Common polymorphisms in the cannabinoid CB2 receptor gene (*CNR2*) are not associated with myocardial infarction and cardiovascular risk factors

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Received March 3, 2008; Accepted April 18, 2008

DOI: 10.3892/ijmm_00000004

Abstract. Myocardial infarction (MI) is a complex disease. Multiple genes and their interaction with various environmental factors influence the pathogenesis of MI that is thought to be tightly regulated by inflammatory pathways. Recent progress in genetic analysis includes the use of large-scale genome-wide association studies that have proven to be powerful tools even in the analysis of multifactorial phenotypes. However, certain genes are only sparsely represented on the available gene chips and additional candidate gene approaches are necessary. One such example is the *CNR2* gene, encoding the cannabinoid receptor 2 (CB2), which has been implicated in mediating anti-inflammatory and anti-atherosclerotic effects *in vivo*. We therefore hypothesized that genetic variations within the *CNR2* gene are associated with the development of MI or classic cardiovascular risk factors. In a large case-control study, 1,968 individuals from the German MI family study were examined with 13 single nucleotide polymorphisms (SNPs) covering *CNR2* and the adjacent genes. The association of these SNPs with MI or cardiovascular risk factors, such as arterial hypertension, obesity, hypercholesterolemia and diabetes mellitus, was determined. In allelic and genotypic models, none of the SNPs showed a significant association with MI. Separate analyses for men and women revealed no gender-specific relationship between common genetic variations within the *CNR2* gene and MI. Moreover, no significant association between *CNR2* gene variants and common cardiovascular risk factors was observed. We therefore provide evidence in a large German population that common polymorphisms within the *CNR2* gene confer no susceptibility to MI or to cardiovascular risk factors.

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Key words: genetics, *CNR2*, myocardial infarction, cardiovascular risk factors, polymorphism

Introduction

Cardiovascular diseases continue to be the leading cause of death in high-income countries (1). Increasing evidence has emerged that inflammatory pathways play a key role in the pathogenesis of coronary artery disease (CAD) and other forms of atherosclerosis (2). CAD is regarded as a chronic inflammatory condition with its maximally exacerbated form of myocardial infarction (MI) following plaque rupture and thrombus formation (2,3). Chronic medical treatment of cardiovascular disease is mainly based on the optimisation of concomitant cardiovascular risk factors, such as arterial hypertension and hypercholesterolemia. However, the direct challenge of the atherosclerotic lesions themselves is less addressed by pharmaceutical therapy. In contrast, statins have been reported to exert favourable pleiotropic properties by mediating anti-inflammatory and immunomodulatory effects (4). Other therapeutics that delay atherosclerotic disease progression, such as plaque stabilizers or immuno-suppressants, are under development and intensive research for pharmaceuticals with anti-inflammatory properties is being carried out (5,6).

Cannabinoids have been known for their anti-inflammatory and immunomodulatory potential for a long time (7,8) and have been introduced in treating autoimmune disorders, such as multiple sclerosis or rheumatoid arthritis (9,10). Recently, low dose oral cannabinoid treatment has been shown to reduce atherosclerosis progression in the apolipoprotein E knockout mouse model (11). The effects of inhibited macrophage chemotaxis and reduced lymphoid cell proliferation seem to be mediated via the cannabinoid

receptor 2 (CB2) (12). CB2 is primarily expressed in cells of the immune system, such as B and T cells and monocytes (12,13) and is found in human and mice atherosclerotic plaques (11). Its blockade by a selective antagonist completely reversed the anti-atherosclerotic effects *in vivo* (11) and, thus, it stands to reason that CB2 is involved in the pathogenesis of atherosclerosis.

These data qualify *CNR2* as a notable candidate gene for CAD. Moreover, recent genetic analyses implicated a common exonic *CNR2* gene polymorphism with systolic and diastolic blood pressure in Japanese men (14). However, *CNR2* is not represented on the readily available genome-wide single nucleotide polymorphism (SNP) arrays (like Illumina Human Hap 300/550 Genotyping BeadChip and Affymetrix GeneChip Human Mapping 500 K array). HapMap data of the gene is scarce, thus a comprehensive analysis of the genomic region is warranted. Therefore, we evaluated 13 common genetic variations within and near the *CNR2* gene concentrating on those with a possible impact on gene function. We assessed their association with MI and common cardiovascular risk factors carrying out a case-control association analysis in 1,968 individuals from the German MI family study.

