

Haplotypes Encompassing the *KIAA0391* and *PSMA6* Gene Cluster Confer a Genetic Link for Myocardial Infarction and Coronary Artery Disease

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Summary

The role of the *KIAA0391* and *PSMA6* genes in predisposing individuals to disease is still not fully understood. We evaluated by molecular beacon-based genotyping assays the roles of five single nucleotide polymorphisms (SNPs) in the chromosomal region 14q13.2 harbouring the *KIAA0391* and *PSMA6* gene cluster in coronary artery disease (CAD) in the Saudi population. Two of the studied SNPs rs8008319 (denoted as 1) and rs7157492 (2), reside in the *KIAA0391* locus, two others rs1048990 (3) and rs12878391 (4) are components of the *PSMA6*, while rs4981283 (5) resides downstream of both genes. In a study involving 1071 patients and 929 controls, none of the studied SNPs showed significant association with CAD. In contrast, two haplotypes consisting of 1A-2G-3C-4A-5A [O.R.(95% C.I.) = 1.49(0.95–2.35); $p = 0.022$] and 1A-2G-3G-4A-5A [2.24(0.84–5.98); $p = 0.031$] conferred risk for both CAD and myocardial infarction (MI) in a five-SNP locus model, while another comprising 1A-2G-3C-4A-5G [2.24(0.84–5.98); $p = 0.079$] showed a borderline association. One haplotype consisting of 1T-2G-3C-4G-5A [0.79(0.59–1.05); $p = 0.015$] exhibited protective properties and another, 1T-2G-3C-4A-5G [0.20(0.03–1.39); $p = 0.073$], showed a similar but weaker trend. Our study identified haplotypes in the chromosomal region encompassing the *KIAA0391* and *PSMA6* genes as a possible genetic link between CAD and MI. These results also suggest that haplotypes may be more informative than individual SNPs in identifying risk factors for disease.

Keywords: Myocardial infarction, coronary artery disease, *KIAA0391*, *PSMA6*, single nucleotide polymorphism, haplotype

Introduction

Coronary artery disease (CAD) is a manifestation of complex events including gene-gene and gene-environment interactions, partly triggered through inflammatory processes (Lusis et al., 2004a, 2004b). Various pathways as well as candidate genes and loci identified thus far for susceptibility to CAD through case-control association studies or linkage analysis appear to be involved in the regulation of inflammatory mechanisms (Pajukanta et al., 2000; Harrap et al., 2002; Hauser

et al., 2004; Wang et al., 2004; Samani et al., 2005). Several of these genes have also been associated with myocardial infarction (MI), an event which underlies such inflammatory processes and is a critical component in the pathogenesis of CAD (Helgadottir et al., 2006, 2007; Yamada, 2006; Yamada et al., 2006). The ubiquitin-proteasome system is one such pathway that regulates the inflammatory processes (Auld & Silver, 2006; Ciechanover, 2006a; Reed, 2006; Wang et al., 2007; Konstantinova et al., 2008) and presumably plays an important role in mechanisms leading to MI (Ciechanover, 2006b; Wang & Maldonado, 2006; Hochrainer & Lipp, 2007). This proteasome is a large multicatalytic proteinase that functions as a central switch within the cell by selectively degrading a multitude of proteins, including metabolic enzymes, transcription factors and cell cycle regulators (Rivett, 1989; Maki et al., 1996; Dimmeler et al., 1999; Salghetti et al., 1999;

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Karin & Delhase, 2000). Not surprisingly therefore, the proteasomal alpha subunit type 6 gene (*PSMA6*), a component of the ubiquitin-proteasome system has been associated with MI (Ozaki et al., 2006; Barbieri et al., 2008), type 2 diabetes mellitus (DM2) (Sjakste et al., 2007b), greater intima-media thickness (Takashima et al., 2007), and possibly atherosclerosis (Takashima et al., 2007). On the other hand, its role in conferring risk for cardiovascular-related events in general has been called into question recently by a number of studies pointing to lack of association with these disorders in some ethnic groups (Sjakste et al., 2007b; Takashima et al., 2007; Banerjee et al., 2008; Bennett et al., 2008).

The *PSMA6* gene is located on chromosome 14q13.2, a region containing microsatellites that have also been implicated in various diseases, including DM2 (Sjakste et al., 2007a), Grave's disease (Sjakste et al., 2004) and familial schizophrenia (Kamnasaran et al., 2003). These disorders have been associated partly with mutations in the *KIAA0391* gene, which also resides in this chromosomal region. Among the familiar single nucleotide polymorphisms (SNPs) recently associated with various cardiovascular disorders in this region are the rs8008319, rs7157492, rs1048990, rs12878391 and rs4981283, which partly reside on either of the two genes. However, to date, it is still not known whether the *KIAA0391* polymorphisms can influence the pathways to CAD or MI. Furthermore, while the *PSMA6* gene codes for a characterized protein, the *KIAA0391* gene (Entrez Gene Database ID 9692) is thought to encode a hypothetical protein (LOC9692). The fact that the two genes form an evolutionarily conserved cluster on this locus, and the fact that both genes have been cited with respect to predisposing individuals to coronary vascular disorders points to a potential linkage of this chromosomal region with manifestation of CAD. We therefore tested the possibility that the above genetic variants in this chromosomal region may predispose individuals to acquiring CAD and its primary risk factor MI, using the Saudi population as a study model.

