Genetic factors for lone atrial fibrillation

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Abstract. Atrial fibrillation (AF) may result from an electric conduction disturbance, increased hemodynamic stress, ischemia, inflammation, or remodeling in atria. Although genetic epidemiological studies have identified several genetic variants as risk factors for AF, the genetic determinants of this condition remain largely unknown. The purpose of the present study was to identify gene polymorphisms that confer susceptibility to lone AF. The study population comprised 1069 unrelated Japanese individuals, including 196 subjects with chronic lone AF and 873 controls. The genotypes for 40 polymorphisms of 32 candidate genes were determined by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. Multivariable logistic regression analysis with adjustment for age, sex, body mass index, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia as well as a stepwise forward selection procedure revealed that the -1306C→T polymorphism of the matrix metalloproteinase 2 gene *(MMP2)* and the -592A→C polymorphism of the interleukin 10 gene *(IL10)* were significantly (false discovery rate of <0.05) associated with the prevalence of AF. The *T* allele of the *MMP2* polymorphism and the *C* allele of the *IL10* polymorphism were a risk factor for and protective factor against AF, respectively. Determination of the genotypes for these polymorphisms may thus prove informative for assessment of the genetic component of AF.

Introduction

Atrial fibrillation (AF) is the most common type of arrhythmia associated with an unfavorable outcome, particularly stroke. The prevalence of AF in the US is >2.2 million (1). AF is responsible for ~15 to 20% of all strokes and is an independent risk factor for stroke, increasing the risk ~5-fold in the US. Given the importance of prevention of stroke, the identification of markers for AF risk is the key, both for risk prediction and for potential intervention to avert cardiogenic embolic stroke.

About 15-30% of AF patients have no underlying disease, with their condition being referred to as lone AF. Idiopathic forms of disease are traditionally not considered genetic in origin. In recent years, however, research attention has focused on the genetic aspects of AF (2) and several studies have shown that familial AF is more common than previously recognized (3-5). Of the 914 patients with AF, 36% had lone AF and a family history of AF was present in 15% of these patients with lone AF (4). In addition, genetic linkage analysis and candidate gene association studies have implicated several loci (10q22-24, 6q14-16, and 5p13) (3,6,7) and candidate genes, including *KCNE1* (8), *KCNE5* (9), and those for connexin 40 (10) and angiotensinogen (11), in predisposition to lone AF. The genetic determinants of lone AF, however, remain largely unknown.

We performed an association study for 40 polymorphisms of 32 candidate genes and lone AF in 1069 Japanese individuals. The purpose of the present study was to identify gene polymorphisms that confer susceptibility to lone AF and thereby to contribute to the personalized prevention of this condition.

Materials and methods

Study population. The subjects with AF comprised 196 consecutive unrelated Japanese individuals (153 men, 43 women) who visited the participating hospitals (Gifu Prefectural Tajimi Hospital, Gifu Prefectural Gifu Hospital, and Hirosaki University Hospital) between October 2002 and March 2005 and who were diagnosed with persistent AF in the absence of a history of structural heart disease, including congestive heart failure, coronary heart disease, or valvular heart disease such as mitral, aortic, tricuspid, or pulmonary stenosis or regurgitation. Structural heart disease was evaluated by measurement of biochemical markers, electrocardiography, examination of

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Table I. Primers, probes, and other PCR conditions for genotyping.

Gene	Polymorphism	Sense primer	Antisense primer
IL10	-592A→C	gTggAAACATgTgCCTgAgAATC	TAAATATCCTCAAAgTTCCCAAgC
IL10	-819T→C	TgTgCTggAgATggTgTACAgT	ATgCTAgTCAggTAgTgCTCAC
BCHE	1615G→A (Ala539Thr)	TACAACTTATTCCATATTTTACAggA	TgTAATTgTTCCAgCgATggAATC
CETP	1061A→G (Ile405Val)	gCTCCAgggAggACTCACCA	gATgCCCACAgCggTgATCAT
SLC26A8	A→G (Ile639Val)	CCAgATTCTTTACACAgAgCgAT	gCAgTTTggCTTgTgTTCATgCT
MMP2	-1306C→T	TCTgggCCATTgTCAATgTTCC	gTgACTTCTgAgCTgAgACCTg
TNF	-238G→A	gTCCTACACACAAATCAgTCAgT	gACACACAAgCATCAAggATACC

