Mitochondrial Haplogroup N9a Confers Resistance against Type 2 Diabetes in Asians

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Because mitochondria play pivotal roles in both insulin secretion from the pancreatic β cells and insulin resistance of skeletal muscles, we performed a large-scale association study to identify mitochondrial haplogroups that may confer resistance against or susceptibility to type 2 diabetes mellitus (T2DM). The study population comprised 2,906 unrelated Japanese individuals, including 1,289 patients with T2DM and 1,617 controls, and 1,365 unrelated Korean individuals, including 732 patients with T2DM and 633 controls. The genotypes for 25 polymorphisms in the coding region of the mitochondrial genome were determined, and the haplotypes were classified into 10 major haplogroups (i.e., F, B, A, N9a, M7a, M7b, G, D4a, D4b, and D5). Multivariate logistic-regression analysis with adjustment for age and sex revealed that the mitochondrial haplogroup N9a was significantly associated with resistance against T2DM (*P* = .0002) with an odds ratio of 0.55 (95% confidence interval 0.40–0.75). Even in the modern environment, which is often characterized by satiety and physical inactivity, this haplogroup might confer resistance against T2DM.

Type 2 diabetes mellitus (T2DM [MIM 125853]) is a complex disorder characterized by impaired insulin secretion from pancreatic β cells and reduced insulin action or insulin resistance in the peripheral tissue. There is a growing body of evidence indicating that mitochondrial dysfunction plays a pivotal role in β -cell dysfunction, as well as in insulin resistance. Mitochondrial metabolism, which produces ATP, is essential in insulin secretion through metabolism-secretion coupling.¹ A pancreatic β -cell line lacking mitochondrial function exhibits impaired insulin secretion,² and mice with pancreatic β -cell–specific knockout of mitochondrial transcription factor Tfam show a diabetic phenotype with severe mtDNA depletion.³ Decreased capacity of the mitochondrial oxidative phosphorylation (OXPHOS) is associated with the insulin resistance found in aged people and in offspring of individuals with T2DM.^{4,5} Microarray studies have shown that insulin resistance and T2DM are associated with decreased expression of genes related to OXPHOS in the skeletal muscle.^{6,7} Therefore, mitochondrial dysfunction can explain not only impaired insulin secretion but also reduced insulin action.

Proteins composing the mitochondrion are encoded by both nuclear DNA and mtDNA. The latter encodes 13 subunits of the OXPHOS machinery and also encodes 2 ribosomal RNA (rRNA) and 22 tRNA genes essential for the translation process in mitochondria.⁸ There are many

lines of evidence indicating that mtDNA is responsible for the pathogenesis of diabetes. A point mutation at nucleotide position 3243 in mitochondrial tRNA-Leu (UUR) is well known to cause maternally inherited diabetes and deafness, as well as mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike (MELAS) episodes in patients with high mutant loads.¹ However, it remains questionable whether mitochondrial dysfunction originating from common mtDNA polymorphisms is responsible for T2DM. In this regard, it should be noted that many epidemiologic studies have reported a maternal excess in the transmission of T2DM.9,10 In addition, a control-region polymorphism, such as the 16189T→C substitution in the noncoding region, is known to be associated with insulin resistance, obesity, and diabetes in both Europeans¹¹ and Asians.^{12,13} A meta-analysis of European studies, however, has indicated that genetic variation of the 16184-16193 poly-C tract is unlikely to have a major role in the cause of T2DM.¹⁴

The geographic region–specific variations of mtDNA haplogroups are now known to have been formed by natural selection, possibly to allow habitation in cold climatic environments.^{15,16} Although mtDNA variations might have permitted our ancestors to adapt to more-northern or colder climates, they are also suggested to play a detrimental role in modern human diseases related to bioenergetics or mitochondrial dysfunction.^{15–17} Therefore,

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some mtDNA haplogroup might actually confer susceptibility to T2DM. It has been very recently reported that there is no evidence of association between common mtDNA polymorphisms and T2DM, at least not in Europeans.¹⁸ Since Asians have different mtDNA haplogroups and since T2DM is the result of complex interactions between genes and the environment, the above finding cannot be extended to the Asian populations. In the present study, we performed a large-scale association study on T2DM and 10 major haplogroups in both Japan and Korea, on the basis of comprehensive analysis of polymorphisms in the coding region of the mitochondrial genome.

Material and Methods

Study Population

The study population comprised 2,906 Japanese and 1,365 Korean subjects. Unrelated Japanese individuals (1,938 men and 968 women) aged ≥40 years were enrolled from the population of individuals who had either visited outpatient clinics of or been admitted to one of the participating hospitals (Gifu Prefectural Gifu, Tajimi, and Gero Hotspring Hospitals) between October 2002 and March 2005. The patients with T2DM had a fasting plasma glucose (FPG) concentration of \geq 7.0 mmol/liter (126 mg/dl) and/or a blood glycosylated hemoglobin (HbA1c) level of \geq 6.5% or were taking antidiabetes medication. T2DM was defined according to the criteria accepted by the World Health Organization. Members of families with diabetes mellitus and sib pairs with this condition were excluded from the study. Although some of the patients with T2DM who were taking antidiabetes medication had a normal HbA1c level or a normal FPG concentration when the blood samples were obtained, they had exhibited abnormally high levels of HbA1c and FPG before starting the antidiabetes medication. We excluded the patients with type 1 diabetes who required insulin within 1 year after the initial diagnosis or episode of diabetic ketoacidosis.

On the basis of these criteria, 1,289 subjects (890 men and 399 women) in the Japanese study population were given diagnoses of T2DM. The control group comprised the remaining 1,617 individuals (1,048 men and 569 women) in the Japanese study population who visited the outpatient clinics of the participating hospitals for an annual health checkup. They had an FPG concentration of <6.1 mmol/liter (110 mg/dl) and a blood HbA1c level of <5.6%, and they had no history of T2DM or of taking antidiabetes medication. The study protocol was approved by the Committee on the Ethics of Human Research of Gifu International Institute of Biotechnology, and written informed consent was obtained from each participant.

Unrelated Korean patients with T2DM were enrolled from the Diabetes Clinic of Seoul National University Hospital (n = 732). Control subjects without diabetes were recruited from the group of individuals who had visited Seoul National University Hospital for a routine annual checkup (n = 633). T2DM was diagnosed according to World Health Organization criteria. Subjects with positive glutamic acid decarboxylase antibodies were excluded. The control subjects without diabetes were selected according to the following criteria: age ≥ 60 years, no past history of diabetes, no diabetes in first-degree relatives, an FPG concentration of <6.1 mmol/liter, and an HbA1c value of <5.8%. The Institutional Review Board of the Clinical Research Institute in Seoul National University Hospital approved the study protocol, and written in-

formed consent for genetic analysis was obtained from each subject. All study subjects were examined in the morning after an overnight fast. The clinical characteristics of Japanese and Korean subjects are shown in tables 1 and 2, respectively.

Selection of Mitochondrial Polymorphisms for Haplogroup Classification

In earlier studies, we aimed to identify mitochondrial SNPs (mtSNPs) associated with age-related conditions, such as longevity,¹⁹ Parkinson disease,^{20,21} and Alzheimer disease, as well as those related to energy metabolism—such as obesity,^{22,23} thinness, and T2DM²²—or to atherosclerosis. For this purpose, we sequenced the entire mitochondrial genomes of 672 individuals belonging to seven different groups, with 96 individuals in each group namely, centenarians, patients with Parkinson disease, patients with Alzheimer disease, young obese or nonobese males, and patients with T2DM with or without severe vascular involvement.²⁴ From our findings, we constructed a human mitochondrial genome polymorphism database (mtSNP). On the basis of these mtSNP data, we have developed a comprehensive mtSNP analysis system that uses fluorescent beads.

By using our mtSNP database and a phylogenetic tree of the Japanese,²⁴ we selected 149 polymorphic sites that have been useful for classification of mitochondrial haplogroups. We selected a further 25 mtSNPs that define 10 major haplogroups (i.e., F, B, A, N9a, M7a, M7b, G, D4a, D4b, and D5) found in this area (table 3). Then, we examined the relationship between these haplogroups and T2DM in the 4,271 participants.

Genotyping of Polymorphisms

Venous blood (7 ml) was collected from each subject into tubes containing 50 mmol/liter EDTA (disodium salt), and genomic DNA was isolated with the use of a commercial kit (Genomix [Talent]). For amplifying mtDNA fragments, we performed 28plex PCR. The reaction mixture (25 μ l) contained 1 ng of genomic DNA, 5 pmol of each primer, 0.2 mmol/liter of each deoxynucleoside triphosphate, 2 mmol/liter MgCl₂, and 1 U of DNA polymerase (FastStart Taq DNA Polymerase [Roche Diagnostics]) in the PCR buffer supplied by the manufacturer. The amplification protocol consisted of an initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 94°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 7 min. The primers used are shown in table 4. Mitochondrial polymorphisms were determined with sequencespecific oligonucleotide probes (G&G Science) by use of suspensionarray technology (Luminex 100 [Luminex]). The methodology used for genotyping was described in detail elsewhere.²⁵ Probes used for haplotyping are shown in tables 5 and 6. To confirm the accuracy of genotyping by this method, we subjected 91 DNA samples whose entire sequence of the mitochondrial genome had been determined by direct sequencing to the Luminex method. In each instance, the genotype determined by the Luminex sequence-specific oligonucleotide-hybridization assay system was identical to that determined by direct sequencing.

Statistical Analysis

Quantitative clinical data were compared between patients with diabetes and control individuals by use of the unpaired Student's *t* test. Qualitative data were compared using the χ^2 test. We performed multivariate logistic-regression analysis to adjust for risk

