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# *Reduced risk of recurrent myocardial infarction in homozygous carriers of the chromosome 9p21 rs1333049 C risk allele in the contemporary percutaneous coronary intervention era: a prospective observational study*

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## *Abstract*

**Objectives:** It is controversial whether chromosome 9p21 single nucleotide polymorphism (SNP), susceptibility variants for acute myocardial infarction (AMI) in the primary prevention setting, is associated with recurrent myocardial infarction (ReMI) in the secondary prevention setting. The purpose of this study is to evaluate the impact of chromosome 9p21 SNP on ReMI in patients receiving secondary prevention programs after AMI.

**Design:** A prospective observational study.

**Setting:** Osaka Acute Coronary Insufficiency Study (OACIS) in Japan.

**Participants:** 2,022 patients from OACIS database.

**Interventions:** Genotyping of the 9p21 rs1333049 variant.

**Primary outcome measures:** ReMI event after survival discharge for 1 year.

**Results:** A total of 43 ReMI occurred during the 1-year follow-up period. Although the rs1333049 C allele had an increased susceptibility to their first AMI in an additive model when compared with 1373 healthy controls (odds ratio 1.20, 95% confidence interval 1.09-1.33, $p= 2.3*10^{-4}$), patients with the CC genotype had a lower incidence of ReMI at 1-year after discharge of AMI (log-rank p=0.005). The adjusted hazard ratios of the CC genotype as compared with CG/GG genotypes was 0.20 (0.06-0.65, p=0.007).




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Subgroup analysis demonstrated that the association between the rs1333049 CC genotype and lower incidence of 1-year ReMI was common to all subgroups.

**Conclusions:** Homozygous carriers of the rs1333049 C allele on chromosome 9p21 showed a reduced risk of 1-year ReMI in the contemporary percutaneous coronary intervention era, although the C allele had conferred susceptibility to their first AMI.

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**Strengths and limitations of this study**

- This is the first study to clearly show a change of the susceptibility risk of the 9p21 variant to acute myocardial infarction between the primary and secondary prevention settings in percutaneous coronary intervention era.
- Data regarding the mechanism and culprit lesion of re-myocardial infarction were not available.

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## INTRODUCTION

Acute myocardial infarction (AMI) is one of the leading causes of death and disability worldwide.[1] AMI is associated with a positive family history as well as several traditional coronary risk factors including diabetes, hypertension, dyslipidemia, and smoking, suggesting that the pathogenesis of AMI has a substantial genetic component.[2] Genome-wide association studies (GWAS) have identified several genetic loci that confer susceptibility to AMI and coronary artery disease (CAD) in the primary prevention setting.[3-7] Among these genetic variants, single nucleotide polymorphisms (SNPs) on chromosome 9p21 are the most common and significant susceptibility risk factors for AMI and CAD, regardless of race.[3-11] However, it remains controversial whether chromosome 9p21 SNPs are associated with recurrent myocardial infarction (ReMI) in post-AMI patients receiving the evidence-based secondary prevention programs.[12-14]

For example, the Italian Genetic study and TexGen registry revealed that 9p21 genetic variation was not associated with ReMI events after early-onset myocardial infarction and acute coronary syndrome (ACS), respectively,[13-14] while the GRACE genetic study showed that risk allele carriers of 9p21 SNPs had a higher incidence of ReMI after ACS.[12] One possible explanation for this discrepancy among these three



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studies is an involvement of in-hospital ReMI as an endpoint. It is reported that 9p21 SNPs increase the risk of AMI onset by promoting the development and progression of coronary plaque deposition, rather than increasing susceptibility to plaque rupture.[9-14] Thus, inclusion of acute phase ReMI might have made the interpretation difficult in these 9p21 variant studies, as most of ReMI occurring during the acute phase of AMI were likely caused by 9p21-independent mechanisms, such as re-occlusion of the culprit lesion and/or thrombosis. Therefore, to simply assess the susceptibility impact of 9p21 to ReMI in the secondary prevention settings, it may be better to include post-AMI patients only who survived the acute stage and received the state of the art secondary prevention program after discharge.

The aim of the present study was to investigate the susceptibility impact of 9p21 genetic variation on ReMI in consecutive 2,022 patients with a first AMI who were registered in the Osaka Acute Coronary Insufficiency Study (OACIS),[15-19] treated with emergent percutaneous coronary intervention (PCI), and discharged alive.

## METHODS

### The OACIS

The OACIS is a multicenter, prospective, observational registry for AMI in Japan that



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was initiated in April 1998 among 25 collaborating hospitals. The OACIS is designed to assess patient demographics including genomic information, therapeutic procedures, and subsequent clinical events in AMI patients. All study candidates were informed about data collection, blood sampling, and genotyping, and provided written informed consent. Research cardiologists and trained research nurses recorded data using a specific reporting form. The diagnosis of AMI was based on the World Health Organization criteria,[20] which required 2 of the following 3 criteria to be met: (1) clinical history of central chest pressure, pain, or tightness lasting ≥30 min; (2) ST segment elevation >0.1 mV in at least one standard or 2 precordial leads; and (3) a rise in serum creatinine phosphokinase concentration to more than twice the normal laboratory value. The OACIS is registered to the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) in Japan (ID: UMIN000004575). Other details of OACIS are described elsewhere.[15-19]

**Study design**

Among 10,074 consecutive AMI patients registered in the OACIS between April 1998 and April 2011, 2,045 patients who had a first AMI, underwent emergent PCI, survived to discharge, and gave a written informed consent to the study were enrolled in the



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present study. Exclusion criteria included a history of previous myocardial infarction or PCI, in-hospital death cases, and lack of written informed consent for genetic study and deoxyribonucleic acid (DNA) sampling. Genomic DNA was extracted from peripheral blood samples using a commercially available kit (QIAamp DNA Blood Midi Kit; Qiagen, Hilden, Germany). Patients were genotyped for the rs1333049 SNP of chromosome 9p21 using the multiplex-polymerase chain reaction-based invader assay as previously described.[21] The reason why we focused on the rs1333049 was that it is the most widely studied 9p21 genetic variants in both the primary and secondary prevention settings.[8,10-12,14] We also confirmed that rs1333049 is in linkage disequilibrium with other major 9p21 SNPs in the OACIS registry (Supplementary Figure 1). Finally, the genotyping success rate for rs1333049 was 98.9% and 2,022 patients were successfully genotyped and analyzed for the susceptibility to ReMI within a year after survival discharge (Figure 1). To validate the association of the rs1333049 SNP with the first AMI, we performed a case–control association study between the present study population and healthy Japanese controls. Control blood samples of healthy Japanese adults (n=1,373, mean age, 38.6 years old, 59% male) were obtained from the Health Science Research Resources Bank (Osaka, Japan). The patient backgrounds and primary preventive medications were not adjusted in this case-control



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association study in the primary prevention setting, since these data were not available in commercially obtained healthy controls and medications before first AMI were not available in our study population in detail.

**Statistical analysis**

Categorical variables were compared by the chi-square test, and continuous variables were compared by the Kruskal-Wallis test. The impact of the rs1333049 genotype on the onset of AMI was assessed in both the primary and secondary prevention settings. The impact of rs1333049 on the onset of AMI was calculated as odds ratios (OR) and 95% confidence intervals (CI) in an additive model (OR per C allele increase). In the secondary prevention analysis, the Kaplan-Meier method was used to estimate event rates. Because the Kaplan-Meier analysis revealed that the incidence of ReMI differed between the CC and CG/GG genotypes of rs1333049 (Figure 2), the differences between CC and CG/GG genotypes were assessed by the log-rank tests. In addition, a Cox regression model was used to compare the 1-year prognostic impacts between the rs1333049 CC and CG/GG genotypes based on the estimate hazard ratios (HR) and 95% CI. Multivariate Cox regression analysis was performed to reduce the confounding effects of variations in patient backgrounds using age, gender, body mass index,



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ST-elevation myocardial infarction, diabetes, hypertension, dyslipidemia, smoking, target lesion, multivessel disease, peak creatinine phosphokinase, and prescription of angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, beta-blockers, calcium channel blockers, statins, diuretics, and dual anti-platelet agents as covariates. Hence, the final multivariate model included all the above mentioned covariates. The gene-drug interactions were evaluated using p for interaction between genotype and each drug tested. Statistical significance was set as $p<0.05$ for comparison of patient background or gene-drug interaction. Bonferroni correction for multiple testing was employed during the secondary prevention analysis and statistical significance was set as $p<0.025$ (0.05 divided by the number of independent testing; log-rank test and multiple Cox regression analysis). All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC) or R software packages version 2.15.1 (R Development Core Team).

## RESULTS

Patient characteristics are shown in Table 1. Median age was 65 years, 76.8% were male, 87.7% had ST-elevation myocardial infarction. No significant differences in patient background based on rs1333049 genotypes were detected.



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In the primary prevention setting, the rs1333049 C allele was associated with increased susceptibility to AMI (OR 1.20 per C allele increase, 95% CI 1.09-1.33, $p=2.3*10^{-4}$; and OR 1.38 CC vs CG/GG, 95% CI 1.17-1.62, $p=8.7*10^{-5}$) compared to 1,373 healthy Japanese controls (Figure 3). The frequencies of the CC, CG, and GG genotypes of rs1333049 were 28.4% (574/2022), 49.3% (997/2022), and 22.3% (451/2022), respectively, among the study population, and 22.4% (307/1373), 52.3% (718/1373), and 25.3% (348/1373), respectively, among the healthy controls.

In the secondary prevention setting, 43 ReMI (4 for CC, 30 for CG, and 9 for GG genotypes) occurred during a 1-year follow-up period after survival discharge for their first AMI. Kaplan-Meier analysis revealed that incidence of ReMI differed between patients with the CC and CG/GG genotypes (log-rank p=0.005) (Figure 2). Multivariate Cox regression analysis revealed that the CC genotype was associated with a lower risk of ReMI after survival discharge compared to the CG/GG genotypes (adjusted HR 0.20, 95% CI 0.06-0.65, p=0.007). Subgroup analysis demonstrated that the association between the rs1333049 CC genotype and lower incidence of 1-year ReMI was common to all subgroups, and no significant gene-drug interactions were detected (Figure 4).



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## DISCUSSION

The present study demonstrated that, in the secondary prevention setting of AMI, homozygous carriers of the rs1333049 risk allele (CC genotype) on chromosome 9p21 had a reduced incidence of ReMI, whereas the C allele did have conferred susceptibility to their first AMI. This result is of clinical importance because this is the first study to clearly show a change of the susceptibility risk of the 9p21 variant to AMI between before and after the first AMI, namely, between the primary and secondary prevention settings.

Historically, 9p21 SNP was identified as a susceptibility variant of CAD with GWAS using data from Wellcome Trust Case Control Consortium in 2007.[3] Many other GWASs have also revealed the same association between 9p21 SNP and CAD and/or myocardial infarction.[3-8] In addition, one report by Chan et al suggested the presence of common pathway to develop CAD and myocardial infarction via 9p21 SNP.[11] Thus, 9p21 SNP is now considered as one of the most robust susceptibility variants of myocardial infarction and/or CAD in the primary prevention setting. To date, three major studies have assessed the association between 9p21 genetic variation and ReMI rates after ACS (Table 2)[12-14]: the Italian Genetic study and TexGen registry reported a lack of association with ReMI events after early-onset myocardial infarction and ACS



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(fraction unkown), respectively,[13-14] while the GRACE genetic study suggested a susceptibility risk of 9p21 SNPs for ReMI after ACS (STEMI 27.2%, non-STEMI 43.3%, and unstable angina 29.5%).[12] Since Buysschaert et al. also reported that the statistical significance of the susceptibility risk of 9p21 disappeared after full adjustment with patient background in the GRACE genetic study, [12] it is possible to interpret the results of these 3 studies as a lack of 9p21 susceptibility to reoccurrence of AMI in post-ACS patients.[12-14] These findings are of clinical significance because they suggested a modification of the genetic risk of 9p21 by secondary prevention programs after ACS. However, these studies only examined the susceptibility impact of 9p21 SNPs to the reoccurrence of AMI without comparison with that to the first AMI (ACS) in their study cohort, which could be a limitation to discuss modification of genetic risk with secondary prevention programs.

In this point of view, it is noteworthy that the present study clearly showed a change of the 9p21 susceptibility risk to AMI between before and after the first onset of AMI in the same population. In the present study, the results showed that the rs1333049 C allele was associated with onset of the first AMI (OR 1.20, 95% CI 1.09-1.33, $p=2.3*10^{-4}$), which was consistent with the results of several previous studies in the primary prevention settings.[3-8] Interestingly, however, the present study also demonstrated that



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patients with the CC genotype had a lower incidence of 1-year ReMI (adjusted HR, 0.20, 95% CI 0.06-0.65, p=0.007) as a novel finding. Thus, the results suggested that the risk of 9p21 variant seen in the primary prevention setting of the present study population was modified in the secondary prevention setting. However, it should be discussed why the 9p21 rs1333049 CC genotype was associated with reduced incidence of ReMI in the present study, while not in the previous 3 studies.[12-14] One possible explanation for this discrepancy between the previous and our studies is that we only include post-AMI patient who were treated with emergent PCI on admission and survived to discharge, whereas all previous studies included all of the patients hospitalized for AMI or ACS and thus include ReMI during the acute stage of ACS as an endpoint. Considering that ReMI occurring during the acute stage of ACS was likely associated with patient or procedure-related backgrounds such as re-occlusion of the culprit lesion due to thrombus or mechanical acute closures rather than genetic background, inclusion of these ReMI might have made the interpretation of the results difficult in the previous studies. In addition, patient selection limited to those treated with primary PCI for the first AMI in the present study might have helped to more clearly elucidate the 9p21-reated susceptibility to ReMI in the secondary prevention cohort.

