Dual matrilineal geographic distribution of Korean type 2 diabetes mellitus-associated -11,377 G adiponectin allele

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Abstract. The present study was performed to identify the susceptible single nucleotide polymorphisms (SNPs) for the prediction of Korean type 2 diabetes mellitus (T2DM) and to clarify the matrilineal origin of Korean T2DM-specific SNPs. Fourteen SNPs from the adiponectin (*ADIPOQ*), hepatocyte nuclear factor 4α, phosphoenolpyruvate carboxykinase 1 and glucokinase genes in the Korean population were analyzed. Only one SNP, -11,377 C/G on the *ADIPOQ* gene, was finally determined to be responsible for the incidence of Korean T2DM (P=0.028). The G-T-T-A haplotype at positions -11,377, +45, +276 and +349 on the *ADIPOQ* gene was also associated with a high incidence of Korean T2DM (P=0.023). In addition, the susceptibility of Korean individuals to T2DM appears to be affected by their matrilineal origin. Of note, the group of Southern origin, consisting of mitochondrial DNA macrohaplogroups F and R, was predisposed to T2DM, whereas the group of Northern origin, consisting of haplogroups A and Y, was resistant to T2DM. This implied that the differential genetics between the two groups, which were formed from the initial peopling of the proto-Korean population via Southern and Northern routes to the present time, may explain their differing susceptibility to T2DM. In conclusion, from Southern Asia Northward, a matrilineal origin of Korean individuals appears to be responsible for the prevalence of Korean T2DM caused by the -11,377 G allele.

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Introduction

Type 2 diabetes mellitus (T2DM), one of the most common types of diabetes, is a multifactorial disease that appears to differ in prevalence based on ethnicity. To date, ~40 susceptibility loci for T2DM have been discovered through genome-wide association studies (1-3); however, the majority of the genetic loci have been investigated in Caucasian European subjects and the results are not always replicable in other ethnic groups, including Asian populations. For example, +45 T/G and +276 G/T single nucleotide polymorphisms (SNPs) of adiponectin (*ADIPOQ, 3q27*) are highly involved SNPs as candidate risk variants for T2DM in Asian populations, whereas these same SNPs are not correlated with a risk of T2DM in Europeans, including Italian, French and Swedish individuals (4-8). Gong *et al* (9) described in detail the ethnic differences in SNPs (-11,391 A/G and -11,377 C/G of *ADIPOQ*) associated with T2DM on the basis of published data obtained from MEDLINE, EMBASE and Science citation index expanded databases. According to this study, a G allele at the position -11,377 of *ADIPOQ* increased the risk of T2DM in European populations, but did not appear to affect the incidence of T2DM in Asian individuals. Therefore, the results from European studies do not appear to coincide with those from other ethnic groups with different genetic backgrounds and environmental factors. Gong *et al* (9) suggested that ethnic differences in the -11,377 G *ADIPOQ* allele in correlation with the susceptibility to T2DM may be due to the combined effect of the subjects' discrete genetic components and somatic compliance affected by environmental factors, as well as the lower size and heterogeneity of the samples examined, which had been formed over long period from initial peopling to the ethnic distribution of the present time. However, the effect of human migration on the peopling of ethnic groups and the formation of ethnicity-specific genetic components has been underestimated in the majority of the association studies between SNPs and the susceptibility to certain diseases, including diabetes.

Gong et al (9) performed a meta-analysis for the association between *ADIPOQ* SNPs and the incidence of T2DM using data from a large population of European and Asian subjects

who had already been screened for SNPs susceptible to T2DM in several studies. The authors proposed that there existed a statistically significant heterogeneity (P=0.043) in seven Asian studies consisting of Chinese and Japanese populations, and a considerable level of homogeneity (P=0.133) was prominent in six European studies, including British, French, German, Swedish and Swiss subjects. The -11,377 G *ADIPOQ* allele was proven to be closely correlated with T2DM in the majority of the European populations screened, but demonstrated varied association with the incidence of T2DM in the Chinese and Japanese ethnic groups. A series of association studies for the identification of T2DM-associated SNPs in a Han Chinese population, which may have consisted of different ethnic groups each with a unique history of initial peopling and residing in different regions of China, produced contradictory results in linking ethnic-specific SNPs with the occurrence of T2DM, suggesting the importance of determining the ethnic origin of subjects in an association study (10). These findings implied that the ethnic-specific physical and genetic background during ethnogenesis may impact the susceptibility to T2DM, even in Asian populations who are expected to be genetically similar to each other, reflecting that the ethnic origin of T2DM-associated SNPs should be a concern when identifying the ethnic-specific SNPs responsible for T2DM. Therefore, it is apparent from several studies that the ethnogenesis of individuals, as well as the sample size and genome heterogeneity, need to be carefully considered in an association study.

To assess the correlation of the genotype as SNPs and haplotypes with the occurrence of T2DM, the present study aimed to identify Korean-specific SNPs and haplotypes, which are associated with a predisposition to T2DM, and to define the matrilineal origin of Korean-specific, T2DM-associated SNPs.

