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Identification of Genetic Factors and Development of Genetic Risk Diagnosis Systems for Cardiovascular Diseases and Stroke

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Background Polymorphisms of *GJA4* and *CYBA* and of *PAI1* and *MMP3* are associated with myocardial infarction (MI) in men and women, respectively. In addition, several polymorphisms associated with restenosis after percutaneous coronary intervention, coronary artery spasm, or hypertension have been identified. More recently, a large genetic epidemiological study was performed to identify additional gene polymorphisms that confer susceptibility to cardiovascular diseases, stroke, and other complex diseases.

Methods and Results The relationship of 202 polymorphisms in 152 candidate genes to MI, hypertension, ischemic or hemorrhagic stroke, metabolic syndrome, type 2 diabetes mellitus, obesity, or in-stent restenosis were examined in 5,000 unrelated Japanese individuals. Of these, 14 polymorphisms related to MI, 8 to atherothrombotic cerebral infarction, 9 to intracerebral hemorrhage, and 10 to subarachnoid hemorrhage were identified. This information was then used to develop risk diagnosis systems to predict the future risk for development of each disease in a given individual.

Conclusions Identification of gene polymorphisms that confer susceptibility to cardiovascular diseases or stroke and the development of genetic risk diagnosis systems may contribute to the personalized prevention of these conditions. (*Circ J* 2006; **70:** 1240–1248)

Key Words: Cardiovascular disease; Genetics; Myocardial infarction; Polymorphism; Stroke

Personalized medicine relies on knowledge obtained from characterization of the human genome and disease biology for the development of preventive, diagnostic, and therapeutic strategies that target the underlying determinants of disease in populations with specific molecular profiles. Personalized medicine is thus based on a redefinition of diseases at the molecular level, so that prevention, diagnosis, and therapy can be targeted. It also refers in part to the ability to individualize therapy through prediction of which patients have a greater chance of benefit or risk from a particular treatment. This approach maximizes the effectiveness and safety of drugs and depends on several factors: the ability to identify key signature biomarkers associated with a particular disease, the availability of a treatment (or treatments) for the disease, and the ability to identify the biomarkers in a particular subject so as to be able to effectively match the treatment with the individual.

Completion of the Human Genome Project has the potential to provide substantial benefits to clinical medicine, including the development of panels of genetic markers for the assessment of disease risk[1] One approach to this goal is to evaluate selected polymorphisms of genes that are potentially associated with a disease, either because they are known to encode proteins related to the disease process or because they are located within chromosomal regions shown to be in linkage disequilibrium with the disease.

Coronary heart disease (CHD) and stroke are complex multifactorial disorders that are thought to result from an interaction between a person's genetic background and various environmental factors. CHD is the single largest killer of men and women in the United States. The total numbers of individuals affected by CHD or by myocardial infarction (MI) in the United States in 2003 were 13.2 million and 7.2 million, respectively. Despite recent advances in therapy for these conditions, nearly 480,000 and 170,000 patients die annually from CHD or MI, respectively[2] In Japan, the total number of individuals with CHD is 0.9 million, and nearly 50,000 people die annually from MI (Ministry of Health, Labor, and Welfare of Japan). Disease prevention is an important strategy for reducing the overall burden of CHD and MI, and the identification of biomarkers for disease risk is the key, both for risk prediction and for potential intervention to reduce the chance of future events.

Stroke is a common and serious disease, with about 700,000 individuals suffering a new or recurrent stroke and nearly 158,000 deaths from stroke-related causes in 2003 in the United States. The prevalence of stroke in the United States is 5.4 million. Of all such events, 88% are ischemic stroke, 9% are intracerebral hemorrhage, and 3% are subarachnoid hemorrhage[2] In Japan, the prevalence of stroke is 1.4 million (62% ischemic stroke, 23% intracerebral hemorrhage, 11% subarachnoid hemorrhage), with nearly 132,000 deaths from this condition each year (Ministry of Health, Labor, and Welfare of Japan). Despite recent advances in acute stroke therapy, stroke remains the leading cause of severe disability and the third leading cause of death, after heart disease and cancer, in Western countries and Japan[3] As for heart disease, the identification of the biomarkers of stroke is important both for risk prediction and for intervention to avert future events.

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Recent Advances in Genetics of Cardiovascular Diseases and Stroke

Several whole-genome linkage analyses of families or sib-pairs[4–7] and various association studies of unrelated individuals[8–17] have identified chromosomal loci linked to or genetic variations that confer susceptibility to CHD or MI. The identified genes include those for integrin, β-3 (*ITGB3*),[8] coagulation factor VII (*F7*),[9] cholesterol ester transfer protein, plasma (*CETP*),[10] gap junction protein, α-4 (*GJA4*), plasminogen activator inhibitor 1 (*PAI1*), matrix metalloproteinase 3 (*MMP3*),[11] lymphotoxin-α (*LTA*),[12] lectin, galactoside-binding, soluble, 2 (*LGALS2*),[13] palladin, mouse, homolog of (*KIAA0992*), v-Ros avian UR2 sarcoma virus oncogene homolog 1 (*ROS1*), taste receptor, type 2, member 50 (*TAS2R50*),[14] arachidonate 5-lipoxygenase-activating protein (*ALOX5AP*),[15] prostaglandin-endoperoxide synthase 2 (*PTGS2*),[16] and leukotriene A4 hydrolase (*LTA4H*).[17] Genetic linkage analysis and candidate gene association studies have also implicated a locus (5q12)[18] and several candidate genes, including those for phosphodiesterase 4D, cAMP-specific (*PDE4D*),[19] *ALOX5AP*[15] and *PTGS2*,[16] in predisposition to stroke. However, the genetic components of MI or those of ischemic or hemorrhagic stroke have not been determined definitively.

