetic mapping of susceptibility loci in both linkage and association studies.

In a step-wise genome-wide association, Ozaki et al. [7] identified a susceptibility locus for MI on chromosome 6p in a Japanese population five SNPs within a 50 kb genomic locus region comprising BAT1 (encoding HLA-B-associated transcript 1), NFKBIL1 (encoding nuclear factor of kappa-light chain gene enhancer in B cells inhibitor-like 1), and LTA (encoding lymphotoxin-alpha) were in high linkage disequilibrium (LD) and significantly associated with MI [7]. Functional data as well as involvement of the gene product in the inflammatory pathways supported further their hypothesis.

In another study, the same group identified the SNP rs7291467 in the LGALS2 gene to be significantly associated with susceptibility to MI, which in vitro affected transcriptional level of the LGALS2 protein [8]. The authors demonstrated binding of LTA protein to LGALS2 and speculated that altered biological availability of the LTA protein might affect disease pathogenesis.

In subsequent studies, controversial results were obtained. Whereas two other groups were able to replicate the original findings in the LTA genomic region [9, 10], others failed to provide additional evidence for the association of the LTA with MI [11–14]. To our knowledge, in case of LGALS2 only one replication study has been published so far reporting no association with MI in a British population [15].

To assess the role of polymorphisms in the LTA and LGALS2 genes for the development of atherosclerosis and MI in European populations, we examined SNPs in the LTA and LGALS2 in two German MI populations with markedly different ascertainment strategies.

Materials and methods

Populations

The first study population (Population 1) consisted of 1,214 unrelated German MI patients from the Regensburg MI Family Study in which families with increased prevalence of MI were ascertained according to previously described criteria [16]. Briefly, the index patient was recruited if he had suffered from MI prior to the age of 60 years, and had had at least one affected first-degree relative with MI or severe coronary artery disease treated with coronary artery bypass grafting or percutaneous coronary intervention before the age of 70 years. Diagnosis of MI was based upon standard WHO criteria [17]. Control individuals

Table 1 Nomenclature of genotyped SNPs

rs Number	Gene	Location	Variation
rs2239527	BAT1	Promoter	−23G→C
rs2071592	NFKBIL1	Promoter	63T→A
rs1800683	LTA	Exon 1	10G→A
rs909253	LTA	Intron 1	252A→G[a]
rs1041981	LTA	Exon 3	804C→A (Thr26Asn)
rs7291467	LGALS2	Intron 1	3279C→T

[a] Not genotyped in this study

(n=1,080) were disease-free participants of this study who were unrelated to the MI patients.

In the second population (Population 2), cases (n=607) were MI patients from the Augsburg MI register, which is a substudy of the WHO MONICA (monitoring of trends and determinants of cardiovascular disease) Survey in the Augsburg region, Germany [18] and controls (n=1,492) were German participants of the WHO MONICA population survey [19]. They had no clinically manifest coronary artery disease.

Diabetes mellitus was defined based on medical history questionnaire and/or intake of oral antidiabetic drugs or use of insulin. Smoking was defined as current smoking or a history of smoking habit. For other cardiovascular risk factors (i.e., hypertension, hyperlipidemia), blood pressure values and lipoprotein values were used in order to eliminate a possible bias introduced by a different risk factor background in the case and control populations as well as by different management strategies in those populations.

The ethics committee of the University of Regensburg approved the study protocol and all participants gave their written informed consent at the time of blood sample collection. The reported investigations were in accordance with the principles of the current version of the Declaration of Helsinki.