Materials and methods

Subjects. Blood samples were collected from 194 normal subjects and 328 T2DM patients who visited Chung-Ang University Hospital (Seoul, South Korea). Unassociated normal subjects were randomly recruited from a population undergoing routine health check-ups and diabetic subjects were randomly recruited from patients at the outpatient clinic of the Department of Metabolic Diseases (Chung-Ang University Hospital). The diabetes status was determined according to the World Health Organization criteria (11). Whole blood samples were collected from all of the subjects, and the fasting levels of glucose, insulin and glycated hemoglobin (HbA1c) were measured. All subjects enrolled in the present study were of full Korean ethnicity and their clinical characteristics are summarized in Table I. Written informed consent was obtained from all non-diabetic and diabetic subjects. The study procedures was approved by the Institutional Review Board of Chung-Ang University Hospital.

Genotyping of polymorphisms. Genomic DNA was extracted from blood samples with EDTA using the Qiagen DNA isolation kit (Qiagen, Chatsworth, CA, USA). The extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and its quality was assessed by agarose gel (Bioneer, Dajeon, South Korea) electrophoresis and staining with ethidium bromide (Bioneer). A total of nine sites on the nuclear genome (two sites on *ADIPOQ*, four sites on hepatocyte nuclear factor (*HNF)*4α, two sites on *PCK*1 and one site on *GCK*) that are already known to be associated with T2DM according to previous studies, were screened to identify mutations or polymorphisms by polymerase chain reaction (PCR)-directed sequencing (12-15). Primer information is presented in Table II. PCR was performed in 50 μl Accupower PCR premix (Bioneer). PCR cycling conditions were as follows: Denaturation at 94˚C for 40 sec, followed by 35 cycles of denaturation for 40 sec, annealing at 54˚C for 40 sec and extension at 72˚C for 40 sec, with a final extension at 72˚C. Following PCR amplification, the PCR products were purified with a Gel/PCR DNA Extraction kit (Real Biotech Corp., Taipei, Taiwan) and then sequenced by a Macrogen Service Center (Macrogen Corp., Seoul, Korea). To identify the SNPs, the sequencing results were analyzed using SeqMan^{TM}II software (DNASTAR, Madison, WI, USA).

Mitochondrial (mt)DNA haplogroup analysis. To determine the mtDNA haplogroup of Korean subjects with the -11,377 G *ADIPOQ* allele, hypervariable region 1 (HV1) and HV2 were analyzed for their DNA sequence. PCR was firstly performed for DNA sequencing using the following conditions: 95˚C for 10 min, 40 cycles of 30 sec at 95˚C, 1 min at the annealing temperature, 1 min at 72˚C, followed by a final extension step of 7 min at 72˚C. Following PCR amplification, PCR products were purified with a Gel/PCR DNA Extraction kit (Real Biotech Corp.) and subjected to TA cloning. Four colonies obtained from TA cloning products were sequenced by a Macrogen Service Center (Macrogen Corp.). Sequencing results were analyzed using SeqMan^{TM}II software (DNASTAR) and compared with the revised Cambridge reference sequence (rCRS) (16). Haplogroups were established with HV1 and HV2 polymorphisms using the well-established web-based programs, Haplogroup Prediction tool and mtDNA Manager (http://mtmanager.yonsei.ac.kr).

Statistical analysis. The frequencies of genotypes or alleles were compared by χ^2 analysis. The odds ratios (OR) and 95% confidence intervals (CIs) with adjustment for age and sex were calculated by logistic regression analysis. P<0.05 was considered to indicate a statistically significant result. Association analyses for allele types and genotypes were conducted using SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) statistics were obtained through the use of Haploview software version 3.2 (Broad Institute, Cambridge, MA, USA) and the Haplo.stats Package for R was used to predict haplotypes and to directly estimate their frequencies for the haplotype association with T2DM (17). Haplo.stats analysis was restricted to haplotypes with an inferred frequency >1%. Principal component analysis (PCA) was performed with the mtDNA haplogroup frequencies of 33 Asian populations using SPSS software, which were obtained from the literature and the data of the present study (18-27).

Table I. Clinical characteristics of Korean T2DM patients and normal control subjects.

Characteristic	T2DM subjects		Normal subjects	
	Male (n=120)	Female (n=208)	Male (n=85)	Female (n=109)
Fasting blood glucose (mg/dl)	140±45.8	125.7±47.2	93.4±9.4	90.56±7.7
Total cholesterol (mg/dl)	176.3±40.7	190.9±38.9	184.9±25.1	185.5±27.3
HDL-cholesterol (mg/dl)	44.5±11	49.5±12.4	50.3±9.9	55.4±11.6
LDL-cholesterol (mg/dl)	103.9±31.9	113.5±30.5	114±23.5	110.1±24.7
HbA1c (%)	7.2±1.6	6.8±1.5	5.1±0.5	4.8±1.3
Triglycerides (mg/dl)	158.6±137.1	114.9±66.7	90±28.6	83.3±34.6

T2DM, type 2 diabetes mellitus; DHL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated hemoglobin.

Table II. Primers for PCR amplification of T2DM-associated genes.