Through screening of 65,671 single nucleotide polymorphisms (SNPs), Ozaki et al[12] found that polymorphisms of *LTA* were associated with susceptibility to MI in a large-scale association study with 1,133 subjects with MI and 1,878 controls. In vitro functional analysis indicated that the *A* allele of the 804C→A (Thr26Asn) polymorphism of *LTA* was associated with increased expression of genes for vascular cell adhesion molecule 1 and selectin E, and that the *G* allele of the 252A→G polymorphism was associated with an increase in the transcriptional activity of *LTA*. These researchers suggested that variants of *LTA* are risk factors for MI, implicating this gene in the pathogenesis. They subsequently showed that the 3279C→T polymorphism of *LGALS2* was associated with the prevalence of MI. LGALS2 plays a role in the secretion of LTA from smooth muscle cells and macrophages, and the identified polymorphism affects the transcriptional activity of *LGALS2*.[13] These results suggested that an LGALS2–LTA axis is important in the pathophysiology of coronary atherosclerosis and MI.

Since 1994, my colleagues and I have been studying the genetic epidemiology of common complex diseases, including cardiovascular diseases, stroke, and osteoporosis. In an association study of MI with a total of 5,061 individuals, including 2,819 subjects with MI and 2,242 controls, we detected 10 polymorphisms in men and 5 in women that were associated with the prevalence of MI.[11] Among these polymorphisms, the 1019C→T (Pro319Ser) polymorphism of *GJA4* was associated with MI in men, with the *T* allele representing a risk factor for this condition. The 242C→T (His72Tyr) polymorphism of the gene for cytochrome b(–245), α subunit (*CYBA*), was also associated with MI in men, with the *T* allele protecting against this condition. The –668/4G→5G polymorphism of *PAI1* and the –1171/5A→6A polymorphism of *MMP3* were associated with MI in women, with the *5G* allele and the *6A* allele, respectively, representing risk factors for this condition.

In a study of restenosis after percutaneous coronary intervention, we examined 2,391 coronary lesions, including 1,390 lesions resulting from plain old balloon angioplasty (POBA) and 1,001 lesions resulting from stent implantation.[20] The *G* allele of the 1648A→G (Lys505Glu) polymorphism of the integrin, α-2, gene (*ITGA2*) protected against restenosis after POBA in men, whereas the *T* allele of the 825C→T (splice variant) polymorphism of the guanine nucleotide-binding protein, β-3, gene (*GNB3*) was a risk factor for this condition in men. The *A* allele of the –863C→A polymorphism of the tumor necrosis factor gene (*TNF*) was a risk factor for in-stent restenosis in men. The *A* allele of the 2445G→A (Ala54Thr) polymorphism of the fatty acid-binding protein 2 gene (*FABP2*) was a risk factor for restenosis after POBA in women, whereas the *5G* allele of the –668/4G→5G polymorphism of *PAI1* was a risk factor for restenosis after either POBA or stent implantation (as it also was for MI) in women.

In a study of coronary artery spasm with 2,188 individuals, including 593 subjects with coronary artery spasm and 1,595 controls, we found that the 242C→T (His72Tyr) polymorphism of *CYBA* was associated with coronary artery spasm (as it was with MI) in men.[21] The –1171/5A→6A polymorphism of *MMP3* was associated with coronary artery spasm (as it was with MI) in women. The *G* allele of the –634C→G polymorphism of the interleukin 6 gene (*IL6*) was also a risk factor for coronary artery spasm in women. The fact that polymorphisms in *CYBA* and *MMP3* were associated with both coronary artery spasm and MI suggests that the former plays an important role in the etiology of the latter in the Japanese population.

In a study of 1,940 individuals, including 1,067 subjects with hypertension and 873 controls, we found that the 825C→T (splice variant) polymorphism of *GNB3* and the 190G→A (Val64Ile) polymorphism of the chemokine, CC motif, receptor 2, gene (*CCR2*) were associated with hypertension in men.[22] The –238G→A polymorphism of *TNF* was associated with this condition in women.

We also examined the relationship of gene polymorphisms to the prevalence of CHD among men or women with or without hypertension, diabetes mellitus, or hyperlipidemia.[23–26] In men at low risk (without hypertension, diabetes mellitus, or hypercholesterolemia), the –219G→T polymorphism of the apolipoprotein E gene (*APOE*) and the 190G→A (Val64Ile) polymorphism of *CCR2* were associated with CHD; in men at high risk (with hypertension, diabetes mellitus, and hypercholesterolemia), the 1019C→T (Pro319Ser) polymorphism of *GJA4* and the –592A→C polymorphism of the interleukin 10 gene (*IL10*) were associated with this condition. In women at low risk, the –1171/5A→6A polymorphism of *MMP3* and the 1018C→T (Thr145Met) polymorphism of glycoprotein Ib, platelet, α polypeptide, gene (*GP1BA*) were associated with CHD, whereas the 3932T→C (Cys112Arg) polymorphism of *APOE* and the 561A→C (Ser128Arg) polymorphism of the gene for selectin E (*SELE*) were associated with this condition in women at high risk.[23] In men with hypertension, the 1186G→C (Ala387Pro) polymorphism of the thrombospondin IV gene (*THBS4*) was associated with CHD, whereas, in men without hypertension, the –219G→T polymorphism of *APOE* and the 190G→A (Val64Ile) polymorphism of *CCR2* were associated with this condition. In women with hypertension, the –482C→T polymorphism of the apolipoprotein C-III gene (*APOC3*) was associated with CHD, whereas the –1171/5A→6A polymorphism of *MMP3* was associated with this condition in women without hypertension.[24] In men with type 2 diabetes

