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Key genes of the interleukin 6 signaling pathway are not associated with coronary artery disease in a large European population

Mark Luedde[1*], Arne S. Schaefer[2*], Natalie Scheller[1], Corinna Doege[3], Hans-Joerg Hippe[1], Michael Nothnagel[4], Nils Haake[5], Norbert Frey[1], Stefan Schreiber[6], Nour Eddine El-Mokhtari[7#]

[1]Department of Internal Medicine III: Cardiology and Angiology, University of Kiel, Kiel, Germany
[2]Institute for Clinical Molecular Biology, University of Kiel, Kiel, Germany
[3]Department of Pediatric Neurology, University of Kiel, Kiel, Germany
[4]Institute of Medical Informatics and Statistics, University of Kiel, Kiel, Germany
[5]Department of Cardiovascular Surgery, University of Kiel, Kiel, Germany
[6]Department of Medicine, Christian-Albrechts-Universität Kiel, Kiel, Germany
[7]Imland Klinikum Rendsburg, Christian-Albrechts-Universität Kiel, Kiel, Germany
Email: [#]nour-eddine.el-mokhtari@imland.de

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ABSTRACT

Background: Recent studies indicate a strong functional relevance of the canonical inflammatory interleukin 6 signaling pathway in coronary artery disease (CAD). A genetic association of this signaling pathway with CAD has not been shown yet. We aimed to assess novel single nucleotide polymorphisms (SNPs) from genes of the Interleukin 6 signaling pathway. Results: To identify novel SNPs that are relevant for CAD, we employed a large-scale population-based case-control association study of 2199 cases and 1715 controls and assessed 73 SNPs from 12 genes out of the *IL*-6 signaling pathway. Results were adjusted to the CAD-related risk factors diabetes, hypertension, Body Mass Index, smoking and sex by logistic regression analysis. In a primary explorative study, we identified 5 SNPs that were significantly associated with CAD (*MAPK*1_rs6928, *MAPK*1_rs9340, *MAPK*1_rs11913721, *MAPK*14_rs7757672, *JAK*1_rs310236). After adjustment to CAD-risk factors, *MAPK*1_rs6928 showed the strongest association with CAD (P 0.0217, Odds Ratio 1.36, Confidence Interval 1.05 - 1.77). To reproduce this result, we performed a replication study employing independent patient and control panels. In this study we could not approve the association of rs6928 with CAD. Conclusion: In conclusion, we did not detect significant associations of SNPs from the *IL*-6 signaling pathway with CAD. Our investigation demonstrates the importance of independent replication studies to verify results from candidate-gene association studies in the quest to discover the underlying pathomechanism of CAD.

Keywords: Coronary Artery Disease; Genetics; Single Nucleotide Polymorphism; Interleukin 6

1. INTRODUCTION

Coronary artery disease (CAD) is one of the leading causes of death worldwide [1]. The cause of the disease, atherosclerosis is a systemic disease that is influenced by environmental and behavioural as well as genetic risk factors. The proportion of heritable factors for the individual risk of coronary artery disease has been estimated 30% - 60% [2]. Thus, unraveling the genetic sequence variants that are associated with an increased risk for CAD is an important goal of genetic research. Despite a multitude of studies, only a few gene variants could be credibly demonstrated to be associated with an increased risk of CAD, that would mostly comprise an effect on LDL-cholesterol [3]. Recent genome-wide association studies (GWAS) [4-6] and metanalyses [7] detected several novel sequence variants that could be linked to an increased risk of CAD. These studies demontrated that the analysis of large study populations is needed to discover candidate sequences with modest size effects [7]. Another possibility to discover novel CAD-related sequence variants is the target-oriented analysis of candidate genes in cardiovascular signaling pathways that may potentially mediate increased risk [3]. Overall, common disease variants could be mapped to 34 distinct loci [8]. These studies have proven to be an

[*]Both authors contributed equally to this work.
[#]Corresponding author.

enormously valuable tool to detect frequent variants with a limited genetic effect [7]. However, GWAS are often restricted when pursuing the goal to discover rare variants with greater genetic effects [9]. This is mainly due to the fact that many GWAS are executed in an underpowered situation due financial limits [9]. In this regard, target-oriented analyses of candidate genes are an important complement to GWAS, since they can use much higher sample numbers and thus provide a more adequate study design to identify rare variants with a stronger genetic effect [9]. Genes that are involved in lipid metabolism [3], stress response [10,11] and inflammation [12,13] have been appealing targets of many studies, however a significant correlation could only be detected in few cases, possibly because study samples have been too small to deliver reproducible data [5].