Table 1. Characteristics of Japanese Patients with T2DM and Controls

	All			Women			Men		
Variable	T2DM (<i>n</i> = 1,289)	Controls $(n = 1,617)$	Р	T2DM (<i>n</i> = 399)	Controls (<i>n</i> = 569)	Р	T2DM (<i>n</i> = 1,289)	Controls $(n = 1,048)$	Р
Age (years)	63.5 ± 11.6 (25-92)	65.5 ± 11.0 (18-95)	<.0001	65.2 ± 11.9 (26-90)	66.1 ± 11.4 (18-95)	.2290	62.7 ± 11.3 (25-92)	65.2 ± 10.8 (22-94)	<.0001
Sex (% female/% male)	30.9/69.1	35.2/64.8	.0140						
BMI (kg/m²)	23.7 \pm 3.5 (13.2-42.6)	$23.1 \pm 3.2 (13.6 - 34.2)$	<.0001	$23.5 \pm 3.9 (13.2 - 39.4)$	$23.0 \pm 3.5 (13.6 - 34.2)$.0270	23.8 \pm 3.3 (14.6-42.6)	$23.2 \pm 3.0 (14.1 - 34.1)$	<.0001
Blood pressure (mmHg):									
Systolic	146 ± 27 (82-256)	142 \pm 26 (70-254)	.0004	150 ± 29 (88-256)	145 ± 26 (89-254)	.0200	145 ± 26 (82-250)	141 ± 25 (70-244)	.0030
Diastolic	77 ± 15 (30-166)	76 ± 15 (31-146)	.0420	77 ± 15 (41-166)	76 ± 15 (38-130)	.1710	77 ± 15 (30-132)	76 ± 14 (31-146)	.1300
Total cholesterol (mmol/liter)	5.21 ± 1.01 (2.26-10.50)	5.24 ± .98 (2.60-9.02)	.6470	5.50 \pm 1.15 (2.94–10.50)	5.43 ± 1.02 (2.81-9.02)	.4300	5.10 \pm .93 (2.26-8.22)	5.12 \pm .95 (2.60-8.87)	.5630
Triglycerides (mmol/liter)	$1.80 \pm 1.37 \; (.15 - 19.62)$	$1.58 \pm 1.04 (.13 - 16.90)$	<.0001	$1.60 \pm .93 (.44 - 7.90)$	$1.42 \pm .84 (.29 - 5.54)$.0170	$1.94 \pm 1.50 \; (.15 - 19.62)$	$1.66 \pm 1.12 (.13 - 16.90)$.0020
HDL cholesterol (mmol/liter)	$1.26 \pm .44 (.42 - 6.01)$	$1.33 \pm .45 (.36 - 9.31)$.0005	$1.40 \pm .43 (.62 - 3.64)$	1.45 ± .38 (.65-3.22)	.1440	$1.20 \pm .42 (.42 - 6.01)$	$1.26 \pm .46 (.36 - 9.31)$.0110
FPG (mmol/liter)	9.32 ± 3.98 (3.80-33.72)	5.40 ± .76 (2.81-6.88)	<.0001	9.44 ± 3.94 (3.63-26.40)	5.41 ± .76 (3.25-6.88)	<.0001	9.27 ± 4.00 (3.80-33.72)	5.40 ± .76 (2.81-6.88)	<.0001
HbA1c (%)	7.5 ± 2.2 (4.4-16.4)	5.3 \pm .4 (3.8-6.4)	<.0001	7.9 ± 2.3 (4.7-15.0)	5.2 ± .4 (3.8-6.4)	<.0001	7.3 ± 2.2 (4.4-16.4)	5.3 \pm .4 (4.1-6.2)	<.0001

Note.—Values are given as means $\pm\,$ SDs, with ranges in parentheses.

Table 2. Characteristics of Korean Patients with T2DM and Controls

	All			Women			Men		
Variable	T2DM (<i>n</i> = 732)	Controls $(n = 633)$	Р	T2DM (<i>n</i> = 393)	Controls (<i>n</i> = 351)	Р	T2DM (<i>n</i> = 339)	Controls (<i>n</i> = 282)	Р
Age (years)	59.5 ± 9.4 (32-83)	64.7 ± 3.6 (60-93)	<.0001	60.0 ± 9.1 (32-81)	64.4 ± 3.4 (60-75)	<.0001	59.0 ± 11.3 (32-83)	64.9 ± 3.8 (60-93)	<.0001
Sex (% female/% male)	53.8/46.3	55.5/44.6	.5295						
BMI (kg/m²)	24.4 \pm 2.8 (16.5-35.0)	23.6 \pm 3.1 (14.7-32.8)	<.0001	24.8 \pm 3.1 (16.5-35.0)	$24.1 \pm 3.2 (14.7 - 32.8)$.0026	24.1 \pm 2.5 (16.5-32.2)	23.0 \pm 2.8 (16.0-32.0)	<.0001
Blood pressure (mmHg):									
Systolic	135 ± 20 (88-200)	128 ± 20 (87-203)	<.0001	135 ± 21 (88-200)	129 ± 20 (88-202)	<.0001	135 \pm 19 (90-199)	128 ± 19 (87-203)	<.0001
Diastolic	81 ± 12 (36-120)	80 ± 11 (51-120)	.0834	80 ± 12 (40-120)	79 ± 11 (51-120)	.1017	82 ± 12 (36-113)	81 ± 11 (51-113)	.4883
Total cholesterol (mmol/liter)	5.15 ± .97 (1.87-9.33)	4.98 ± .91 (2.47-8.74)	.0011	5.29 ± .98 (2.68-9.33)	$5.10 \pm .89 (3.20 - 8.09)$.0063	$5.00 \pm .94 (1.87 - 8.97)$	4.84 ± .92 (2.47 -8.74)	.0039
Triglycerides (mmol/liter)	1.88 ± 1.28 (.36-12.23)	$1.39 \pm .70 (.36 - 5.83)$	<.0001	$1.83 \pm 1.15 (.42 - 11.41)$	1.42 ± .71 (.36-5.83)	<.0001	$1.93 \pm 1.43 (.36 - 12.23)$	$1.36 \pm .68 (.50-4.92)$	<.0001
HDL cholesterol (mmol/liter)	$1.23 \pm .05 (.34 - 2.60)$	$1.20 \pm .07 (.52 - 2.52)$.1463	$1.28 \pm .05 (.60 - 2.60)$	$1.19 \pm .05 (.60 - 2.26)$	<.0001	$1.16 \pm .02 (.34 - 2.31)$	1.21 ± .09 (.52-2.52)	.0497
FPG (mmol/liter)	8.54 ± 2.53 (3.74-21.29)	4.96 ± 0.49 (3.69-6.05)	<.0001	8.61 ± 2.55 (3.74-18.37)	4.95 ± .49 (3.85-6.05)	<.0001	8.47 ± 2.51 (3.96-21.29)	4.97 ± .50 (3.69-6.05)	<.0001
HbA1c (%)	$8.0 \pm 1.6 (4.2 - 14.4)$	5.3 ± .3 (4.1-5.8)	<.0001	$8.1 \pm 1.6 (4.2 - 14.4)$	5.3 ± .3 (4.1-5.8)	<.0001	$7.9 \pm 1.6 (4.4 - 14.3)$	5.3 ± 1.3 (4.1-5.8)	<.0001

Note.—Values are given as means \pm SDs, with ranges in parentheses.

Table 3. Polymorphic Sites Characteristic to 10 Major Haplogroups

Haplogroup	Polymorphism(s) ^a
F	3970C→T (ND1: syn), 13928G→C (ND5: S531T), 10310G→A (ND3: syn)
В	8272 (9-bp deletion in noncoding region)
A	663A→G (125 rRNA), 8794C→T (ATP6: H90Y)
N9a	5231G→A (ND2: syn), 12358A→G (ND5: T8A), 12372G→A (ND5: syn)
M7a	2772C→T (16S rRNA), 4386T→C (tRNA-Gln)
M7b	4071C→T (ND1: syn), 4048G→A (ND1: D248N), 6680T→C (C01: syn), 12811T→C (ND5: Y159H)
G	709G→A (12S rRNA), 4833A→G (ND2: T122A), 5108T→C (ND2: syn)
D4a	4883C→T (ND2: syn), 5178C→A (ND2: L237M), 3010G→A (16S rRNA), 14979T→C (Cytb: I78T), ^b 8473T→C (ATP8: syn
D4b	4883C→T (ND2: syn), 5178C→A (ND2: L237M), 3010G→A (16S rRNA), 1382A→C (12Ss rRNA)
D5	4883C→T (ND2: syn), 5178C→A (ND2: L237M), 10397A→G (ND3: syn)

^a syn = Synonymous mutation.

^b Ctyb = cytochrome b.

factors, with T2DM as a dependent variable and independent variables including age, sex (0 = female and 1 = male), and genotype of each mtSNP. The *P* value, odds ratio (OR), and 95% CI were calculated. Unless indicated otherwise, a *P* value <.05 was considered statistically significant. Because of multiple comparisons of haplogroups, we applied Bonferroni correction. Since we examined 10 haplogroups, we divided .05 by 10 to get .005. Thus, a *P* value <.005 was considered statistically significant.

Results

The characteristics of the 2,906 Japanese subjects are shown in table 1. BMI, systolic and diastolic blood pressure, serum concentration of triglycerides, FPG concentration, and blood HbA1c level were significantly higher in patients with T2DM than in the controls (P < .05). Age,

female:male ratio, and serum concentration of high-density lipoprotein (HDL) cholesterol were lower in the patients with diabetes than in the controls (P < .05).

The characteristics of the 1,365 Korean subjects are shown in table 2. The subjects with diabetes were significantly younger than the controls (P < .05). BMI, systolic blood pressure, serum concentrations of total cholesterol and triglycerides, FPG concentration, and blood HbA1c level were significantly higher in the subjects with T2DM than in the controls (P < .05).

Ten common mtDNA haplogroups accounted for 72.4% and 68.2% of haplogroups in Japanese and Korean subjects, respectively (table 7). When we combined Japanese and Korean subjects, multivariate logistic-regression analysis with adjustment for age and sex (table 8) showed that the subjects in the mitochondrial haplogroup N9a had a

Table 4. Primers Used for 28-Plex PCR

		Pri	imer				
		Forward		Reverse			
Fragment	Position	Sequence (5'→3')	Position	Sequence (5'→3')	Product (bp)		
1	631	ACATCACCCCATAAACAAATAggTT	931	gCTTCTATTgACTTgggTTAATCg	301		
2	1272	AgCAAACCCTgATgAAggCTAC	1781	TATATCTATTgCgCCAggTTTCAAT	510		
3	2698	AgAggCgggCATgACACAgCA	3066	gATCACgTAggACTTTAATCgTTgA	369		
4	3215	CCAAgAACAgggTTTgTTAAgATg	3569	ggggTTCATAgTAgAAgAgCgAT	355		
5	3611	TCCTATTTATTCTAgCCACCTCTAg	3862	ATCATATTATggCCAAgggTCATg	252		
6	3916	gAgTCCgAACTAgTCTCAggCT	4255	gAgggggAATgCTggAgATTgTA	340		
7	4344	TCgAACCCATCCCTgAgAATCC	4577	gTTTATTTCTAggCCTACTCAggTAA	234		
8	4623	TCCACAgAAgCTgCCATCAAgTA	4940	gAgAgTgAggAgAAggCTTACgT	318		
9	4989	CAgCTACgCAAAATCTTAgCATAC	5257	TTgggCAAAAAgCCggTTAgCg	269		
10	5921	ACTATTCTCTACAAACCACAAAgAC	6284	TgTTCAACCTgTTCCTgCTCCg	364		
11	6535	CAgACCgCAACCTCAACACCAC	6807	gTgTgTCTACgTCTATTCCTACTg	273		
12	7567	CTAAATCCTATATATCTTAATggCAC	7895	ATTggTggCCAATTgATTTgATggT	329		
13	8153	ggggTATACTACggTCAATgCTC	8530	TCATTTTggTTCTCAgggTTTgTTAT	378		
14	8628	CAAATATCTCATCAACAACCgACTA	8994	CAgggCTATTggTTgAATgAgTAg	367		
15	9044	TAATTggAAgCgCCACCCTAgC	9414	ggCCTTggTATgTgCTTTCTCgT	371		
16	9673	gAAACCAAATAATTCAAgCACTgCT	9987	ACCCTCATCAATAgATggAgACAT	315		
17	10277	ACCCCTACCATgAgCCCTACAA	10515	gTgAgATggTAAATgCTAgTATAATAT	239		
18	10983	TCACAATCATggCAAgCCAACgC	11280	AgTgAgCCTAgggTgTTgTgAg	298		
19	11667	TCCAAACCCCCTgAAgCTTCAC	12137	AAgAggAAAACCCggTAATgATgT	471		
20	12274	AggATAACAgCTATCCATTggTCT	12545	gTggCTCAgTgTCAgTTCgAgAT	272		
21	12582	AgACTACTTCTCCATAATATTCATCC	12858	gTATAggATTgCTTgAATggCTgC	277		
22	13077	CCACTCAAgCACTATAgTTgTAgC	13591	TCAgggAggTAgCgATgAgAgTA	515		
23	13711	gCCggAAgCCTATTCgCAggAT	13980	CAggTTTTggCTCgTAAgAAggC	270		
24	14217	CTAATCAACqCCCATAATCATACAA	14562	qTCqqqTqTqTTATTATTCTqAATTT	346		
25	14829	TCCgCATgATgAAACTTCggCT	15175	ggCCCCTCAgAATgATATTTggC	347		
26	15257	gACAgTCCCACCTCACACgAT	15600	gggACggATCggAgAATTgTgT	344		
27	15696	TTCgCCCACTAAgCCAATCACTT	16037	TCCCCATgAAAgAACAgAgAATAgT	342		
28	16421	ATATCCCgCACAAgAgTgCTACT	45	TggAgAgCTCCCgTgAgTggTT	194		