Table 1 Multivariate Logistic Regression Analysis of MI With Adjustment for Age, Sex, BMI, and the Prevalence of Smoking, Hypertension, Diabetes Mellitus, and Hypercholesterolemia

Variable	*p value*	*OR (95%CI)*
Conventional risk factors		
Sex (male)	*<0.0001*	*5.37 (4.45–6.51)*
Hypercholesterolemia	*<0.0001*	*3.16 (2.66–3.76)*
Diabetes mellitus	*<0.0001*	*2.62 (2.20–3.13)*
Hypertension	*<0.0001*	*2.20 (1.84–2.63)*
Genetic factors		
FABP2 (AA + GA vs GG)	*0.0006*	*1.34 (1.13–1.59)*
AKAP10 (GG vs AA + AG)	*0.0007*	*1.93 (1.32–2.83)*
IPF1 (4G4G + 3G4G vs 3G3G)	*0.0008*	*0.72 (0.59–0.87)*
GP1BA (TT + CT vs CC)	*0.0018*	*1.36 (1.12–1.65)*
MTHFR (TT vs CC + CT)	*0.0019*	*1.40 (1.13–1.73)*
ACDC (GG + CG vs CC)	*0.0094*	*1.25 (1.06–1.47)*
F7 (AA + GA vs GG)	*0.0096*	*0.70 (0.54–0.92)*
TNF (AA + CA vs CC)	*0.0103*	*0.78 (0.65–0.94)*
LPL (GG + CG vs CC)	*0.0181*	*0.79 (0.64–0.96)*
TNFSF4 (GG + AG vs AA)	*0.0252*	*1.26 (1.03–1.53)*
AGER (AA vs GG + GA)	*0.0292*	*0.50 (0.26–0.91)*
PAX4 (TT + CT vs CC)	*0.0313*	*0.65 (0.44–0.96)*
TNF (TT vs CC + CT)	*0.0371*	*1.60 (1.03–2.49)*
CETP (AA vs CC + CA)	*0.0450*	*1.20 (1.00–1.44)*

MI, myocardial infarction; BMI, body mass index; OR, odds ratio; CI, confidence interval.

mellitus, the 1019C→T (Pro319Ser) polymorphism of *GJA4* was associated with CHD; in men without type 2 diabetes mellitus, the –863C→A polymorphism of *TNF*, the –219G→T polymorphism of *APOE*, and the 1019C→T (Pro319Ser) polymorphism of *GJA4* were associated with this condition. In women with type 2 diabetes mellitus, the 2445G→A (Ala54Thr) polymorphism of *FABP2* was associated with CHD, whereas, in women without type 2 diabetes mellitus, the –482C→T polymorphism of *APOC3* was associated with this condition[25] In men with hypercholesterolemia, the 994G→T (Val279Phe) polymorphism of the phospholipase A2, group VII, gene (*PLA2G7*), the 242C→T (His72Tyr) polymorphism of *CYBA*, and the 1100C→T polymorphism of *APOC3* were associated with CHD; in men without hypercholesterolemia, the –863C→A polymorphism of *TNF* was associated with this condition. Finally, in women with hypercholesterolemia, the 1019C→T (Pro319Ser) polymorphism of *GJA4* was associated with CHD, whereas the –260C→T polymorphism of the monocyte differentiation antigen CD14 gene (*CD14*) was associated with this condition in women without hypercholesterolemia[26] These observations suggest that polymorphisms associated with CHD may differ among men and women or among individuals with different risk factors. Stratification of subjects on the basis of conventional risk factors may thus be important in order to achieve personalized prevention of CHD or MI with the use of genetic information.

The results of these genetic association studies raise 2 important questions: (1) Why did the polymorphisms associated with MI or CHD differ between men and women? Although the molecular mechanisms responsible for these differences remain unclear, there are several possibilities. A role for genetic factors in the greater risk of mortality from CHD in women aged <65 years than in men of the same age was demonstrated in a study of Swedish twins[27] This observation suggests that genetic factors may play a more important role in the development of MI in women aged <65 years than in similarly aged men. Given that, in general, the total risk for CHD and MI in women lags behind that in men by approximately 10 years, the mechanisms underlying the risk for these conditions in women may differ from those in men at each age. The sex difference in the association of polymorphisms with MI may be attributable, at least in part, to the difference in estrogen–estrogen receptor signaling between men and women[28] given that estrogen exerts various favorable effects on vascular wall and vasomotor function, including stimulation of the production of nitric oxide and prostaglandin I_2 as well as inhibition of the release of endothelin-1 by vascular endothelial cells[29] Furthermore, given that the polymorphisms examined in our studies likely represent only a small proportion of those potentially associated with MI, it remains possible that further investigation will uncover polymorphisms that are associated with MI in both men and women. (2) Why did SNPs associated with CHD differ among subjects with different conventional coronary risk factors? Given that the effects of single polymorphisms on the development of CHD are small, the association between a polymorphism and CHD might be influenced by age, sex, or the presence of conventional risk factors including hypertension, diabetes mellitus, and hyperlipidemia. Furthermore, given that conventional coronary risk factors themselves may have genetic components, there may be interactions between genes related to CHD and those related to conventional risk factors.