The *IL* (interleukin)-6-like cytokines *IL*-6, *IL*-11, LIF (leukaemia inhibitory factor), OSM (oncostatin M), ciliary neurotrophic factor, cardiotrophin-1 and cardiotrophin-like cytokine are an essential family of cytokines that play a role as regulators of the acute phase response and inflammation [14]. They act through binding to membrane receptors containing gp130-like receptors, subsequently activating the canonical *JAK* (Janus Kinase)-*STAT* (signal transducers and activators of transcription)-pathway as well as the mitogen-activated protein (*MAPK*) cascade [14]. In the heart, the *IL*-6/*JAK*/*STAT*-signaling pathway plays a fundamental role in ischemia, stress-induced remodeling and cardiomyopathy [15,16]. Moreover, *IL*-6 seems to have an impact on plaque development and morphology in atherosclerosis [17]. Consistently, *IL*-6 serum levels are elevated in patients with CAD [18,19]. It thus seems possible that genetic variants of *IL*-6 are associated with an increased risk of CAD, however several reports on this correlation have provided inconclusive results: While Georges and coworkers could demonstrate an association between the interleukin-6 gene polymorphism (−174 G/C) and susceptibility to myocardial infarction [20], this association could not be confirmed in another study [21].

However, a novel report suggests that assessing the interaction between *IL*-6 and the coagulation factor 2 receptor (F2R) haplotypes modulates the risk of myocardial infarction [22]. These data implicate, that a candidate gene approach that comprises simoultaneous analysis of genetic variants of *IL*-6 and interacting molecules, might offer more conclusive information about the relevance of *IL*-6 in CAD-related risk prediction. We therefore performed a population based case-control study assessing the association of 73 Single nucleotide polymorphisms (SNPs) of 12 candidate genes from the *IL*-6 pathway with CAD. In a second approach, suspect SNPs were verified in a replication study on a different study and control cohorts.

2. MATERIALS AND METHODS

2.1. Study Population

CAD patients involved in this study were recruited from a population-representative collection of unrelated Germans (**Table 1**). The PopGen biobank (population based assessment of genetic defects [23]), which represents a large population from the northernmost German region Schleswig Holstein, was the source of recruitment. Due to small migration rates, this region offers the advantage of a homogenous genetic structure. Study subjects were identified by screening of all cardioangiogramms from any of the five cardiac catheterizations laboratories of the recruitment area. Subjects who were assigned to the patient cohort, had a significant CAD (at least 70% stenosis in one major epicardial coronary vessel), as demonstrated by coronary catheterization. Following catheterization, more than 90% of the patients had undergone a coronary revas-cularization procedure (percutaneous coronary intervention or coronary artery bypass grafting). The screening period ranged from 01/1997 until 10/2004. An age > 65 years at diagnosis was an exclusion criterion. The patient cohort was further subdivided into two groups: patients younger than 55 years (1096 patients) were obtained for an explorative study, patients 55 years or older (1103 patients) were obtained for an independent replication study.

Control subjects for the explorative study (636 subjects) were randomly identified on the basis of data that were supplied by local population registries. Sub-

Table 1. characteristics of patients in the study and control groups.

	Explorative study		Replication study	
Cohort	**Case CAD < 55 years**	**Control Healthy subjects**	**Case CAD, 55 - 65 years**	**Control MICK**
Subjects	1096	636	1103	1079
Male (%)	917 (83.7)	319 (50.2)	828 (75.1)	1079 (100)
Female (%)	179 (16.3)	317 (49.8)	275 (24.9)	0 (0)
Mean age at recruitment (SD)	54.2 (5.97)	62.2 (7.27)	63.3 (6.02)	57.0 (6.31)
Mean age at diagnosis (SD)	48.2 (5.67)	-	57.0 (6.4)	-
Smoker (%)	869 (81.1)*	324 (51.0)	754 (68.7)*	190 (17.7)
Diabetes mellitus (%)	196 (18.1)*	31 (4.9)	218 (19.8)	0 (0)
Hypertension† (%)	786 (73.2)*	276 (43.5)	830 (75.7)*	607 (56.3)
BMI ≥ 30 (%)	323 (30.1)	95 (15.0)	275 (25.3)	(18.5)

*P < 0.05, †Blood pressure ≥ 140 mmHg systolic or ≥90 mmHg diastolic. An SD: Standard deviation, BMI: Body mass index.

jects with known CAD were excluded from this group. Information on age, gender and general health status was obtained to adequately match controls and patients. For the second replication study, matched controls were obtained from the Metabolic intervention Cohort Kiel (MICK) [24], an independent cohort of the Max Rubner Federal Research Institute of Nutrition and Food. This representative population cohort (1079 subjects) was recruited between 2003 and 2006 in the area of Kiel, Schleswig Holstein, Germany and comprised male subjects that were between 45 and 65 years old. To adjust both studies to these risk factors, logistic regression analysis was applied. Of note, the replication study was not adjusted for diabetes, since this disease was an exclusion criterion for the MICK cohort. Written informed consent was obtained from all participants. Recruitment and experimental protocols were approved by the institutional ethics review board and data protection authorities.