Materials and methods

Study population. All MI patients were participants of the German MI family study. Selection criteria and study details were described previously (15,16). In brief, we identified MI families from all parts of Germany with the accumulation of premature MI and/or severe CAD, e.g. percutaneous coronary intervention or coronary bypass surgery. The index patient always suffered from MI before the age of 60 years. The diagnosis of MI was established by a 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). Control individuals were recruited from unaffected spouses of MI family members who had no genetic relationship to the cases.

The present case-control study included 1,040 unrelated MI patients and 928 unrelated, unaffected control subjects (cardiovascular disease-free married-in spouses, sisters-in-law, and brothers-in-law). Individuals with a history of CAD, cerebrovascular accidents (transient ischemic attack or stroke) or peripheral artery disease were excluded from the control group. Written informed consent was obtained from each of the subjects and the local Ethics Committee approved the research protocol.

Definition of cardiovascular risk factors. Individuals with a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or regular intake of established blood pressure lowering drugs (diuretics, ß-blockers, angiotensin converting enzyme inhibitors, angiotensin 2-blockers, calcium channel blockers, vasodilators, α-blockers) were classified as hypertensive. Individuals who reported a current or former smoking habit were classified as smokers. Hypercholesterolemia was defined as low density lipoprotein (LDL) cholesterol ≥160 mg/dl or use of lipid lowering therapy (statins, fibrates). Diabetes was defined as self-reported diabetes mellitus or the regular intake of anti-diabetic medication. Obesity was defined as a body mass index (BMI) ≥30 kg/m^2.

Phenotyping. At recruitment, each of the patients was studied with a standardized interview, clinical examination and biochemical as well as molecular analyses, as previously described (16-18). A validation of cardiovascular events at study entry was performed by a review of medical records. Resting blood pressure was taken according to the MONICA guidelines after participants had been resting in a sitting position (19). Body weight in kilograms and height in meters were determined with subjects wearing light clothing. Asservation of serum was carried out from non-fasting individuals. Serum levels of low- and high-density lipoprotein cholesterol were measured by standard enzymatic methods (20). Baseline characteristics of all the individuals are shown in Table I.

SNPs and genotyping methods. Among several polymorphisms previously identified within *CNR2*, we focused on those with a possible impact on gene function and used an exon-centred approach in our SNP selection with a dense coverage of the coding second exon. Other selection criteria for the 13 SNPs were: i) minor allele frequency (MAF) of >0.05 in a Caucasian population according to HapMap Project http://www.hapmap.org/ (21), ii) compatibility with our genotyping platform and iii) evidence of validation status. To determine the extent of LD beyond the boundaries of the gene, SNPs in the 5' and 3' flanking sequences and the neighbouring genes were included.

Genomic DNA was extracted from EDTA blood samples by standard procedures. Pre-designed and custom-made Taq Man® SNP genotyping assays (Applied Biosystems) were used (Table II). PCR reaction and post-PCR endpoint plate read was carried out according to the manufacturer's instructions using the Applied Biosystems 7900HT real-time PCR system. Sequence detection system software (SDS 2.2) was used to assign genotypes applying the allelic discrimination test performed by a person blinded to the affection status (22). Case and control DNA was genotyped together on the same plate. Duplicates of samples (15%) were employed to assess intra-plate and inter-plate genotyping quality. There were no discrepancies between the duplicates. The overall genotyping call-rate was >98% for all SNPs. For the association analyses, individuals with <10% genotypes were excluded (n= 27); the genotyping rate in the remaining individuals was 99.8%.

Statistical analyses. To determine whether the genotypes of cases and controls of all *CNR2* SNPs deviated from the Hardy-Weinberg equilibrium, actual and predicted genotype counts of cases and controls were compared by the exact test (23). Differences in allele frequencies between the MI cases and the controls were analyzed employing the χ^2-test. To account for an uneven distribution of baseline factors across genotypes, a logistic regression model with genotype, age and gender as explanatory variables was developed. Genotypes were coded for the dominant and recessive effect. Odds ratios (OR) with their 95% confidence intervals (CI)

Table I. Anthropometric data of the study population.