Methods

Study Subjects

We recruited a total of 1071 candidates (784 males and 287 females; age 61.6 ± 0.3 yr) of Saudi descent with documented coronary vessel disease as our patient group (CAD). The inclusion criterion for CAD was the presence of angiographically determined narrowing of the coronary vessels by at least 50%. Exclusion criteria were major cardiac rhythm disturbances, incapacitating or life-threatening illness, major psychiatric illness or substance abuse, history of cerebral vascular disease, neurological disorders, and administration of psychotropic medication. A second group of 929 individuals

(463 male and 466 female; mean age, 49.4 ± 0.6 yr) served as the angiographed control (CON) group. These individuals were sequentially and randomly selected among candidates visiting the Catheterization Clinic of the King Faisal Specialist Hospital and Research Centre for diagnostic or follow-up procedures related to rheumatic heart disease (RHD) or idiopathic dilated cardiomyopathy (DCM), and those who reported with chest pain, but were established to have no significant coronary stenosis by angiography. Exclusion criteria for this group included, among others, the existence of diseases such as cancer, autoimmune disease, or any other disorders likely to influence the variables being studied. The important clinical and demographic features of these individuals are summarized in Table 1. This study was performed in accordance with the regulations laid down by the Hospital Ethics Committee and all participants provided informed consent.

Genotyping by Molecular Beacon Assays

We selected five SNPs (rs8008319, rs7157492, rs1048990, rs12878391 and rs4981283) within a 100-kb region of chromosome 14q13.2 containing both the *KIAA0391* (Ensembl ID: ENSG00000100890) and the *PSMA6* (Ensembl ID: ENSG00000100902) genes. Allele-specific molecular beacons and primers were designed to encompass the five SNPs using Beacon designer 6.0 (PREMIER Biosoft International, Palo Alto, CA, USA) (Alsmadi et al., 2006) (Table 2), and outsourced from Metabion GmbH (Metabion, Martinsried, Germany). Blood was procured from all candidates following their signing of informed consent. High throughput molecular beacon genotyping assays were employed in clear optical 384-well PCR reaction plates on the ABI Prism 7900 HT sequence detection system (Applied Biosystems, Foster City, CA, USA). PCR mixtures consisted of 1X qPCR mastermix plus (Eurogentec, Seraing, Belgium), $0.5 \mu\text{M}$ forward/reverse primers, $0.25 \mu\text{M}$ of each molecular beacon probe, and 20 ng of genomic DNA template. Reaction tubes were subjected to initial 2-min incubation at 50°C , employing uracil-N-glycosylase to eliminate carryover amplicon contaminants, followed by 10-min incubation at 95°C for activating *Taq* polymerase. Thermocycling conditions were 95°C denaturation for 10 sec, 58°C annealing for 30 sec, and 72°C extension for 30 sec for 40 cycles. De-ionized water was included as negative control (no template) in each assay.

Fluorescence data was collected during each annealing step of the cycle at which point the molecular beacon ought to be bound to its complementary target amplicon. Results were displayed as an amplification plot for fluorescence versus cycle number. Genotype calling of DNA samples was determined by measuring the threshold cycle (Ct) value for each sample. This is the cycle at which the signal is first reported above

Table 1 Clinical features and demographics of the studied cases.

	Controls			CAD		
	All	Male	Female	All	Male	Female
Gender	929	463(49.8)	466(50.2)	1071	784(73.2)	287(26.8)
Age	49.4 ± 0.6	50.3 ± 0.9	48.5 ± 0.7	61.6 ± 0.3	50.2 ± 0.4	48.5 ± 0.7
BMI	29.3 ± 0.8	28.4 ± 0.4	30.3 ± 1.5	29.1 ± 0.5	28.4 ± 0.7	30.3 ± 0.4
MI	269(29.0)	157(33.9)	112(24.0)	1016(94.9)*	748(95.4)	268(93.4)
FH	271(29.2)	127(27.4)	144(30.9)	370(34.5)	276(35.2)	94(32.8)
HTN	647(69.6)	326(70.4)	321(68.9)	924(86.3)*	666(84.9)	258(89.9)
DM2	318(34.2)	146(31.5)	172(36.9)	734(68.5)*	515(65.7)	219(76.3)
HLPD	124(13.3)	55(11.9)	69(14.8)	364(34.0)*	263(33.5)	101(35.2)
VD						
One	0(0.0)	0(0.0)	0(0.0)	586(54.7)*	410(52.3)	176(61.3)
Two	0(0.0)	0(0.0)	0(0.0)	157(14.7)*	119(15.2)	38(13.2)
>Two	0(0.0)	0(0.0)	0(0.0)	328(30.6)*	255(32.5)	73(25.4)
Smoking						
Never	789(84.9)	338(73.0)	451(96.8)	763(71.2)	485(61.9)	278(96.9)
Current	109(11.7)	99(21.4)	10(2.1)	213(19.9)*	208(26.5)	5(1.7)
Unknown	31(3.3)	26(5.6)	5(1.1)	95(8.9)	91(11.6)	4(1.4)

The table shows the important clinical features of the studied groups. The numbers in brackets are the percentages of the given values. CAD, coronary artery disease; DM2, type 2 diabetes mellitus; VD, diseased vessels; FH, family history; MI, myocardial infarction; BMI, body mass index; HLPD, hyperlipidaemia; HTN, hypertension. *P-value by χ^2 test is <0.001 versus control for all variables.

Table 2 Allele-specific molecular beacons and primers for the studied SNPs.

