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Longevity-associated NADH dehydrogenase subunit-2 polymorphism and serum electrolyte levels in middle-aged obese Japanese men

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Abstract

Mitochondrial DNA 5178 cytosine/adenine polymorphism, which is also called NADH dehydrogenase subunit-2 237 leucine/methionine (ND2-237 Leu/Met) polymorphism is associated with Japanese longevity. This polymorphism is widely associated with blood pressure, serum lipid levels, hematological parameters, intraocular pressure, and serum protein fraction levels. However, there have been no reports on the association between ND2-237 Leu/Met polymorphism and serum electrolyte levels. To investigate this relationship, we performed an association study in 321 healthy middle-aged Japanese men. Crude data showed that serum sodium levels and serum chloride levels were significantly lower in men with ND2-237 Met than in those with ND2-237 Leu ($P = 0.021$ and 0.003, respectively). Cigarette consumption and body mass index were significantly and positively associated with serum chloride levels ($P = 0.002$ and 0.008, respectively) and hemoglobin levels were significantly and negatively associated with them ($P = 0.007$) in ND2-237 Leu genotypic men. In men with ND2-237 Met, only hemoglobin levels were significantly and negatively associated with serum chloride levels ($P = 0.025$). After adjusting for covariates, only in male obese (body mass index ≥ 25) subjects, serum sodium and chloride levels remained significantly lower, and serum calcium levels appeared to be significantly higher in ND2-237 Met than in ND2-237 Leu ($P = 0.013$, <0.001, and 0.046, respectively). Longevity-associated NADH dehydrogenase subunit-2 polymorphism may influence serum electrolyte levels in middle-aged obese Japanese men.

Keywords: Longevity-associated mitochondrial DNA polymorphism; NADH dehydrogenase; Serum sodium levels; Serum chloride levels

1. Introduction

Physiological factors, such as body composition, thirst perception, renal function and hormonal systems are involved in aging-associated changes in water and electrolyte balance (Miller, 2003). Therefore, in order to better understand the mechanisms of aging, it is important to detect genetic factors affecting serum electrolyte levels. Mitochondrial DNA, which plays a role in aging (Attardi, 2002) is potentially one of these genetic factors.

Several longevity-associated mitochondrial DNA polymorphisms have been reported (Ivanova et al., 1998; Tanaka et al., 1998; De Benedictis et al., 1999; Ross et al., 2001; Niemi et al., 2003). NADH dehydrogenase subunit-2 237

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leucine/methionine polymorphism (ND2-237 Leu/Met), which results from mitochondrial DNA 5178 cytosine/adenine (Mt5178 C/A) polymorphism is reportedly associated with longevity in the Japanese population (Tanaka et al., 1998). Tanaka et al. (1998) reported that the frequency of ND2-237 Met was significantly higher in Japanese centenarians than in the general population. Several case–control studies have shown that individuals with ND2-237 Leu are more susceptible to adult-onset diseases (Wang et al., 2001; Ohkubo et al., 2002; Mukae et al., 2003; Takagi et al., 2004) than those with ND2-237 Met. In contrast, individuals with ND2-237 Met were found to be resistant to atherosclerosis (Kokaze et al., 2001, 2003a; Matsunaga et al., 2001). Moreover, this longevity-associated polymorphism may be associated with blood pressure (Kokaze et al., 2004a), serum lipid levels (Kokaze et al., 2001, 2003b), hematological parameters (Kokaze et al., 2005), intraocular pressure (Kokaze et al., 2004b), or serum protein fraction levels (Kokaze et al., 2002, 2003a). This genetic factor thus widely affects physiological and biochemical conditions. However, to the best of our knowledge, the relationship between ND2-237 Leu/Met polymorphism and serum electrolyte levels in healthy individuals has not been reported.

We, therefore investigated the relationship between longevity-associated ND2-237 Leu/Met polymorphism and serum sodium, potassium, chloride and calcium levels in middle-aged Japanese men.

2. Subjects and methods

2.1. Subjects

Participants were recruited from individuals visiting the Mito Red Cross Hospital for medical check-ups. The study was performed according to the Declaration of Helsinki (revised in Edinburgh, 2000), and was approved by the Ethical Committee of Kyorin University School of Medicine. Written informed consent was obtained from 602 volunteers before participation. In total, 79 volunteers were excluded from analysis for lack of data, and 111 were excluded for having chronic disease or taking medication. After excluding 91 healthy women, 321 men (mean age $\pm$ S.D.; 53.0 $\pm$ 7.7 years) were enrolled in this study.

2.2. Serum electrolyte levels and other biochemical data

Determination of blood chemical data were as previously reported (Kokaze et al., 2001). Briefly, plasma samples were obtained after a minimum 12-h fasting period. Blood chemical data were measured by routine methods at the Mito Red Cross Hospital. Serum electrolyte levels were measured by autoanalyzer (HITACHI 7600-110S). Body mass index (BMI) was calculated as the ratio of subject weight (kg) to the square of subject height (m). Drinking and smoking frequency were ascertained using an original questionnaire. Habitual smoking was evaluated as the number of cigarettes consumed per day.

2.3. Genotyping

Genotyping methods were as described previously (Kokaze et al., 2001). Briefly, DNA was extracted from peripheral white blood cells. The genomic region coding the NADH dehydrogenase subunit 2 was amplified using polymerase chain reaction (PCR), and the PCR products were digested with *Alu*I. The absence of an *Alu*I site was designated as ND2-237 Met (Mt5178A), and the presence of this restriction site was designated as ND2-237 Leu (Mt5178C).

2.4. Statistical analyses

All statistical analyses were performed with SAS statistical software, version 8.2 for Windows (SAS Institute Inc., Cary, NC). In analysis of covariates (ANCOVA), the ND2-237 Leu/Met genotypes (ND2-237 Leu = 0, ND2-237 Met = 1) were numerically coded. Age, BMI, hemoglobin level, creatinine level, drinking frequency (ex- or non-drinkers = 0; 'several times per month' drinkers = 1; 'several times per week' drinkers = 2; daily drinkers = 3), and cigarettes per day were included as covariates. Moreover, in ANCOVA, subjects were divided into three subgroups by BMI: "BMI $<$ 22" (n = 107); "22 $\leq$ BMI $<$ 25" (n = 134); and "BMI $\geq$ 25" (n = 80). The cut-off points were determined based on reports regarding optimal BMI (Tokunaga et al., 1991) or classification of obesity (Matsuzawa et al., 2000). P-values $<$ 0.05 were considered to be statistically significant.

3. Results

Crude data (Table 1) showed that serum sodium and chloride levels were significantly lower in men with ND2-237 Met than in those with ND2-237 Leu (P = 0.021 and 0.003, respectively).

Multiple regression analysis for serum chloride level was performed (Table 2). Cigarette consumption and BMI were significantly and positively associated with serum chloride levels (P = 0.002 and 0.008, respectively) and hemoglobin levels were significantly and negatively associated with serum chloride levels (P = 0.007) in ND2-237 Leu genotypic men. In men carrying ND2-237 Met, hemoglobin levels were significantly and negatively associated with serum chloride levels (P = 0.025). We did not designate significant variables associated with serum sodium levels in either genotype (data not shown).

ANCOVA was used to adjust serum electrolyte levels for drinking frequency, cigarette consumption, serum creatinine levels, hemoglobin levels, BMI, and age, in three BMI