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ORIGINAL INVESTIGATION

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Association of the mitochondrial DNA 5178 A/C polymorphism with serum lipid levels in the Japanese population

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Abstract As one approach to exploring whether the mitochondrial DNA 5178 adenine/cytosine (mt5178 A/C) polymorphism is associated with atherosclerosis, we genotyped 461 healthy Japanese individuals and studied the relationship of mt5178 A/C genotypes to serum lipid levels. Blood specimens were obtained after at least a 12-h fasting period from the subjects. The mt5178 A/C was genotyped by the polymerase chain reaction/restriction fragment length polymorphism method. The relative frequency of mt5178 A was 41.6% (192/461) and of mt5178 C was 58.4% (269/461). After adjustments for age and body mass index, the high-density lipoprotein cholesterol concentration in males carrying mt5178 A was significantly higher than that in males carrying mt5178 C (P=0.026). The tryglyceride (TG) concentration in females carrying mt5178 A was significantly lower than that in females carrying mt5178 C (P=0.012). This difference in the TG level between the two genotypes was more evident in postmenopausal females than in premenopausal females. Mt5178 A seems to have an antiatherogenic effect. This is the first genetic epidemiological report on the association of mt5178 A/C polymorphism with serum lipid levels in the Japanese population.

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Introduction

Aging leads to various cellular and tissular oxidative damage. Mitochondria may be strongly involved in the production of reactive oxygen species, the pathogenic agent of many adult degenerative diseases (Lenaz et al. 2000). Considerable numbers of single nucleotide polymorphisms in the mitochondrial genome have been reported, and over 50 pathogenic mitochondrial DNA (mtDNA) base substitutions and hundreds of mtDNA rearrangement mutations (deletions and insertions) have been identified in a variety of degenerative diseases (Wallace 1999). These facts have driven us to ask whether mtDNA genotypes affect the aging process and life span, and which genotype is related to them. Tanaka et al. (1998) have focused a great deal of their interest on the mitochondrial DNA 5178 adenine/cytosine (mt5178 A/C) polymorphism in the NADH dehydrogenase subunit 2 (ND2) coding region and have reported that the mitochondrial genotype mt5178 A might be associated with longevity. Substitution of adenine by cytosine at mtDNA 5178 results in an amino acid change of methionine to leucine at ND2 237 (ND2–237 M/L). They have shown that the frequency of mt5178 A is significantly higher in Japanese centenarians than in the general population of Japan, whereas other mtDNA polymorphisms associated with longevity have been reported in a European people (Ivanova et al. 1998; De Benedictis et al. 1999). Moreover, they have demonstrated that individuals with mt5178 C are more susceptible to adult-onset diseases than those with mt5178 A. The mt5178 A polymorphism is reported to be relatively rare all over the world (Cann et al. 1987). However, Tanaka et al. (1998) and Shimokata et al. (2000) have revealed a high frequency of mt5178 A among Japanese population. These results may be associated with the finding that Japan is characterized by the highest life expectancy at birth among all countries. However, the mechanisms by which the ND2 gene polymorphism affects longevity or the occurrence of adult-onset diseases have not been disclosed. Tanaka et al. (2000) have speculated that the

mt5178 A genotype is likely to confer resistance to adult-onset diseases by suppressing obesity and atherosclerosis. Therefore, as one approach to examining whether mt5178 A/C polymorphism is associated with atherosclerosis, we have genotyped 461 healthy Japanese individuals and studied the relationship of mt5178 A/C genotypes to serum lipid levels. This is the first study reporting genotypic effects of the ND2 locus on serum lipid levels in the Japanese population.

Subjects and methods

Subjects

Volunteers were recruited from the people entering the Mito Red Cross Hospital for a medical check-ups. The study was performed according to the Declaration of Helsinki of 1975, and written informed consent was obtained from volunteers before participation. Only persons found to be healthy with respect to their medical history and not taking antihypertensive, antihyperlipidemic, and antidiabetic medications were studied. Subjects in this study included 461 Japanese, viz., 362 males and 99 females (mean age ± SD: 53.5±8.2 years).

