Published in final edited form as:
Ann Hum Genet. 2011 July ; 75(4): 475–482. doi:10.1111/j.1469-1809.2011.00646.x.

Association of SNP rs17465637 on chromosome 1q41 and rs599839 on 1p13.3 with Myocardial Infarction in an American Caucasian Population

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Summary

Recent genome-wide single nucleotide polymorphism (SNP) association studies (GWAS) have identified a number of SNPs that were significantly associated with coronary artery disease (CAD) and myocardial infarction (MI). However, many independent replication studies in other populations are needed to unequivocally confirm the GWAS association. To assess GWAS association, we have established a case-control cohort consisting of 1,231 well-characterized MI patients and 560 controls without detectable coronary stenosis, all selected from the Cleveland Genebank population. The Genebank cohort has a sufficient power to detect the association between MI and four GWAS SNPs, including rs17465637 within the *MIA3* gene, rs2943634 (intergenic), rs6922269 in *MTHFD1L*, and rs599839 near *SORT1*. SNPs were genotyped by TaqMan assays and follow-up multivariate logistic regression analysis with incorporation of significant covariates showed significant association with MI for *MIA3* SNP rs17465637 (*P-adj*=0.0034) and *SORT1* SNP rs599839 (*P-adj*=0.009). The minor allele G of rs599839 was also associated with a decreased LDL-C level of 5–9 mg/dL per allele, but not with HDL-C or triglyceride levels. No association for MI or lipid levels was found for SNPs rs2943634 and rs6922269 (*P-adj*>0.05). Our results establish two SNPs, rs17465637 in *MIA3* and rs599839 near *SORT1* as significant risk factors for MI in the American Genebank Caucasian population.

Keywords

genome-wide association study (GWAS); single nucleotide polymorphism (SNP); myocardial infarction (MI); coronary artery disease; genetics; LDL; SNP rs17465637; SNP rs599839

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Introduction

Coronary artery disease (CAD), along with its leading complication of myocardial infarction (MI), is the leading cause of mortality and disability worldwide (Lloyd-Jones et al., 2009). CAD is considered to be a complex disease that is caused by multiple genetic factors, environmental factors, and their interactions (Topol et al., 2006; Wang, 2005b; Wang, 2005a). As genetic factors play a substantial role in inherited risk for CAD/MI (Nora et al., 1980), it is important to identify these specific molecular and genetic determinants. Large scale genome-wide association studies (GWAS) with 500,000 to 1,000,000 single nucleotide polymorphisms (SNPs) have been developed as a popular strategy for identifying genetic factors for common, complex disease traits such as CAD and MI.

Recent GWAS have identified a number of candidate genetic loci for CAD and MI (Erdmann et al., 2009; Helgadottir et al., 2007; Kathiresan et al., 2009; McPherson et al., 2007; Samani et al., 2007; Samani et al., 2009; Tregouet et al., 2009). One limitation of GWAS is a high rate of false positives; thus, rigorous replication studies in many independent populations from different independent research groups are needed to replicate the findings. To date, the very first chromosome 9p21 locus for CAD and MI identified by GWAS has been replicated in more than 30 independent studies. GWAS also reported a number of other candidate loci that confer risk of or protection from CAD and MI, including SNPs rs17465637 on chromosome 1q41, rs2943634 on 2q36.3, rs6922269 on 6q25.1, rs599839 on 1p13.3, rs9818870 on 3q22.3 and rs501120 on 10q11.21 (Erdmann et al., 2009; Kathiresan et al., 2009; Samani et al., 2007; Samani et al., 2009). However, further replication studies are needed to establish the unequivocal association between these SNPs and CAD/MI using other independent populations. We have established a well-characterized case-control population of 1,231 MI patients and 560 non-CAD controls (Shen et al., 2007), which has a sufficient power for assessing the association between MI and four GWAS SNPs: rs17465637 within the *MIA3* gene, rs2943634 (intergenic), rs6922269 in *MTHFD1L*, and rs599839 near *SORT1*. The aim of this study was to determine whether any of these four SNPs was associated with risk of MI in our U.S. Caucasian population from the Northeast Ohio area (Cleveland Clinic Genebank population).

