Brief Report

Women With Mitochondrial Haplogroup N9a Are Protected Against Metabolic Syndrome

Masashi Tanaka,[1] Noriyuki Fuku,[1] Yutaka Nishigaki,[1] Hitoshi Matsuo,[2] Tomonori Segawa,[2] Sachiro Watanabe,[2] Kimihiko Kato,[3] Kiyoshi Yoko,[3] Masafumi Ito,[4] Yoshinori Nozawa,[4] and Yoshiji Yamada[5]

To identify mitochondrial haplogroups that confer resistance against or susceptibility to metabolic syndrome, we performed a large-scale association study on 1,337 unrelated Japanese individuals, including 871 subjects with metabolic syndrome and 466 control subjects. Metabolic syndrome was diagnosed according to modified National Cholesterol Education Program Adult Treatment Panel III guidelines, using the cutoff point for obesity as a BMI of ≥25 kg/m^2 instead of waist circumference. 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, G1, G2, D5, and D4. Multivariate logistic regression analysis revealed that the haplogroup N9a was significantly associated with resistance against metabolic syndrome in women with an odds ratio (OR) of 0.21 (95% CI 0.07–0.58, $P = 0.0042$). Women with haplogroups G1 and D5 tended to be resistant against metabolic syndrome with an OR of 0.22 (0.06–0.68, $P = 0.0129$) for G1 and with an OR of 0.32 (0.10–0.96, $P = 0.0469$) for D5, respectively. These results indicate that mitochondrial haplogroup N9a may be a protective factor against metabolic syndrome in Japanese women. *Diabetes* 56:518–521, 2007

Metabolic syndrome is a complex, multifactorial disorder in which interactions among various genetic and environmental influences play an important role. Metabolic syndrome is characterized by visceral obesity, hyperlipidemia, hypertension, and hyperglycemia and is pathophysiologically linked with insulin resistance. Persistence of these features of metabolic syndrome leads to the development of atherosclerosis and type 2 diabetes and, finally, to cardiovascular and cerebrovascular events that result in disability and even death. Mitochondrial dysfunction is postulated to be involved in both insulin resistance in the skeletal muscle and impaired insulin secretion from pancreatic β-cells (1).

Maternally inherited variations in the mitochondrial genome have resulted from the sequential addition of new mutations during the expansion of *Homo sapiens* from Africa to Asia and Europe over the last 200,000 years (2). Because the mutational rate of the mitochondrial genome is ~10 times higher than that of the nuclear genome, the ancient mitochondrial polymorphisms that have accumulated during the relatively short history of *Homo sapiens* may contribute to the metabolic characteristics of modern humans (3). It is reasonable to speculate that the mitochondrial haplogroups detected in various regions of the world have been selected through the adaptation to cold climates and nutritional conditions (3–5). Lines of evidence supporting functional differences among mitochondrial haplogroups have been provided by studies on sperm motility (6), survival rate after sepsis (7), and susceptibility to Parkinson's disease (8).

In earlier studies we aimed to identify mitochondrial single nucleotide polymorphisms (mtSNPs) associated with age-related conditions, such as longevity (9), Parkinson's disease (10,11), and Alzheimer's disease, with those related to energy metabolism, such as obesity (12), thinness (13), and type 2 diabetes (12), or with atherosclerosis. For this purpose, we sequenced the entire mitochondrial genome of 672 individuals (14). From our findings we constructed a human mtSNP database (http://www.giib.or.jp/mtsnp/index_e.shtml). On the basis of this database we have developed a comprehensive mtSNP analysis system by use of fluorescent beads. We have now performed a large-scale association study on metabolic syndrome and 10 major haplogroups (F, B, A, N9a, M7a, M7b, G1, G2, D5, and D4) in Japan based on the comprehensive analysis of mtSNPs. Our aim was to predict the genetic risk for metabolic syndrome and thereby contribute to the primary prevention of this condition.

RESEARCH DESIGN AND METHODS

The study population comprised 1,337 unrelated Japanese individuals (883 men, 454 women), aged ≥40 years, who either visited outpatient clinics of or were admitted to one of the participating hospitals (Gifu Prefectural Gifu, Tajimi, and Gero Hotspring Hospitals) between October 2002 and March 2005. Diagnosis was based on a modified version of the definition of metabolic syndrome proposed by the Adult Treatment Panel III (15). In this modified version, which was also used in the West of Scotland Coronary Prevention Study (16) and the Women's Health Study (17), BMI replaces waist circum-

From the [1]Department of Genomics for Longevity and Health, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; the [2]Department of Cardiology, Gifu Prefectural Gifu Hospital, Gifu, Japan; the [3]Department of Cardiovascular Medicine, Gifu Prefectural Tajimi Hospital, Tajimi, Japan; the [4]Gifu International Institute of Biotechnology, Kakamigahara, Japan; and the [5]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu, Japan.