The 9p21 locus is adjacent to the tumor suppressor genes CDKN2A and CDKN2B.[8]



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Although the mechanism by which variation in the 9p21 locus increases AMI susceptibility in the primary prevention setting remains unclear,[8] the evidence-based secondary prevention programs might have masked the susceptibility risk of the 9p21 rs1333049 C allelle to ReMI after ACS in the present study (Figure 2), possibly via stabilizing coronary plaques.[9-14,22-23] Indeed, Do et al. reported that the impact of 9p21 genetic variation can be modified by increasing the dietary intake of vegetables,[24] suggesting a role of secondary prevention programs including dietary practice. Thus, further studies are warranted to investigate whether and how the secondary prevention programs with evidence-based medication and lifestyle modification can reduce the risk of ReMI in patients with 9p21 genetic variants in the near future. In particular, the potential interaction of the rs1333049 SNP with secondary prevention medications warrants further investigation, because gene-drug interactions have already been detected in cardiovascular patients treated with warfarin, clopidogrel, and statin.[25-27]

The present study has several limitations that warrant mention. First, because our study population only consisted of patients who provided written informed consent at survival discharge, there may have been selection bias as well as survival bias since high risk patients carrying C allele might have died more frequently than patients with GG genotype during hospitalization. Second, our study lacked a replication cohort. However,



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it is usually difficult to have a validation cohort in a prospective observational study design.[28] Indeed, all studies presented in Table 2 did not include a replication cohort. Third, therapeutic regiment of AMI such as of coronary stenting and dual-antiplatelet therapy advanced and varied across the ages. Forth, data regarding the mechanism and culprit lesion of ReMI were not available. Since ReMI can occur through a variety of mechanisms such as acute stent thrombosis of culprit lesion, excessive intimal proliferation of stented vessels, and plaque rupture of new atherosclerotic lesion, detailed analysis for the mechanism of ReMI is ideal. Finally, it is possible that unmeasured confounding factors influenced the study outcomes due to the inherent nature of observational registry. The data should be interpreted within the context of these potential limitations.

## Conclusions

We demonstrated that homozygous carriers of AMI susceptibility variant rs1333049 SNP C allele on chromosome 9p21 showed a reduced risk of 1-year ReMI after survival discharge, suggesting a modification of genetic susceptibility of AMI with secondary prevention programs.



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**Contributors**

All authors contributed to the work as follows: (1) made substantial contributions to the study concept and design, acquisition of data, or analysis and interpretation of data; (2) drafted the article or revised it critically by providing intellectual content; and (3) approved the final version of the manuscript to be published.

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**Competing interests**

Dr. Komuro has received research grants and speaker's fees from Takeda Pharmaceutical Company, Astellas Pharma, DAIICHI SANKYO COMPANY, Boehringer Ingelheim, Novartis Pharma and Shionogi. No other authors have relationships with industry to disclose or financial associations that might pose a conflict of interest in connection with the submitted article.

**Ethics approval**

The study protocol complied with the Helsinki Declaration and the guidelines for genomic/genetic research issued by the Japanese government. The study was approved by the institutional ethical committee of each participating institution.

**Patient consent**

Obtained.

**Provenance and peer review**

Not commissioned; externally peer reviewed.



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**Data sharing statement**

Data and documentation for the OACIS registry are available at the study coordination office, Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan.

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studies to the next level. *J Am Coll Cardiol* 2007;50:930-932.

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Table 1. Patient Background Based on rs1333049 Genotype

| Parameter | Overall (n=2022) | CC (n=574) | CG (n=997) | GG (n=451) | p-value |
|---|---|---|---|---|---|
| Age, years | 65 (57-73) | 65 (57-73) | 65 (57-73) | 65 (58-73) | 0.986 |
| Male, % | 76.8 | 78.6 | 75.2 | 77.8 | 0.265 |
| BMI, kg/m$^2$ | 23.8 (21.9-25.9) | 23.9 (21.8-25.7) | 23.7 (21.8-25.8) | 23.8 (22.0-26.0) | 0.653 |
| STEMI, % | 87.7 | 88.1 | 86.6 | 89.6 | 0.272 |
| Coronary risk factor | | | | | |
| Diabetes, % | 31.6 | 33.0 | 29.7 | 33.7 | 0.227 |
| Hypertension, % | 60.1 | 61.0 | 59.5 | 60.3 | 0.829 |
| Dyslipidemia, % | 46.5 | 43.8 | 47.2 | 48.5 | 0.267 |
| Smoking, % | 64.3 | 63.6 | 64.4 | 65.2 | 0.868 |
| CAG Findings | | | | | |
| Target lesion | | | | | 0.153 |
| Left main trunk, % | 1.0 | 1.0 | 1.0 | 1.1 | |
| LAD, % | 45.1 | 43.2 | 43.7 | 50.3 | |
| Diagonal branch, % | 2.9 | 3.0 | 2.7 | 3.1 | |
| RCA, % | 35.8 | 37.3 | 35.6 | 34.4 | |
| LCx, % | 14.7 | 14.8 | 16.5 | 10.6 | |
| Graft, % | 0.1 | 0.3 | 0.0 | 0.0 | |
| Unkown, % | 0.4 | 0.3 | 0.4 | 0.4 | |
| Stenting, % | 88.8 | 90.1 | 87.6 | 90.0 | 0.207 |
| Multivessel disease, % | 40.2 | 38.6 | 40.1 | 42.4 | 0.473 |
| Peak CPK, IU/L | 2269 (1027-4006) | 2304 (1005-4087) | 2242 (1026-4041) | 2345 (1104-3882) | 0.898 |
| Medication at discharge | | | | | |
| ACEI, % | 44.6 | 46.2 | 44.2 | 43.5 | 0.650 |
| ARB, % | 40.4 | 38.2 | 41.4 | 41.0 | 0.425 |
| Beta-blocker, % | 62.0 | 59.9 | 62.5 | 63.4 | 0.466 |
| Ca-blocker, % | 13.5 | 13.2 | 13.2 | 14.4 | 0.814 |
| Statin, % | 53.5 | 50.7 | 54.1 | 55.7 | 0.249 |
| Diuretics, % | 24.7 | 22.6 | 26.0 | 24.4 | 0.333 |
| Dual anti-platelet, % | 80.8 | 80.8 | 80.6 | 80.9 | 0.990 |

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Categorical variables are presented as percentage and continuous variables are presented as the median (25-75 percentiles). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAG, coronary angiography; CPK, creatine phosphokinase; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; and STEMI, ST-elevation myocardial infarction.

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Table 2. Summary of studies examining the association between 9p21 variants and re-myocardial infarction events after acute coronary syndrome

| | OACIS Registry | GRACE Genetic study | Italian Genetic study | TexGen Registry |
|---|---|---|---|---|
| Reference | — | 12 | 13 | 14 |
| Year | — | 2010 | 2011 | 2012 |
| SNP | rs1333049 | rs1333049 | rs1333040 | rs1333049 |
| Pt number | 2,022 | 2,942 | 1,508 | 2,067 |
| Design | Prospective | Prospective | Prospective | Prospective |
| Follow-up | 1 year | 6 months | 9.95 years | 3.2 years |
| Population | Japan | UK, Belgium, Poland | Italy | USA |
| Background disease | MI (STEMI 87.7%, non-STEMI 12.3%) | ACS (STEMI 27.2%, non-STEMI 43.3%, UA 29.5%) | Early-onset MI | ACS (fraction unkown) |
| PCI | 100% | 47.5% | 0% | 63.6% |
| End-point | ReMI after survival discharge | ReMI including in-hospital events | ReMI including in-hospital events | ReMI including in-hospital events |
| Conclusion | Low event rate with homozygous carriers of risk allele | High event rate with risk allele carriers (univariate) | No association | No association |
| Replication | None | None | None | None |

ACS, acute coronary syndrome; CAD, coronary artery disease; DES, drug-eluting stent; MI, myocardial infarction; PCI, percutaneous coronary intervention; Pt, partcipants; ReMI, recurrence of myocardial infarction; STEMI, ST-elevation myocardial infarction; UA, unstable angina.



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Figure Legends

Figure 1. Patient selection flow chart. AMI, acute myocardial infarction; DNA, deoxyribonucleic acid; MI, myocardial infarction; OACIS, Osaka Acute Coronary Insufficiency Study; and PCI, percutaneous coronary intervention.

Figure 2. Kaplan-Meier estimates of re-myocardial infarction event.

Figure 3. Impact of the rs1333049 genotype on the onset and 1-year re-myocardial infarction. AMI, acute myocardial infarction; CI, confidence interval; HR, hazard ratio (CC vs CG/GG); OR, odds ratio (C vs G per allele); and ReMI, re-myocardial infarction.

Figure 4. Subgroup analysis of the impact of rs1333049 genotype on 1-year re-myocardial infarction rate. ACEI, angiotensin-converting enzyme inhibitor; ARB,



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angiotensin receptor blocker; CI, confidence interval; DAPT, dual anti-platelet therapy; HR, hazard ratio; NA, not assessed due to insufficient number of events in the subgroup analysis; and STEMI, ST-elevation myocardial infarction.

Supplementary Figure 1. Linkage disequilibrium of chromosome 9p21 single nucleotide polymorphisms in the present study population

Linkage disequilibrium was evaluated by D prime (left) and R squared (right) using PLINK and Haploview softwares.

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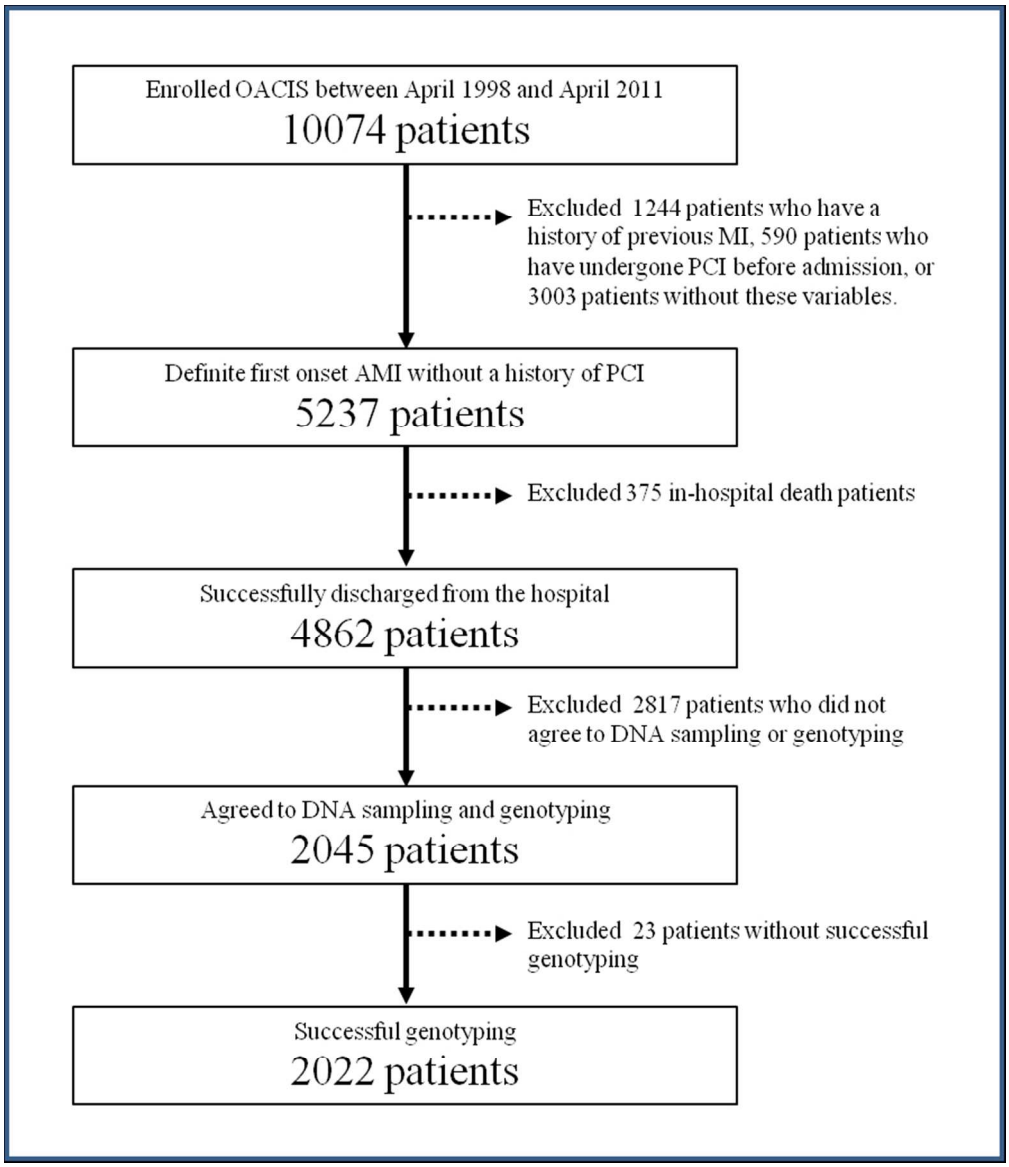
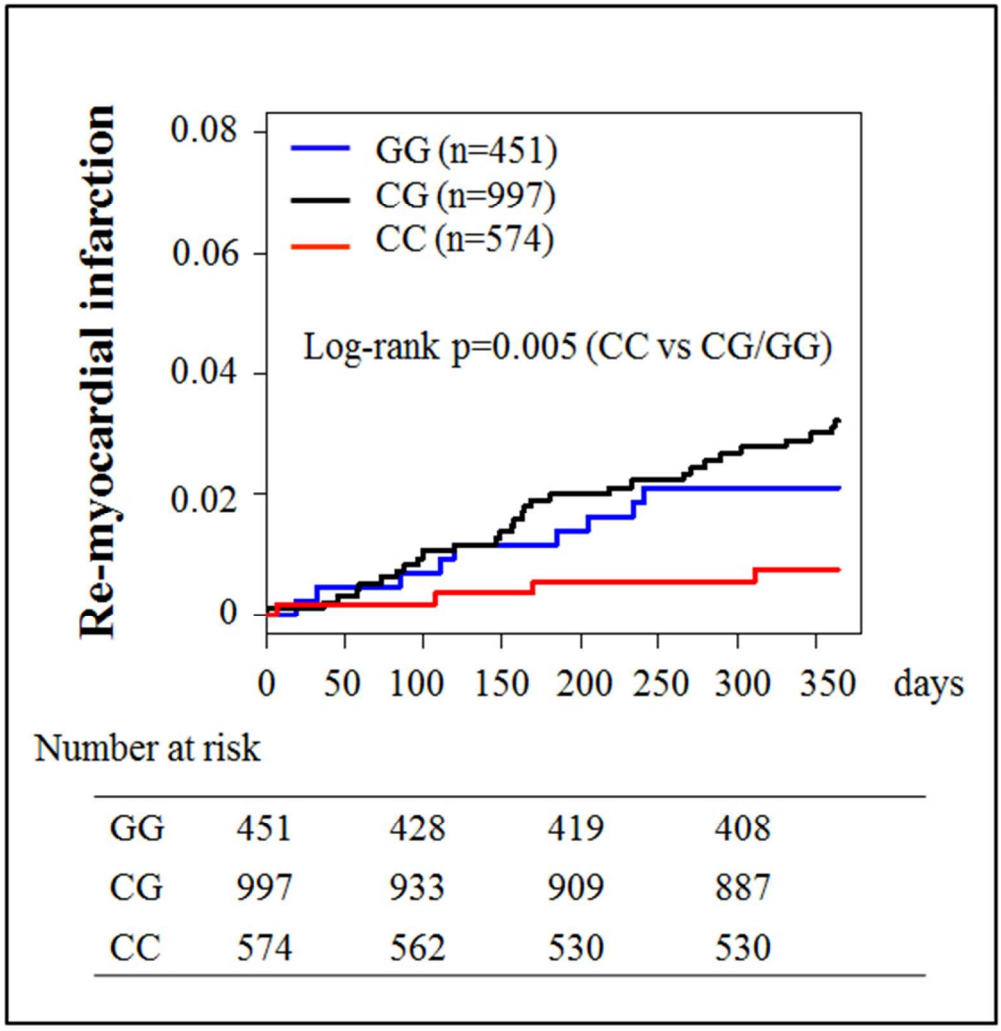


