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Association of genetic variants of the α-kinase 1 gene with type 2 diabetes mellitus in a longitudinal population-based genetic epidemiological study

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Abstract. Previously, our studies identified nine genes and the chromosomal region 3q28 as susceptibility loci for myocardial infarction, ischemic stroke or chronic kidney disease in individuals by genome-wide or candidate gene association studies. The present study examined the possible association of 13 polymorphisms at these 10 loci with the prevalence of type 2 diabetes mellitus (DM) in community-dwelling individuals. Study subjects comprised 6,027 individuals (797 subjects with type 2 DM and 5,230 controls) who were recruited to the Inabe Health and Longevity Study, a longitudinal genetic epidemiological study of atherosclerotic, cardiovascular and metabolic diseases. The subjects were recruited from individuals who visited for an annual health checkup and they were followed up each year (mean follow-up, 5 years). Longitudinal analysis with a generalized estimating equation and with adjustment for age, gender and body mass index (BMI) revealed that rs2116519 (C→T) of *FAM78B* (P=0.0188), as well as rs2074379 (G→A, P=0.0121) and rs2074388 (A→G, P=0.0053) of *ALPK1* were significantly (P<0.05) associated with the prevalence of type 2 DM. Longitudinal analysis with a generalized linear mixed-effect model and with adjustment for age, gender and BMI among all the individuals revealed that rs2116519, rs2074379 and rs2074388 were significantly associated with fasting plasma glucose level (P=0.0352, 0.0017 and 0.0010, respectively) and to blood glycosylated hemoglobin (hemoglobin A_{1c}) content (P=0.0065, 0.0090 and 0.0079, respectively). Similar analysis among individuals not taking antidiabetic medication revealed that rs2074379 and rs2074388 were associated with the fasting plasma glucose level (P=0.0073 and 0.0042, respectively) and blood hemoglobin A_{1c} content (P=0.0142 and 0.0126, respectively), whereas rs2116519 was associated with blood hemoglobin A_{1c} content only (P=0.0470). *ALPK1* may thus be a susceptibility gene for type 2 DM.

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Key words: type 2 diabetes mellitus, genetics, polymorphism, genetic epidemiology, longitudinal study

Introduction

The prevalence of type 2 diabetes mellitus (DM) is increasing rapidly worldwide, with >170 million individuals currently affected (1,2) and 439 million adults (7.7% of all adults) predicted to be affected by 2030 (2). The major site of this emerging epidemic is expected to be Asia, mainly as a result of changes in nutrition and other lifestyle factors (3). Given that type 2 DM increases the risk for cardiovascular disease and long-term mortality, the health care burden imposed by this condition is a matter of urgent concern (4,5). Aggressive strategies for disease prevention and early detection will be key to tackling this global issue. Approximately 95% of patients with DM have type 2 DM, with characteristics that range from insulin resistance with relatively minor insulin deficiency to insulin deficiency with relatively minor insulin resistance (6). The several mechanisms that have been suggested for the pathogenesis of type 2 DM include an increase in the production of nonesterified fatty acids, inflammatory cytokines or adipokines, and dysfunction of mitochondria for insulin resistance and glucotoxicity, lipotoxicity and amyloid formation for β-cell dysfunction (1). Although a sedentary lifestyle and overeating appear to be triggering factors, genetic factors are also indicated in the pathogenesis of type 2 DM, as a positive family history is associated with a 2.4-fold increase in the risk (1,7).

Previous genome-wide association studies (GWASs) have indicated numerous loci and genes in the predisposition to type 2 DM in various ethnic groups (8-17). Although *KCNQ1* (13,14) and *UBE2E2* (15) were identified as susceptibility genes for type 2 DM in Japanese individuals, the

genes that contribute to genetic susceptibility to this condition remain to be identified.