Table 5. Probe Set A for Haplotyping

Table 5.	FIDDE SE	t A for Haptotyping
Position	Purpose ^a	Sequence $(5' \rightarrow 3')$
681	а	TgTAATCTTACTgAgAgCTAAT
681	b	TgTAATCTTACTAAgAgCTAA
752	а	CgTgCTTgATgCTTATTCCTTTTgA
856	а	AAAgTTTAACTAggCTATACTA
1310	а	CgTCTTTACgTggATACTTgC
1382	а	ggCTATCgTAgTTgTCTggg
1442	а	AACTAAgCACTCTATTCTCAgT
1647 2766	p	AggAgATTTCAACTTAACTTgA gACCTgTgggTTTATTAggTA
3010	a a	ATCAggACATCCCAATggTg
3010	b	ATCAggACATCCCgATggT
3027	а	TqCAqCCqCTATCAAAqq
3458	р	gCCATAAAACTCTTCACCAA
3496	а	CCCTAAAACCCTCCACATc
3497	а	CCTAAAACCCgTCACATC
3644	а	ggATTgAgTAAgCggCT
3667	р	TAgTTTgAgTTTgATgCTCA
4048	а	CTAggAACAACATATAACgCACTC
4071	a b	gACAAAATATgTTgTATAgAgTTC
4071 4086	a	ACAAAATATgTTgTgTAgAgTTC AAgTAgggTCTTggTAACAAAATA
4386	a	qqTqTqqTAqqTqqCAC
4386	b	gggTgTgATAggTggC
4491	а	CTggCCCAACCCATCATCTA
4505	а	gTCATCTACTCTACTATCTTTg
4541	а	CAgCgCTAAgCTCACACTgA
4833	а	AggTTACCCAAggCgCCCCT
4895	а	CCATCTCAATCATgTACCAA
4895	b	ATCTCAATCATATACCAAATC
5108 5147	a a	TTATCCTAACTACCACCgCA CTCCAqCACCACCACC
5178	a	TgAAACAAgATAACATgAC
5178	b	CTqAAACAAqCTAACATqA
5231	а	TCCCTAggAggCCTACCCCC
5964	а	ATgCgCCgAATAgTAggTAT
6023	а	TggCTggCCCAgTTCggCT
6086	а	CgTCACAgCCCACgCATTTg
6086	Ь	AgATTATTACAAATgCATgggCT
6253	а	CTCgCATCTgCTACAgTggA
6689 6752	a a	TggTTCTTTTTTTCCAgAgTAgT CAATTggCTTCCTggggTT
6752	b	CAATTggCTTCCTAgggTTT
8272	a	CTCTAgAgggggTAgAggTggTgCT
8272	b	TgggCTCTAgAggTggTgCTAT
8392	а	gTAATTATggTgggTCATACg
8684	а	ATCATTTgTTTTgAgATTAgTTT
8701	b	AgTgTTgTgTATggTTATCAT
8731	р	TgATTAAggATACTAgTATAAgAg
8784	а	TAACCTCCTCgggCTCCTgC
8793 8794	a a	CggACTCCTgCCCCACTCA TggTgTAAATgAgTAAggCAgg
8829	a	CAACTATCTATAAATCTAgCC
9123	a	gATTTCTAggATAgTTAgTAgAAT
8794	а	TggTgTAAATgAgTAAggCAgg
8829	а	CAACTATCTATAAATCTAgCC
9123	а	gATTTCTAggATAgTTAgTAgAAT
9219	р	ATCACATgCCTATCATATAgTA
9296	а	CTAATgACCTCCggTCTAgCC
9755	а	TCAgAgTACTTCgAATCTCCC
9774	p	ATgCCgTCggAAATggTgA
9950 10310	a a	ATTTTgTAgATgTggTCTgACTA ACTATTAgTggTAggTTAgTT
10310	a	TACCAATTCAgCCCAgTCTAAT
10397	m m	ACTATATACCAATTCAgCCCAgTCTAAT
10400	n	ACTATATACCAATTCggTTCAgT
		(continued)

Table 5. (co	ontinued)
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Position	Purpose ^a	Sequence $(5' \rightarrow 3')$		
11084	а	gTggCTgTgAATgCTATAATTA		
11215	а	TACTTCCTATTCTATACCCTAg		
11215	Ь	TACTTCCTATTCTACACCC		
11963	а	AggACTCAACATACTAATCACA		
12063	р	ACCCTCATgTTCATACACCT		
12501	а	CAACAATATTCATATgCCTAg		
12501	b	ACAACAATATTCATgTgCCT		
12705	а	CTACTCATTTTCCTAATTACCA		
12775	р	TAggAATTATATCCTTCTTgC		
12811	а	TCATCAgTTgATgACACgCCC		
13105	а	ATgAgTAAgAAgACTCCTgC		
13143	а	TggATTAgTgggCTgTTTTC		
13156	р	CTAAgCATAgTgTTAgAgTTTg		
13263	а	TATTATgAgTCCTAgCTgACTTg		
13563	а	gCCTgAgCCCTgTCTAT		
13928	а	ggATTCTACCCTACCATCA		
13928	Ь	gATTCTACCCTAgCATCA		
14343	р	gTgggTgAAAgAgTATgATg		
14476	а	CTgTAgTATATCCAAAAACAACC		
14893	а	TgCATggCTAggAACAgTCCT		
14893	Ь	gCATggCTAggAATAgTCC		
14927	а	ATgAAAAggCggCTgAgg		
14944	а	gAgTgATgTgggCAATTgAT		
14979	а	AAggTAgCggATggTTCAgC		
15067	а	TATATTACggATCATTCCTCTAC		
15346	а	CACCTCCTATTCTTACACgAAA		
15440	а	ACgCCCTCggCCTACTTCT		
15487	а	TATTCTCACCTgACCTCCT		
15497	а	CCAgACCTCCTAAgCgAC		
15524	а	TTATACCCTAgCCgACC		
15535	а	CCAACCCCTTAAATACCCCTC		
15535	b	AACCCCTTAAACACCCCTCC		
15826	р	gTTggTATTAggATTAggATTgTT		
15860	а	gAgTATTTTgTTTTCAACTAgggA		
15874	а	AggCCCATTTgAgCATTTTgTT		
15924	а	gTTTTCATCTCCggCTTACAAg		
16519	а	TTCCTACTTCAgggCCATAAAg		
16519	Ь	TCCTACTTCAgggTCATAAAgC		

NOTE.—Probes used for the first set of hybridization. ^a Purposes for probes are as follows: *a*, for detecting polymorphism; *b*, for detecting wild type; *p*, for verifying PCR product; *m*, for detecting macrohaplogroup M; and *n*, for detecting macrohaplogroup N.

significantly reduced risk of T2DM (OR 0.55 [95% CI 0.40– 0.75], P = .0002), whereas those in haplogroup F or D5 tended to have an increased risk of T2DM. We performed multiple-regression analysis of haplogroup N9a associated with T2DM, with adjustment not only for age and sex but also for BMI, systolic and diastolic blood pressure, total cholesterol, triglycerides, and HDL cholesterol (table 9). Even after adjustment for these parameters, logistic-regression analysis demonstrated that haplogroup N9a is an independent protective factor against T2DM for all Korean subjects (P = .017, OR 0.47 [95% CI 0.24–0.86]), Korean men (P = .023, OR 0.36 [95% CI 0.14–0.83]), all Japanese subjects (P = .048, OR 0.57 [95% CI 0.32–0.98]), and Japanese women (P = .030, OR 0.19 [95% CI 0.03–0.69]).

We examined the relationships of the three mtSNPs that were used for determination of the haplotype N9a to the prevalence of T2DM in all populations, by multivariable