Gene	Probe 1	Probe 2	Annealing (˚C)	Cycles
IL10	CCCCgCCTgTACTgTAggAA	ACCCCgCCTgTCCTgTAggA	60	50
IL10	TACAggTgATgTAACATCTCTg	gAggCACAgAgATATTACATCA	60	50
BCHE	CACTCCCATTCTgCTTCATCA	CACTCCCATTCTgTTTCATCAAT	60	50
CETP	AgCTCCgAgTCCATCCAgAg	ggAAgCTCTggACggACTCg	60	50
SLC26A8	AAgCATCCTCCATTAACCTg	TgAATCAggTTAACggAggATg	60	50
MMP2	CACCCAgCACTCCACCTCT	AgAgCTAAAgAggTAgAgTgC	60	50
TNF	CTCCCTgCTCCgATTCCgT	CCCTCggAATCAgAgCAgg	60	50

chest X-rays, echocardiography, radioisotope analysis, coronary angiography, and left ventriculography. Patients with hyperthyroidism, chronic lung disease, or excessive alcohol intake (>100 g of pure ethanol per day) were also excluded from the study.

Control subjects comprised 873 individuals (302 men, 571 women) who visited the participating hospitals for an annual health checkup and who had no history of AF or other significant supraventricular or ventricular arrhythmias or of taking antiarrhythmics.

The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Gifu Prefectural Tajimi Hospital, Gifu Prefectural Gifu Hospital, Hirosaki University School of Medicine, and Mie University School of Medicine. Written informed consent was obtained from each participant.

Selection of polymorphisms. With the use of public databases, we selected 32 candidate genes that have been characterized and were suggested to be associated with AF on the basis of a comprehensive overview of inflammation, oxidative and hemodynamic stress, hypertension, atherosclerosis, matrix metabolism, and other metabolic factors. We further selected 40 polymorphisms of these genes, most located in the promoter region, exons, or splice donor or acceptor sites of introns, that might be expected to result in changes in the function or expression of the encoded protein (data not shown).

Genotyping of polymorphisms. Venous blood (7 ml) was collected into tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 40 polymorphisms were determined (G&G Science, Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with analysis by suspension array technology (Luminex 100 flow cytometer, Luminex, Austin, TX). Primers, probes, and other conditions for genotyping are shown in Table I. Detailed methodology for genotyping was described previously (12).

Statistical analysis. Clinical data were compared between subjects with or without AF by the unpaired Student's t-test. Qualitative data were compared by the Chi-square test. Allele frequencies were estimated by the gene counting method, and the Chi-square test was used to identify departure from Hardy-Weinberg equilibrium. In the initial screen, the genotype distribution of each autosomal polymorphism was compared between subjects with or without AF by the Chi-square test (3x2); for polymorphisms on the X chromosome, allele frequencies were compared by the Chi-square test (2x2). The false discovery rate (FDR) was calculated by the method of Benjamini and Hochberg (13). Calculation of the FDR is an approach to dealing with the problems associated with multiple comparisons and provides a measure of the expected proportion of false positives among data. The FDR threshold is determined from the observed P-value distribution and is adaptive to the signal level in data. The FDR differs from a P-value, and much higher FDRs than P-values can be tolerated. Initial screening of polymorphisms with the Chi-square test was followed by application of multivariable logistic regression analysis and a stepwise forward selection procedure for a more rigorous evaluation of association. The FDR was calculated at each step of the statistical analysis. In the initial screen (Chi-square test), the FDR was calculated from the distribution of

Table II. Baseline characteristics of the study subjects (n=1069).

Characteristic	Atrial fibrillation (n=196)	Controls (n=873)	P
Age (years)	67.8±10.1	60.3±10.8	0.0001
Sex (male/female)	153/43	302/571	<0.0001
Body mass index (kg/m^2)	23.0±3.4	24.0±3.3	<0.0001
Smoking (%)	41.8	16.1	<0.0001
Hypertension (%)	44.4	29.2	<0.0001
Diabetes mellitus (%)	22.5	9.7	<0.0001
Hypercholesterolemia (%)	35.7	26.0	<0.0001

Data for age and body mass index are expressed as means ± SD. Smoker: smoking of ≥10 cigarettes daily. Hypertension: systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg (or both), or taking of antihypertensive medication. Diabetes mellitus: fasting plasma glucose concentration of ≥6.93 mmol/l (126 mg/dl) or hemoglobin A_{1c} content of ≥6.5% (or both), or taking of antidiabetes medication. Hypercholesterolemia: serum total cholesterol concentration of ≥5.72 mmol/l (220 mg/dl) or taking of lipid-lowering medication.