Our previous studies identified nine genes and chromosomal region 3q28 as susceptibility loci for myocardial infarction, ischemic stroke or chronic kidney disease in Japanese individuals by genome-wide (18-20) or candidate gene (21-23) association studies. As type 2 DM is an important risk factor for these diseases (24-26), we hypothesized that certain single-nucleotide polymorphisms (SNPs) at these 10 loci may contribute to the genetic susceptibility by affecting the susceptibility to type 2 DM. The present study examined the possible association of 13 SNPs at the 10 loci with the prevalence of type 2 DM in community-dwelling Japanese individuals.

Materials and methods

Study population. Study subjects comprised 6,027 community-dwelling individuals (797 subjects with type 2 DM and 5,230 controls) who were recruited to a population-based cohort study (Inabe Health and Longevity Study) in Inabe (Mie, Japan). The Inabe Health and Longevity Study is a longitudinal genetic epidemiological study of atherosclerotic, cardiovascular and metabolic diseases (27-33). Detailed methods for recruitment of study subjects and collection of medical examination data were described previously (27).

Individuals with DM were defined as those with a fasting plasma glucose concentration of ≥126 mg/dl (6.93 mmol/l), with a blood glycosylated hemoglobin (hemoglobin A_{1c}) content of ≥6.5%, or who were taking antidiabetic medication. Type 2 DM was defined according to the criteria accepted by the World Health Organization and described previously (6,34). 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, or other metabolic or endocrinological diseases were excluded from the study. Individuals taking drugs that cause secondary DM were also excluded. Control individuals had a fasting plasma glucose level of <110 mg/dl (6.05 mmol/l), a blood hemoglobin A_{1c} content of <6.2% and no history of DM or of taking antidiabetic medication.

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

Selection and genotyping of polymorphisms. The 13 SNPs examined in the present study were selected from our previous genome-wide (18-20) or candidate gene (21-23) association studies and were described previously (27). Wild-type (ancestral) and variant alleles of the SNPs were determined from the SNP database (dbSNP, National Center for Biotechnology Information, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/SNP).

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 a DNA extraction kit (SMITEST EX-R&D; Medical and Biological Laboratories, Co., Ltd., Nagoya, Japan). Genotypes of the 13 SNPs were determined at G&G Science Co., Ltd., (Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex Corp., Austin, TX, USA). Primers, probes and other conditions for genotyping of SNPs examined in the present study were described previously (27), as was the detailed genotyping methodology (35).

Statistical analysis. Quantitative data were compared between subjects with type 2 DM and controls using the unpaired Student's t-test. Categorical data were compared with the χ^2 test. The associations of 13 SNPs to the prevalence of type 2 DM, to fasting plasma glucose level or blood hemoglobin A_{1c} content were examined in a 5-year longitudinal cohort study. Longitudinal changes in the prevalence of type 2 DM were compared between the two groups (dominant or recessive genetic model) by a generalized estimating equation (36) and with adjustment for age, gender and body mass index (BMI). Longitudinal changes in fasting plasma glucose level or blood hemoglobin A_{1c} content in all the individuals or in individuals not taking antidiabetic medication were compared between the two groups (dominant or recessive model) in a generalized linear mixed-effect model (37) with adjustment for age, gender and BMI. Age-related changes in the prevalence of type 2 DM or in fasting plasma glucose level or blood hemoglobin A_{1c} content were estimated with quadratic curves controlling for the observation year. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed with R software version 3-0-2 (The R Project for Statistical Computing) and JMP Genomics version 6.0 (SAS Institute, Inc., Cary, NC, USA).

Results

Subject characteristics. Characteristics of subjects with type 2 DM and controls in the cross-sectional analysis in March 2014 are shown in Table I. Age, the frequency of males and BMI were significantly greater in subjects with type 2 DM compared to the controls.

Associations with type 2 DM. The associations of the 13 SNPs to the prevalence of type 2 DM were analyzed with a generalized estimating equation and with adjustment for age, gender and BMI (Table II). The rs2116519 (C→T) SNP of the *FAM78B* gene (recessive model), as well as rs2074379 (G→A, dominant model) and rs2074388 (A→G, dominant model) of *ALPK1* were significantly ($P<0.05$) associated with the prevalence of type 2 DM.

