Association of genetic variants of the α-kinase 1 gene with myocardial infarction in community-dwelling individuals

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Abstract. We previously demonstrated that rs2074380 (G→A, Gly870Ser) and rs2074381 (A→G, Asn916Asp) of the α-kinase 1 gene (*ALPK1*) were significantly associated with chronic kidney disease (CKD) in individuals with diabetes mellitus. As CKD is a significant risk factor for coronary heart disease, we hypothesized that rs2074380 and rs2074381 of *ALPK1* may contribute to the genetic susceptibility to myocardial infarction (MI) through affecting the susceptibility to CKD. The aim of the present study was to investigate a possible association of rs2074380 and rs2074381 with MI in community-dwelling individuals. The study subjects comprised 5,771 community-dwelling individuals (41 subjects with MI and 5,730 controls) who were recruited to a population-based cohort study in Inabe, Japan. The comparison of allele frequencies and genotype distributions using the Chi-square test revealed that rs2074380 and rs2074381 were significantly associated with MI (P<0.05). The multivariable logistic regression analysis with adjustment for covariates demonstrated that rs2074380 (P=0.0354, dominant model) and rs2074381 (P=0.0438, dominant model) were significantly associated with MI, with the minor *A* and *G* alleles, respectively, being protective against this condition. A haplotype analysis of these polymorphisms indicated that the frequency of the major haplotype, G (rs2074380)-A (rs2074381), was significantly higher (permutation P=0.012), whereas that of the minor haplotype A-G was significantly lower (P=0.020), in subjects with MI compared to that observed among controls. Therefore, *ALPK1* may be a susceptible locus for MI.

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Introduction

Myocardial infarction (MI) is a major health problem, due to its significant contribution to global morbidity and mortality (1,2). Disease prevention is an important strategy for reducing the overall burden of MI. In addition to several conventional risk factors, including hypertension, diabetes mellitus, dyslipidemia and chronic kidney disease (CKD) (3,4), the significance of genetic factors and of the interaction between genetic and environmental factors was previously demonstrated in genetic epidemiological and genome-wide association studies (GWASs) (5-11). Although several loci and genes that confer susceptibility to MI have been identified in Caucasian populations by previous GWASs (8-11), the genetic variants associated with MI in Japanese individuals have not yet been definitively identified.

We demonstrated in a previous GWAS that rs2074380 (G→A, Gly870Ser) and rs2074381 (A→G, Asn916Asp) of the α-kinase 1 gene (*ALPK1*) were significantly associated with CKD in Japanese individuals (12). As CKD is crucial in the development of atherosclerotic disease, including MI, we hypothesized that rs2074380 and rs2074381 of *ALPK1* may contribute to the genetic susceptibility to MI through affecting the predisposition to CKD. The aim of the present study was to investigate a possible association of these polymorphisms with MI in community-dwelling Japanese individuals.

Materials and methods

Study population. The study population comprised 5,771 community-dwelling Japanese individuals (41 subjects with MI and 5,730 controls) who were recruited to a population-based cohort study in Inabe (Mie, Japan) between 2010 and 2012. The subjects with MI (37 men and 4 women) underwent coronary angiography and left ventriculography. The diagnosis of MI was based on typical electrocardiographic changes and on increased serum activity of creatine kinase (MB isozyme) and concentration of troponin T. The diagnosis was confirmed by the presence of wall motion abnormality on left ventriculography and by the identification of the responsible stenosis in

Table I. Baseline characteristics of the patients with MI and controls.

