Genetic Epidemiological Studies of Longevity-Associated Mitochondrial DNA 5178 C/A Polymorphism*

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Abstract

Mitochondrial DNA 5178 cytosine/adenine (Mt5178 C/A) polymorphism is associated with longevity in the Japanese population, and the Mt5178A genotype may confer antiatherogenic advantages. Individuals with the Mt5178A genotype may be more resistant to adult-onset diseases, such as myocardial infarction, cerebrovascular diseases and type 2 diabetes, than those with the Mt5178C genotype. Moreover, Mt5178 C/A polymorphism has been reported to be associated with blood pressure, serum lipid levels, hematological parameters, intraocular pressure, serum protein fraction levels and serum electrolyte levels in healthy Japanese individuals. Differences in the influence of habitual drinking or habitual smoking on health status between the Mt5178C genotype and the Mt5178A genotype have been reported. The individual modification of drinking habits or smoking habits based on the genotyping of Mt5178 C/A may promote improved health and lead to the establishment of personalized prevention strategies for adult-onset diseases.

Key words: mitochondrial DNA polymorphism, longevity, habitual drinking, habitual smoking

Introduction

Genetic epidemiology is the integration of genetic information into epidemiologic research and is useful in clarifying the influences of the interactions between lifestyle and genetic factors on the risk of important human diseases (1). Moreover, the individual modification of lifestyle based on genetic susceptibility may have the potential to establish personalized prevention strategies for lifestyle-related adult-onset diseases. Mitochondrial DNA 5178 cytosine/adenine (Mt5178 C/A) polymorphism, which results in NADH dehydrogenase subunit-2 237 leucine/methionine (ND-237 Leu/Met) polymorphism, is a candidate genetic marker of adult-onset disease susceptibility in the Japanese population. This polymorphism was originally reported to be associated with longevity in Japanese people (2), with the frequency of the Mt5178A genotype being significantly higher in centenarians than in the general population. There have also been reports of genetic differences between the Mt5178 C/A genotypes with regard to susceptibility to adult-

onset diseases (3–6), with the frequency of the Mt5178C genotype being significantly higher in patients with myocardial infarction (3, 4), cerebrovascular disorders (5), or type 2 diabetes (6) than in normal controls. In addition, the mean age at onset of diabetes is significantly lower in patients with the Mt5178C genotype than in those with the Mt5178A genotype (6), while ultrasonography revealed that the mean intima-media thickness in the bilateral carotid arteries is significantly larger in type 2 diabetic patients with the Mt5178C genotype than in those with the Mt5178A genotype (7). Thus, individuals with the Mt5178A genotype appear to be resistant to atherogenic diseases and tend to experience increased longevity (Fig. 1). From the viewpoint of the primary prevention of atherosclerotic diseases, it is crucial to investigate differences between the Mt5178C genotype and the Mt5178A genotype with regard to biophysical and biochemical statuses among healthy Japanese people. We thus conducted cross-sectional studies, the protocols of which were approved by the Ethical Committee of Kyorin University, and reported the associations of longevity-associated Mt5178 C/A polymorphism with blood pressure (8), serum lipid levels (9–11), hematological parameters (12), intraocular pressure (13), serum protein fraction levels (14, 15) and serum electrolyte levels (16) in the Japanese population.

Mt5178 C/A polymorphism and blood pressure

Blood pressure is a crucial factor in the pathology of age-related circulatory diseases. Therefore, we investigated the

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Fig. 1 Postulated scheme of association of Mt5178 C/A polymorphism with longevity. The Mt5178A genotype may be protective against adult-onset diseases, such as atherogenic diseases and type 2 diabetes. This effect may make it more possible for individuals with the Mt5178A genotype to realize long life than for those with the Mt5178C genotype.

association between Mt5178 C/A polymorphism and blood pressure (8). In healthy Japanese women, the diastolic blood pressure (DBP) was significantly higher in those with the Mt5178A genotype than in those with the Mt5178C genotype. After adjusting for age, body mass index (BMI), habitual drinking, and habitual smoking, this difference remained statistically significant. However, whether the higher DBP in Mt5178A genotypic women is related to longevity warrants further longitudinal studies. In healthy Japanese men, Mt5178 C/A polymorphism was found to be not associated with blood pressure. However, Mt5178 C/A polymorphism may be indirectly associated with systolic blood pressure (SBP) and DBP through drinking frequency. After adjusting for age, BMI, and habitual smoking, SBP was confirmed to be significantly higher in daily drinkers with the Mt5178C genotype than in occasional drinkers and nondrinkers with the Mt5178C genotype. Moreover, DBP was observed to be significantly higher in daily drinkers with the Mt5178C genotype than in occasional drinkers and nondrinkers with the Mt5178C genotype. However, in men with the Mt5178A genotype, no significant differences in blood pressure among daily drinkers, occasional drinkers and nondrinkers were observed. Mt5178 C/A polymorphism may influence the blood-pressure-increasing effects of alcohol consumption.