Oligo name	5' tag	3' tag	Sequence
rs8008319A	FAM	DABCYL	CGCGATCAACCAAAGCTGTAAC <u>A</u> CTAAGTTTTCTACTAGATCGCG
rs8008319T	TET	DABCYL	CGCGATCAACCAAAGCTGTAAC <u>T</u> CTAAGTTTTCTACTAGATCGCG
rs8008319F	-	-	CAGATTGCTTGAGCTTAGG
rs8008319R	-	-	GCTGGTGGTTTTATGAGAG
rs7157492A	FAM	DABCYL	CGCGATCAAATTTAACCCCGAT <u>A</u> GTAATCAGAATAAAGATCGCG
rs7157492G	TET	DABCYL	CGCGATCAAATTTAACCCCGAT <u>G</u> GTAATCAGAATAAAGATCGCG
rs7157492F	-	-	CCGCACCTGGCAAAATATG
rs7157492R	-	-	CTGATTTGGGGTTTTCTGTTTCC
rs1048990C	FAM	DABCYL	CGCGATCTAAAGTAGTGCTT <u>C</u> TACCAACATGTCCCGGATCGCG
rs1048990G	TET	DABCYL	CGCGATCTAAAGTAGTGCTT <u>G</u> TACCAACATGTCCCGGATCGCG
rs1048990F	-	-	CTGGTGCGGGAGCTACGG
rs1048990R	-	-	GCAACCTGGTTCACCTACCTAC
rs12878391A	FAM	DABCYL	CGCGATCCTCTCACTCTCT <u>A</u> GTGCTCTGGTTTGATCGCG
rs12878391G	TET	DABCYL	CGCGATCCTCTCACTCTCT <u>G</u> TGCTCTGGTTTGATCGCG
rs12878391F	-	-	CGCTTGAGTCTGCCTTG
rs12878391R	-	-	CTAGAGAACAGTTTGGGGTAAC
rs4981283A	FAM	DABCYL	CGCGATCTGGAAATCTAAATCC <u>A</u> TGTGAATTATGTTGATCGCG
rs4981283G	TET	DABCYL	TCGGATCTGGAAATCTAAATCC <u>G</u> TGTGAATTATGTTGATCGCG
rs4981283F	-	-	GGGAATACAGCGGTGAGC
rs4981283R	-	-	GCAATGACCTGTCAAATGAG

For the molecular beacon sequences, underlined bold bases represent the allelic variants for each SNP. The beacon probes were designed using the Beacon designer 6.0 and mfold for appropriate probe folding (Alsmadi et al., 2006).

the baseline fluorescence, set at 10% above the average value of the first 18 cycles. Genotyping was based on positive Ct values for FAM, TET, or both. Positive Ct values were normally detected between cycles 25–30, and indicated the presence of the specific allele(s). Sequence-validated genotyping controls were included in quadruplicate for each SNP with every run, to control assay performance and positional effects if any, accomplishing a 95% genotyping success rate with 100% concordance and accuracy.

Statistical Analysis

Categorical variables were analyzed by the Chi-Square test. Comparison of genotypes and alleles between different groups for continuous dependent variables was done by Analysis of Variance (ANOVA) or Student's test as appropriate. Logistic regression analysis was used to compute odds ratios and their 95% confidence intervals. Haplotype-based association analysis was performed using the haplo.stats package (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm) in the R Statistical Computing software (<http://www.r-project.org/>). Odds ratios for the haplotypes were calculated using as reference the baseline haplotype 1A-2A-3C-4A-5A, and the Haplotype Score statistics for the association of a haplotype with the binary trait was calculated as in Schaid et al. (2002) and Lake et al. (2003). Significance of association was determined between haplotypes and the case-control status - a binomial trait denoting whether or not a patient had CAD. All other statistical analyses were performed using the SPSS software version 14 (SPSS Inc., Chicago, IL, USA), and data are expressed as mean ± SEM. Associations with a two-tailed *p* value <0.05 was considered statistically significant.

Results

Genotyping and Coronary Heart Disease

The present study examined the potential relevance of 5 SNPs in a conserved 14q13.2 chromosomal region harbouring the *KIAA0391* and *PSMA6* genes. These include two SNPs, rs8008319 (hereafter also denoted as 1) and rs7157492 (2), residing on the *KIAA0391* gene, two others, rs1048990 (3) and rs12878391 (4), which are components of the downstream *PSMA6* gene, and a fifth, rs4981283 (5) which is found downstream of both genes (Fig. 1). Three of these (rs7157492, rs12878391 and rs4981283) are tag SNPs. Furthermore, no gene has been allotted to the rs4981283 SNP to date. Figure 2 portrays the linkage disequilibrium (LD) structure of the five studied SNPs, indicating that these SNPs were in moderate LD ($D = 0.186-0.648$; $r^2 = 0.002-0.407$) with each other (see also Supplementary data).

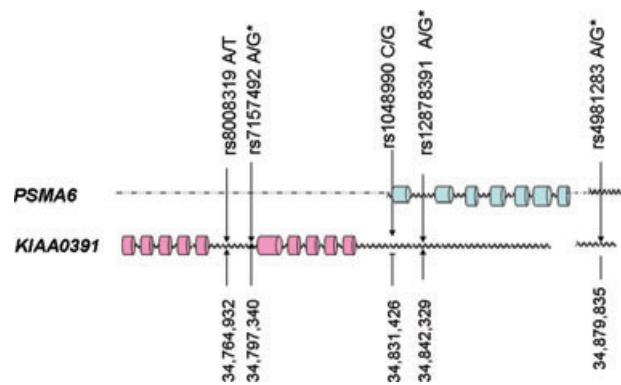


Figure 1 Schematic presentation of the conserved *KIAA0391* and *PSMA6* gene loci on chromosome 14q13.2.