Address correspondence and reprint requests to Masashi Tanaka, MD, PhD, Department of Genomics for Longevity and Health, Tokyo Metropolitan Institute of Gerontology, Tokyo, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan. E-mail: mtanaka@tmig.or.jp.

Received for publication 8 August 2006 and accepted in revised form 3 November 2006.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db06-1105.

mtDNA, mitochondiral DNA; mtSNP, mitochondrial single nucleotide polymorphism.

DOI: 10.2337/db06-1105

TABLE 1
Characteristics of subjects

Characteristics	All subjects			Women			Men		
	Metabolic syndrome	Control subjects	*P*	Metabolic syndrome	Control subjects	*P*	Metabolic syndrome	Control subjects	*P*
Age (years)	65.2 ± 10.1	65.9 ± 12.5	0.266	68.3 ± 10.0	64.1 ± 13.8	0.0227	63.7 ± 9.8	66.9 ± 11.6	0.0169
Sex (female/male)	33.0/67.1	35.8/64.2	0.2892						
BMI (kg/m^2)	25.3 ± 3.3	21.4 ± 2.2	<0.0001	25.2 ± 3.8	21.1 ± 2.4	<0.0001	25.4 ± 3.2	21.5 ± 2.1	<0.0001
Smoker (%)*	22.7	15	0.0006	5.9	4.2	0.419	31	21.1	0.0015
Hypertension (%)†	86.3	60.3	<0.0001	89.5	60.5	<0.0001	84.8	60.2	<0.0001
Systolic blood pressure (mmHg)	150.7 ± 27.7	133.7 ± 22.8	<0.0001	154.9 ± 30.1	134.4 ± 23.0	<0.0001	148.6 ± 26.1	133.3 ± 22.7	<0.0001
Diastolic blood pressure (mmHg)	81.9 ± 15.6	69.6 ± 9.2	<0.0001	81.4 ± 16.2	70.2 ± 10.0	<0.0001	82.2 ± 15.2	69.3 ± 8.8	<0.0001
Hypercholesterolemia (%)‡	61.6	43.8	<0.0001	67.9	53.9	0.0029	58.6	38.1	<0.0001
Total cholesterol (mmol/l)	5.37 ± 1.00	5.2 ± 0.91	0.013	5.62 ± 1.01	5.47 ± 0.87	0.0047	5.27 ± 0.98	5.06 ± 0.90	0.0189
Triglycerides (mmol/l)	2.3 ± 1.55	0.99 ± 0.33	<0.0001	2.02 ± 1.02	0.97 ± 0.32	<0.0001	2.43 ± 1.73	1.01 ± 0.33	<0.0001
HDL cholesterol (mmol/l)	1.13 ± 0.40	1.56 ± 0.36	<0.0001	1.24 ± 0.33	1.76 ± 0.38	<0.0001	1.08 ± 0.42	1.46 ± 0.31	<0.0001
Diabetes (%)§	64.7	8.8	<0.0001	60.6	9	<0.0001	67.1	8.7	<0.0001
Fasting blood glucose (mmol/l)	8.24 ± 3.8	4.99 ± 0.63	<0.0001	8.16 ± 3.63	4.99 ± 0.59	<0.0001	8.29 ± 3.88	4.98 ± 0.66	<0.0001
A1C (%)	7.14 ± 2.15	5.66 ± 1.76	<0.0001	7.22 ± 1.93	5.94 ± 2.41	0.0742	7.10 ± 2.26	5.54 ± 1.39	<0.0001

Data are means ± SD or percent. *Defined as smoking ≥10 cigarettes/day. †Defined as having systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, both, or taking antihypertensive medication. ‡Defined as having serum total cholesterol ≥220 mg/dl (5.72 mmol/l) or taking lipid-lowering medication. §Defined as having fasting blood glucose ≥126 mg/dl (6.93 mmol/l) or A1C ≥6.5%, both, or taking antidiabetic medication.

