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Multi-allelic haplotype association identifies novel information different from single-SNP analysis: A new protective haplotype in the *LRP8* gene is against familial and early-onset CAD and MI

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Abstract

Our previous studies identified a functional SNP, R952Q in the *LRP8* gene, that was associated with increased platelet activation and familial and early-onset coronary artery disease (CAD) and myocardial infarction (MI) in American and Italian Caucasian populations. In this study, we analyzed four additional SNPs near R952Q (rs7546246, rs2297660, rs3737983, rs5177) to identify a specific *LRP8* SNP haplotype that is associated with familial and early-onset CAD and MI. We employed a case–control association design involving 381 premature CAD and MI probands and 560 controls in GeneQuest, 441 individuals from 22 large pedigrees in GeneQuest II, and 248 MI patients with family history and 308 controls in an Italian cohort. Like R952Q, *LRP8* SNPs rs7546246, rs2297660, rs3737983, and rs5177 were significantly associated with early-onset CAD/MI in both population-based and family-based association studies in GeneQuest. The results were replicated in the GeneQuest II family-based population and the Italian population. We then carried out a haplotype analysis for all five SNPs including R952Q. One common haplotype (TCCGC) was significantly associated with CAD ($P = 4.0 \times 10^{-11}$) and MI ($P = 6.5 \times 10^{-12}$) in GeneQuest with odds ratios of 0.53 and 0.42, respectively. The results were replicated in the Italian cohort ($P = 0.004$, OR = 0.71). The sib-TDT analysis also showed significant association between the TCCGC haplotype and CAD in GeneQuest II ($P = 0.001$). These results suggest that a common *LRP8* haplotype TCCGC confers a significant protective effect on the development of familial, early-onset CAD and/or MI.

Keywords

LRP8; Haplotype; SNPs; Association study

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1. Introduction

Coronary artery disease (CAD) and myocardial infarction (MI) are the most common cause of sudden death. In the United States, more than 2200 Americans die of CAD/MI each day, i.e. an average of 1 death every 39 s (Roger et al., 2012). A family history of early-onset CAD/MI and related mortality is an independent risk factor for the development of CAD/MI (Guttmacher et al., 2004). The family history and twin studies have confirmed that genetic factors are one of the most importance risk contributor for the development of CAD and MI (Shah, 2007).

Genome-wide linkage analysis in 428 families with early-onset CAD and MI identified a genetic linkage to chromosome 1p36 (Wang et al., 2004), and follow-up candidate genes analysis revealed that SNP R952Q in the low density lipoprotein receptor-related protein 8 (*LRP8*) gene was significantly associated with familial and early-onset CAD and MI in American Caucasian populations using both population-based and family-based design (Shen et al., 2007). Functional studies showed that SNP R952Q was a functional SNP that results in increased phosphorylation of p38 MAPK (Shen et al., 2007). We found that *LRP8* SNP R952Q showed significant association with CAD in a population of 381 CAD probands from the GeneQuest families with familial and early-onset CAD and MI and 560 controls without stenosis detectable by coronary angiography (Shen et al., 2007). We also found that R952Q was associated with CAD in the full GeneQuest cohort including probands and other family members by sib-TDT analysis. These results were replicated in two additional independent Caucasian populations, including a cohort of large CAD/MI families with a total of 441 individuals from 22 Caucasian families and the average pedigree size of 20 people, and an Italian cohort of 248 individuals with a family history of MI and 308 Italian controls (Shen et al., 2007). In the Italian population with ApoE concentration data available, we further showed that *LRP8* SNP R952Q may determine the ApoE concentrations and be associated with risk of MI with an additive effect to *APOE* epsilon2/epsilon3/epsilon4 genotype (Martinelli et al., 2009). Interestingly, the R952Q variant in *LRP8* also showed significant association with increased platelet activation at two concentrations of the ADP agonist (Shen et al., 2007).

Using the HapMap data, we found that *LRP8* gene contains five linkage disequilibrium (LDs) or haplotype blocks, and SNP R952Q is located in the fifth LD (LD5) at the 3′-terminus of the gene. In this study, we further analyzed the association between *LRP8* and CAD/MI by incorporating haplotype analysis of other four SNPs in LD5 and found that a common haplotype of LD5 in the *LRP8* gene confers a highly protective role in the development of familial and early-onset CAD and/or MI.