2.2. Object of Investigation

In a population-based case-control study 73 tagging Single-Nucleotide-Polymorphisms (tSNPs) out of 12 candidate genes of the *IL*-6 pathway were assessed for association with CAD. Selection of tSNPs was aligned to linkage disequilibrium analyses and haplotype distribution models from HapMap CEU-genotypes, one of 11 populations in HapMap phase 3 from the Centre de' Etude du Polymorphism Humain (CEPH, http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?pop=1409). The Haploview 3.2 software [25] was employed for this selection. Significant SNPs were assessed in a second replication study.

2.3. Genotyping

After extraction from blood samples (QIAGEN Flexi Gene DNA Kit, QIAGEN), genomic DNA was amplified by whole genome amplification (GenomiPhi, Amersham, Uppsala, Sweden). Genotyping was performed on an automated platform employing the TaqMan and SNPlex Genotyping Systems (Both Applied biosystems, Foster City, CA, USA). The process was facilitated by the use of Tecan Genesis RSP 150 pipetting robots (TECAN, Maennedorf, Switzerland). Detection of flourescence signals and generation of genotypes was carried out employing the ABI PRISM® 7900 HT Sequence Detector System (Applied Biosystems). The obtained data were further processed using the Data collection software v2.0 and analysed using the GeneMapper analysis Software v3.5.1 (Applied Biosystems) with default settings. Genotypes which gave significant association signals were additionally reviewed manually.

2.4. Statistical Analysis

To rule out possible systematic errors like insufficient genotyping [26] or diverging binding activities of probes in homozygos and heterozygos carriers of an allel, markers were assessed for deviations from the Hardy-Weinberg-equilibrium [27] in controls before employment in the study (http://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa2.pl,α=0.05). Association-case-control analysis was performed using Pearson's chi-squared test [28]. Signficance was verified by a Wald test and by an odds ratio test. Logistic regression analysis was performed to assess the influence of covariants like sex, hypertension, body mass index (BMI), smoking and diabetes. Power calculation was performed for uncorrected χ^2 tests as implemented for an independent and retrospective case-control study design in the software PS Power and Sample Size Calculations [29]. If the true odds ratio for disease in exposed subjects relative to unexposed subjects is 1.8, a putative risk allele of a minor allele frequency (MAF) of 5% given, in our explorative study, we were able to reject the null hypothesis that this odds ratio equals 1 with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. For more common alleles, e.g. 40% frequency in the general population, we were able to detect a genetic effect of 1.3 (Supplementary **Figure S1**).

3. RESULTS

3.1. Study Population

In this study we aimed to identify variants that were associated with CAD. In a primary explorative study 73 tagging SNPs from 12 candidate genes (**Figure 1**, **Table 1**) within the *IL*-6 signaling pathway were tested for association with CAD in a large population of 1096 early-onset CAD patients (age of disease onset < 55 years) and 636 ethnically matched healthy controls (**Table 2**). SNPs that gave significant evidence for an association with CAD were subsequently replicated in a second independent study population. This population comprised 1103 patients with age at disease onset between 55 and 65 years. The control cohort for this study population consisted of 1079 healthy, ethnically matched men (mean age of 57 years) who were tested free of diabetes. Collectively, we detected a significant preponderance of classical CAD-related risk factors (diabetes, hypertension, Body Mass Index, smoking and sex) in both study populations vs. control groups.

3.2. Explorative Study

In the primary explorative study, 1086 out of 1096 CAD patients and 631 out of 636 healthy controls were

Table 2. 73 tSNPs of 12 genes from the *IL*-6 pathway that were assesseds in the explorative study.