Materials and Methods

Study population

We conducted a case-control association study involving a total population of 1,231 unrelated MI patients and 560 normal controls as previously reported by us (Shen et al., 2007). The study subjects were selected from the Cleveland Clinic Genebank program, which ascertained patients who were evaluated at the Cardiac Catheterization Laboratories. Clinical diagnosis of CAD and MI was carried out by a panel of cardiologists. Individuals with a stenosis of more than 70% or with a history of revascularization procedures (percutaneous coronary angioplasty – PTCA, coronary artery bypass graft - CABG) were classified as CAD patients. CAD patients with a previous diagnosis of MI were classified as MI patients (Shen et al., 2007; Wang et al., 2004). A myocardial infarction was diagnosed on the basis of chest pain of ≥ 30-minute duration, electrocardiogram patterns consistent with patterns of acute MI, and significant elevation of cardiac enzymes (Shen et al., 2007; Wang et al., 2004). All Caucasian patients who were enrolled in the first year of the program (a total of 1,231 patients) were selected for this study. The 560 controls were chosen from Cleveland Genebank, and included individuals who underwent coronary angiography. Only Caucasian individuals without atherosclerotic lesions detected by angiography were included. Every participant had fasted blood drawn, a lipid profile completed, glucose levels measured, and each subject completed a health questionnaire as well. This study was approved by the Cleveland Clinic Institutional Review Board on Human Subject Research.

Informed consent was obtained from all participants, and the investigation conformed to the guidelines of the Declaration of Helsinki.

Isolation of genomic DNA and SNP genotyping

Human genomic DNA was isolated from whole blood with the Puregene Kits (Gentra). SNP genotyping was performed using the 5' nuclease discrimination assay (Taqman Assays, Applied Biosystems) on an ABI PRISM 7900HT Sequence Detection System (as previously described by us) (Abdullah et al., 2008; Hu et al., 2008). The quality of SNP genotyping was verified by direct DNA sequence analysis of 36 samples. The results from the Taqman Assay were 100% in agreement with those from the sequencing analysis. In addition, all of the DNA samples for case and control subjects were run in the same batches.

Statistical analysis

Allelic association of a SNP with a disease trait was assessed with Pearson's 2x2 contingency table Chi-square test, implemented within SAS Ver 9.00 (SAS Institute Inc) as described previously (Shen et al., 2007; Shen et al., 2008; Xu et al., 2010). Odds ratios and 95% confidence intervals (CI) were estimated through SAS Ver 9.00. Multivariate logistic regression analysis was also performed using SAS Ver 9.00 to test relationships between SNPs and to account for significant covariates as described (gender, age, smoking, body mass index, hypertension, diabetes, total cholesterol, and triglyceride level) (Shen et al., 2007; Shen et al., 2008; Xu et al., 2010). All SNPs were tested for Hardy-Weinberg equilibrium among the normal controls using a chi-square test with one degree of freedom from an online software program (http://www.genes.org.uk/software/hardy-weinberg.shtml). Point-wise statistical significance was adjusted for multiple tests with the Bonferroni method.

Power analysis was estimated using two group Chi-square tests of two unequal proportions and performed using the nQuery Advisor 7.0 using reported ORs for individual SNPs (Samani et al., 2007) and their minor allele frequencies for the U.S. residents with northern and western European ancestry (HapMap Public Release #28 http://hapmap.ncbi.nlm.nih.gov/). Our Genebank case-control cohort has a power of 73%, 74%, 73%, and 77% for detecting MI association for SNPs rs17465637, rs2943634, rs6922269, and rs599839, respectively.

Results

Significant association of SNPs rs17465637 in *MIA3* and rs599839 near *SORT1* with MI in an American Caucasian population

We performed a case-control study with 1,231 MI patients and 560 controls from the Cleveland Genebank population. The same study population was used in a previous study to evaluate the association between an *LRP8* SNP and platelet aggregation/MI (Shen et al., 2007). All study subjects were of American Caucasian descent. The average age at onset for case subjects was 60.6±12.1 years, and the average age at examination for control subjects was 53.5±12.1 years. As expected, the male/female ratio and the rates of smoking, hypertension, diabetes, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were higher in the case population than in the control population.

Four SNPs (rs17465637 within the *MIA3* gene, rs2943634 (intergenic), rs6922269 in *MTHFD1L*, and rs599839 near *SORT1*) were genotyped in the study population. None of the SNPs demonstrated deviation from Hardy-Weinberg equilibrium (Table 1) ($P_{HW} > 0.01$). After adjusting for the significant clinical covariates of age, gender, smoking, hypertension, diabetes, BMI, total cholesterol, and triglyceride levels, a significant association was

identified for SNPs rs17465637 in *MIA3* and rs599839 near *SORT1* with MI; *P-adj*=0.0034 and *P-adj*=0.009, respectively (Table 1). In both cases, the minor allele conferred a protective effect on MI with an adjusted OR of 0.75.