Table 6. Probe Set B for Haplotyping

PositionPurpose*Sequence (5'-3')663agCTAATAgAAAggCCAggA709agAACTCACTggAATggggAT827aAACAgCAgTgATTAgCTTTA1891aTITCATAAggGCTgTCGTAgT1438aAgCACTCTACTCTTAGTTACT1664aAACTTAACTGACACTCTGA2772agTTAggACTgTAgTTG2835aTGCTCgAAgTTAAgTTTG3243agATTACCggGCCTGCAT3254aTTTAGTAGGCCACACTGTGAG3421agCAAAgGCCCACACTTGTG3546aCTTAGCTCTCACTATCG3546aCTTAGTCTCACTATCG3714agAATTGTTGgGCCACTGCT3744bgAATTGTTGGCCAGTGCTG3759agCCATCATTCTACTGTGCACTAGCG3700agGCTTGTGTGTGTGTAGAGAG3750agCCGTCTTGTGTGTAGAGAG4688aTTGTGAGTCCCAGAGTTACGG4688aTTGTGAGTCCCAGAAGTTACCC4850aCTGGAATAGGGAAGTTACCC4850aCTGACATCGGTCTTA5127aATTCTAATGCCCTAATAGG6146aGCTTTGGATAGGAAGGTTAGG6146aGCTTGGATAGGTAGTTG6179aGGCCCCGATATAGGAACTAGTTG6188aCTCCCAATTGGATTAGG6146aGCTTTGGATAGGAACTAGTTG6146aGCTCCTATTGGAAGGTTG6146aGCTTGGCATAAGGAACTAGTA6146aGCTCCTATTGGTAGGTT7600aTAGAGGTGAAGAGGCCT7614aAGATGTGGTGAAG	Table 0.	TIODE DE	
709 a GAACTCACT3GJAT3G3GAT 827 a AACAGCAGTGATAGCCTTA 1391 a TTTCATAAG3GGTGTCGTAG 1438 a AGCACTCACTCTTAJGTTACT 1664 a AACTTAACTTGACCACTCTGA 2772 a GTTTAGGAGTTAGCCACTCTGA 2835 a TGTCGGAGTTAGCCACTCTGA 3243 a GATTACCGGGCCCACCTGTG 3244 a GCAAAGGCCCCACACTTGGTAG 3537 a CCCCGACTTGGCCCTC 3546 a CTTAGCTCTCACTTAGC 3696 a GCTCGCAGTGGCCACTCGG 3714 a GAATTGTTGGCTCACGCACTGGT 3714 b GAATTGTTGGCACTGCT 3714 b GAGTTGTAGAAATAAGGCA 3700 a GCCTCATTCACTGTCACTACAC 3714 b GAAGTGTAAGAGAATAAGGCA 3715 a GCCACATTCACTGTCACTACTGCT 3716 gCAGTTGTAGAGAATAAGGCACTACTGGCT 3717 b GAAGTGTGAGTAGAGCACTACGGCACTACAC 3714 b GAAGTGTGAGTGCACTAC	Position	Purpose ^a	Sequence (5'→3')
827 a ÄACAgCAg TgATTA GCCTTTA 1391 a TTTCATAAggCTGTGTAgT 1438 a AgCACTCTACTTAGTTTACT 1664 a AACTTAACTTGACCACTGTA 2772 a GTTTAggACCTGTAGTAGT 2835 a TGCTGgAggTTAGTTTG 2842 a GATAACGGGCCCAACATTGTG 2842 a GCACAgGCCCAACATGG 3537 a CCCCGACTTGGCTC 3546 a CTTAGTGCACATCAG 3714 b gAgATTGTTGGGAATCAGG 3714 b gAgATTGTTGGGAATTAGG 3714 b gAgATTGTTGGAGAATAAGGA 3759 a GCCATCATTCTACTGTCAACA 3970 a GGCATCATTCAGTGAAATTAGGA 4688 a TTGTGAGAATAAGGAA 4538 a AACTAGCCGAGAGGAATTAGGA 4520 a TTCTGAGTCAGAGAGAGAGA 4688 a TGTGGAGAAGAGAGAGAAGAGAGA 4688 a TGTGGAGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	663	а	gCTAATAgAAAggCCAggA
1391 a TITCATAAgggCTgTCgTAgT 1438 a AqCACTCTACTCTTAgTTTACT 1664 a AACTTAACTTGACCACTCTGA 2772 a gTTTAgACCGTGTAgTTTG 2835 a TGCTCgGAGTTAGACTACTCTGA 2843 a gATTACGGGCCTGCCAT 3254 a TTTTAAGTTTATGCAATTACCG 3421 a gCAAgGCCCAACATGTAG 3537 a CCCCGACTTAGCCA 3546 a CTTAGCTCTCACTATCG 3546 a CTTAGCTCACATTGCT 3546 a CCTAGCATGCCAACAG 3714 a gAgATTGTTGGTGATGCACAG 3759 a gCCATCATTCTACTGTCACACAG 3700 a gCGTTGTAGAACAACAG 4688 a TTGTGAAGAGAATAAGGAGAG 4715 a TTATAGGTCAGAAGTAGAG 4820 a TCTGAACTCGGTCAGAGTAGTAG 4820 a TCTGAATAGGAGAGCAGTAGAG 61146 a GCTTTGCACTGTAGTAGAG 6018 a GCTCCATATTGGAACTGGTGTG	709	а	gAACTCACTggAATggggAT
1438 a AgCACTCTACTCTAgTTTACT 1664 a AACTTAACTTGACCACTCTGA 2772 a GTTTAGGACCTGTAGGTTTG 2835 a TGCTCGAGAGTTAGGTTTG 3243 a GATTACCGGGCCTGCCAT 3254 a TTTTAGGACTTGCTGCAT 3254 a TTTTAGGACCTTGCTGCAT 3537 a CCCCGACCTTGGCTC 3546 a CTTAGTCACAATGTGC 3547 a GCCCGACTTGGCTGCT 3546 a CTTAGTCACAATGGCAACTGGC 3714 a GAGATGTTGGGCCAACTGGCT 3714 b GAGATGTTGGCAACAAGGGGAACTTAGCG 3759 a GCCATCATCTACTGCAACAA 3700 a GGCATCAATAGGGAACTTAGCGAAGGGGAACTTAGCGGAGGAACTTAGCGGGAACTTAGCGGAGGAACTTAGCGGAGGAACTAAGGGAGGAACTAAGGGAGGAACTAGGGGGAACTTAGCGGAGAACTAGGGGGAACTTAGCGGAGAACCCCCGAGAAGAGAGCAACTGCGGAGAAGAGCACTTGGCGAGAGAGA	827	а	
1664 a AACTTAACTTgACCACTCTgA 2772 a gTTTAggACCTgTAggTTTg 2835 a TgCTCggAggTgAgTTCTg 2843 a gATTACCggGCCTqCAT 3254 a TTTTAAgTTTTATgCAATTACCg 3421 a gCAAAggCCCCAACATTGTAG 3537 a CCCCgACTTGGCAT 3546 a CTTAgCTCTCACTATCG 3696 a gCGCAGTCATTCTACTGC 3714 a gAgATTGTTggGCACTGCT 3714 b gAgATTGTTggGCACTGCT 3714 b gAgATTGTTggGCACTGGCT 3714 b gAgATTGTTGGGAACTTAGC 3970 a gCCATCATTCTGCTGACACA 3970 a gCGTTGTGTGTGAACACA 3970 a GCGGTTGTGTGTGAACACA 3970 a GCGTTGTGTGTGAACACA 3970 a GCGTTGTGTGTGTGAACACA 3970 a GCGCACTATGTGTGTGACACAGAACTAGGGAACTAGGGAACACA 4820 a TTTGAAGAGAACAAAAAAAAAAAAAAAAAAAAAAAAAAA		а	333 3 3 3
2772 a gTTAggACCTgTAggTTTg 2835 a TgCTGgAggTTGAgTTCTg 3243 a gATTACCgggCCTgCCAT 3254 a TTTTAAgTTTTATGCAATTACCg 3241 a gCAAAggCCCCAACATTGTAG 3537 a CCCCGACTTGGCTC 3546 a CTTAGCTCTCACTATCGC 3696 a gCTGCGAGTGCCAATCAG 3714 b gAgATTGTTTgGCACTGCT 3714 b gAgATTGTTGGAACACAG 3970 a gCCATCATTCTACTGTCAACA 3970 a gCGTTGTGTGTGAAGAG 4688 a TTGTGAGTGCAGAGAGAG 4538 a AAAATCAGTGCCAGAGAG 4688 a TTGTGAGTCCAGAGAGAG 4715 a TTATGGTCCTAGAGAGAGAG 4820 a TTCTGAGTCCAGAGAGAGAG 4820 a GTCTGAATAGGGAGAGGAGG 6018 a GCCTCCTATTCCAATAGGAGGAGG 6146 a GCTTGGAACTAGGAGGGG 6146 b GCTTGGAAGAGCCCT			
2835 a TgCTCggAggTTgAgTTCTg 3243 a gATTACCgggCCCTgCCAT 3254 a TTTTAAgTTTTATgCAATTACCg 3421 a gCAAAggCCCCAACATTGAG 3537 a CCCCgACTTgGCTCC 3546 a CTTAgCTCTCACTATCGC 3546 a CCCCgACTTgGCTCC 3547 a gAgATTGTTgGGCACTGCT 3714 a gAgATTGTTgGCACTGCT 3759 a GCCATCATTCTACTGTCACAA 3970 a gGCATCATCATCTGCAACAA 3970 a gGCATCATCATTGTCACAA 3970 a gGCATCATTGTCACAACAGGAACTTAGCG 4688 a TTGTGAAAgAGAATAAggCAA 4538 a AAAAATCAGTGCAGAAG 4688 a TTGTGAAGAGAGTAGAGGAACTTAGCG 4688 a TTGTGAAGAGAGAGTTAGAG 4680 a CTGCAATAGGAGACTTAGAG 4715 a ATTCTGAGTCAGAGAGCACTTAGC 4820 a CTGCAATAGGAGACTTAGA 6018 a GCTCCCAATATGGACTTA			
3243 a gATTACCgggCCCTgCAT 3254 a TTTTAAgTTTTAGCAATTACCg 3421 a GCAAAggCCCCAACATTGIAg 3537 a CCCCgACTTgGCTCC 3546 a CTTAGCTTCACTATCgC 3696 a gCTCGCAgTgCgCCAATCAg 3714 b gAgATTgTT1gggCACTgCT 3759 a gCCATCATTCTACTGTCAACA 3970 a gCATTGAAgAATAAgGGA 4538 A AAAAATCAgTgCgAACTTAgCg 4685 a GTGTTGAAgAATAAgGCgA 4715 a TTATAGTTGTGAAGAAGTTAGCG 4820 a TTGTGAAGAAGTAGGGAAGTTAGCG 4883 a ACTAGCCCAAAGTTACCGGAGAGAGTTAGCG 4883 a ACTAGCCCAAGAGTTAGCGAGAGTTAGA 6005 a gGCTGCATATCGAACTAGTGAGTTAG 6146 a gCTTTGCAACCGGAGTAGTGAGTTG 6146 a GCTTCCTATTGAACCGAGCCCT 8188 A AACTAGTGGTGAGTTG 7698 CTGCTTCTAGTGCACAGAACCCCT 8188 A AACTACTG			
3254aTTTTAAgTTTATgCAATTACCg3421agCAAAggCCCCAACATTgTAg3537aCCCCgACTTgCTC3546aCTTAgCTCTCACTATCgC3696agCTCGCAGTGGCCAATCATG3714agAgATTgTTgggCACTGCT3714bgAgATTGTTggGCACTGGT3714bgAgATTGTTTggGCACTGGT3759agCCATCATTCTACTGTCAACA3970agGCATGATAAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA			
3421 a gCAAAggCCCCAACATTgTAg 3537 a CCCCgACCTTgGCTCC 3546 a CTTAgCTCTCATTCGC 3696 a gCTCGCAgTgCgCCATCAg 3714 b gAgATTgTTggGCACTGCT 3759 a gCCATCATTCTACTGTCAACA 3970 a gGCATGATTGTGAAGAATAAggCgA 4538 a AAAAATCAgTgCgAACTTAGCG 4655 a GCggTGCTTGTGTGAGAAC 4715 a TTATgGTTCATGCCGgAG 4820 a TTCTgAAGAAgAGATGCTATA 4820 a TTCTgAGTCCCAGAAGAGTTAGA 6005 a gGTCGAATAAggAACTTAGA 6018 a GCTCCTTATTCGACTAA 6018 a GCTCCCTATTGGACTAGT 6146 b gCTTTGGCAGAGAGCCCT 6146 b GCTTTGGTATAGGTT 6146 a GCTCCTATTGGACTAGTGC 7698 CTGCTTCCTAGTCCTGATTGTGTGT 7698 CTGCTTCCTAGTCCTGTTTAG 7698 CTGCTCTAAAGGAGACCCCT 8188 AAACATG			
3537 a CCCCGACCTTgGCTCC 3546 a CTTAGCTCTCACTATCGC 3696 a GCTCGCAGTGCGCAATCAg 3714 a GAATTGTTTGGGCAATCAG 3714 a GAATTGTTTGGGCAATCAG 3714 b GAATTGTTTGGGCAATCAG 3714 b GAATTGTTTGGCCAACAACA 3759 a GCCATCATTCTACTGTCTGCT 3714 b GAATTGTTGGAACAACAACAACAACAG 3970 a GGCATCATTCTACTGTCACAACAACAACAACAACAACAACAACAACAACAACAAC			
3546 a CTTAGCTCTCACTATCgC 3696 a gCTCgCAgTgCgCCAATCAg 3714 a gAgATTgTTTgggCTACTgCT 3714 b gAgATTgTTTgggCTACTgCT 3714 b gAgATTgTTTgggCTACTgCT 3759 a gCCATCATTCACTgTCAACA 3970 a gCTTTgAAgAATAAggCgA 4538 a AAAAATCAgTgCgAACTTAgCg 4655 a gCggTTgCTTgTgTgAggA 4688 a TTGTgAgTCCCAgAAgTTACCC 4820 a TTCGAGTCCAgAAgTTACCC 4850 a CTGACATCCGGCTGCTT 4883 a AACTAGCCCCTATCTCAAT 5127 a ATTCCTACTCAGCAGAGGAG 6018 a gCTCGAATAAgAgAGATTAGA 6005 a gGTTgGCCAGATAGGCTAGTTG 6146 b gCTTTgGCAACTGACTGAGT 6179 a GTGCCCGATATAGCGTGTGT 6188 a CACCCATATTGTAGCTGTGAGT 7600 a TAGACTACTTGTGCTGTAGTT 7861 a ACGAGTGGTTAGTTGGT			5 55 5 5
