Antiatherogenic Mitochondrial Genotype in Patients With Type 2 Diabetes

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OBJECTIVE — To evaluate the significance of a longevity-associated mitochondrial genotype (Mt5178A) derived from a C → A transversion at nucleotide position 5178 of mitochondrial DNA, which causes a Leu-to-Met substitution within the NADH dehydrogenase subunit 2 gene, in type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS — Mt5178 typing was done by polymerase chain reaction–restriction fragment-length polymorphism with the restriction enzyme *Alu*I in 1,148 type 2 diabetic Japanese subjects, and the results were compared with the clinical characteristics. Then, the association of Mt5178 type with early atherosclerotic changes of the bilateral carotid arteries on ultrasonography was assessed in 412 diabetic subjects randomly selected from the original 1,148 type 2 diabetic subjects, while maintaining the same frequency of Mt5178A and Mt5178C.

RESULTS — The frequency of Mt5178A in the type 2 diabetic subjects (454 of 1,148; 40%) was not different from that previously found in healthy blood donors (114 of 252; 45%). Clinical characteristics regarding diabetes were not significantly different between the Mt5178A group ($n = 454$) and the Mt5178C group ($n = 694$). However, the mean intima-media thickness (IMT) at six sites in the bilateral carotid arteries was significantly smaller in the Mt5178A group than in the Mt5178C group (0.906 ± 0.018 vs. 0.995 ± 0.021 mm, mean ± SEM, $P = 0.022$), and the Mt5178 type was significantly correlated with both the mean IMT and the presence of plaque on multiple regression analysis and discriminant analysis.

CONCLUSIONS — The Mt5178A genotype may be unrelated to the etiology of type 2 diabetes. However, Mt5178A seems to have an antiatherogenic effect, at least in type 2 diabetic individuals.

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An epidemiological study on coronary heart disease has indicated that longevity is more strongly associated with maternal rather than paternal age at death (1). This suggests that the maternally transmitted mitochondrial genotype may influence susceptibility to adult-onset diseases, thus affecting life span. We have previously analyzed mitochondrial DNA (mtDNA) in Japanese centenarians and found several mutations that showed a higher frequency in centenarians than in healthy control subjects. Accordingly, we reported that a mitochondrial genotype, Mt5178A, derived from the C→A transversion at nucleotide 5178 of mtDNA that causes a Leu-to-Met substitution within the NADH dehydrogenase subunit 2 (ND2) gene, was related to longevity in the Japanese population (2). Although our data suggest that Mt5178A may promote resistance to adult-onset diseases, we have not identified the particular disease for which people with Mt5178A show resistance. Because type 2 diabetes is an important adult-onset disease and because a major factor influencing the life span of diabetic patients is hyperglycemia-induced atherosclerosis leading to ischemic heart disease (IHD) or cerebrovascular disease (CVD) (3), we evaluated the association between Mt5178A and early atherosclerosis of the carotid artery in Japanese subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

— A total of 1,148 Japanese subjects with type 2 diabetes (740 men and 408 women, aged 20–84 years; mean ± SEM 60 ± 0.5) diagnosed by the previous World Health Organization criteria in 1985 were recruited from the outpatient clinic of Juntendo University Hospital, Tokyo. All subjects gave written informed consent before enrollment in the study, which was approved by the Ethics Committee of Juntendo University.