Blood chemical and physical data, drinking, and smoking habits

Plasma samples were obtained after at least a 12-h fasting period, with informed consent. Serum concentrations of total cholesterol (TC) were measured on a Determiner L TC II (Kyowa Medex; Allain et al. 1974). Serum lipids concentrations were analyzed by routine methods at Mito Red Cross Hospital. Serum concentrations of triglyceride (TG) were measured by Determiner L TG II (Kyowa Medex; Fossati and Prencipe 1982). Serum concentrations of high-density lipoprotein cholesterol (HDLC) were measured by Determiner L HDL-C (Kyowa Medex; Sugiuchi et al. 1995). Low-density lipoprotein cholesterol (LDLC) concentrations were calculated according to the Friedewald formula (Friedewald et al. 1972). Other blood chemical data were measured by routine methods at Mito Red Cross Hospital. The values presented for both systolic blood pressure and diastolic blood pressure were the means of two measurements obtained by physicians. The body mass index (BMI) was defined as the ratio of weight (in kilograms) to height (in meters) squared. A survey of drinking and smoking habits was performed by questionnaire. Drinking habits were defined as alcohol drinking one or more times per week. The clinical characteristics of male and female subjects are summarized in Table 1.

Genotyping

DNA was isolated from whole blood by using the DNA Extractor WB kit (Wako Pure Chemical Industries, Japan). The oligonucleotide primers used to determine the mt5178 A/C genotype were: forward 5'-CTTAGCATACTCCTCAATTACCC-3' and reverse 5'-CTGAATTCTTCGATAATGGCCCA-3'. The polymerase chain reaction (PCR) was performed with 50 ng genomic DNA in buffer containing 1.5 mM $MgCl_2$, 1.25 mM dNTPs, and 0.5 µl Amplitaq (GeneAmp, Perkin Elmer, USA) DNA polymerase. After an initial denaturation at 94°C for 5 min, PCR was conducted through 40 cycles in the following steps: denaturation at 94°C for 30 s, annealing at 60°C for 60 s, and polymerase extension at 72°C for 90 s. After cycling, a final extension at 72°C for 10 min was performed. The PCR product was then digested with the restriction enzyme *Alu*I (Nippon Gene, Japan) and electrophoresed in 1.5% agarose gel. Gels were stained with ethidium bromide, visualized under UV light, and photographed. The absence of the *Alu*I site was designated as mt5178 C, and the presence of this restriction enzyme cutting site was designated mt5178 A.

Statistical analyses

Statistical analyses were performed by using SAS statistical software, version 6.12 (SAS Institute, Cary, N.C.). The gender difference in genotype distribution was tested by the chi-square test. Differences in BMI and serum lipid levels between mt5178 A/C genotypes were evaluated by using the least square means calculated from the general linear regression model procedure. Age and BMI were taken into account in these analyses. In regression analyses, the mt5178 genotypes were numerically coded, with 0 for mt5178 A and 1 for mt5178 C.

Table 1 Clinical characteristics of study subjects. Age is given as the mean±SD. For drinking habits (%) and current smokers (%), *P* values were calculated by using the chi-square test. Body mass index (*BMI*), systolic blood pressure, diastolic blood pressure, fasting glucose, uric acid, creatinine, total cholesterol, high-density lipoprotein (*HDL*) cholesterol, low-density lipoprotein (*LDL*) cholesterol, and triglyceride are given as mean±SE. All *P* values show the significant difference between males and females

Characteristic	Males (*n*=362)	Females (*n*=99)	*P* value
Age (years)	52.96 ±7.63	55.60 ±9.83	–
Drinking habits[a](%)	64.67	18.36	0.001
Current smokers (%)	40.74	3.06	<0.001
BMI (kg/m^2)	23.26 ±0.14	21.93 ±0.27	<0.001
Systolic blood pressure (mmHg)	123.21 ±0.77	119.52 ±1.46	0.026
Diastolic blood pressure (mmHg)	72.35 ±0.50	68.49 ±0.94	<0.001
Fasting glucose (mmol/l)	5.544±0.092	5.249±0.171	0.134
Uric acid (mmol/l)	35.23 ±0.37	26.79 ±0.70	<0.001
Creatinine (µmol/l)	71.16 ±0.50	51.36 ±0.95	<0.001
Total cholesterol (mmol/l)	5.259±0.046	5.681±0.087	<0.001
HDL cholesterol (mmol/l)	1.450±0.021	1.691±0.040	<0.001
LDL cholesterol[b] (mmol/l)	3.148±0.044	3.482±0.083	<0.001
Triglyceride (mmol/l)	1.451±0.036	1.114±0.069	<0.001