ference. On the basis of the recent recognition of a need to revise BMI criteria for obesity in Japanese and other Asian populations (18), we set the cutoff point for obesity as a BMI of ≥25 kg/m^2. Participants were thus diagnosed with metabolic syndrome if they had three or more of the following five components: *1*) a BMI of ≥25 kg/m^2, *2*) a serum concentration of triglycerides of ≥1.65 mmol/l (150 mg/dl), *3*) a serum concentration of HDL cholesterol of <1.04 mmol/l (40 mg/dl) for men or <1.30 mmol/l (50 mg/dl) for women, *4*) a systolic blood pressure of ≥130 mmHg and diastolic blood pressure of ≥85 mmHg, and *5*) a fasting plasma glucose level of ≥6.05 mmol/l (110 mg/dl). On the basis of these criteria, 871 subjects (584 men, 287 women) were diagnosed with metabolic syndrome. The control subjects comprised a total of 466 individuals (299 men, 167 women) who visited the outpatient clinics of the participating hospitals for an annual health checkup. They had none of the five criteria of metabolic syndrome described above or any history of obesity, hyperlipidemia, dyslipidemia, hypertension, or diabetes. Individuals with metabolic or endocrinologic diseases or those taking drugs that cause secondary diabetes were excluded from the study. The study protocol was approved by the Committee on the Ethics of Human Research of Gifu International Institute of Biotechnology, and informed consent was obtained from each participant.

Selection of mitochondrial polymorphisms for haplogroup classification. With the use of our human mtSNP database (http://www.giib.or.jp/mtsnp/index_e.shtml) and the phylogenetic tree of Japanese (14), we selected 149 polymorphic sites that have been useful for classification of mitochondrial haplogroups. We further selected 25 mtSNPs that define 10 major haplogroups (A, B, D4, D5, F, G1, G2, N9a, M7a, and M7b) found in the Japanese population (Supplementary Table 1, available at http://dx.doi.org/10.2337/db06-1105). We examined the relationship between these haplogroups and metabolic syndrome in the 1,337 participants of the present study.

Genotyping of polymorphisms. Total DNA was extracted from blood samples, and fragments of mitochondrial DN (mtDNA) were amplified from the total DNA by 28-plex PCR. Mitochondrial polymorphisms were determined with sequence-specific oligonucleotide probes by use of suspension array technology (Luminex 100; Luminex, Austin, TX). Detailed methodology for genotyping was described in the legend for Supplementary Table 1.

Statistical analysis. Quantitative clinical data were compared between patients with metabolic syndrome and control subjects by the unpaired Student's *t* test. Qualitative data were compared by the χ^2 test. We performed multivariate logistic regression analysis to adjust risk factors, with metabolic syndrome as a dependent variable and independent variables including age, sex, smoking status, and genotype of each mtSNP. The *P* value, odds ratio (OR), and 95% CI were calculated. Unless indicated otherwise, a *P* value of <0.05 was considered statistically significant. Because of multiple comparisons of haplogroups, we applied Bonferroni's correction. Since we examined 10 haplogroups, we divided 0.05 by 10 to get 0.005. Thus, a *P* value of <0.005 was considered statistically significant.

RESULTS

The characteristics of the 1,337 subjects are shown in Table 1. Neither age nor female-to-male ratio was different between subjects with metabolic syndrome and control subjects. BMI, triglycerides, fasting plasma glucose concentration, and A1C were significantly higher in subjects with metabolic syndrome than in control subjects. HDL cholesterol was significantly lower in metabolic syndrome subjects than in control subjects. The prevalences of smoking, hypertension, hypercholesterolemia, and diabetes were significantly higher in subjects with metabolic syndrome than in control subjects.

Multivariate logistic regression analysis with adjustment for age, sex, and smoking status revealed that the mitochondrial haplogroup N9a was related to resistance against metabolic syndrome and that three haplogroups, i.e., N9a, G1, and D5, were related to resistance against metabolic syndrome in women (Table 2), on the basis of a *P* value of <0.05. However, because of the multiple comparisons of haplogroups, we considered a *P* value of <0.005 to be significant for such associations. On the basis of this criterion, the haplogroup N9a was significantly associated with resistance against metabolic syndrome for women.