2. Materials and methods

2.1. Study subjects

We carried out both population-based case–control association studies and family-based association studies. Three independent European-descent study populations were used in this study, including GeneQuest, GeneQuest II and an Italian population. The sample size, structure, clinical characteristics and the criteria for diagnosis of CAD and MI were all described previously (Shen et al., 2007). The GeneQuest and GeneQuest II families were ascertained at Cleveland Clinic. The Italian cohort was enrolled in the Verona Heart Study, Italy. Only Caucasian study subjects from these populations were selected for the present study to avoid confounding of the ethnic factor.

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This study was approved by local Institutional Review Boards on Human Subject Research, and written informed consent was obtained from all participants.

2.2. Genotyping of SNPs

In addition to SNP R952Q, four additional SNPs within the last *LRP8* LD block (LD5) (data not shown) were selected based on a minor allele frequency of >30% and availability by ABI assay-on-demand. Three SNPs were located in exons (rs2297660, rs3737983 and rs5177) and one SNP was in an intron (rs7546246). Whole blood was drawn from each participant and genomic DNA was isolated from the blood using standard protocols. The SNP genotyping assays were purchased from ABI (Applied Biosystems, Foster City, CA, USA). SNP genotyping was carried out as previously described by us (Shen et al., 2009). High-throughput SNP genotyping was performed on an ABI PRISM 7900HT Sequence Detection System. The PCR Automatic allele calling was carried out by ABI PRISM 7900HT data collection and analysis software version 2.1.

To ensure the quality of SNP genotyping by TaqMan assays, direct DNA sequence analysis was used to genotype SNP rs2297660 in the entire GeneQuest cohort and other four SNPs in randomly selected 32 samples. The results from TaqMan assays matched completely the sequencing data. Direct DNA sequence analysis was performed using an ABI PRISM 3100 Genetic Analyzer (ABI, Foster City, CA, USA).

2.3. Statistical analysis

Genotyping data from each SNP was tested for Hardy–Weinberg equilibrium among the CAD and/or MI patients and controls using Haploview software version 3.0.

Association of a SNP or a SNP haplotype with a disease trait was assessed using Chi-square tests (SAS Ver 9.00 or Haploview version 3.0). Odds ratios and 95% confidence intervals were estimated using the Chi-square test (SAS Ver 9.00). Empirical *P*-values were calculated using 10,000 Monte Carlo simulations by the CLUMP program. Haplotypes were estimated using PHASE software. Pairwise linkage disequilibrium (LD) was estimated using Haploview software version 3.0. Haplotype data was subjected to permutation analysis (with 1000 to 10,000,000 simulations) using Haploview software. Sib-TDT analysis was carried out using the TDT/S-TDT program 1.1.

3. Results

3.1. Association analysis between four new SNPs in LRP8 block LD5 and risk of CAD and MI in the GeneQuest population and an Italian MI cohort with family history

Four SNPs within *LRP8* LD5, including rs7546246, rs2297660, rs3737983 and rs5177, were in Hardy–Weinberg equilibrium in all populations genotyped in this study ($P > 0.10$). Allelic association analysis for the four SNPs was then carried out. Significant association was identified between each SNP and CAD after adjusting for possible confounding effects of gender, age, hypertension, diabetes and plasma total cholesterol levels ($Padj = 0.001–0.012$, Table 1). Significant association was also identified between each SNP and MI ($Padj = 0.003–0.012$, Table 1). The association remained after a permutation analysis ($Pemp < 0.05$) or even after a non-conservative Bonferroni correction ($P < 0.05$). These results suggest that SNPs rs7546246, rs2297660, rs3737983 and rs5177 in the *LRP8* gene are associated with early-onset CAD and MI in an American Caucasian population.

In order to further establish the association of *LRP8* SNPs with CAD and MI, we studied an independent Caucasian population with familial MI from Italy. We have genotyped the same four SNPs in the Italian cohort. All four SNPs showed significant association with MI after

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adjusting for gender, age, hypertension, diabetes and plasma total cholesterol levels (P = 0.0008–0.009, Table 1).

In a combined population with the GeneQuest and Italian study subjects, the significant P level for association between each *LRP8* SNP and CAD/MI decreased markedly (*Padj* = 0.0001–0.003, Table 1).