Analysis of mitochondrial genotype

Mitochondrial DNA was extracted from peripheral white blood cells using a DNA extraction kit (Mag Extractor; Toyobo, Osaka, Japan). The Mt5178 genotype was assayed by polymerase chain reaction (PCR)–restriction fragment-length polymorphism analysis with the restriction enzyme *Alu*I, as previously described (2). Briefly, PCR was performed using two primers [(5′ to 3′): TAA ACT CCA GCA CCA CGA C (5178-forward) and GGT GGA GTA TAG GCG TAG (5178-reverse)], and then the PCR products were incubated with *Alu*I for 3 h at 37°C. After agarose gel electrophoresis, the

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Abbreviations: CVD, cerebrovascular disease; IHD, ischemic heart disease; IMT, intima-media thickness; mtDNA, mitochondrial DNA; ND2, NADH dehydrogenase subunit 2; PCR, polymerase chain reaction; ROS, reactive oxygen species.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics and carotid IMT of the subjects undergoing ultrasonography*

	Mt5178C	Mt5178A	*P*
n	243	169	
Age (years)	59 ± 0.5	60 ± 0.6	NS
Sex (M/F)	160/83 (66%/34%)	112/57 (66%/34%)	
Duration of diabetes (years)	12 ± 0.4	12 ± 0.4	NS
Onset age of diabetes (years)	47 ± 0.5	48 ± 0.6	NS
Family history of diabetes (%)			NS
Father's side	19	42	
Mother's side	26	20	
Siblings	25	19	
Children	2	2	
BMI (kg/m^2)	23 ± 0.2	23 ± 0.2	NS
Treatment of diabetes (%)			NS
Diet alone	9	15	
Sulfonylurea	31	24	
α-Glucosidase inhibitor	55	50	
Biguanide	10	5	
Troglitazone	3	4	
Insulin	42	44	
Treatment of hyperlipidemia (%)			NS
Statins	11	14	
Fibrates	3	3	
HbA_{1c} (%)	7.6 ± 0.4	7.1 ± 0.4	NS
Serum total cholesterol (mg/dl)	194 ± 1.5	188 ± 1.9	<0.04
HDL cholesterol (mg/dl)	55 ± 0.6	55 ± 0.8	NS
Triglyceride (mg/dl)	158 ± 5.0	158 ± 10	NS
Carotid IMT (mm)	0.995 ± 0.021	0.906 ± 0.018	<0.03

Data are means ± SEM unless otherwise indicated.

Mt5178 genotype was detected by ethidium bromide staining.

Carotid ultrasonography

High-resolution *B*-mode ultrasonography of the carotid arteries was performed using an echotomographic system with an electrical linear transducer (mid-frequency ≥10 MHz). The extracranial carotid arteries were scanned bilaterally in three different longitudinal projections (anterior-oblique, lateral, and posterior-oblique) and in the transverse projection. The use of these projections allowed the common carotid artery, carotid bulb, and parts of the internal and external carotid arteries to be scanned. In each longitudinal projection, three measurements of the intima-media thickness (IMT) were made, i.e., at the site of greatest thickness, 1 cm upstream, and 1 cm downstream from the thickest point. Then, the values for all six points on both the right and left carotid arteries were averaged, and this mean value was used for each subject. The reproducibility of this method of IMT measurement has been previously demonstrated (4). The IMT was measured, and carotid plaque was assessed in 412 of the diabetic subjects who were randomly selected from the original 1,148 type 2 diabetic subjects, while maintaining the same frequencies of Mt5178A and Mt5178C.

Statistical analysis

Data are means ± SEM. The statistical significance of the differences in mean values and frequencies was determined by the Student's *t* test and the χ^2 test, respectively. To assess the relationship of Mt5178 type to carotid IMT and carotid plaque, multiple regression and discriminant analyses were performed.

RESULTS — The frequencies of Mt5178A and Mt5178C in our Japanese type 2 diabetic subjects were not significantly different from those previously reported in healthy control subjects. The number of diabetic subjects with Mt5178A was 454 (40%), and the number of diabetic subjects with Mt5178C was 694 (60%) in the present study vs. 45 and 55% in our previous study (2). The mean serum total cholesterol level was within the normal range in both groups, but the mean total cholesterol level of the Mt5178A group was significantly lower than that of the Mt5178C group (189 ± 2.0 vs. 194 ± 1.5 mg/dl, $P < 0.05$). None of the other clinical parameters differed between the two groups.