Characteristics	MI (n=41)	Controls (n=5,730)	P-value
Age (years)	64.7±1.9	52.2±0.2	<0.0001
Gender			
Male	37	3,137	
Female	4	2,593	<0.0001
Body mass index (kg/m^2)	24.5±0.5	22.9±0.0	0.0028
Current or former smoker (%)	46.3	39.1	0.3476
Hypertension[a] (%)	75.6	26.7	<0.0001
Systolic blood pressure (mmHg)	125.8±2.5	119.6±16.0	0.0124
Diastolic blood pressure (mmHg)	76.0±11.6	73.6±12.2	0.2256
Diabetes mellitus[b] (%)	36.6	7.9	<0.0001
Fasting plasma glucose (mmol/l)	6.1±1.2	5.5±1.1	0.0009
Blood hemoglobin A_{1c} (%)	6.2±0.7	5.7±0.6	<0.0001
Dyslipidemia[c] (%)	75.6	50.4	0.0010
Serum total cholesterol (mmol/l)	4.5±0.7	5.2±0.8	0.0006
Serum triglycerides (mmol/l)	1.5±1.0	1.3±0.9	0.0515
Serum HDL-cholesterol (mmol/l)	1.3±0.3	1.7±0.4	<0.0001
Serum LDL-cholesterol (mmol/l)	2.7±0.9	3.2±0.8	<0.0001
Chronic kidney disease (%)	37.5	7.1	<0.0001
Serum creatinine (μmol/l)	81.6±24.1	64.5±15.2	<0.0001
eGFR (ml/min/1.73 m^2)[d]	68.4±20.8	80.1±15.2	<0.0001

Quantitative data are expressed as means ± standard deviation. [a]Systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, or on antihypertensive medication. [b]Fasting plasma glucose concentration of ≥6.93 mmol/l, hemoglobin A_{1c} content of ≥6.9%, or on antidiabetic medication. [c]Serum concentration of triglycerides of ≥1.65 mmol/l, serum concentration of high density lipoprotein (HDL)-cholesterol of <1.04 mmol/l, serum concentration of low density lipoprotein (LDL)-cholesterol of ≥3.64 mmol/l, or on antidyslipidemic medication. [d]eGFR (ml/min/1.73 m^2) = 194 x [age (years)]$^{-0.287}$ x [serum creatinine (mg/dl)]$^{-1.094}$ x (0.739 if female). MI, myocardial infarction; eGFR, estimated glomerular filtration rate.

any of the major coronary arteries or in the left main trunk by coronary angiography. The control subjects comprised 5,730 individuals (3,137 men and 2,593 women) without a history of coronary heart disease, aortic aneurysm or peripheral arterial occlusive disease, ischemic or hemorrhagic stroke or other cerebrovascular disease, or other atherosclerotic, thrombotic, embolic or hemorrhagic disorders.

The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committees of Human Research of Mie University Graduate School of Medicine and Inabe General Hospital. Written informed consent was obtained from all the subjects.

Genotyping of polymorphisms. Venous blood (5 ml) was collected into tubes containing 50 mmol/l EDTA (disodium salt; Terumo Corp., Tokyo, Japan). Peripheral blood leukocytes were isolated and genomic DNA was extracted from these cells with a DNA extraction kit (SMITEST EX-R&D; Medical and Biological Laboratories Co., Ltd., Nagoya, Japan). The polymorphism genotypes were determined at G&G Science Co., Ltd. (Fukushima, Japan) by the Multiplex Bead-based assay (Luminex Corp., Austin, TX, USA), which combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology, as previously described (12). The detailed genotyping methodology was previously described (13).

Statistical analysis. Quantitative data were compared between subjects with MI and controls by the unpaired Student's t-test. Categorical data were compared by the Chi-square test. The allele frequencies were estimated by the gene counting method and the Chi-square test was used to identify departures from the Hardy-Weinberg equilibrium. The genotype distributions (3x2) and allele frequencies (2x2) of each polymorphism were compared between subjects with MI and controls by the Chi-square test.

A multivariable logistic regression analysis was performed, with MI as a dependent variable and age, gender (0, female; 1, male), body mass index (BMI), serum concentration of creatinine, prevalence of hypertension, diabetes mellitus, dyslipidemia and genotype of each polymorphism as the independent variables. The P-value, odds ratio (OR) and 95% confidence interval (CI) were calculated. Each genotype was assessed according to dominant (0, wild-type homozygote;

Table II. Association of rs2074380 and rs2074381 of *ALPK1* with MI, as determined by the Chi-square test.