	All individuals (n=1,968)			Males (n=1,072)			Females (n=896)		
	MI cases (n=1,040)	Controls (n=928)	P	MI cases (n=752)	Controls (n=320)	P	MI cases (n=288)	Controls (n=608)	P
Male gender (%)	72.3	34.5	<0.001	-	-	-	-	-	-
Age at first MI in years (range)	50.8±8.0 (24-65)	-	-	49.8±7.8 (24-64)	-	-	53.2 ± 8.0 (26-65)	-	-
Age at inclusion in years (range)	58.4±8.3 (29-82)	56.0±9.2 (29-74)	<0.001	57.9±8.6 (29-82)	58.1±9.7 (32-74)	n.s.	59.7±7.5 (36-74)	55.0±8.8 (29-69)	<0.001
Systolic BP (mmHg)	138±20	133±18	<0.001	136±18	137±17	n.s.	142±22	131±18	<0.001
Diastolic BP (mmHg)	82±10	82±10	n.s.	82±10	83±10	n.s.	83±10	81±10	0.048
Total cholesterol (mg/dl)	227±46	238±43	<0.001	226±46	236±43	<0.001	232±45	238±44	n.s.
LDL cholesterol (mg/dl)	152±43	146±35	0.002	152±43	148±34	n.s.	151±43	146±36	0.038
HDL cholesterol (mg/dl)	50±13	61±15	<0.001	48±12	54±14	<0.001	56±15	64±15	<0.001
Hypercholesterolemia (%)	82.9	39.0	<0.001	81.2	42.5	<0.001	84.7	37.2	<0.001
Lipid lowering therapy (%)	64.7	8.5	<0.001	63.6	10.0	<0.001	67.7	7.7	<0.001
BMI (kg/m^2)	27.3±3.6	26.7±4.2	<0.001	27.3±3.2	27.2±3.4	n.s.	27.3±4.4	26.4±4.5	0.003
Obesity (%)	20.9	18.5	n.s.	18.8	17.2	n.s.	26.2	19.2	0.018
Hypertension (%)	51.7	47.8	n.s.	49.0	52.7	n.s.	59.0	45.1	<0.001
Anti-hypertensive therapy (%)	87.5	34.9	<0.001	86.3	37.5	<0.001	90.6	33.6	<0.001
Type 2 diabetes (%)	15.9	5.2	<0.001	14.4	7.2	0.001	19.8	4.1	<0.001
Smoking (%)	70.8	50.9	<0.001	77.4	71.9	n.s.	53.5	39.9	<0.001

Data are expressed as mean ± standard deviation. BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; BMI, body mass index; hypercholesterolemia is defined as low density lipoprotein (LDL) cholesterol ≥160 mg/dl or use of lipid lowering therapy (statins, fibrates); obesity is defined as BMI >30 kg/m^2; hypertension is defined as BP ≥140/90 mmHg or taking antihypertensive therapy which is defined as the regular intake of established blood pressure lowering drugs (diuretics, ß-blockers, angiotensin converting enzyme inhibitors, angiotensin 2-blockers, calcium channel blockers, vasodilators, α-blockers); type 2 diabetes is defined as self-reported diabetes mellitus or the regular intake of antidiabetic medication and smoking is defined as a current or former smoking habit.