Genetic analyses

Genomic DNA was isolated from whole blood samples using the PureGene DNA Purification System Blood Kit (Gentra, Minneapolis, MN, USA). The DNA samples were genotyped using 5′ exonuclease TaqMan technology (Applied Biosystems, Foster City, CA, USA) with differently fluorescence-labeled probes. Altogether, 4 SNPs in the LTA genomic region and 1 SNP in the LGALS2 gene were studied (Table 1). For all SNPs analyzed, Custom TaqMan SNP Genotyping Assays (Applied Biosystems) were used. The LTA SNP LTA intron 1 252A→G (rs909253) from the work of Ozaki et al. was not genotyped for technical reasons. Furthermore, almost complete LD in the previous studies made it possible to use the neighboring SNPs as surrogate markers for the SNP rs909253. For each genotyping experiment, 10 ng DNA was used in a total volume of 5 μl containing 1× TaqMan Universal PCR Master Mix (Applied Biosystems). PCR reaction and post-PCR endpoint plate read was carried out following the

Table 2 Baseline clinical characteristics of study populations

	Population 1		Population 2	
	MI cases (N=1,214)	Controls (N=1,080)	MI cases (N=607)	Controls (N=1,492)
Age at inclusion, years	58.7±8.6	57.4±9.7*	56.3±7.2	51.4±13.7*
Gender, percent male	68.8	35.3*	87.3	51.1*
Systolic blood pressure, mmHg	137.6±19.6	134.6±18.0*	132.2±16.9	134.5±19.9*
Diastolic blood pressure, mmHg	82.2±10.1	82.2±10.0	84.2±10.3	80.6±11.7*
Body mass index, kg/m^2	27.4±3.6	26.7±4.1*	28.5±3.7	26.9±4.1*
Total cholesterol, mg/dl	227.0±47.1	237.3±43.2*	224.1±44.1	234.2±44.2*
LDL cholesterol, mg/dl	151.2±43.4	146.6±35.8*	133.0±38.0	144.4±43.1*
HDL cholesterol, mg/dl	50.1±13.2	60.1±15.2*	47.1±14.3	53.8±16.5*
Diabetes mellitus[a], %	16.3	6.0*	14.9	4.1*
Smoking[b], %	69.4	50.3*	N.A.	26.6

Values are means±standard deviation and percentages unless stated otherwise. To convert values for total cholesterol, HDL cholesterol, and LDL cholesterol to millimoles per liter, divide by 38.66.

*Significant different between the case and control groups (P≤0.01)

[a] Defined as history of diabetes mellitus or intake of antidiabetic medication

[b] Former or current smoking

Table 3 Genetic analyses and association tests in two study populations

SNP	Genotype distributions		MAF		Recessive model		Dominant model		Codominant model	Trend test (Armitage's)	
	MI cases	Controls	MI cases	Controls	OR (95% CI)	*P* value	OR (95% CI)	*P* value	*P* value	OR (95% CI)	*P* value
Population 1											
BAT1 promoter −23G→C rs2239527	555;540;107 (46.2;44.9;8.9)	488;460;117 (45.8;43.2;11.0)	0.31	0.33	0.79 (0.60–1.04)	0.097	0.99 (0.84–1.16)	0.87	0.24	0.94 (0.83–1.07)	0.38
NFKBIL1 promoter 63T→A rs2071592	580;527;97 (48.2;43.7;8.1)	495;460;107 (46.6;43.3;10.1)	0.30	0.32	0.78 (0.59–1.04)	0.094	0.94 (0.80–1.11)	0.46	0.24	0.92 (0.81–1.04)	0.19
LTA exon 1 10G→A rs1800683	542;548;107 (45.3;45.8;8.9)	481;460;120 (45.3;43.4;11.3)	0.32	0.33	0.77 (0.58–1.01)	0.060	1.00 (0.85–1.18)	0.98	0.14	0.95 (0.84–1.07)	0.40
LTA exon 3 804C→A rs1041981	544;546;105 (45.5;45.7;8.8)	490;463;120 (45.6;43.2;11.2)	0.32	0.33	0.77 (0.58–1.01)	0.057	1.01 (0.85–1.19)	0.95	0.13	0.95 (0.84–1.08)	0.41
LGALS2 intron 1 3279C→T rs7291467	447;568;183 (37.3;47.3;15.4)	382;503;168 (36.3;47.0;16.7)	0.39	0.40	0.95 (0.76–1.19)	0.660	0.96 (0.81–1.14)	0.61	0.84	0.96 (0.86–1.09)	0.56
Population 2											
BAT1 promoter −23G→C rs2239527	303;249;47 (50.6;41.6;7.8)	698;594;130 (49.1;41.8;9.1)	0.29	0.30	0.85 (0.60–1.20)	0.340	0.94 (0.78–1.14)	0.54	0.60	0.99 (0.82–1.20)	0.37
NFKBIL1 promoter 63T→A rs2071592	313;241;41 (52.6;40.5;6.9)	767;599;110 (52.0;40.5;7.5)	0.27	0.28	0.92 (0.63–1.33)	0.650	0.97 (0.81–1.18)	0.79	0.90	0.97 (0.83–1.13)	0.69
LTA exon 1 10G→A rs1800683	304;256;44 (50.3;42.4;7.3)	716;599;131 (49.5;41.4;9.1)	0.28	0.30	0.79 (0.55–1.13)	0.180	0.97 (0.80–1.17)	0.74	0.41	0.94 (0.81–1.09)	0.40
LTA exon 3 804C→A rs1041981	298;255;44 (49.9;42.7;7.4)	717;605;129 (49.4;41.7;8.9)	0.29	0.30	0.82 (0.57–1.16)	0.260	0.98 (0.81–1.19)	0.84	0.52	0.95 (0.82–1.11)	0.52
LGALS2 intron 1 3279C→T rs7291467	227;279;97 (37.6;46.3;16.1)	488;684;251 (34.3;48.1;17.6)	0.39	0.42	0.90 (0.69–1.16)	0.390	0.86 (0.71–1.05)	0.15	0.33	0.90 (0.79–1.04)	0.15