No significant allelic association was detected for intergenic SNP rs2943634 and *MTHFD1L* SNP rs6922269 with MI (Table 1).

Significant association of SNP rs599839 near *SORT1* with plasma LDL-C levels

Further statistical analysis was performed for the association between the four SNPs and plasma LDL-C, HDL-C, and triglyceride levels in the study population. General linear model analysis showed no significant Bonferroni-corrected *P*-values for HDL-C and triglyceride concentrations under an additive, dominant, or recessive model (Tables 2–4). Interestingly, for LDL-C levels, one of the SNPs, rs599839 near *SORT1*, showed significant association in an additive model in 1,231 MI cases and the combined population of 1,791 subjects with *P-adj* values of 0.0015 and 0.0003, respectively (Table 3). In the population of 560 controls, the association was significant before adjustment for significant clinical covariates (*P-obs*=0.0009). However, this significance was reduced to *P-adj*=0.017 after adjustment, which is higher than the accepted threshold for Bonferroni-corrected significance (0.05/4=0.013). The association remained significant under a dominant model (*P-adj*=0.000038 and 0.000034 in 1,231 MI cases and the combined population, respectively) but not under a recessive model (best *P-adj*=0.012). Each minor allele G decreases LDL-C levels by 4.87–9.19 mg/dL (AA=103.71 +/− 35.88; GA=98.84 +/− 34.35; GG=89.65 +/− 32.43).

DISCUSSION

In this study, we assessed four SNPs (rs17465637 within the *MIA3* gene, rs2943634 (intergenic), rs6922269 in *MTHFD1L*, and rs599839 near *SORT1*) for their association with MI in an American Caucasian population. These four SNPs have previously been identified as being associated with CAD and/or MI by GWAS (WTCCC CAD and German MI studies, Figure 1) (Samani et al., 2007). The recruited MI cases and control subjects used in this study were carefully ascertained and very strict criteria were used to define both the MI phenotype and the normal phenotype. All the control subjects showed no detectable stenosis, as verified by angiography. Two of the SNPs, rs17465637 in *MIA3* and rs599839 near *SORT1*, demonstrated significant allelic association with MI after adjustment by multivariate logistic regression analysis incorporating the significant covariates of age, gender, smoking, hypertension, diabetes, BMI, total cholesterol, and triglyceride levels (Table 1). SNP rs599839 was also associated with plasma LDL-C levels. The minor allele G of rs599839 confers a protective effect on MI and is associated with decreased LDL-C levels.

SNP rs17465637 on chromosome 1q41 is located in the *MIA3* gene, which encodes melanoma inhibitor protein 3 required for export of collagen VII (COL7A1) from the endoplasmic reticulum (Saito et al., 2009), and appears to be a tumor suppressor of malignant melanoma (Arndt & Bosserhoff, 2007). *MIA3* may be involved in facilitating the migration of monocytic cells through fibrinogene or human microvascular endothelial cells (Arndt et al., 2007), which may increase the risk of plaque formation. However, it remains to be further established whether *MIA3* is directly related to CAD and MI.

SNP rs599839 on chromosome 1p13.1 is located in the 3’ untranslated region of the *PSRC1* gene and is near the *SORT1* gene. SNP rs599839 has been reported to be associated with LDL-C levels (Sandhu et al., 2008; Willer et al., 2008; Kleber et al., 2010). The minor allele G was associated with increased expression levels of *SORT1* mRNA and that overexpression of *SORT1* led to a significant increase in LDL uptake into cells (Linsel-Nitschke et al.,

2010). A very recent study further showed that overexpression of *SORT1* resulted in a decrease in total plasma cholesterol and LDL-C levels (Musunuru et al., 2010). The study also demonstrated that knockdown of *SORT1* expression by siRNA caused a 46 percent increase in total cholesterol and a more than twofold increase in LDL-C levels (Musunuru et al., 2010). Thus, *SORT1* appears to be the causal gene for reduced LDL-C levels at the 1p13.3 locus, and may lower the risk of MI by decreasing the LDL-C levels.