Table III. Polymorphisms related (FDR <0.05) to atrial fibrillation as revealed by the Chi-square test.

Gene	Polymorphism	P	FDR
IL10	-592A→C	0.0008	0.0320
IL10	-819T→C	0.0008	0.0160
BCHE	1615G→A (Ala539Thr)	0.0013	0.0173
CETP	1061A→G (Ile405Val)	0.0023	0.0230
SLC26A8	A→G (Ile639Val)	0.0023	0.0184
MMP2	-1306C→T	0.0072	0.0480
TNF	-238G→A	0.0076	0.0434

FDR, false discovery rate.

Table IV. Genotype distributions (percent) of polymorphisms related to atrial fibrillation.

Gene	Polymorphism	Atrial fibrillation (n=196)	Controls (n=873)
IL10	-592A→C		
	AA	48.5	41.6
	AC	46.9	45.3
	CC	4.6	13.2
IL10	-819T→C		
	TT	48.5	41.7
	TC	46.9	45.1
	CC	4.6	13.2
BCHE	1615G→A (Ala539Thr)		
	GG	67.4	73.1
	GA	26.0	24.9
	AA	6.6	2.0
CETP	1061A→G (Ile405Val)		
	AA	22.4	24.1
	AG	58.2	45.8
	GG	19.4	30.1
SLC26A8	A→G (Ile639Val)		
	AA	47.5	52.1
	AG	49.5	39.4
	GG	3.0	8.5
MMP2	-1306C→T		
	CC	76.0	84.7
	CT	22.5	14.9
	TT	1.5	0.4
TNF	-238G→A		
	GG	99.0	95.4
	GA	1.0	4.6
	AA	0.0	0.0

P-values for the 40 polymorphisms. Polymorphisms with an FDR of <0.05 were further examined by multivariable logistic regression analysis with adjustment for covariates, with AF as a dependent variable and independent variables including age, sex (0 = woman, 1 = man), body mass index (BMI), smoking status (0 = nonsmoker, 1 = smoker), metabolic variables (0 = no history of hypertension, diabetes mellitus, or hypercholesterolemia; 1 = positive history), and genotype of each polymorphism. Each genotype was assessed according to dominant (0 = wild-type homozygote, 1 = heterozygote = variant homozygote), recessive (0 = wild-type homozygote = heterozygote, 1 = variant homozygote), and additive [(0, 0) = wild-type homozygote, (1, 0) = heterozygote, (0, 1) = variant homozygote] genetic models, and the P-value, odds ratio, and 95% confidence interval were calculated. The additive genetic models each comprised two groups: heterozygotes versus wild-type homozygotes for the additive 1 model, and variant homozygotes versus wild-type homozygotes for the additive 2 model. We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as of other covariates on AF. Given the multiple comparisons of genotypes with AF, we adopted the criterion of FDR <0.05 for significant association at each step of the statistical analysis. For other clinical background data, a P-value of <0.05 was considered statistically significant. Statistical significance was examined by two-sided tests, which were performed with JMP version 5.1 software (SAS Institute, Cary, NC).

Results

The baseline characteristics of the study subjects are shown in Table II. Age, the frequency of men, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia were greater, whereas BMI was smaller, in subjects with AF than in controls. Evaluation of genotype distributions or allele frequencies by the Chi-square test

Table V. Multivariable logistic regression analysis of polymorphisms related to atrial fibrillation with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia.

Gene	Polymorphism	Dominant		Recessive	
		P (FDR)	OR (95% CI)	P (FDR)	OR (95% CI)
IL10	-592 A→C	0.1826 (0.2638)		**0.0039 (0.0338)**	0.33 (0.15-0.67)
IL10	-819T→C	0.1868 (0.2556)		**0.0039 (0.0254)**	0.33 (0.15-0.67)
BCHE	1615G→A (Ala539Thr)	0.0466 (0.0808)	1.48 (1.00-2.18)	**0.0129 (0.0335)**	3.27 (1.28-8.34)
CETP	1061A→G (Ile405Val)	0.9336 (0.9336)		0.0294 (0.0588)	0.62 (0.40-0.95)
SLC26A8	A→G (Ile639Val)	0.4542 (0.5134)		0.0342 (0.0635)	0.38 (0.14-0.87)
MMP2	-1306C→T	**0.0042 (0.0218)**	1.91 (1.22-2.97)	**0.0176 (0.0381)**	7.26 (1.27-37.49)
TNF	-238G→A	**0.0060 (0.0223)**	0.12 (0.02-0.44)		