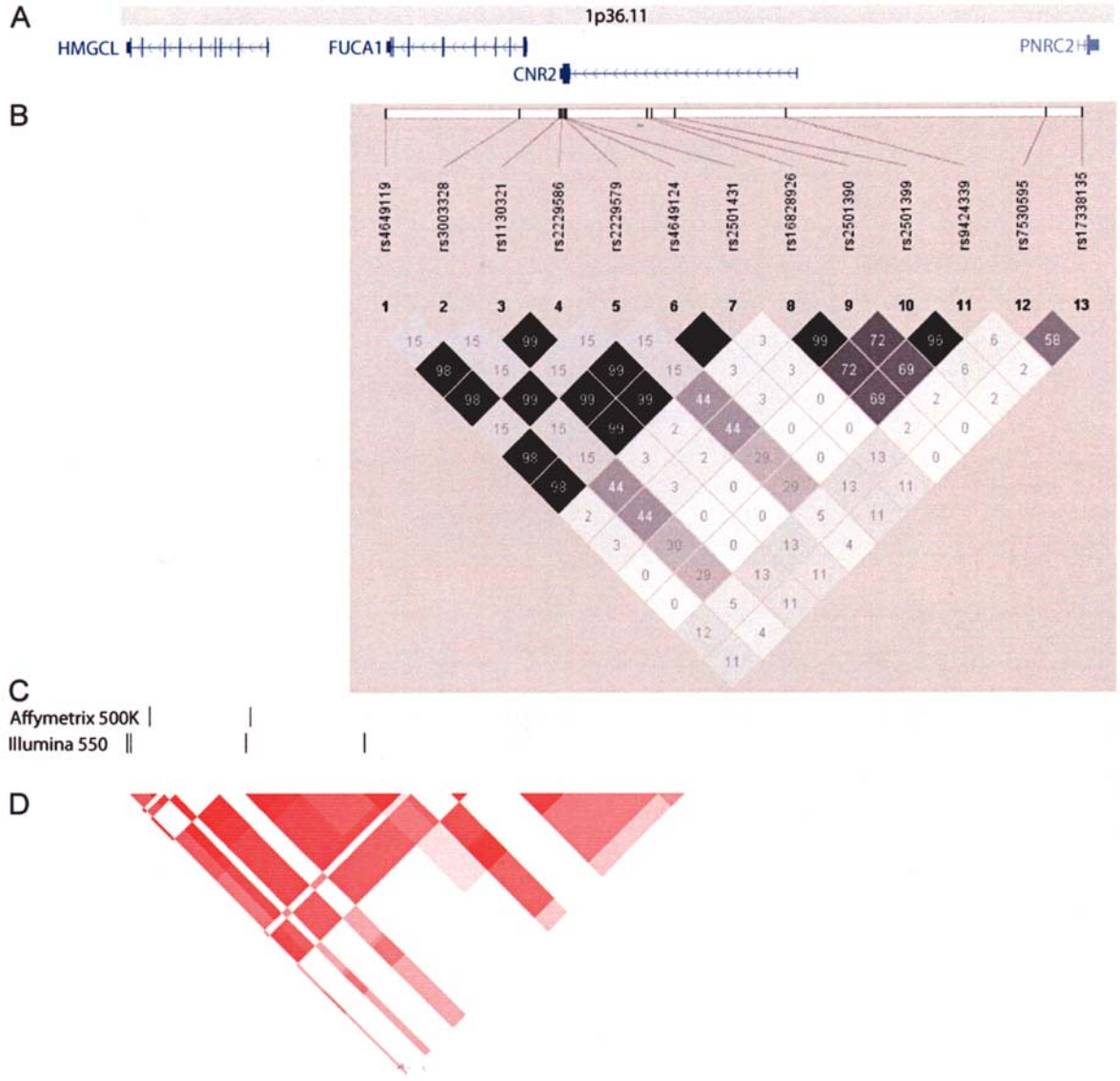

Figure 1. A schematic representation of the *CNR2* gene locus and its LD pattern. (A) The ~165 kb genomic region on chromosome 1p36.11 contains *CNR2* and neighbouring genes (*HMGCL*, 3-hydroxylmethyl-3-methylglutaryl-Coenzyme A; *FUCA1*, α-L fucosidase; *PNRC2*, proline-rich nuclear receptor co-activator 2). *CNR2* is oriented from right to left. Horizontal lines correspond to introns and vertical bars depicting the exons. (B) Distribution of the 13 genotyped SNPs and representation of LD structure in the *CNR2* region. Pairwise r^2-values (multiplied by 100) between markers are shown. Black denotes perfect LD with $r^2=1$; white denotes no LD with $r^2=0$; grey denotes r^2 intermediate LD between 0 and 1. Note that there is a region of strong LD between SNPs rs4649119 and rs2501431 including the *CNR2* coding region. (C) Coverage of depicted genomic region with the SNP marker from common genome-wide SNP arrays. SNP markers represented on the Affymetrix GeneChip Human Mapping 500 K array set and Illumina HumanHap 550 Genotyping BeadChip are shown as vertical lines. Note that no array SNP marker is present in the *CNR2* gene. (D) LD structure from HapMap phase II data (release 21).

from the Wald χ^2-test are reported. A two-sided P-value of <0.05 indicated statistical significance.

Furthermore, associations between *CNR2* SNPs and cardiovascular risk factors were analysed employing the χ^2-test for differences in allele frequencies. Risk factors were treated as qualitative traits with either the presence or absence of the appropriate risk factor definition. To test for the association of rs2501431 and arterial hypertension, additional analyses were performed. Blood pressure levels between the three genotypes were compared by a one-way analysis of variance and the Turkey-HSD *post hoc* test. Descriptive P-values of a 2-tailed t-test for independent samples are reported for the dominant (GG + AG vs. AA) and recessive (GG vs. AA + AG) model. Moreover, logistic regression models for a dominant and recessive effect on the prevalence of hypertension with adjustment for age, gender and anti-hypertensive therapy were performed. Analyses were carried out using JMP IN 5.1 (SAS Institute Inc., Cary, NC, USA), SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and plink v0.99s (24).

