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Association between *PPAR-alpha* Gene Polymorphisms and Myocardial Infarction

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ABSTRACT

Peroxisome proliferator activated receptor alpha (PPAR α) regulates the expression of genes that are involved in lipid metabolism, tissue homeostasis, and inflammation. Consistent rodent and human studies suggest a link between PPAR α function and cardiovascular disease, qualifying PPAR α as a candidate gene for coronary artery disease. We comprehensively evaluated common genetic variations within the PPAR α gene and assessed their association with myocardial infarction.

First, we characterized the linkage disequilibrium within the PPAR α gene in an initial case-control sample of 806 individuals from the Regensburg Myocardial Infarction Family Study using a panel of densely spaced single nucleotide polymorphisms (SNPs) across the gene. Single SNP analysis showed significant association with the disease phenotype (OR=0.74, P =0.012, 95% CI=0.61-0.94 for rs135551). Moreover, we identified a protective 3-marker haplotype with an association trend for myocardial infarction (OR=0.76, P =0.067, 95% CI=0.56-1.92). Subsequently, we were able to confirm the single SNP and haplotype association results in an independent second case-control cohort with 667 cases from the Regensburg Myocardial Infarction Family Study and 862 control individuals from the WHO MONICA Augsburg project (OR=0.87, P =0.046, 95% CI=0.72-0.99 for rs135551; OR=0.80, P =0.034, 95% CI=0.65-0.98 for the 3-marker haplotype, respectively).

From these cross-sectional association results, we provide evidence that common variations in the PPAR α gene may influence the risk of myocardial infarction in a European population.

214 words

INTRODUCTION

Myocardial infarction (MI) is considered a complex disease. Its development and progression underlies interactions between environmental and genetic influences [1]. Genetic association studies of candidate genes focus on the identification of genetic variations that contribute to or protect against the development of the disease [2].

Peroxisome proliferator activated receptor alpha (PPAR α), a member of the nuclear receptor superfamily, qualifies as an interesting candidate gene for atherosclerotic disease. PPAR α is a ligand-activated transcription factor that regulates the transcription of genes involved in lipid metabolism, tissue homeostasis and proteins that initiate an inflammatory response central to atherosclerotic plaque formation [3]. Ligands for PPAR α include fibrates, a substance class of lipid lowering drugs that has been shown to reduce the risk of cardiovascular events [4;5]

Previously, genetic examinations of the *PPAR α* locus have mainly focussed on the L162V polymorphism, which is the only functional variant that has so far been shown to be associated with changes in serum concentrations of lipids and lipoproteins, and with left ventricular hypertrophy [6-9]. Yet, association studies between the L162V variant and atherosclerosis are limited and provide inconsistent results [10].

However, to date, no comprehensive evaluation of common variations within the *PPAR α* gene and their association with MI was performed. Therefore, the aims of our study were (1) to assess the linkage disequilibrium (LD) structure of the *PPAR α* gene and to identify possible haplotypes in an initial case-control sample, (2) to perform single SNP and haplotype analyses for association with MI, and (3) to validate available positive results in an independent second case-control sample.

MATERIALS AND METHODS

Study population

All MI patients were participants of the Regensburg MI Family Study. Selection criteria have been described previously [11;12]. In brief, we identified MI families, in which the index patient suffered from MI at the age of 60 years or younger and at least one more family member had been affected with MI and/or severe coronary artery disease (CAD), e.g. percutaneous coronary intervention or coronary bypass surgery). The diagnosis of MI was established by review of medical records according to the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) diagnostic criteria (<http://www.ktl.fi/publications/monica/manual/index.htm>). All MI patients and all of their available siblings and spouses were contacted and invited to participate in the study. Medical history, risk factor evaluation, clinical examination with anthropometric measurements, and blood samples were obtained from all participants.

For the initial case-control study, 433 unrelated MI patients fulfilling our index criteria and 373 unrelated unaffected control subjects were randomly selected to establish genotyping and to analyse gene structure. Control individuals consisted of cardiovascular disease-free married-in spouses, sisters-in-law, and brothers-in-law, from the Regensburg MI Family Study. For the second sample, we identified an additional 667 unrelated MI patients from the Regensburg MI Family Study (age of MI in men <60 years, in women <70 years) and 862 controls from the Third WHO-MONICA Augsburg survey 1994/1995. Individuals with a history of CAD, cerebrovascular accidents (transient ischemic attack or stroke) or peripheral artery disease were excluded from the control group. The MONICA Augsburg survey is a large population-based sample of all German residents of the Augsburg area that consists of individuals aged 25 to 74 years, with about 300 subjects per 10-year age increment and gender. The Augsburg project was part of the international collaborative World Health Organization MONICA study [13].