[a]Drinking habits were defined as alcohol drinking one or more times per week
[b]LDL cholesterol concentrations were calculated according to the Friedewald formula (Friedewald et al. 1972)

Table 2 Mt5178 A/C genotypes in subjects. The numbers of subjects are listed, with the percentage in *parentheses*. There was no gender difference in genotypic distribution. The distribution of genotype frequencies did not significantly differ between premenopausal and postmenopausal phases

Genotype	Males	Females	Premenopausal females	Postmenopausal females	Total
mt5178 A	148 (40.9)	44 (44.4)	20 (50.0)	24 (40.7)	192 (41.6)
mt5178 C	214 (59.1)	55 (55.6)	20 (50.0)	35 (59.3)	269 (58.4)
Total	362 (100)	99 (100)	40 (100)	59 (100)	461 (100)

Results

Genotype frequencies of mt5178 A/C

Genotypic frequencies for mt5178 A/C are shown by gender in Table 2. The relative frequency of mt5178 A was 41.6% (192/461), and that of mt5178 C was 58.4% (269/461). There was no gender difference in genotypic distribution according to the chi-square test.

Relationship between the mt5178 A/C polymorphism and serum lipid levels

Table 3 shows the least square means, adjusted for age and BMI, of TC, HDLC, LDLC, and TG in the studied groups. In both sexes, the least square means of TC and LDLC were lower and that of HDLC was higher in the mt5178 A group than in the mt5178 C group. However, these differences did not reach statistical significance. The TG level in females carrying mt5178 A was significantly lower than that in females carrying mt5178 C (P=0.012). Moreover, after adjustments for age, BMI, drinking habits, and smoking habits, the TG level in females carrying mt5178 A was also significantly lower than that in females carrying mt5178 C (P=0.013; data not shown). The HDLC level in males carrying mt5178 A was significantly higher than that in males carrying mt5178 C (P=0.026). However, after adjustments for age, BMI, drinking habits, and smoking habits, the HDLC concentration in males was not significantly associated with mt5178 A/C genotypes (P=0.078; data not shown).

Table 4 shows the least square means, adjusted for age and BMI, of serum lipid levels in premenopausal and postmenopausal females. In premenopausal females, there is no significant difference in serum lipid levels between mt5178 A and mt5178 C. However, in postmenopausal females, the TG level is significantly higher in the mt5178 A group than in the mt5178 C group (P=0.023). After adjustments for age, BMI, drinking habits, and smoking habits, the TG concentration in postmenopausal females is also associated with mt5178 A/C genotypes (P=0.040; data not shown).

Discussion

This is the first genetic epidemiological report of the association of the mt5178 A/C polymorphism with serum lipid levels in the Japanese population. Genotypic distributions of mt5178 A/C polymorphism do not differ between our results and others reports. Shimokata et al. (2000) have reported that the proportion of mt5178 A is

Table 3 BMI, drinking habits, smoking habits, and serum lipid levels by mt5178 A/C genotypes. Age is given as mean±SD. For drinking habits (%) and current smokers (%), *P* values were calculated by using the chi-square test. Body mass index (BMI) is given as least square mean±SE adjusted for age. Total cholesterol, high-density lipoprotein (*HDL*) cholesterol, low-density lipoprotein (*LDL*) cholesterol, and triglyceride are given as least square means±SE adjusted for age and BMI. All *P* values show the significant difference between mt5178 A and mt5178 C

Characteristic	Males			Females		
	mt5178 A (n=148)	mt5178 C (n=214)	*P* value	mt5178 A (n=44)	mt5178 C (n=55)	*P* value
Age (years)	52.5 ±7.7	53.3 ±7.6	–	56.3 ±7.9	55.1 ±11.2	–
Drinking habits[a] (%)	66.09	63.16	0.544	13.96	21.82	0.304
Current smokers (%)	43.06	37.32	0.335	2.33	3.64	0.828
BMI (kg/m^2)	23.39 ±0.22	23.16 ±0.18	0.431	21.60 ±0.43	22.18 ±0.38	0.327
Total cholesterol (mmol/l)	5.218±0.073	5.286±0.060	0.479	5.629±0.127	5.723±0.112	0.590
HDL cholesterol (mmol/l)	1.503±0.031	1.414±0.025	0.026	1.740±0.062	1.652±0.055	0.296
LDL cholesterol[b] (mmol/l)	3.063±0.070	3.206±0.057	0.118	3.450±0.117	3.507±0.104	0.715
Triglyceride (mmol/l)	1.432±0.058	1.464±0.048	0.670	0.963±0.079	1.232±0.070	0.012