3.2. Family-based TDT analysis further implicates the association of LRP8 SNPs with CAD

We then genotyped SNPs rs7546246, rs2297660, rs3737983 and rs5177 in the full GeneQuest population, including probands and other family members (Shen et al., 2007; Wang et al., 2004). Sib-TDT analysis showed that the four SNPs were significantly associated with CAD (P = 0.00005–0.04; Table 2). However, after stringent Bonferroni correction, SNP rs2297660 failed to show a significant association with CAD.

Similarly, in the second family-based GeneQuest II study population, sib-TDT analysis showed that all four SNPs were significantly associated with CAD (P = 0.004–0.016; Table 2). After Bonferroni correction, only SNPs rs2297660 and rs5177 remained significant for association with CAD. Together, family-based association studies partially validated the conclusion from the population-based studies that SNPs rs7546246, rs2297660, rs3737983 and rs5177 in *LRP8* LD5 were associated with the risk of CAD.

3.3. Identification of a haplotype in LRP8 with a protective role in CAD and MI

Haplotype analysis was carried out for genotyping data of SNPs rs7546246, rs2297660, rs3737983 and rs5177 as well as R952Q reported previously (Shen et al., 2007). A total of 20 out of 32 (i.e. 2^5) expected haplotypes were identified in the GeneQuest population and analyzed for their association with CAD or MI. We identified one haplotype, TCCGC carrying protective alleles from all five SNPs, which conferred highly significant protection against CAD (*Pobs* = 4.0×10^{-11}, OR = 0.53, *Pemp* < 1.0×10^{-7}; Table 3) and MI (*Pobs* = 6.5×10^{-12}, OR = 0.42, *Pemp* < 1.0×10^{-7}; Table 3). This protective haplotype was also detected in the Italian cohort and was significantly associated with resistance to the development of MI (*Pobs* = 0.004, OR = 0.71, *Pemp* = 0.008; Table 3). The association was strengthened in the combined GeneQuest and Italian populations (*Pobs* = 3.0×10^{-11}, OR = 0.61, *Pemp* < 1×10^{-7}; Table 3).

We also performed a family-based association analysis with sib-TDT for the protective haplotype in GeneQuest II and found that it was significantly associated with CAD (P = 0.001; Table 4).

4. Discussion

We previously reported that the sequence variant R952Q, a functional SNP in the *LRP8* gene, was significantly associated with familial and early-onset CAD and MI in two independent populations including an American Caucasian GeneQuest population and an Italian population (Shen et al., 2007). To provide additional evidence to support the association between the *LRP8* variant and familial and early-onset CAD and MI, we extended the previous study from a single R952Q SNP to the LD block where R952Q resides (LD5). We analyzed four additional SNPs in LD5, rs7546246, rs2297660, rs3737983 and rs5177, which are in LD with SNP R952Q. As expected, significant association was found for each SNP with familial and early-onset CAD and MI in both GeneQuest and the Italian population (Tables 1 and 2). We conducted an extended SNP haplotype analysis with genotyping data for all five *LRP8* SNPs. Haplotypes were estimated using an accelerated expectation–maximization algorithm, which gives highly accurate population frequency estimate of the phased haplotypes based on the maximum likelihood as determined from the

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unphased input (Barrett et al., 2005). Interestingly, we identified one protective haplotype TCCGC for association with CAD/MI in the GeneQuest, GeneQuest II and Italian populations. Haplotype TCCGC conferred a protective effect against CAD/MI (OR = 0.42–0.71) (Tables 3 and 4). Haplotype TCCGC was common and had a population frequency of 59.5%–64.6% in the control populations, and a frequency of 38.8%–57.6% in the CAD/MI populations (Table 3). These results demonstrate that extended SNP haplotype analysis can provide important insights into the relation between the SNP patterns and CAD/MI that is beyond what single point SNP analysis can reveal. Together, these results provide supportive genetic evidence that *LRP8* is a susceptibility gene for familial and early-onset CAD and MI.

