PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Hara, Masahiko; Sakata, Yasuhiko; Nakatani, Daisaku; Suna, Shinichiro; Usami, Masaya; Matsumoto, Sen; Ozaki, Kouichi; Nishino, Masami; Sato, Hiroshi; Kitamura, Tetsuhisa; Nanto, Shinsuke; Hamasaki, Toshimitsu; Tanaka, Toshihiro; Hori, Ma; Komuro, Issei

VERSION 1 - REVIEW

REVIEWER	Mitsuru Ohishi Department of Cardiovascular Medicine and Hypertension, Graduate School of Medical and Dental Sciences, Kagoshima University, JAPAN
REVIEW RETURNED	10-May-2014

GENERAL COMMENTS	The main purpose of this study performed by Hara et al. was to investigate the impact of the 9p21 rs1333049 variant on recurrent MI(ReMI) in patients with acute myocardial infarction (AMI). The authors demonstrated that susceptibility to acute coronary events conferred by chromosome 9p21 variants is discernable before and after the first experience of AMI. This finding is of clinical significance because 9p21 SNPs have been consistently shown to be associated with increased cardiovascular risk regardless of age, gender and race, and a paradoxical impact of a genetic variant on disease susceptibility has not been described in a similar cohort. However,there are several limitations to be addressed. Major comment 1. The patients in homozygous carriers of the rs1333049 C risk allele had lower ReMI event rate than in other allele carriers. Why the C allele showed this inverse effect between primary and secondary prevention settings. Because this is the novel and key finding which may attract physicians attention, speculation of the mechanism is mandatory in the discussion section in my opinion. minor comments 1. If possible, how many percentages of the patients with ReMI had re-occulusion of the culprit lesion? Although authors state these data are not available, I think it's better to show even prelimintary data as

	a supplementary material. 2. The authors stated that genetic factor is linked with plaque progression. Please specify if LDL values are controlled during the follow-up. 3. Even though I understand that some physicians and geneticists do not adjust patient backgrounds in a case-control target-SNP comparison study in the primary prevention setting, I reccomend to state this (no adjustment) in a study limitation section. 4. What the baseline covariates, which showed significant univariate relationship with ReMI? I would like to know the rationale for selecting covariates in the final multivariate model.

REVIEWER	Heribert Schunkert German Heart Centre, Cardiology
REVIEW RETURNED	06-Jun-2014

GENERAL COMMENTS	The authors studied the risk allele at the chromosome 9p21 locus for association with recurrent myocardial infarction. In the first part of their study the authors confirmed the repeatedly published association between the chromosome 9p21 locus and myocardial infarction. Subsequently they investigated as to whether this risk allele also affects the chance of a recurrent myocardial infarction. The aims of the study are clear and the paper is written very well. BMJ Open 2014-005438 by Hara et al 2 Major Comments: The initial observation of association between the chromosome 9p21 locus and myocardial infarction risk was obtained in thousands of cases and controls. The current research question of association between the very same risk locus and recurrent myocardial infarction is only based on 43 subjects, who had such recurrent event. Thus, the authors should either seek for independent replication in a much larger sample or discuss in detail the fairly low power to detect a true association. Only six individuals with the CC genotype (homozygous for the risk allele) experienced a myocardial infarction. Even single digit changes in this group would have profound impact on the conclusions. The authors may elaborate on this point and discuss in detail the limitation of their paper.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Major comment
1.
The patients in homozygous carriers of the rs1333049 C risk allele had lower ReMI event rate than in other allele carriers. Why the C allele showed this inverse effect between primary and secondary prevention settings. Because this is the novel and key finding which may attract physicians attention, speculation of the mechanism is mandatory in the discussion section in my opinion.
…We greatly appreciate this comment. It is very difficult to speculate the discrepancy of 9p21 risks between the primary and secondary prevention settings. However, we hypothesize that this discrepancy could be explained by the mechanism shown in the Supplementary Figure 2, which is newly provided in the revised manuscript. In the Figure, blue and yellow bars indicate the susceptibility risks of G (dominant risk) and C (additive risk) alleles, respectively. The left panel (A) explains the risk of first acute myocardial infarction (AMI) in the primary prevention setting, and the right panel (B) explains the risk of re-myocardial infarction in the secondary prevention setting. 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, the risk of C allele overwhelmed the G allele risk, making an “additive risk model” of C allele, in which the total risk increases as the number of C allele increased. On the other hand, in the secondary prevention setting, 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. We revised the discussion section according to this hypothesis and provided Supplementary Figure 2 for the discussion of this matter. Thank you very much again for your great advice. (Page 16, line 7-10) (Page 34, caption of Supplementary Figure 2)

minor comments
1.
If possible, how many percentages of the patients with ReMI had re-occulusion of the culprit lesion? Although authors state these data are not available, I think it's better to show even preliminary data as a supplementary material.
…Thank you very much for pointing out this very important issue. We totally agree that it is very important to consider whether ReMI occurs due to re-occlusion of target lesion or not. Unfortunately, however, such data are not available in the present study. We acknowledged this in the study limitation section in the revised manuscript. (Page 18, line 12-16)