[a]Drinking habits were defined as alcohol drinking one or more times per week
[b]LDL cholesterol concentrations were calculated according to the Friedewald formula (Friedewald et al. 1972)

Table 4 Body mass index (*BMI*), drinking habits, smoking habits, and serum lipid levels in premenopausal and postmenopausal females by mt5178 A/C genotypes. Age is given as mean±SD. For drinking habits (%) and current smokers (%), *P* values were calculated by using the chi-square test. BMI is given as least square mean±SE adjusted for age. Total cholesterol, high-density lipoprotein (*HDL*) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride are given as least square means±SE adjusted for age and BMI. All *P* values show the significant difference between mt5178 A and mt5178 C

Characteristic	Premenopausal females			Postmenopausal females		
	mt5178 A (*n*=20)	mt5178 C (*n*=20)	*P* value	mt5178 A (*n*=24)	mt5178 C (*n*=35)	*P* value
Age (years)	52.5 ±7.7	53.3 ±7.6	–	56.3 ±7.9	55.1 ±11.2	–
Drinking habits[a] (%)	10.00	30.00	0.236	16.66	17.14	0.760
Current smokers (%)	5.00	10.00	0.548	0.00	0.00	–
BMI (kg/m^2)	21.48 ±0.66	21.67 ±0.64	0.839	21.67 ±0.59	22.48 ±0.49	0.300
Total cholesterol (mmol/l)	5.460±0.164	5.689±0.159	0.322	5.780±0.177	5.730±0.146	0.829
HDL cholesterol (mmol/l)	1.876±0.090	1.806±0.088	0.586	1.637±0.081	1.561±0.067	0.474
LDL cholesterol[b] (mmol/l)	3.193±0.143	3.445±0.140	0.219	3.666±0.164	3.533±0.135	0.533
Triglyceride (mmol/l)	0.857±0.091	0.958±0.089	0.434	1.041±0.115	1.393±0.095	0.023

[a]Drinking habits were defined as alcohol drinking one or more times per week

42.1% and of mt5178 C is 57.9%, with no gender difference for 641 males and 649 females in Japan.

The important findings of this study were, first, that the TG concentration in females carrying mt5178 A was significantly lower than that in females carrying mt5178 C, and moreover, that this difference in the TG level between the two genotypes was clearer in postmenopausal females than in premenopausal females. Secondly, in the Japanese population, the HDLC concentrations were higher in men carrying mt5178 A than in men carrying mt5178 C. However, after adjustment for drinking and smoking habits, there was no significant association between the HDLC level and mtDNA genotype (*P*=0.078). This calls for further investigation. In Japanese patients with type 2 diabetes, the total cholesterol level in the mt5178 A group was significantly lower than that in the mt5178 C group (Matsunaga et al. 2001). However, in our study subjects, from whom we excluded diabetes patients, the total cholesterol levels did not differ between two groups.

Many previous studies have revealed that HDLC is highly associated with morbidity in coronary heart disease (Wilson 1994; Assmann et al. 1996; Goldbourt et al. 1997; Wannamethee et al. 2000). Furthermore, over the past few decades, a considerable number of studies have been conducted on the contribution of hypertriglyceridemia to atherosclerosis (Kaplan 1989; Matsuzawa et al. 1989; Haffner et al. 1992). TG-rich lipoproteins have been shown to be independent risk factors for the progression of coronary artery disease (Hodis et al. 1994). Jensen et al. (1990) have reported that the serum concentration of TG increases significantly as a consequence of menopause. After menopause, high TG, high LDLC, and high blood pressure are important all-risk factors for coronary heart disease and for cerebrovascular problems (Brocher and Arwidson 1998).

The results of our study support the speculation of Tanaka et al. (2000) that the mitochondrial genotype, mt5178 A, which predisposes resistance to adult-onset diseases including coronary heart disease and cerebrovascular diseases, is one of the genetic factors associated with longevity. The possible mechanism by which mt5178 A is associated with higher HDLC levels in men and lower triglyceride levels in postmenopausal women remains unsolved. Hegele et al. (1997) have reported that another mitochondrial DNA polymorphism, namely the mtDNA +16517 genotype, is associated with plasma TG concentration. They speculate that this mtDNA polymorphism might affect the mitochondrial β-oxidation cycle of fatty acid derived from plasma TG metabolism. Moreover, the reports that methionine residues may constitute an important antioxidant defense mechanism (Levine et al. 1996) and that mtDNA may be a major target of free radical attack (Sastre et al. 2000) should be noted. In this sense, the association of mt5178 A/C (ND2–237 M/L) with longevity deserves further investigations from various aspects.