Genotype distributions are numbers (percentages)
MAF, minor allele frequency; *OR*, odds ratio; *CI*, confidence interval

manufacturer's instructions using the Applied Biosystems 7900HT Real-Time PCR System. Sequence Detection System software version 2.2 (Applied Biosystems) was used to assign genotypes applying the allelic discrimination test. Case and control DNA was genotyped together on same plates. Duplicates of samples (15%) were employed to assess intraplate and interplate genotype quality. No genotyping discrepancies were detected. The overall call rate was 98.0%. A person without knowledge of affection status performed assignment of genotypes.

Statistical analyses

Discrete variables were compared by the chi-square test. Continuous variables were evaluated using the one-way analysis of variance (ANOVA). Genotype distributions in the studied populations were compared to predicted distributions under HW equilibrium. LD was analyzed using D' and r^2 [20]. Genotypic association tests between cases and controls assuming dominant, recessive or allele dose (trend) genetic models were performed using logistic regression analysis, and rare alleles were defined as risk alleles. Haplotypes were reconstructed using the EM algorithm [21] and haplotype association was tested using a regression-based method [22]. Assuming the risk allele frequency of 0.3, genotypic relative risk ratio ≥1.5 and population disease prevalence of 5–10%, a statistical power of >90% was calculated for both samples to detect a significant association ($P<0.05$) in dominant, recessive, multiplicative, and additive models of inheritance.

Results

Populations characteristics

Demographic characteristics of the study populations are given in Table 2. Due to different ascertainment strategies and medical treatment bias, there were significant differences between the case and control populations. The case populations were slightly older, predominantly male, had higher body mass index (BMI), and there were more diabetic individuals and smokers among them. Blood pressure and lipid values were similar, although significantly different due to the large sample size. Differences between blood pressure and lipid values between the cases and controls in both study populations were attributable to different cardiovascular risk factor profiles and management strategies.

Genetic analyses

Genotype distributions in both control groups were in accordance with the HW equilibrium for all 4 BAT1-NFKBIL1-LTA genomic region markers as well as for the SNP in the LGALS2 gene. High degree of LD between the SNPs in the BAT1-NFKBIL1-LTA region was found (D'= 0.97–0.99; r^2=0.94–0.99). Genotype distributions, minor allele frequencies, and results of recessive, dominant, codominant, and additive models of association testing in the BAT1-NFKBIL1-LTA region and LGALS2 gene are shown in Table 3.