The figure shows the position of the studied SNPs on the two genes and their corresponding chromosomal nucleotide position. Two of the SNPs, rs8008319 and rs7157492, are resident on the *KIAA0391* gene sequence, while two others rs1048990 and rs12878391 are components the *PSMA6* gene. The rs4981283 SNP is currently denoted as intergenic, but no gene has been allotted to it yet. *Indicates tag SNPs.

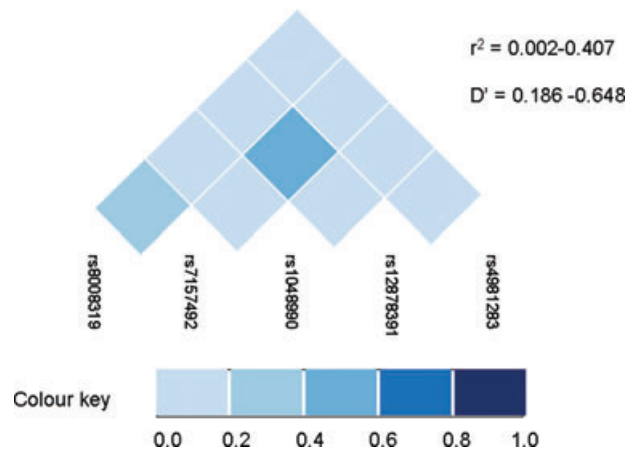


Figure 2 The linkage disequilibrium (LD) structure of the studied five SNPs. D' , coefficient of linkage disequilibrium; R' , regression coefficient.

The summary of genotyping results is given in Table 3. Briefly, the SNP rs7157492 conferring an nt135,835 A>G change in intron 5 of the *KIAA0391* gene exhibited a weak causative association ($p = 0.059$) with CAD. Thereby, the GG genotype [Odds ratio (95% Confidence Interval) = 1.52(1.02–2.27); $p = 0.042$] conferred risk for the disease. On the other hand, both the mutant TT genotype [0.63(0.41–0.97); $p = 0.036$] and the T allele [0.82(0.68–1.00); $p = 0.052$] of rs8008319 also in intron 5 of the *KIAA0391* exhibited protective tendencies against CAD. The other three SNPs, rs1048990 at position nt113 in exon 1 of the *PSMA6* gene, rs12878391 at position nt11016 in intron 1

SNP	CON (n = 929)	CAD (n = 1071)	P-value	O.R. (95% C.I.)
rs8008319			0.112	
AA	752(80.9)	884(82.6)	-	-
AT	128(13.8)	149(13.9)	0.925	0.99(0.77–1.28)
TT	50(5.4)	37(3.5)	0.036	0.63(0.41–0.97)
A allele	1630(87.7)	1919(89.6)	-	-
T allele	229(12.3)	222(10.4)	0.052	0.82(0.68–1.00)
rs7157492			0.059	
AA	685(73.7)	790(73.8)	-	-
AG	204(22.0)	211(19.7)	0.328	0.90(0.72–1.12)
GG	40(4.3)	70(6.5)	0.042	1.52(1.02–2.27)
A allele	1575(84.7)	1792(83.7)	-	-
G allele	284(15.3)	349(16.3)	0.376	1.08(0.91–1.28)
rs1048990			0.627	
CC	564(60.7)	643(60.0)	-	-
CG	301(32.4)	342(31.9)	0.972	1.00(0.82–1.20)
GG	64(6.9)	86(8.0)	0.347	1.18(0.84–1.66)
C allele	1430(76.9)	1629(76.1)	-	-
G allele	429(23.1)	512(23.9)	0.534	1.05(0.91–1.22)
rs12878391			0.502	
AA	693(74.6)	822(76.8)	-	-
AG	173(18.6)	179(16.7)	0.249	0.88(0.69–1.10)
GG	63(6.8)	70(6.5)	0.718	0.94(0.66–1.34)
A allele	1562(84.0)	1824(85.2)	-	-
G allele	297(16.0)	317(14.8)	0.306	0.91(0.77–1.09)
rs4981283			0.772	
AA	519(55.9)	584(54.5)	-	-
AG	252(27.1)	293(27.4)	0.755	1.03(0.84–1.27)
GG	158(17.0)	194(18.1)	0.478	1.08 (0.86–1.39)
A allele	1291(69.4)	1459(68.2)	-	-
G allele	568(30.6)	682(31.8)	0.376	1.06(0.93–1.21)

The table displays the distribution of the genotype and alleles for the 5 SNPs. O.R., odds ratio.

of the *PSMA6* and the intergenic SNP rs4981283 at chromosomal position nt 34,879,835 showed no delineable trends with respect to CAD (Table 3).

We further tested for possible association of the SNPs with the extent of disease as measured by the number of disease vessels. Within the CAD group, 586 (54.7%) had one-vessel, 157 (14.7%) had two-vessel and 328 (30.6%) had more than two-vessel disease. Logistic regression analysis showed no significant relationship for the individual SNPs with increasing number of vessels, pointing to a lack of association with the degree of CAD. It should be noted that none of the SNPs deviated significantly ($p > 0.1$) from the Hardy-Weinberg equilibrium (see also Supplementary Data).

Gene Polymorphism, Confounding Factors and CAD

As shown in Table 1, our patient population displayed a much higher proportion of MI (94.9% vs 29.0%), hyperlipidaemia

(34.0% vs 13.3%), hypertension (86.3% vs 69.6%) and DM2 (68.5% vs 34.2%) compared to controls. There was no remarkable variation by gender in the distribution of these confounders, as well as in the extent of the disease among the patients (depicted as single, double or more than two diseased vessels in the table). We further explored the likelihood of the confounding variables, such as DM2, hypertension, obesity and MI on admission influencing the impact of the SNPs on CAD. The test for association of the individual risk factors with the genotypes revealed no positive association of these SNPs with any of the variables, pointing to a dissociation of these confounders from potential influence of the gene variants on disease manifestation in the study population.