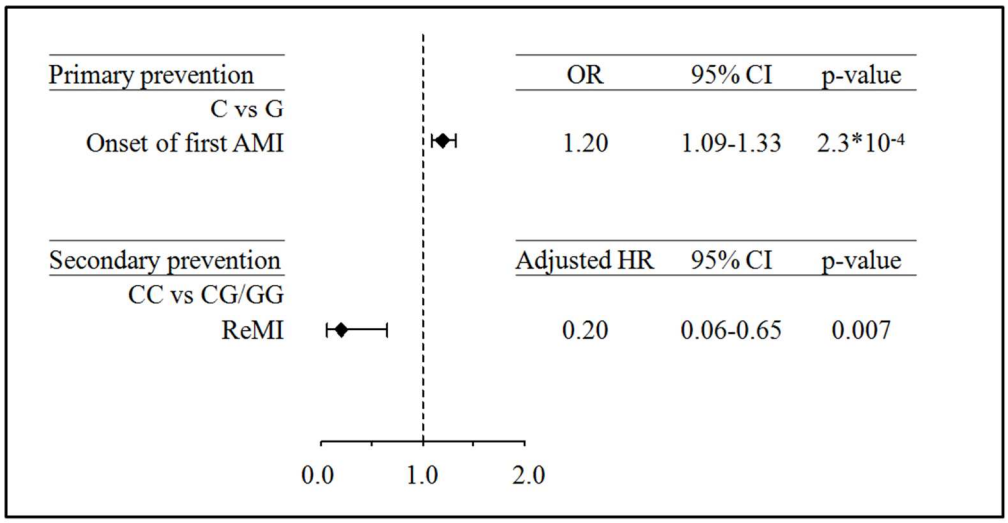

Patient selection flow chart. AMI, acute myocardial infarction; DNA, deoxyribonucleic acid; MI, myocardial infarction; OACIS, Osaka Acute Coronary Insufficiency Study; and PCI, percutaneous coronary intervention.
151x174mm (150 x 150 DPI)




Number at risk

| | | | | |
|---|---|---|---|---|
| GG | 451 | 428 | 419 | 408 |
| CG | 997 | 933 | 909 | 887 |
| CC | 574 | 562 | 530 | 530 |

Kaplan-Meier estimates of re-myocardial infarction event.
152x156mm (96 x 96 DPI)




Impact of the rs1333049 genotype on the onset and 1-year re-myocardial infarction. AMI, acute myocardial infarction; CI, confidence interval; HR, hazard ratio (CC vs CG/GG); OR, odds ratio (C vs G per allele); and ReMI, re-myocardial infarction.
277x143mm (96 x 96 DPI)

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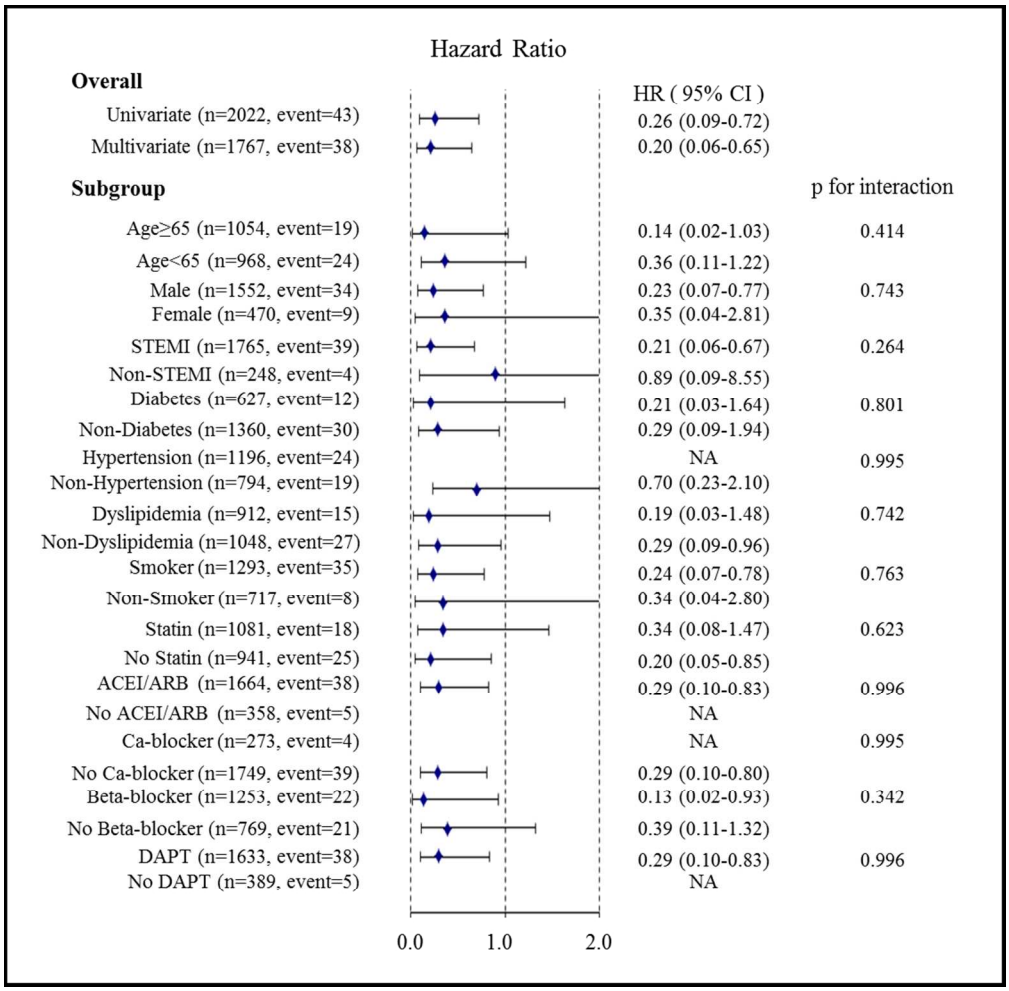
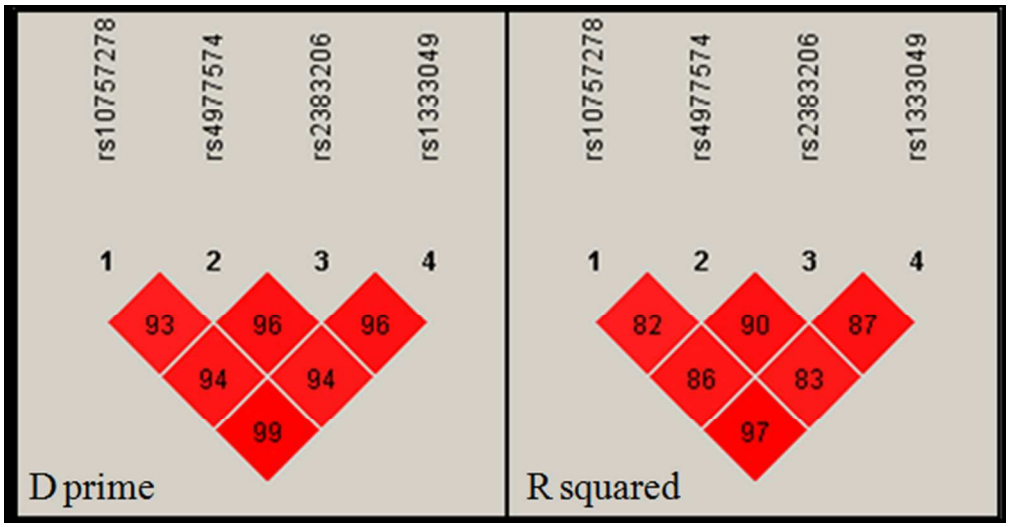



Subgroup analysis of the impact of rs1333049 genotype on 1-year re-myocardial infarction rate. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; DAPT, dual anti-platelet therapy; HR, hazard ratio; NA, not assessed due to insufficient number of events in the subgroup analysis; and STEMI, ST-elevation myocardial infarction.
190x186mm (150 x 150 DPI)




Linkage disequilibrium of chromosome 9p21 single nucleotide polymorphisms in the present study population. Linkage disequilibrium was evaluated by D prime (left) and R squared (right) using PLINK and Haploview softwares.

235x121mm (67 x 67 DPI)

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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias | 9 | Describe any efforts to address potential sources of bias |
| Study size | 10 | Explain how the study size was arrived at |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding |
| | | (*b*) Describe any methods used to examine subgroups and interactions |
| | | (*c*) Explain how missing data were addressed |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy |
| | | (*e*) Describe any sensitivity analyses |

Continued on next page



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| | | |
|---|---|---|
| **Results** | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed |
| | | (b) Give reasons for non-participation at each stage |
| | | (c) Consider use of a flow diagram |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders |
| | | (b) Indicate number of participants with missing data for each variable of interest |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included |
| | | (*b*) Report category boundaries when continuous variables were categorized |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses |
| **Discussion** | | |
| Key results | 18 | Summarise key results with reference to study objectives |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results |
| **Other information** | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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BMJ Open

# Reduced risk of recurrent myocardial infarction in homozygous carriers of the chromosome 9p21 rs1333049 C risk allele in the contemporary percutaneous coronary intervention era: a prospective observational study

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| | |
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| Primary Subject Heading: | Cardiovascular medicine |
| Secondary Subject Heading: | Genetics and genomics |
| Keywords: | Acute myocardial infarction, Secondary prevention, Single nucleotide polymorphism, 9p21 |
| | |

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Original Research Article

# *Reduced risk of recurrent myocardial infarction in homozygous carriers of the chromosome 9p21 rs1333049 C risk allele in the contemporary percutaneous coronary intervention era: a prospective observational study*

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**Key Words:** 9p21, Acute myocardial infarction, Secondary prevention, and Single nucleotide polymorphism.

Running Title: 9p21 SNP and ReMI

Total word count of the text: 2820 words

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## *Abstract*

**Objectives:** It is controversial whether chromosome 9p21 single nucleotide polymorphism (SNP), susceptibility variants for acute myocardial infarction (AMI) in the primary prevention setting, is associated with recurrent myocardial infarction (ReMI) in the secondary prevention setting. The purpose of this study is to evaluate the impact of chromosome 9p21 SNP on ReMI in patients receiving secondary prevention programs after AMI.

**Design:** A prospective observational study.

**Setting:** Osaka Acute Coronary Insufficiency Study (OACIS) in Japan.

**Participants:** 2,022 patients from OACIS database.

**Interventions:** Genotyping of the 9p21 rs1333049 variant.

**Primary outcome measures:** ReMI event after survival discharge for 1 year.

**Results:** A total of 43 ReMI occurred during the 1-year follow-up period. Although the rs1333049 C allele had an increased susceptibility to their first AMI in an additive model when compared with 1373 healthy controls (odds ratio 1.20, 95% confidence interval 1.09-1.33, $p= 2.3*10^{-4}$), patients with the CC genotype had a lower incidence of ReMI at 1-year after discharge of AMI (log-rank p=0.005). The adjusted hazard ratios of the CC genotype as compared with CG/GG genotypes was 0.20 (0.06-0.65, p=0.007).




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Subgroup analysis demonstrated that the association between the rs1333049 CC genotype and lower incidence of 1-year ReMI was common to all subgroups.

**Conclusions:** Homozygous carriers of the rs1333049 C allele on chromosome 9p21 showed a reduced risk of 1-year ReMI in the contemporary percutaneous coronary intervention era, although the C allele had conferred susceptibility to their first AMI.

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**Strengths and limitations of this study**

- This is the first study to clearly show a change of the susceptibility risk of the 9p21 variant to acute myocardial infarction between the primary and secondary prevention settings in percutaneous coronary intervention era.
- Data regarding the mechanism and culprit lesion of re-myocardial infarction were not available.
- Replication studies with a larger sample are warranted to confirm our observations.

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## INTRODUCTION

Acute myocardial infarction (AMI) is one of the leading causes of death and disability worldwide.[1] AMI is associated with a positive family history as well as several traditional coronary risk factors including diabetes, hypertension, dyslipidemia, and smoking, suggesting that the pathogenesis of AMI has a substantial genetic component.[2] Genome-wide association studies (GWAS) have identified several genetic loci that confer susceptibility to AMI and coronary artery disease (CAD) in the primary prevention setting.[3-7] Among these genetic variants, single nucleotide polymorphisms (SNPs) on chromosome 9p21 are the most common and significant susceptibility risk factors for AMI and CAD, regardless of race.[3-11] However, it remains controversial whether chromosome 9p21 SNPs are associated with recurrent myocardial infarction (ReMI) in post-AMI patients receiving the evidence-based secondary prevention programs.[12-14]

For example, the Italian Genetic study and TexGen registry revealed that 9p21 genetic variation was not associated with ReMI events after early-onset myocardial infarction and acute coronary syndrome (ACS), respectively,[13-14] while the GRACE genetic study showed that risk allele carriers of 9p21 SNPs had a higher incidence of ReMI after ACS.[12] One possible explanation for this discrepancy among these three



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studies is an involvement of in-hospital ReMI as an endpoint. It is reported that 9p21 SNPs increase the risk of AMI onset by promoting the development and progression of coronary plaque deposition, rather than increasing susceptibility to plaque rupture.[9-14] Thus, inclusion of acute phase ReMI might have made the interpretation difficult in these 9p21 variant studies, as most of ReMI occurring during the acute phase of AMI were likely caused by 9p21-independent mechanisms, such as re-occlusion of the culprit lesion and/or thrombosis. Therefore, to simply assess the susceptibility impact of 9p21 to ReMI in the secondary prevention settings, it may be better to include post-AMI patients only who survived the acute stage and received the state of the art secondary prevention program after discharge.