Genetic Epidemiological Study in Gifu and Aomori Prefectures

In 2002, we began another genetic epidemiological study to identify gene polymorphisms that confer susceptibility to ischemic or hemorrhagic stroke, MI, hypertension, instent restenosis, type 2 diabetes mellitus, metabolic syndrome, or obesity in a total of 5,000 residents of Gifu and Aomori Prefectures. We selected 152 candidate genes that might be expected to be associated with these various conditions on the basis of a comprehensive overview of vascular biology (from the viewpoint of hypertension, atherosclerosis, arterial spasm, or arterial aneurysm), platelet function, leukocyte, lymphocyte, and monocytes–macrophage biology, coagulation and fibrinolysis cascades, neurological factors (from the viewpoint of regulation of the circulation, blood pressure, or endocrine function), as well as lipid and adipose tissue metabolism, insulin and glucose metabolism, the function of pancreatic β-cells, peripheral insulin sensitivity, hepatic glucose production, homocysteine metabolism, and other metabolic factors. We further selected 202 polymorphisms of these genes (mostly located in the promoter region, exons, or splice donor or acceptor sites of introns) that might be expected to result in changes in function or expression of the encoded protein[30] Genotypes of the 202 polymorphisms were determined by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with the use of suspension array technology[31] Determination of a total of approximately 1 million genotypes for these polymorphisms has already been completed. We have examined the relationship of these 202 polymorphisms in the 152 candidate genes to the selected disorders and have identified various polymorphisms that confer susceptibility to each disease. Furthermore, we have begun a whole-genome association study for atherothrombotic cerebral infarction with microarray chips that include 500,000 SNPs evenly

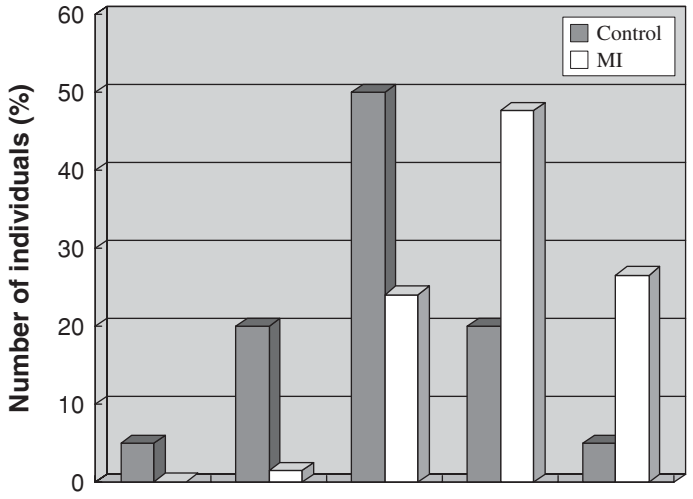

Fig 1. Prediction of the risk for myocardial infarction (MI).

Factor (odds ratio)	No	Yes
Conventional risk factor		
Hypercholesterolemia (3.16)	1	**3.16**
Diabetes mellitus (2.62)	1	**2.62**
Hypertension (2.20)	1	**2.20**
Smoking (1.71)	1	**1.71**
Genetic factor		
FABP2 (1.34)	1	**1.34**
AKAP10 (1.93)	**1**	1.93
IPF1 (0.72)	**1**	0.72
GP1BA (1.36)	1	**1.36**
MTHFR (1.40)	**1**	1.40
ACDC (1.25)	1	**1.25**
F7 (0.70)	**1**	0.70
TNF (0.78)	**1**	0.78
LPL (0.79)	**1**	0.79
TNFSF4 (1.26)	1	**1.26**
AGER (0.50)	**1**	0.50
PAX4 (0.65)	1	**0.65**
TNF (1.60)	**1**	1.60
CETP (1.20)	1	**1.20**

Prediction probability **70%**

Improve-ment of lifestyle

Medical treatment

Factor (odds ratio)	No	Yes
Conventional risk factor		
Hypercholesterolemia (3.16)	**1**	3.16
Diabetes mellitus (2.62)	**1**	2.62
Hypertension (2.20)	**1**	2.20
Smoking (1.71)	**1**	1.71
Genetic factor		
FABP2 (1.34)	1	**1.34**
AKAP10 (1.93)	**1**	1.93
IPF1 (0.72)	**1**	0.72
GP1BA (1.36)	1	**1.36**
MTHFR (1.40)	**1**	1.40
ACDC (1.25)	1	**1.25**
F7 (0.70)	**1**	0.70
TNF (0.78)	**1**	0.78
LPL (0.79)	**1**	0.79
TNFSF4 (1.26)	1	**1.26**
AGER (0.50)	**1**	0.50
PAX4 (0.65)	1	**0.65**
TNF (1.60)	**1**	1.60
CETP (1.20)	1	**1.20**

Prediction probability **15%**

Fig 2. Personalized prevention of myocardial infarction. Conventional and genetic risk factors exhibited by the hypothetical patient are shown in bold.

distributed throughout the entire genome. The goal of the Gifu–Aomori study is to develop personalized prevention systems for stroke, MI, hypertension, in-stent restenosis, type 2 diabetes mellitus, metabolic syndrome, and obesity, and thereby to contribute to primary, personalized prevention of these conditions. This approach has the potential to improve the health and extend the healthy life expectancy of Japanese individuals.