Gene	Chromosome	Reference sequence	Base pair position (bp)
IL27RA	19 (p13.12)	rs6511905	14003616
		rs11881500	14010814
		rs2306190	14023676
		rs6928	20439558
		rs9340	20439907
MAPK1	22 (q11.21 - q11.22)	rs2298432	20447743
		rs9610470	20533693
		rs11913721	20534689
		rs2069824	22538472
		rs1800795	22539885
IL-6	7 (p15.3)	rs2069832	22540673
		rs2069843	22543234
		rs2069860	22544278
		rs851034	36095623
		rs851024	36107542
		rs851023	36114198
MAPK14	6 (p21.31)	rs2145362	36137133
		rs851006	36173163
		rs12200998	36190660
		rs7757672	36193303
		rs2272087	37713088
		rs1053005	37719436
		rs2293152	37735055
STAT3	17 (q21.2)	rs3816769	37751799
		rs9912773	37764060
		rs744166	37767727
		rs10471960	55272131
		rs1900173	55275763
IL6ST	5 (q11.2)	rs11574780	55279795
		rs7719246	55280534
		rs10940495	55298417
		rs6870870	55330085
		rs310244	65017898
		rs2780819	65020894
		rs188698	65030605
		rs310228	65034253
JAK1	1 (p31.3)	rs2256298	65042703
		rs310236	65045878
		rs3790541	65053588
		rs310202	65061565
		rs310199	65062143
		rs40419	67551358
		rs706713	67558478
		rs7713645	67563082
		rs34300	67592001
		rs10940160	67598983
		rs2161120	67599952
PIK3R1	5 (q13.1)	rs10515074	67601949
		rs1819987	67602809
		rs34306	67614501
		rs1550805	67619563
		rs1445760	67628772
		rs1043526	67630080
		rs9291926	67635412
		rs4727666	106099429
		rs1526083	106104027
PIK3CG	7 (q22.3)	rs849367	106110105
		rs4730205	106123200
		rs12667819	106140608
		rs849412	106143853
PTPN11	12 (q24.13)	rs11066301	111334092
		rs2301756	111353496

Continued

		rs11066323	111386081
PTPN22	1 (p13.2)	rs2476601	114089610
		rs952146	151182001
		rs4845618	151213088
		rs7549250	151217409
		rs7518199	151220492
IL6R	1 (q21.3)	rs4845623	151228850
		rs4845374	151240020
		rs8192284	151240043
		rs2229238	151250969
		rs7526293	151257282

successfully genotyped. All 73 tested tSNPs reached a call rate of ≥95% and a P_{HWE} > 0.01. Three out of these 73 SNPs (rs2069843, *IL*-6; rs2069860, *IL*-6; rs11574780, *IL6ST*) did not meet the required minor allele frequency (minAF) of >5% and were therefore excluded from further analysis (**Table S1**). In the next stage of the study, association analyses and subsequent adjustment for CAD-related risk factors resulted in the detection of 5 SNPs that showed significant association with CAD (**Table 3**). Interestingly, three out of these five SNPs were located in the *MAPK*1 gene encoding the Mitogen-activated protein kinase 1, a member of the extracellular signalregulated kinase (ERK) family. Significant associations before risk factor adjustment are shown in supplementary **Table 2**. After risk factor adjustment, rs6928, *MAPK*1 showed the strongest association with CAD under the dominant model both for homozygous and heterozygous carriers of the rare allele (P = 0.0217, OR 1.36 (95%