The associations between the prevalence of type 2 DM and age analyzed longitudinally with a generalized estimating equation according to SNP genotype are shown in Fig. 1. The prevalence of type 2 DM was greater in subjects with the *CC* genotype of rs2116519 of *FAM78B* compared to the combined group of subjects with the *TT* or *TC* genotypes from 40 to 90 years of age (Fig. 1A), in the combined group of subjects with the *AG* or *GG* genotypes of rs2074379 of *ALPK1* compared to those with the *AA* genotype (Fig. 1B) and in the combined group of subjects with the *AG* or *GG* genotypes of

Table I. Characteristics of the subjects with type 2 diabetes mellitus and controls: Cross-sectional analysis in March 2014.

Parameter	Diabetes mellitus (n)	Controls (n)	P-value
No. of subjects	797	5230	
Age, years	61.9±10.5 (797)	53.0±12.9 (5230)	<0.0001
Gender, % (male/female)	70.5/29.5 (797)	53.3/46.7 (5230)	<0.0001
Height, cm	162.6±9.7 (760)	162.5±9.1 (5194)	0.7605
Weight, kg	64.6±13.5 (758)	60.4±11.8 (5194)	<0.0001
Body mass index, kg/m^2	24.3±3.9 (758)	22.8±3.3 (5194)	<0.0001
Waist circumference, cm	84.9±9.9 (683)	79.8±9.0 (4922)	<0.0001
Alcohol drinking, %	50.2 (797)	48.0 (5230)	0.2437
Current or former smoking, %	55.5 (797)	44.2 (5230)	<0.0001
Systolic blood pressure, mmHg	127±18 (753)	120±16 (5192)	<0.0001
Diastolic blood pressure, mmHg	77±12 (753)	74±12 (5192)	<0.0001
Mean blood pressure, mmHg	94±13 (753)	89±12 (5192)	<0.0001
Ocular tension, right, mmHg	14.1±3.2 (246)	13.4±2.9 (1815)	0.0005
Functional vital capacity, l	3.14±0.78 (255)	3.32±0.81 (1988)	0.0009
FEV1%	80.4±6.3 (255)	81.4±6.6 (1988)	0.0287
Serum albumin, g/l	44.1±3.6 (613)	44.7±2.5 (3599)	<0.0001
Serum total cholesterol, mmol/l	5.10±1.00 (784)	5.23±0.87 (5166)	0.0001
Serum triglycerides, mmol/l	1.49±1.06 (772)	1.23±0.82 (5164)	<0.0001
Serum HDL-cholesterol, mmol/l	1.51±0.42 (771)	1.68±0.45 (5163)	<0.0001
Serum LDL-cholesterol, mmol/l	3.13±0.87 (770)	3.18±0.79 (5162)	0.1294
Fasting plasma glucose, mg/dl	132.4±40.3 (789)	95.8±8.6 (5167)	<0.0001
Blood hemoglobin A_{1c}, %	6.65±1.27 (621)	5.54±0.33 (3842)	<0.0001
Blood urea nitrogen, mmol/l	6.27±3.48 (612)	5.03±1.54 (3489)	<0.0001
Serum creatinine, μmol/l	109.5±182.9 (767)	68.3±45.3 (4809)	<0.0001
eGFR, ml min^{-1} 1.73 m^{-2}	71.2±23.6 (767)	77.5±15.2 (4809)	<0.0001
Serum uric acid, μmol/l	340±84 (759)	324±86 (4772)	<0.0001
Serum C-reactive protein, μg/l	2515±14479 (295)	981±3758 (1818)	0.0001
White blood cells, $10^3/\mu$l	5.90±2.19 (554)	5.31±1.57 (4053)	<0.0001
Red blood cells, $10^4/\mu$l	438±50 (556)	437±44 (4067)	0.4542
Hemoglobin, g/l	139±18 (556)	138±15 (4067)	0.0549
Hematocrit, %	40.6±4.8 (555)	40.2±4.2 (4063)	0.0656
Platelets, $10^4/\mu$l	21.0±5.9 (551)	22.5±5.3 (4017)	<0.0001

Quantitative data are mean ± standard deviation. FEV1, forced expiratory volume in 1 sec; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate (ml min^{-1} 1.73 m^{-2}) = 194 x [age (years)]$^{-0.287}$ x [serum creatinine (mg/dl)]$^{-1.094}$ x [0.739 if female].