3696 a gCTGCAgTgCgCCAATCAg 3714 a gAgATTgTTTgggCCACTgCT 3714 b gAgATTgTTTgggCCACTgCT 3759 a gCCATCATTCACTgTCAACA 3970 a ggCTATgAAgAATAAggCgA 4538 a AAAAATCAGTGCAACA 3970 a ggCTTgAAgAATAAggCgA 4688 a TTGTGATGCAACA 4538 a AAAATCAGTGCAACAGAACTTAACGGAA 4688 a TTGTGAGTCCCAGAAGTTACCC 4820 a TTGCAGTCCCAGAAGTTACCC 4850 a CTGACATCCGGCTATCTCAAT 5127 a ATTCCTACTGCAGAAGTTACCC 4883 a AACTAGCCCCATTCTCAAT 5127 a ATTCCTACTACTGACTAAG 6018 a gCTCCAATAAggAGACTTAGA 6018 a gCTCCCAATAAGGGTTAGTC 6146 b gCTTTGCTAGTCTGTGTAGTC 7698 p CTgCTTCTAGTCTGTATGC 7698 p CTgCTTCTAGTCTGTAAGCTGAGTAGTGAGACAGA 8450 a CACCA			
3714 b gAgATTgTTTgggCTACTgCT 3759 a gCCATCATTCTACTgTCAACA 3970 a gGCATCATTCACTgTCAACA 3970 a gGCATCATTCACTgTCAACA 3970 a gGCATCATTCACTgTCAACA 3970 a gGCATCATTCACTGTCACTAGCG 4538 a AAAAATCAgTCAGTGTGTGTGTGT 4688 a TTGTGAGTCCAGAAGTTACCC 4820 a TTCTGAGTCCAGAAGTTACCC 4850 a CTGACATCCGGTCGTT 4883 a AACTAGCCCATACTCAAT 5127 a ATTCCTACTGACTGAATAAGAGACCTAAT 6005 a gGCTCCAATAGGACCTAAT 6146 a gCTTTgCAACTGACTAGT 6146 a GCTTTGCAACTGACTAGTTG 6146 a GCTTCCTATTGTGACTACTACT 7600 a TAgACCACTACTGTGTAGTC 6179 a GTGCCCCAATATGGTGTATGC 7681 a ACGATGTGTATGACTACTACT 7600 a TAgACCTACTGTGTATGC 7881 a AACATGTGG	3696	а	gCTCgCAgTgCgCCAATCAg
3759agCCATCATTCTACTGTCACCA3970aggCTATgAAgAATAAggCgA4538aAAAAATCAgTgCgAACTTAgCg4655agCgGTTgCTTGTTGTGTGAgAG4688aTTGTTGATGAGAGAGAG4715aTTATgGTTCATTGCCGgAG4820aTTCTGAGTCCCGAAAGTTACCC4850aCTGACATCCGGTCTGCTT4883aAACTAGCCCCTATCTCAAT5127aATTCCTACTGCGACTAAGGAGG6018agCTCGAATAAGAGAGAGCGAGC6146agCTTTGGCACTGACTAGTTG6146bgCTTTGGCACTGACTAGTTG6146bgCTTTGGCACTGACTAGTTG6680aCTCCCCATATTGTACCGAGC7698pCTGCTTCCTAGTCCTGTATGC7698pCTGCTTCCTAGTCCTGTATGC7861aACGAGTGGTGTAGTTG8188aAAACTGTGGTGAAGCCCT8188aAACTAGTGGTGTGAGGTGTG8251aAggTAAAACGGGTGTGGTG8450aCCCCAACTAAAAATATTAGACACA8453aCCCCAACTAAAAATATTAGACACA8453aCCCCAACTAAAAATATTAGACACA8453aCCCCAACTAAAAATATTAGGCACA8454aACCTCCTTATGAGCAGAGGAA8955aCCCCCTTATGAGCAGAGAGAATT9900aAGATGTGTAGTAGAACTAGAATT9912aCCGCCTGATACGGTAGATT10310bTATTAGTGAGAGAACTAGAATT10400mTACCAATTCAGTCACATT10400mTACCAATTCAGTCACATT10400mTACCAATTCAGTGCGG <trr><</trr>	3714	а	gAgATTgTTTgggCCACTgCT
3970 a ggCTATgAAgAATAAggCgA 4538 a AAAAATCAgTgCgAACTTAgCg 4655 a gCgTTgTTgTgTgAggA 4688 a TTgTTgAAgAggATggCTATT 4715 a TTATgTTGATGAGAgGA 4820 a TTGTGAGTCCCAgAAgTTACCC 4850 a CTGACATCCGgTCGTT 4883 a AACTAGCCCTATCTCAAT 5127 a ATTCCTACTACTGGACTAA 6005 a ggCTCGAATAAggAgACTTAGA 6018 a gCCTCCTATTCGAACTGACTAGT 6146 a gCTTTgCAACTGACTAGTAGT 6146 a GCTTTGGCACTGACTAGTGT 6146 a GCTTCTATTGACGACTAGTAGT 6680 a CTCCCATATTGTACCTACTACT 6680 a CTCCCATATTGTACCTACTACT 7600 a TAGACTACTGTGTTAGCG 8188 a AAACTGTGTTTAGCGGTGTT 8188 a AACTGTGGTTAACGGTGTGT 8250 a CCCCAACTAACAGGTGTGT 8450 a CACCCAACTAACAGTGTGTAT	3714	Ь	
4538 a AAAAATCAgTgCgAACTTÁgCg 4655 a gCggTTgCTTgTgTgAggA 4688 a TTgTTgAAgAggATggCTATT 4715 a TTATgTTGAAgAggATggCTATT 4715 a TTGTGAGTCCCAgAAGTTACCC 4820 a CTGACATCCgGTCTGCTT 4833 a AACTAGCCCCTATCTCAAT 5127 a ATTCTACTACTCGACTTAA 6005 a gGCTCGAATAAggAGACTTAGA 6018 a gCCTCCTATTCGAACTGACTAGTC 6146 a gCTTTgGCACTGACTAGTTG 6146 a GCTTTGGCACTAGTGTGTGTTG 61680 a CTCCCATATTGTAACCTACTACT 61680 a CTCCCATATTGTAGCTGTATGC 6188 a AAACTGTGTTTAGCTGTATGC 7698 p CTGCTCTAGTCCTGATTGCCACAGA 8200 a ACGATGGTGATACGGTGTTTAG 8450 a CCCCAACTAACAGTGAAGCC		а	
4655 a gCggTTgCTTgTgTgTgAggA 4688 a TTgTTgAAgAgAgATggCTATT 4715 a TTATgTTACTTgCCCggAg 4820 a TTCTgAGTCCATGCCGgAG 4820 a TTCTgAGTCCAGAAGTTACCC 4850 a CTgACATCCggTCTGCTT 4883 a AACTAGCCCTATCTCAAT 5127 a ATTCTACTACTCGACTTAA 6005 a ggCTGAATAAggAgACTTAAA 6018 a GCCTCTTATTCGAACCGACTAGA 6018 a GCCTCCTATTGACTGACTAGT 6146 a GCTTTgGCAACTGACTAGTAGT 6146 a GCTTTGGCAACTGACTAGTGT 6146 a GCTTCTTATGAACCGAGCCT 6146 b GCTTCTTATGTACCGTATTGC 6680 a CTCCCATATTGTACCGTATT 6680 a CTCCCATATTGTACCTTGTAGC 7698 p CTgCTCCTAGTCCTTATT 8188 a AAACTgTGGCAACAGACCCT 8188 a AACGAGTGGTAAAAGTGTGG 8251 a GGCCAACTAACAGAAT			
4688 a TTgTTgAAgAgAgTgCTATT 4715 a TTATgTTGCCTgCCGgAg 4820 a TTCTgAGTCCATGCCGgAG 4820 a TTCTGAGTCCAGAAGTTACCC 4850 a CTGACATCCGGTCGCTT 4883 a AACTAGCCCTATCTCAAT 5127 a ATTCCTACTACTCGACTTAA 6005 a gGCTCGATAAggAGACTTAAA 6018 a GCTCCTTATTCGAACCGACTAGA 6018 a GCTCCTTATTCGAACCGAGC 6146 a GCTTTgCAACTGACTAGTTG 6146 a GCTTTGGCAACTGACTAGTTG 6146 a GCTTCTTATCGAACCGAGCC 6146 a GCTTCTTATGCACTGATTG 6680 a CTCCCATATTGTACCTGTATTG 6680 a CTCCCATATTGTACCTGTATGC 7698 p CTGCTTCAGTCGTATG 8188 a AAACTGTGGTGAAGTGTG 8251 a AggGTAAATACggTGTATTA 8450 a CCCCAACTAAAAATATTAGACCAC 8453 a CCCCAACTAAAAATATTAGCACC			
4715aTTATggTTCATTgCCCggAg4820aTTCTgAgTCCATGCCGAAGTTACCC4850aCTgACATCCggTCTgCTT4883aAACTAgCCCTATCTCAAT5127aATTCCTACTCGACTGAACGAGACCGAGC6005aggCTCgAATAAggAgACTTAGA6018agCTCTgGAATAAggAgACTTAGA6018agCTTTgGCAACTgGTAGTTC6146bgCTTTgGCAACTGGCTAGTTC6146bgCTTTgGCAACTGGCTGAGTTG6680aCTCCCATATTGTAACCTACTACT7600aTAgACCTACTTGTGC7698pCTgCTTCCTAGTCCTGTATGC7698pCTgCTTCCTAGTCGTGTAGC7861aACGAGTGGGCAGAAGCCCCT8188aAAACTGTGGTGTAGGTGTG8250aACGATGGGCATGAAGGTGTG8251aAggGTAAATACggGTCCTATT8383aTggGCCATACGAGTGTGAGAGCACA8453aCCCAACTAAAAATATTAGACACA8453aCCCCAACTAAAAATATTAGACACA8456aATCCCCTTATGTAGTGAACAGA856aCCCCATACTAGTTATCATCGAA8955aCCCCATACTAGTAGAGAGACAGA8955aCCCCTTATGAGTAGAACTAGAATT9922aCGCCTGATATGAGTAGTGTT10310bTATTAGTGAGAATGAGTT10400mTACCAATTGAGTAGTAGTT10454aGACTCATTAATGAGAATGAGTT10410aTTTTGTTAGTGGTGG11202aCATCACCACCACCAGGTAACAATAGAGTT10454aGACTCATTAACCTATTGCCCCAAT10454aGAC			
4820aTTCTgAgTCCCAgAAgTTACCC4850aCTgACATCCggTCTgCTT4883aAACTAgCCCTATCTCAAT5127aATTCCTACTACTCGACTTAA6005aggCTCgAATAAggAgACTTAgA6018agCCTCTTATTCGAACCGAGC6146agCTTTgGCAACTgGTAgTTC6146bgCTTTgGCAACTGACTAGTTC6147agTGCCCCGATATAGCTTGTGC6148aGCTCCTATTGTAACTACTACT6149agTGCCCCGATATAGCTGTGTGC6179agTGCCCCGATATGGCTGTAGTC6180aCTCCCATATTGTAACCTACTACT6000aTAGACCTACTTGTGCC7698pCTGCTTCCTAGTCTGTATGC7698pCTGCTTCCTAGTGAGTGTG8200aACGATGGGCATGAAGCCCCT8188aAAACTGTGGTGAAGAGCTGTG8251aAggGTAAATACGGGTGTG8251aAggGCATAAGgGTGTGTGTAGT8450aCCCCAACTAAAAATATTAGACACA8453aCCCCAACTAAAAATATAAACACC8453aCCCCAACTAAAAATATTAGACACA8453aCCCCATACTAGTTATCATTGGCACAA8456aATCCCCTTATGAGAGGACACA8955aCCCCATACTAGTTAGAACTAGAATT990aAgATGATAAGTGTAGAACTAGAATT9915aGGCCTGATACTGACAGTGAGAATT9922aCCGCCTATATGATAGTAGTAAAGTCAATA10400mTACCAATTCAGTCAGTCT10410aTTTTGTTAGTGATAA10454aGACTCATACACTATGGTGGT10454aGACTCATACACC			
4850aCTgACATCCggTCTgCTT4883aAACTAgCCCTATCTQAT5127aATTCCTACTACTACTQACTTAA6005aggCTCgAATAAggAgACTTAA6018agCCTCCTTATTCGAACCGAGC6146agCTTTgGCAACTGGTAGTTC6146bgCTTTgGCAACTGGCTAGTTC6146bgCTCCQATATAGCGTTT6146bgCTCCCATGTGCTGGCTGGTGC6179agTgCCCCCGATATAGCGTTT6680aCTCCCATATTGTAACCTACTACT7600aTAgACCTACTGTGCTGTGC7698pCTgCTTCCTAGTCCTGTATGC7861aACGAggTCAACGACCCT8188aAAACTgTgGTGTGCCACAGA8200aACGATggGCATGAAGTGTG8251aAggTAAATACggTGTGTATT8383aTggGCCATACGgTGGTGTATT8383aTggGCCATACGGTGACCACA8450aCCCCAACTAAAAATACTAAACAC8453aCCCAACTAAAAATATTAGACACA8453aCCCAACTAACAATATAGGGCACA8456aATCCCCTTATGAGAGGCACA8955aCCCCTTATGAGAGGCACA8955aCCCCTTATAGTAAGAGCCAGC9833aGACTTCACGTCATGGACATT10310bTATTAGTGAGAGTAGATT10400mTACCAATTCAGCTCAGTCT10410aTTTTGTTAAGTAGGTGTGGT10454aGACTCATTAATTAGAATGGTGCGT10400mTACCAATTCAGCTAGGTGGG11722aTAATGAGAATGAGTGGCGT11017aTGATGAGAATGAGTGCGT1202			