LD structure in the *CNR2* region was determined from pairwise r^2-values between markers calculated with the program Haploview (http://www.broad.mit.edu/mpg/haploview (25).

Results

Association analyses of CNR2 variants with MI. The *CNR2* gene consists of two exons, whereby only exon 2 translates into the CB2 protein. We genotyped 13 SNPs covering a region of ~120 kb focusing on *CNR2* exon 2 and including six SNPs

Table II. Characteristics of genotyped CNR2 SNP markers.

SNP (dbSNP)	Position on chr. 1 (hg18)	Function	Call rate (1,968 individuals)	Flanking sequence
rs4649119	24,044,130	3' near *FUCA1*	98.93%	AAGCTATAAGCAGTATAAGTTGAAA(C/G)TAGGAAAAGAAAGCTATACCATGTT
rs3003328	24,066,413	Intron *FUCA1*	99.75%	AGTTCTCAGAGAGAGTCAGACCCTTC(C/T)TGGACACTTCCTACGTAAAAGGGAT
rs1130321	24,073,111	3' UTR *CNR2*	99.34%	AGTCACCCTTGCCACTGAGGACCGAG(A/G)ACTATGCTATGATGAGGATTAAGGT
rs2229586	24,073,490	3' UTR *CNR2*	98.88%	AGTCCCAGACACCTAGACACGGACCC(C/G)TTTTTGCTGATGAGTGTTGGGACTG
rs2229579	24,073,749	H316Y *CNR2*	99.29%	AGGCCCCTCACACACTTCTTCCAGT(A/G)AGCCAGGCAGTGATGGGCAGAGGAG
rs4649124	24,073,944	L251L *CNR2*	99.24%	AACCAACAGATGAGGAGCACAGCCA(A/G)CACTAGCCCTAGGGTCTTGGCCAAC
rs2501431	24,074,230	G155G *CNR2*	98.98%	CTAGTGCTGAGAGGACCCACATGAT(A/G)CCCAGGGTCACCAGTGCCCTTCCAC
rs16828926	24,087,717	Intron 1 *CNR2*	99.19%	TACAGATGTAAGAGTAAAAGAGAAGC(A/G)GGAGTCTTTCTGAACCTATTCTGGG
rs2501390	24,088,383	Intron 1 *CNR2*	99.14%	AATCTCTCAGAGCTGGAGGTAAGGA(A/G)GAGAATGATACCCTGCCTGTGGCTA
rs2501399	24,092,345	Intron 1 *CNR2*	99.19%	TTCCTTCTGCATCATCATCTCCAGC(A/C)TCTCTCCTGGGCAGAGCAGAGCTAA
rs9424339	24,110,549	Intron 1 *CNR2*	99.39%	AGGACCCAGGAGCCTCCCTCACGCTT(A/T)TGTTCTGTGTGTCCTCTGGGTCAAT
rs7530595	24,153,693	5' near *PNRC2*	99.14%	TGGTCTTACATGTAAATAGATGGCTC(C/T)GATATACCATTATTTTGGGGGGAGT
rs17338135	24,159,484	Intron 1 *PNRC2*	98.93%	AGACATTTTTATATATTTAAGATGGG(C/T)TTAGACTGTTAGGGCTTGTGAGAAC

encompassing the 3' and 5' UTR and neighbouring genes (Fig. 1, Table II). All of the 13 SNPs met the Hardy-Weinberg expectations (P=0.05 level, exact test). Strong linkage disequilibrium (LD) was present between SNPs rs4649119 and rs2501431 including the *CNR2* coding region as previously shown by Karsak *et al* and supported by HapMap data (Fig. 1) (21,26).

We found no statistically significant differences of allele and genotype distributions between the MI cases and the controls (Table III). Likewise, no significant association between the 13 *CNR2* SNPs and MI was observed in the dominant and recessive genetic model (Table III). To test for gender-specific influences of *CNR2* variants on MI, we performed analyses in men and women separately, where no gender-specific effects could be detected (data not shown).

Association analyses of CNR2 variants with cardiovascular risk factors. We investigated whether the *CNR2* variants conferred susceptibility to classic CAD related risk factors such as obesity, arterial hypertension, hypercholesterolemia and diabetes mellitus.

A comparison of the allele frequencies between the individuals with and without the respective quantitative trait showed no significant effect of the *CNR2* variants on the prevalence of obesity, arterial hypertension and diabetes mellitus (Table IV). Only hypercholesterolemia displayed a marginal significant association with rs3003328 (P=0.045) and rs2229586 (P=0.044). However, the two SNPs are in strong LD (r^2=0.997) and the two have a relatively low MAF (for rs3003328 and rs2229586 in cases and controls 0.097 and 0.109 as well as 0.096 and 0.107, respectively).