Platelet activation is involved in physiological hemostasis and atherothrombosis. We were the first to establish the significant association between the *LRP8* gene and platelet activation in CAD and MI patients (Shen et al., 2007). Later, Robertson et al. showed that compared with littermate controls, homozygous $LRP8^{-/-}$ knockout mice showed decreased platelet activation and prolonged carotid artery occlusion time in response to ADP and thrombin stimulation (Robertson et al., 2009). Recently, Quinn et al. observed reduced leukocyte rolling, and decreased platelet activation and thrombosis in response to stimulation by human neutrophil peptides (HNPs) (Quinn et al., 2011). HNPs are molecules released by activated neutrophils and involved in platelet activation and form cell formation in atherosclerosis (Quinn et al., 2011). In 2003, Huo et al. showed that circulating activated platelets can increase atherosclerosis in $ApoE^{-/-}$ background (Huo et al., 2003). These studies suggest that *LRP8* variation increases the risk of CAD and MI by increasing platelet activation and thrombus formation, which may be associated with increased p38MAP kinase phosphorylation (activation), a property identified for *LRP8* SNP R952Q. With the same reasoning, the protective haplotype TCCGC of LRP8 contains the common R allele associated with less p38 MAPK activation than the Q allele, which may lead to less platelet activation, thrombosis and atherosclerosis, explaining the protective effect for CAD and MI.

5. Conclusions

In conclusion, multiple lines of evidence from both population-based and family-based association studies from three independent populations support the finding that the most common TCCGC haplotype at the 3′-terminal block of the *LRP8* gene (LD5) confers a protective role in the development of familial and early-onset CAD and/or MI.

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Abbreviations

CAD	coronary artery disease
MI	myocardial infarction
LRP8	low density lipoprotein receptor-related protein 8
SNP	single nucleotide polymorphism
LD	linkage disequilibrium
TDT	transmission/disequilibrium test

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MAF	minor allele frequency
OR	odds ratio

References

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21 (2):263–265. [PubMed: 15297300]

Guttmacher AE, Collins FS, Carmona RH. The family history—more important than ever. N Engl J Med. 2004; 351 (22):2333–2336. [PubMed: 15564550]

Huo Y, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med. 2003; 9 (1):61–67. [PubMed: 12483207]

Martinelli N, et al. Additive effect of LRP8/APOER2 R952Q variant to APOE epsilon2/epsilon3/epsilon4 genotype in modulating apolipoprotein E concentration and the risk of myocardial infarction: a case–control study. BMC Med Genet. 2009; 10:41. [PubMed: 19439088]

Quinn KL, et al. Human neutrophil peptides mediate endothelial–monocyte interaction, foam cell formation, and platelet activation. Arterioscler Thromb Vasc Biol. 2011; 31 (9):2070–2079. [PubMed: 21817096]

Robertson JO, Li W, Silverstein RL, Topol EJ, Smith JD. Deficiency of LRP8 in mice is associated with altered platelet function and prolonged time for in vivo thrombosis. Thromb Res. 2009; 123 (4):644–652. [PubMed: 18706682]

Roger VL, et al. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation. 2012; 125 (1):e2–e220. [PubMed: 22179539]

Shah SH. Gene polymorphisms and susceptibility to coronary artery disease. Pediatr Blood Cancer. 2007; 48 (7):738–741. [PubMed: 17262794]

Shen GQ, et al. An LRP8 variant is associated with familial and premature coronary artery disease and myocardial infarction. Am J Hum Genet. 2007; 81 (4):780–791. [PubMed: 17847002]

Shen GQ, Abdullah KG, Wang QK. The TaqMan method for SNP genotyping. Methods Mol Biol. 2009; 578:293–306. [PubMed: 19768602]

Wang Q, et al. Premature myocardial infarction novel susceptibility locus on chromosome 1P34–36 identified by genomewide linkage analysis. Am J Hum Genet. 2004; 74 (2):262–271. [PubMed: 14732905]

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Table 1

Association analysis of four SNPs in the *LRP8* gene with CAD and MI.