The clinical characteristics of the 412 diabetic subjects randomly selected from the original 1,148 type 2 diabetic subjects, while maintaining the same frequencies of Mt5178A ($n = 169$, 40%) and Mt5178C ($n = 243$, 60%), are shown in Table 1. Similar to the original population, the serum total cholesterol level was significantly lower in the Mt5178A group than in the Mt5178C group (188 ± 1.9 vs. 194 ± 1.5 mg/dl, $P < 0.04$). However, the mean IMT value of the Mt5178A group was significantly smaller than that of the Mt5178C group (0.906 ± 0.018 vs. 0.995 ± 0.021 mm, $P < 0.03$), although the other clinical characteristics did not differ between the two groups. Multiple regression analysis of the relationship between mean IMT and all parameters listed in Table 1 showed that the mean IMT was significantly correlated with sex, age, and Mt5178 type. Similarly, discriminant analysis showed that the existence of carotid plaque was significantly correlated with age, Mt5178 type, and the serum triglyceride level. The partial regression coefficients of these factors calculated by the stepwise forward selection method are shown in Table 2.

CONCLUSIONS — We analyzed the Mt5178 type in a large number of type 2 diabetic Japanese subjects and found equal frequencies of Mt5178A and Mt5178C in diabetic subjects and healthy subjects (2). Furthermore, because clinical characteristics regarding diabetes did not differ between the two groups with Mt5178A and Mt5178C, this genotype may not be associated with the onset and/or progression of type 2 diabetes. However, the Mt5178 type was significantly associated with carotid IMT and carotid plaque, so this genotype may be related to early atherosclerosis, at least in type 2 diabetic individuals.

In the present study, we evaluated the association of Mt5178 type with carotid IMT and carotid plaque, because these markers have been identified as major risk factors for IHD and CVD in both

Table 2—*Multiple regression analysis of factors influencing carotid IMT and plaque*

	Partial regression coefficient	*P*
Carotid IMT		
Constant	0.3866	<0.0001
Sex (0 = female, 1 = male)	0.0929	0.0018
Age (years)	0.0111	<0.0001
Mt5178 (0 = C, 1 = A)	–0.0948	<0.0001
Carotid plaque		
Constant	–5.3697	<0.0001
Age (years)	0.0685	<0.0001
Mt5178 (0 = C, 1 = A)	–0.5721	0.0142
Triglyceride (mg/dl)	0.0025	0.0263

Partial regression coefficients for carotid plaque were determined using logistic regression model.

cross-sectional and longitudinal prospective studies (5,6). To date, the largest prospective study was performed on 7,983 subjects aged 55 years and showed that the odds ratios for stroke and myocardial infarction per 0.163-mm increase in mean carotid IMT at the baseline were ~1.4–1.6 during a 2.7-year (mean) follow-up period (7). Thus, the difference of 0.09 mm in mean IMT shown by the two groups in the present study may associate with a difference in longevity over a sufficient period of time. Previously, we reported that carotid IMT increases with age and that diabetes accelerates this increment of IMT by ~15–20 years (4,8). Interestingly, the present study suggests that Mt5178A retards the age-dependent increase in IMT. As shown in Table 2, the absolute values of the Mt5178A partial regression coefficients for both IMT and plaque were eight to nine times higher than those of age; the deceleration rate of age-dependent atherosclerotic change by Mt5178A was ~8–9 years.