The aim of the present study was to investigate the susceptibility impact of 9p21 genetic variation on ReMI in consecutive 2,022 patients with a first AMI who were registered in the Osaka Acute Coronary Insufficiency Study (OACIS),[15-19] treated with emergent percutaneous coronary intervention (PCI), and discharged alive.

## METHODS

### The OACIS

The OACIS is a multicenter, prospective, observational registry for AMI in Japan that



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was initiated in April 1998 among 25 collaborating hospitals. The OACIS is designed to assess patient demographics including genomic information, therapeutic procedures, and subsequent clinical events in AMI patients. All study candidates were informed about data collection, blood sampling, and genotyping, and provided written informed consent. Research cardiologists and trained research nurses recorded data using a specific reporting form. The diagnosis of AMI was based on the World Health Organization criteria,[20] which required 2 of the following 3 criteria to be met: (1) clinical history of central chest pressure, pain, or tightness lasting ≥30 min; (2) ST segment elevation >0.1 mV in at least one standard or 2 precordial leads; and (3) a rise in serum creatinine phosphokinase concentration to more than twice the normal laboratory value. The OACIS is registered to the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) in Japan (ID: UMIN000004575). Other details of OACIS are described elsewhere.[15-19]

**Study design**

Among 10,074 consecutive AMI patients registered in the OACIS between April 1998 and April 2011, 2,045 patients who had a first AMI, underwent emergent PCI, survived to discharge, and gave a written informed consent to the study were enrolled in the



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present study. Exclusion criteria included a history of previous myocardial infarction or PCI, in-hospital death cases, and lack of written informed consent for genetic study and deoxyribonucleic acid (DNA) sampling. Genomic DNA was extracted from peripheral blood samples using a commercially available kit (QIAamp DNA Blood Midi Kit; Qiagen, Hilden, Germany). Patients were genotyped for the rs1333049 SNP of chromosome 9p21 using the multiplex-polymerase chain reaction-based invader assay as previously described.[21] The reason why we focused on the rs1333049 was that it is the most widely studied 9p21 genetic variants in both the primary and secondary prevention settings.[8,10-12,14] We also confirmed that rs1333049 is in linkage disequilibrium with other major 9p21 SNPs in the OACIS registry (Supplementary Figure 1). Finally, the genotyping success rate for rs1333049 was 98.9% and 2,022 patients were successfully genotyped and analyzed for the susceptibility to ReMI within a year after survival discharge (Figure 1). To validate the association of the rs1333049 SNP with the first AMI, we performed a case–control association study between the present study population and healthy Japanese controls. Control blood samples of healthy Japanese adults (n=1,373, mean age, 38.6 years old, 59% male) were obtained from the Health Science Research Resources Bank (Osaka, Japan). The patient backgrounds and primary preventive medications were not adjusted in this case-control



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association study in the primary prevention setting, since these data were not available in commercially obtained healthy controls and medications before first AMI were not available in our study population in detail.

**Statistical analysis**

Categorical variables were compared by the chi-square test, and continuous variables were compared by the Kruskal-Wallis test. The impact of the rs1333049 genotype on the onset of AMI was assessed in both the primary and secondary prevention settings. The impact of rs1333049 on the onset of AMI was calculated as odds ratios (OR) and 95% confidence intervals (CI) in an additive model (OR per C allele increase). In the secondary prevention analysis, the Kaplan-Meier method was used to estimate event rates. Because the Kaplan-Meier analysis revealed that the incidence of ReMI differed between the CC and CG/GG genotypes of rs1333049 (Figure 2), the differences between CC and CG/GG genotypes were assessed by the log-rank tests. In addition, a Cox regression model was used to compare the 1-year prognostic impacts between the rs1333049 CC and CG/GG genotypes based on the estimate hazard ratios (HR) and 95% CI. Multivariate Cox regression analysis was performed to reduce the confounding effects of variations in patient backgrounds using age, gender, body mass index,



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ST-elevation myocardial infarction, diabetes, hypertension, dyslipidemia, smoking, target lesion, multivessel disease, peak creatinine phosphokinase, and prescription of angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, beta-blockers, calcium channel blockers, statins, diuretics, and dual anti-platelet agents as covariates. Hence, the final multivariate model included all the above mentioned covariates regardless of the univariate results shown in Supplementary Table 1 because we assumed that even non-significant differences in these covariates could be confounders and should be adjusted. The gene-drug interactions were evaluated using p for interaction between genotype and each drug tested. Statistical significance was set as $p<0.05$ for comparison of patient background or gene-drug interaction. Bonferroni correction for multiple testing was employed during the secondary prevention analysis and statistical significance was set as $p<0.025$ (0.05 divided by the number of independent testing; log-rank test and multiple Cox regression analysis). All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC) or R software packages version 2.15.1 (R Development Core Team).

## RESULTS

Patient characteristics and medications at discharge are shown in Table 1. Median age



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was 65 years, 76.8% were male, 87.7% had ST-elevation myocardial infarction. No significant differences in patient background based on rs1333049 genotypes were detected.

In the primary prevention setting, the rs1333049 C allele was associated with increased susceptibility to AMI (OR 1.20 per C allele increase, 95% CI 1.09-1.33, $p= 2.3*10^{-4}$; and OR 1.38 CC vs CG/GG, 95% CI 1.17-1.62, $p=8.7*10^{-5}$) compared to 1,373 healthy Japanese controls (Figure 3). The frequencies of the CC, CG, and GG genotypes of rs1333049 were 28.4% (574/2022), 49.3% (997/2022), and 22.3% (451/2022), respectively, among the study population, and 22.4% (307/1373), 52.3% (718/1373), and 25.3% (348/1373), respectively, among the healthy controls.

In the secondary prevention setting, 43 ReMI (4 for CC, 30 for CG, and 9 for GG genotypes) occurred during a 1-year follow-up period after survival discharge for their first AMI. Kaplan-Meier analysis revealed that incidence of ReMI differed between patients with the CC and CG/GG genotypes (log-rank p=0.005) (Figure 2). Multivariate Cox regression analysis revealed that the CC genotype was associated with a lower risk of ReMI after survival discharge compared to the CG/GG genotypes (adjusted HR 0.20, 95% CI 0.06-0.65, p=0.007). Subgroup analysis demonstrated that the association between the rs1333049 CC genotype and lower incidence of 1-year



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ReMI was common to all subgroups, and no significant gene-drug interactions were detected (Figure 4).

## DISCUSSION

The present study demonstrated that, in the secondary prevention setting of AMI, homozygous carriers of the rs1333049 risk allele (CC genotype) on chromosome 9p21 had a reduced incidence of ReMI, whereas the C allele did have conferred susceptibility to their first AMI. This result is of clinical importance because this is the first study to clearly show a change of the susceptibility risk of the 9p21 variant to AMI between before and after the first AMI, namely, between the primary and secondary prevention settings.

Historically, 9p21 SNP was identified as a susceptibility variant of CAD with GWAS using data from Wellcome Trust Case Control Consortium in 2007.[3] Many other GWASs have also revealed the same association between 9p21 SNP and CAD and/or myocardial infarction.[3-8] In addition, one report by Chan et al. suggested the presence of common pathway to develop CAD and myocardial infarction via 9p21 SNP.[11] Thus, 9p21 SNP is now considered as one of the most robust susceptibility variants of myocardial infarction and/or CAD in the primary prevention setting. To date, three



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major studies have assessed the association between 9p21 genetic variation and ReMI rates after ACS (Table 2)[12-14]: the Italian Genetic study and TexGen registry reported a lack of association with ReMI events after early-onset myocardial infarction and ACS (fraction unkown), respectively,[13-14] while the GRACE genetic study suggested a susceptibility risk of 9p21 SNPs for ReMI after ACS (STEMI 27.2%, non-STEMI 43.3%, and unstable angina 29.5%).[12] Since Buysschaert et al. also reported that the statistical significance of the susceptibility risk of 9p21 disappeared after full adjustment with patient background in the GRACE genetic study, [12] it is possible to interpret the results of these 3 studies as a lack of 9p21 susceptibility to reoccurrence of AMI in post-ACS patients.[12-14] These findings are of clinical significance because they suggested a modification of the genetic risk of 9p21 by the secondary prevention programs after ACS. However, these studies only examined the susceptibility impact of 9p21 SNPs to the reoccurrence of AMI without comparison with that to the first AMI (ACS) in their study cohort, which could be a limitation to discuss modification of genetic risk with secondary prevention programs.

In this point of view, it is noteworthy that the present study clearly showed a change of the 9p21 susceptibility risk to AMI between before and after the first onset of AMI in the same population. In the present study, the results showed that the rs1333049 C allele



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was associated with onset of the first AMI (OR 1.20, 95% CI 1.09-1.33, $p=2.3*10^{-4}$), which was consistent with the results of several previous studies in the primary prevention settings.[3-8] Interestingly, however, the present study also demonstrated that patients with the CC genotype had a lower incidence of 1-year ReMI (adjusted HR, 0.20, 95% CI 0.06-0.65, p=0.007) as a novel finding. These observations suggested that the risk of 9p21 variant seen in the primary prevention setting of the present study population was modified in the secondary prevention setting. Although it is speculative, it is considered that modification of genetic risk of rs1333049 C allele might have unmasked the risk of rs1333049 G allele in the secondary prevention setting (Supplementary Figure 2). However, it should be discussed why the 9p21 rs1333049 CC genotype was associated with reduced incidence of ReMI in the present study, while not in the previous 3 studies.[12-14] One possible explanation for this discrepancy between the previous and our studies is that we only include post-AMI patient who were treated with emergent PCI on admission and survived to discharge, whereas all previous studies included all of the patients hospitalized for AMI or ACS and thus include ReMI during the acute stage of ACS as an endpoint. Considering that ReMI occurring during the acute stage of ACS was likely associated with lesion- or procedure-related backgrounds such as re-occlusion of the culprit lesion due to thrombus or mechanical acute closures



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rather than genetic background, inclusion of these ReMI might have made the interpretation of the results difficult in the previous studies. In addition, the patient selection limited to those treated with primary PCI for the first AMI in the present study might have clearly elucidated the 9p21-reated susceptibility to ReMI in the secondary prevention cohort.

The 9p21 locus is adjacent to the tumor suppressor genes CDKN2A and CDKN2B.[8] Although the mechanism by which variation in the 9p21 locus increases AMI susceptibility in the primary prevention setting remains unclear,[8] the evidence-based secondary prevention programs might have masked the susceptibility risk of the 9p21 rs1333049 C allelle to ReMI after ACS in the present study (Figure 2), possibly via stabilizing coronary plaques.[9-14,22-23] Indeed, Do et al. reported that the impact of 9p21 genetic variation can be modified by increasing the dietary intake of vegetables,[24] suggesting a role of secondary prevention programs including dietary practice. Thus, further studies are warranted to investigate whether and how the secondary prevention programs with evidence-based medication and lifestyle modification can reduce the risk of ReMI in patients with 9p21 genetic variants in the near future. In particular, the potential interaction of the rs1333049 SNP with secondary prevention medications warrants further investigation, because gene-drug interactions have already been



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detected in cardiovascular patients treated with warfarin, clopidogrel, and statin.[25-27]

The present study has several limitations that warrant mention. First, because our study population only consisted of patients who provided written informed consent at survival discharge, there may have been selection bias as well as survival bias since high risk patients carrying C allele might have died more frequently than patients with GG genotype during hospitalization. Second, the number of recurrent myocardial infarction was relatively small and our study lacked a replication cohort to validate our observations. Therefore, replication studies with a larger sample are warranted to confirm our observations. However, it is often difficult to have a validation cohort in a prospective observational study design.[28] Indeed, all studies presented in Table 2 did not include a replication cohort. Third, therapeutic regiment of AMI such as of coronary stenting and dual-antiplatelet therapy advanced and varied across the study period. Forth, data regarding the mechanism and culprit lesion of ReMI were not available. Since ReMI can occur through a variety of mechanisms such as acute stent thrombosis of culprit lesion, excessive intimal proliferation of stented vessels, and plaque rupture of new atherosclerotic lesion, detailed analysis for the mechanism of ReMI is ideal. Fifth, patient backgrounds and primary preventive medications were not adjusted in the case-control association study in the primary prevention setting. Finally, it is possible



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that unmeasured confounding factors influenced the study outcomes due to the inherent nature of observational registry. The data should be interpreted within the context of these potential limitations.

## Conclusions

We demonstrated that homozygous carriers of AMI susceptibility variant rs1333049 SNP C allele on chromosome 9p21 showed a reduced risk of 1-year ReMI after survival discharge, suggesting a modification of genetic susceptibility of AMI with secondary prevention programs.

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**Contributors**

All authors (MH, YS, DN, SS, MU, SM, KO, MN, HS, TK, SN, TH, TT, MH, and IK) participated in the study conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the manuscript

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**Competing interests**

Dr. Komuro has received research grants and speaker's fees from Takeda Pharmaceutical Company, Astellas Pharma, DAIICHI SANKYO COMPANY, Boehringer Ingelheim, Novartis Pharma and Shionogi. No other authors have relationships with industry to disclose or financial associations that might pose a conflict of interest in connection with the submitted article.

**Ethics approval**

The study protocol complied with the Helsinki Declaration and the guidelines for genomic/genetic research issued by the Japanese government. The study was approved



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by the institutional ethical committee of each participating institution.

**Patient consent**

Obtained.

**Provenance and peer review**

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**Data sharing statement**

No additional data available.