Written informed consent was obtained from all subjects, and the local ethics committee approved the study.

Phenotyping

At recruitment, all patients were studied by comparable design as previously described including a standardized interview, clinical examination, and biochemical as well as molecular analyses either at a visit at a study center (patients from the MONICA Augsburg study) or at a home visit by a physician (patients from rehabilitation centers) [12;14;15]. Validation of cardiovascular events at study entry was performed by review of medical records. Resting blood pressure was taken according to MONICA guidelines after participants had been resting in a sitting position [16]. Body weight in kilogram and height in meters were determined with subjects wearing light clothing. Blood samples were taken from non-fasting individuals. Serum levels of low- and high-density lipoprotein cholesterol and triglycerides were measured by standard enzymatic methods [17]. Characteristics of both cohorts are shown in Table 1.

SNPs and Genotyping Methods

Seventeen SNPs were selected from public databases (dbSNP, <http://www.ncbi.nlm.nih.gov/SNP/>) based on the following criteria: (1) minor allele frequency of >0.05 in a Caucasian population (according to HapMap Project population data, <http://www.hapmap.org/>), (2) average coverage target of one SNP per 5 kb of the *PPAR α* gene and flanking sequences, (3) evidence of validation status, and (4) compatibility with our genotyping platform. To allow to determine the extend of LD beyond the boundaries of the gene, one SNP each in the 5' intergenic region and in the 3' UTR was included.

Genomic DNA was extracted from EDTA blood samples by standard procedures. High throughput genotyping was performed on the ABI 7900 platform according to the manufacturer's protocol using the ABI assay on-demand or by-design service. Genotyping quality was ensured by repetition of 10% (n=233) of all genotypes in independent PCR reactions. Genotypes did not differ between duplicates. For five duplicates, the definite genotype call was obtained in one of the assays with an undetermined call in the other. The overall genotyping call-rate was $>98\%$ for all SNPs. In individuals from the second sample, only rs135557, rs135551, rs135543, and rs135539 were genotyped.

Statistical Analyses

For each of the 17 SNPs, we tested whether the observed genotype frequencies in MI cases and controls deviated from Hardy-Weinberg equilibrium ($P = 0.05$ level). Single SNP association analyses were performed in both case-control samples. Allele and genotype frequencies were compared using the χ^2 test and Armitage test for trend reporting odds ratios (OR) and 95% confidence intervals (CI). The Armitage's trend test under the assumption of an additive mode of inheritance allows the reduction of genotype comparisons to one degree of freedom [18]. Additionally, pair-wise LD was determined applying the standard definition of r^2 [19]. A haplotype block was defined as a region in which all pair-wise r^2 values were >0.4 . Haplotypes and their frequencies were inferred from phase-unknown genotype data using PHASE version 2.1 [20;21]. Uncertain haplotypes with a probability of correct phase or allele $<90\%$ were excluded from the analyses. The calculated haploblock A as well as the number of copies of hap2 were tested for disease association by a χ^2 test. For interaction analyses all pair-wise combinations of SNPs were tested in a population-based case/control setting implemented in plink v0.99s [22]. A two-sided P -value of <0.05 indicated statistical significance. Multiple logistic regression analyses were used in the multivariate model of the matched cohort adjusting for age, total cholesterol/HDL cholesterol ratio, systolic blood pressure, body mass index, diabetes mellitus and smoking status with P -values from Effect Wald test. Total cholesterol/HDL cholesterol ratio was chosen because it has been shown to be superior to the use of total cholesterol or LDL cholesterol alone in the prediction of CAD risk and is less affected by ongoing lipid lowering therapy [23-25]. Association results were not corrected for multiple testing.