Gene	Primer name	Locus	Primer sequence	Product size (bp)
ADIPOQ	A_pr	Promoter	5'-CACTTGCCCTGCCTCTGTC-3'	
			5'-TATGCCTATTCTGTCTCTTGTGGA-3'	296
	A_ex	Exon 2 and intron 3	5'-CATGGGAAAATTAGAGGAGTGTC-3'	
			5'-GGAATAGGGATGAGGGTGAAGATG-3'	767
PCK1	P_J1	Promoter	5'-GCCAAGCCCCCTCCACTCCCTAAA-3'	428
			5'-CGCCCCCTGCCCCGATCTAAAC-3'	
	P_J2	Exon	5'-GGGGCAGGGCTATTCTTTTCCACA-3'	596
			5'-GGGGGCCGAGCTTACCAGTTG-3'	
HNF-4α	H_J1	P2 promoter	5'-CCGCTTTCATTCGTCTCATAACAG-3'	387
			5'-GAGACCGAGGCAGAGATAGAGAAA-3'	
	H_J2	P1 promoter	5'-CCCGCCGGTATTTGGTCTC-3'	358
			5'-TCTGCCCTGCTTAGCTTTATTCTG-3'	
	H_J4	Intron 1	5'-CTCCCAGGCCCTTCCAGTCC-3'	368
			5'-TCACCCCACCCATACAACCTACAT-3'	
	H_J5	Intron 2	5'-GCTCATGGCCCCAAGGTCTGTCT-3'	345
			5'-AACGGCCTGGGGTCAATCTCCTAT-3'	
GCK	G_pr	Promoter	5'-GACTGTGTCTCTCACATCCT-4'	258
			5'-TAAGGCGATTGAGTGGTC-4'	
HV1	FN15977	15977	5'-CCACCATTAGCACCCAAAGC-4'	423
	RN16399	16399	5'-TCAAGGGACCCCTATCTGA-4'	
HV2	FN29	29	5'-CTCACGGGAGCTCTCCAT-4'	353
	R381	381	5'-GCTGGTGTTAGGGTTCTTTG-4'	

T2DM, type 2 diabetes mellitus; *ADIPOQ*, adiponectin; HNF, hepatocyte nuclear factor; PCK1, phosphoenolpyruvate carboxykinase 1; GCK, glucokinase; HV, hypervariable region; PCR, polymerase chain reaction.

Results

Identification of T2DM-associated SNPs in a Korean population. DNA sequences of nine regions on *ADIPOQ*, *HNF4α*, phosphoenolpyruvate carboxykinase (*PCK*)1 and glucokinase (*GCK*) genes that are well established as closely associated with the incidence of T2DM in several ethnic groups, were analyzed to identify the SNPs susceptible to Korean T2DM. As a result, fourteen SNPs of the four genes were identified in 328 Korean T2DM patients and 194 normal individuals, and statistically analyzed to define a correlation with the risk of T2DM (Table III).

The fourteen SNPs were located in variable regions, including the promoter, exon and intron of each gene (Table III). Five SNPs from *ADIPOQ* were separately located on the promoter (-11,426 and -11,377), exon 2 (+45), and intron 2 (+276 and +349) regions, and seven SNPs from *HNF4α* were on the promoter (-4031, -3927 and +43582), intron 1 (+45980

Table III. Allele frequencies of SNPs in Korean T2DM subjects and normal individuals.

dbSNPs	Genes (locations)	Alleles	Allele frequencies				Genotypes	Genotype frequencies		*HWE* P-value
			T2DM	Normal	OR (95%CI)	P-value		OR (95%CI)	P-value	
rs16861194	*ADIPOQ*	A	0.77	0.75	0.98.	0.882	AA	0.70	0.419	0.992
	-11,426	G	0.23	0.25	(0.71-1.34)		AG and GG	(0.30-1.66)		
rs266729	*ADIPOQ*	C	0.73	0.80	1.51	0.028[a]	CC	1.75	0.017[a]	0.158
	-11,377	G	0.27	0.20	(1.05-2.18)		CG and GG	(1.10-2.76)		
rs2241766	*ADIPOQ*	G	0.72	0.67	1.10	0.567	GG	0.87	0.553	0.662
	+45	T	0.28	0.33	(0.79-1.55)		GT and TT	(0.56-1.36)		
rs1501299	*ADIPOQ*	G	0.69	0.71	1.14	0.441	GG	1.08	0.741	0.206
	+276	T	0.31	0.29	(0.82-1.60)		GT and TT	(0.69-1.68)		
rs2241767	*ADIPOQ*	A	0.75	0.82	1.00	0.993	AA	1.17	0.514	<0.001
	+349	G	0.25	0.18	(0.69-1.45)		AG and GG	(0.73-1.86)		
rs1884613	*HNF4α*	C	0.49	0.51	1.03	0.852	CC	0.73	0.527	0.480
	-4,031	G	0.51	0.49	(0.75-1.41)		CG and GG	(0.28-1.91)		
rs1884614	*HNF4α*	C	0.49	0.53	1.33	0.077	CC	1.45	0.166	0.151
	-3,927	T	0.51	0.47	(0.97-1.81)		CT and TT	(0.86-2.45)		
rs2425640	*HNF4α*	G	0.78	0.77	0.96	0.813	GG	0.84	0.704	0.911
	+43,582	A	0.22	0.23	(0.70-1.32)		GA and AA	(0.34-2.06)		
rs2071197	*HNF4α*	G	0.53	0.52	1.00	0.994	GG	0.97	0.947	0.965
	+45,980	A	0.47	0.48	(0.73-1.36)		GA and AA	(0.37-2.52)		
rs2071198	*HNF4α*	A	0.98	0.98	1.40	0.541	AA	1.66	0.359	0.581
	+46,029	T	0.02	0.02	(0.48-4.08)		AT and TT	(0.52-5.34)		
rs3212183	*HNF4α*	T	0.93	0.92	1.09	0.625	TT	0.84	0.776	0.169
	+55,462	C	0.07	0.08	(0.78-1.51)		TC and CC	(0.25-2.85)		
rs3212184	*HNF4α*	C	0.97	0.98	1.18	0.342	CC	3.33	0.215	0.612
	+55,484	G	0.03	0.02	(0.84-1.64)		CG and GG	(0.50-22.34)		
rs1799884	*GCK*	G	0.83	0.81	0.97	0.845	GG	0.82	0.680	0.070
	-30	A	0.17	0.19	(0.70-1.33)		GA and AA	(0.33-2.07)		
rs1042521	*PCK1*	A	0.62	0.56	0.98	0.890	AA	1.18	0.500	0.327
	-232	G	0.38	0.44	(0.71-1.34)		AG and GG	(0.73-1.88)		