4883aAACTAgCCCCTATCTCAAT5127aATTCCTACTACTCGACTTAA6005aggCTCGAATAAggAgACTTAA6005agCTCGTATTCGAACCGAGC6146agCTTTgCAACTGGACCGAGC6146bgCTTGGCAACTGACTAGTTC6146bgCTTGGCAACTGGCTAGTTC6146bgTTGCCCCGATATAGCGTTT6179aGTGCCCCGATATAGCGTTT680aCTCCCATATTGTAACCTACTACT7600aTAGACTACTGTGTGTGC7698pCTGCTTCCTAGTCCTGTATGC7861aACGAgGTCAACGACCCT8188aAAACTGTGGTTGAAGCCCCT8188aAAACTGTGGTTGAAGCCCTG8251aAggTAATACGGTGTG8251aAggGTAATACGGTGTGG8450aCACCCAACTAAAAATACTAAACAC8453aCCCAACTAAAAATACTAAACAC8453aCCCAACTAAAAATATAGAGCACA8473aAACTACCACCTACCCCCCTC8701aAgTGTTGTGTAGGCAACA8456aATCCCCTTATGAGCAGCACA8955aCCCCATACTAGTTATCATCGAA9090aAgATGATAAGTGAGAACATGAATT9242aCTATCATATAGTAAGTAGTAGTTT10310bTATTAGTGACCAGTCGT932aCCGCCTGATACTAGTAGTTATA10400mTACCAATTCAGTCAGTCGT10410aTTTTGTTAACTATGTACAAT10454aGACTCACTACTATAGTAGAATCAATA10454aGACTCACCCCCTTATGTGAGT10456aATTATGAGAATGAGTGGT10456			
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6018 a GCCTCCTTATTCGAACCGAGC 6146 a GCTTTGGCAACTGGCTAGTTC 6146 b GCTTTGGCAACTGACTAGTTC 6146 b GCTTTGGCAACTGACTAGTTC 6179 a GTGCCCCGATATAGCGTTT 6680 a CTCCCATATTGTAACCTACTACTACT 7600 a TAGACCTACTTGTGC 7698 p CTGCTTCCTAGTCCTGTATGC 7861 a ACGAGGTCAACGACCCCT 8188 a AAACTGTGGTTTGCCCACAGA 8200 a ACGATGGGTTAACGGGTGGTATTG 8383 a TGGCCCAACTAAAAGTGTAGACCA 8450 a CACCCAACTAAAAATACTAAACACA 8453 a CCCAACTAAAAATACTAAACACA 8453 a CCCAACTAAAAATACTAAACACA 8453 a CCCCAACTAAAAATATAGACACA 8453 a CCCCAACTAACAACAACAA 8473 a AACTACCACCTACCCCCCCC 8701 a AGTGTTGTATGAGGACAA 8955 a CCCCCATACTAGTACACAACAA 8955 a <			5
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7861aACgAggTCAACgACCCCT8188aAAACTgTggTTTgCCCCACAgA8200aACgATgggCATgAAgCTgTg8251aAgggTAAATACgggTCCTATT8383aTgggCCATACggTgTATTAg8450aCACCCAACTAAAAATACTAAACAC8453aCCCAACTAAAAATATTAgACACA8473aAACTACCACCTACCCCCCTC8701aAgTgTTgTgTATggCTATCATT8762aCCCCATACTAAGAGACAA8955aCCCCATACTAGAGAGAGAAA9090aAgATgATAgTgTgTgTgGgAggAA9115aggATAGTCAGTAAAGTCAGAAAATAT9242aCTATCATATAGTAAAGCCCAGC9833agACTTCACGTCATTGGCACAA10310bTATTAGTGCAGTCATTGTT10373aTCCTTTTTGTAGTCATTGATAAT10400mTACCAATTCAGCTCAGTCT10410aTTTTGTTTAAACTATGTACAAATCATA10454aGACTCATTAAATTAGACAATCATA1016aGATAGTGGTGAGTAGATAGTGAGCGT11722aTAATGAGAATGATGGCG11722aTCATCACCCATATCCCCATT12092aCCACCCACTACTATCCCCTT12092bTATCCCCCATTCTCCCCTT12092bTATCCCCCATTCTCCCCT12358aGACACACACCACCCT			
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8453aCCCAACTAAAAATATTAgACACA8473aAACTACCACCTACCCCCCCCC8701aAgTgTTgTgTATggCTATCATT8762aCCTTAATCATTTTACTgCCACAA8856aATCCCCTTATgAgCAggCAGCA8955aCCCCATACTAgTTATCATCgAA9090aAgATgATAAgTgTggAggA9115aggATAgTCAgTAgAACTAgAATT9242aCTATCATATAgTAAAgCCCAgC9833agACTTCACGTCATCATTGGCA9932aCCgCCTgATACTgACATTT10310bTATTAgTgGCAggTAGTGTT10400mTACCAATTCAgCTCAGTCT10410aTTTTgTTTAAACTATGTACCAAT10454agATAgTggTTCGTGg1166agATAgTggTTCGTGg11696aATTATgAgAATgATTGGC11722aTAATGAgAATGATTGGCG12026aCACTCACCCACCACGTTAACA12092aCTATCCCCATTATCCCCTT12092bTATCCCCCATTATCCCCTT12092bTATCCCCCATTATCCCCT12358agCACACTACTATAAGCACCCTT	8383	а	
8473 a AACTACCACCTACCCCCTC 8701 a AgTgTTgTgTATggCTATCATT 8762 a CCTTAATCATTTTACTgCCACAA 8856 a ATCCCCTTATgAgCAgCACA 8955 a CCCCATACTAGTTATCATCGAA 9090 a AgATgATAAgTgTgAgAggA 9115 a ggATAGTCAgTAGAACTAGAATT 9242 a CTATCATATAGTAAAGCCAGC 9833 a gACTTCACGTCATTGGCAGC 9833 a GACTTCACGTCATTGGCAGC 9833 a GACTTCACGTCATTGGCAGC 9833 a GACTTCACGTCATTAGTAAGCCCAGC 9833 a GACTTCACGTCATCATTGGCT 9932 a CCGCCTGATACTGACATTT 10310 b TATTAGTGGCAGTTAGTTGTTT 10373 a TCCTTTTTGTAGTCAGTCT 10400 m TACCAATTCAGTCAGTCT 10454 a GACTCATTAAATTATGACAATCATAT 1016 a GATAGTGGTGAGT 11017 a TGATAGAGATGATGGTGGT 12026 a CAC	8450	а	
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8762 a CCTTAATCATTTTACTgCCACAA 8856 a ATCCCCTTATgAgCAggCACA 8955 a CCCCATACTAgTTATCATCgAA 9090 a AgATgATAAgTgTggAgggA 9115 a ggATAgTCAgTAgAACTAgAATT 9242 a CTATCATATAGTAAAGCCAgC 9833 a gACTTCACGTCATTGGCAGC 9833 a GACTTCACGTCATTGGCAGC 9833 a GACTTCACGTCATTGGCT 9932 a CCgCCTgATACTGACATTGGCT 10310 b TATTAGTGGCAGGTTAGTTGTT 10373 a TCCTTTTTGTAGTCATAA 10400 m TACCAATTCAGCTCAGTCT 10410 a TTTTGTTAACTATGTACAAT 10454 a GACTCATTAAATTATGACAATCATA 11016 a GATAGTGGTGGT 11696 a ATTATGAGAATGATTGCGT 11722 a TAATGAGAGTGAGGCGT 11722 a TAATGAGAGTGAGGCGT 12026 a CATCACCCACCACGTTAACA 12092 a CTATCCCCCATT		а	
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9115 a ggATAgTCAgTAgAACTAgAATT 9242 a CTATCATATAgTAAAgCCAgC 9833 a gACTTCACgTCATCATTagTAAAgCCCAgC 9932 a CCgCCTgATACTgACATTgGTT 10310 b TATTAgTggCAggTTAgTTgTT 10373 a TCCTTTTTgTAgTCATTCATA 10400 m TACCAATTCAgCTCAgTCT 10410 a TTTTgTTAACTATGTACCAAT 10454 a gACTCATTAAATTATgACAATCATA 1016 a gATAgTggTTCGTTgGTg 11017 a TgATAgTggTTCgTgg 11696 a ATTATgAgAATgATTgCgC 11722 a TAATgAggATgTAgTCGT 12026 a CATCACCCACCACGTTAACA 12092 a CTATCCCCATTATCCCCATT 12092 a CTATCCCCCATTATCCCCATT 12092 b TATCCCCCATTCTCCCCTT 12092 b TATCCCCCATTCTCCCCTT 12358 a GCACACTACTATAAGCACCCCT			
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10400mTACCAATTCAGCTCAGTCT10410aTTTTGTTTAAACTATGTACCAAT10454agACTCATTAAATTATGACAATCATA11016agATAgTgGTTCATTgGATA11017aTGATAGTGGTGG11696aATTATGAGAATGATGGGC11722aTAATGAGAGTGTGAGTCGGT12026aCACTCACCCACCACGTTAACA12095aTCATACACCTATCTCCCATT12092bTATCCCCCATTATCCTCC12092bTATCCCCCATTATCCTCC12092bTATCCCCCATTATCCTCCT12358agCACACTACTATAGCCACCCT	10310	b	TATTAgTggCAggTTAgTTgTT
10410 a TTTTgTTTAAACTATgTACCAAT 10454 a gACTCATTAAATTATgACAATCATA 11016 a gATAgTggTTCATTggATA 11017 a TgATAgTggTTCgCTgg 11696 a ATTATgAgAATgATGCGC 11722 a TAATgAggATgTgAgTCgT 12026 a CACTCACCACCACGTAACA 12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCCATTATCCTCC 12092 b TATCCCCCATTCTCCTCT 12358 a GCACACTACTATAGCCACCCT	10373	а	TCCTTTTTgTAgTCATTCATA
10454 a gACTCATTAAATTATgACAATCATA 11016 a gATAgTggTTCATTggATA 11017 a TgATAgTggTTCGCTgg 11696 a ATTATgAgAATgATTgCgC 11722 a TAATgAggATgTgAgTCGgT 12026 a CACTCACCACCACGTAACA 12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCATTATCCTCC 12092 b TATCCCCCATTCTCCCTCT 12038 a GCACACTACTATAGCCACCCT	10400	т	5 5
11016 a gATAgTggTTCATTggATA 11017 a TgATAgTggTTCgCTgg 11696 a ATTATgAgAATgATTgCgC 11722 a TAATgAggATgTgAgTCGgT 12026 a CACTCACCACCACGTAACA 12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCATTATCCTCC 12092 b TATCCCCCATTATCCTCT 12092 b TATCCCCCATTCTCCTCT 12358 a gCACACTACTATAgCCACCCT		а	
11017 a TgATAgTggTTCgCTgg 11696 a ATTATgAgAATgATTgCgC 11722 a TAATgAggATgTgAgTCCgT 12026 a CACTCACCACCACGTTAACA 12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCATTATCCTCC 12092 b TATCCCCCATTCTCCCTT 12358 a GCACACTACTATAGCCACCCT			
11696 a ATTATGAGAATGATTGCGC 11722 a TAATGAGGATGTGAGTCCGT 12026 a CACTCACCACCACGTGACACA 12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCATTATCCTCC 12092 b TATCCCCCATTCTCCCTCT 12358 a GCACACTACTATAGCCACCCT			
11722 a TAATgAggATgTgAgTCCgT 12026 a CACTCACCACCACGTTAACA 12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCCATTATCCTCC 12092 b TATCCCCCATTCTCCCTCT 12358 a GCACACTACTATAGCCACCCT			
12026 a CACTCACCACCACGTTAACA 12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCCATTATCCTCC 12092 b TATCCCCCATTCTCCTCT 12358 a gCACACTACTATAGCCACCT			
12085 a TCATACACCTATCTCCCATT 12092 a CTATCCCCCATTATCCTCC 12092 b TATCCCCCATTCTCCTCT 12358 a gCACACTACTATAGCCACCCT			
12092aCTATCCCCCATTATCCTCC12092bTATCCCCCATTCTCCTCCT12358agCACACTACTATAgCCACCCT			J J
12092bTATCCCCCATTCTCCTCCT12358agCACACTACTATAgCCACCCT			
12358 a gCACACTACTATAgCCACCCT			
(continued)			
			(continued)