ELECTRONIC DATABASE INFORMATION

<http://www.ktl.fi/publications/monica/manual/index.htm>

<http://www.hapmap.org/>

<http://www.ncbi.nlm.nih.gov/SNP/>

RESULTS

PPAR α linkage disequilibrium mapping and haplotype structure

We characterised the LD and haplotype structure of the *PPAR α* gene by genotyping a case-control sample of 806 individuals with (n=433) and without (n=375) MI for 17 SNPs (Table 1). There were 14 intronic SNPs, one SNP was positioned in exon 6, and one SNP was located in the 5' intergenic region and the 3' UTR, respectively, yielding in an average marker distance of 5.6 kb. All 17 SNPs had minor allele frequencies (MAF) >0.05 and met Hardy-Weinberg expectations in MI cases and controls in both study samples ($P=0.05$ level). Haplotypes and their frequencies were constructed from phase-unknown genotype data. A haplotype block was defined as a region in which all pair-wise r^2 values were >0.4. A total of four haplotype blocks were identified (block A-D, Fig. 1). The first haploblock (haploblock A) comprised rs135551, rs135543 and rs135539 and spanned part of intron 2 (6,246 bp); the haploblocks B, C and D were built up by two (rs4253681 and rs7364220), three (rs4823613, rs5766741, rs4253728) and four SNPs (rs4253754, rs4253760, rs4253778, rs11704979) SNPs, respectively. Haploblock B covered part of intron 3, haploblock C spanned most of intron 4, whereas haploblock D commenced within intron 6 and reached across *PPAR α* boundaries into the 3' UTR (Fig. 1). Reconstruction of haploblock A revealed 3 common haplotypes with a frequency >0.10 which were termed hap1, hap 2, and hap3 with frequencies of 54.3%, 28.7% and 12.3%, respectively. They comprised 95.3% of total chromosomes of the screening sample population.

Association of PPAR α variants with MI in the initial case-control cohort

Initially, we carried out single marker association tests with all 17 SNPs the first cohort was genotyped for calculating odds ratios (OR) from comparisons of allele and genotype frequencies in cases and controls (Table 2). We found a significant association between rs135551 and MI ($P=0.012$, OR=0.74, 95% CI=0.61-0.94; Armitage's trend test). Additionally, an association trend between rs135543 and MI could be observed resulting from an overrepresentation of the rare alleles in control individuals ($P=0.082$, OR=0.81, 95% CI=0.68-1.02; Armitage's trend test). The effect was more pronounced in carriers of 2 copies of the rare alleles as reflected in the homozygous model (22 vs. 11; $P=0.005$, OR=0.49, 95% CI=0.29-0.81 for rs135551,

and $P=0.025$, $OR=0.59$, $95\% CI=0.38-0.94$ for rs135543, respectively; data not shown).

As both SNPs are parts of haploblock A, we next performed SNP haplotype association analysis using the three most common haplotypes hap1 – hap3. Haplotype analyses may provide more power to detect association with disease than single marker analyses alone [26]. In the initial sample, we found no statistical significant association between haplotypes hap1 – hap3 and MI (Table 3). However, hap2, which is defined by the combination of the rare alleles, showed a trend for association with MI ($P=0.067$, $OR=0.76$, $95\% CI=0.56-1.02$, Table 3). Hap2 was more frequently present in control individuals, thus corresponding to a protective haplotype. Additionally, as for single SNP analyses, we observed a significant association between the number of copies of hap2 and MI. Individuals homozygous for hap2 were at significantly reduced risk for MI than individuals with no or only one copy of the protective haplotype ($P=0.003$, $OR=0.44$, $95\% CI=0.25-0.77$, and $P=0.020$, $OR=0.52$, $95\% CI=0.23-0.91$, respectively, Fig. 2). Importantly, individuals with 0, 1 or 2 copies of hap2 did not differ with respect to age (54.5 ± 10 , 53.8 ± 10.1 , 56.8 ± 8.3 years), blood pressure levels (systolic 134 ± 19 , 135 ± 17 , 136 ± 16 mmHg; diastolic 81 ± 10 , 82 ± 9 , 82 ± 9 mmHg), body mass index (26.9 ± 3.6 , 27.6 ± 4.1 , 27.5 ± 4.1 kg/m²), or serum lipid concentrations (LDL cholesterol 146 ± 36 , 148 ± 34 , 147 ± 40 mg/dL; HDL cholesterol 54 ± 14 , 54 ± 14 , 55 ± 15 mg/dL, triglycerides 165 ± 90 , 180 ± 112 , 180 ± 114 mg/dL respectively). The observed gender difference (71.9, 74.6, 58.1% male, $P=0.033$) did not influence the results after correction in a logistic regression model ($P=0.025$, $OR=0.48$, $95\% CI=0.25-0.91$).

