eliminate these confounding influences on the presence of CAD, logistic regression analysis was performed for the four SNPs in the risk haplotype block. After adjusting for confounding conventional risk factors such as sex, age, BMI, HT, HL, DM, and smoking, subjects with GG genotype for SNP4 and GG genotype for SNP6 were still at decreased risk for CAD compared with those having C-allele for SNP4 and A-allele for SNP6 ($P = 0.0040$, OR: 2.20, 95%CI: 1.29-3.77 and $P = 0.029$, OR: 1.82, 95%CI: 1.07-3.12, respectively) (Table V).

Tendency of associations between Mrf-2 SNPs and severity of CAD: To evaluate whether Mrf-2 SNPs were associated with the severity of CAD, individuals in the CAD group who had previously undergone coronary artery bypass graft surgery

Table V. Logistic Regression Analysis for Mrf-2 SNPs and CAD

		P	Odds ratio	95%CI
SNP4	CC+CG/GG	0.0040*	2.20	1.29-3.77
	CC/CG/GG	0.0010*	1.56	1.20-2.03
SNP6	AA+AG/GG	0.029*	1.82	1.07-3.12

Odds ratios (OR) were calculated using three genetic models. CC+CG/GG and AA+AG/GG are dominant models for SNP4 and SNP6, respectively. OR for each SNP was adjusted simultaneously for sex, age, BMI HT, HL, smoking, and DM. * indicates statistical significance. SNP4 and SNP6 were indicated to be potential independent predictors for susceptibility to CAD after eliminating confounding factors.

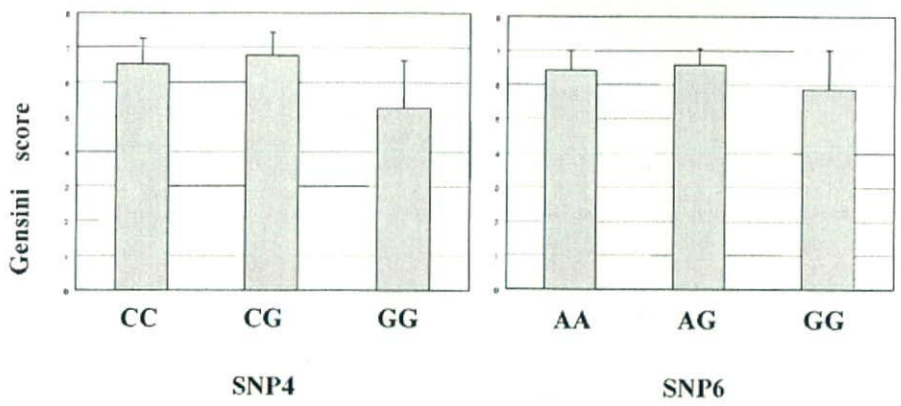

Figure 3. Mrf-2 SNPs and severity of CAD. The carriers of GG genotype in SNP4 and GG genotype in SNP6 had slightly less severe coronary artery diseases, although statistical significance was not observed.

(CABG) or percutaneous coronary intervention (PCI) were excluded, and only the patients who underwent a coronary arteriogram for the first time without revascularization were selected for this analysis.

The associations between genotypes of Mrf-2 and the modified Gensini score,[19,20] which is an index of coronary atherosclerosis severity and is computed by assigning the severity score to each coronary stenosis according to the degree of luminal narrowing and its geographic importance, were investigated. Although the differences among Mrf-2 polymorphisms and severity of CAD did not reach statistical significance, individuals with GG genotype for SNP4 and GG for SNP6 had lower Gensini scores, which was relevant to the correlations that these SNPs were negatively associated with susceptibility to CAD (Figure 3).

No genetic variations were found in exon3 and exon4: Exon 3 is the only exon included in the haplotype block consisting of SNP4-6, and exon 4 is located close to SNP6, therefore, we screened exon3 (226 bp), exon4 (231 bp) and their exon-intron boundaries to investigate whether there was functional SNP in these exon regions by comparing sequences of 100 randomly chosen samples of CAD subjects. No SNP was found in these regions.

DISCUSSION

In the present study, we evaluated whether the polymorphisms of the Mrf-2 gene were associated with susceptibility to CAD. We first demonstrated that genetic variations of Mrf-2 in SNP4 (rs2893880) and SNP6 (rs7087507) themselves and in the haplotype block, consisting of SNP4 (rs2893880), SNP5 (rs10740055), SNP6 (rs7087507), and SNP7 (rs10761600) were significantly associated with susceptibility to CAD. Carrying G for SNP4, C for SNP5, G for SNP6, and A for SNP7 was much more likely to play protective roles against CAD.

It is well accepted that the pathogenesis of CAD is multi-factorial. In addition to the conventional coronary risk factors, other factors such as vasoconstriction,[21] and platelet function[22,23] are also well established. Furthermore, studies have indicated a greater genetic risk in monozygotic twins compared with dizygotic ones, and adoption studies showed evidence that much of the excess risk was genetic rather than environmental for CAD.[24,25] A number of initial studies attempted to address the possibility that polymorphisms at various candidate genes might be associated with the pathogenesis of CAD and some concluded there was a significant association between them. For example, polymorphisms in renin-angiotensin system gene polymorphisms (angiotensin-type receptor genes, angiotensin II converting enzyme genes) are well known to be associated with hypertension,[26] CAD[27-31] and MI,[32-34] though dissenting opinions still exist.

In the case of transcriptional factors, only a few of them were concluded to be associated with the presence of CAD. Within them, controversial data of associations such as the findings between mutations in the myocyte enhancer factor (MEF) gene and the presence of myocardial infarction (MI) have been reported.[35-38] The contribution of genetic polymorphisms to the pathogenesis of CAD still remains to be definitively established. Our findings provided the first evidence for disease relevance of the polymorphisms of a novel transcription factor, Mrf-2.