The higher incidence of atherosclerotic disease in diabetic subjects compared with nondiabetic subjects is thought to be associated with abnormal lipid metabolism and enhancement of oxidative stress by the diabetic state. The serum total cholesterol level of the Mt5178A group was significantly lower than that of the Mt5178C group in the present study. Because tricarboxylic acid cycle and respiratory chain of mitochondria are major pathways for utilization of acetyl-CoA, which is a source of cholesterol synthesis, investigation of the relationship between these mitochondrial functions and Mt5178 genotype may help us to understand the mechanism behind the lower total cholesterol level in the Mt5178A group. We previously reported an equation for estimating the annual change in mean IMT: [mean IMT increase (mm/year = 0.000501 × (total cholesterol – HDL cholesterol) (mg/dl) + 0.01285 × HbA_{1c} (%) + 0.0315 × mean IMT at baseline (mm) – 0.158], which was based on data obtained by a twice-yearly measurement of IMT for >3 years in ~1,000 patients (8). Because the coefficient of non–HDL cholesterol was very small, the 6 mg/dl difference of total cholesterol between the two groups in the present study may not influence the mean IMT. Furthermore, it was recently reported on univariate analysis, but not on multivariate analysis, that the serum total cholesterol and LDL cholesterol levels showed a correlation with the mean IMT change over 3.1 years (9). Altogether, it remains unclear whether the antiatherogenic effect of Mt5178A can be explained by a change of lipid metabolism, so further investigation of the relationship between the Mt5178 type and lipid metabolism is required. Long-term diabetic hyperglycemia induces abnormal activation of protein kinase C (10), formation of advanced glycation end products (11), and activation of the polyol pathway (12). Although all of these metabolic changes increase reactive oxygen species (ROS) (13–15), the major intracellular source of ROS in diabetes has not yet been elucidated. Nishikawa et al. (16) recently reported that enhancement of ROS in cultured bovine aortic endothelial cells under high-glucose conditions was prevented by an inhibitor of electron transport chain complex II, by an uncoupler of oxidative phosphorylation, by uncoupling protein-1, and by manganase superoxide dismutase. These data suggest that the increase of ROS in the diabetic state may primarily be related to mitochondrial dysfunction. Thus, it would be very interesting to investigate whether mitochondrial genotype Mt5178A, which causes Leu-to-Met substitution in ND2 of electron transport chain complex I, has an antioxidant effect; such a study should provide important information on the mechanism of the antiatherogenic effect of Mt5178A in diabetic subjects.

Several gene polymorphisms have been shown to be related to an increase of carotid IMT in diabetic subjects. Both methylenetetrahydrofolate reductase (C677T) polymorphism causing Ala-to-Val substitution and paraoxonase (Gln192Arg) polymorphism have been reported to show a positive association with carotid IMT (17,18). However, recent studies showed a negative result (19,20), so it remains unclear whether these genotypes can influence carotid IMT. An insertion/deletion polymorphism in intron 16 of the ACE gene was previously reported as a risk factor for myocardial infarction (21), and it was reported that the maximum carotid IMT in type 2 diabetic subjects with the D-positive genotype (ID + DD) (1.101 ± 0.557 mm) was greater than that in subjects with the II genotype (0.991 ± 0.362 mm) (22). Therefore, the influence of the Mt5178 genotype on carotid IMT may be similar regarding the ACE genotype.

The present study focused on the association of Mt5178 type with the development of type 2 diabetes and with early atherosclerotic changes in diabetic individuals. However, it would also be interesting to assess whether this genotype is associated with diabetic microangiopathy, because oxidative stress is involved in the onset of microangiopathy, as well as macroangiopathy. Our preliminary investigation showed no significant difference in the frequency of retinopathy, nephropathy, peripheral neuropathy, or macroangiopathy (including IHD and CVD) between the Mt5178A group and the Mt5178C group (data not shown). Thus, further investigation of these complications is required to better evaluate the association of Mt5178 genotype with microangiopathy and macroangiopathy.

In conclusion, the mitochondrial ge-

notype Mt5178A associated with longevity may not have an antidiabetic effect, but it seems to have an antiatherogenic effect, at least in type 2 diabetic patients. Large-scale cross-sectional and longitudinal prospective studies including nondiabetic subjects are needed to clarify the significance and mechanism of the antiatherogenic effect of the Mt5178A genotype.

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