Gene	Polymorphisms	dbSNP	MI (n=41)	Controls (n=5,730)	P-value (genotype)	P-value (allele)
ALPK1	G→A	rs2074380			0.0198	0.0051
	GG		40 (97.6)	4,842 (84.5)		
	GA		1 (2.4)	850 (14.8)		
	AA		0 (0)	38 (0.7)		
HW P-value			0.9370	0.9167		
ALPK1	A→G	rs2074381			0.0275	0.0075
	AA		40 (97.6)	4,888 (85.3)		
	AG		1 (2.4)	811 (14.2)		
	GG		0 (0)	31 (0.5)		
HW P-value			0.9370	0.6725		

Parenthetical data represent percentage values. *ALPK1,* α-kinase 1 gene; MI, myocardial infarction; dbSNP, single-nucleotide polymorphism database; HW, Hardy-Weinberg.

Table III. Multivariable logistic regression analysis of rs2074380 and rs2074381 of *ALPK1* and MI.

Gene	dbSNP	Dominant		Recessive	
		P-value	OR (95% CI)	P-value	OR (95% CI)
ALPK1	rs2074380 (G→A)	0.0354	0.1 (0.0-0.5)	0.8928	-
ALPK1	rs2074381 (A→G)	0.0438	0.1 (0.0-0.6)	0.8999	-

Multivariable logistic regression analysis was performed following adjustment for age, gender, body mass index, serum concentration of creatinine and the prevalence of hypertension, diabetes mellitus and dyslipidemia. *ALPK1*, α-kinase 1 gene; MI, myocardial infarction; OR, odds ratio; CI, confidence interval; dbSNP, single-nucleotide polymorphism database.

1, heterozygote and variant homozygote) and recessive (0, wild-type homozygote and heterozygote; 1, variant homozygote) genetic models.

A stepwise forward selection procedure was also performed to investigate the effects of genotypes as well as those of other covariates on MI. In this analysis, each genotype was assessed according to a dominant model on the basis of statistical significance in the multivariable logistic regression analysis. The P-values for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. P<0.05 was considered to indicate a statistically significant difference. Statistical significance was assessed by two-sided tests performed with JMP and JMP Genomics software, version 6.0 (SAS Institute, Inc., Cary, NC, USA). Linkage disequilibrium and haplotype analysis of the polymorphisms was performed with SNPAlyze software, version 6 (Dynacom, Yokohama, Japan).

Results

Study population. The characteristics of the study subjects are presented in Table I. Age, number of male subjects, BMI and the prevalence of hypertension, diabetes mellitus, dyslipidemia and CKD were higher among subjects with MI compared to those among controls.

Genotype distribution and allele frequencies. The comparison of genotype distribution and allele frequencies by the Chi-square test between subjects with MI and controls revealed that rs2074380 (G→A, Gly870Ser) and rs2074081 (A→G, Asn916Asp) were significantly associated with MI (P<0.05). The genotype distributions of the two polymorphisms were in Hardy-Weinberg equilibrium among subjects with MI and controls (Table II).

Multivariable logistic regression analysis and stepwise forward selection procedure. The multivariable logistic regression analysis, following adjustment for age, gender, BMI, serum concentration of creatinine and prevalence of hypertension, diabetes mellitus and dyslipidemia, demonstrated that rs2074380 (dominant model) and rs2074381 (dominant model) were significantly associated with MI (P<0.05), with the minor *A* and *G* alleles of rs2074380 and rs2074381, respectively, being protective against this condition (Table III).

A stepwise forward selection procedure revealed that hypertension, serum concentration of creatinine, diabetes

Table IV. Effects of genotype and other characteristics on the prevalence of MI as determined by a stepwise forward selection procedure.

Characteristics	P-value	R^2
Hypertension	<0.0001	0.0899
Serum creatinine concentration	0.0001	0.0307
Diabetes mellitus	0.0002	0.0296
Gender (male)	0.0004	0.0268
Age	0.0005	0.0261
rs2074380 of *ALPK1* (dominant model)	0.0017	0.0208

MI, myocardial infarction; R^2, contribution rate.

mellitus, male gender, age and rs2074380 of *ALPK1* (dominant model) were significant (P<0.05) and independent determinants of MI (Table IV).

Haplotype analysis. Given that rs2074380 and rs2074381 of *ALPK1* were in linkage disequilibrium [standard linkage disequilibrium coefficient $(r^2)=0.938$; P<0.0001], we performed a haplotype analysis for these polymorphisms. The haplotype analysis revealed that the frequency of the major haplotype, G (rs2074380)-A (rs2074381), was significantly higher (P<0.05), whereas that of the minor haplotype, A-G, was significantly lower in subjects with MI compared to that in controls (Table V).