Prediction of the Genetic Risk for MI

We have studied the relationship of polymorphisms to the prevalence of MI in a total of 3,483 Japanese individuals (1,913 men, 1,570 women), including 1,192 subjects (926 men, 266 women) with MI and 2,291 controls (987 men, 1,304 women).[32] Multivariable logistic regression analysis of MI with adjustment for age, sex, body mass index (BMI), and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia revealed that 14 polymorphisms were related ($p<0.05$) to the prevalence of MI (Table 1). The prediction probability, which reflects an individual's susceptibility to MI, was calculated from the results of the multivariable logistic regression analysis (Fig 1). We divided control individuals into 5 groups on the basis of the risk level determined by the prediction probability: 5% in the lowest risk group, the next 20% in the relatively low risk group, the next 50% in the average risk group, the next 20% in the relatively high risk group, and the last 5% in the highest risk group. The subjects with MI were then divided into these risk groups on the basis of their prediction probabilities. The distribution of subjects with MI shifted to the right, indicating that these individuals are at higher risk than are controls. For prevention of MI, it is thus important to reduce the risk level, especially

Table 2 Multivariate Logistic Regression Analysis of Atherothrombotic Cerebral Infarction With Adjustment for Age, Sex, BMI, and the Prevalence of Smoking, Hypertension, Diabetes Mellitus, and Hypercholesterolemia

Variable	*p value*	*OR (95%CI)*
Conventional risk factors		
Sex (male)	*<0.0001*	*1.94 (1.58–2.39)*
Hypertension	*<0.0001*	*2.58 (2.10–3.18)*
Diabetes mellitus	*<0.0001*	*1.85 (1.49–2.30)*
Genetic factors		
IL6 (CC vs GG + GC)	*0.0006*	*1.41 (1.16–1.73)*
MTHFR (TT vs CC + CT)	*0.0020*	*1.48 (1.15–1.89)*
IPF1 (4G4G + 3G4G vs 3G3G)	*0.0019*	*0.70 (0.57–0.88)*
TNFSF4 (GG vs AA + AG)	*0.0050*	*2.81 (1.35–5.77)*
ITGB2 (TT + CT vs CC)	*0.0137*	*1.27 (1.05–1.54)*
THBS2 (GG + TG vs TT)	*0.0419*	*1.29 (1.01–1.64)*
MMP12 (GG + AG vs AA)	*0.0450*	*1.57 (1.00–2.43)*
ANXA5 (TT vs CC + TC)	*0.0514*	*0.13 (0.01–0.66)*

Abbreviations see in Table 1.

in individuals at relatively high or high risk. Simulation of the personalized prevention of MI is shown in Fig 2. The patient has hypercholesterolemia, diabetes mellitus, and hypertension and is a smoker with the indicated genotypes for the 14 polymorphisms related to MI. The prediction probability of the patient is 70%, which is in the high-risk range for predisposition to MI. With counseling on how to reduce this risk and if the patient stops smoking and is treated for hypercholesterolemia, diabetes mellitus, and hypertension, the prediction probability would be reduced to 15%, which is in the average-risk range. The genetic factors cannot be changed. The effects of conventional risk factors do not necessarily disappear immediately even if they are eliminated. However, this system may simulate the effects of improvement in lifestyle and medical treatment and thereby contribute to the personalized prevention of MI.

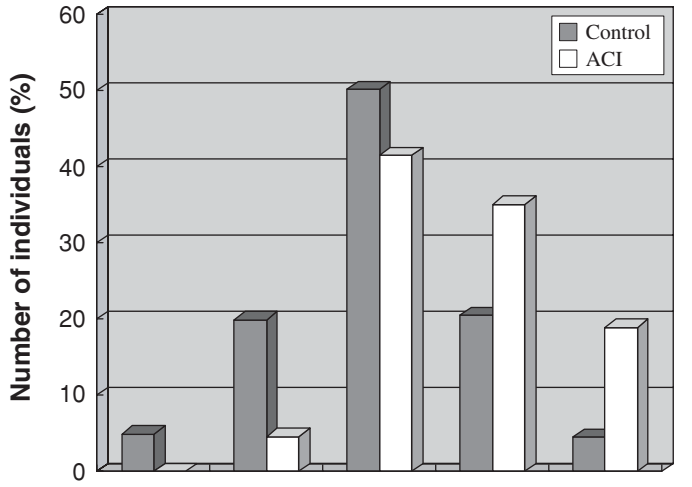

Fig 3. Prediction of the risk for atherothrombotic cerebral infarction (ACI).

Factor (odds ratio)	**No**	**Yes**
Conventional risk factor		
Hypertension (2.58)	1	**2.58**
Diabetes mellitus (1.85)	1	**1.85**
Smoking (1.80)	1	**1.80**
Genetic factor		
IL6 (1.41)	1	**1.41**
MTHFR (1.48)	**1**	1.48
IPF1 (0.70)	**1**	0.70
TNFSF4 (2.81)	1	**2.81**
ITGB2 (1.27)	**1**	1.27
THBS2 (1.29)	1	**1.29**
MMP12 (1.57)	**1**	1.57
ANXA5 (0.13)	**1**	0.13

Prediction probability **43%**

Improve-ment of lifestyle

Medical treatment

Factor (odds ratio)	**No**	**Yes**
Conventional risk factor		
Hypertension (2.58)	**1**	2.58
Diabetes mellitus (1.85)	**1**	1.85
Smoking (1.80)	**1**	1.80
Genetic factor		
IL6 (1.41)	1	**1.41**
MTHFR (1.48)	**1**	1.48
IPF1 (0.70)	**1**	0.70
TNFSF4 (2.81)	1	**2.81**
ITGB2 (1.27)	**1**	1.27
THBS2 (1.29)	1	**1.29**
MMP12 (1.57)	**1**	1.57
ANXA5 (0.13)	**1**	0.13

Prediction probability **8%**

Fig 4. Personalized prevention of atherothrombotic cerebral infarction. Conventional and genetic risk factors exhibited by the hypothetical patient are shown in bold.