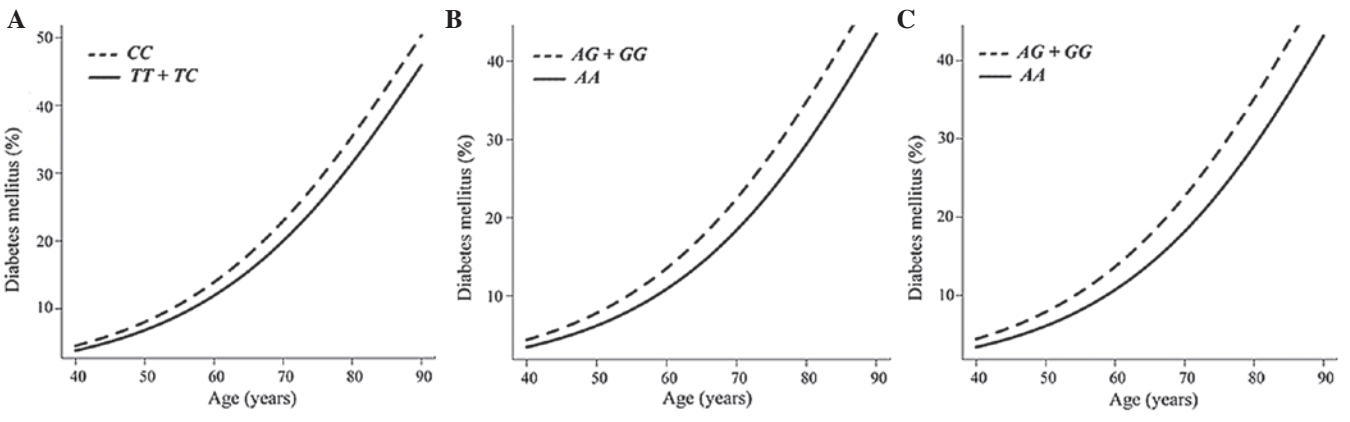

Figure 1. Longitudinal analysis of the associations between the prevalence of type 2 diabetes mellitus and age according to the genotype for (A) rs2116519 of *FAM78B* (*TT + TC* vs. *CC*), (B) rs2074379 of *ALPK1* (*AA* vs. *AG + GG*) or (C) rs2074388 of *ALPK1* (*AA* vs. *AG + GG*), with a generalized estimating equation.

Table II. Associations of polymorphisms with type 2 diabetes mellitus analyzed for 5-year longitudinal data with a generalized estimating equation.