[a]Significant P-values (P<0.05). T2DM, type 2 diabetes mellitus; *ADIPOQ*, adiponectin; HWE, Hardy-Weinberg equilibrium; SNPs, single nucleotide polymorphisms; HNF, hepatocyte nuclear factor; PCK1, phosphoenolpyruvate carboxykinase 1; GCK, clucokinase; OR, odds ratio; 95% CI, 95% confidence interval.

and +46029) and intron 2 (+55462 and +55484) regions. The two remaining SNPs identified on *GCK* and *PCK1* were at the -30 and -232 sites on the promoter region of the each gene, respectively. All SNPs identified were concordant with variants that are well established as potential genetic makers correlated with the prevalence of T2DM in European and Japanese populations (6,7,28,29). The allele frequencies of the fourteen SNPs are provided in Table III. Among them, 13 SNPs, except +349 SNP on *ADIPOQ*, were in HWE. Severe violation of the HWE (P<0.001) on the +349 SNP was observed in the normal Korean group, while it was not observed in the T2DM patients.

To identify the Korean T2DM-specific SNPs, odds ratios (ORs) and 95% confidence intervals (CIs), according to the distribution of allelotypes, were calculated by multivariate statistical analysis, compensating for gender and age among the subjects examined (Table III). The results demonstrated that only one SNP, -11,377 C/G of *ADIPOQ*, was closely associated with the incidence of T2DM (P=0.028), implying that the -11,377 G allele may be a putative genetic marker to predict the predisposition of Korean individuals to T2DM. The correlation of the -11,377 G allele with the risk of Korean individuals for T2DM was confirmed by genotype analysis (Table III). According to the genotype analysis, Korean subjects of the CG or GG genotypes at the -11,377 site of *ADIPOQ* are 1.75x more vulnerable to developing T2DM than Korean individuals of the CC type (P=0.017; 95% CI, 1.10-2.76). However, all other SNPs (except for the SNP at the -11,377 site of *ADIPOQ*) demonstrated no statistical difference between T2DM and normal subjects (P>0.05).

Identification of the T2DM-associated ADIPOQ haplotype in a Korean population. Although none of the 13 SNPs except for -11,377 C/G of *ADIPOQ* were associated with T2DM, haplotypes, a set of closely linked SNPs on the same gene, may be

Table IV. *ADIPOQ* haplotypes and risk of T2DM in Korean individuals.

Haplotype -11,426/-11,377/+45/+276/+349	Freq.	OR	95% CI	P-value
+45/+276				
TG	0.395	1	Reference haplotype	
GG	0.302	1.02	(0.69-1.50)	0.929
TT	0.303	1.18	(0.81-1.73)	0.389
+45/+276/+349				
TGA	0.396	1	Reference haplotype	
GGA	0.078	0.67	(0.32-1.41)	0.293
GGG	0.223	1.08	(0.72-1.63)	0.712
TTA	0.304	1.17	(0.80-1.71)	0.423
-11,426/-11,377				
AC	0.529	1	Reference haplotype	
AG	0.232	0.91	(0.43-1.92)	0.803
GC	0.236	0.75	(0.34-1.68)	0.483
-11,377/+45/+276/+349				
CTGA	0.256	1	Reference haplotype	
CGGA	0.069	0.8	(0.33-1.93)	0.619
CGGG	0.206	1.26	(0.77-2.05)	0.353
CTTA	0.227	1.15	(0.69-1.89)	0.595
GGGG	0.016	1.03	(0.21-5.07)	0.969
GTGA	0.139	1.59	(0.87-2.89)	0.132
GTTA	0.0379	2.55	(1.14-5.73)	0.023[a]
-11,426/-11,377/+45/+276/+349				
ACTGA	0.241	1	Reference haplotype	
ACGGA	0.104	0.82	(0.20-3.37)	0.788
ACGGG	0.104	1.47	(0.26-8.19)	0.660
ACTTA	0.074	0.49	(0.11-2.21)	0.356
AGGGA	0.020	0.18	(0.01-4.25)	0.290
AGGGG	0.013	4.54	(0.02-896.53)	0.575
AGTGA	0.132	0.45	(0.13-1.53)	0.202
AGTTA	0.068	2.78	(0.36-21.27)	0.326
GCGGA	0.037	0.44	(0.04-4.53)	0.494
GCGGG	0.026	0.3	(0.02-3.76)	0.351
GCTGA	0.037	0.41	(0.04-4.15)	0.451
GCTTA	0.141	0.77	(0.23-2.59)	0.676

[a]Significant P-values (P<0.05); OR, odds ratio; 95% CI, 95% confidence interval; T2DM, type 2 diabetes mellitus; *ADIPOQ*, adiponectin; SNPs, single nucleotide polymorphisms.