Prediction of the Genetic Risk for Ischemic or Hemorrhagic Stroke

We have studied the relationship of polymorphisms to the prevalence of stroke in a total of 3,151 Japanese individuals: 1,141 stroke patients (636 with atherothrombotic cerebral infarction, 282 with intracerebral hemorrhage, and 223 with subarachnoid hemorrhage) and 2,010 controls.[30] Multivariate logistic regression analysis of atherothrombotic cerebral infarction with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia revealed that 8 polymorphisms were related to the prevalence of this condition (Table 2). Calculation of the prediction probability for atherothrombotic cerebral infarction revealed that, compared with the distribution of control individuals, the distribution of subjects with this condition shifted to the right (Fig 3), showing that subjects with atherothrombotic cerebral infarction have a higher risk level than do controls. Simulation of the personalized prevention of atherothrom-

Table 3 Multivariate Logistic Regression Analysis of Intracerebral Hemorrhage With Adjustment for Age, Sex, BMI, and the Prevalence of Smoking, Hypertension, Diabetes Mellitus, and Hypercholesterolemia

Variable	*p value*	*OR (95%CI)*
Conventional risk factors		
Sex (male)	*<0.0001*	*1.97 (1.48–2.62)*
Hypertension	*<0.0001*	*2.42 (1.83–3.20)*
Hypercholesterolemia	*<0.0001*	*0.46 (0.33–0.65)*
Genetic factors		
IL6 (CC vs GG + GC)	*0.0014*	*1.56 (1.19–2.06)*
TNF (AA + CA vs CC)	*0.0041*	*0.64 (0.47–0.86)*
CD14 (TT + CT vs CC)	*0.0051*	*1.62 (1.17–2.29)*
FBN1 (CC + TC vs TT)	*0.0117*	*1.47 (1.09–1.99)*
PECAM1 (GG + CG vs CC)	*0.0169*	*1.49 (1.08–2.09)*
UCP1 (CC + AC vs AA)	*0.0182*	*0.61 (0.40–0.91)*
CPB2 (AA vs GG + GA)	*0.0318*	*0.48 (0.23–0.89)*
LIPC (AA + GA vs GG)	*0.0330*	*1.43 (1.04–2.01)*
CCL5 (GG vs CC + CG)	*0.0460*	*0.13 (0.01–0.62)*

Abbreviations see in Table 1.

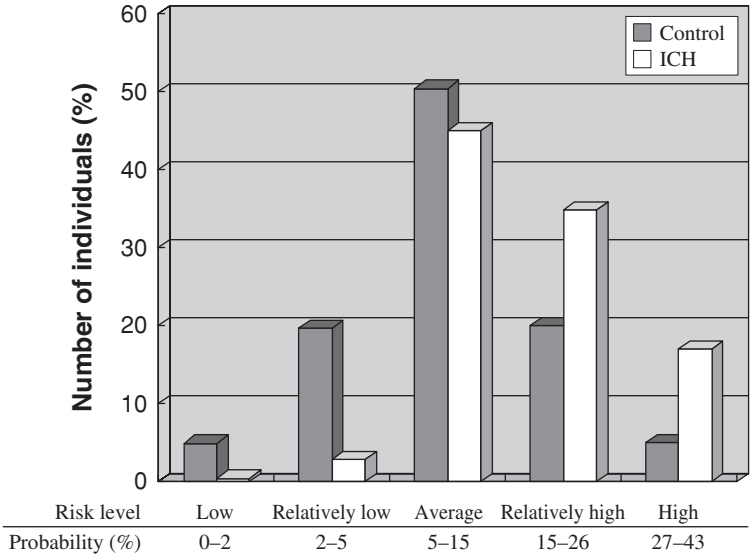

Fig 5. Prediction of the risk for intracerebral hemorrhage (ICH).

Factor (odds ratio)	No	Yes
Conventional risk factor		
Hypertension (2.42)	1	**2.42**
Hypercholesterolemia (0.46)	**1**	0.46
Genetic factor		
IL6 (1.56)	1	**1.56**
TNF (0.64)	**1**	0.64
CD14 (1.62)	1	**1.62**
FBN1 (1.47)	1	**1.47**
PECAM1 (1.49)	**1**	1.49
UCP1 (0.61)	**1**	0.61
CPB2 (0.48)	**1**	0.48
LIPC (1.43)	1	**1.43**
CCL5 (0.13)	**1**	0.13

Prediction probability **22%**

Improvement of lifestyle

Medical treatment

Factor (odds ratio)	No	Yes
Conventional risk factor		
Hypertension (2.42)	**1**	2.42
Hypercholesterolemia (0.46)	**1**	0.46
Genetic factor		
IL6 (1.56)	1	**1.56**
TNF (0.64)	**1**	0.64
CD14 (1.62)	1	**1.62**
FBN1 (1.47)	1	**1.47**
PECAM1 (1.49)	**1**	1.49
UCP1 (0.61)	**1**	0.61
CPB2 (0.48)	**1**	0.48
LIPC (1.43)	1	**1.43**
CCL5 (0.13)	**1**	0.13

Prediction probability **9%**

Fig 6. Personalized prevention of intracerebral hemorrhage. Conventional and genetic risk factors exhibited by the hypothetical patient are shown in bold.