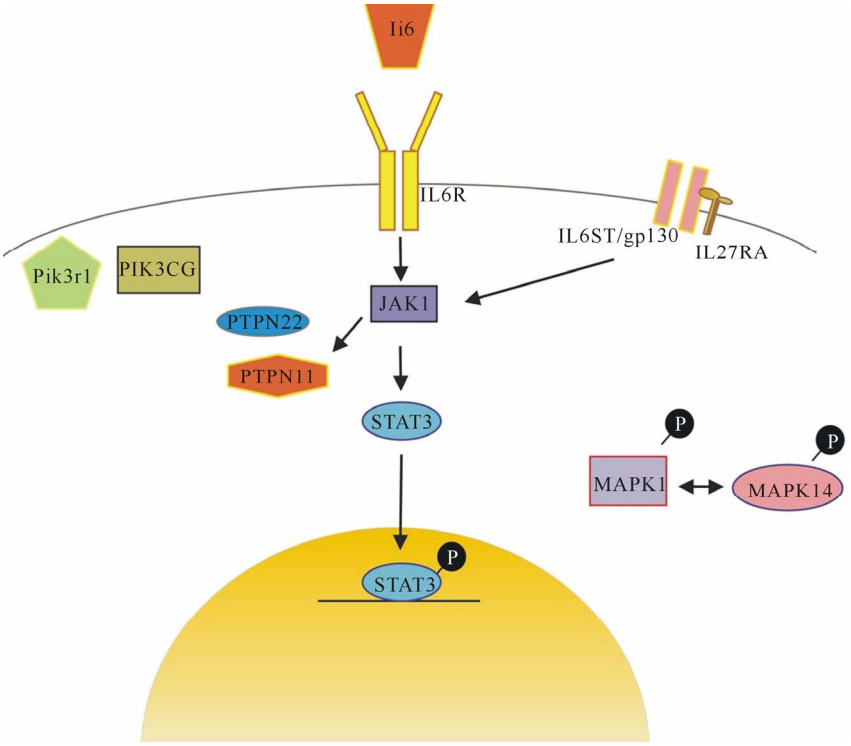

Figure 1. Proteins involved in interleukin 6 (*IL*-6)—related signaling that were assessed for genetic variants. *IL*-6: interleukin 6; *IL6R*: interleukin 6 receptor; *IL6ST*/gp130: Glycoprotein 130. *IL27RA*: Interleukin Receptor 27 subunit alpha; *JAK*1: Janus Kinase1; *STAT*3: Signal transductor and activator of transcription 3; *PIK*3r1: Phosphatidylinositol 3-kinase regulatory subunit alpha; *PIK3CG*: Phosphatidy-linositol-4, 5-bisphosphate 3-kinase catalytic subunit gamma isoform; *PTPN*11: Protein tyrosine phosphatase, non-receptor type 11; *PTPN*22: Protein tyrosine phosphatase, non-receptor type 22; *MAPK*1: Mitogen-activated protein kinase 1; *MAPK*14: Mitogen-activated protein kinase 14.

C.I. 1.05 - 1.77) after risk factor adjustment, **Table 3**). This association was accompanied by a higher frequency of homozygous and heterozygous carriers of the rare allele in the cases (27.0% and 50.4%, respec-

tively) compared to the controls (25.4% and 45.7%, respectively; **Table 4**). The other 4 SNPs showed association in the recessive model (rs7757672, *MAPK*14) in the additive model (rs310236, *JAK*1), or in both models (rs9340, *MAPK*1 and rs11913721 *MAPK*1).

3.3. Replication Study

In the next step of the study, SNP rs6928 (*MAPK*1), which exhibited the strongest association signal in the explorative study was tested for association in a replication study that was carried out in an independent case-control population. In this study, all 1103 CAD patients and 1001 out of 1079 healthy controls were successfully genotyped. The results met the quality criteria CR ≥ 95%, P_{HWE} > 0.01, MAF > 5% (**Table 5**). The significant association of rs6928 with CAD, that was detected in the explorative study, was not reproduced in the replication (**Table 6**). Consistently, highly similar allele frequencies between cases and controls contradicted a prominent genetic role of this SNP in CAD (**Table 7**).

4. DISCUSSION

In this multigenic candidate gene association study, we assessed the association of 12 candidate genes from the Interleukin-6 signaling pathway with coronary artery disease. Many studies have proven, that *IL*-6 plays a prominent role in the pathogenesis of atherosclerosis and CAD [15,17,30,31]. Consistently, genetic polymorphisms of *IL*-6 have been demonstrated to affect the onset and clinical course of CAD [32,33]. The functional relevance of two polymorphisms in the promoter region of the *IL*-6 gene, *IL*6-174G_C and -572G_were explained by their ability to increase serum *IL*6-levels in different situations of physiological stress, e.g. status after coronary bypass graft surgery [34]. These data imply that the inflammatory response to stress situations

Table 3. Explorative study: significant association of 5 tSNPs with coronary artery disease.

	Dominant		Additive		Recessive	
Gene_SNP	P	OR (95-CI)	P	OR (95-CI)	P	OR (95-CI)
*MAPK*1_rs6928	0.0217	1.36 (1.05 - 1.77)	0.0369	1.19 (1.01 - 1.40)	0.2546	1.17 (0.90 - 1.52)
*MAPK*1_rs9340	0.1052	0.82 (0.64 - 1.04)	0.0158	0.82 (0.69 - 0.96)	0.0147	0.69 (0.51 - 0.93)
*MAPK*1_rs11913721	0.1006	0.82 (0.64 - 1.04)	0.0150	0.82 (0.69 - 0.96)	0.0142	0.68 (0.51 - 0.93)
*MAPK*14_rs7757672	0.2514	0.79 (0.52 - 1.18)	0.1490	1.14 (0.95 - 1.36)	0.0118	1.35 (1.07 - 1.70)
*JAK*1_rs310236	0.1620	1.29 (0.90 - 1.84)	0.0468	1.19 (1.00 - 1.42)	0.0751	1.24 (0.98 - 1.56)

Association Statistics are shown for the case and control group of coronary artery disease (CAD). Given are the odds ratios (OR), their 95% confidence intervals (CI 95%) and the P values which were obtained from a Wald test (autosomal-dominant, additive and recessive models). Values are given after adjustment for the covariates diabetes, hypertension, BMI, smoking and gender in a logistic regression model. All other markers showed no significance at the 5% test level under either of the models. Siginficant associations are highlighted in yellow.

Table 4. Significant SNPs from the explorative study.