Gene	Additive 1		Additive 2	
	P (FDR)	OR (95% CI)	P (FDR)	OR (95% CI)
IL10	0.6655 (0.7210)		0.0037 (0.0962)	0.32 (0.14-0.66)
IL10	0.0037 (0.0481)		**0.0037 (0.0481)**	0.32 (0.14-0.66)
BCHE	0.1974 (0.2566)		**0.0090 (0.0293)**	3.50 (1.36-9.00)
CETP	0.4085 (0.4828)		0.2020 (0.2501)	
SLC26A8	0.1641 (0.2510)		0.0686 (0.1115)	
MMP2	**0.0132 (0.0312)**	1.78 (1.12-2.79)	**0.0126 (0.0364)**	8.07 (1.41-41.80)
TNF	**0.0060 (0.0260)**	0.12 (0.02-0.44)		

FDR, false discovery rate; OR, odds ratio; CI, confidence interval. FDR values of <0.05 are shown in bold.

Table VI. Genotypes and other characteristics associated with atrial fibrillation as determined by a stepwise forward selection procedure.

Characteristic	R^2	P	FDR
Sex	0.1237	<0.0001	<0.0001
Age	0.0588	<0.0001	<0.0001
Hypercholesterolemia	0.0163	<0.0001	<0.0001
IL10 (-592 A→C, recessive)	0.0114	0.0006	0.0021
Body mass index	0.0100	0.0014	0.0039
MMP2 (-1306C→T, dominant)	0.0098	0.0016	0.0037
Diabetes mellitus	0.0054	0.0189	0.0378

R^2, contribution rate; FDR, false discovery rate.

revealed that seven polymorphisms were related (FDR <0.05) to the prevalence of AF (Table III). The genotype distributions of these polymorphisms among subjects with AF and controls are shown in Table IV. The genotype distributions of these polymorphisms among control individuals were in Hardy-Weinberg equilibrium.

The seven polymorphisms related to AF by the Chi-square test were further examined by multivariate logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia (Table V). The analysis revealed that the -592A→C and -819T→C polymorphisms of the interleukin 10 gene (*IL10*; recessive model and both recessive and additive 2 models, respectively), the 1615G→A (Ala539Thr) polymorphism of the butyrylcholinesterase gene (*BCHE*; recessive and additive 2 models), the -1306C→T polymorphism of the matrix metalloproteinase 2 gene (*MMP2*; dominant, recessive, and additive 1 and 2 models), and the -238G→A polymorphism of the tumor necrosis factor gene (*TNF*; dominant and additive 1 models) were significantly (FDR <0.05) associated with the prevalence of AF. The *A* allele of the 1615G→A polymorphism of *BCHE* and the *T* allele of the -1306C→T polymorphism of *MMP2* were risk factors for AF, whereas the C alleles of the -592A→C and -819T→C polymorphisms of *IL10* and the *A* allele of the -238G→A polymorphism of *TNF* were protective against this condition.

Finally, we performed a stepwise forward selection procedure to examine the effects of genotypes for the identified genes, age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia on AF (Table VI). Each genotype was examined according to dominant or recessive models on the basis of statistical

significance in the multivariate logistic regression analysis. In descending order of statistical significance, sex, age, hypercholesterolemia, *IL10* genotype (-592A→C, recessive model), BMI, *MMP2* genotype (-1306C→T, dominant model), and diabetes mellitus significantly (FDR <0.05) and independently affected the prevalence of AF.

Discussion

Several genetic variants have been found to confer predisposition to AF, either by altering atrial electrophysiological properties or by contributing to a structural background susceptible to arrhythmia (10,14,15). Herein we showed that the *T* allele of the -1306C→T polymorphism of *MMP2* is a risk factor for AF, whereas the C allele of the -592A→C polymorphism of *IL10* is protective against this condition. Our results thus implicate *MMP2* and *IL10* as candidate loci for genetic susceptibility to AF.

Electrical remodeling manifested by changes in transmembrane ionic currents and shortening of the atrial effective refractory period promotes the persistence of AF (16-19). An underlying structural remodeling might occur before, during, or after electrical remodeling and plays a pivotal role in progression of sustained AF (16). Contractile remodeling, manifested by a decrease in atrial contractility, occurs before structural remodeling such as the development of fibrosis and atrial dilation (20).

