Association between polymorphisms of the α-kinase 1 gene and type 2 diabetes mellitus in community-dwelling individuals

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Abstract. We previously demonstrated that the α-kinase 1 gene (*ALPK1*) is a susceptibility locus for chronic kidney disease in individuals with diabetes mellitus (DM) by a genome-wide association study. Although genetic variants of *ALPK1* have been associated with chronic kidney disease in individuals with DM, whether *ALPK1* is a susceptibility locus for DM has not been elucidated. The purpose of the present study was to investigate a possible association of the rs2074388 (A→G, Asp565Gly) or rs2074379 (A→G, Ile732Met) variants of *ALPK1* with type 2 DM in community-dwelling individuals. The study subjects comprised 5,959 community-dwelling individuals (495 subjects with type 2 DM and 5,464 controls) who were recruited to a population-based cohort study in Inabe, Mie, Japan. The comparisons of allele frequencies or genotype distributions using the Chi-square test revealed that the rs2074388 and rs2074379 variants of *ALPK1* were significantly associated with type 2 DM ($P<0.05$). A multivariable logistic regression analysis with adjustment for age, gender, body mass index and smoking status revealed that the rs2074388 ($P=0.0051$; odds ratio, 1.32) and rs2074379 ($P=0.0058$; odds ratio, 1.32) variants were significantly associated with type 2 DM. The haplotype analysis of these polymorphisms revealed that the frequency of the major haplotype, A (rs2074388)-A (rs2074379), was significantly lower, whereas that of the minor haplotype G-G was significantly higher in subjects with type 2 DM compared to controls. Thus, *ALPK1* may be a susceptible gene for type 2 DM in community-dwelling Japanese individuals.

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Introduction

The incidence of type 2 diabetes mellitus (DM) is rapidly increasing and has become one of the most common chronic diseases worldwide (1). According to recent estimates, the prevalence of type 2 DM will increase to 439 million adults (7.7%) by 2030 (2). Asia in particular is a major site of emerging epidemics, mainly due to the transition in nutritional habits and changes in lifestyle (2). Since type 2 DM is associated with an increased incidence of cardiovascular disease and long-term mortality, it imposes a significant economic burden on health-care wordwide (3,4). Furthermore, the contribution of intensive lifestyle intervention or glycemic control to the reduction of cardiovascular mortality remain controversial (5,6). Therefore, aggressive disease prevention and early detection should be a global strategy priority.

Family history is one of the major risk factors for type 2 DM (7), suggesting that genetic factors significantly contribute to the development and progression of type 2 DM (8). To date, genome-wide association studies (GWASs) have identified various loci and ~40 genes associated with predisposition to type 2 DM (9-13); however, the genes that contribute to genetic susceptibility to type 2 DM in Japanese individuals have yet to be definitively identified.

We previously demonstrated that the α-kinase 1 gene (*ALPK1*) is a susceptibility locus for chronic kidney disease in Japanese individuals with DM by a GWAS (14). Although genetic variants of *ALPK1* have been associated with chronic kidney disease in individuals with DM, whether *ALPK1* is a susceptibility locus for DM has not been elucidated. Thus, we conducted an association study for the rs2074388 (A→G, Asp565Gly) or rs2074379 (A→G, Ile732Met) variants of *ALPK1* and type 2 DM in community-dwelling Japanese individuals, in order to provide a basis for the personalized prevention of this disease.

Materials and methods

Study population. The study population comprised 5,959 community-dwelling individuals (495 subjects with type 2 DM and 5,464 controls) who were recruited to a

Table I. Characteristics of subjects with type 2 diabetes mellitus (DM) and controls.

Characteristics	DM	Controls	P-value
No. of subjects	495	5,464	
Age (years)	60.2±9.6	51.9±12.8	<0.0001
Gender (male/female, %)	74.9/25.1	53.6/46.4	<0.0001
Body mass index (kg/m^2)	24.8±3.8	22.8±3.3	<0.0001
Current or former smoker (%)	48.7	38.5	<0.0001
Hypertension (%)	58.2	25.3	<0.0001
Systolic blood pressure (mmHg)	128±17	119±16	<0.0001
Diastolic blood pressure (mmHg)	78±12	73±12	<0.0001
Dyslipidemia (%)	69.3	47.0	<0.0001
Serum total cholesterol (mmol/l)	5.14±0.94	5.18±0.84	0.2324
Serum triglycerides (mmol/l)	1.52±1.09	1.23±0.84	<0.0001
Serum HDL-cholesterol (mmol/l)	1.49±0.39	1.67±0.44	<0.0001
Serum LDL-cholesterol (mmol/l)	3.25±0.89	3.23±0.81	0.9922
Chronic kidney disease (%)	14.8	7.1	<0.0001
Fasting plasma glucose (mmol/l)	7.85±2.43	5.34±0.48	<0.0001
Blood hemoglobin A_{1c} (%)	6.6±1.3	5.2±0.3	<0.0001
Serum creatinine (μmol/l)	59.6±14.8	56.0±13.4	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	77.5±17.8	80.0±15.1	0.0002

