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# LETTERS TO THE EDITOR

## Association of Gene Polymorphisms With Coronary Artery Disease in Low- or High-Risk Subjects Defined by Conventional Risk Factors

We read with interest the careful study by Hirashiki et al. (1), who, upon examining 37 single nucleotide polymorphisms (SNPs) in 31 candidate genes, reported a significant association of coronary artery disease (CAD) with SNPs in the apolipoprotein E, connexin-37, stromelysin-1, and endothelial nitric oxide synthase (eNOS) genes. A polymorphism in the promoter ($T^{-786}C$) of the latter gene was recently shown to bear functional consequences (2) because the C allele creates a binding site for a replication protein A-1 that acts as a repressor of gene transcription (3). Accordingly, essential hypertensive patients carrying this allele exhibited a blunted forearm vasodilation in response to acetylcholine (4). This allele was shown to be significantly associated with coronary vasospasm and myocardial infarction (MI) in Japanese patients (2,5) and with multivessel CAD in consecutive Caucasian patients undergoing coronary angiography because of suspected CAD (6). Thus, the results of Hirashiki et al. (1) in the Japanese patient population appear to be fully confirmatory of earlier reports (5), including our study of Caucasians (6), that were overlooked.

Based on the presence or absence of hypertension, hypercholesterolemia, and diabetes mellitus, subjects in the Hirashiki et al. study (1) were divided into a high- and a low-risk group, respectively, yet about 74% of their CAD patients had myocardial infarction (MI) and therefore cannot be considered at low risk. Indeed, according to the Third Report of the National Cholesterol Education Program Expert Panel (Adult Treatment Panel III) (7), these individuals would represent high-risk patients (e.g., subjects with a >20% risk of cardiovascular events in the next 10 years). Moreover, as the investigators correctly pointed out, their population comprises MI survivors. Thus, it remains unclear whether the SNPs that showed a significant association with CAD in a population that mainly comprised high-risk MI survivors represented markers of survival or of predisposition to CAD (e.g., protective or nefarious genetic variants). In our consecutive patients, a minority (45.6%) had a history of MI (6). Accordingly, although the mortality for MI might be higher in Western countries than in Japan, the majority of our patient population did not comprise MI survivors.

The significant association of the $^{-786}C$ allele with CAD was confirmed by Hirashiki et al. also in their high-risk men; however, the researchers did not comment on this important finding. Of interest, we tested the hypothesis that the genetic predisposition to generate less nitric oxide (NO) was more detrimental under conditions with blunted NO availability, such as age older than 60 years, hypercholesterolemia, cigarette smoking, and low high density lipoprotein (HDL) cholesterol. With such analysis we found not only a significant association of multivessel CAD with the $^{-786}C$ allele but also, more importantly, a marked increase in the relative risk of multivessel CAD from 1.7 (95% confidence interval: 1.11 to 2.55, $p = 0.014$) in the overall population to 3.61 (1.63 to 8.0, $p = 0.002$) when at least four such conditions coexisted. Thus, one of the SNPs that was found to be associated with CAD by Hirashiki et al. (1) does not seem to increase the risk of CAD independently of other risk factors. Instead, it appears to deeply interact with environmental factors that blunt NO availability.

It must be kept in mind that association studies are peculiarly prone to false positive results, particularly when controls are not randomly selected, as in the study by Hirashiki et al. (1). Based on dividing the patients into low- and high-risk groups, the investigators pinpointed SNPs that were significantly associated with CAD in low- and high-risk groups of men and women. As the investigators correctly acknowledged, the large number of comparisons that were made opened up the possibility of chance findings; furthermore, no information on power of the subgroup analyses was furnished. Nonetheless, the independent replication by Hirashiki et al. in a genetically unrelated population of our report of an association of the $^{-786}C$ allele with multivessel CAD lends strong support to our conclusion that this SNP is an important genetic factor predisposing to the development of multivessel CAD.