2.
The authors stated that genetic factor is linked with plaque progression. Please specify if LDL values are controlled during the follow-up.
…We also appreciate this question. We speculate that LDL values were well-controlled during follow-up because 46.5% of all patients had a history of dyslipidemia and 53.5% received statin treatment at discharge as shown in Table 1 and because many other patients are assumed to receive statin treatment at the outpatient clinics after discharge based on the secondary prevention guidelines of AMI. However, because this is just a speculation, we only stated that medications at discharge were shown in Table 1 in the result section of the revised manuscript. (Page 12, last sentence)

3.
Even though I understand that some physicians and geneticists do not adjust patient backgrounds in a case-control target-SNP comparison study in the primary prevention setting, I reccomend to state this (no adjustment) in a study limitation section.

…Thank you very much for the great advice. We revised the manuscript following your recommendation by adding the above mentioned limitation in the study limitation section of the revised manuscript. (Page 18, line 16-18)

4.
What the baseline covariates, which showed significant univariate relationship with ReMI? I would like to know the rationale for selecting covariates in the final multivariate model.
…Thank you for the question. We would like to show you the data of univariate Cox regression results for ReMI in the Supplementary Table 1. Among all covariates tested, only smoking status showed statistically significant association with ReMI in the present study possibly due to lower event rate of PCI era.
The final multivariate model included all the tested covariates in the Supplementary Table 1 regardless of the univariate results. We thought that these covariates should be adjusted because these were likely to impact on atherosclerotic events to our knowledge. We acknowledged this in the statistical analysis section. (Page 12, line 5-8) (Page 32 Supplementary Table 1)

Reviewer: 2
Major Comments:
The initial observation of association between the chromosome 9p21 locus and myocardial infarction risk was obtained in thousands of cases and controls. The current research question of association between the very same risk locus and recurrent myocardial infarction is only based on 43 subjects, who had such recurrent event. Thus, the authors should either seek for independent replication in a much larger sample or discuss in detail the fairly low power to detect a true association. Only six individuals with the CC genotype (homozygous for the risk allele) experienced a myocardial infarction. Even single digit changes in this group would have profound impact on the conclusions. The authors may elaborate on this point and discuss in detail the limitation of their paper.
…We greatly appreciate this thoughtful comment. We agree that independent replication studies with a larger sample are warranted. We discussed this issue in the discussion section of the revised manuscript (Page 18, line 6-9). We also acknowledged this in the summary of “Strengths and limitations of this study” section (Page 6, line 7). However, we would greatly appreciate if you could kindly understand that the statistical significance was demonstrated under consideration of the relatively small number of recurrent myocardial infarction. To further validate our results, we also performed sensitivity analysis by excluding each and every one of 43 subjects with re-myocardial infarction and confirmed the robustness of the study result just to be safe (See the data below). Thank you very much again for this important comment.

Total (data shown in the manuscript)
Adjusted hazard ratio (aHR) of CC genotype = 0.1988 (95% confidence interval [CI]: 0.06091-0.6487), p-vlaue = 0.00742

Sensitivity analysis
Excluding re-myocardial infarction case 1.
aHR = 0.319 (95% CI: 0.03161 - 0.5501), p-value = 0.00543
Excluding re-myocardial infarction case 2.
aHR = 0.1988 (95% CI: 0.06091 - 0.6487), p-value = 0.00742
Excluding re-myocardial infarction case 3.
aHR = 0.1318 (95% CI: 0.03159 - 0.5497), p-value = 0.00542
Excluding re-myocardial infarction case 4.
aHR = 0.1328 (95% CI: 0.03184 - 0.5542), p-value = 0.00561
Excluding re-myocardial infarction case 5.