Table 4 Multivariate Logistic Regression Analysis of Subarachnoid Hemorrhage With Adjustment for Age, Sex, BMI, and the Prevalence of Smoking, Hypertension, Diabetes Mellitus, and Hypercholesterolemia

Variable	*p value*	*OR (95%CI)*
Conventional risk factors		
Hypercholesterolemia	*0.0017*	*0.55 (0.37–0.79)*
Smoking	*0.0050*	*1.74 (1.18–2.55)*
Sex (male)	*0.0079*	*0.64 (0.46–0.89)*
Hypertension	*0.0092*	*1.53 (1.11–2.10)*
Genetic factors		
PKD1-like (AA + GA vs GG)	*0.0005*	*5.78 (2.07–15.01)*
TNF (AA + CA vs CC)	*0.0006*	*1.69 (1.25–2.28)*
CAPN10 (AA + GA vs GG)	*0.0014*	*1.87 (1.26–2.73)*
MTHFR (TT vs CC + CT)	*0.0050*	*1.68 (1.16–2.41)*
UCP3 (TT + CT vs CC)	*0.0066*	*1.51 (1.12–2.05)*
OLR1 (CC + GC vs GG)	*0.0083*	*0.65 (0.47–0.89)*
PAX4 (TT + CT vs CC)	*0.0144*	*0.23 (0.06–0.63)*
TGFBR2 (TT + CT vs CC)	*0.0213*	*0.71 (0.52–0.95)*
IL10 (CC vs AA + AC)	*0.0313*	*0.53 (0.28–0.91)*
CCL5 (AA vs GG + GA)	*0.0382*	*0.59 (0.35–0.95)*

Abbreviations see in Table 1.

botic cerebral infarction is shown in Fig 4. The patient has hypertension and diabetes mellitus and is a smoker with the indicated genotypes for the 8 polymorphisms related to atherothrombotic cerebral infarction. The prediction probability of the patient is 43%, which is in the relatively high-risk range for predisposition to this condition. With counseling on how to reduce this risk and if the patient stops smoking and is treated for hypertension and diabetes mellitus, the prediction probability would be reduced to 8%, which is in the relatively low-risk range.

Multivariate logistic regression analysis of intracerebral hemorrhage with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia revealed that 9 polymorphisms were related to the prevalence of this condition (Table 3). Calculation of the prediction probability revealed that the distribution of subjects with intracerebral hemorrhage shifted to the right compared with that of controls (Fig 5), indicating that subjects with intracerebral hemorrhage have a higher risk level than do control individuals. Simulation of the personalized prevention of intracerebral hemorrhage is shown in Fig 6. The patient has hypertension and the indicated genotypes for the 9 polymorphisms related to intracerebral hemorrhage. The prediction probability of the patient is 22%, which is in the relatively high-risk range for predisposition to this condition. With counseling on how to reduce this risk and if the patient is treated for hypertension, the prediction probability would be reduced to 9%, which is in the average-risk range.

Finally, multivariate logistic regression analysis of subarachnoid hemorrhage with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia revealed that 10 polymorphisms were related to the prevalence of this condition (Table 4). Again, calculation of the prediction probability revealed that the distribution of subjects with subarachnoid hemorrhage shifted to the right compared with that of controls (Fig 7), showing that subjects with subarachnoid hemorrhage have a higher risk level than do control individuals. Simulation of the personalized prevention of subarachnoid hemorrhage is shown in Fig 8. The patient has hypertension and is a smoker with the indicated genotypes for the 10 polymorphisms related to subarachnoid hemorrhage. The prediction probability of the patient is 35%, which is in the high-risk range for predisposition to this condition. With counseling on how to reduce this risk and if the patient stops smoking and is treated for hypertension, the prediction probability would be reduced to 17%, which is in the relatively high risk range.

Development of a Personalized Prevention System for MI and Stroke

A simulated personalized prevention system for MI and stroke is shown in Fig 9. Conventional risk factors are evaluated by laboratory examination. In addition, genetic factors are evaluated by genotyping of various polymorphisms. The risk diagnosis system predicts the future risk for MI or each type of stroke in each individual by calculating the prediction probability from the results of both laboratory examination and genetic analysis. Furthermore, this system predicts how the risk level will decrease if conventional and treatable risk factors, including hypertension, diabetes

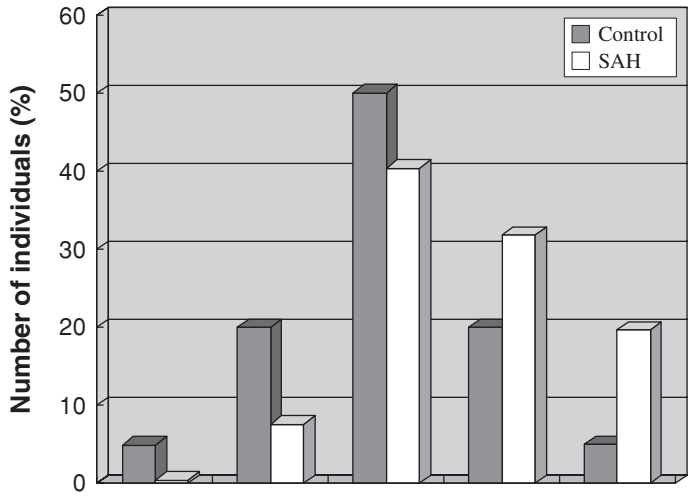

Fig 7. Prediction of the risk for subarachnoid hemorrhage (SAH).