A stepwise forward selection procedure revealed that

TABLE 2
Multivariate logistic regression analysis of haplogroups associated with metabolic syndrome with adjustment for age, sex, and prevalence of smoking

Variable	*P*	OR (95% CI)
All subjects		
Conventional risk factors		
Age	0.5558	
Sex	0.8721	
Smoking	0.0020	1.6 (1.2–2.2)
Genetic risk factors		
Haplogroup N9a	0.0135	0.52 (0.31–0.88)
Women		
Conventional risk factors		
Age	0.0002	0.13 (0.05–0.37)
Smoking	0.2258	
Genetic risk factors		
Haplogroup N9a	0.0042	0.21 (0.07–0.58)
Haplogroup G1	0.0129	0.22 (0.06–0.68)
Haplogroup D5	0.0496	0.32 (0.10–0.96)
Men		
Conventional risk factors		
Age	0.0001	6.0 (2.4–15.0)
Smoking	0.0142	1.5 (1.1–2.1)
Genetic risk factors		
Haplogroup N9a	0.3622	0.7 (0.4–1.4)
Haplogroup G1	0.5510	1.3 (0.6–3.0)
Haplogroup D5	0.8524	0.9 (1.5–2.0)

haplogroup N9a is a protective factor against metabolic syndrome (Table 3). For men, however, none of these haplogroups were associated with metabolic syndrome. For women, a stepwise forward selection procedure revealed that age and haplogroups N9a, G1, and D5 were independent risk or protective factors against metabolic syndrome. The total contribution of haplogroups N9a, G1, and D5 to protection against metabolic syndrome was 0.0361, suggesting that mitochondrial genome polymorphisms are important genetic factors influencing the susceptibility to metabolic syndrome for women.

DISCUSSION

We examined the relationship between metabolic syndrome and each of 10 major mitochondrial haplogroups in a large-scale association study of 1,337 individuals. Three of these haplogroups, i.e., N9a, G1, and D5, were significantly associated with resistance against metabolic syndrome in women. The present observation in Japanese subjects need to be replicated in other populations to confirm whether these haplogroups influence metabolic characteristics of individuals. In the present study, we adopted the criteria of BMI $\geq$25 kg/m^2 instead of waist circumference. If we adopt different criteria of metabolic syndrome, relations of mitochondrial haplogroups to this condition might differ from those observed in the present study.

TABLE 3
Effects of haplogroups and other characteristics on metabolic syndrome assessed by a stepwise forward selection procedure

Variable	*P* value	R^2
All subjects		
Smoking	0.0006	0.0068
Haplogroup N9a	0.0147	0.0034
Women		
Age	0.0002	0.0227
Haplogroup N9a	0.0022	0.0156
Haplogroup G1	0.0061	0.0126
Haplogroup D5	0.0293	0.0079
Smoking	0.1626	0.0033

Most of the mtSNPs characteristic of haplogroup N9a are synonymous mutations including 5231G→A and 12372G→A, which were used for the present genotyping. Possible candidates for functional polymorphisms in this haplogroup are 150C→T and 338C→T, which are in the noncoding region of the mitochondrial genome. The 150C→T replacement was originally reported in Italian centenarians (19). Also, we reported this replacement to be associated with healthy longevity in both Finland and Japan (20). Thus, the 150C→T might confer resistance against metabolic syndrome in women.

Among haplogroup N9a-specific mtSNPs, the 12358A→G causing the Thr8Ala replacement in the NADH dehydrogenase subunit 5 (ND5) would seem to be one of the potentially functional polymorphisms (Supplementary Tables 5 and 6). This subunit seems to be essential for the function of Complex I, because various missense mutations in the *ND5* gene have been reported in mitochondrial diseases. Therefore, it seems possible that the Thr8Ala replacement caused by the 12358A→G might also affect the function of the ND5 subunit and Complex I. The actual effect of the mtSNP 12358A→G (ND5: T8A) on mitochondrial function remains to be examined.

Among the mtSNPs characteristic of haplogroup G1, the 15497G→A (Cyt*b*: G251S) seems to be a functional polymorphism. Our previous survey as a part of the National Institute for Longevity Longitudinal Study on Aging revealed that the 15497G→A (Cyt*b*: G251S) was associated with obesity in a middle-aged and elderly Japanese population (21). This observation suggests that the 15497A is associated with simple obesity without hypertension, hyperglycemia, or hypercholesterolemia, which are components of metabolic syndrome. Thus, the present result showing that the haplogroup G1 with 15497A conferred resistance against metabolic syndrome is consistent with our previous observation that the metabolism of both glucose and cholesterol is relatively normal in women with the 15497A allele (21).