The notion that inflammation contributes to at least some types of AF is supported by genetic studies (21), the frequent occurrence of AF after cardiac surgery (22), and an association of AF with pericarditis (23). Inflammatory stimuli may lead to structural remodeling of atria that promotes progression and persistence of AF. Marked inflammatory infiltrates, myocyte necrosis, and fibrosis have been demonstrated in atrial biopsies of patients with lone AF (24). An increase in the plasma concentration of C-reactive protein observed in AF patients may reflect an inflammatory process that promotes the persistence of this condition (25). A population-based study also showed that the plasma concentration of C-reactive protein was significantly greater in patients with persistent lone AF than in individuals in normal sinus rhythm (26). These observations suggest that inflammatory changes may contribute to atrial structural remodeling and increase the propensity for AF persistence.

IL10 is a major anti-inflammatory cytokine that plays an important role in regulation of the immune system. It deactivates the inflammatory response mediated by macrophages and lymphocytes and inhibits the production of proinflammatory cytokines (27-31). An increased production of IL10 might thus result in better control of inflammatory responses induced by chronic vessel damage and reduce the risk of atherogenic complications (32). A previous study of the -592A→C polymorphism of *IL10* showed that the circulating level of IL10 was significantly higher in individuals with the *CC* genotype than in those with the *AA* genotype (33). The *A* allele of this polymorphism is associated with reduced IL10 production by monocytes and macrophages compared with the *C* allele, suggesting that the -592A→C polymorphism may affect the expression of *IL10* (34,35). The precise mechanisms by which genetic variants of *IL10* might modulate atrial electrophysiological properties or the structural background conferring susceptibility to AF remain unknown. However, the results of our study suggest that the association of the -592A→C polymorphism of *IL10* with the prevalence of AF may be attributable to anti-inflammatory effects of IL10 on atrial tissue.

Members of the matrix metalloproteinase (MMP) family of proteolytic enzymes mediate turnover of extracellular matrix components and are inhibited by tissue inhibitors of metalloproteinases (TIMPs) (36,37). MMPs play an important role in pathological myocardial remodeling (38,39). MMP2 (gelatinase) possesses the ability to degrade several interstitial proteins including basement membrane components such as type IV collagen (40). Mechanical stretch, a hallmark of arterial hypertension that leads to vessel wall remodeling and induces formation of reactive oxygen species by NAD(P)H oxidase, increases the expression and activity of MMPs, and reactive oxygen species contribute to vascular remodeling associated with arterial hypertension through MMP activation (41). Given that the extracellular matrix not only provides a supportive scaffold for myocytes and maintains the structural integrity of the heart but also cooperates with myocytes in activation conduction, changes in extracellular matrix components in the atrium have been considered likely to contribute to the development of sustained AF (42,43). Remodeling of the atrial extracellular matrix, manifested by selective down-regulation of *TIMP2* expression and up-regulation of *MMP2* expression, has been associated with the development of sustained AF (43). The markedly increased expression of *MMP2* in the atria of patients with AF thus suggests the importance of this enzyme not only in extracellular matrix remodeling but also in the development of atrial dilation during progression of AF (43,44). In this study we showed that the -1306C→T polymorphism of *MMP2* is associated with AF, with the *T* allele representing a risk factor for this condition. To clarify the effect of the -1306C→T polymorphism on the expression of MMP2, we measured the serum activity of MMP2 in 46 individuals. There was no significant relation between this polymorphism and the enzyme activity (data not shown). The functional relevance of the -1306C→T poly-morphism of *MMP2* to the etiology of AF thus remains to be elucidated.

Although we have provided evidence for a genetic basis of predisposition to AF, there are some limitations of our study: i) Measurements of parameters of structural remodeling in atria, such as left atrial size, were not performed in AF patients or control subjects. ii) Given the multiple comparisons of genotypes with AF, we adopted a level of FDR <0.05 for association. It is not possible, however, to exclude completely potential statistical errors such as false positives. iii) It is also possible that the -1306C→T polymorphism of *MMP2* and the -592A→C polymorphism of *IL10* are in linkage disequilibrium with polymorphisms in the same or nearby genes that are actually responsible for the development of AF. (iv) The functional relevance of the association of the -1306C→T polymorphism of *MMP2* or the -592A→C polymorphism of *IL10* with AF remains to be determined.

In conclusion, our present results suggest that genetic variants of *MMP2* and *IL10* may be a risk factor for or protective factor against AF, respectively, in the Japanese

population. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic component of AF and may contribute to the personalized prevention of this condition.

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