SNP Haplotyping and CAD

We were interested in establishing further the potential impact of haplotypes generated from these SNPs as predisposing

Haplotype 1-2-3-4-5	Control	MI	Pooled	P-value	O.R. (95% C.I.)
A-G-C-A-A	2.04	3.02	2.49	0.022	1.49(0.95–2.36)
A-G-C-A-G	0.22	0.63	0.48	0.084	2.60(0.64–10.59)
A-G-G-A-A	0.45	1.03	0.79	0.031	2.24(0.84–5.98)
T-G-C-A-G	0.54	0.07	0.29	0.073	0.20(0.03–1.39)
T-G-C-G-A	6.29	4.25	5.18	0.015	0.79(0.59–1.05)

Table 4 Association of haplotypes in the *KIAA0391* and *PSMA6* gene cluster with coronary heart disease.

The table shows the relative frequencies of the associated haplotype constructs from combinations of the alleles of the studied SNPs. Number 1 denotes the SNP rs8008319, 2 denotes rs7157492, 3 denotes rs1048990, 4 denotes rs12878391 and 5 denotes rs4981283. The most frequent haplotype A-A-C-A-A was employed to determine the relative frequencies of the associated haplotypes as described in the Statistical analysis procedure (See also Supplementary data file). Frequencies for the two independent groups and the groups pooled together are given as percentage. O.R., odds ratio; C.I. confidence interval.

factors to the disease. Interestingly, the test for marker–trait association for these SNPs identified 5 haplotypes generated by a five-SNP locus model that displayed an association with the disease (Supplementary data). Two of these haplotypes, 1A-2G-3C-4A-5A [1.49(0.95–2.35); $p = 0.022$] and 1A-2G-3G-4A-5A [2.24(0.84–5.98); $p = 0.031$] conferred risk for CAD (Table 4). One other haplotype comprising 1A-2G-3C-4A-5G [2.24(0.84–5.98); $p = 0.079$] pointed to a borderline association with the disease. Furthermore, a haplotype consisting of 1T-2G-3C-4G-5A [0.79(0.59–1.05); $p = 0.015$] exhibited protective properties while another, 1T-2G-3C-4A-5G [0.20(0.03–1.39); $p = 0.073$] showed a weaker tendency.

We then analyzed the data to evaluate the possible interaction with the confounding variables. Most interestingly, the two haplotypes 1A-2G-3C-4A-5A and 1A-2G-3G-4A-5A conferring risk for CAD were even more significantly associated with MI (with p -values of 0.002 and 0.009 respectively) in an independent analysis (results not published). No similar relationships were delineable for the other confounders, pointing to lack of influence by these variables on the relationship of the investigated SNPs with CAD in the present study.

Discussion

The present study investigates the potential association of five SNPs in a 100-kb region of chromosome 14 (14q13.2), encompassing the *PSMA6* and *KIAA0391* gene cluster in predisposing individuals to CAD. The findings of the study suggested that at the SNP level, only the rs7157492 SNP might confer risk for CAD. Interestingly, this SNP constitutes an intronic component of the *KIAA0391* gene. Furthermore, another SNP (rs8008319) in the same region of the *KIAA0391* gene pointed to a protective rather than causative effect. None

of the SNPs constituting part of the *PSMA6* gene was found to be associated with CAD, possibly precluding these SNPs individually as predisposing factors for the disease, at least with respect to the *PSMA6* gene. It is also worth mentioning that no delineable relationship was found between any of the studied SNPs and the number of diseased vessels, suggesting that the relevance of these variants, if any, may be limited to predicting primarily the presence rather than the extent of the disease.

Very limited information is currently available on the impact of polymorphic changes in this chromosomal locus on CAD manifestation. In fact, the existing data remain at best inconsistent, probably implicating both the *KIAA0391* and the *PSMA6* genes in cardiovascular risk factors, such as MI (Ozaki et al., 2006), DM2 (Barbieri et al., 2008) and changes in intima thickness (Takashima et al., 2007), rather than CAD per se. Of the SNPs involved in the present study, the rs1048990 appears to be the most extensively studied to date. This SNP was associated with MI in the Japanese population in a study by Ozaki and colleagues (Ozaki et al., 2006). In contrast, a study by Bennett et al. (2008) pointed to the risk of rs1048990 for MI as unlikely to be conspicuous in the Western population, due to its rarity in these ethnic groups. Additionally, Banerjee and colleagues (Banerjee et al., 2008) were unable to establish its association with MI in the Indian population. Our present results are also not in concordance with the findings of Ozaki and colleagues pertaining to the role of this SNP in CAD. Having said that, it should be pointed out that a meta-analysis by Bennett et al. (2008) showing a combined odds ratio of 1.15 (95% CI: 1.08–1.21) per G allele of the rs1048990, supported the notion of its association with MI. Comparison of this analysis with our findings indicates corresponding values of 1.05 (95% CI: 0.91–1.22) possibly pointing to the SNP eventually presenting a risk for CAD in our study population. Notably, the G allele frequency in our population was 0.23,

which is comparatively lower than in the Japanese population (Ozaki et al., 2006) in which it was significantly associated with MI. This low prevalence may explain its lack of association with disease in our population. Furthermore, apart from the genotype -8C/G (SNP rs1048990) itself, Sjakste and colleagues (Sjakste et al., 2007b) also described an association of a haplotype C-110/G-8 of the *PSMA6* gene with a higher risk for DM2. In our study, rs1048990 was not associated with DM2, or any other confounding factor, possibly pointing to inter-ethnic differences with respect to its impact as a risk for cardiovascular-related disorders, in general. Hence, the inconsistencies in the literature only confirm the assertion that the functional role of this SNP remains at best to be deciphered.