References

Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20: 470–475

Assmann G, Schulte H, Eckardstein A von, Huang Y (1996) High-density lipoprotein as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. Atherosclerosis 124 (Suppl): S11–S20

Brocher ML, Arwidson P (1998) Coronary heart disease risk factors in women. Eur Heart J (Suppl A):A45–A52

Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31–36

De Benedictis G, Rose G, Carrieri G, De Luca M, Falcone E, Passarino G, Bonafé M, Monti D, Baggio G, Bertolini S, Mari D, Mattace R, Franceschi C (1999) Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. FASEB J 13:1532–1536

Fossati P, Prencipe L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 28:2077–2080

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502

Goldbourt U, Yaari S, Medalie JH (1997) Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality. A 21-year follow-up of 8000 men. Arterioscler Thromb Vasc Biol 17:107–113

Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP (1992) Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes 41:715–722

Hegele RA, Zinman B, Hanley AJG, Harris S, Connelly PW (1997) A common mtDNA polymorphism associated with variation in plasma triglyceride concentration. Am J Hum Genet 60:1552–1555

Hodis HN, Mack WJ, Azen SP, Alaupovic P, Pogoda JM, LaBree L, Hemphill LC, Kramsch DM, Blankenhorn DH (1994) Triglyceride- and cholesterol-rich lipoproteins have a differential as assessed by quantitative coronary angiography in a controlled trial of lovastatin. Circulation 90:42–49

Ivanova R, Lepage V, Charron D, Schächter F (1998) Mitochondrial genotype associated with French Caucasian centenarians. Gerontology 44:349

Jensen J, Nilas L, Christiansen C (1990) Influence of menopause on serum lipids and lipoproteins. Maturias 12:321–331

Kaplan NM (1989) The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglycedemia, and hypertension. Arch Intern Med 149:1514–1520

Lenaz G, D' Aurelio M, Merlo PM, Genova ML, Ventura B, Bovina C, Formiggini G, Parenti CG (2000) Mitochondrial bioenergetics in aging. Biochem Biophys Acta 1459:397–404

Levine RL, Mosoni L, Berlett BS, Stadman ER (1996) Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci USA 93:15036–15040

Matsunaga H, Tanaka Y, Tanaka M, Gong JS, Zhang J, Nimiyama T, Ogawa O, Ogihara T, Yamada Y, Yagi K, Kawamori R (2001) Antiatherogenic mitochondrial genotype in patients with type 2 diabetes. Diabetes Care 24:500–503

Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K (1989) Pathophysiology and pathogenesis of visceral fat obesity. Obes Res 2 (Suppl):S187–S194

Sastre J, Pallardo FV, Garcia de la Asuncion J, Vina J (2000) Mitochondria, oxidative stress and aging. Free Radic Res 32:189–198

Shimokata H, Yamada Y, Nakagawa M, Okubo R, Saido T, Funakoshi A, Miyasaka K, Ohta S, Tsujimoto G, Tanaka M, Ando F, Niino N (2000) Distribution of genetic disease-related genotypes in the National Institute for Longevity Science, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 10:S46–S55

Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, Miyauchi K (1995) Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated alpha-cyclodextrin. Clin Chem 41:717–723

Tanaka M, Gong JS, Zhang J, Yoneda M, Yagi K (1998) Mitochondrial genotype associated with longevity. Lancet 351:185–186

Tanaka M, Gong JS, Zhang J, Yamada Y, Yagi K (2000) Mitochondrial genotype associated with longevity and its inhibitory effect on mutagenesis. Mech Ageing Dev 116:65–76

Wallace DC (1999) Mitochondrial diseases in man and mouse. Science 283:1482–1488

Wannamethee SG, Shaper AG, Ebrahim S (2000) HDL-cholesterol, total choresterol, and the risk of stroke in middle-aged British men. Stroke 31:1882–1888

Wilson PW (1994) Established risk factors and coronary heart disease: the Framingham study. Am J Hypertens 7:S7–S12