more informative in studying T2DM in Korean individuals. All 12 SNPs on the *ADIPOQ* and *HNF4α* that were identified in the T2DM and normal groups were thus combined to determine the haploblock through LD analysis. In the case of *HNF4α*, a haploblock that consisted of -4,031 C/G and -3,927 was found to be highly linked to T2DM (D'=1), but haplotypes from various combinations of a total of seven SNPs, including two SNPs involved in the haploblock, were all proven not to be associated with the incidence of T2DM (data not shown). By contrast, the +45 T/G, +276 G/T and +349 A/G SNPs of *ADIPOQ* were found to be highly linked, as determined by the LD test (D'=1). There was low LD between the SNPs on the promoter and the SNPs on the exon and intron (Fig. 1). Considering the results of the LD analysis, haplotype analysis was performed for the haploblock combined with +45 T/G, +276 G/T and +349 A/G SNPs. The four haplotypes (T-G-A, G-G-A, G-G-G and T-T-A) with a frequency >1% were not associated with T2DM (Table IV). Although other SNPs were not in the high LD block, an additional 25 haplotypes with a frequency of >1% were detected in *ADIPOQ* through the Haplo Stats package for ambiguous haplotypes (Table IV). Of the haplotypes, the most common haplotype was handled as a reference haplotype to analyze the correlation of the haplotypes with Korean T2DM. The results of the haplotype analysis demonstrated that the G-T-T-A haplotype

Table V. Distribution of mitochondrial DNA haplogroup frequencies in Korean individuals with the -11,377 GG genotype of *ADIPOQ*.

Characteristic	Haplogroup	Sub-haplogroup	T2DM (n=23)	Normal (n=15)
Mostly found in Northeast Asia	A	A5a	-	1 (7%)
	D	D4 (D4, D4a, D4b1, D4b2b, D4c, D4e1, D4i, D4j*, D4j3, D4k)	8 (35%)	4 (27%)
		D5a2		
	G	G2a1	2 (9%)	1 (7%)
	Y	Y Y1	-	3 (20%)
		Sub total	10 (43%)	9 (60%)
Mostly found in Southeast Asia	M	M7 (M7a1, M7b2 M7c1)	7 (30%)	4 (27%)
		M51*		
		M37a		
		M10b		
	N	N9 (N9a, N9b)	1 (4%)	1 (7%)
	B	B4 (B4a, B4b1)	3 (13%)	1 (7%)
		B5b2		
	F	F3a	1 (4%)	-
	R	R30a*	1 (4%)	-
		Sub total	13 (57%)	6 (40%)

T2DM, type 2 diabetes mellitus; ADIPOQ, adiponectin.

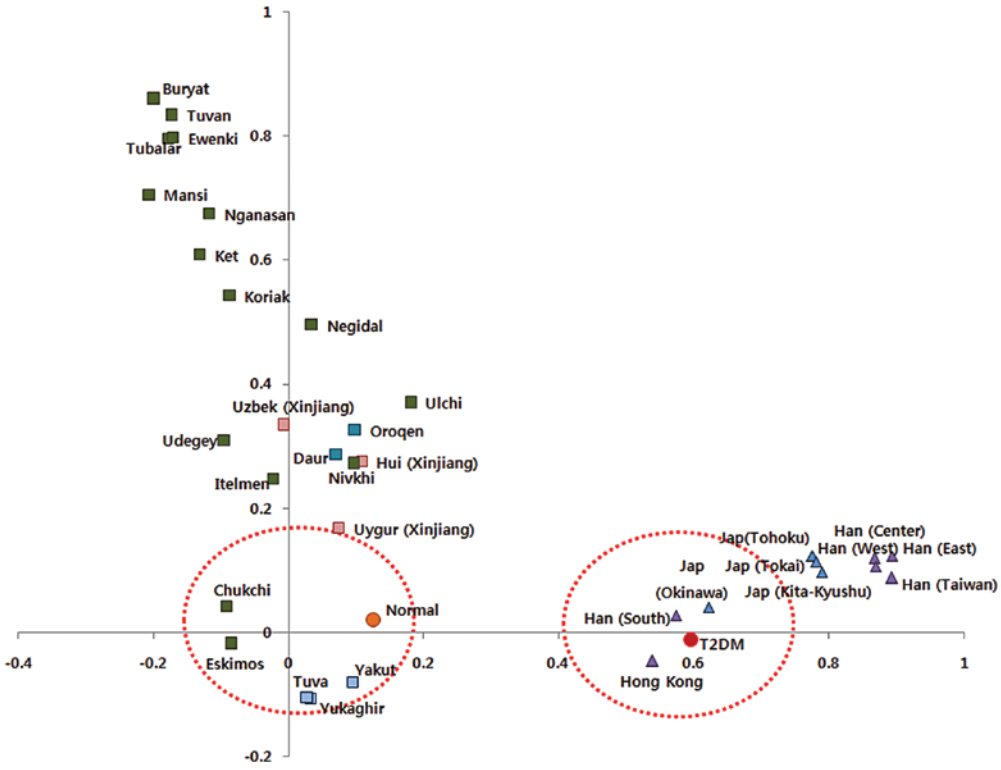

Figure 1. Principal component analysis of mtDNA haplogroup frequencies in Korean T2DM and normal subjects in 33 Asian populations. The Principal component analysis of mtDNA haplotype frequencies demonstrated that Southern Asian populations, including those from China, Okinawa and Hong Kong, were largely grouped with Korean T2DM subjects, whereas northern Asian populations, including Eskimo, Yakut and Chukchi, tended to be grouped with normal Korean subjects. T2DM, type 2 diabetes mellitus; mtDNA, mitochondrial DNA.