Gene_SNP	nco	ca	n11co	n11ca	n12co	n12ca	n22co	n22ca	F22co	F12co	F22ca	F12ca
*MAPK*1_rs6928	630	1082	182	245	288	545	160	292	0.254	0.457	0.27	0.504
*MAPK*1_rs9340	630	1071	214	395	295	512	121	164	0.192	0.468	0.153	0.478
*MAPK*1_rs11913721	631	1075	214	396	296	514	121	165	0.192	0.469	0.153	0.478
*MAPK*14_rs7757672	631	1075	285	535	294	441	52	99	0.082	0.466	0.092	0.41
*JAK*1_rs310236	630	1068	256	473	294	477	80	118	0.127	0.467	0.11	0.447

Numeric (N) and proportionate (F) frequency of the allelic genotypes 11, 12 and 22 in controls (co) and cases (ca), with 1 being the more frequent and 2 the less frequent allel.

Table 5. Replication study: quality criteria oft he most significant SNP from the explorative study.

Gene_SNP	nco	nca	CR	Chi Quadrat	HWE	minAF co	minAF ca
*MAPK*1_rs6928	1001	1103	1	0.601	0.728927548	0. 50	0.49

nco: number of controls, nca: Number of cases, CR: call rate, HWE: Hardy Weinberg Equilibrium, minAFco: minor allel frequency in controls, minAFca: minor allel frequency in cases.

Table 6. Replication Study: No association of *MAPK*1_rs6928 with CAD.

	Dominant		Additive		Recessive	
Gene_SNP	P	OR (95-CI)	P	OR (95 - CI)	P	OR (95-CI)
*MAPK*1_rs6928	0.657	0.95 (0.76 - 1.19)	0.590	0.96 (0.84 - 1.11)	0.662	0.95 (0.76 - 1.19)

Association Statistics are shown for the case and control group of coronary artery disease (CAD). Given are the odds ratios (OR). Their 95% confidence intervals (CI 95%) and the P values which were obtained from a Wald test (autosomal-dominant, additive and recessive models). Values are given after adjustment for the covariates, hypertension, BMI, smoking and gender in a logistic regression model.

Table7. Numeric (N) and proportionate (F) frequency of the allelic genotypes 11, 12 and 22 of rs6928 in controls (co) and cases (ca), with 1 being the more frequent and 2 the less frequent allel.

Gene_SNP	nco	nca	n11co	n11ca	n12co	n12ca	n22co	n22ca	F22co	F12co	F22ca	F12ca
*MAPK*1_rs6928	1001	1103	256	298	495	540	250	265	0.25	0.495	0.24	0.49

in CAD is influenced by genetic variants, underlining the necessity of the candidate gene approach to systematically evaluate the functional relevance of a molecule in CAD. Since *IL*-6 is embedded in a canonical signaling pathway, and exerts it's functions through many distinct molecules, we chose a multigenic approach to cover 12 important mediators of *IL*6-signaling in a single approach.

In the explorative study, we found five SNPs to be associated nominal significantly with CAD, with rs6928 (*MAPK*1) showing the strongest association. Additionally, two other *MAPK*1 SNPs showed significant associations in this part of the study (rs9340 and rs11913721).

While in our explorative study SNPs in the *MAPK*1 gene were significantly associated with CAD, we could not reproduce this result in the replication study. These conflicting results might not be caused by population stratification, based on undiscovered subpopulations, such as a different ethnic background or age [35]. The populations employed in this study were selected as genetically homogenous as previously described [36-38]. However, the population of CAD patients was on average almost nine years older in the replication study than in the explorative study. It is generally believed that genetic influences on the onset and course of a disease are overbalanced by environmental and behavioural factors in older patients more than in younger patients [37]. The lack of association of rs6928 with CAD in the replication study could be due to this effect. For common alleles, we were able to detect a genetic effect of 1.3 (**Figure S1**). Thus, we might have missed associations of common variants which confer a genetic risk of <1.3.

Recently, genome wide association studies (GWAS) have discovered several novel variants that were associated with CAD [6,7,39]. The high relevance of these investigations is based on their ability to discover novel associations in loci that were not assumed to be functionally relevant for CAD or even not protein-coding. However, the variants discovered explain only 4% of interindividual variants in disease risk and only up to 13% of the total heredity of CAD [8], underlining the need for further genetic studies. The limited size of study populations assessed in GWAS leads to the identification of mainly frequent variants, leaving a "gap" in the search for rare variants [9]. "Classical" candidate gene studies like ours maintain a strong supplemental value, since large study populations can easily be assessed to discover genetic variants of potentially higher impact. Moreover, by simultaneously addressing *IL*-6 and interacting molecules, we believe that we can offer an integrated view on the genetic relevance of this canonical signaling pathway in CAD. Our study might be a starting point for the genetic assessment of other *IL*6-related molecules in CAD to finally provide new insights in the pathogenetic mechanisms of this complex disease.