For the BAT1-NFKBIL1-LTA genomic region, the minor allele frequency of the studied SNPs was very similar (MAF 0.29–0.33) due to high degree of LD and slightly lower in the case populations. In both case-control populations, no statistically significant association could be found using different genetic inheritance models (Table 3). The lowest OR and P value were observed in the Regensburg MI Family Study population for the SNP rs1041981 [OR 0.77 (95% CI 0.58–1.01), P=0.057] using the recessive inheritance model.

The SNP rs7291467 in the LGALS2 gene was not associated with MI in the Regensburg MI Family Study (OR=0.96, P=0.56) or in the KORA-MONICA population (OR=0.90, P=0.15; for details see Table 3).

When men and women were analyzed separately, no significant association between the SNPs in the BAT1-NFKBIL1-LTA region and LGALS2 gene with MI was identified (data not shown).

The structure of the most abundant haplotypes with their frequencies and association tests between the case and control groups is shown in Table 4. Two most frequent haplotypes in the study of Ozaki et al. (CAAA and GTGC) had a very low frequency in our populations (0.005 and 0.002, respectively). None of the estimated haplotypes was associated with MI.

Table 4 Haplotype structures, frequencies, and case-control comparisons

Haplotype	Population 1				Population 2			
	MI cases	Controls	OR	P	MI cases	Controls	OR	P
CTGC	0.68	0.67	–	–	0.71	0.70	–	–
GAAA	0.29	0.31	0.93	0.25	0.26	0.27	0.96	0.56
GTAA	0.02	0.01	1.42	0.15	0.01	0.02	0.78	0.34

Numbers for cases and controls are population haplotype frequencies.

Discussion

A Japanese study showed strong association of LTA and LGALS2 SNPs with MI. In our study, however, this association could not be replicated in two different MI populations of European descent. Several confounding factors, which are inherent to association studies of complex human traits may be responsible for these discrepant findings [23].

The most straightforward explanation may be sought in genetic differences of studied populations. Indeed, the risk allele frequencies of the LTA and LGALS2 genes are markedly different among historically distant populations (Table 5). Even within populations with similar genetic background, e.g., British and German populations, the differences in allele frequency make it potentially difficult to extrapolate genetic findings from one population to another. In this study, we also demonstrate a striking difference in the frequency of prevalent haplotypes in the LTA genomic region between the Japanese and Caucasian populations. Furthermore, variable usefulness and transferability of LD structure in different populations for tagging genomic regions of interest has been described [24]. Genetic diversity in different populations may be especially relevant to the LTA genomic region, which lies within the HLA region and has an erratic pattern of LD structure with both short LD islands, as well as long-range haplotypes (Supplementary Fig. 1). Consequently, significantly associated markers in the original Japanese population might tag efficiently causal variants, whereas in other populations the same markers would fail in this respect. As a good example, the original LTA finding of Ozaki et al. was replicated in another Japanese study [9], but yet another Japanese study failed to confirm the association [14]. In the study of Iwanaga et al. [9], the dominant model of inheritance was the most significant finding contrary to the recessive model presented in the work by Ozaki et al. Our negative LTA data add to the available evidence that in the populations of Caucasian descent, any significant association of the LTA SNPs with MI seems unlikely [11–13]. Although one study involving 400 British trio families reported a positive association for the SNP rs1041981, another subsequent larger trio study in the UK could not replicate this finding [12]. Recently, a metanalysis of all so far published studies dealing with the role of the LTA SNPs in the pathogenesis of MI revealed no association for the recessive model and a weak association for the dominant model in 10,996 MI cases and 7,329 controls [13]. The authors concluded that the common polymorphisms in the LTA gene were not strongly associated with susceptibility to CAD.