Gene or locus	SNP	Genotype	Diabetes mellitus, n (%)	Control, n (%)	P-value (dominant)[a]	P-value (recessive)[b]
FAM78B	rs2116519 (C→T)	*TT*	553 (29.0)	5783 (31.0)	0.0648	0.0188[c]
		TC	950 (49.8)	9438 (50.5)		
		CC	403 (21.1)	3459 (18.5)		
3q28	rs9846911 (A→G)	*AA*	1642 (86.1)	16272 (87.1)	0.7924	0.9172
		AG	251 (13.2)	2304 (12.3)		
		GG	13 (0.7)	104 (0.6)		
ALPK1	rs2074379 (G→A)	*AA*	807 (42.3)	8680 (46.5)	0.0121[c]	0.2027
		AG	901 (47.3)	8180 (43.8)		
		GG	198 (10.4)	1820 (9.7)		
ALPK1	rs2074380 (G→A)	*GG*	1613 (84.6)	15797 (84.6)	0.6579	0.3014
		GA	287 (15.1)	2736 (14.6)		
		AA	6 (0.3)	147 (0.8)		
ALPK1	rs2074381 (A→G)	*AA*	1646 (86.4)	15937 (85.3)	0.4558	0.1330
		AG	258 (13.5)	2622 (14.0)		
		GG	2 (0.1)	121 (0.6)		
ALPK1	rs2074388 (A→G)	*AA*	799 (41.9)	8687 (46.5)	0.0053[c]	0.1492
		AG	906 (47.5)	8169 (43.7)		
		GG	201 (10.5)	1824 (9.8)		
BTN2A1	rs6929846 (T→C)	*CC*	1507 (79.1)	14566 (78.0)	0.6322	0.9904
		CT	373 (19.6)	3850 (20.6)		
		TT	26 (1.4)	264 (1.4)		
THBS2	rs8089 (T→G)	*TT*	1559 (81.8)	15393 (82.4)	0.7768	0.4105
		TG	329 (17.3)	3120 (16.7)		
		GG	18 (0.9)	167 (0.9)		
PDX1	rs146021107 (G→-)	*GG*	540 (28.3)	5303 (28.4)	0.9381	0.6936
		G-	924 (48.5)	9284 (49.7)		
		--	442 (23.2)	4093 (21.9)		
F7	rs6046 (G→A)	*GG*	1665 (87.4)	16285 (87.2)	0.6075	0.1153
		GA	234 (12.3)	2313 (12.4)		
		AA	7 (0.4)	82 (0.4)		
LLGL2	rs1671021 (G→A)	*AA*	1393 (73.1)	13813 (73.9)	0.7072	0.5812
		AG	487 (25.6)	4490 (24.0)		
		GG	26 (1.4)	377 (2.0)		
ILF3	rs2569512 (G→A)	*GG*	834 (43.8)	8200 (43.9)	0.8400	0.4642
		GA	878 (46.1)	8442 (45.2)		
		AA	194 (10.2)	2038 (10.9)		
CELSR1	rs6007897 (C→T)	*TT*	1838 (96.4)	18151 (97.2)	0.1157	ND
		TC	68 (3.6)	529 (2.8)		
		CC	0 (0)	0 (0)		

[a]Dominant, AA vs. AB + BB (A, major allele; B, minor allele). [b]Recessive, AA + AB vs. BB. [c]P<0.05. Prevalence of type 2 diabetes mellitus was compared between two groups (dominant or recessive model) for each polymorphism with adjustment for age, gender and body mass index. SNP, single-nucleotide polymorphism; ND, not determined.

rs2074388 of *ALPK1* compared to those with the *AA* genotype (Fig. 1C).

As three SNPs were significantly associated with type 2 DM, the associations of these SNPs to fasting plasma glucose level or blood hemoglobin A_{1c} content in all the individuals or individuals not taking antidiabetic medication were analyzed with a generalized linear mixed-effect model and with adjustment for age, gender and BMI (Table III). The rs2116519 SNP

Table III. Associations of polymorphisms to fasting plasma glucose level or blood hemoglobin A_{1c} content in all individuals or individuals not taking antidiabetic medication, analyzed for 5-year longitudinal data with a generalized linear mixed-effect model.