Another SNP studied herein, rs7157492, has also been previously discussed as a potential risk for cardiovascular disorders, and was noted by Ozaki et al. (2006) as being associated with MI. Interestingly, in our study the heterozygous A/G genotype, but not the minor G allele of this SNP, was associated with CAD. Thus, it appears indeed that rs7157492 may constitute a predisposing factor to both CAD and MI.

The variations in the reported impact of the studied SNPs on cardiovascular disease may pertain to a variety of factors. Firstly, although the linkage of this chromosomal region with both cardiovascular-related (Ozaki et al., 2006; Sjakste et al., 2007b; Barbieri et al., 2008) and other diseases (Kamnasaran et al., 2003; Sjakste et al., 2004) in general has been discussed primarily with respect to the *PSMA6* polymorphism, it should be noted that some of the associated SNPs reside on the *KIAA0391* gene. To our knowledge, however, virtually no evidence is currently available in the literature directly implicating this gene in CAD. Secondly, it seems likely that, although the impact of these alterations may not be evident at the individual SNP level, CAD could manifest itself as a combined outcome of an interaction involving several gene variants, especially given the fact that CAD is a complex polygenic disorder. To this end, we sought to investigate the role of haplotypes constructed from the studied SNPs in this gene cluster. Interestingly, we identified two distinct haplotypes as conferring risk for CAD, and another showing a borderline association with the disease. Even more important perhaps was the observation that these haplotypes were also implicated in MI. While MI is known to be a primary indicator for the events leading to CAD, currently there is a lack of genetic-based evidence linking the former with the latter. In the present study, apart from underscoring the importance of haplotypes as markers for CAD, our observations also point to some of them constituting a strong link between CAD and MI. It is particularly noteworthy in this regard that these combinations of alleles predisposing individuals to both CAD and MI were constructed from the same SNPs that individually lacked delineable relationship with the disease.

An important question remains as to the functional nature of the potential gene(s) involved in CAD and MI. Based on the currently defined role of *PSMA6* in inflammatory processes, Ozaki et al. (Ozaki et al., 2006) have postulated that the risk-conferring G allele of the SNP -8C/G may exacerbate inflammation by enhancing the transcription of *PSMA6* through the activation of nuclear factor- κ B protein (Ozaki et al., 2006). However, while such a mechanism might explain the involvement of the inflammatory processes in the manifestation of MI, it does not appear to pertain to the process(es) by which this change may be involved under the present circumstances, since it was not linked with MI or CAD. Accordingly, the *PSMA6* gene does not seem to meet the criteria, particularly since the findings of various studies are also not consistent for any of the potentially functional SNPs relative to its sequence. On the other hand, such a mechanism might involve functional changes in the *KIAA0391* gene. To date, the only cardiac-related event associated with this gene is DM2, but no speculations have been advanced yet on the possible mechanisms involved. Moreover, the two SNPs displaying any form of link with CAD in our study were both intronic to the *KIAA0391* gene, rendering them unlikely candidates from a functional point of view. Hence, this novel finding of haplotypes predisposing individuals concomitantly to MI and CAD strongly points to the probability of yet undefined functional or regulatory entities in this gene cluster as the decisive players in events leading to the disease. The fact that various studies have linked microsatellites in this region with MI (Zintzaras & Kitsios, 2006) and that no coding SNP in the *KIAA0391* gene has been assigned to these observations lend support to this notion. Further studies are therefore necessary to establish whether or not this phenomenon is attributable to a common mechanism or to interdependent pathways leading to the manifestation of CAD.

There is increasingly evidence that haplotypes may be more predictive than individual SNPs as predisposing risk factors for complex diseases such as CAD^{30–33}. It is noteworthy that our study clearly differentiates the potential impact of the studied SNPs individually from that of their ensuing haplotypes on both CAD and MI as a confounder to the former. Therefore, the present findings bolster the value of haplotyping as a more informative approach than individual SNP genotyping for deciphering risk factors for such diseases. In conclusion, our study identified the chromosomal region encompassing the *KIAA0391* and *PSMA6* genes as presenting a risk for CAD, and as harbouring common haplotypes predisposing humans to both MI and CAD. These results point to a discernible link at the genetic level for CAD and MI, its important risk factor, possibly implicating common pathway(s) leading to both diseases.