Factor (odds ratio)	No	Yes
Conventional risk factor		
Hypercholesterolemia (0.55)	**1**	0.55
Smoking (1.74)	1	**1.74**
Hypertension (1.53)	1	**1.53**
Genetic factor		
PKD1-like (5.78)	1	**5.78**
TNF (1.69)	1	**1.69**
CAPN10 (1.87)	**1**	1.87
MTHFR (1.68)	**1**	1.68
UCP3 (1.51)	1	**1.51**
OLR1 (0.65)	**1**	0.65
PAX4 (0.23)	**1**	0.23
TGFBR2 (0.71)	**1**	0.71
IL10 (0.53)	**1**	0.53
CCL5 (0.59)	**1**	0.59

Prediction probability **35%**

Improve-ment of lifestyle

Medical treatment

Factor (odds ratio)	No	Yes
Conventional risk factor		
Hypercholesterolemia (0.55)	**1**	0.55
Smoking (1.74)	**1**	1.74
Hypertension (1.53)	**1**	1.53
Genetic factor		
PKD1-like (5.78)	1	**5.78**
TNF (1.69)	1	**1.69**
CAPN10 (1.87)	**1**	1.87
MTHFR (1.68)	**1**	1.68
UCP3 (1.51)	1	**1.51**
OLR1 (0.65)	**1**	0.65
PAX4 (0.23)	**1**	0.23
TGFBR2 (0.71)	**1**	0.71
IL10 (0.53)	**1**	0.53
CCL5 (0.59)	**1**	0.59

Prediction probability **17%**

Fig 8. Personalized prevention of subarachnoid hemorrhage. Conventional and genetic risk factors exhibited by the hypothetical patient are shown in bold.

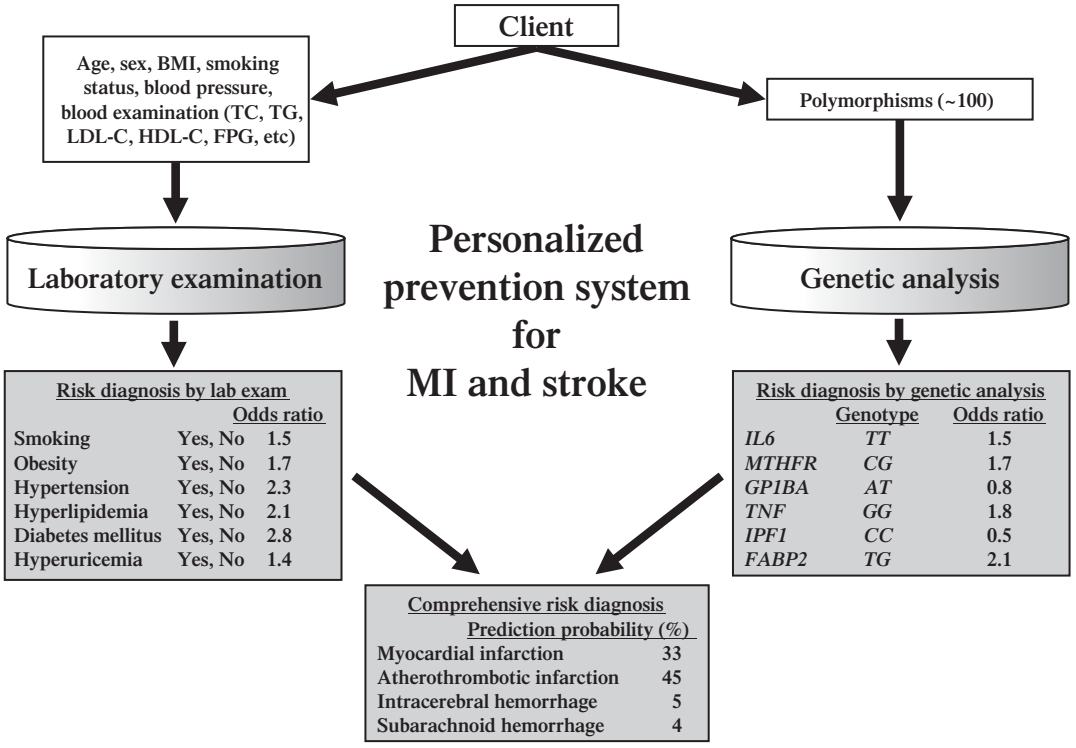

Fig 9. Simulated personalized prevention system for myocardial infarction and stroke. BMI, body mass index; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose.

mellitus, hyperlipidemia, and smoking, are reduced or eliminated.

Conclusion

Various gene polymorphisms that confer susceptibility to MI or stroke have been identified. Genotyping of these polymorphisms may prove informative for prediction of the genetic risk for MI or stroke, and thereby contribute to personalized prevention of these conditions. Given that the polymorphisms examined are likely represent only a small proportion of those potentially associated with MI or stroke, further comprehensive analyses, such as whole-genome association studies, will be required to identify susceptibility genes for these conditions. Validation of findings of association between polymorphisms and MI or stroke will

require their replication with independent subject panels before clinical application. Haplotype and diplotype analyses will be required to confirm the association of a gene with the development of each disease. It will also be important to determine the role of each polymorphism in disease pathogenesis by examining its effect on gene transcription or protein structure or function. Clarification of ethnic divergence of gene polymorphisms associated with MI or stroke will be facilitated by international collaborations.

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