Gene (SNP)	Dominant model[a]		P-value	Recessive model[a]		P-value
All individuals						
FAM78B (rs2116519, C→T)	*TT* (6336)	*TC* + *CC* (14250)		*TT* + *TC* (16724)	*CC* (3862)	
Fasting plasma glucose, mg/dl	99.9±16.4	100.5±18.3	0.0352[b]	100.3±17.5	100.5±19.0	0.2251
Blood hemoglobin A_{1c}, %	5.69±0.59	5.71±0.65	0.0065[b]	5.70±0.63	5.70±0.66	0.4079
ALPK1 (rs2074379, G→A)	*AA* (9484)	*AG* + *GG* (11099)		*AA* + *AG* (18568)	*GG* (2018)	
Fasting plasma glucose, mg/dl	99.8±15.7	100.8±19.4	0.0017[b]	100.3±17.6	101.0±19.0	0.5509
Blood hemoglobin A_{1c}, %	5.68±0.57	5.72±0.69	0.0090[b]	5.70±0.62	5.74±0.73	0.0502
ALPK1 (rs2074388, A→G)	*AA* (9486)	*AG* + *GG* (11100)		*AA* + *AG* (18561)	*GG* (2025)	
Fasting plasma glucose, mg/dl	99.8±15.6	100.8±19.4	0.0010[b]	100.3±17.6	101.2±19.1	0.4149
Blood hemoglobin A_{1c}, %	5.68±0.57	5.72±0.69	0.0079[b]	5.70±0.62	5.74±0.73	0.0417
Individuals without antidiabetic medication						
FAM78B, (rs2116519, C→T)	*TT* (6260)	*TC* + *CC* (14045)		*TT* + *TC* (16495)	*CC* (3810)	
Fasting plasma glucose, mg/dl	99.6±15.8	100.0±17.2	0.1087	99.8±16.6	99.9±17.6	0.2482
Blood hemoglobin A_{1c}, %	5.68±0.57	5.69±0.61	0.0470[b]	5.68±0.60	5.68±0.62	0.3992
ALPK1, (rs2074379, G→A)	*AA* (9370)	*AG* + *GG* (10935)		*AA* + *AG* (18318)	*GG* (1987)	
Fasting plasma glucose, mg/dl	99.4±15.0	100.2±18.1	0.0073[b]	99.8±16.6	100.5±18.3	0.5845
Blood hemoglobin A_{1c}, %	5.66±0.54	5.70±0.65	0.0142[b]	5.68±0.59	5.72±0.71	0.1134
ALPK1, (rs2074388, A→G)	*AA* (9369)	*AG* + *GG* (10936)		*AA* + *AG* (18311)	*GG* (1994)	
Fasting plasma glucose, mg/dl	99.4±15.0	100.3±18.2	0.0042[b]	99.8±16.6	100.6±18.3	0.4372
Blood hemoglobin A_{1c}, %	5.66±0.54	5.70±0.65	0.0126[b]	5.68±0.59	5.72±0.71	0.0947

[a]Values in parentheses are numbers of measurements. [b]P<0.05. Data for fasting plasma glucose level and blood hemoglobin A_{1c} content are means ± standard deviation. Fasting plasma glucose level and blood hemoglobin A_{1c} content were compared between two groups (dominant or recessive model) for each polymorphism with adjustment for age, gender and body mass index. SNP, single-nucleotide polymorphism.