In particular, rs2501431, previously shown to be associated with blood pressure levels in Japanese men (14), displayed in our study population no significant difference in the prevalence of arterial hypertension dependent on the genotype (Table V). The exclusion of individuals with anti-hypertensive medication or adjustment for medication intake, age and gender in a logistic regression model did not influence this finding (Table V). Moreover, systolic or diastolic blood pressure levels were not significantly influenced by the rs2501431 variant regardless of whether a comparison of the genotypes applied or a dominant or recessive model was assumed (Table V).

Discussion

In recent years, increasing evidence has been presented highlighting the role of inflammation in the pathogenesis of atherosclerosis and its complications (2). The early atherosclerotic lesions are dominated by immune cells and disease progression is accelerated by their effector molecules (3). The cannabinoids are well recognized for their anti-inflammatory properties (8). Recently, CB2 was found in human atherosclerotic plaques, where it exhibited clear anti-atherosclerotic effects (11) and therefore represents a good candidate gene for CAD.

In the present case-control association study, we comprehensively evaluated the relationship of 13 common genetic variations within the *CNR2* gene with MI and their potential influence on the established cardiovascular risk

Table III. Summary of association analysis between CNR2 SNPs and myocardial infarction.

	MI cases (n=1,040)			Controls (n=928)			Difference in allele frequency		Dominant model[a]		Recessive model[a]	
SNP	Genotype	MAF	HWE	Genotype	MAF	HWE	(Allele 2 vs. 1)		(Genotype 22+12 vs. 11)		(Genotype 22 vs. 11+12)	
(dbSNP)	11/12/22 (n)	(%)	P	11/12/22 (n)	(%)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
rs4649119	333/508/180	42.5	0.609	301/457/157	42.1	0.498	1.02 (0.89-1.15)	0.813	1.08 (0.88-1.32)	0.492	1.12 (0.87-1.45)	0.375
rs3003328	831/183/9	9.8	0.861	738/164/16	10.7	0.058	0.91 (0.74-1.12)	0.382	0.98 (0.77-1.25)	0.879	0.58 (0.24-1.41)	0.228
rs1130321	339/505/179	42.2	0.749	302/461/154	41.9	0.343	1.01 (0.89-1.15)	0.875	1.05 (0.85-1.29)	0.656	1.15 (0.89-1.49)	0.284
rs2229586	336/504/178	42.2	0.700	300/462/153	42.0	0.278	1.01 (0.89-1.15)	0.864	1.06 (0.86-1.30)	0.607	1.15 (0.89-1.48)	0.295
rs2229579	831/181/9	9.8	1.000	736/163/16	10.7	0.056	0.91 (0.74-1.12)	0.350	0.97 (0.76-1.25)	0.834	0.58 (0.24-1.40)	0.227
rs4649124	339/505/179	42.2	0.749	300/461/154	42.0	0.342	1.01 (0.89-1.14)	0.921	1.04 (0.84-1.27)	0.734	1.15 (0.89-1.48)	0.300
rs2501431	339/505/179	42.2	0.749	301/462/153	41.9	0.309	1.01 (0.89-1.15)	0.871	1.05 (0.85-1.29)	0.649	1.16 (0.89-1.50)	0.269
rs16828926	744/249/24	14.6	0.531	655/238/23	15.5	0.801	0.93 (0.78-1.11)	0.434	0.95 (0.76-1.18)	0.632	1.30 (0.70-2.43)	0.409
rs2501390	746/250/23	14.5	0.705	657/234/25	15.5	0.449	0.93 (0.78-1.11)	0.395	0.98 (0.79-1.22)	0.852	1.06 (0.57-1.98)	0.848
rs2501399	685/302/35	18.2	0.833	597/286/35	19.4	0.916	0.93 (0.78-1.09)	0.343	0.95 (0.78-1.17)	0.636	0.97 (0.57-1.62)	0.892
rs9424339	683/304/35	18.3	0.835	598/285/32	19.1	0.831	0.95 (0.81-1.12)	0.537	0.94 (0.77-1.15)	0.533	1.05 (0.62-1.79)	0.852
rs7530595	322/506/192	43.6	0.799	305/439/173	42.8	0.501	1.03 (0.91-1.18)	0.605	1.09 (0.88-1.34)	0.433	0.92 (0.72-1.17)	0.484
rs17338135	329/492/198	43.6	0.567	287/447/184	44.4	0.689	0.97 (0.85-1.10)	0.609	0.95 (0.77-1.17)	0.614	0.97 (0.76-1.23)	0.790

For the *CNR2* locus, 13 genotyped SNPs and their rs numbers from the 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. HWE denotes testing of deviation from the Hardy-Weinberg equilibrium for each patient group. Odds ratios (OR) with 95% confidence interval (CI) and P-values are given. [a]P-value from logistic regression analysis for the dominant and recessive model adjusted for age and gender.