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References

- Alsmadi, O. A., Al-Kayal, F., Al-Hamed, M. & Meyer, B. F. (2006) Frequency of common HFE variants in the Saudi population: a high throughput molecular beacon-based study. *BMC Med Genet* **7**, 43–49.
- Auld, K. L. & Silver, P. A. (2006) Transcriptional regulation by the proteasome as a mechanism for cellular protein homeostasis. *Cell Cycle* **5**, 1503–1505.
- Banerjee, I., Gupta, V., Ahmed, T., Faizaan, M., Agarwal, P. & Ganesh, S. (2008) Inflammatory system gene polymorphism and the risk of stroke: a case-control study in an Indian population. *Brain Res Bull* **75**, 158–165.
- Barbieri, M., Marfella, R., Rizzo, M. R., Boccardi, V., Siniscalchi, M., Schiattarella, C., Siciliano, S., Lemme, P. & Paolisso, G. (2008) The -8 UTR C/G polymorphism of PSMA6 gene is associated with susceptibility to myocardial infarction in type 2 diabetic patients. *Atherosclerosis* **201**, 117–123.
- Bennett, D. A., Xu, P., Clarke, R., Zondervan, K., Parish, S., Palmer, A., Cardon, L., Peto, R., Lathrop, M. & Collins, R. (2008) The exon 1–8C/G SNP in the PSMA6 gene contributes only a small amount to the burden of myocardial infarction in 6946 cases and 2720 controls from a United Kingdom population. *Eur J Hum Genet* **16**, 480–486.
- Ciechanover, A. (2006a) Intracellular protein degradation: from a vague idea thru the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. *Hematology Am Soc Hematol Educ Program* **1–12**, 505–506.
- Ciechanover, A. (2006b) The ubiquitin proteolytic system: from a vague idea, through basic mechanisms, and onto human diseases and drug targeting. *Neurology* **66**, S7–S19.
- Dimmeler, S., Breitschopf, K., Haendeler, J. & Zeiher, A. M. (1999) Dephosphorylation targets Bcl-2 for ubiquitin-dependent degradation: a link between the apoptosome and the proteasome pathway. *J Exp Med* **189**, 1815–1822.
- Harrap, S. B., Zammit, K. S., Wong, Z. Y., Williams, F. M., Bahlo, M., Tonkin, A. M. & Anderson, S. T. (2002) Genome-wide linkage analysis of the acute coronary syndrome suggests a locus on chromosome 2. *Arterioscler Thromb Vasc Biol* **22**, 874–878.
- Hauser, E. R., Crossman, D. C., Granger, C. B., Haines, J. L., Jones, C. J., Mooser, V., Mcadam, B., Winkelmann, B. R., Wiseman, A. H., Muhlestein, J. B., Bartel, A. G., Dennis, C. A., Dowdy, E., Estabrooks, S., Eggleston, K., Francis, S., Roche, K., Clevenger, P. W., Huang, L., Pedersen, B., Shah, S., Schmidt, S., Haynes, C., West, S., Asper, D., Booze, M., Sharma, S., Sundseth, S., Middleton, L., Roses, A. D., Hauser, M. A., Vance, J. M., Pericak-Vance, M. A. & Kraus, W. E. (2004) A genomewide scan for early-onset coronary artery disease in 438 families: the GENECARD Study. *Am J Hum Genet* **75**, 436–447.
- Helgadóttir, A., Manolescu, A., Helgason, A., Thorleifsson, G., Thorsteinsdóttir, U., Gudbjartsson, D. F., Gretarsdóttir, S., Magnusson, K. P., Gudmundsson, G., Hicks, A., Jonsson, T., Grant, S. F., Sainz, J., O'Brien, S. J., Sveinbjornsdóttir, S., Valdimarsson, E. M., Matthiasson, S. E., Levey, A. I., Abramson, J. L., Reilly, M. P., Vaccarino, V., Wolfe, M. L., Gudnason, V., Quyyumi, A. A., Topol, E. J., Rader, D. J., Thorgeirsson, G., Gulcher, J. R., Hakonarson, H., Kong, A. & Stefansson, K. (2006) A variant of the gene encoding leukotriene A4 hydrolase confers ethnicity-specific risk of myocardial infarction. *Nat Genet* **38**, 68–74.
- Helgadóttir, A., Thorleifsson, G., Manolescu, A., Gretarsdóttir, S., Blondal, T., Jonasdóttir, A., Jonasdóttir, A., Sigurdsson, A., Baker, A., Palsson, A., Masson, G., Gudbjartsson, D. F., Magnusson, K. P., Andersen, K., Levey, A. I., Backman, V. M., Matthiasdóttir, S., Jonsdóttir, T., Palsson, S., Einarsdóttir, H., Gunnarsdóttir, S., Gylfason, A., Vaccarino, V., Hooper, W. C., Reilly, M. P., Granger, C. B., Austin, H., Rader, D. J., Shah, S. H., Quyyumi, A. A., Gulcher, J. R., Thorgeirsson, G., Thorsteinsdóttir, U., Kong, A. & Stefansson, K. (2007) A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* **316**, 1491–1493.
- Hochrainer, K. & Lipp, J. (2007) Ubiquitylation within signaling pathways in- and outside of inflammation. *Thromb Haemost* **97**, 370–377.
- Kamnasaran, D., Muir, W. J., Ferguson-Smith, M. A. & Cox, D. W. (2003) Disruption of the neuronal PAS3 gene in a family affected with schizophrenia. *J Med Genet* **40**, 325–332.
- Karin, M. & Delhase, M. (2000) The I kappa B kinase (IKK) and NF-kappa B: key elements of proinflammatory signalling. *Semin Immunol* **12**, 85–98.
- Konstantinova, I. M., Tsimokha, A. S. & Mittenberg, A. G. (2008) Role of proteasomes in cellular regulation. *Int Rev Cell Mol Biol* **267**, 59–124.
- Lake, S. L., Lyon, H., Tantisira, K., Silverman, E. K., Weiss, S. T., Laird, N. M. & Schaid, D. J. (2003) Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. *Hum Hered* **55**, 56–65.
- Lusis, A. J., Fogelman, A. M. & Fonarow, G. C. (2004a) Genetic basis of atherosclerosis: part I: new genes and pathways. *Circulation* **110**, 1868–1873.
- Lusis, A. J., Fogelman, A. M. & Fonarow, G. C. (2004b) Genetic basis of atherosclerosis: part II: clinical implications. *Circulation* **110**, 2066–2071.
- Maki, C. G., Huibregtse, J. M. & Howley, P. M. (1996) In vivo ubiquitination and proteasome-mediated degradation of p53(1). *Cancer Res* **56**, 2649–2654.
- Ozaki, K., Sato, H., Iida, A., Mizuno, H., Nakamura, T., Miyamoto, Y., Takahashi, A., Tsunoda, T., Ikegawa, S., Kamatani, N., Hori, M., Nakamura, Y. & Tanaka, T. (2006) A functional SNP in PSMA6 confers risk of myocardial infarction in the Japanese population. *Nat Genet* **38**, 921–925.
- Pajukanta, P., Cargill, M., Viitanen, L., Nuotio, I., Kareinen, A., Perola, M., Terwilliger, J. D., Kempas, E., Daly, M., Lilja, H., Rioux, J. D., Brettin, T., Viikari, J. S., Ronnema, T., Laakso, M., Lander, E. S. & Peltonen, L. (2000) Two loci on chromosomes 2 and X for premature coronary heart disease identified in early- and late-settlement populations of Finland. *Am J Hum Genet* **67**, 1481–1493.
- Reed, S. I. (2006) The ubiquitin-proteasome pathway in cell cycle control. *Results Probl Cell Differ* **42**, 147–181.
- Rivett, A. J. (1989) The multicatalytic proteinase of mammalian cells. *Arch Biochem Biophys* **268**, 1–8.
- Salghetti, S. E., Kim, S. Y. & Tansey, W. P. (1999) Destruction of Myc by ubiquitin-mediated proteolysis: cancer-associated and transforming mutations stabilize Myc. *Embo J* **18**, 717–726.
- Samani, N. J., Burton, P., Mangino, M., Ball, S. G., Balmforth, A. J., Barrett, J., Bishop, T. & Hall, A. (2005) A genomewide linkage study of 1,933 families affected by premature coronary