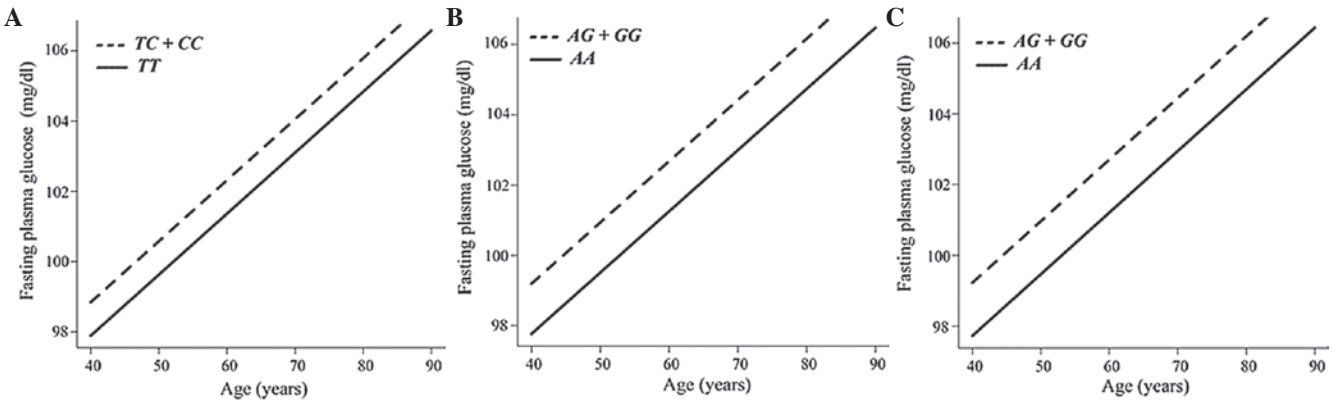

Figure 2. Longitudinal analysis of the associations between fasting plasma glucose level and age according to the genotype for (A) rs2116519 of *FAM78B* (*TT* + *TC* vs. *CC*), (B) rs2074379 of *ALPK1* (*AA* vs. *AG* + *GG*) or (C) rs2074388 of *ALPK1* (*AA* vs. *AG* + *GG*), with a generalized linear mixed-effect model among all the individuals.

of *FAM78B*, as well as rs2074379 and rs2074388 of *ALPK1* were significantly (P<0.05) associated with fasting plasma glucose level and blood hemoglobin A_{1c} content in a dominant model among all the individuals. Among individuals not taking antidiabetic medication, rs2116519 of *FAM78B* was significantly associated with blood hemoglobin A_{1c} content in a dominant model, whereas rs2074379 and rs2074388 of *ALPK1* were significantly associated with fasting plasma glucose level and blood hemoglobin A_{1c} content in a dominant model.

The associations between fasting plasma glucose level and age analyzed longitudinally according to genotype in all the individuals with a generalized linear mixed-effect model are shown in Fig. 2. Fasting plasma glucose level was greater in the combined group of individuals with the *TC* or *CC* genotypes of

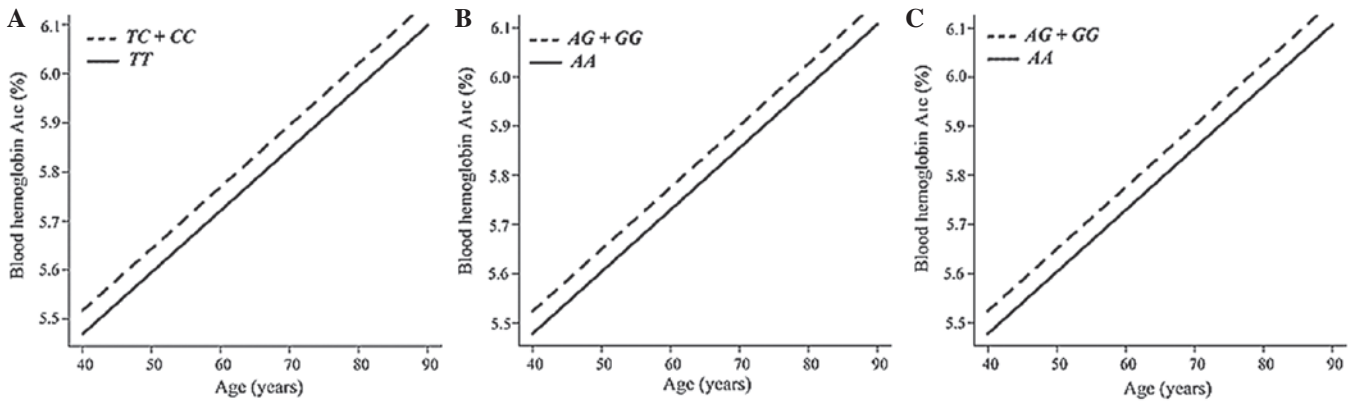

Figure 3. Longitudinal analysis of the associations between blood hemoglobin A_{1c} content and age according to the genotype for (A) rs2116519 of *FAM78B* (*TT* + *TC* vs. *CC*), (B) rs2074379 of *ALPK1* (*AA* vs. *AG* + *GG*) or (C) rs2074388 of *ALPK1* (*AA* vs. *AG* + *GG*,), with a generalized linear mixed-effect model among all the individuals.

rs2116519 of *FAM78B* compared to those with the *TT* genotype from 40 to 90 years of age (Fig. 2A), in the combined group of individuals with the *AG* or *GG* genotypes of rs2074379 of *ALPK1* compared to those with the *AA* genotype (Fig. 2B) and in the combined group of individuals with the *AG* or *GG* genotypes of rs2074388 of *ALPK1* compared to those with the *AA* genotype (Fig. 2C).