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Appendix

Supplementary Figure

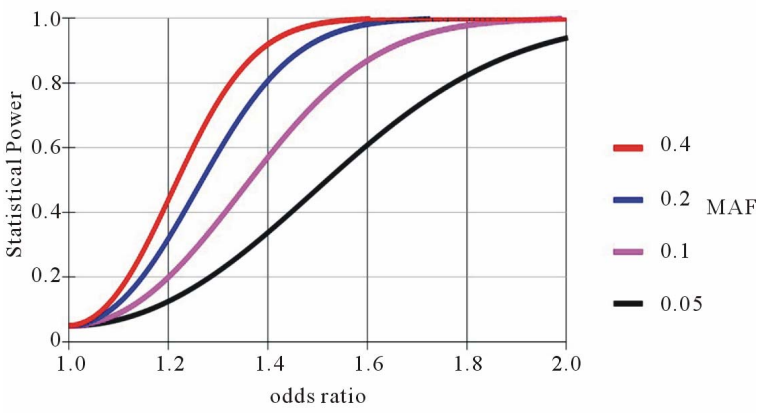

Figure S1. Statistical power of the finemapping study. The guaph shows the statistical power of the case-control population of the explorative study (1096 cases, 636 controls) of the finemapping in relation to the minor allele frequency (MAF) of the putative risk alleles in the controls and different genetic effects (odds ratio). A power of 0.8 is regarded as statistically significant.

Supplementary Table

Table S1. Quality criteria of the primary study for all 73tested tSNPs. tSNPs that did not meet all quality criteria are marked in yellow rows.

Gene_SNP	Nco	Nca	CR	Chi Quadrat	HWE	minAF co	minAF ca
*IL27RA*_rs6511905	631	1070	0.99	0.064	0.076242471	0.25	0.26
*IL27RA*_rs11881500	631	1071	0.99	0.161	0.261265308	0.29	0.29
*IL27RA*_rs2306190	631	1082	1	0.096	0.107249474	0.42	0.43
*MAPK*1_rs6928	630	1082	1	4.891	0.033721819	0.48	0.52
*MAPK*1_rs9340	630	1071	0.99	3.814	0.285986453	0.43	0.39
*MAPK*1_rs2298432	631	1073	0.99	1.186	0.050294602	0.39	0.41
*MAPK*1_rs9610470	631	1084	1	3.096	0.66273949	0.25	0.23
*MAPK*1_rs11913721	631	1075	0.99	3.759	0.305161687	0.43	0.39
*IL6*_rs2069824	630	1084	1	0.004	0.073713428	0.08	0.08
*IL6*_rs1800795	631	1073	0.99	0.127	0.75765008	0.43	0.43
*IL6*_rs2069832	631	1073	0.99	0.318	0.671851745	0.42	0.43
*IL6*_rs2069843	631	1084	1	0.862	0.68584196	0.02	0.02
*IL6*_rs2069860	631	1083	1	2.041	0.936339137	0	0.01
*MAPK*14_rs851034	631	1073	0.99	0.032	0.41542216	0.29	0.29
*MAPK*14_rs851024	631	1081	1	0.037	0.013700599	0.48	0.49
*MAPK*14_rs851023	631	1084	1	0.067	0.600194839	0.14	0.14
*MAPK*14_rs2145362	631	1075	0.99	0	0.458401824	0.11	0.11
*MAPK*14_rs851006	631	1075	0.99	0.029	0.753696554	0.27	0.26
*MAPK*14_rs12200998	631	1073	0.99	0.112	0.013935742	0.48	0.49
*MAPK*14_rs7757672	631	1075	0.99	1.24	0.047287746	0.32	0.3
*STAT*3_rs2272087	628	1067	0.99	0.207	0.942574621	0.16	0.17
*STAT*3_rs1053005	631	1074	0.99	0.493	0.539027211	0.19	0.2
*STAT*3_rs2293152	631	1073	0.99	0.289	0.475558137	0.41	0.4
*STAT*3_rs3816769	631	1081	1	0.528	0.861645375	0.35	0.36
*STAT*3_rs9912773	631	1084	1	0.003	0.974796647	0.26	0.26
*STAT*3_rs744166	630	1082	1	1.125	0.65561739	0.41	0.43
*IL6ST*_rs10471960	631	1081	1	0.091	0.848028064	0.12	0.12
*IL6ST*_rs1900173	631	1082	1	0.002	0.367532043	0.08	0.07
*IL6ST*_rs11574780	631	1084	1	2.764	0.684369975	0.05	0.04
*IL6ST*_rs7719246	631	1084	1	0.026	0.977089227	0.13	0.13
*IL6ST*_rs10940495	631	1081	1	0.013	0.568879468	0.27	0.28
*IL6ST*_rs6870870	630	1076	0.99	0.013	0.373900264	0.41	0.41
*JAK*1_rs310244	631	1071	0.99	0.62	0.343039886	0.25	0.24
*JAK*1_rs2780819	631	1076	1	1.643	0.752061213	0.29	0.27
*JAK*1_rs188698	631	1083	1	1.769	0.690302262	0.36	0.34
*JAK*1_rs310228	631	1074	0.99	0.761	0.365615004	0.14	0.13
*JAK*1_rs2256298	631	1082	1	0.961	0.070162807	0.28	0.27
*JAK*1_rs310236	630	1068	0.99	2.47	0.756739144	0.36	0.33
*JAK*1_rs3790541	631	1077	1	1.771	0.823662205	0.11	0.1