None of the other SNP markers was significantly associated with MI. In particular, we did not find a relation between rs1800206, the only SNP resulting in an amino acid exchange (L162V), and MI (Table 2). We further performed comprehensive testing for epistatic interaction between any of the 17 SNPs and found a statistically significant interaction with an asymptotic p -value threshold below 0.01 between rs135539 and rs11704979 ($P=0.001$, data not shown). To avoid missing an effect that was not detected by single SNP association analyses, we investigated the other three haploblocks B, C and D for possible haplotype association. These analyses did not reveal significant association results (data not shown). For further association analyses, we therefore focussed on haplotype block A as well as on the single markers contributing to or surrounding it.

Confirmation of association between MI and *PPAR* α variants in an independent second case-control sample

In order to corroborate the data, we sought to validate our results in an independent second case-control cohort. Therefore, additional 1,529 individuals (667 MI cases from the Regensburg MI Family Study, 862 controls from the WHO MONICA Augsburg project) were genotyped for all SNPs of the 3-marker haplotype and one 5' adjacent SNP. In this second cohort, we were able to confirm single SNP association result for rs135551 ($P=0.046$, OR=0.87, 95% CI=0.72-0.99, Armitage's trend test, Table 4). Moreover, for rs135543 as well as hap2, which both showed a trend for association in the initial sample, now significant association with MI could be observed resulting from an over-representation in control individuals ($P=0.014$, OR=0.84, 95% CI=0.71-0.96, and $P=0.034$, OR=0.80, 95% CI=0.65-0.98, respectively; Tables 3 and 4).

DISCUSSION

We comprehensively evaluated the LD structure of common genetic variations within the *PPAR α* gene and assessed their potential relation with MI. In a two-step approach, we initially used a case-control sample to investigate the LD structure of the gene and to construct common haplotypes as well as to explore the association between single SNPs and haplotypes and MI. We found significant association of a single SNP marker (rs135551) with MI and a trend for association of the adjacent SNP (rs135543). Moreover, we identified a common three marker haplotype (hap2), consisting of the rare alleles of rs135551, rs135543 and rs135539, that was significantly associated with the disease phenotype. Consecutively, we were able to confirm our results in an independent second case-control sample. Here, we detected significant association with MI for the same two single SNP markers, rs135551 and rs135543, and hap2. Hap2 is over-represented in control individuals, thus it suggests protective properties against MI with an OR of 0.80 ($P=0.034$, 95% CI=0.65-0.98).

The mechanisms by which hap2 or its single marker variants influence the atherosclerotic process remain unclear. *PPAR α* is extensively expressed in vascular cells, such as endothelial and smooth muscle cells or monocytes/macrophages [27]. *PPAR α* activation prompts anti-inflammatory effects at multiple stages of the atherosclerotic pathway [28-30]. Thus, polymorphisms in the *PPAR α* gene may affect this sequence via a functional mutation resulting for example in a defective protein or altered trans-activation or -repression potentials.

However, the genetic variations found in this study to be associated with the disease phenotype are positioned in intron 2. They neither insert a potential alternative splice site (visual checking for GT-AG consensus sites) nor do they include a consensus sequence for binding of an established transcription factor (analyzed by TRANSFAC 7.0 Public) [31]. It is therefore unlikely that one of these SNPs itself represents the causal mutation. We assume that an untested proximate marker in LD with hap2 might be a functional variant. SNP markers of hap2 are part of a weak but distinct LD block covering the promoter region and exon 1 to intron 2 as shown by HapMap data. Marker rs135557, located upstream to transcription start site, is not associated with MI in our study. Hence, the postulated causal mutation could be located between the core promoter region and intron 2 of the *PPAR α* gene, but is unlikely to be located

within the neighbouring gene. A possible effect of such a functional variant could be altered gene expression, as seen with the exonic *PPAR α* L162V missense mutation, where the V allele possesses higher transcriptional activation in vitro [6;32]. In our study, the L162V variant did not show any association with MI. Other studies examining the impact of the L162V variant on cardiovascular disease have provided inconsistent results. In the LOCAT study, the V162 allele was associated with reduced progression of angiographically assessed diffuse atherosclerosis, whereas the prospective Northwick Park Heart Survey found no impact on the L162V variant on the risk for ischemic heart disease [10]. In a study by Tai et al., 827 men from the Veterans Affairs HDL Intervention trial (VA-HIT) showed a reduction of cardiovascular events in V162 carriers only in the presence of insulin resistance or diabetes mellitus [33].