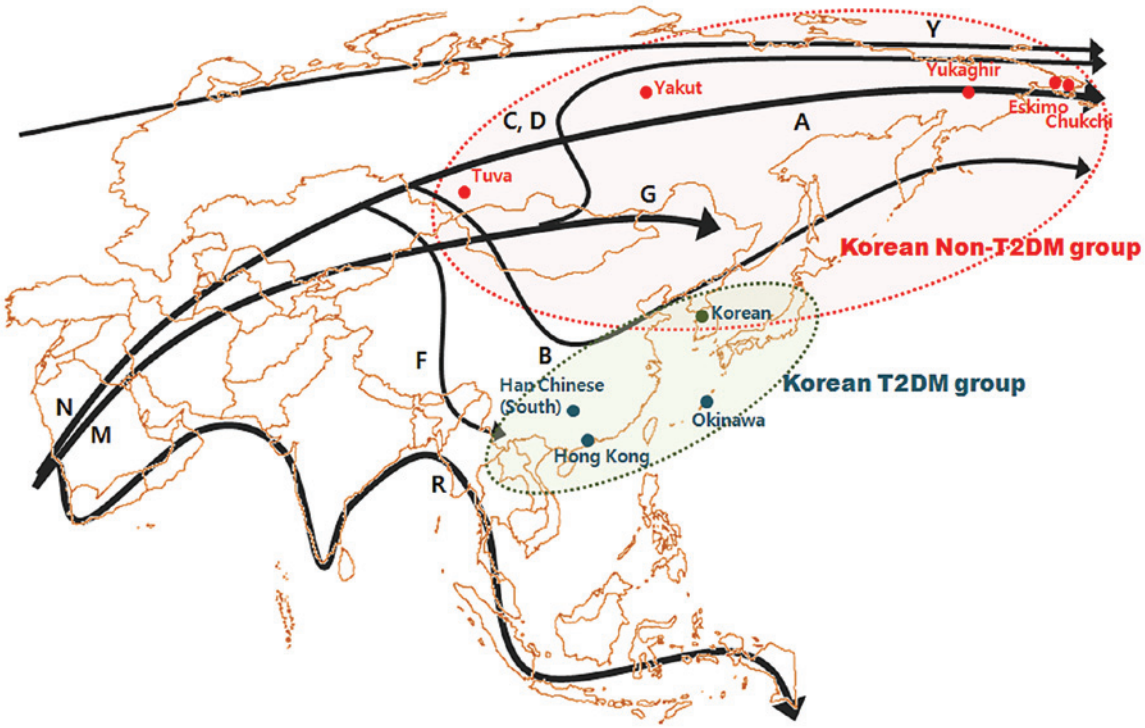

Figure 2. Migratory history and geographical distribution of mtDNA haplogroups attributed to peopling of the Korean population. The black arrows represent migration routes and divergence of the mtDNA haplogroup from Africa to Asia. Dotted lines represent areas that were proven to be correlated with normal and T2DM Korean groups based on the principal component analysis results. T2DM, type 2 diabetes mellitus; mtDNA, mitochondrial DNA.

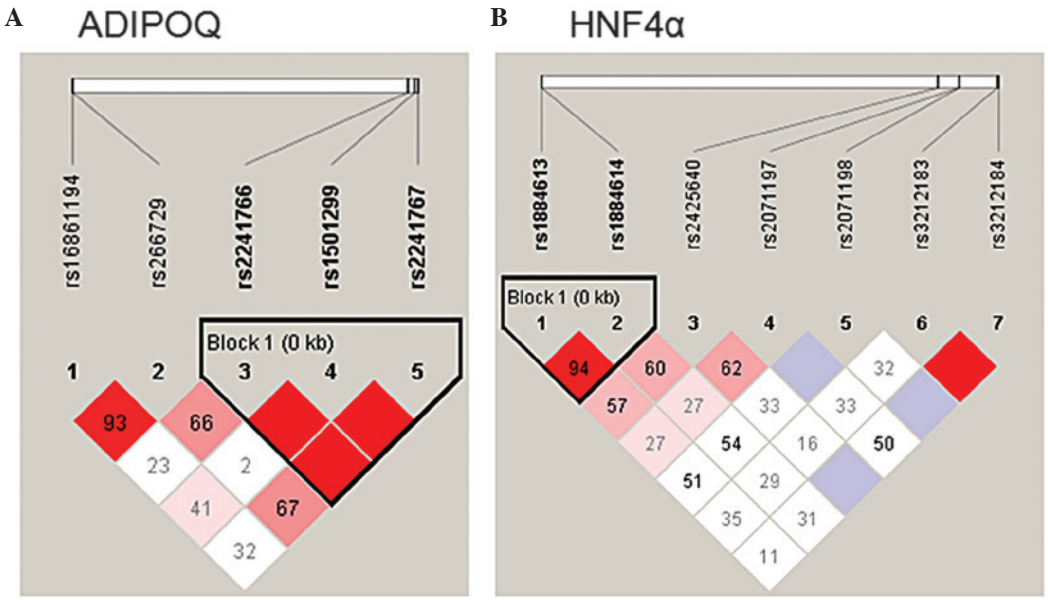

Figure 3. Linkage disequilibrium for the single nucleotide polymorphisms on (A) *ADIPOQ* and (B) *HNF4α* identified in Koreans. *ADIPOQ*, adiponectin; HNF, hepatocyte nuclear factor.

at the -11,377, +45, +276 and +394 SNP positions in *ADIPOQ* was closely correlated with Korean T2DM (OR, 2.55; 95% CI, 1.14-5.73; P=0.023). These results indicated that Koreans with the G-T-T-A haplotype of *ADIPOQ* are more susceptible to T2DM than Korean individuals with any other haplotype.