Continued

*JAK*1_rs310202	631	1080	1	1.175	0.401503008	0.22	0.2
*JAK*1_rs310199	630	1073	0.99	1.13	0.80223942	0.29	0.27
*PIK3R*1_rs40419	631	1079	1	0.072	0.228159001	0.42	0.43
*PIK3R*1_rs706713	630	1083	1	0.031	0.649598965	0.25	0.25
*PIK3R*1_rs7713645	631	1072	0.99	0.282	0.010619532	0.48	0.49
*PIK3R*1_rs34300	631	1075	0.99	0.679	0.44089187	0.14	0.15
*PIK3R*1_rs10940160	631	1083	1	0.207	0.475357019	0.46	0.46
*PIK3R*1_rs2161120	631	1078	1	0.093	0.449440035	0.44	0.45
*PIK3R*1_rs10515074	631	1084	1	0.166	0.370104169	0.21	0.21
*PIK3R*1_rs1819987	631	1079	1	0	0.822559441	0.4	0.4
*PIK3R*1_rs34306	631	1082	1	0.605	0.459896984	0.15	0.16
*PIK3R*1_rs1550805	631	1083	1	0.5	0.135883362	0.09	0.1
*PIK3R*1_rs1445760	631	1081	1	0.876	0.767125459	0.47	0.49
*PIK3R*1_rs1043526	631	1084	1	0.26	0.920419684	0.14	0.14
*PIK3R*1_rs9291926	629	1072	0.99	0.187	0.597732407	0.49	0.5
*PIK3CG*_rs4727666	631	1082	1	0.658	0.456708563	0.18	0.19
*PIK3CG*_rs1526083	631	1072	0.99	1.409	0.771314385	0.4	0.38
*PIK3CG*_rs849367	631	1084	1	0.005	0.48951446	0.1	0.1
*PIK3CG*_rs4730205	631	1082	1	0.163	0.642496287	0.31	0.32
*PIK3CG*_rs12667819	631	1081	1	1.367	0.736860173	0.46	0.44
*PIK3CG*_rs849412	630	1083	1	0.013	0.37651594	0.14	0.14
*PTPN*11_rs11066301	630	1080	1	0.085	0.270192315	0.46	0.45
*PTPN*11_rs2301756	631	1082	1	3.34	0.251633002	0.11	0.09
*PTPN*11_rs11066323	631	1076	1	0.496	0.982054572	0.08	0.07
*PTPN*22_rs2476601	631	1083	1	1.255	0.373272239	0.12	0.11
*IL6R*_rs952146	631	1075	0.99	3.38	0.579127752	0.4	0.37
*IL6R*_rs4845618	631	1075	0.99	0.399	0.397402393	0.44	0.45
*IL6R*_rs7549250	630	1083	1	0.285	0.587533209	0.44	0.45
*IL6R*_rs7518199	631	1074	0.99	0.044	0.199284606	0.39	0.39
*IL6R*_rs4845623	631	1076	1	0.038	0.089966564	0.4	0.4
*IL6R*_rs4845374	631	1074	0.99	0.402	0.224824771	0.17	0.16
*IL6R*_rs8192284	631	1082	1	0.311	0.237837658	0.39	0.38
*IL6R*_rs2229238	631	1073	0.99	1.015	0.722613569	0.19	0.2
*IL6R*_rs7526293	631	1075	0.99	1.687	0.447643437	0.2	0.22

nco: number of controls, nca: Number of cases, CR: call rate, HWE: Hardy Weinberg Equilibrium, minAFco: minor allel frequency in controls, minAFca: minor allel frequency in cases.