In our previous study, we reported that haplogroup D, characterized by the 5178C→A (ND2: L237M) polymorphism, is associated with longevity and that this polymorphism confers resistance against age-related diseases (9). In addition to the 5178C→A, individuals with haplogroup D5 carry the 150C→T transition in the noncoding region of their mitochondrial genome. We recently reported that the 150C→T transition was more frequently detected in both Japanese centenarians and the Finnish nanogerians (>90 years) than in the respective control populations (20). The protective effect of haplogroup D5 against metabolic syndrome reported here is consistent with our previous observation that the 150C→T is associated with longevity.

Because there are no recombination mechanisms in mitochondria, all of the mtSNPs in each haplogroup or subhaplogroups are tightly linked to each other. Therefore, it is possible that some of the mtSNPs examined in our present study are in linkage disequilibrium with functional polymorphisms of other mitochondrial genes that are actually responsible for the resistance against metabolic syndrome. For women, haplogroups N9a, G1, and D5 were associated with resistance against metabolic syn-

drome, whereas these associations were not detected in men. There is other evidence supporting the idea that the association of genetic variation with obesity is stronger in women than in men (22,23). As shown in Table 2, old age was associated with an increased risk for metabolic syndrome in women, whereas younger age was associated with an increased risk for this condition in men. This difference in the effect of age on the risk of metabolic syndrome between women and men might provide us a clue to understand the sex difference in the effects of mitochondrial haplogroups. The possible cause of metabolic syndrome in women would be related to the biologically defined and age-dependent decrease in tissue metabolisms. In men, the cause of metabolic syndrome might be related to extrinsic factors, such as smoking, satiety, and physical inactivity. These differences might explain the present observation that women but not men were distinctly influenced by the differences among mitochondrial haplogroups.

Several association studies have previously examined the relationship between nuclear gene polymorphisms and metabolic syndrome. Yamada et al. (24) showed that a nuclear single nucleotide polymorphism in the promoter region of the apolipoprotein A-V gene is associated with metabolic syndrome. Genotyping of these mtSNPs and nuclear single nucleotide polymorphisms may prove informative for predicting the genetic risk for metabolic syndrome and may thereby contribute to the primary prevention of metabolic syndrome.

ACKNOWLEDGMENTS

This work was supported in part by a grant-in-aid (no. A-15200051 to M.T.) from the Ministry of Education, Culture, Sports, Science, and Technology; by a grant from the Third-Term Comprehensive 10-year Strategy for Cancer Control (to M.T.); by grant 17A-10 from the program Research Grants for Nervous and Mental Disorders of the Ministry of Health, Labor, and Welfare (to M.T.), by grants for scientific research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (no. 15209021 to Y.Y), and by the Gifu Prefectural Science and Technology Promotion Center (to Y.Y.).

APPENDIX

In addition to the authors, the following individuals participated in the study: Y. Matsuno and M. Tomita (Gifu Prefectural Gifu Hospital, Gifu); M. Oguri, T. Hibino, and T. Kameyama (Gifu Prefectural Tajimi Hospital, Tajimi); S. Tanihata (Gifu Prefectural Gero Hotspring Hospital, Gero); and nursing and laboratory staff of the participating hospitals.