Quantitative data are presented as means ± standard deviation. HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate.

population-based cohort study in Inabe, Mie, Japan, between 2010 and 2012. DM was defined as a fasting plasma glucose level of ≥6.93 mmol/l (126 mg/dl), a blood glycosylated hemoglobin (hemoglobin A_{1c}) content of ≥6.9%, or administration of antidiabetic medication. Type 2 DM was defined according to the criteria of the World Health Organization previously described (15,16). Individuals with type 1 DM, maturity-onset diabetes of the young, DM associated with mitochondrial diseases or single gene disorders, pancreatic diseases including severe pancreatitis and pancreatic tumors, other metabolic or endocrinologic diseases, or severe liver and renal dysfunction, were excluded from the study. Individuals on medication that may cause secondary DM were also excluded. The control individuals had a fasting plasma glucose level of <6.05 mmol/l (110 mg/dl) and a blood hemoglobin A_{1c} content of <6.2% and had no history of DM or of receiving antidiabetic medication.

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

Genotyping of polymorphisms. Venous blood (5 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), peripheral blood leukocytes were isolated and genomic DNA was extracted from these cells with the SMITEST EX-R&D DNA extraction kit (Medical and Biological Laboratories, Co., Ltd, Nagoya, Japan). The genotypes of rs2074388 and rs2074379 variants of *ALPK1* were determined at G&G Science Co., Ltd. (Fukushima, Japan) by the multiplex bead-based Luminex assay, a method that combines polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Genotyping involved PCR amplification, hybridization, streptavidin-phycoerythrin reaction and measurement of fluorescence. The genotyping methodology was previously described in detail (14,17).

Statistical analysis. Quantitative data were compared between subjects with type 2 DM and controls using the unpaired Student's t-test and categorical data were compared using the Chi-square test. The allele frequencies were estimated with the gene-counting method and the Chi-square test was used to identify departures from Hardy-Weinberg equilibrium. Genotype distributions and allele frequencies of the rs2074388 and rs2074379 variants of *ALPK1* were compared between subjects with type 2 DM and controls using the Chi-square test. A multivariable logistic regression analysis was performed with type 2 DM as a dependent variable and independent variables including age, gender (0, female; 1, male), body mass index, smoking status (0, non-smoker; 1, current or former smoker) and *ALPK1* genotype; the P-values, odds ratios and 95% confidence intervals were calculated. The *ALPK1* genotype was assessed according to dominant (0, wild-type homozygote; 1, heterozygote and variant homozygote) and recessive (0, wild-type homozygote and heterozygote; 1, variant homozygote) genetic models. We investigated the linkage disequilibrium between the rs2074388 and rs2074379 variants of *ALPK1* and the association of the haplotypes of these polymorphisms to type 2 DM. P<0.05 was considered to indicate statistically significant differences. Statistical significance was assessed using two-sided tests performed with JMP

Table II. Comparison of genotype distributions or allele frequencies of the rs2074388 and rs2074379 variants of *ALPK1* by the Chi-square test between subjects with type 2 diabetes mellitus (DM) and controls.

Genotypes	DM[a]	Controls[a]	P-value (genotype)	P-value (allele)
rs2074388			0.0244	0.0095
AA	197 (39.8)	2517 (46.1)		
AG	239 (48.3)	2393 (43.8)		
GG	59 (11.9)	554 (10.1)		
Hardy-Weinberg P-value	0.2953	0.6723		
rs2074379			0.0304	0.0111
AA	198 (40.0)	2516 (46.0)		
AG	238 (48.1)	2397 (43.9)		
GG	59 (11.9)	551 (10.1)		
Hardy-Weinberg P-value	0.3283	0.5689		

[a]The numbers in parentheses are percentages. *ALPK1*, α-kinase 1 gene.

Table III. Multivariable logistic regression analysis of the rs2074388 and rs2074379 variants of *ALPK1* and type 2 diabetes mellitus with adjustment for age, gender, body mass index and smoking status.

	Dominant		Recessive	
Genotypes	P-value	OR (95% CI)	P-value	OR (95% CI)
rs2074388 (A→G)	0.0051	1.32 (1.09-1.61)	0.1693	-
rs2074379 (A→G)	0.0058	1.32 (1.08-1.60)	0.1259	-

OR, odds ratio; CI, confidence interval; *ALPK1*, α-kinase 1 gene.

Table IV. Association of *ALPK1* haplotypes with type 2 diabetes mellitus (DM).

		Frequency		Chi-square	Permutation
Haplotype	Overall frequency	DM	Controls	P-value	P-value
A-A	0.6754	0.6394	0.6787	0.0115	0.015
G-G	0.3226	0.3596	0.3193	0.0094	0.010
G-A	0.0011	0.0010	0.0011	0.9353	0.652
A-G	0.0008	$1.6x10^{-12}$	0.0009	0.3407	0.358

Haplotypes consist of the A→G (rs2074388) and A→G (rs2074379) of *ALPK1*. *ALPK1*, α-kinase 1 gene.