Mt5178 C/A polymorphism and serum lipid levels

Longevity-associated Mt5178 C/A polymorphism may be associated with serum lipid levels in the Japanese population (9–11). In healthy men, after adjusting for age and BMI, the high-density lipoprotein cholesterol levels were significantly higher in those with the Mt5178A genotype than in those with the Mt5178C genotype (9). However, after adjusting for habitual drinking, this significant difference disappeared. We therefore investigated the influence of the interaction between Mt5178 C/A polymorphism and drinking frequency on serum lipid level in healthy middle-aged male subjects (10). Mt5178 C/A polymorphism may interact with daily drinking to

influence serum triglyceride (TG) level. Moreover, this polymorphism may interact with habitual smoking to influence serum TG level. In men with the Mt5178A genotype, daily drinking decreased TG level and cigarette consumption increased it, while in men with the Mt5178C genotype, such effects of daily drinking and cigarette consumption were not observed. Further analyses revealed the influence of the interaction between Mt5178 C/A polymorphism and cigarette smoking on serum TG level in subjects with BMI $\leq$ 22, and on serum total cholesterol (TC) level and serum low-density lipoprotein cholesterol (LDLC) level in subjects with BMI $>$ 22 (11). In subjects with BMI $>$ 22, the TC levels were significantly lower in nonsmokers with the Mt5178A genotype than in smokers with the Mt5178A genotype, while the LDLC levels were lower in nonsmokers with the Mt5178A genotype than in those with the Mt5178C genotype or in smokers with the Mt5178A genotype. Although no influence of the interaction between Mt5178 C/A polymorphism and habitual smoking on TG level in subjects with BMI $>$ 22 was observed, TG levels were significantly higher in smokers with the Mt5178A genotype than in nonsmokers with the Mt5178A genotype as well as in nonsmokers with the Mt5178C genotype. In healthy women, TG levels were significantly lower in those with the Mt5178A genotype than in those with the Mt5178C genotype (9). This difference in TG level between the Mt5178C genotype and the Mt5178A genotype was more evident in postmenopausal women than in premenopausal women. These findings support the postulate that the Mt5178A genotype exerts an antiatherogenic effect. However, habitual smoking may deprive Japanese Mt5178A genotypic men having BMI $>$ 22 of their antiatherogenic advantage.

Mt5178 C/A polymorphism and hematological parameters

We analyzed data on hematological parameters from the viewpoint of thrombogenesis (12). No significant differences in blood parameters were observed between Mt5178 C genotypic

men and Mt5178A genotypic men. However, in subjects with BMI $\leq$ 23, this polymorphism modulated the effects of habitual smoking on hematological parameters. Red blood cell (RBC) counts were significantly lower in smokers with the Mt5178A genotype than in nonsmokers with the Mt5178A genotype. Platelet counts were significantly higher in smokers with the Mt5178C genotype than nonsmokers with the Mt5178C genotype. Cigarette consumption was strongly associated with RBC count for men with the Mt5178A genotype, and was associated with platelet count for those with the Mt5178C genotype. These data suggest that Mt5178 C/A polymorphism influences the effects of cigarette smoking on hematological parameters in healthy men with BMI $\leq$ 23.

Mt5178 C/A polymorphism and intraocular pressure

We reported that lifestyle is associated with intraocular pressure (IOP) in middle-aged Japanese men (17). From a biophysical standpoint, analysis of the influence of the interaction between Mt5178 C/A polymorphism and lifestyle on IOP was thus investigated. The mean IOP was significantly higher in men with the Mt5178C genotype than in those with the Mt5178A genotype (13). This difference in mean IOP between the Mt5178C genotype and the Mt5178A genotype remained evident after adjusting for age, BMI, blood pressure, habitual smoking and habitual drinking. Moreover, the influence of the interactions between Mt5178 C/A polymorphism and habitual smoking or habitual drinking on IOP was observed. Habitual smoking was significantly associated with IOP in men with the Mt5178A genotype, while daily alcohol consumption was significantly associated with IOP in those with the Mt5178C genotype.