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Table 1. Patient Background Based on rs1333049 Genotype

| Parameter | Overall (n=2022) | CC (n=574) | CG (n=997) | GG (n=451) | p-value |
|---|---|---|---|---|---|
| Age, years | 65 (57-73) | 65 (57-73) | 65 (57-73) | 65 (58-73) | 0.986 |
| Male, % | 76.8 | 78.6 | 75.2 | 77.8 | 0.265 |
| BMI, kg/m$^2$ | 23.8 (21.9-25.9) | 23.9 (21.8-25.7) | 23.7 (21.8-25.8) | 23.8 (22.0-26.0) | 0.653 |
| STEMI, % | 87.7 | 88.1 | 86.6 | 89.6 | 0.272 |
| Coronary risk factor | | | | | |
| Diabetes, % | 31.6 | 33.0 | 29.7 | 33.7 | 0.227 |
| Hypertension, % | 60.1 | 61.0 | 59.5 | 60.3 | 0.829 |
| Dyslipidemia, % | 46.5 | 43.8 | 47.2 | 48.5 | 0.267 |
| Smoking, % | 64.3 | 63.6 | 64.4 | 65.2 | 0.868 |
| CAG Findings | | | | | |
| Target lesion | | | | | 0.153 |
| Left main trunk, % | 1.0 | 1.0 | 1.0 | 1.1 | |
| LAD, % | 45.1 | 43.2 | 43.7 | 50.3 | |
| Diagonal branch, % | 2.9 | 3.0 | 2.7 | 3.1 | |
| RCA, % | 35.8 | 37.3 | 35.6 | 34.4 | |
| LCx, % | 14.7 | 14.8 | 16.5 | 10.6 | |
| Graft, % | 0.1 | 0.3 | 0.0 | 0.0 | |
| Unkown, % | 0.4 | 0.3 | 0.4 | 0.4 | |
| Stenting, % | 88.8 | 90.1 | 87.6 | 90.0 | 0.207 |
| Multivessel disease, % | 40.2 | 38.6 | 40.1 | 42.4 | 0.473 |
| Peak CPK, IU/L | 2269 (1027-4006) | 2304 (1005-4087) | 2242 (1026-4041) | 2345 (1104-3882) | 0.898 |
| Medication at discharge | | | | | |
| ACEI, % | 44.6 | 46.2 | 44.2 | 43.5 | 0.650 |
| ARB, % | 40.4 | 38.2 | 41.4 | 41.0 | 0.425 |
| Beta-blocker, % | 62.0 | 59.9 | 62.5 | 63.4 | 0.466 |
| Calcium-blocker, % | 13.5 | 13.2 | 13.2 | 14.4 | 0.814 |
| Statin, % | 53.5 | 50.7 | 54.1 | 55.7 | 0.249 |
| Diuretics, % | 24.7 | 22.6 | 26.0 | 24.4 | 0.333 |
| Dual anti-platelet, % | 80.8 | 80.8 | 80.6 | 80.9 | 0.990 |

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Categorical variables are presented as percentage and continuous variables are presented as the median (25-75 percentiles). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAG, coronary angiography; CPK, creatine phosphokinase; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; and STEMI, ST-elevation myocardial infarction.

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Table 2. Summary of studies examining the association between 9p21 variants and re-myocardial infarction events after acute coronary syndrome

| | OACIS Registry | GRACE Genetic study | Italian Genetic study | TexGen Registry |
|---|---|---|---|---|
| Reference | — | 12 | 13 | 14 |
| Year | — | 2010 | 2011 | 2012 |
| SNP | rs1333049 | rs1333049 | rs1333040 | rs1333049 |
| Pt number | 2,022 | 2,942 | 1,508 | 2,067 |
| Design | Prospective | Prospective | Prospective | Prospective |
| Follow-up | 1 year | 6 months | 9.95 years | 3.2 years |
| Population | Japan | UK, Belgium, Poland | Italy | USA |
| Background disease | MI (STEMI 87.7%, non-STEMI 12.3%) | ACS (STEMI 27.2%, non-STEMI 43.3%, UA 29.5%) | Early-onset MI | ACS (fraction unkown) |
| PCI | 100% | 47.5% | 0% | 63.6% |
| End-point | ReMI after survival discharge | ReMI including in-hospital events | ReMI including in-hospital events | ReMI including in-hospital events |
| Conclusion | Low event rate with homozygous carriers of risk allele | High event rate with risk allele carriers (univariate) | No association | No association |
| Replication | None | None | None | None |

ACS, acute coronary syndrome; CAD, coronary artery disease; DES, drug-eluting stent; MI, myocardial infarction; PCI, percutaneous coronary intervention; Pt, partcipants; ReMI, recurrence of myocardial infarction; STEMI, ST-elevation myocardial infarction; UA, unstable angina.



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**Figure Legends**

Figure 1. Patient selection flow chart. AMI, acute myocardial infarction; DNA, deoxyribonucleic acid; MI, myocardial infarction; OACIS, Osaka Acute Coronary Insufficiency Study; and PCI, percutaneous coronary intervention.

Figure 2. Kaplan-Meier estimates of re-myocardial infarction event.

Figure 3. Impact of the rs1333049 genotype on the onset and 1-year re-myocardial infarction. AMI, acute myocardial infarction; CI, confidence interval; HR, hazard ratio (CC vs CG/GG); OR, odds ratio (C vs G per allele); and ReMI, re-myocardial infarction

Figure 4. Subgroup analysis of the impact of rs1333049 genotype on 1-year re-myocardial infarction rate. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; DAPT, dual anti-platelet therapy; HR, hazard ratio; NA, not assessed due to insufficient number of events in the subgroup analysis; and STEMI, ST-elevation myocardial infarction.

Supplementary Figure 1. Linkage disequilibrium of chromosome 9p21 single nucleotide polymorphisms in the present study population. Linkage disequilibrium was evaluated by D prime (left) and R squared (right) using PLINK and Haploview softwares.

Supplementary Figure 2. A possible mechanism for the differential impact of rs1333049



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genotypes between the primary and secondary prevention settings. (A) Risk of first acute myocardial infarction (AMI) in the primary prevention setting. (B) Risk of re-myocardial infarction (ReMI) in the secondary prevention setting. Blue and yellow bars indicate the susceptibility risks of G (dominant risk) and C (additive risk) alleles, respectively. In this model, it is assumed that the risk of rs1333049 G allele was not changed between primary and secondary prevention settings, while the risk of rs1333049 C allele was reduced by the secondary prevention programs after AMI. In the primary prevention setting (panel A), the risk of C allele overwhelmed the G allele risk, making an "additive risk model" of C allele, in which the total risk increased as the number of C allele increased. On the other hand, in the secondary prevention setting (panel B), the risk of rs1333049 C allele was reduced and then the risk of G allele became highlighted, making a so-called "dominant risk model" of rs1333049 G allele.

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Original Research Article

# *Reduced risk of recurrent myocardial infarction in homozygous carriers of the chromosome 9p21 rs1333049 C risk allele in the contemporary percutaneous coronary intervention era: a prospective observational study*

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## *Abstract*

**Objectives:** It is controversial whether chromosome 9p21 single nucleotide polymorphism (SNP), susceptibility variants for acute myocardial infarction (AMI) in the primary prevention setting, is associated with recurrent myocardial infarction (ReMI) in the secondary prevention setting. The purpose of this study is to evaluate the impact of chromosome 9p21 SNP on ReMI in patients receiving secondary prevention programs after AMI.

**Design:** A prospective observational study.

**Setting:** Osaka Acute Coronary Insufficiency Study (OACIS) in Japan.

**Participants:** 2,022 patients from OACIS database.

**Interventions:** Genotyping of the 9p21 rs1333049 variant.

**Primary outcome measures:** ReMI event after survival discharge for 1 year.

**Results:** A total of 43 ReMI occurred during the 1-year follow-up period. Although the rs1333049 C allele had an increased susceptibility to their first AMI in an additive model when compared with 1373 healthy controls (odds ratio 1.20, 95% confidence interval 1.09-1.33, $p= 2.3*10^{-4}$), patients with the CC genotype had a lower incidence of ReMI at 1-year after discharge of AMI (log-rank p=0.005). The adjusted hazard ratios of the CC genotype as compared with CG/GG genotypes was 0.20 (0.06-0.65, p=0.007).




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Subgroup analysis demonstrated that the association between the rs1333049 CC genotype and lower incidence of 1-year ReMI was common to all subgroups.

**Conclusions:** Homozygous carriers of the rs1333049 C allele on chromosome 9p21 showed a reduced risk of 1-year ReMI in the contemporary percutaneous coronary intervention era, although the C allele had conferred susceptibility to their first AMI.

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**Strengths and limitations of this study**

- This is the first study to clearly show a change of the susceptibility risk of the 9p21 variant to acute myocardial infarction between the primary and secondary prevention settings in percutaneous coronary intervention era.
- Data regarding the mechanism and culprit lesion of re-myocardial infarction were not available.
- Replication studies with a larger sample are warranted to confirm our observations.

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## INTRODUCTION

Acute myocardial infarction (AMI) is one of the leading causes of death and disability worldwide.[1] AMI is associated with a positive family history as well as several traditional coronary risk factors including diabetes, hypertension, dyslipidemia, and smoking, suggesting that the pathogenesis of AMI has a substantial genetic component.[2] Genome-wide association studies (GWAS) have identified several genetic loci that confer susceptibility to AMI and coronary artery disease (CAD) in the primary prevention setting.[3-7] Among these genetic variants, single nucleotide polymorphisms (SNPs) on chromosome 9p21 are the most common and significant susceptibility risk factors for AMI and CAD, regardless of race.[3-11] However, it remains controversial whether chromosome 9p21 SNPs are associated with recurrent myocardial infarction (ReMI) in post-AMI patients receiving the evidence-based secondary prevention programs.[12-14]

For example, the Italian Genetic study and TexGen registry revealed that 9p21 genetic variation was not associated with ReMI events after early-onset myocardial infarction and acute coronary syndrome (ACS), respectively,[13-14] while the GRACE genetic study showed that risk allele carriers of 9p21 SNPs had a higher incidence of ReMI after ACS.[12] One possible explanation for this discrepancy among these three



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studies is an involvement of in-hospital ReMI as an endpoint. It is reported that 9p21 SNPs increase the risk of AMI onset by promoting the development and progression of coronary plaque deposition, rather than increasing susceptibility to plaque rupture.[9-14] Thus, inclusion of acute phase ReMI might have made the interpretation difficult in these 9p21 variant studies, as most of ReMI occurring during the acute phase of AMI were likely caused by 9p21-independent mechanisms, such as re-occlusion of the culprit lesion and/or thrombosis. Therefore, to simply assess the susceptibility impact of 9p21 to ReMI in the secondary prevention settings, it may be better to include post-AMI patients only who survived the acute stage and received the state of the art secondary prevention program after discharge.

The aim of the present study was to investigate the susceptibility impact of 9p21 genetic variation on ReMI in consecutive 2,022 patients with a first AMI who were registered in the Osaka Acute Coronary Insufficiency Study (OACIS),[15-19] treated with emergent percutaneous coronary intervention (PCI), and discharged alive.

## METHODS

### The OACIS

The OACIS is a multicenter, prospective, observational registry for AMI in Japan that



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was initiated in April 1998 among 25 collaborating hospitals. The OACIS is designed to assess patient demographics including genomic information, therapeutic procedures, and subsequent clinical events in AMI patients. All study candidates were informed about data collection, blood sampling, and genotyping, and provided written informed consent. Research cardiologists and trained research nurses recorded data using a specific reporting form. The diagnosis of AMI was based on the World Health Organization criteria,[20] which required 2 of the following 3 criteria to be met: (1) clinical history of central chest pressure, pain, or tightness lasting ≥30 min; (2) ST segment elevation >0.1 mV in at least one standard or 2 precordial leads; and (3) a rise in serum creatinine phosphokinase concentration to more than twice the normal laboratory value. The OACIS is registered to the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) in Japan (ID: UMIN000004575). Other details of OACIS are described elsewhere.[15-19]

**Study design**

Among 10,074 consecutive AMI patients registered in the OACIS between April 1998 and April 2011, 2,045 patients who had a first AMI, underwent emergent PCI, survived to discharge, and gave a written informed consent to the study were enrolled in the



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present study. Exclusion criteria included a history of previous myocardial infarction or PCI, in-hospital death cases, and lack of written informed consent for genetic study and deoxyribonucleic acid (DNA) sampling. Genomic DNA was extracted from peripheral blood samples using a commercially available kit (QIAamp DNA Blood Midi Kit; Qiagen, Hilden, Germany). Patients were genotyped for the rs1333049 SNP of chromosome 9p21 using the multiplex-polymerase chain reaction-based invader assay as previously described.[21] The reason why we focused on the rs1333049 was that it is the most widely studied 9p21 genetic variants in both the primary and secondary prevention settings.[8,10-12,14] We also confirmed that rs1333049 is in linkage disequilibrium with other major 9p21 SNPs in the OACIS registry (Supplementary Figure 1). Finally, the genotyping success rate for rs1333049 was 98.9% and 2,022 patients were successfully genotyped and analyzed for the susceptibility to ReMI within a year after survival discharge (Figure 1). To validate the association of the rs1333049 SNP with the first AMI, we performed a case–control association study between the present study population and healthy Japanese controls. Control blood samples of healthy Japanese adults (n=1,373, mean age, 38.6 years old, 59% male) were obtained from the Health Science Research Resources Bank (Osaka, Japan). The patient backgrounds and primary preventive medications were not adjusted in this case-control



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association study in the primary prevention setting, since these data were not available in commercially obtained healthy controls and medications before first AMI were not available in our study population in detail.

**Statistical analysis**

Categorical variables were compared by the chi-square test, and continuous variables were compared by the Kruskal-Wallis test. The impact of the rs1333049 genotype on the onset of AMI was assessed in both the primary and secondary prevention settings. The impact of rs1333049 on the onset of AMI was calculated as odds ratios (OR) and 95% confidence intervals (CI) in an additive model (OR per C allele increase). In the secondary prevention analysis, the Kaplan-Meier method was used to estimate event rates. Because the Kaplan-Meier analysis revealed that the incidence of ReMI differed between the CC and CG/GG genotypes of rs1333049 (Figure 2), the differences between CC and CG/GG genotypes were assessed by the log-rank tests. In addition, a Cox regression model was used to compare the 1-year prognostic impacts between the rs1333049 CC and CG/GG genotypes based on the estimate hazard ratios (HR) and 95% CI. Multivariate Cox regression analysis was performed to reduce the confounding effects of variations in patient backgrounds using age, gender, body mass index,



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ST-elevation myocardial infarction, diabetes, hypertension, dyslipidemia, smoking, target lesion, multivessel disease, peak creatinine phosphokinase, and prescription of angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, beta-blockers, calcium channel blockers, statins, diuretics, and dual anti-platelet agents as covariates. Hence, the final multivariate model included all the above mentioned covariates regardless of the univariate results shown in Supplementary Table 1 because we assumed that even non-significant differences in these covariates could be confounders and should be adjusted. The gene-drug interactions were evaluated using p for interaction between genotype and each drug tested. Statistical significance was set as $p<0.05$ for comparison of patient background or gene-drug interaction. Bonferroni correction for multiple testing was employed during the secondary prevention analysis and statistical significance was set as $p<0.025$ (0.05 divided by the number of independent testing; log-rank test and multiple Cox regression analysis). All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC) or R software packages version 2.15.1 (R Development Core Team).