aHR = 0.2035 (95% CI: 0.06222 - 0.6655), p-value = 0.00845
Excluding re-myocardial infarction case 6.
aHR = 0.2054 (95% CI: 0.06285 - 0.6715), p-value = 0.00882
Excluding re-myocardial infarction case 7.
aHR = 0.2051 (95% CI: 0.06277 - 0.6703), p-value = 0.00874
Excluding re-myocardial infarction case 8.
aHR = 0.2044 (95% CI: 0.06255 - 0.668), p-value = 0.00860
Excluding re-myocardial infarction case 9.
aHR = 0.2074 (95% CI: 0.06347 - 0.6775), p-value = 0.0092
Excluding re-myocardial infarction case 10.
aHR = 0.2057 (95% CI: 0.06294 - 0.6723), p-value = 0.00887
Excluding re-myocardial infarction case 11.
aHR = 0.1988 (95% CI: 0.06091 - 0.6487), p-value = 0.00742
Excluding re-myocardial infarction case 12.
aHR = 0.2065 (95% CI: 0.06321 - 0.6746), p-value = 0.00901
Excluding re-myocardial infarction case 13.
aHR = 0.1988 (95% CI: 0.06091 - 0.6487), p-value = 0.00742
Excluding re-myocardial infarction case 14.
aHR = 0.2039 (95% CI: 0.06239 - 0.6664), p-value = 0.00849
Excluding re-myocardial infarction case 15.
aHR = 0.1988 (95% CI: 0.06091 - 0.6487), p-value = 0.00742
Excluding re-myocardial infarction case 16.
aHR = 0.2036 (95% CI: 0.06228 - 0.6655), p-value = 0.00844
Excluding re-myocardial infarction case 17.
aHR = 0.2043 (95% CI: 0.06251 - 0.6676), p-value = 0.00857
Excluding re-myocardial infarction case 18.
aHR = 0.1988 (95% CI: 0.06091 - 0.6487), p-value = 0.00742
Excluding re-myocardial infarction case 19.
aHR = 0.2029 (95% CI: 0.06207 - 0.6631), p-value = 0.00829
Excluding re-myocardial infarction case 20.
aHR = 0.2051 (95% CI: 0.06274 - 0.6703), p-value = 0.00874
Excluding re-myocardial infarction case 21.
aHR = 0.2043 (95% CI: 0.06251 - 0.6678), p-value = 0.00859
Excluding re-myocardial infarction case 22.
aHR = 0.2061 (95% CI: 0.06307 - 0.6735), p-value = 0.00895
Excluding re-myocardial infarction case 23.
aHR = 0.2058 (95% CI: 0.06298 - 0.6724), p-value = 0.00887
Excluding re-myocardial infarction case 24.
aHR = 0.2047 (95% CI: 0.06264 - 0.6687), p-value = 0.00863
Excluding re-myocardial infarction case 25.
aHR = 0.2036 (95% CI: 0.06227 - 0.6654), p-value = 0.00844
Excluding re-myocardial infarction case 26.
aHR = 0.2061 (95% CI: 0.06308 - 0.6736), p-value = 0.00895
Excluding re-myocardial infarction case 27.
aHR = 0.2027 (95% CI: 0.06200 - 0.6624), p-value = 0.00825
Excluding re-myocardial infarction case 28.
aHR = 0.2039 (95% CI: 0.06237 - 0.6666), p-value = 0.00851
Excluding re-myocardial infarction case 29.
aHR = 0.2031 (95% CI: 0.06213 - 0.6636), p-value = 0.00832
Excluding re-myocardial infarction case 30.
aHR = 0.2039 (95% CI: 0.06240 - 0.6664), p-value = 0.00849
Excluding re-myocardial infarction case 31.

aHR = 0.2060 (95% CI: 0.06305 - 0.6733), p-value = 0.00893
Excluding re-myocardial infarction case 32.
aHR = 0.2051 (95% CI: 0.06275 - 0.6701), p-value = 0.00873
Excluding re-myocardial infarction case 33.
aHR = 0.2052 (95% CI: 0.06278 - 0.6707), p-value = 0.00877
Excluding re-myocardial infarction case 34.
aHR = 0.2038 (95% CI: 0.06237 - 0.6662), p-value = 0.00849
Excluding re-myocardial infarction case 35.
aHR = 0.2045 (95% CI: 0.06256 - 0.6684), p-value = 0.00862
Excluding re-myocardial infarction case 36.
aHR = 0.2059 (95% CI: 0.06296 - 0.6731), p-value = 0.00892
Excluding re-myocardial infarction case 37.
aHR = 0.2020 (95% CI: 0.06180 - 0.6603), p-value = 0.00812
Excluding re-myocardial infarction case 38.
aHR = 0.2043 (95% CI: 0.06252 - 0.6678), p-value = 0.00858
Excluding re-myocardial infarction case 39.
aHR = 0.2043 (95% CI: 0.06252 - 0.6676), p-value = 0.00857
Excluding re-myocardial infarction case 40.
aHR = 0.2059 (95% CI: 0.06300 - 0.6728), p-value = 0.0089
Excluding re-myocardial infarction case 41.
aHR = 0.2046 (95% CI: 0.06259 - 0.6687), p-value = 0.00864
Excluding re-myocardial infarction case 42.
aHR = 0.2069 (95% CI: 0.06329 - 0.6764), p-value = 0.00914
Excluding re-myocardial infarction case 43.
aHR = 0.2009 (95% CI: 0.06144 - 0.6568), p-value = 0.00792

VERSION 2 – REVIEW

REVIEWER	Mitsuru Ohishi Department of Cardiovascular Medicine and Hypertension, Graduate School of Medical and Dental Sciences, Kagoshima University, JAPAN
REVIEW RETURNED	22-Jul-2014

- The reviewer completed the checklist but made no further comments.