	Allele		MAF		OR	*P*			
	Risk	**Minor**	**Cases**	**Controls**		**HW**	**Observed**	**Adjusted**	**Empirical**
GeneQuest CAD (N = 381) and control (N = 560)									
rs7546246	C	C	0.461	0.399	1.29	0.38	**0.011**	**0.002**	**0.012**
rs2297660	A	A	0.446	0.386	1.28	0.47	**0.014**	**0.007**	**0.017**
rs3737983	T	T	0.474	0.402	1.34	0.14	**0.003**	**0.001**	**0.003**
rs5177	G	G	0.436	0.389	1.22	0.19	**0.047**	**0.012**	**0.049**
GeneQuest MI (N = 183) and control (N = 560)									
rs7546246	C	C	0.470	0.399	1.33	0.38	**0.018**	**0.003**	**0.019**
rs2297660	A	A	0.467	0.386	1.40	0.47	**0.006**	**0.008**	**0.007**
rs3737983	T	T	0.486	0.402	1.41	0.14	**0.005**	**0.003**	**0.005**
rs5177	G	G	0.449	0.389	1.28	0.19	**0.029**	**0.012**	**0.033**
Italian MI (N = 248) and control (N = 308)									
rs7546246	C	C	0.424	0.338	1.44	0.36	**0.004**	**0.007**	**0.005**
rs2297660	A	A	0.439	0.331	1.58	0.53	**3.0×10^{-4}**	**8.0×10^{-4}**	**3.0×10^{-4}**
rs3737983	T	T	0.416	0.350	1.32	0.64	**0.027**	**0.009**	**0.030**
rs5177	G	G	0.421	0.347	1.37	0.29	**0.012**	**0.008**	**0.015**
Combined (CAD/MI = 629; control = 868)									
rs7546246	C	C	0.445	0.378	1.32	0.46	**3.0×10^{-4}**	**8.0×10^{-4}**	**7.0×10^{-4}**
rs2297660	A	A	0.446	0.367	1.39	0.21	**2.0×10^{-5}**	**1.0×10^{-4}**	**2.0×10^{-4}**
rs3737983	T	T	0.449	0.384	1.31	0.15	**4.0×10^{-4}**	**1.0×10^{-4}**	**5.0×10^{-4}**
rs5177	G	G	0.432	0.374	1.27	0.15	**0.002**	**0.003**	**0.002**

MAF: minor allele frequency; OR: odds ratio; *P*-HW: *P*-value for Hardy–Weinberg disequilibrium analysis; *P*-observed: uncorrected *P*-value; *P*-adjusted: *P*-value obtained after adjustment for gender, age, hypertension, diabetes and plasma total cholesterol levels; *P*-empirical: permutation *P*-value calculated using 10,000 Monte Carlo simulations.

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Table 2

Sib-TDT analysis of *LRP8* SNPs for association with CAD in GeneQuest and GeneQuest II.

	Risk allele	Minor alleles	*Z*-score	*P*-value
GeneQuest				
rs7546246	C	181	2.98	**0.002**
rs2297660	A	190	1.89	**0.040**
rs3737983	T	194	3.46	**3.0×10^{-4}**
rs5177	G	181	3.81	**5.0×10^{-5}**
GeneQuest II				
rs7546246	C	67	2.15	**0.016**
rs2297660	A	72	2.70	**0.004**
rs3737983	T	67	2.15	**0.016**
rs5177	G	72	2.70	**0.004**

Minor alleles: the number of minor alleles among affected sibs across all sibships.

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Table 3

Association analysis of a protective haplotype TCCGC of *LRP8* with CAD and MI in GeneQuest and the Italian population.

Populations	Haplotype	Frequency		OR	*P*	
		Cases	Controls		Observed	Empirical
GeneQuest CAD and control	TCCGC	0.439	0.595	0.53	**4.0×10^{-11}**	**$<1 \times 10^{-7}$**
GeneQuest MI and control	TCCGC	0.388	0.595	0.42	**6.5×10^{-12}**	**$<1 \times 10^{-7}$**
Italian MI and control	TCCGC	0.576	0.646	0.71	**0.004**	**0.008**
Combined	TCCGC	0.490	0.613	0.61	**3.0×10^{-11}**	**$<1 \times 10^{-7}$**

P-observed: uncorrected *P*-value; *P*-empirical: permutation *P*-value calculated using 1000 to 10,000,000 simulations.

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Table 4

Sib-TDT analysis for association between a *LRP8* SNP haplotype TCCGC and CAD in GeneQuest II.

Type of haplotype	No. of Families	*Z*-Score	*P*-value
TCCGC	148	3.033	**0.001**