Table 6. (continued)

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Position	Purpose ^a	Sequence (5'→3')
12372	а	AgCCACCCTAACCCTAACTTCC
12406	а	ATCCTTACCACCCTCATTAACC
12753	а	ACAACCTATTCCAgCTgTTC
12753	Ь	ACAACCTATTCCAACTgTTC
13437	а	TCAAAACCATACCCCTCAC
13512	а	ggTTTCTACTCCAAggACCAC
13759	а	TgTTTggAAgggggATgTgggg
13879	а	CAAACTTAAAATAAAACCCCCA
13942	а	ATCACACACCgCgCAAT
14287	а	ATAATTTATgAAggggAggggT
14308	а	TAATAgTgTAgggAgCTgAAT
14364	а	AggTAggATTggTgTTgTgg
14914	а	TgAggCgTCTggCgAgT
14996	а	gAggCgCCATTggTgTgAAg
15047	а	ACACATCggACgAAgCCTATA
15314	а	gCTgCTAgggCTgTAATAATg
15422	а	ACCCTTACTACACAgTCAAAgA
15508	а	AggCgACCCAgATAATTAT
15508	b	gCgACCCAgACAATTATAC
15850	а	TCAATTAgggAgACggTTggTA
15883	а	ACAAAATACTCAAATgAgCCTgT
Nee		

NOTE.—Probes used for the second set of hybridization.

^a Purposes for probes are as follows: *a*, for detecting polymorphism; *b*, for detecting wild type; *p*, for verifying PCR product; and *m*, for detecting macrohaplogroup M.

logistic-regression analysis with adjustment for age and sex. All three mtSNPs were significantly associated with resistance against T2DM (5231G \rightarrow A: P = .0001, OR 0.54 [95% CI 0.40–0.74]; 12358A→G: *P* = .0026, OR 0.62 [95% CI 0.46–0.84]; and 12372G \rightarrow A: P = .0005, OR 0.59 [95%) CI 0.44–0.79]). The slight differences in the *P* values and ORs among these mtSNPs are due to the occurrence of the same replacement in different haplogroups (homoplasy or parallel mutations). The first mtSNP (5231G \rightarrow A) was detected not only in haplogroup N9a but also in subhaplogroup D4k3. The second mtSNP (12358A \rightarrow G) was not detected in some of the subjects with haplogroup N9a, probably because of a revertant substitution. In addition, the second mtSNP was also detected in subhaplogroup D4b2b2 (tentative nomenclature). The third mtSNP (12372G \rightarrow A) was detected not only in haplogroup N9a but also in subhaplogroup D4h. Thus, the combined analysis of these three mtSNPs is essential for accurate identification of haplogroup N9a.

Japanese subjects in haplogroup F had a significantly increased risk of T2DM (OR 1.53 [95% CI 1.16–2.04], P = .0032), whereas those in haplogroup N9a tended to have a reduced risk for the disease. In particular, Japanese women in haplogroup N9a had a significantly reduced risk of T2DM (OR 0.27 [95% CI 0.10–0.62], P = .0042), whereas those in haplogroup F or A tended to have an increased risk of T2DM.

Korean subjects in haplogroup N9a had a significantly reduced risk of T2DM (OR 0.43 [95% CI 0.24–0.77], P = .0048), whereas those in haplogroup D5 or subhaplogroup

Table 7. Haplogroup Distribution in Controls and Patients with T2DM

	No. of Controls (%)			No. of Patients with T2DM (%)			
Haplogroup	Japanese	Korean	Total	Japanese	Korean	Total	
F	96 (5.9)	61 (9.6)	157 (7.0)	112 (8.7)	71 (9.7)	183 (9.1)	
В	196 (12.1)	98 (15.5)	294 (13.1)	152 (11.8)	113 (15.4)	265 (13.1)	
Α	102 (6.3)	46 (7.3)	148 (6.6)	100 (7.8)	63 (8.6)	163 (8.1)	
N9a	79 (4.9)	40 (6.3)	119 (5.3)	41 (3.2)	19 (2.6)	60 (3.0)	
M7a	115 (7.1)	9 (1.4)	124 (5.5)	92 (7.1)	17 (2.3)	109 (5.4)	
M7b	68 (4.2)	18 (2.8)	86 (3.8)	45 (3.5)	21 (2.9)	66 (3.3)	
G	188 (11.6)	43 (6.8)	231 (10.3)	141 (10.9)	65 (8.9)	206 (10.2)	
D4a	152 (9.4)	38 (6.0)	190 (8.4)	111 (8.6)	32 (4.4)	143 (7.1)	
D4b	109 (6.7)	29 (4.6)	138 (6.1)	83 (6.4)	54 (7.4)	137 (6.8)	
D5	62 (3.8)	34 (5.4)	96 (4.3)	59 (4.6)	58 (7.9)	117 (5.8)	
Others ^a	450 (27.8)	217 (34.3)	667 (29.6)	353 (27.4)	219 (29.9)	573 (28.3)	
Total	1,617 (100)	633 (100)	2,250 (100)	1,289 (100)	732 (100)	2,021 (100)	

^a Seventeen other haplogroups with low frequencies, including haplogroups N9b, Y, M10–M12, M7c, M8a, Z, C, and D4d–D4n (except for D4f and D4i).

D4b tended to have an increased risk of the disease. Korean men in haplogroup N9a had a significantly reduced risk of T2DM (OR 0.28 [95% CI 0.11–0.62], P = .0031), whereas those in haplogroup D4b had a significantly increased risk of T2DM (OR 3.55 [95% CI 1.65–8.34], P = .0019).

We then examined whether the risk of T2DM with haplogroup N9a was related to age; systolic blood pressure; diastolic blood pressure; serum concentration of total cholesterol, triglycerides and/or HDL cholesterol; FPG concentration; or HbA1c level. None of the parameters, other than FPG and HbA1c, showed significant differences between the subjects with haplogroup N9a and those without it. The FPG (mean \pm SD) concentration was significantly lower in the individuals with haplogroup N9a than in those with other haplogroups (6.5 \pm 3.0 mmol/liter vs. 7.1 \pm 3.1 mmol/liter, P = .021). The HbA1c level was significantly lower in individuals with haplogroup N9a than in those with other haplogroups (6.1% \pm 1.5% vs. 6.8% \pm 1.9%, P = .002).

Discussion

We examined the relationships between T2DM and each of 10 major mitochondrial haplogroups in a large-scale association study in the Japanese and Korean populations. Haplogroup N9a was significantly associated with reduced susceptibility to T2DM.

Mitochondrial haplogroup N9a has a great diversity in the whole of China and Korea. In Japan, this haplogroup was not detected in aboriginal Ainu and Ryukyuans but only in mainland Honshu Japanese. This distribution suggests that this haplogroup was derived from the new immigrant, or Yayoi, people. These so-called mammoth hunters who had adapted to extremely cold climates in Siberia migrated back to the northern part of China ~6,000 years ago. A part of this continental population immigrated into Japan through the Korean peninsula ~2,900 years ago, and this immigration started the Yayoi period. Haplogroup N9a was not detected in tooth DNA from the remains of an individual from the Japanese Neolithic period, known as the "Jomon" period, whereas N9a was recently detected in the Yayoi remains at the Kuma-Nishioda site in the northern part of Kyushu Island (K. Shinoda [National Science Museum, Tokyo], personal communication). Thus, haplogroup N9a might be one of the mitochondrial haplogroups that had been selected for adaptation to cold climates. This historical character of haplogroup N9a might be relevant to resistance against T2DM by individuals who carry this haplogroup. These hypotheses, however, must be examined further by functional analysis of this haplogroup.

Most of the mtSNPs characteristic to haplogroup N9a are synonymous substitutions, including $5231G \rightarrow A$ and $12372G \rightarrow A$, which were used for the present genotyping.