Table IV. Summary of association analysis between CNR2 SNPs and cardiovascular risk factors.

	Obesity		Hypertension		Hypercholesterolemia		Diabetes mellitus	
	affected (n=387)	unaffected (n=1,572)	affected (n=1,493)	unaffected (n=475)	affected (n=1,224)	unaffected (n=744)	affected (n=213)	unaffected (n=1,755)
SNP	Allele 2 vs. 1		Allele 2 vs. 1		Allele 2 vs. 1		Allele 2 vs. 1	
(dbSNP)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
rs4649119	1.12 (0.96-1.31)	0.164	1.07 (0.93-1.25)	0.353	1.06 (0.93-1.20)	0.411	1.05 (0.86-1.29)	0.627
rs3003328	1.15 (0.89-1.47)	0.290	1.10 (0.86-1.40)	0.458	0.81 (0.66-1.00)	0.045	0.93 (0.67-1.31)	0.695
rs1130321	1.13 (0.96-1.32)	0.145	1.08 (0.93-1.25)	0.304	1.05 (0.92-1.20)	0.485	1.06 (0.87-1.30)	0.551
rs2229586	1.13 (0.96-1.32)	0.148	1.09 (0.94-1.27)	0.237	1.04 (0.91-1.18)	0.584	1.06 (0.86-1.30)	0.594
rs2229579	1.16 (0.90-1.49)	0.252	1.08 (0.85-1.38)	0.531	0.81 (0.65-0.99)	0.044	0.94 (0.67-1.33)	0.738
rs4649124	1.13 (0.96-1.32)	0.136	1.08 (0.93-1.26)	0.290	1.05 (0.92-1.19)	0.498	1.06 (0.86-1.30)	0.579
rs2501431	1.13 (0.97-1.33)	0.129	1.08 (0.93-1.25)	0.311	1.05 (0.92-1.20)	0.477	1.06 (0.87-1.30)	0.565
rs16828926	1.00 (0.80-1.25)	0.989	0.91-0.75-1.12)	0.385	0.88 (0.74-1.06)	0.173	0.95 (0.71-1.26)	0.703
rs2501390	0.99 (0.79-1.23)	0.903	0.92 (0.76-1.13)	0.441	0.87 (0.72-1.03)	0.111	0.96 (0.73-1.28)	0.797
rs2501399	0.94 (0.76-1.15)	0.537	0.97 (0.81-1.17)	0.774	0.86 (0.73-1.02)	0.081	1.08 (0.84-1.39)	0.553
rs9424339	0.93 (0.76-1.14)	0.487	1.02 (0.84-1.23)	0.854	0.86 (0.73-1.02)	0.079	1.11 (0.86-1.43)	0.429
rs7530595	1.10 (0.94-1.29)	0.224	1.07 (0.93-1.25)	0.343	0.99 (0.87-1.13)	0.901	1.07 (0.87-1.31)	0.525
rs17338135	0.98 (0.83-1.15)	0.771	0.95 (0.82-1.10)	0.483	1.01 (0.88-1.15)	0.939	0.94 (0.76-1.15)	0.534

Odds ratios (OR) with 95% confidence interval (CI) and P-values are given.

Table V. Summary of association analysis between rs2501431 and arterial hypertension.

	rs2501431 genotype				Dominant model		Recessive model	
	AA	AG	GG	P	GG + AG	P	AA + AG	P
Hypertension (%) (n=1968)	74.3	76.4	76.9	n.s.[a]	76.5	0.862[b]	75.5	0.996[b]
Systolic blood pressure (mmHg)	134.8±20.2	136.0±17.6	136.0±19.8	n.s.[c]	136.0±18.1	0.220[d]	135.5±18.6	0.698[d]
Diastolic blood pressure (mmHg)	81.9±10.0	80.0±9.7	82.8±10.3	n.s.[c]	82.2±9.8	0.612[d]	82.0±10.0	0.202[d]
Anti-hypertensive therapy (%)	60.2	63.3	65.2	n.s.[a]	63.8	0.124[a]	62.1	0.293[a]
BP ≥140/90 mmHg without anti-hypertensive therapy (%) (n=728)	35.3	35.6	33.6	n.s.[a]	35.1	0.957[a]	35.5	0.704[a]