Discussion

We previously demonstrated that rs2074380 and rs2074381 of *ALPK1* were significantly associated with CKD, with the *A* and *G* alleles of rs2074380 and rs2074381, respectively, being protective against CKD (12). The postmortem immunohistochemical staining of human kidneys demonstrated that the expression of ALPK1 was increased in the renal tubular epithelial cells of kidneys with diabetic glomerulosclerosis compared to that in normal kidneys, suggesting that *ALPK1* may be key to the development of diabetic nephropathy (12). In the present study, we demonstrated that rs2074380 and

rs2074381 of *ALPK1* were significantly associated with the prevalence of MI in community-dwelling Japanese individuals, with the minor *A* and *G* alleles of rs2074380 and rs2074381, respectively, being protective against this condition. Our previous (12) and present results suggested that the *A* allele of rs2074380 and the *G* allele of rs2074381 were protective against CKD and MI and that the association of these polymorphisms to MI may be attributable, at least in part, to their effects on the susceptibility to CKD.

ALPK1 belongs to a recently identified α-kinase family and exhibits no detectable sequence homology to conventional protein kinases (14). ALPK1 is expressed in various human tissues, including the heart and kidney (15) and was shown to be crucial in protein sorting and polarization in epithelial cells (16). ALPK1 may act synergistically with monosodium urate monohydrate crystals to promote the production of proinflammatory cytokines through the activation of nuclear factor-κB and mitogen-activated protein kinase (ERK1/2 and p38) signaling in cultured HEK293 cells (17), indicating that ALPK1 may contribute to the inflammatory process associated with the development of gout. Since the onset of MI is likely precipitated by activated inflammation at atherosclerotic plaques in the coronary arterial wall (18,19), the association of *ALPK1* to MI may be attributable to its effect on vascular inflammation.

Our previous GWAS on CKD demonstrated that the overexpression of ALPK1 resulted in upregulation of the expression of cystatin C in cultured HEK293 T cells (12). Cystatin C is an inhibitor of cysteine proteases and recognized as a sensitive marker of renal dysfunction (20). Cystatin C was shown to be associated with inflammation, regardless of renal function. The serum concentrations of cystatin C were correlated with those of C-reactive protein and fibrinogen in 990 subjects with coronary heart disease from the Heart and Soul Study (21) and in subjects with renal dysfunction from the Cardiovascular Health Study (22). Furthermore, the serum concentrations of cystatin C were associated with the severity of coronary heart disease (23) and the risk of secondary cardiovascular events (24). Those observations suggested that the correlation of ALPK1 to MI may be mediated by the effects of cystatin C on the development of vascular inflammation. Therefore, those observations (17-19,21-24) suggested that *ALPK1* may contribute to the development of MI through the acceleration of vascular inflammation.

Our present study had several limitations: i) as the subjects were recruited among community-dwelling individuals who

Table V. Association of *ALPK1* gene haplotypes to MI.

Haplotype	Overall frequency	Frequency		Chi-square P-value	Permutation P-value
		MI	Controls		
G-A	0.9197	0.9878	0.9192	0.0227	0.012
A-G	0.0757	0.0122	0.0762	0.0291	0.020
A-A	0.0046	3.4×10^{-17}	0.0046	0.5371	0.302

The haplotypes consist of the G→A (rs2074380) and A→G (rs2074381) polymorphisms, respectively, of *ALPK1*. *ALPK1*, α-kinase 1 gene; MI, myocardial infarction.

visited the health care center of Inabe General Hospital for an annual health checkup, the number of subjects with MI was limited; ii) as the results of the present study were not replicated, validation of our findings may require their replication in additional independent subject panels or ethnic groups; iii) the molecular mechanisms underlying the effects of rs2074380 and rs2074381 of *ALPK1* on the development of MI were not determined.

In conclusion, the present study suggests that *ALPK1* may be a susceptibility locus for MI in Japanese individuals. Since multiple variants, each exerting a limited effect, may ultimately prove to be responsible for a significant fraction of the genetic component of MI, further identification of MI susceptibility genes may allow more accurate assessment of the genetic component of this condition.

Acknowledgements

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