Genomics software, version 6.0 (SAS Institute, Cary, NC, USA). Linkage disequilibrium and haplotype analysis of these polymorphisms were performed with SNPAlyze software, version 6 (Dynacom Co., Ltd., Yokohama, Japan).

Results

Subject characteristics. The baseline characteristics of the subjects are presented in Table I. Age, the frequency of male gender, body mass index and the prevalence of smoking, hypertension, dyslipidemia and chronic kidney disease were higher among subjects with type 2 DM compared to controls.

Comparison of genotype distributions and allele frequencies. The comparison of genotype distributions or allele frequencies using the Chi-square test revealed that the rs2074388 and rs2074379 variants of *ALPK1* were significantly associated with type 2 DM ($P<0.05$) (Table II). The genotype distributions of the two polymorphisms were in Hardy-Weinberg equilibrium among subjects with type 2 DM and controls.

Multivariable logistic regression analysis. A multivariable logistic regression analysis with adjustment for age, gender, body mass index and smoking status revealed that rs2074388 (dominant model) and rs2074379 (dominant model) were

significantly associated with type 2 DM, with the *G* alleles of the two polymorphisms representing risk factors for this disease (Table III).

Haplotype analysis. As the rs2074388 and rs2074379 variants of *ALPK1* were in linkage disequilibrium [standard linkage disequilibrium coefficient (r^2)=0.9908, P<0.0001], we performed a haplotype analysis for these polymorphisms. That analysis revealed that the frequency of the major haplotype, A (rs2074388)-A (rs2074379), was significantly lower (P<0.05), whereas that of the minor haplotype G-G was significantly higher in subjects with type 2 DM compared to controls (Table IV).

Association of ALPK1 polymorphisms with plasma glucose and hemoglobin A_{1c}. Finally, we assessed the association of rs2074388 or rs2074379 to fasting plasma glucose level or blood hemoglobin A_{1c} content among all individuals, or individuals not on antidiabetic medication. There were no significant differences in the fasting plasma glucose level or blood hemoglobin A_{1c} content among the of rs2074388 or rs2074379 genotypes (data not shown).

Discussion

We previously demonstrated that *ALPK1* is a susceptibility locus for chronic kidney disease in individuals with DM by a GWAS (14). In this study, we demonstrated that the rs2074388 and rs2074379 variants of *ALPK1* were significantly associated with the prevalence of type 2 DM in community-dwelling Japanese individuals, with the *G* alleles of the two polymorphisms representing risk factors for this disease.

ALPK1 is a member of a newly discovered protein kinase family that exhibits no sequence homology to the conventional protein kinases (18). It functions in apical transport by phosphorylating myosin-1a and is a putative candidate for the regulation of intracellular trafficking processes by phosphorylation (19). ALPK1 may act synergistically with monosodium urate monohydrate crystals in promoting 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, suggesting that ALPK1 may contribute to the inflammatory process associated with the development of gout (20).

Impaired insulin secretion and increased insulin resistance are key components of type 2 DM (21). Although the contributions of these factors to the onset and progression of type 2 DM may differ between Caucasian and Asian populations, both factors are essential for the diagnostic and therapeutic strategies of type 2 DM (22). Recent studies demonstrated that proinflammatory cytokines (interleukin-1β and tumor necrosis factor) exert deleterious effects on insulin secretion and insulin resistance (23,24). In addition, signal pathways activated by proinflammatory cytokines, such as nuclear factor-κB signaling, were shown to be responsible for these conditions (25). As chronic inflammation may be an important factor in the development of type 2 DM, the effects of the rs2074388 or rs2074379 variants of *ALPK1* on the acceleration of the inflammatory process may account for their association with type 2 DM.

Although the rs2074388 and rs2074379 variants of *ALPK1* were significantly associated with the prevalence of type 2 DM, either polymorphism was not associated with fasting plasma glucose level or blood hemoglobin A_{1c} content among all individuals or individuals not on antidiabetic medication. Although the reason for this discrepancy has not been fully elucidated, there are possible explanations: i) the number of subjects with type 2 DM not on antidiabetic medication was limited; ii) information regarding drug compliance obtained by a questionnaire was incomplete; and iii) either polymorphism was not associated with fasting plasma glucose level among control individuals.

Our study had certain limitations: i) as the results of the present study were not replicated, validation of our findings may require their replication with other independent subject panels or ethnic groups; ii) it is possible that rs2074388 or rs2074379 are in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of type 2 DM; iii) although the rs2074388 and rs2074379 variants of *ALPK1* were significantly associated with the prevalence of type 2 DM, either polymorphism was not associated with fasting plasma glucose level or blood hemoglobin A_{1c} content; and iv) the functional relevance of the rs2074388 or rs2074379 variants of *ALPK1* to the pathogenesis of type 2 DM has not been determined.

In conclusion, our results suggest that *ALPK1* is a susceptibility gene for type 2 DM in community-dwelling Japanese individuals. Determination of the genotypes for the polymorphisms of *ALPK1* may prove informative for the assessment of the genetic risk for type 2 DM in the Japanese population.

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