Dual matrilineal geographic distribution of the -11,377 GG genotype of ADIPOQ in a Korean population. As demonstrated in Tables III and IV, the -11,377 GG genotype of *ADIPOQ* was closely linked with Korean T2DM. Of the 38 subjects demonstrating the -11,377 GG genotype of *ADIPOQ*, 24 subjects were T2DM patients and 14 were T2DM negative. Korean-specific susceptibility to T2DM was determined by varied mutations on the genome that were accumulated during the formation of the Korean population. To analyze the cause of differential susceptibility to T2DM in Korean subjects with the identical

-11,377 GG genotype of *ADIPOQ*, the matrilineal origin of the 38 subjects was traced by determining haplogroups of mtDNA, which are diverged with consecutive mutational steps on the genome, and reliably reflect the ancient migration history and recent admixture of populations. The mtDNA haplogroups of 38 subjects were determined through sequencing of the HV1 (16024~16380) and HV2 (47~360) regions of mtDNA. The haplogroup frequencies of Korean T2DM and normal groups are summarized in Table V. Based on these haplogroup assignments, subjects were divided into two large lineages with the Southern (M7, N9, B, R and F) and the Northern (D, G, A and Y) haplogroup complexes of East Asia, reflecting that the Korean population was formed by a mixture of dual matrilineal origins via Northward and Southward routes.

Nine mtDNA haplogroups (A, D, G, Y, M, N, B, F and R) were identified in 38 subjects demonstrating the -11,377 GG genotype of *ADIPOQ*. The nine mtDNA haplogroups were divided into 15 subclades (A5a, D4, D5a2, G2a1, Y, Y1, M7, M51*, M37a, M10b, N9, B4, B5b2, F3a and R30a; Table V). Of them, the A, D and Y haplogroups were widespread in Northeast Asia, Northeast Siberia and America, representing the Northern origin of Asian populations, including Eskimo, Na-denes, Chukchis, Nivkhs and Ainus populations (30,31). A total of 19/38 subjects (50%) demonstrated these haplogroups. The M, N, B, F and R haplogroups are found in Southeast Asia, including Bangladesh, Indonesia, Malaysia, Vietnam and Japan (32), representing the Southern origin of the Asian population. Those haplogroups were also found in 19/38 subjects (50%) in the present study. This result is in concordance with the theory that the Korean population was formed by dual matrilineal origins via Northward and Southward routes (33,34). The Northern haplogroups account for 57 and 40% of the T2DM and normal groups, respectively, whereas the Southern haplogroups constituted 43 and 60%, respectively. In particular, haplogroups A5a and Y, which are mostly prevalent in Northeast Asia and Southeast Siberia, were found exclusively in the normal subjects (Table V). Southeast Asian-prevalent mtDNA lineages represented by haplogroups B, F3a and R were found almost exclusively in Korean subjects with T2DM (Table V). These findings suggested that a matrilineal lineage of Southern origin affects susceptibility to T2DM among Korean subjects with the -11,377 GG genotype of *ADIPOQ*.

In addition, haplogroups of the Korean subjects were investigated by principal component analysis (PCA) based on data retrieved from the literature (18-27), in which haplogroups of 33 Asian populations were investigated. In the PCA plot, Korean subjects with T2DM demonstrated a close correlation with South Asian populations, including China, Okinawa and Hong Kong individuals (Fig. 2), whereas normal Korean subjects were grouped together with Northern Asian populations, including Chukchi, Eskimo, Tuva, Yukaghir and Yakut. This result indicated that Koreans with an identical -11,377 GG *ADIPOQ* genotype would have a differential susceptibility to T2DM depending on matrilineal origin.

Discussion

ADIPOQ is located on chromosome 3q27 and encodes adiponectin proteins. Adiponectin is specifically and abundantly secreted by adipocytes and circulates in the blood at high concentrations (35). It is also known as 'adipocyte complement related protein 30' (*ACRP30*) and 'gelatin-binding protein 28' (*GBP28*) (36). It has previously been demonstrated that plasma concentrations of adiponectin are reduced in obese individuals, as compared with lean subjects (37), and increasing concentrations of adiponectin enhance insulin sensitivity by reducing the risk for T2DM (38,39). Therefore, *ADIPOQ* has been suggested as a causative gene for metabolic diseases, including T2DM.

The -11,377 C/G SNP of *ADIPOQ* is located in the promoter region of *ADIPOQ* and may have a functional role in the regulation of adiponectin expression (7,8). The promoter region of *ADIPOQ* has four SP1 binding sites that control *ADIPOQ* expression. The -11,377 C/G SNP is located in one of the SP1 binding sites on the *ADIPOQ* promoter. It alters the sequence for the SP1 binding site and thereby may inhibit promoter activity, resulting in a reduction of *ADIPOQ* expression (40,41).