The associations between blood hemoglobin A_{1c} content and age analyzed longitudinally according to genotype in all the individuals with a generalized linear mixed-effect model are shown in Fig. 3. Blood hemoglobin A_{1c} was greater in the combined group of individuals with the *TC* or *CC* genotypes of rs2116519 of *FAM78B* compared to those with the *TT* genotype from 40 to 90 years of age (Fig. 3A), in the combined group of individuals with the *AG* or *GG* genotypes of rs2074379 of *ALPK1* compared to those with the *AA* genotype (Fig. 3B) and in the combined group of individuals with the *AG* or *GG* genotypes of rs2074388 of *ALPK1* compared to those with the *AA* genotype (Fig. 3C).

Discussion

As genetic factors and interactions between multiple genes and environmental factors are important in the development of type 2 DM (1,7), prediction of the risk for type 2 DM on the basis of genetic variants would be beneficial for personalized prevention of this condition. In the present study, rs2074379 and rs2074388 of *ALPK1* were significantly associated with the prevalence of type 2 DM in a longitudinal genetic epidemiological study, with the minor *G* allele of each SNP representing a risk factor for this condition. Our previous study showed that *ALPK1* is a susceptibility locus for chronic kidney disease in individuals with DM by a GWAS (20). We also observed that genetic variants of *ALPK1* were associated with type 2 DM in a previous cross-sectional analysis of the Inabe Health and Longevity Study (28). The present results in the longitudinal population-based study are consistent with the previous observations in the cross-sectional study (28) and they validate the association of genetic variants of *ALPK1* with type 2 DM.

ALPK1 functions in apical transport by phosphorylating myosin 1a in epithelial cells and is indicated in the regulation of intracellular trafficking processes by phosphorylation (38). 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 (extracellular signal-regulated kinase 1/2 and p38) signaling in cultured HEK293 cells, suggesting that ALPK1 may contribute to the inflammatory process associated with the development of gout (39).

Impaired insulin secretion and increased insulin resistance are key components of type 2 DM (40). Although the contributions of these factors to the onset and progression of type 2 DM may differ between Caucasian and Asian populations, the two factors are significant for diagnostic and therapeutic strategies targeted to this disease (41). Previous studies have shown that proinflammatory cytokines (interleukin-1β and tumor necrosis factor) detrimentally affect insulin secretion and resistance (42,43). Additionally, signaling pathways activated by proinflammatory cytokines, including those mediated by nuclear factor-κB, have been identified to impair insulin secretion or to promote insulin resistance (44). As chronic inflammation may play an important role in the development of type 2 DM, the effects of rs2074379 and rs2074388 of *ALPK1* on the inflammatory process may account for the association of this gene with type 2 DM.

rs2116519 of *FAM78B* was also associated with the prevalence of type 2 DM, as well as to fasting plasma glucose level and blood hemoglobin A_{1c} content among all the individuals or to blood hemoglobin A_{1c} content among the individuals not taking antidiabetic medication. *FAM78B* is located at chromosome 1q24.1, a region previously suggested to harbor a susceptibility locus for type 2 DM (45), although the function of this gene remains unclear.

There are certain limitations to the present study: i) As the results were not replicated, validation of these findings requires their replication with other independent subject panels or ethnic groups; ii) rs2074379 or rs2074388 are possibly in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are responsible for the development of type 2 DM; and iii) the functional relevance of rs2074379 or rs2074388 of *ALPK1* to the pathogenesis of type 2 DM has not been determined.

In conclusion, the present results suggest that *ALPK1* is a susceptibility gene for type 2 DM in community-dwelling

Japanese individuals. Determination of genotypes for the polymorphisms of *ALPK1* may prove informative for assessment of the genetic risk for type 2 DM in the Japanese population.

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