Some possible limitations should be considered. MI cases from both, the initial and the second sample, were recruited from the Regensburg MI Family Study. Our sampling strategy included MI families from all over Germany with at least two living siblings where the index patient was affected with MI under the age of 60 years and the sibling suffered from MI or severe coronary artery disease. The use of our index criteria could possibly introduce a bias for MI survival and familial form of the disease. However, the fact that all MI cases are from unrelated families of Caucasian ethnicity from all parts of Germany excludes a geographical selection bias. Most important, replication of our results with an independent control group supports the validity of our findings. Yet, we cannot support our findings with prospective data from an unselected sample and hence our results may not be generalizable to the general population.

A second possible limitation is the number of female MI patients included in our study. It is very probable that the lack of statistical power in the female subsample can be attributed to the lower number (~30% of the male subgroup). However, gender-specific effects are unlikely because both, allelic and genotype association tests point to the same direction and have the same order of magnitude, i.e. relative risk reduction of about 20% in male and female MI patients. Furthermore, in none of the previously published studies gender-specific effects could be observed [10;33;34].

We anticipate the possibility that our findings may represent a “false positive” result – the probably most common problem of many genetic association studies [35]. In

particular, our association results did not withstand adjustments for multiple testing. However, we were able to conduct an independent confirmation study that corroborated our initial findings, which suggest our findings are not a type 1 statistical error. To place our findings in context, there have been two recently conducted whole genome association studies on MI and CAD both employing the Affymetrix GeneChip® Human Mapping 500K Array Set [36]. However, this chip provided no coverage of the region between rs135557, rs135551, and rs135543, where we saw the association signal [36]. In these studies, the remaining *PPAR α* gene region was covered by a total of 12 SNP markers, four of which were also included in our work (rs4253662, rs12330015, rs4253754, and rs4253778). Neither the German “Cardiogenics” MI sample nor the British “WTCCC” MI/CAD sample revealed association with any of these SNP markers at a *P*-value <0.05 [36]. Therefore, the results from these large whole genome screens neither support nor impair our findings.

In conclusion, our results suggest that genetic variations in the *PPAR α* gene, in addition to established genetic and environmental factors, may influence the risk of MI in a European population. Our findings support further investigations to address the role of *PPAR α* in cardiovascular disease and in the future detailed analysis of this gene may be warranted.

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TABLES

Table 1. Anthropometric data of 806 individuals from the initial sample and 1,529 individuals from the second sample

	Initial sample			Second sample		
	MI Cases (n=433)	Controls (n=373)	P	MI Cases (n=667)	Controls (n=862)	P
Male gender (%)	73.2	69.2	n.s.	74.2	70.6	n.s.
Age at first MI in years (range)	49.7 ± 7.5 (25-60)	-	-	50.3 ± 8.5 (24-69)	-	-
Age at inclusion in years (range)	59.4 ± 9.4 (35-82)	59.7 ± 9.4 (32-91)	n.s.	57.6 ± 9.0 (29-82)	50.2 ± 11.9 (25-69)	<0.001
Systolic BP (mmHg)	134 ± 18	136 ± 18	n.s.	138 ± 19	137 ± 19	n.s.
Diastolic BP (mmHg)	81 ± 10	82 ± 10	0.043	83 ± 11	83 ± 11	n.s.
Hypertension (%)	47.7	52.4	n.s.	51.6	43.7	0.003
Antihypertensive therapy (%)	86.6	42.4	<0.001	87.1	15.8	<0.001
Total cholesterol (mg/dl)	225 ± 43	239 ± 42	<0.001	228 ± 48	237 ± 45	<0.001
LDL cholesterol (mg/dl)	143 ± 36	150 ± 33	0.031	156 ± 46	148 ± 43	0.001
HDL cholesterol (mg/dl)	52 ± 14	57 ± 15	<0.001	49 ± 13	51 ± 16	0.003
Triglycerides (mg/dl)	162 ± 92	182 ± 108	0.006	n.a.	n.a.	n.a.
Lipid lowering therapy (%)	64.2	10.7	<0.001	64.8	3.0	<0.001
BMI (kg/m ²)	27.5 ± 3.9	26.9 ± 3.7	0.023	27.3 ± 3.5	27.2 ± 3.9	n.s.
Obesity (%)	21.7	17.4	n.s.	22.1	20.1	n.s.
Type 2 diabetes (%)	16.4	7.4	<0.001	15.1	3.5	<0.001
Ever Smoked (%)	74.1	59.5	<0.001	72.0	59.3	<0.001