Data for systolic and diastolic blood pressure are mean ± SD. [a]P-value from χ^2-test with 2x2 comparisons between genotypes and for the dominant (GG + AG vs. AA) and recessive (GG vs. AA + AG) model; n.s. denotes no statistical significance between any two of the three genotypes. [b]P-value from logistic regression analysis for the prediction of prevalent hypertension in the dominant (GG + AG vs. AA) and recessive (GG vs. AA + AG) models adjusted for anti-hypertensive therapy, age and gender. [c]P-values from one-way analysis of variance and Turkey-HSD post hoc test; n.s. denotes no statistical significance between any two of the three genotypes. [d]Descriptive p-value of a 2-tailed t-test for independent samples for the dominant (GG + AG vs. AA) and recessive (GG vs. AA + AG) model.

factors such as obesity, arterial hypertension, hypercholesterolemia and diabetes mellitus. However, we found no significant association of common *CNR2* sequence variants with MI. Moreover, our results indicate no relevant influence of the investigated *CNR2* polymorphisms on classic cardiovascular risk factors. In contrast to the results of Yamada *et al* where Japanese men without anti-hypertensive therapy carrying the rare GG genotype of rs2501431 demonstrated significantly higher systolic and diastolic blood pressure levels than those with the AA or AG genotype (14), we did not see an association of the exon SNP rs2501431 with prevalent arterial hypertension or systolic and diastolic blood pressure levels in our study population. Besides the varying ethnic background, a possible explanation for this discrepancy may be the different recruitment strategies of the study populations. While Yamada *et al* examined a population-based cohort randomly selected from residents of two cities in central Japan, our ascertainment approach was to recruit individuals with a strong familial history of CAD from all over Germany with a concomitant accumulation of classic cardiovascular risk factors including arterial hypertension.

Our study has several strengths. Although we report a negative association result, the study is characterized by stringent criteria for study design together with a careful phenotypic and genotypic assessment on the basis of a biologically plausible hypothesis. Furthermore, the candidate gene approach is designed to allow a comprehensive analysis of the complete *CNR2* gene region and to largely exclude that a major effect from variations in this gene was missed. Although unbiased large-scale genome-wide association studies using several thousands of SNP markers have only recently become available [and proven to identify genomic regions for susceptibility of complex phenotypes such as CAD and MI (27-29)], they do not fully cover all genes. Specifically, the *CNR2* gene is not covered by Illumina HumanHap 300/550 Genotyping BeadChip and Affymetrix GeneChip Human Mapping 500 K array and might thus escape the attention if tested only with these whole genome arrays.

A possible limitation of the study is the recruitment of our MI patients. We included only patients who survived at least one MI and had another sibling affected with CAD or MI, therefore we have a selection bias for survival of MI and a familial form of the disease. In contrast, however, the family-based character of our selection strategy with its high genetic background enables us to investigate even small genetic effects. Using married-in spouses or their relatives as control subjects minimizes the risk of a population stratification between affected MI patients and unaffected individuals. In addition, the control group was validated as being free from any signs and symptoms of cardiovascular disease including peripheral artery or cerebrovascular disease during the five-year follow-up period.

In conclusion, our results suggest that genetic variations in the *CNR2* gene have no influence on the risk of MI in a German population. Furthermore, common *CNR2* sequence variants are not significantly implicated in the development of classic cardiovascular risk factors, such as arterial hypertension.

Acknowledgements

We appreciate the invaluable contribution of participants of the German MI family study. We gratefully acknowledge the excellent technical assistance of Dagmar Glatz, Martina Köhler, Silke Muyrers, Josef Simon and Michaela Vöstner. This study was supported by grants from the National Genome Network (NGFN, 01GS0417), Deutsche Forschungsgemeinschaft (He1921/9-1), the Vaillant-Stiftung (C.H. and H.S.) and the 'Deutsche Stiftung für Herzforschung'. Electronic database information: http://www.ktl.fi/publications/monica/manual/index.htm, http://www.hapmap.org/, http://www.ncbi.nlm.nih.gov/SNP/ and http://www.broad.mit.edu/mpg/haploview/.

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