Some genetic polymorphisms have a direct effect on the occurrence or development of a disease, whereas others have a modifying effect. We previously reported the full length of the Mrf-2 gene consisting of 11 exons and including two nonoverlapped isoforms. The two isoforms differ at the 5' end and share the same sequence from exon5. Exon 1α (specific for Mrf-2α), together with exon5-10 constitute the shorter isoform named Mrf-2α whose open reading frame was 2835 bp, and exon 1-10 constitute the longer one named Mrf-2β whose open reading frame was 3564 bp. SNPs involved in this study were located in the second and third intron regions of the Mrf-2β gene, and no coding SNP was observed in exon3 caught in the 4 disease associated SNPs and exon4, therefore, these findings suggest the intronic polymorphisms in the Mrf-2β gene are much more likely to have a modifying effect by influencing the expression level of Mrf-2 by themselves or by other unknown causative genetic variations located within or nearby the haplotype block. Since the two isoforms conserve the same sequences from exon5 and the Mrf-2 α specific exon located between exon 4 and exon 5, there are 2 possibilities: one could be that these intronic SNPs of Mrf-2β might be related to probable variations in the promoter region of the Mrf-2α specific exon, as yet unidentified; the other is that they might also regulate the upstream promoter of Mrf-2β as these SNPs are located in the second and third intron region, and by which influence the Mrf-2 expression level and regulate its function. In addition, Mrf-2 is a gene of approximately 200 kb, and the haplotype block containing the disease associated SNPs is located in the mid portion of the Mrf-2 gene, which excluded the possibility that other nearby genes were functionally causative concerning the associations between the Mrf-2 variations and susceptibility to CAD in the present study.

The Mrf-2 SNPs exhibited a tendency to be associated with the severity of CAD with GG genotype for SNP4 and GG genotype for SNP6 had slightly lower Gensini scores. However, these findings did not reach statistical significance. One probable reason might be a reduced statistical power due to the small available sample size because only the CAD patients who underwent coronary arteriogram for the first time without revascularization were selected for this analysis. Individuals who had previously undergone CABG or PCI were excluded, which also indicated another possibility that the reduced statistical power was due to the

fact that the more serious patients were excluded from this analysis.

Within the population studied, the definition of CAD was based on coronary angiography, while the control subjects were defined on the basis of having a normal ECG pattern and having no medical history of coronary artery disease or stroke. It remained a possibility that the control group might not be completely free from significant coronary atherosclerosis. However, if it were not for the ethics reason, we would have obtained much clearer results by performing coronary angiography in the control group to exclude the subjects with more than 50% narrowing. Therefore, the results on the associations of Mrf-2 SNPs with CAD in the present study were considered to be reliable.

The positive disease associations were adjusted by performing logistic regression analysis to eliminate conventional confounding risk factors. The significant differences that remained indicated genetic variations of the Mrf-2 may contribute risk to CAD independent from traditional coronary risk factors. Within the studied population, assessments on Mrf-2 variations and each of the traditional coronary risk factors revealed weak associations of Mrf-2 SNPs with DM and BMI. However, statistical significance was not attained after multiple analysis, which might be a result of 1) reduced statistical power due to the small available investigating sample size; and 2) the definition of DM for the CAD and its control groups was based on only one time FBS level or HbA1c value, which was inadequate for obtaining more reliable results. Thus, the possibility that they are associated cannot be excluded, and this should be confirmed in future studies.

At present, although we could not provide definite evidence to elucidate the mechanisms of how the Mrf-2 gene contributes to the pathogenesis of CAD, there are a number of possible explanations. First, the phenotypic change (ie, differentiation and dedifferentiation) of SMCs has recently been considered critical in the pathogenesis of atherosclerotic lesions. Since Mrf-2 was cloned as a key regulator for smooth muscle cell differentiation,[4] we hypothesized that Mrf-2 might be involved in the pathogenesis of atherosclerosis via its regulation of SMC differentiation. This question would be resolved by constructing a targeting mouse model of Mrf-2 and examining the changes of SMCs. Second, it was reported that mice lacking the Mrf-2 gene exhibited severe growth retardation, reduced weight gain, and reduced lipid accumulation,[8,13,14] which indicate the possibility that the association between Mrf-2 and CAD might be mediated partially through metabolic syndrome and DM. Third, Mrf-2 is a transcriptional factor which preferentially binds to the sequence AATAC/T and is expressed in the cardiovascular system. Thus, it is conceivable that this gene product might regulate other important vasoactive and pathogenetic molecules, which should be investigated in future studies. Fourth, Mrf-2 was shown to be an early marker for nephrogenic tissues, and broad expression of Mrf-2α was observed in lung, heart, brown adi-

pose tissue, brain and kidney, which suggested widespread biological functions. Gene targeting of Mrf-2 indicated that the homozygous mutant showed reduced viability, displayed female and male reproductive organ abnormalities, and adrenal gland abnormalities,[8,13] suggesting that Mrf-2 might presumably play important roles in the endocrine system through which it has an effect on the pathogenesis of CAD.

Overall, the present results demonstrate for the first time disease associations of the Mrf-2 SNPs with the presence of CAD. One limitation of the present study is the number of subjects studied. To validate the present findings, more individuals need to be recruited. In addition, much more basic research should be conducted to elucidate the mechanisms of the Mrf-2 gene. Furthermore, a prospective study would be a more effective means and should be performed to obtain reliable information on the clinical utility of this possible genetic risk factor of CAD.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Yuki Itoh, Ms. Eri Fujiu, Ms. Chika Masuo, Ms. Yasuko Kubono, and Ms. Yukino Sato for their excellent technical assistance.

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