Data are expressed as mean ± standard deviation. BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; n.a., not available; lipid lowering therapy is defined as regular intake of statins or fibrates, BMI, body mass index; obesity defined as BMI >30 kg/m²; hypertension defined as BP ≥140/90 mmHg; antihypertensive therapy is defined as regular intake of established blood pressure lowering drugs (diuretics, beta-blockers, angiotensin converting enzyme inhibitors, angiotensin 2-blockers, calcium channel blockers, vasodilators, alpha-blockers), type 2 diabetes defined as self-reported diabetes mellitus or regular intake of antidiabetic medication.

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Table 2. Summary of association analyses of the initial case-control sample

SNP	Genotype MI cases (n)				Genotype controls (n)				Allele 2 vs. Allele 1		Armitage's trend test	
	11	12	22	MAF	11	12	22	MAF	OR (95% CI)	P	OR (95% CI)	P
rs135557	129	220	80	0.44	100	185	85	0.48	0.86 (0.71-1.05)	0.141	0.86 (0.70-1.05)	0.137
rs135551	218	183	29	0.28	165	160	45	0.34	0.76 (0.62-0.94)	0.013	0.74 (0.61-0.94)	0.012
rs135543	197	194	40	0.32	158	158	54	0.36	0.83 (0.68-1.02)	0.079	0.81 (0.68-1.02)	0.082
rs135539	137	208	83	0.44	102	194	75	0.46	0.90 (0.74-1.09)	0.285	0.90 (0.73-1.09)	0.281
rs881740	330	93	7	0.12	299	69	3	0.10	1.26 (0.92-1.73)	0.142	1.30 (0.92-1.70)	0.141
rs4253662	357	63	4	0.08	289	56	8	0.10	0.81 (0.57-1.14)	0.215	0.79 (0.59-1.14)	0.234
rs4253681	301	121	3	0.15	279	86	3	0.13	1.23 (0.92-1.64)	0.160	1.23 (0.93-1.70)	0.139
rs7364220	260	148	18	0.22	246	114	11	0.18	1.23 (0.96-1.57)	0.104	1.24 (0.96-1.59)	0.100
rs12330015	337	79	2	0.10	302	62	3	0.09	1.08 (0.77-1.51)	0.656	1.06 (0.77-1.53)	0.651
rs4823613	208	181	35	0.30	196	147	24	0.27	1.16 (0.93-1.50)	0.181	1.17 (0.93-1.46)	0.176
rs5766741	217	170	36	0.29	196	152	21	0.26	1.12 (0.90-1.40)	0.303	1.17 (0.90-1.41)	0.298
rs4253728	217	179	34	0.29	198	151	21	0.26	1.14 (0.92-1.42)	0.238	1.17 (0.92-1.44)	0.230
rs1800206	380	45	2	0.06	328	42	1	0.06	0.97 (0.64-1.47)	0.870	0.99 (0.64-1.47)	0.871
rs4253754	265	147	15	0.21	239	118	12	0.19	1.10 (0.86-1.40)	0.460	1.09 (0.86-1.42)	0.452
rs4253760	274	133	17	0.20	240	114	14	0.19	1.03 (0.80-1.32)	0.841	1.03 (0.80-1.32)	0.842
rs4253778	272	137	15	0.20	238	113	15	0.20	1.01 (0.79-1.30)	0.937	0.99 (0.79-1.30)	0.937
rs11704979	324	96	6	0.13	294	71	5	0.11	1.18 (0.87-1.61)	0.287	1.16 (0.87-1.61)	0.287

For the *PPAR α* locus, 17 genotyped SNPs and their rs numbers from dbSNP database are shown. Numbers (n) for each genotype (11/12/22) and minor allele frequencies (MAF) are listed separately for MI cases and controls. Odds ratios (OR) with 95% confidence interval (CI) and P-values are given for allelic association and genotypic association based on the Armitage test for trend with significant P-values in bold print.