## RESULTS

Patient characteristics and medications at discharge are shown in Table 1. Median age



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was 65 years, 76.8% were male, 87.7% had ST-elevation myocardial infarction. No significant differences in patient background based on rs1333049 genotypes were detected.

In the primary prevention setting, the rs1333049 C allele was associated with increased susceptibility to AMI (OR 1.20 per C allele increase, 95% CI 1.09-1.33, $p= 2.3*10^{-4}$; and OR 1.38 CC vs CG/GG, 95% CI 1.17-1.62, $p=8.7*10^{-5}$) compared to 1,373 healthy Japanese controls (Figure 3). The frequencies of the CC, CG, and GG genotypes of rs1333049 were 28.4% (574/2022), 49.3% (997/2022), and 22.3% (451/2022), respectively, among the study population, and 22.4% (307/1373), 52.3% (718/1373), and 25.3% (348/1373), respectively, among the healthy controls.

In the secondary prevention setting, 43 ReMI (4 for CC, 30 for CG, and 9 for GG genotypes) occurred during a 1-year follow-up period after survival discharge for their first AMI. Kaplan-Meier analysis revealed that incidence of ReMI differed between patients with the CC and CG/GG genotypes (log-rank p=0.005) (Figure 2). Multivariate Cox regression analysis revealed that the CC genotype was associated with a lower risk of ReMI after survival discharge compared to the CG/GG genotypes (adjusted HR 0.20, 95% CI 0.06-0.65, p=0.007). Subgroup analysis demonstrated that the association between the rs1333049 CC genotype and lower incidence of 1-year



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ReMI was common to all subgroups, and no significant gene-drug interactions were detected (Figure 4).

## DISCUSSION

The present study demonstrated that, in the secondary prevention setting of AMI, homozygous carriers of the rs1333049 risk allele (CC genotype) on chromosome 9p21 had a reduced incidence of ReMI, whereas the C allele did have conferred susceptibility to their first AMI. This result is of clinical importance because this is the first study to clearly show a change of the susceptibility risk of the 9p21 variant to AMI between before and after the first AMI, namely, between the primary and secondary prevention settings.

Historically, 9p21 SNP was identified as a susceptibility variant of CAD with GWAS using data from Wellcome Trust Case Control Consortium in 2007.[3] Many other GWASs have also revealed the same association between 9p21 SNP and CAD and/or myocardial infarction.[3-8] In addition, one report by Chan et al. suggested the presence of common pathway to develop CAD and myocardial infarction via 9p21 SNP.[11] Thus, 9p21 SNP is now considered as one of the most robust susceptibility variants of myocardial infarction and/or CAD in the primary prevention setting. To date, three



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major studies have assessed the association between 9p21 genetic variation and ReMI rates after ACS (Table 2)[12-14]: the Italian Genetic study and TexGen registry reported a lack of association with ReMI events after early-onset myocardial infarction and ACS (fraction unkown), respectively,[13-14] while the GRACE genetic study suggested a susceptibility risk of 9p21 SNPs for ReMI after ACS (STEMI 27.2%, non-STEMI 43.3%, and unstable angina 29.5%).[12] Since Buysschaert et al. also reported that the statistical significance of the susceptibility risk of 9p21 disappeared after full adjustment with patient background in the GRACE genetic study, [12] it is possible to interpret the results of these 3 studies as a lack of 9p21 susceptibility to reoccurrence of AMI in post-ACS patients.[12-14] These findings are of clinical significance because they suggested a modification of the genetic risk of 9p21 by the secondary prevention programs after ACS. However, these studies only examined the susceptibility impact of 9p21 SNPs to the reoccurrence of AMI without comparison with that to the first AMI (ACS) in their study cohort, which could be a limitation to discuss modification of genetic risk with secondary prevention programs.

In this point of view, it is noteworthy that the present study clearly showed a change of the 9p21 susceptibility risk to AMI between before and after the first onset of AMI in the same population. In the present study, the results showed that the rs1333049 C allele



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was associated with onset of the first AMI (OR 1.20, 95% CI 1.09-1.33, $p=2.3*10^{-4}$), which was consistent with the results of several previous studies in the primary prevention settings.[3-8] Interestingly, however, the present study also demonstrated that patients with the CC genotype had a lower incidence of 1-year ReMI (adjusted HR, 0.20, 95% CI 0.06-0.65, p=0.007) as a novel finding. These observations suggested that the risk of 9p21 variant seen in the primary prevention setting of the present study population was modified in the secondary prevention setting. Although it is speculative, it is considered that modification of genetic risk of rs1333049 C allele might have unmasked the risk of rs1333049 G allele in the secondary prevention setting (Supplementary Figure 2). However, it should be discussed why the 9p21 rs1333049 CC genotype was associated with reduced incidence of ReMI in the present study, while not in the previous 3 studies.[12-14] One possible explanation for this discrepancy between the previous and our studies is that we only include post-AMI patient who were treated with emergent PCI on admission and survived to discharge, whereas all previous studies included all of the patients hospitalized for AMI or ACS and thus include ReMI during the acute stage of ACS as an endpoint. Considering that ReMI occurring during the acute stage of ACS was likely associated with lesion- or procedure-related backgrounds such as re-occlusion of the culprit lesion due to thrombus or mechanical acute closures



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rather than genetic background, inclusion of these ReMI might have made the interpretation of the results difficult in the previous studies. In addition, the patient selection limited to those treated with primary PCI for the first AMI in the present study might have clearly elucidated the 9p21-reated susceptibility to ReMI in the secondary prevention cohort.

The 9p21 locus is adjacent to the tumor suppressor genes CDKN2A and CDKN2B.[8] Although the mechanism by which variation in the 9p21 locus increases AMI susceptibility in the primary prevention setting remains unclear,[8] the evidence-based secondary prevention programs might have masked the susceptibility risk of the 9p21 rs1333049 C allelle to ReMI after ACS in the present study (Figure 2), possibly via stabilizing coronary plaques.[9-14,22-23] Indeed, Do et al. reported that the impact of 9p21 genetic variation can be modified by increasing the dietary intake of vegetables,[24] suggesting a role of secondary prevention programs including dietary practice. Thus, further studies are warranted to investigate whether and how the secondary prevention programs with evidence-based medication and lifestyle modification can reduce the risk of ReMI in patients with 9p21 genetic variants in the near future. In particular, the potential interaction of the rs1333049 SNP with secondary prevention medications warrants further investigation, because gene-drug interactions have already been



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detected in cardiovascular patients treated with warfarin, clopidogrel, and statin.[25-27]

The present study has several limitations that warrant mention. First, because our study population only consisted of patients who provided written informed consent at survival discharge, there may have been selection bias as well as survival bias since high risk patients carrying C allele might have died more frequently than patients with GG genotype during hospitalization. Second, the number of recurrent myocardial infarction was relatively small and our study lacked a replication cohort to validate our observations. Therefore, replication studies with a larger sample are warranted to confirm our observations. However, it is often difficult to have a validation cohort in a prospective observational study design.[28] Indeed, all studies presented in Table 2 did not include a replication cohort. Third, therapeutic regiment of AMI such as of coronary stenting and dual-antiplatelet therapy advanced and varied across the study period. Forth, data regarding the mechanism and culprit lesion of ReMI were not available. Since ReMI can occur through a variety of mechanisms such as acute stent thrombosis of culprit lesion, excessive intimal proliferation of stented vessels, and plaque rupture of new atherosclerotic lesion, detailed analysis for the mechanism of ReMI is ideal. Fifth, patient backgrounds and primary preventive medications were not adjusted in the case-control association study in the primary prevention setting. Finally, it is possible



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that unmeasured confounding factors influenced the study outcomes due to the inherent nature of observational registry. The data should be interpreted within the context of these potential limitations.

## Conclusions

We demonstrated that homozygous carriers of AMI susceptibility variant rs1333049 SNP C allele on chromosome 9p21 showed a reduced risk of 1-year ReMI after survival discharge, suggesting a modification of genetic susceptibility of AMI with secondary prevention programs.

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**Contributors**



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All authors (MH, YS, DN, SS, MU, SM, KO, MN, HS, TK, SN, TH, TT, MH, and IK) participated in the study conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the manuscript.

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**Competing interests**

Dr. Komuro has received research grants and speaker's fees from Takeda Pharmaceutical Company, Astellas Pharma, DAIICHI SANKYO COMPANY, Boehringer Ingelheim, Novartis Pharma and Shionogi. No other authors have relationships with industry to disclose or financial associations that might pose a conflict of interest in connection with the submitted article.

**Ethics approval**



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The study protocol complied with the Helsinki Declaration and the guidelines for genomic/genetic research issued by the Japanese government. The study was approved by the institutional ethical committee of each participating institution.

**Patient consent**

Obtained.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

**Data sharing statement**

No additional data available.

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Table 1. Patient Background Based on rs1333049 Genotype

| Parameter | Overall (n=2022) | CC (n=574) | CG (n=997) | GG (n=451) | p-value |
|---|---|---|---|---|---|
| Age, years | 65 (57-73) | 65 (57-73) | 65 (57-73) | 65 (58-73) | 0.986 |
| Male, % | 76.8 | 78.6 | 75.2 | 77.8 | 0.265 |
| BMI, kg/m$^2$ | 23.8 (21.9-25.9) | 23.9 (21.8-25.7) | 23.7 (21.8-25.8) | 23.8 (22.0-26.0) | 0.653 |
| STEMI, % | 87.7 | 88.1 | 86.6 | 89.6 | 0.272 |
| Coronary risk factor | | | | | |
| Diabetes, % | 31.6 | 33.0 | 29.7 | 33.7 | 0.227 |
| Hypertension, % | 60.1 | 61.0 | 59.5 | 60.3 | 0.829 |
| Dyslipidemia, % | 46.5 | 43.8 | 47.2 | 48.5 | 0.267 |
| Smoking, % | 64.3 | 63.6 | 64.4 | 65.2 | 0.868 |
| CAG Findings | | | | | |
| Target lesion | | | | | 0.153 |
| Left main trunk, % | 1.0 | 1.0 | 1.0 | 1.1 | |
| LAD, % | 45.1 | 43.2 | 43.7 | 50.3 | |
| Diagonal branch, % | 2.9 | 3.0 | 2.7 | 3.1 | |
| RCA, % | 35.8 | 37.3 | 35.6 | 34.4 | |
| LCx, % | 14.7 | 14.8 | 16.5 | 10.6 | |
| Graft, % | 0.1 | 0.3 | 0.0 | 0.0 | |
| Unkown, % | 0.4 | 0.3 | 0.4 | 0.4 | |
| Stenting, % | 88.8 | 90.1 | 87.6 | 90.0 | 0.207 |
| Multivessel disease, % | 40.2 | 38.6 | 40.1 | 42.4 | 0.473 |
| Peak CPK, IU/L | 2269 (1027-4006) | 2304 (1005-4087) | 2242 (1026-4041) | 2345 (1104-3882) | 0.898 |
| Medication at discharge | | | | | |
| ACEI, % | 44.6 | 46.2 | 44.2 | 43.5 | 0.650 |
| ARB, % | 40.4 | 38.2 | 41.4 | 41.0 | 0.425 |
| Beta-blocker, % | 62.0 | 59.9 | 62.5 | 63.4 | 0.466 |
| Calcium-blocker, % | 13.5 | 13.2 | 13.2 | 14.4 | 0.814 |
| Statin, % | 53.5 | 50.7 | 54.1 | 55.7 | 0.249 |
| Diuretics, % | 24.7 | 22.6 | 26.0 | 24.4 | 0.333 |
| Dual anti-platelet, % | 80.8 | 80.8 | 80.6 | 80.9 | 0.990 |

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Categorical variables are presented as percentage and continuous variables are presented as the median (25-75 percentiles). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAG, coronary angiography; CPK, creatine phosphokinase; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; and STEMI, ST-elevation myocardial infarction.

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Table 2. Summary of studies examining the association between 9p21 variants and re-myocardial infarction events after acute coronary syndrome

| | OACIS Registry | GRACE Genetic study | Italian Genetic study | TexGen Registry |
|---|---|---|---|---|
| Reference | — | 12 | 13 | 14 |
| Year | — | 2010 | 2011 | 2012 |
| SNP | rs1333049 | rs1333049 | rs1333040 | rs1333049 |
| Pt number | 2,022 | 2,942 | 1,508 | 2,067 |
| Design | Prospective | Prospective | Prospective | Prospective |
| Follow-up | 1 year | 6 months | 9.95 years | 3.2 years |
| Population | Japan | UK, Belgium, Poland | Italy | USA |
| Background disease | MI (STEMI 87.7%, non-STEMI 12.3%) | ACS (STEMI 27.2%, non-STEMI 43.3%, UA 29.5%) | Early-onset MI | ACS (fraction unkown) |
| PCI | 100% | 47.5% | 0% | 63.6% |
| End-point | ReMI after survival discharge | ReMI including in-hospital events | ReMI including in-hospital events | ReMI including in-hospital events |
| Conclusion | Low event rate with homozygous carriers of risk allele | High event rate with risk allele carriers (univariate) | No association | No association |
| Replication | None | None | None | None |

ACS, acute coronary syndrome; CAD, coronary artery disease; DES, drug-eluting stent; MI, myocardial infarction; PCI, percutaneous coronary intervention; Pt, partcipants; ReMI, recurrence of myocardial infarction; STEMI, ST-elevation myocardial infarction; UA, unstable angina.