Ozaki et al. [7] stated in their original paper that the distribution of the allele and genotype frequencies in over 65,000 markers excluded any significant stratification in the tested population. They also used two different control groups without differences in risk allele frequencies, but an independent case replication sample was not provided [25]. Our own and analyses published by Wittke-Thompson et al. [26] of the original publication by Ozaki et al. showed that the genotype distributions in their MI cases and their control group 1 deviated significantly from the HW equilibrium. While departure from HW equilibrium was borderline significant in control 2 group, both control samples together present with highly significant deviation from HW equilibrium. A deviation from HW equilibrium may be expected in a case group due to ascertainment for the causal mutation. However, in control populations, any deviation from HW equilibrium requires very cautious interpretation of the results and might reflect selection for the tested loci. The LTA genomic region is located within the very polymorphic HLA region, which is known not only for its genomic complexity but also for allele frequency differences in various populations demonstrated also in our study, and haplotype-specific long-range LD pattern [27, 28]. Selection pressures and assortative mating may further amplify these problems. Thus, case-control association studies addressing genetic variations in this

Table 5 Allele frequencies of LTA (rs1800683) and LGALS2 (rs7291467) SNPs in different populations

Source	Population	rs1800683	rs7291467
Ozaki et al. [7, 8]	MI cases	0.41	0.31
	Controls	0.37	0.36
Yamada et al. [14]	MI cases	0.42	NA
	Controls	0.40	NA
dbSNP	Japanese	0.37	0.31
Germany[a]	MI cases	0.28	0.39
	Controls	0.30	0.42
UK [13, 15]	MI cases	0.36	0.40
	Controls	0.34	0.40
dbSNP	Caucasian	0.35	0.37
dbSNP	African	0.52	0.71

MI cases, myocardial infarction patients; *Controls*, control population; *NA*, not available; *dbSNP*, SNP database at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp

[a] Data from current study

region may be particularly sensitive to deviations from HW equilibrium. Consequently, genome-wide tests for stratification might not capture potential locus-specific stratification. In our populations, we did not detect any deviations from HW equilibrium implying that the above-mentioned restraints do not apply. Previous experiments demonstrated that the population stratification does not apply to control groups used in this study.

Recently, a study by Mangino et al. [15] failed in a British population to replicate the association of LGALS2 and MI in a Japanese population reported by Ozaki et al. [8]. It has been the first published replication study for this genomic region so far. Independent replication studies in different Japanese populations are not yet available. In their report, Ozaki et al. described a significant association of the SNP rs7291467 with MI. Although in our study the putative risk TT genotype was also slightly underrepresented in both case populations, the results were far from being significant for any of the genetic models tested.

There is accumulating evidence that genetic association studies should be performed separately in men and women, as genetic effects may differ in each sex. Indeed, recent data by Asselbergs et al. [29] suggest that even for LGALS2, a gender-specific effect on MI risk should be considered. Unfortunately, only few populations so far have been ascertained strictly as male or female ones. This limitation also applies to our study populations. Nevertheless, we performed a secondary separate analysis in men and women only. Both in the BAT1-NFKBIL1-LTA region and the LGALS2 gene, these analyses showed similar negative association results as the primary analyses in the entire study samples.

There may be several limitations of this study. First, we evaluated the association of MI and SNPs that were relevant in the Japanese population. It is possible that other markers might more efficiently tag the disease relevant variants. A comprehensive approach would require more complete SNP coverage of this genomic region. However, functional data from the original work by Ozaki et al. supported the hypothesis that the markers studied here might be not only associated with the disease, but also directly involved in its pathogenesis. Furthermore, if genotypic relative risk were in reality much smaller than those reported in the original Japanese population, our study might fail to detect such weak effects. However, practical implications of marginally increased genotypic relative risks detectable only in extremely large populations are at least questionable, as they are unlikely to point to important disease pathways.

In conclusion, our study supports the available evidence that in populations of European descent, the reported SNPs in the LTA genomic region and in the LGALS2 gene are not susceptibility markers for MI. Furthermore, we suggest that these SNPs are unlikely to play a causal role in the pathogenesis of MI in most if not all populations. In some populations, however, they still might serve as genetic mapping proxies for the variants actually relevant to susceptibility for MI and the pathogenesis of coronary atherosclerosis.

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