REFERENCES

1. Lowell BB, Shulman GI: Mitochondrial dysfunction and type 2 diabetes. *Science* 307:384–387, 2005
2. Macaulay V, Hill C, Achilli A, Rengo C, Clarke D, Meehan W, Blackburn J, Semino O, Scozzari R, Cruciani F, Taha A, Shaari NK, Raja JM, Ismail P, Zainuddin Z, Goodwin W, Bulbeck D, Bandelt HJ, Oppenheimer S, Torroni A, Richards M: Single, rapid coastal settlement of Asia revealed by analysis of complete mitochondrial genomes. *Science* 308:1034–1036, 2005
3. Wallace DC: A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 39:359–407, 2005
4. Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, Sukernik RI, Olckers A, Wallace DC: Natural selection shaped regional mtDNA variation in humans. *Proc Natl Acad Sci U S A* 100:171–176, 2003
5. Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC: Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* 303:223–226, 2004
6. Montiel-Sosa F, Ruiz-Pesini E, Enriquez JA, Marcuello A, Diez-Sanchez C, Montoya J, Wallace DC, Lopez-Perez MJ: Differences of sperm motility in mitochondrial DNA haplogroup U sublineages. *Gene* 368C:21–27, 2006
7. Baudouin SV, Saunders D, Tiangyou W, Elson JL, Poynter J, Pyle A, Keers S, Turnbull DM, Howell N, Chinnery PF: Mitochondrial DNA and survival after sepsis: a prospective study. *Lancet* 366:2118–2121, 2005
8. Pyle A, Foltynie T, Tiangyou W, Lambert C, Keers SM, Allcock LM, Davison J, Lewis SJ, Perry RH, Barker R, Burn DJ, Chinnery PF: Mitochondrial DNA haplogroup cluster UKJT reduces the risk of PD. *Ann Neurol* 57:564–567, 2005
9. Tanaka M, Gong JS, Zhang J, Yoneda M, Yagi K: Mitochondrial genotype associated with longevity. *Lancet* 351:185–186, 1998
10. Ikebe S, Tanaka M, Ozawa T: Point mutations of mitochondrial genome in Parkinson's disease. *Brain Res Mol Brain Res* 28:281–295, 1995
11. Tanaka M: Mitochondrial genotypes and cytochrome b variants associated with longevity or Parkinson's disease. *J Neurol* 249 (Suppl. 2):11–18, 2002
12. Guo LJ, Oshida Y, Fuku N, Takeyasu T, Fujita Y, Kurata M, Sato Y, Ito M, Tanaka M: Mitochondrial genome polymorphisms associated with type-2 diabetes or obesity. *Mitochondrion* 5:15–33, 2005
13. Fuku N, Oshida Y, Takeyasu T, Guo LJ, Kurata M, Yamada Y, Sato Y, Tanaka M: Mitochondrial ATPase subunit 6 and cytochrome b gene polymorphisms in young obese adults. *Biochem Biophys Res Commun* 290:1199–1205, 2002
14. Tanaka M, Cabrera VM, Gonzalez AM, Larruga JM, Takeyasu T, Fuku N, Guo LJ, Hirose R, Fujita Y, Kurata M, Shinoda K, Umetsu K, Yamada Y, Oshida Y, Sato Y, Hattori N, Mizuno Y, Arai Y, Hirose N, Ohta S, Ogawa O, Tanaka Y, Kawamori R, Shamoto-Nagai M, Maruyama W, Shimokata H, Suzuki R, Shimodaira H: Mitochondrial genome variation in eastern Asia and the peopling of Japan. *Genome Res* 14:1832–1850, 2004
15. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 285:2486–2497, 2001
16. Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J: Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. *Circulation* 108:414–419, 2003
17. Ridker PM, Buring JE, Cook NR, Rifai N: C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14,719 initially healthy American women. *Circulation* 107:391–397, 2003
18. Kanazawa M, Yoshiike N, Osaka T, Numba Y, Zimmet P, Inoue S: Criteria and classification of obesity in Japan and Asia-Oceania. *Asia Pac J Clin Nutr* 11 (Suppl. 8):S732–S737, 2002
19. Zhang J, Asin-Cayuela J, Fish J, Michikawa Y, Bonafe M, Olivieri F, Passarino G, De Benedictis G, Franceschi C, Attardi G: Strikingly higher frequency in centenarians and twins of mtDNA mutation causing remodeling of replication origin in leukocytes. *Proc Natl Acad Sci U S A* 100:1116–1121, 2003
20. Niemi AK, Moilanen JS, Tanaka M, Hervonen A, Hurme M, Lehtimaki T, Arai Y, Hirose N, Majamaa K: A combination of three common inherited mitochondrial DNA polymorphisms promotes longevity in Finnish and Japanese subjects. *Eur J Hum Genet* 13:166–170, 2005
21. Okura T, Koda M, Ando F, Niino N, Tanaka M, Shimokata H: Association of the mitochondrial DNA 15497G/A polymorphism with obesity in a middle-aged and elderly Japanese population. *Hum Genet* 113:432–436, 2003
22. Borecki IB, Bonney GE, Rice T, Bouchard C, Rao DC: Influence of genotype-dependent effects of covariates on the outcome of segregation analysis of the body mass index. *Am J Hum Genet* 53:676–687, 1993
23. Comuzzie AG, Blangero J, Mahaney MC, Mitchell BD, Hixson JE, Samollow PB, Stern MP, MacCluer JW: Major gene with sex-specific effects influences fat mass in Mexican Americans. *Genet Epidemiol* 12:475–488, 1995
24. Yamada Y, Kato K, Hibino T, Yokoi K, Matsuo H, Segawa T, Watanabe S, Ichihara S, Yoshida H, Satoh K, Nozawa Y: Prediction of genetic risk for metabolic syndrome. *Atherosclerosis*. In press