Previously, numerous molecular epidemiological studies have been performed to identify the T2DM-specific *ADIPOQ* SNPs and their ethnic specificity. These investigations have identified T2DM-associated *ADIPOQ* SNPs, which are commonly found in several ethnic groups, including -11,377 C/G (rs266729), +45 T/G (rs2241766), +276 G/T (rs1501299) and +349 C/G (rs2241767). However, these SNPs, either independently or as a haplotype, do not always demonstrate a correlation with the occurrence of T2DM (42). In the present study, only one SNP, -11,377 C/G on the *ADIPOQ* gene, among the 14 SNPs identified from four genes, was closely associated with T2DM in Korean subjects. This result is consistent with previously reported results on Korean individuals with T2DM (43); however, these data are not concordant with those from studies on other Asian populations, including Japanese and Chinese (9). Rather, +45 T and +276 T alleles of *ADIPOQ*, which were proven not to be correlated with the incidence of Korean T2DM in the present study, were reported to demonstrate a strong correlation with T2DM in Japanese and Chinese individuals. That is, in several studies, the -11,377 G allele of *ADIPOQ* was not associated with T2DM in Japanese and Chinese subjects (28,44-47). Of note, in Caucasoid Europeans, including French and Swedish subjects, living geographically distant from East Asia and having genetic lineages that are different from those of mongoloids, including Japanese and Chinese populations who are expected to be physically and genetically similar to Koreans, the -11,377 G allele was found to be associated with T2DM, as is the case of the Korean subjects in the present study (6,7). Furthermore, 38 Korean subjects who were all expected to be T2DM patients since they all had the -11,377 G allele of *ADIPOQ* that inhibited promoter activity, were classified into two discrete groups: One for T2DM patients and the other for non-diabetic normal subjects. The results implied that the identical SNP on the *ADIPOQ* promoter may function differently in the occurrence of T2DM according to ethnic-specific genetic background, and ethnic-specific SNPs alone; therefore, it may be insufficient to predict the predisposition to T2DM in various ethnic groups, suggesting additional genetic markers are required.

Haplotype analysis of *ADIPOQ* has also been performed in several ethnic groups to determine susceptible haplotypes

associated with T2DM (45,48,49). The T-G haplotype at the +45 and +276 sites of *ADIPOQ* is most frequently reported as a genetic marker associated with T2DM in various ethnic groups, but the correlation between the haplotype and the incidence of T2DM remains controversial (50,51). The present study identified that the T-G haplotype at positions +45 and +276 is not associated with the prevalence of Korean T2DM. By contrast, the present haplotype analysis, using various combinations of SNPs that were supposed to be associated with the incidence of T2DM, demonstrated that the G-T-T-A haplotype at SNP positions -11,377, +45, +276 and +349 is closely associated with T2DM in Korean subjects. These results strongly suggested that population ethnicity should be considered to identify the specific SNP or haplotype as a pre-diagnostic marker for T2DM prevalence.

The present study focused on matrilineal migration in the peopling of the Korean population as a candidate factor involved in the differential susceptibility of SNPs associated with T2DM. Koreans geographically belong to a Northeast Asian group, and anthropological and archeological evidence suggests that the early Korean population was related to Mongolian ethnic groups who inhabited the broad area of the Altai Mountains and Lake Baikal regions of Southeast Siberia (52). However, recent studies through hierarchical genotyping of Y-chromosomal DNA and mtDNA, which are reliable indicators of migration events in the peopling history of populations, revealed that the Korean population contains lineages typical of both Southern and Northern East Asian populations, although genetic components of Koreans are overall more similar to those of the Northeast Asians than Southeast Asians (32,33). In the present study, the mtDNA haplogroup composition of Korean T2DM and normal subjects also demonstrated dual lineages, including Southeast and Northeast Asian lineages. T2DM patients were mainly composed of M, B, R and F haplogroups of the Southeast Asian-prevalent mtDNA lineages, whereas the haplogroup composition of normal individuals was skewed toward Northeast Asian-prevalent mtDNA lineages consisting of A, D, G and Y haplogroups. These findings suggested that a matrilineal lineage with Southern origin increases susceptibility to T2DM in Korean subjects with an identical -11,377 GG *ADIPOQ* genotype. Furthermore, the PCA results supported the finding that the Southern matrilineal origin of the -11,377 GG genotype affects the incidence of T2DM in Korean individuals. That is, Korean subjects with T2DM lay entirely within the cluster of Southeast Asian populations, including China, Okinawa and Hong Kong, whereas populations clustered with T2DM negative Korean subjects were predominantly distributed over Northeast Asia. This result also indicated that Korean subjects with the identical -11,377 GG genotype would have different susceptibilities to T2DM according to their matrilineal origin.

In conclusion, the -11,377 C/G SNP of *ADIPOQ* appeared to be closely associated with T2DM in Korean subjects and the G-T-T-A haplotype in *ADIPOQ*, a combination of four SNPs at the -11,377, +45, +276 and +349 sites, may be regarded as a predictive factor for Korean T2DM. Additionally, the prevalence of T2DM in Korean subjects demonstrating identical allelotypes (-11,377 G allele of *ADIPOQ*) appeared to be affected by their matrilineal origin.

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