SNP rs17465637 in *MIA3* was identified as a probable genetic locus for CAD only after combining the Wellcome Trust Case Consortium study and the German MI family study (more than 80% probability of a true association) (Samani et al., 2007). SNP rs599839 near *SORT1* was also identified as a probable genetic locus after combining the two studies (Samani et al., 2007). A follow-up study by the Myocardial Infarction Genetics Consortium replicated the association between rs17465637 and MI (Kathiresan et al., 2009) (Figure 1). However, a study by the Coronary Artery Disease Consortium (Samani et al., 2009) and another study with an African-American population and a U.S. Caucasian population (Bressleret al., 2010) failed to confirm the association (Figure 1). Our study provides a timely reassessment and provides new evidence that supports the association between SNP rs17465637 and MI. The association between SNP rs599839 near *SORT1* and CAD was replicated in the Coronary Artery Disease Consortium study (Samani et al., 2009), but not in another replication study with an African-American population and a U.S. Caucasian population (Bressleret al., 2010). In the Ludwigshafen Risk and Cardiovascular Health replication study, the association between SNP rs599839 and CAD/MI was confirmed (Kleber et al., 2010). Our study further replicated the association of SNP rs599839 near *SORT1* with MI in an American Caucasian population. In our Genebank population, the other two SNPs, intergenic SNP rs2943634 and SNP rs6922269 in *MTHFD1L*, did not exhibit any association with MI although the 95% confidence intervals for ORs for these two SNPs overlap with those in the original GWAS studies (Figure 1). Our results are more consistent with a recent replication study in Austria and another study in the U.S. that did not detect association between rs6922269 and rs2943634 and CAD (Muendlein et al., 2009; Bressleret al., 2010) (Figure 1).

One limitation of this study is that, as in many other human genetics studies, the sample size is fixed. And the phenotypes under study (MI, lipid levels) are complex and involve multiple small-to modest-sized effects of genes and polygenic/environmental backgrounds. As such, the results may be biased. Furthermore, the relatively small sample size with a limited power of 73% to 77% and the small effect of their contribution to MI may explain why SNPs rs2943634 and rs6922269 did not show significant association with MI.

In conclusion, we found significant associations of SNP rs17465637 in the *MIA3* gene on chromosome 1q41 and SNP rs599839 near the *SORT1* gene on chromosome 1p13.3 with MI in an American Caucasian population. We also found a significant association between SNP rs599839 and LDL-C levels in the same population. However, no significant association was found with MI for intergenic SNP rs2943634 on 1q36.3 and rs6922269 in *MTHFD1L* on 6q25.1. These results represent an important expansion of GWAS findings into an American Caucasian Genebank population.

Acknowledgments

This work was supported by the National Institutes of Health Grants P50 HL81011, the Cleveland Clinic Summer Internship to Shaker Heights High School student A.Z.W., the American Heart Association grants (09BGIA2460022, 11SDG5510001, and 11IRG5570046), the China National Basic Research Program (973 program 2007CB512002), China Nation 863 Scientific Program (2006AA02Z476), the Hubei Province Natural Science Key Program (2008CDA047), and the Key Academic Program Leader Award of Wuhan City (200951830560).

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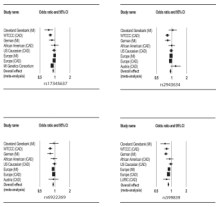

Figure 1.
Forest plot of SNP effect estimates for CAD/MI. The data from original GWAS and follow-up replication studies are summarized fro 4 SNPs, including rs17465637 within the *MIA3* gene, rs2943634 (intergenic), rs6922269 in *MTHFD1L*, and rs599839 near *SORT1*. The X-axis represents the odds ratios (ORs) and 95% confidence intervals (CI) as well as the weight (marked as a filled box) from different studies, including Cleveland Genebank (this study), WTCCC (CAD) and German (MI) (Samani et al., 2007), Europe (MI) and Europe (CAD) (Samani et al., 2009), African-American (CAD) and USA (CAD) (Bressleret al., 2010), Austria (CAD) (Muendlein et al., 2009), MI Genetics Consortium (Kathiresan et al., 2009), and LURIC (CAD) (Kleber et al., 2010). The overall effect of each SNP from meta-analysis of the combined data is shown as a filled diamond. The effect of each SNP was based on the minor allele, thus conversion was made for some studies. The plot was created using the CMA program (http://www.meta-analysis.com/pages/features/forest_plots.html).