Mt5178 C/A polymorphism and serum protein fraction levels

From a gerontological point of view, the difference between the Mt5178 C genotype and the Mt5178A genotype with regard to serum protein fraction levels is particularly interesting. Alpha-1, alpha-2, and beta globulin proportions were significantly higher in women with the Mt5178A genotype than in those with the Mt5178C genotype (14). Alpha-1, alpha-2, and beta globulin levels were also significantly higher in Mt5178A genotypic women than in Mt5178C genotypic women. These differences in serum protein fraction levels between the Mt5178 C/A genotypes were more evident in premenopausal women than in postmenopausal women. However, no such differences in serum protein fraction levels were observed in men. Considering the influence of cigarette smoking on serum protein fraction levels, we investigated whether there are differences in the influences of habitual smoking on serum protein fraction levels between Mt5178C genotypic men and Mt5178A genotypic men (15). In men with the Mt5178C genotype, the alpha-1 and alpha-2 globulin levels were significantly higher in smokers than in nonsmokers, and the influence of smoking on these globulin levels depended on daily cigarette consumption. However, in men with the Mt5178A genotype, no significant differences were observed in alpha-1

or alpha-2 globulin level between smokers and nonsmokers. Considering that the levels of alpha-1 antitrypsin, alpha-1 acid glycoproteins and alpha-2 macroglobulin are reportedly associated with the degree of atherosclerosis (18), these findings suggest that habitual smoking exacerbates atherosclerosis in men with the Mt5178C genotype, and that Mt5178A genotypic men are resistant to the aggravating effect of cigarette smoking on atherosclerosis. However, further investigation is required to elucidate the Mt5178 C/A polymorphism-related differences in serum protein levels between men and women.

Mt5178 C/A polymorphism and serum electrolyte levels

To determine the influence of age-related changes in mitochondrial functions on the regulation of serum electrolyte levels, we investigated the relationships between Mt5178 C/A genotype and serum electrolyte levels (16). Crude data showed that serum sodium and chlorine levels were significantly lower in men with the Mt5178A genotype than in those with the Mt5178C genotype. Cigarette consumption and BMI significantly and positively associated with serum chlorine level, while hemoglobin level significantly and negatively associated with serum chlorine level in Mt5178C genotypic men. In men with the Mt5178A genotype, only hemoglobin level significantly and negatively associated with serum chlorine level. In obese (BMI $\geq$ 25) subjects, after adjusting for covariates, the serum sodium and chlorine levels remained significantly lower, and the serum calcium levels were significantly higher in those with the Mt5178A genotype than in those with the Mt5178C genotype. Longevity-associated Mt5178 C/A polymorphism may influence serum electrolyte levels in middle-aged obese Japanese men.

Prospects for research on longevity-associated Mt5178 C/A polymorphism

In addition to other case-control studies on the association between Mt5178 C/A polymorphism and adult-onset diseases (3–7), we have reported the association of Mt5178 C/A polymorphism with biophysical and biomedical statuses in the healthy Japanese population (8–16). Although the lack of association between Mt5178 C/A polymorphism and six types of cancer (i.e., esophageal cancer, stomach cancer, colorectal cancer, lung cancer, breast cancer, and malignant lymphoma) has been reported (19), this polymorphism has also been reported to be associated with type 1 diabetes (20), bipolar disorder (21) and eating behavior (22). However, the mechanisms by which Mt5178 C/A polymorphism exerts biophysical, biochemical and medical effects remain unknown. Mitochondria are both sources and targets of reactive oxygen species (ROS) (23). Mitochondrial dysfunction and mitochondrial DNA damage, which are brought by ROS, are reportedly associated with cardiovascular diseases and their risk factors, namely, atherosclerosis, hypercholesterolemia, and diabetes (24). Levine et al. reported that methionine residues may act as an endogenous antioxidant (25). It is thought that the differences in enzymatic function and sensitivity to ROS between

Fig. 2 Effects of interaction between Mt5178 C/A polymorphism and habitual drinking or habitual smoking on biophysical and biochemical properties.

ND2-237Leu and ND2-237Met result in differences in health status or the prevalence of adult-onset diseases. However, further investigation into these mechanisms is required.

We have reported differences in the influences of habitual drinking on blood pressure (8), serum lipid levels (10) and IOP (13), and differences in the influences of habitual smoking on serum lipid levels (10, 11), RBC count (12), IOP (13) and serum protein fraction levels (15) between the Mt5178C genotype and the Mt5178A genotype (Fig. 2). Therefore, the individual modification of drinking or smoking habits based on the Mt5178 C/A genotype may improve health conditions, such as blood pressure and serum lipid levels. Further investigation into the association between Mt5178 C/A polymorphism and health status may contribute to the establishment of

personalized prevention strategies for lifestyle-related diseases.

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