Table 3. Frequencies of hap1 – hap3, and association results with MI in the initial and second case-control samples

Haploblock A		hap1	hap2	hap3
Initial sample (n=708)*				
No of haplotypes (frequency)	MI Cases	424 (0.55)	197 (0.26)	104 (0.14)
	Controls	345 (0.53)	210 (0.32)	70 (0.11)
	OR (95% CI)	1.11 (0.78-1.59)	0.76 (0.56-1.02)	1.29 (0.90-1.84)
	<i>P</i>	0.549	0.067	0.167
Second sample (n=1488)*				
No of haplotypes (frequency)	MI Cases	727 (0.56)	312 (0.24)	198 (0.15)
	Controls	884 (0.53)	460 (0.27)	238 (0.14)
	OR (95% CI)	1.20 (0.93-1.55)	0.80 (0.65-0.98)	1.06 (0.84-1.33)
	<i>P</i>	0.159	0.034	0.643

P^{adj} *P*-value from multivariate model (adjusted for age, total cholesterol/HDL cholesterol levels, systolic blood pressure, body mass index, diabetes mellitus, smoking). *From the initial, second and matched sample n=98, n=41, and n=113 individuals were excluded due to improbable haplotype reconstruction. Significant *P*-values are presented in bold print.

Table 4. Summary of association analyses of the second case-control sample

SNP	Genotype MI cases (n)				Genotype controls (n)				Allele 2 vs. Allele 1		Armitage's trend test	
	11	12	22	MAF	11	12	22	MAF	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
rs135557	192	345	121	0.45	247	432	176	0.46	0.95 (0.82-1.10)	0.496	0.95 (0.82-1.10)	0.488
rs135551	365	253	49	0.26	407	359	67	0.30	0.85 (0.72-0.99)	0.047	0.87 (0.72-0.99)	0.046
rs135543	342	260	61	0.29	383	384	91	0.33	0.82 (0.70-0.96)	0.014	0.84 (0.71-0.96)	0.014
rs135539	214	334	116	0.43	246	430	175	0.46	0.88 (0.76-1.00)	0.078	0.87 (0.76-1.01)	0.075

For the *PPARα* locus, 4 genotyped SNPs and their rs numbers from dbSNP database are shown. Numbers (n) for each genotype (11/12/22) and minor allele frequencies (MAF) are listed separately for MI cases and controls. Odds ratios (OR) with 95% confidence interval (CI) and *P*-values are given for allelic association and genotypic association based on the Armitage test for trend with significant *P*-values in bold print.

LEGENDS TO FIGURES

Figure 1. Schematic representation of the *PPAR α* locus. **(A)** Location of *PPAR α* (NM_005036) and neighbouring genes in a region of ~147 kb on human chromosome 22q13.3. Exon positions are indicated as vertical lines. **(B)** Relative positions of genotyped SNPs. Position of the 17 SNPs and corresponding rs numbers from dbSNP are shown with arrows. **(C)** Relative positions of calculated haplotypes. Haploblocks are indicated as block A – D, where block A includes hap1 – hap3 focussed on in the paper. Haplotypes were determined from pair-wise LD comparisons with $r^2 > 0.4$ using PHASE version 2.1 [20;21]. **(D)** Pair-wise r^2 from HapMap release #20 data. Lighter shading indicates low values (low LD) and darker shading indicates higher values (strong LD). Haploblock A does not reach into the neighbouring gene FLJ27365.

Figure 2. Odds ratios for MI depending on the number of hap2 copies in the initial sample (with subjects carrying no hap2 copy as reference group). Individuals carrying 1 or 2 copies of the protective haplotype hap2 are at significantly reduced risk for MI as compared to individuals with no copy of hap2 (overall P -value=0.012). Complete haplotypes could be inferred from 708 individuals.