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Supplementary Table 1. Results of univariate Cox regression analysis for re-myocardial infarction

| Parameter | Hazard Ratio | 95% CI | p value |
|---|---|---|---|
| Age | 0.984 | 0.958-1.009 | 0.206 |
| Male | 1.127 | 0.541-2.350 | 0.750 |
| BMI | 0.999 | 0.913-1.09 | 0.981 |
| STEMI | 1.388 | 0.496-3.883 | 0.533 |
| Coronary risk factor | | | |
| Diabetes | 0.872 | 0.446-1.703 | 0.688 |
| Hypertension | 0.843 | 0.462-1.539 | 0.578 |
| Dyslipidemia | 0.622 | 0.331-1.168 | 0.140 |
| Smoking | 2.423 | 1.124-5.222 | 0.024 |
| Target lesion | | | |
| Left main trunk | 2.533 | 0.337-19.034 | 0.366 |
| LAD | 1 (reference) | (reference) | (reference) |
| Diagonal branch | 0.892 | 0.119-6.706 | 0.912 |
| RCA | 1.434 | 0.745-2.758 | 0.281 |
| LCx | 0.902 | 0.333-2.446 | 0.840 |
| Graft | 0.000 | 0.000-Inf | 0.998 |
| Multivessel disease | 1.072 | 0.5851- 1.966 | 0.821 |
| Peak CPK | 0.9999 | 0.9998-1.0000 | 0.365 |
| Medication at discharge | | | |
| ACEI | 1.572 | 0.861-2.870 | 0.141 |
| ARB | 0.701 | 0.371-1.327 | 0.275 |
| Beta-blocker | 0.641 | 0.352-1.165 | 0.144 |
| Calcium-blocker | 0.630 | 0.225-1.764 | 0.380 |
| Statin | 0.617 | 0.337-1.130 | 0.118 |
| Diuretics | 0.712 | 0.331-1.536 | 0.387 |
| Dual anti-platelet | 1.794 | 0.706-4.558 | 0.219 |

Abbreviations are same as in Table 1.

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Figure Legends

Figure 1. Patient selection flow chart. AMI, acute myocardial infarction; DNA, deoxyribonucleic acid; MI, myocardial infarction; OACIS, Osaka Acute Coronary Insufficiency Study; and PCI, percutaneous coronary intervention.

Figure 2. Kaplan-Meier estimates of re-myocardial infarction event.

Figure 3. Impact of the rs1333049 genotype on the onset and 1-year re-myocardial infarction. AMI, acute myocardial infarction; CI, confidence interval; HR, hazard ratio (CC vs CG/GG); OR, odds ratio (C vs G per allele); and ReMI, re-myocardial infarction.

Figure 4. Subgroup analysis of the impact of rs1333049 genotype on 1-year re-myocardial infarction rate. ACEI, angiotensin-converting enzyme inhibitor; ARB,



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angiotensin receptor blocker; CI, confidence interval; DAPT, dual anti-platelet therapy; HR, hazard ratio; NA, not assessed due to insufficient number of events in the subgroup analysis; and STEMI, ST-elevation myocardial infarction.

Supplementary Figure 1. Linkage disequilibrium of chromosome 9p21 single nucleotide polymorphisms in the present study population. Linkage disequilibrium was evaluated by D prime (left) and R squared (right) using PLINK and Haploview softwares.

Supplementary Figure 2. A possible mechanism for the differential impact of rs1333049 genotypes between the primary and secondary prevention settings. (A) Risk of first acute myocardial infarction (AMI) in the primary prevention setting. (B) Risk of re-myocardial infarction (ReMI) in the secondary prevention setting. Blue and yellow bars indicate the susceptibility risks of G (dominant risk) and C (additive risk) alleles, respectively. In this model, it is assumed that the risk of rs1333049 G allele was not changed between primary and secondary prevention settings, while the risk of rs1333049 C allele was reduced by the secondary prevention programs after AMI. In the



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primary prevention setting (panel A), the risk of C allele overwhelmed the G allele risk, making an "additive risk model" of C allele, in which the total risk increased as the number of C allele increased. On the other hand, in the secondary prevention setting (panel B), the risk of rs1333049 C allele was reduced and then the risk of G allele became highlighted, making a so-called "dominant risk model" of rs1333049 G allele.

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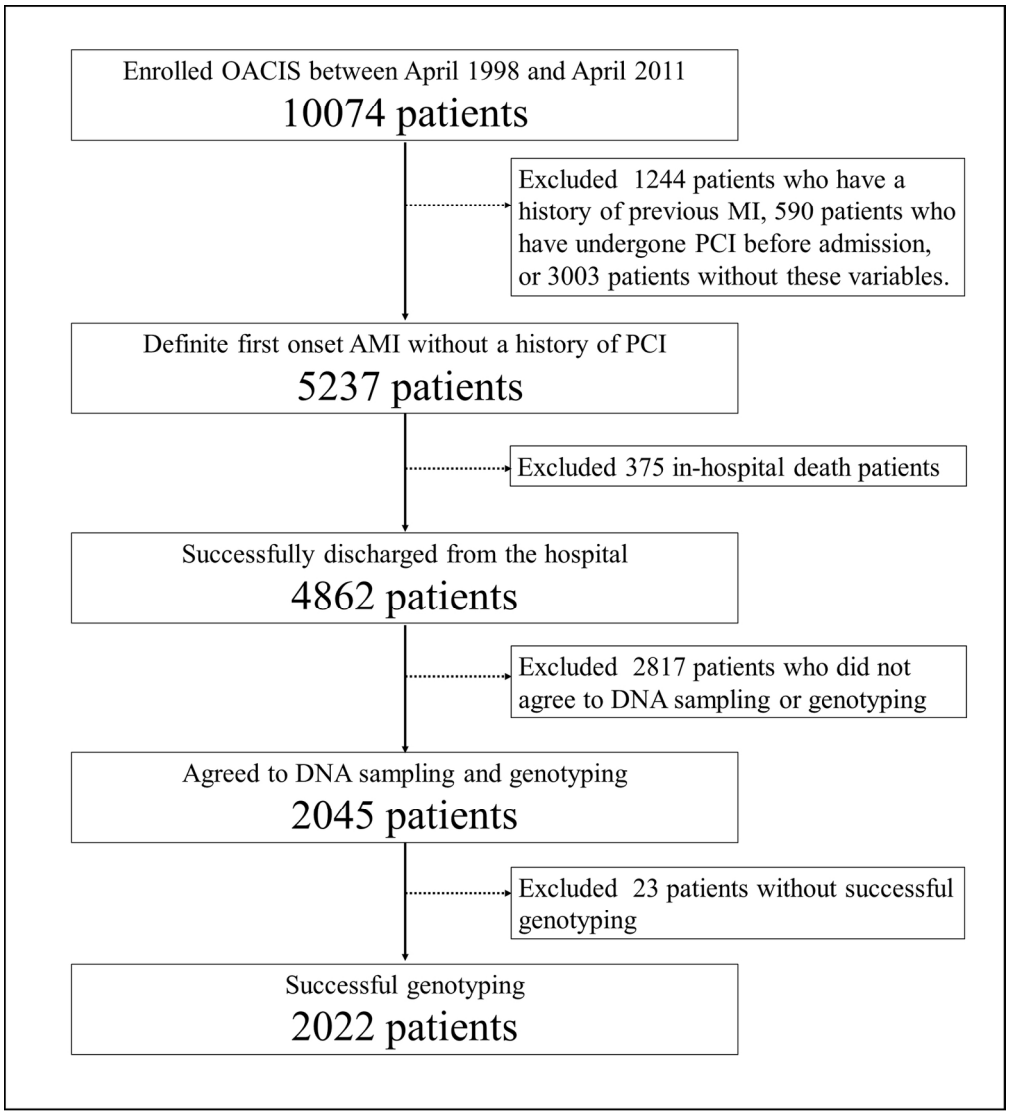


Patient selection flow chart. AMI, acute myocardial infarction; DNA, deoxyribonucleic acid; MI, myocardial infarction; OACIS, Osaka Acute Coronary Insufficiency Study; and PCI, percutaneous coronary intervention.
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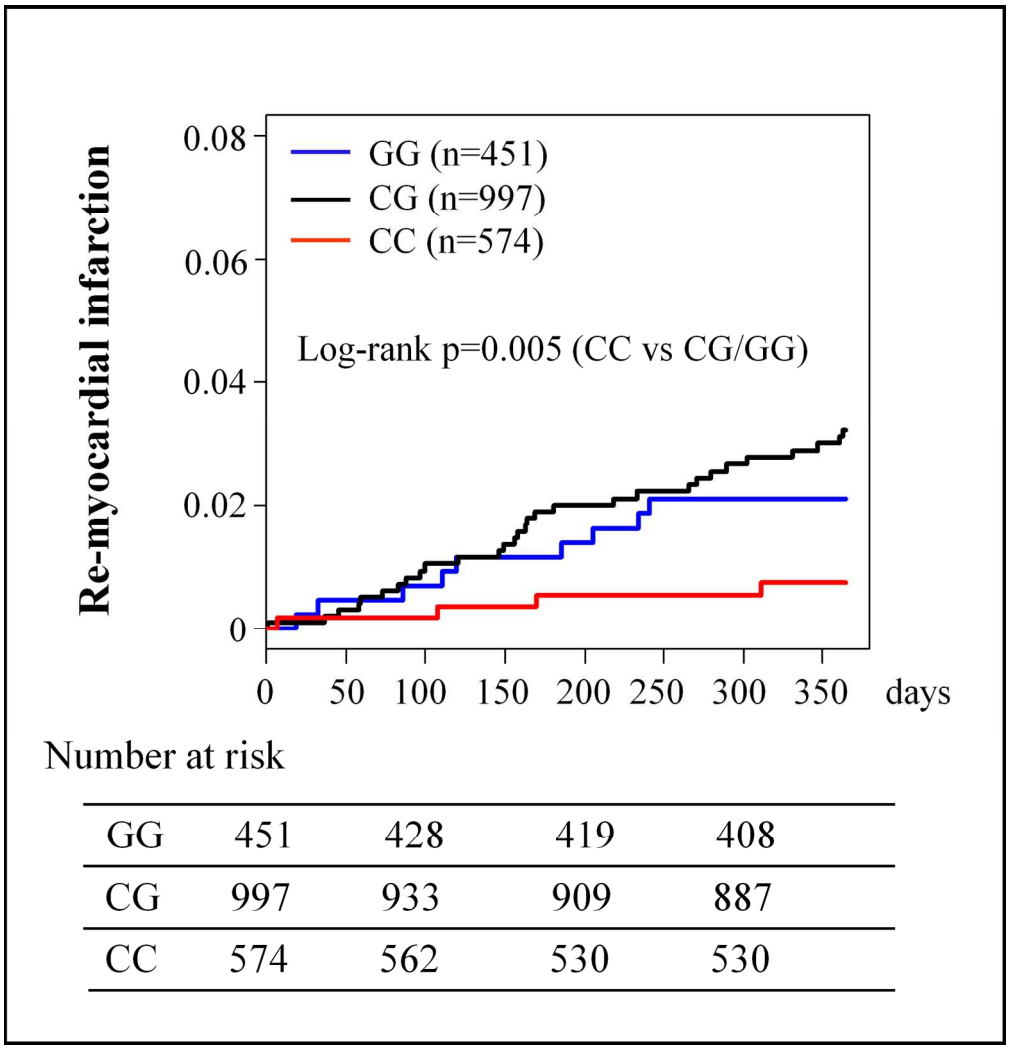
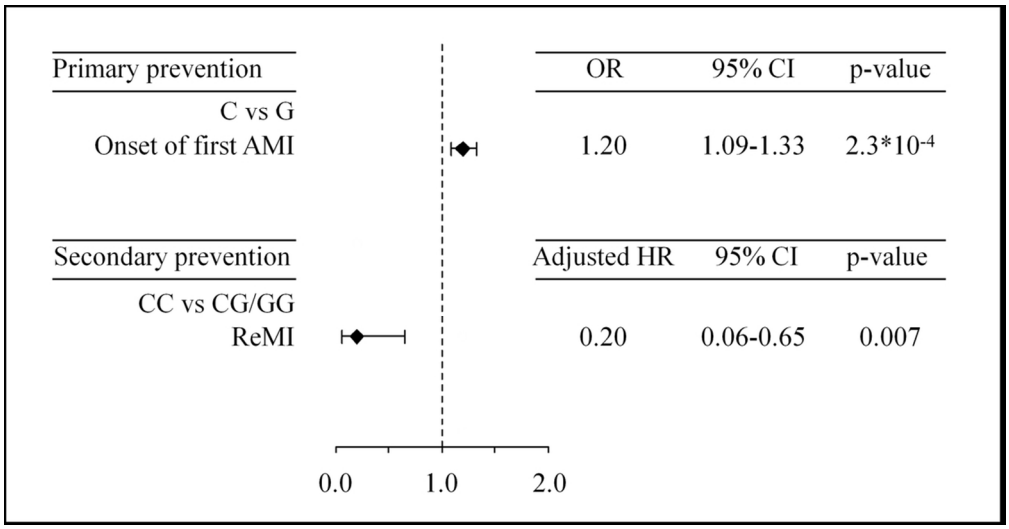



Number at risk

| | | | | |
|---|---|---|---|---|
| GG | 451 | 428 | 419 | 408 |
| CG | 997 | 933 | 909 | 887 |
| CC | 574 | 562 | 530 | 530 |

Kaplan-Meier estimates of re-myocardial infarction event.
172x178mm (300 x 300 DPI)




Impact of the rs1333049 genotype on the onset and 1-year re-myocardial infarction. AMI, acute myocardial infarction; CI, confidence interval; HR, hazard ratio (CC vs CG/GG); OR, odds ratio (C vs G per allele); and ReMI, re-myocardial infarction.
103x53mm (300 x 300 DPI)