- artery disease: The British Heart Foundation (BHF) Family Heart Study. *Am J Hum Genet* **77**, 1011–1020.
- Schaid, D. J., Rowland, C. M., Tines, D. E., Jacobson, R. M. & Poland, G. A. (2002) Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* **70**, 425–434.
- Sjakste, T., Eglite, J., Sochnevs, A., Marga, M., Pirags, V., Collan, Y. & Sjakste, N. (2004) Microsatellite genotyping of chromosome 14q13.2–14q13 in the vicinity of proteasomal gene PSMA6 and association with Graves' disease in the Latvian population. *Immunogenetics* **56**, 238–243.
- Sjakste, T., Kalis, M., Poudziunas, I., Pirags, V., Lazdins, M., Groop, L. & Sjakste, N. (2007a) Association of microsatellite polymorphisms of the human 14q13.2 region with type 2 diabetes mellitus in Latvian and Finnish populations. *Ann Hum Genet* **71**, 772–776.
- Sjakste, T., Poudziunas, I., Ninio, E., Perret, C., Pirags, V., Nicaud, V., Lazdins, M., Evanss, A., Morrison, C., Cambien, F. & Sjakste, N. (2007b) SNPs of PSMA6 gene—investigation of possible association with myocardial infarction and type 2 diabetes mellitus. *Genetika* **43**, 553–559.
- Takashima, N., Shioji, K., Kokubo, Y., Okayama, A., Goto, Y., Nonogi, H. & Iwai, N. (2007) Validation of the association between the gene encoding proteasome subunit alpha type 6 and myocardial infarction in a Japanese population. *Circ J* **71**, 495–498.
- Wang, J. & Maldonado, M. A. (2006) The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. *Cell Mol Immunol* **3**, 255–261.
- Wang, Q., Rao, S., Shen, G. Q., Li, L., Moliterno, D. J., Newby, L. K., Rogers, W. J., Cannata, R., Zirzow, E., Elston, R. C. & Topol, E. J. (2004) Premature myocardial infarction novel susceptibility locus on chromosome 1P34–36 identified by genomewide linkage analysis. *Am J Hum Genet* **74**, 262–271.
- Wang, X., Guerrero, C., Kaiser, P. & Huang, L. (2007) Proteomics of proteasome complexes and ubiquitinated proteins. *Expert Rev Proteomics* **4**, 649–665.
- Yamada, Y. (2006) Identification of genetic factors and development of genetic risk diagnosis systems for cardiovascular diseases and stroke. *Circ J* **70**, 1240–1248.
- Yamada, Y., Matsuo, H., Segawa, T., Watanabe, S., Kato, K., Hibino, T., Yokoi, K., Ichihara, S., Metoki, N., Yoshida, H., Satoh, K. & Nozawa, Y. (2006) Assessment of genetic risk for myocardial infarction. *Thromb Haemost* **96**, 220–227.
- Zintzaras, E. & Kitsios, G. (2006) Identification of chromosomal regions linked to premature myocardial infarction: a meta-analysis of whole-genome searches. *J Hum Genet* **51**, 1015–1021.

Supporting Information

Additional Supporting Information may be found in the online version of the article:

CAD_Supplementary_Data The file summarizes the results of the haplotype-based association analysis. The most frequent haplotype in the study population was AACAA showing a pooled haplotype frequency of 40.70%. This haplotype was used as the reference for determining the relative frequencies of the associated haplotypes as described in the Statistical analysis procedure. The haplotypes showing significant association with CAD are highlighted. The confidence interval is presented as OR low and OR upper.

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