ABBREVIATIONS

PPAR α – Peroxisome proliferator activated receptor alpha

SNP – single nucleotide polymorphism

MI – myocardial infarction

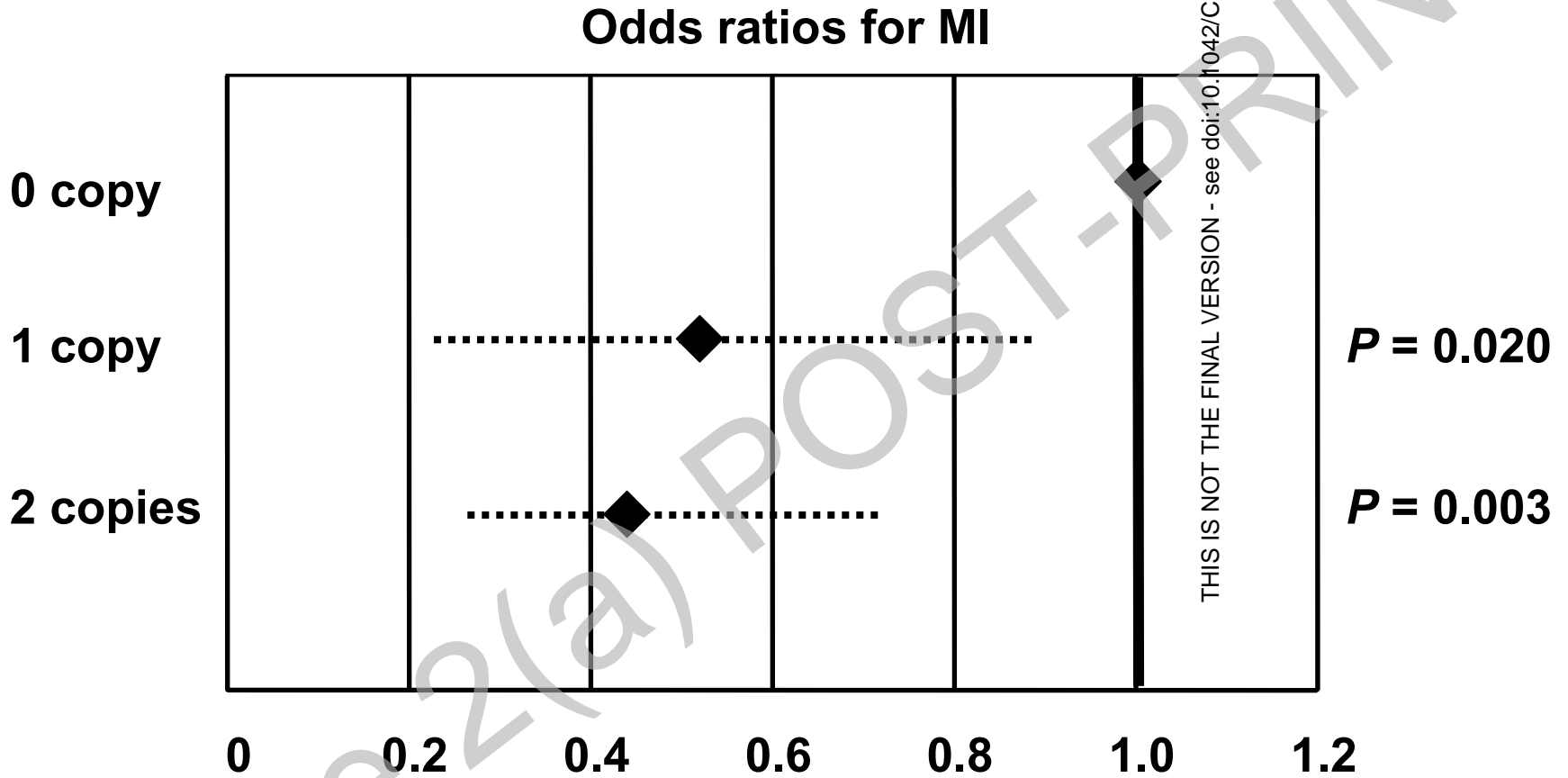
LD – linkage disequilibrium

CAD – coronary artery disease

OR - odds ratio

MAF - minor allele frequency

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Reinhard-W. Figure 2