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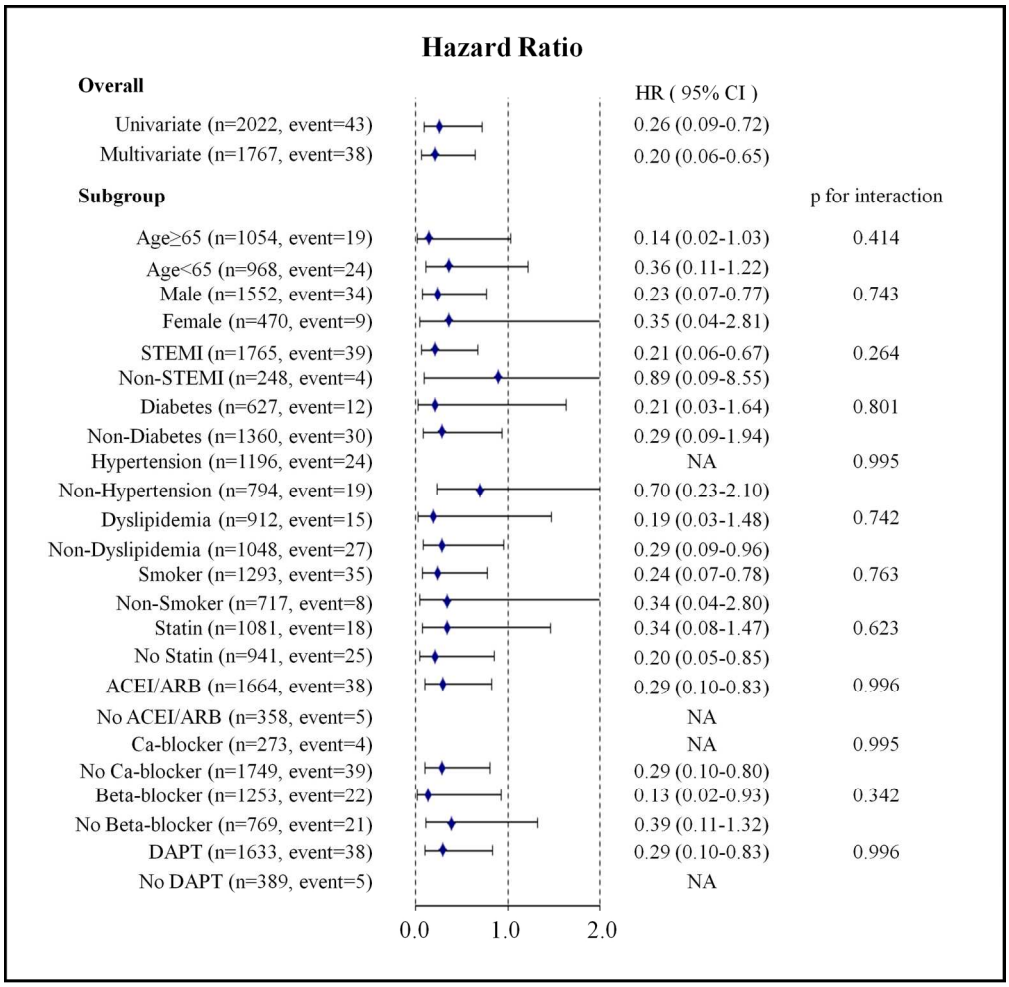


Subgroup analysis of the impact of rs1333049 genotype on 1-year re-myocardial infarction rate. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; DAPT, dual anti-platelet therapy; HR, hazard ratio; NA, not assessed due to insufficient number of events in the subgroup analysis; and STEMI, ST-elevation myocardial infarction.
170x166mm (300 x 300 DPI)

Supplementary Table 1. Results of univariate Cox regression analysis for re-myocardial infarction

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|---|---|---|---|
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| Male | 1.127 | 0.541-2.350 | 0.750 |
| BMI | 0.999 | 0.913-1.09 | 0.981 |
| STEMI | 1.388 | 0.496-3.883 | 0.533 |
| Coronary risk factor | | | |
| Diabetes | 0.872 | 0.446-1.703 | 0.688 |
| Hypertension | 0.843 | 0.462-1.539 | 0.578 |
| Dyslipidemia | 0.622 | 0.331-1.168 | 0.140 |
| Smoking | 2.423 | 1.124-5.222 | 0.024 |
| Target lesion | | | |
| Left main trunk | 2.533 | 0.337-19.034 | 0.366 |
| LAD | 1 (reference) | (reference) | (reference) |
| Diagonal branch | 0.892 | 0.119-6.706 | 0.912 |
| RCA | 1.434 | 0.745-2.758 | 0.281 |
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| Graft | 0.000 | 0.000-Inf | 0.998 |
| Multivessel disease | 1.072 | 0.5851- 1.966 | 0.821 |
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| Medication at discharge | | | |
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| Statin | 0.617 | 0.337-1.130 | 0.118 |
| Diuretics | 0.712 | 0.331-1.536 | 0.387 |
| Dual anti-platelet | 1.794 | 0.706-4.558 | 0.219 |

Abbreviations are same as in Table 1.

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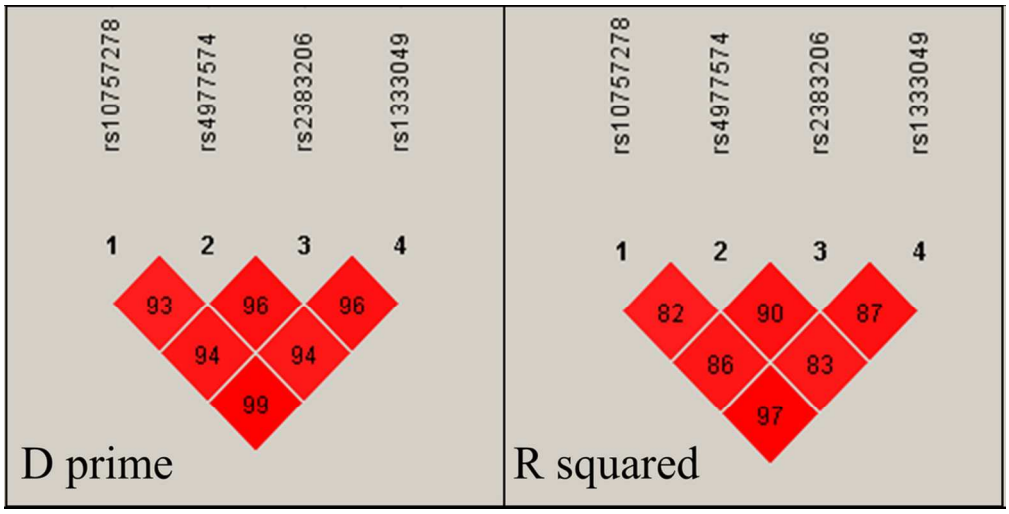


Linkage disequilibrium of chromosome 9p21 single nucleotide polymorphisms in the present study population. Linkage disequilibrium was evaluated by D prime (left) and R squared (right) using PLINK and Haploview softwares.
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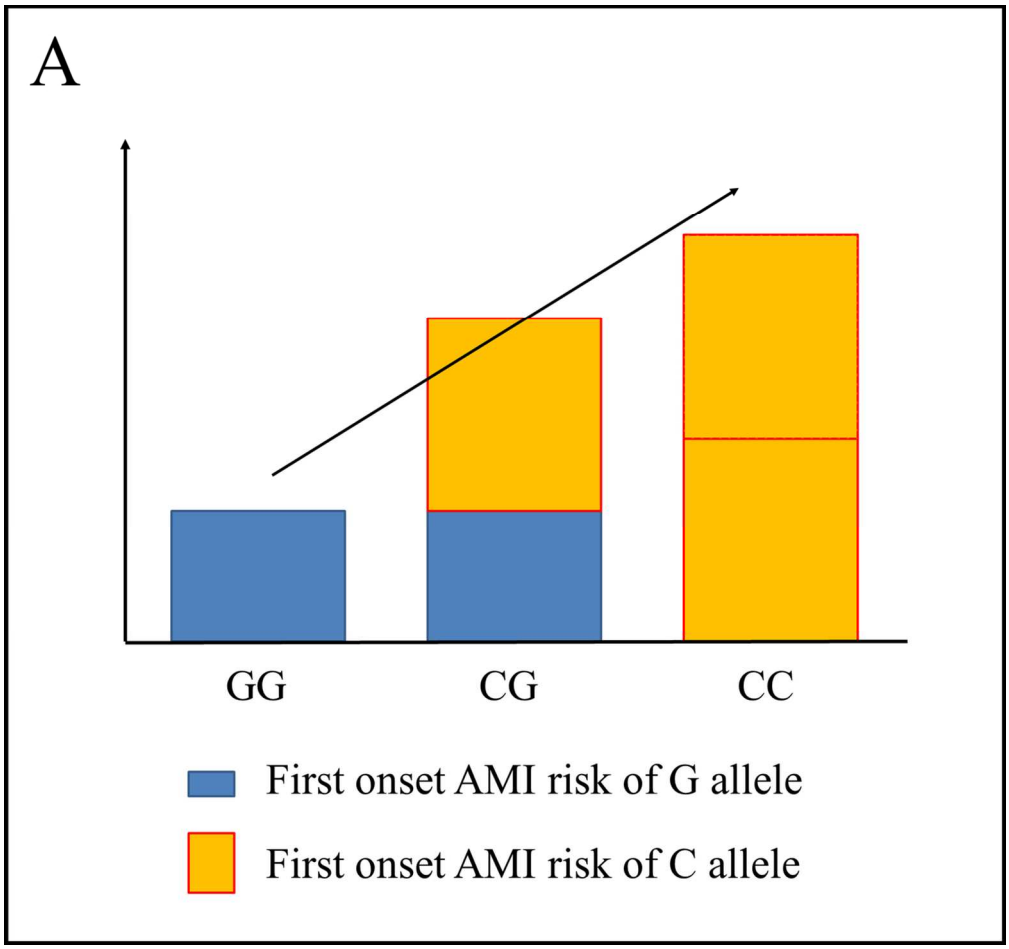
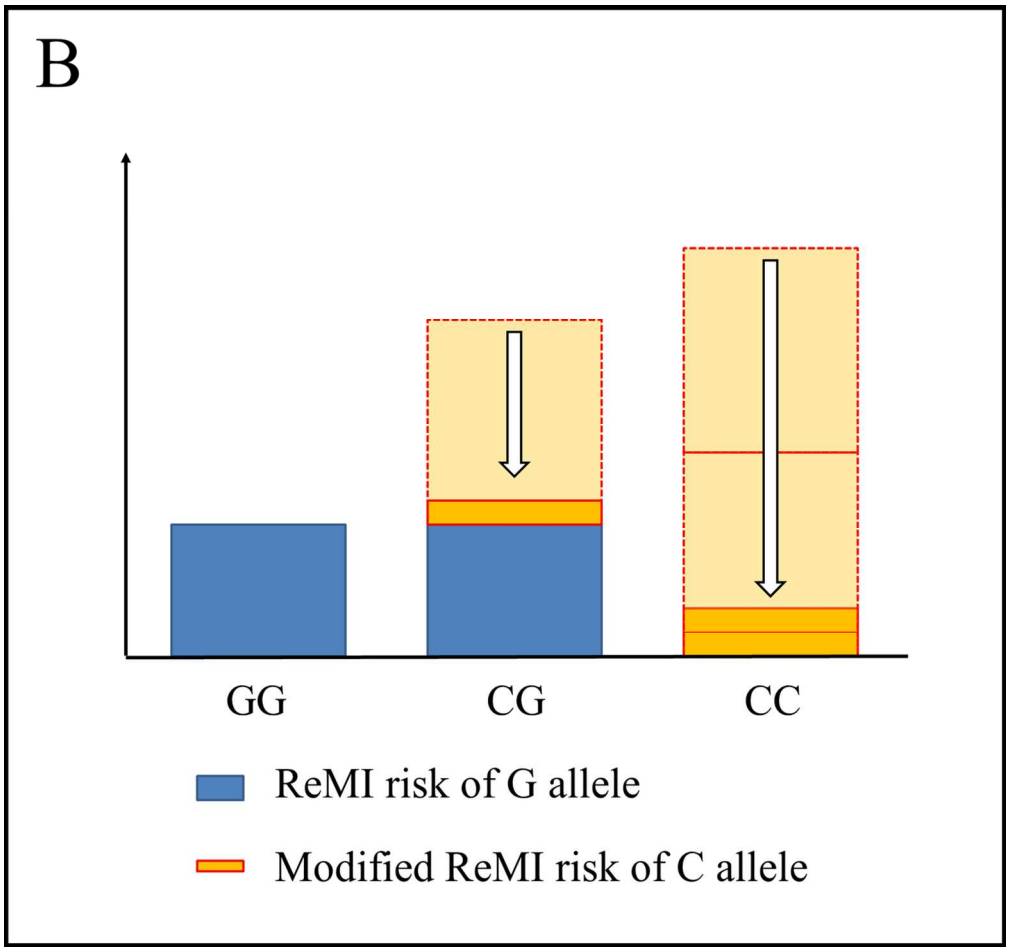



A possible mechanism for the differential impact of rs1333049 genotypes between the primary and secondary prevention settings. (A) Risk of first acute myocardial infarction (AMI) in the primary prevention setting. (B) Risk of re-myocardial infarction (ReMI) in the secondary prevention setting. Blue and yellow bars indicate the susceptibility risks of G (dominant risk) and C (additive risk) alleles, respectively. In this model, it is assumed that the risk of rs1333049 G allele was not changed between primary and secondary prevention settings, while the risk of rs1333049 C allele was reduced by the secondary prevention programs after AMI. In the primary prevention setting (panel A), the risk of C allele overwhelmed the G allele risk, making an "additive risk model" of C allele, in which the total risk increased as the number of C allele increased. On the other hand, in the secondary prevention setting (panel B), the risk of rs1333049 C allele was reduced and then the risk of G allele became highlighted, making a so-called "dominant risk model" of rs1333049 G allele.
119x112mm (300 x 300 DPI)




A possible mechanism for the differential impact of rs1333049 genotypes between the primary and secondary prevention settings. (A) Risk of first acute myocardial infarction (AMI) in the primary prevention setting. (B) Risk of re-myocardial infarction (ReMI) in the secondary prevention setting. Blue and yellow bars indicate the susceptibility risks of G (dominant risk) and C (additive risk) alleles, respectively. In this model, it is assumed that the risk of rs1333049 G allele was not changed between primary and secondary prevention settings, while the risk of rs1333049 C allele was reduced by the secondary prevention programs after AMI. In the primary prevention setting (panel A), the risk of C allele overwhelmed the G allele risk, making an "additive risk model" of C allele, in which the total risk increased as the number of C allele increased. On the other hand, in the secondary prevention setting (panel B), the risk of rs1333049 C allele was reduced and then the risk of G allele became highlighted, making a so-called "dominant risk model" of rs1333049 G allele.
119x112mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias | 9 | Describe any efforts to address potential sources of bias |
| Study size | 10 | Explain how the study size was arrived at |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding |
| | | (*b*) Describe any methods used to examine subgroups and interactions |
| | | (*c*) Explain how missing data were addressed |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy |
| | | (*e*) Describe any sensitivity analyses |

Continued on next page



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| | | |
|---|---|---|
| **Results** | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed |
| | | (b) Give reasons for non-participation at each stage |
| | | (c) Consider use of a flow diagram |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders |
| | | (b) Indicate number of participants with missing data for each variable of interest |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included |
| | | (*b*) Report category boundaries when continuous variables were categorized |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses |
| **Discussion** | | |
| Key results | 18 | Summarise key results with reference to study objectives |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results |
| **Other information** | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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