Table 8.	Multivariate Logistic-Regression Analysis
of Haplog	roups Associated with T2DM with
Adjustme	nt for Age and Sex in Japanese and
Korean Po	opulations

Population and Haplogroup	Р	OR (95% CI)
Japanese and Korean subjects:		
N9a	.0002	.55 (.4075)
F	.0114	1.34 (1.07-1.67)
D5	.0475	1.33 (1.00-1.76)
Japanese and Korean women:		
N9a	.0035	.43 (.2474)
Japanese subjects:		
F	.0032	1.54 (1.16-2.04)
N9a	.0206	.63 (.4393)
Japanese women:		
N9a	.0042	.27 (.1062)
F	.0163	1.79 (1.11-2.89)
Α	.0407	1.67 (1.02-2.72)
Korean subjects:		
N9a	.0048	.43 (.24–.77)
D5	.0483	1.60 (1.01-2.57)
D4b	.0365	1.66 (1.04-2.81)
Korean men:		
N9a	.0031	.28 (.1162)
D4b	.0019	3.55 (1.65-8.34)

NOTE.—Bold font indicates haplogroups with P < .005.

Table 9. Multivariate Logistic-Regression Analysisof Haplogroup N9a Associated with T2DM

Population and Variable	Р	OR (95% CI)
Japanese subjects:		
Age	.0003	.22 (.1049)
Sex	.0088	1.38 (1.08-1.75)
BMI	<.0001	.13 (.0535)
Triglycerides	.0051	.07 (.00941)
HDL cholesterol	.0255	14.5 (1.52-167)
Haplogroup N9a	.0478	.57 (.3298)
Korean subjects:		
Age	<.0001	1,066 (321–3765)
BMI	.0458	.42 (.1898)
Systolic blood pressure	<.0001	.10 (.05–.21)
Triglycerides	<.0001	.01 (.000904)
Haplogroup N9a	.0166	.47 (.2486)
Japanese men:		
BMI	.0019	.16 (.0551)
Triglycerides	.0114	.08 (.0151)
Korean men:		
Age	<.0001	997 (184–6169)
BMI	.0075	.19 (.0563)
Systolic blood pressure	<.0001	.06 (.0221)
Triglycerides	.0012	.007 (.000312
Haplogroup N9a	.0233	.36 (.1483)
Japanese women:		
Age	.0028	.12 (.0347)
BMI	.0158	.19 (.0572)
Haplogroup N9a	.0298	.19 (.0369)
Korean women:		
Age	<.0001	273 (68.8–1186)
Systolic blood pressure	<.0001	.14 (.0537)
Triglycerides	<.0001	.001 (.000102
HDL cholesterol	<.0001	.03 (.00614)
N. TI I		D.117 . 11

NOTE.—The analysis was adjusted for age, sex, BMI, systolic and diastolic blood pressure, triglycerides, HDL and cholesterol.

Possible candidates for functional polymorphisms in the noncoding region of this haplogroup are 150C \rightarrow T and 338C \rightarrow T. The 150C \rightarrow T substitution was originally reported to occur in Italian centenarians.²⁶ Also, we reported this substitution to be associated with healthy longevity in both Finland and Japan.²⁷ Thus, 150C→T might confer resistance against T2DM. Among haplogroup N9a-specific mtSNPs in the coding region, the mtSNP 12358A→G causing the T8A replacement in nicotinamide adenine dinucleotide dehydrogenase subunit 5 (MTND5 [MIM 516005]) may be considered a potentially functional polymorphism. It seems possible that this T8A replacement might influence the function of the ND5 and complex I. The actual effect of the 12358A→G (ND5: T8A) on mitochondrial function remains to be examined. The metabolic characteristics of individuals with haplogroup N9a with both 150C→T and 12358A→G should be examined for better understanding of the mechanisms underlying their resistance against T2DM.

We detected a significant association between haplogroup N9a and a reduced risk of T2DM in all subjects (OR 0.55), and especially low ORs in Japanese women (0.27) and Korean men (0.28) were obtained. Although we cannot exclude the possibility that these associations resulted

from the reduced statistical power due to the decreased sample size of subgroups, these sex- and region-specific associations suggest that cultural factors, including nutritional and social customs, modify the protective effect of haplogroup N9a against T2DM. According to the Wallace theory, adaptation to a cold climate might involve uncoupling of electron transfer with ATP production, to increase heat production.^{15,16} Thus, increased mitochondrial respiration and energy expenditure is essential to meet the ATP requirement. Such an uncoupling phenotype would be protective against the development of obesity and, consequently, T2DM. However, at present, we do not have evidence that N9a is associated with lean body status. Alternatively, the uncoupling phenotype might be related to decreased mitochondrial oxidative stress, which might in turn exert a protective effect against T2DM. Further functional analysis of cybrids carrying haplogroup N9a will be necessary to verify these hypotheses.

The mitochondrial genome variation is so large that a given haplogroup may consist of various subhaplogroups carrying unique and presumably functional mtSNPs. The frequency of each subhaplogroup is sometimes only a few percent. Therefore, large-scale association studies are necessary for elucidating the impact of each subhaplogroup on the susceptibility to various common diseases.

Although haplogroup F was significantly associated with a risk of T2DM in Japanese subjects (OR 1.53 [95% CI 1.16–2.04], P = .0032), this association was not confirmed in Korean individuals. To explain this discrepancy, we hypothesize certain interactions between mitochondrial haplogroups and nuclear polymorphisms and/or environmental factors. Alternatively, the difference in the results between the Japanese and Korean subjects could be ascribable to the difference in the subhaplogroup frequencies between the two countries and to the functional differences among certain subhaplogroups. Our success in detecting a significant association of haplogroup N9a with resistance against T2DM in both Japanese and Korean individuals could be ascribable to the homogeneity of haplogroup N9a (coalescence age of $14,000 \pm 5,000$ years ago) compared with the heterogeneity of haplogroup F (coalescence age of $47,000 \pm 9,000$ years ago). Further biomedical and functional studies on mitochondrial polymorphisms should be conducted in conjunction with human phylogenetic studies.

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Web Resources

Accession numbers and URLs for data presented herein are as follows:

mtSNP, http://www.giib.or.jp/mtsnp/index_e.shtml

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi .nlm.nih.gov/Omim/ (for T2DM and MTND5)

References

- 1. Maechler P, Wollheim CB (2001) Mitochondrial function in normal and diabetic β -cells. Nature 414:807–812
- 2. Soejima A, Inoue K, Takai D, Kaneko M, Ishihara H, Oka Y, Hayashi JI (1996) Mitochondrial DNA is required for regulation of glucose-stimulated insulin secretion in a mouse pancreatic beta cell line, MIN6. J Biol Chem 271:26194–26199
- 3. Silva JP, Kohler M, Graff C, Oldfors A, Magnuson MA, Berggren PO, Larsson NG (2000) Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes. Nat Genet 26:336–340
- 4. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 300:1140–1142
- Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 350: 664–671
- 6. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, et al (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34:267–273
- Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, et al (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci USA 100:8466–8471
- 8. Poyton RO, McEwen JE (1996) Crosstalk between nuclear and mitochondrial genomes. Annu Rev Biochem 65:563–607
- 9. Alcolado JC, Alcolado R (1991) Importance of maternal history of non-insulin dependent diabetic patients. BMJ 302:1178–1180
- 10. Thomas F, Balkau B, Vauzelle-Kervroedan F, Papoz L (1994) Maternal effect and familial aggregation in NIDDM: the CODIAB study. CODIAB-INSERM-ZENECA Study Group. Diabetes 43:63–67
- 11. Poulton J, Luan J, Macaulay V, Hennings S, Mitchell J, Wareham NJ (2002) Type 2 diabetes is associated with a common mitochondrial variant: evidence from a population-based case-control study. Hum Mol Genet 11:1581–1583
- 12. Kim JH, Park KS, Cho YM, Kang BS, Kim SK, Jeon HJ, Kim SY, Lee HK (2002) The prevalence of the mitochondrial DNA

16189 variant in non-diabetic Korean adults and its association with higher fasting glucose and body mass index. Diabet Med 19:681–684

- 13. Weng SW, Liou CW, Lin TK, Wei YH, Lee CF, Eng HL, Chen SD, Liu RT, Chen JF, Chen IY, et al (2005) Association of mitochondrial deoxyribonucleic acid 16189 variant (T→C transition) with metabolic syndrome in Chinese adults. J Clin Endocrinol Metab 90:5037–5040
- 14. Chinnery PF, Elliott HR, Patel S, Lambert C, Keers SM, Durham SE, McCarthy MI, Hitman GA, Hattersley AT, Walker M (2005) Role of the mitochondrial DNA 16184-16193 poly-C tract in type 2 diabetes. Lancet 366:1650–1651
- Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, et al (2003) Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci USA 100:171–176
- Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303:223–226
- 17. Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 39:359–407
- Saxena R, de Bakker PI, Singer K, Mootha V, Burtt N, Hirschhorn JN, Gaudet D, Isomaa B, Daly MJ, Groop L, et al (2006) Comprehensive association testing of common mitochondrial DNA variation in metabolic disease. Am J Hum Genet 79:54–61
- Tanaka M, Gong JS, Zhang J, Yoneda M, Yagi K (1998) Mitochondrial genotype associated with longevity. Lancet 351: 185–186
- Ikebe S, Tanaka M, Ozawa T (1995) Point mutations of mitochondrial genome in Parkinson's disease. Brain Res Mol Brain Res 28:281–295
- 21. Tanaka M (2002) Mitochondrial genotypes and cytochrome b variants associated with longevity or Parkinson's disease. J Neurol Suppl 2 249:II11–II18
- 22. Guo LJ, Oshida Y, Fuku N, Takeyasu T, Fujita Y, Kurata M, Sato Y, Ito M, Tanaka M (2005) Mitochondrial genome polymorphisms associated with type-2 diabetes or obesity. Mitochondrion 5:15–33
- 23. Fuku N, Oshida Y, Takeyasu T, Guo LJ, Kurata M, Yamada Y, Sato Y, Tanaka M (2002) Mitochondrial ATPase subunit 6 and cytochrome b gene polymorphisms in young obese adults. Biochem Biophys Res Commun 290:1199–1205
- 24. Tanaka M, Cabrera VM, Gonzalez AM, Larruga JM, Takeyasu T, Fuku N, Guo LJ, Hirose R, Fujita Y, Kurata M, et al (2004) Mitochondrial genome variation in eastern Asia and the peopling of Japan. Genome Res 14:1832–1850
- 25. Itoh Y, Mizuki N, Shimada T, Azuma F, Itakura M, Kashiwase K, Kikkawa E, Kulski JK, Satake M, Inoko H (2005) Highthroughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. Immunogenetics 57:717–729
- 26. Zhang J, Asin-Cayuela J, Fish J, Michikawa Y, Bonafe M, Olivieri F, Passarino G, De Benedictis G, Franceschi C, Attardi G (2003) Strikingly higher frequency in centenarians and twins of mtDNA mutation causing remodeling of replication origin in leukocytes. Proc Natl Acad Sci USA 100:1116–1121
- 27. Niemi AK, Moilanen JS, Tanaka M, Hervonen A, Hurme M, Lehtimaki T, Arai Y, Hirose N, Majamaa K (2005) A combination of three common inherited mitochondrial DNA polymorphisms promotes longevity in Finnish and Japanese subjects. Eur J Hum Genet 13:166–170