Table 1

Allelic association of 4 GWAS SNPs with MI in an American Genebank Caucasian population.

SNP	Chromosomal location (gene)	Minor Allele	Frequency		OR (95% CI)[a]	*P* value*			Adjusted OR (95% CI)[a]
			Control	Case		HW[b]	Observed[c]	Adjusted[d]	
rs17465637	1q41 (*MIA3*)	A	29.37	26.68	0.88 (0.75–1.02)	0.577	0.097	**0.0034**	0.75 (0.62–0.91)
rs2943634	2q36.3 (intergenic)	A	32.66	33.50	1.04 (0.89–1.21)	0.032	0.626	0.596	1.05 (0.87–1.26)
rs6922269	6q25.1 (*MTHFD1L*)	A	26.14	25.84	0.98 (0.84–1.16)	0.718	0.852	0.718	0.96 (0.78–1.18)
rs599839	1p13.1 (near *SORT1*)	G	22.89	19.76	0.83 (0.70–0.99)	0.980	0.041	**0.009**	0.75 (0.61–0.93)

[a] OR, odds ratio, CI, confidence interval.

[b] HW, *P* value for Hardy-Weinberg disequilibrium test.

[c] Observed, nominal *P*-value.

[d] Adjusted, *P* value obtained after adjustment for gender, age, smoking, body mass index, hypertension, diabetes, total cholesterol, and triglyceride level.

* A *P* value of ≤0.0125 was considered to be statistically significant after adjusting for multiple tests with the Bonferroni method.

Table 2

Assessment of association between the 4 SNPs and HDL-C levels in the Genebank population assuming an additive model.

HDL-C	*P* value*					
	1231 MI cases		560 controls		Combined	
SNP	***P-ob***	***P-adj***	***P-ob***	***P-adj***	***P-ob***	***P-adj***
rs17465637	0.7225	0.7137	0.2325	0.7508	0.9371	0.9854
rs2943634	0.3281	0.5749	0.6522	0.9120	0.7648	0.8643
rs6922269	0.7018	0.9339	0.9287	0.9076	0.8768	0.8928
rs599839	0.3653	0.1434	0.8596	0.0166	0.1923	0.0175

P-ob was obtained from general linear modeling where the predictor is coded with the number of risk alleles, but without any other covariates.

P-adj was obtained from general linear modeling after adjustment for gender, age, smoking, diabetes, hypertension, total cholesterol, triglyceride level and MI.

*A *P* value of ≤0.0125 was considered to be statistically significant after adjusting for multiple tests with the Bonferroni method.

Table 3

Assessment of association between 4 SNPs and LDL-C levels in the Genebank population assuming an additive model.

LDL-C	*P* value*					
	1231 MI cases		560 controls		Combined	
SNP	***P-ob***	***P-adj***	***P-ob***	***P-adj***	***P-ob***	***P-adj***
rs17465637	0.6063	0.8613	0.1815	0.6724	0.9477	0.7817
rs2943634	0.3283	0.8985	0.2793	0.8799	0.8835	0.7508
rs6922269	0.0476	0.6028	0.8742	0.2456	0.1019	0.2371
rs599839	0.0002	0.0015	0.0009	0.0173	0.000009	**0.0003**

P-adj was obtained from general linear modeling after adjustment for gender, age, smoking, diabetes, hypertension, total cholesterol, triglyceride level and MI.

*A *P* value of ≤0.0125 was considered to be statistically significant after adjusting for multiple tests with the Bonferroni method.

Table 4

Assessment of association between 4 SNPs and triglyceride levels (TG) in the Genebank population assuming an additive model.

TG	*P* value*					
	1231 MI cases		560 controls		Combined	
SNP	***P-ob***	***P-adj***	***P-ob***	***P-adj***	***P-ob***	***P-adj***
rs17465637	0.5234	0.3656	0.8580	0.6907	0.5581	0.6900
rs2943634	0.3439	0.1672	0.2097	0.2671	0.1567	0.1141
rs6922269	0.8617	0.3953	0.7195	0.6214	0.7649	0.2806
rs599839	0.9752	0.2294	0.4206	0.0343	0.7801	0.0283

P-adj was obtained from general linear modeling after adjustment for gender, age, smoking, diabetes, hypertension, total cholesterol and MI.

*A *P* value of ≤0.0125 was considered to be statistically significant after adjusting for multiple tests with the Bonferroni method.