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## REPLY

We thank Drs. Rossi and Maiolino for their comments regarding our paper (1). Beyond 2020, it is likely that genetic identification

of the risk factors will lead to gene-informed personalized prevention (2). It is thus important to identify genes that confer susceptibility to coronary artery disease (CAD). Furthermore, stratification of subjects based on conventional risk factors for CAD and prospective cohort studies are also important to accomplish personalized medicine. In our study (1), low-risk individuals were defined as those who did not have any of the three major risk factors (hypertension, diabetes mellitus, and hypercholesterolemia). Given that the study is a cross-sectional association study, the classification of subjects as low-risk or high-risk was not based on the prevalence of myocardial infarction (MI) after follow-up of 10 years. Although a survivor bias could not be excluded completely, it was likely to be small in our study because of a relatively low prevalence of fatal MI (approximately 20%) in Japan. The variant alleles of four single nucleotide polymorphisms (SNPs) significantly associated with CAD are thus markers of predisposition to CAD.

Although the $^{-786}$T→C SNP of the endothelial nitric oxide (eNOS) gene was related to CAD in the total population and in high-risk men, the p values were 0.0484 (additive model) and 0.0369 (dominant model), respectively (1). In an initial screening of the 112 polymorphisms for association with MI in 909 subjects in our previous study (3), the $^{-786}$T→C SNP of eNOS gene was related to MI in men and women. In a large-scale association study, however, this SNP was not significantly associated with MI in men or women (3). The characteristics of ideal association study include a large sample size, small p values, an association that makes biological sense, and alleles that affect the gene product in a physiologically meaningful way (4). Two studies with a p value of $<0.01$ or a single study with a p value of $<0.001$ other than the first positive study is strongly predictive of future replication (5). Given that we adopted a criterion of a p value of $<0.005$ as statistical significance for association (1), the relation of the $^{-786}$T→C SNP of eNOS gene with CAD was considered not to be significant. Although this SNP was shown to be associated with coronary artery spasm (6), the relation of this SNP with CAD remains to be elucidated.

In a stepwise forward selection procedure, four SNPs significantly associated with CAD were all statistically independent of age, smoking, or hyperuricemia as well as hypertension, diabetes mellitus, and hypercholesterolemia. As Drs. Rossi and Maiolino point out, the presence of hypertension, diabetes mellitus, and hypercholesterolemia may blunt nitric oxide availability. However, the $^{1019}$C→T SNP of the connexin-37 gene in high-risk men and the $^{3932}$C→T SNP of the apolipoprotein E gene in high-risk women were statistically independent of these risk factors.

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## Therapeutic Transcutaneous Ultrasound: Long Overdue Therapy

The recent report by Miyamoto et al. (1) presents the results of an experimental study evaluating the coronary vessel dilatory effect of transcutaneous low-frequency ultrasound. The investigators used a quite sophisticated animal model and demonstrated by various means that transcutaneous ultrasound induces vasodilation in canine coronary arteries. The magnitude of the effect was similar to that found with intracoronary nitrates. The researchers very correctly concluded that this phenomenon may be used as a therapeutic tool to reduce myocardial ischemia in patients with acute coronary syndromes.

Interestingly, in 1980 we first performed experimental and later clinical studies using approximately similar doses of transcutaneous ultrasound. The ultrasound frequency in our study was 790 to 910 kHz, with the ultrasound of 0.2 to 0.45 W/cm$^2$. The first case of transcutaneous ultrasound therapy in a patient was performed in early 1981 and later published in a USSR patent (2).

We completely agree with the editorial comments of Drs. McPherson and Holland (3) who postulated that this effect of ultrasound is strictly mechanical (acoustic radiation, streaming, and cavitation). Recently, we demonstrated that similar effects can be achieved with other forms of electromagnetic radiation (4,5).

In conclusion, we want to congratulate the investigators on a very interesting study, and we believe that further preclinical and clinical data may prove the safety and efficacy of this long overdue form of therapy, namely transcutaneous therapeutic ultrasound for treatment of ischemia.

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