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Phenotypic linkage between single-nucleotide polymorphisms of β_3-adrenergic receptor gene and NADH dehydrogenase subunit-2 gene, with special reference to eating behavior

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Abstract

The β_3-adrenergic receptor gene (*BAR-3*) allelic variant (Trp64Arg and Arg64Arg) is correlated with obesity or non-insulin-dependent diabetes mellitus. The mitochondrial NADH dehydrogenase subunit-2 gene (*ND2*) variant (Mt5178A) is associated with longevity or less susceptibility to adult-onset diseases. The frequencies of both the variants are high among the Japanese population. Cross-sectional analysis of these variants was conducted to determine if they correlated well with life-style-related phenotypes and nutrient intake. The body fat rate in the *BAR-3* variant + *ND2* variant group was higher than those rates in the *BAR-3* normal + *ND2* variant, *BAR-3* normal + *ND2* normal. The *BAR-3* normal + *ND2* variant group preferred much carbohydrate and less animal protein compared with other three groups. A combination of SNPs of the nuclear *BAR-3* and the mitochondrial *ND2* genes may affect eating behavior besides the biochemical and metabolic process of signal transduction and electron transfer system.

Keywords: Single nucleotide polymorphisms; β_3-Adrenergic receptor gene; NADH dehydrogenase subunit-2 gene; Combination of SNPs; Eating behavior

Since analysis of SNP (single nucleotide polymorphism) is important in order to know the relation between an individual's hereditary background and a given phenotype, it has attracted much attention in the medical field. Among the well-known SNPs, the β_3-adrenergic receptor gene (*BAR-3*) allelic variant (Trp64Arg and Arg64Arg) has been recognized as one of "thrifty genes" [1]. The *BAR-3* allelic variant is a famous SNP associated with obesity because individuals with this variant have a lower resting metabolic rate than those without the variant [2], and also associated with non-insulin-dependent diabetes mellitus [3]. It is also well known that the Japanese population has a frequency (0.20) of the *BAR-3* allelic variant next to that (0.31) of the Pima Indians, which is the ethnic group richest in *BAR-3* allelic variant [4]. There are some reports about the relation between *BAR-3* variant and body-mass index [5], blood pressure, and blood triacylglycerol levels [6]. Furthermore, recent papers have reported about the interaction of the *BAR-3* variant with the other gene SNPs [7–9]. Mentuccia et al. [7] reported that the variant of the human type 2 deiodinase gene with the *BAR-3* variant increased body-mass index. Ishii et al. [8] studied about the effect of interaction of *BAR-3* variant and *Hin*dIII-lipoprotein lipase gene variant on body-mass index.

In addition to *BAR-3* variant, the mitochondrial NADH dehydrogenase subunit-2 gene (*ND2*) variant Mt5178A is also found abundant specifically in Japanese population (0.45) and is more frequently observed in Japanese centenarians [10,11]. Tanaka et al. [11] reported that individuals with the normal *ND2* gene Mt5178C were more susceptible to adult-onset diseases

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than those with this *ND2* variant, because the frequency of Mt5178C increased more markedly than that of *ND2* variant among old patients over 46 years old; whereas, among the young patients under the age of 46, the frequency of both genotypes was almost the same [11]. People with *ND2* variant, who were frequently observed in Japanese centenarians, have been believed to be able to avoid adult-onset diseases.

Thus, each of the nuclear *BAR-3* and the mitochondrial *ND2* SNPs is participating in the life-style related diseases. In the present report, we investigated the association of both gene variations with body fat rate and body-mass index in order to examine whether the interaction of these 2 SNPs affects certain phenotypes. Moreover, the nutrient intake profile was investigated for the purpose of assessing whether the interaction of these 2 SNPs can determine eating behavior itself in life-style. As a result, we found that the *BAR-3* normal + *ND2* variant group preferred much carbohydrate and less animal protein than the other three groups.

Materials and methods

Materials. This trial was conducted following approval from the Research Ethics Committee of the Siebold University of Nagasaki and the written consent of the students, who were sufficiently informed about the experiment.

The nuclear and mitochondrial genomic DNAs were extracted from buccal mucosal cells of 87 female students (21–33 years old) recruited in our university by using a polyester fiber-tipped applicator swab (Falcon). DNAs on the surface of a swab after air-drying for at least 2 h were dissolved in phosphate-buffered saline and purified using QIAamp DNA Blood Mini Kit (Qiagen) as templates of polymerase chain reaction (PCR) to amplify the SNP-site fragments indicated below: the set of primers, 5′-CGCCCAATACCGCCAACAC-3′ and 5′-CCACCAGGAGTCCCATCACC-3′, for "Trp64Arg of *BAR-3*" [12] and another set of primers, 5′-ATCCATCATAGCAGGCAGTT-3′ and 5′-GAGTAGATTAGGCGTAGGTA-3′, for "Mt5178A of *ND2*" [10] were synthesized by Sigma Genosys, Tokyo. Taq DNA polymerase (Promega), *Bst*NI (New England Biolabs), and *Alu*I (TaKaRa Bio) were purchased for polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method.

Analysis. SNPs of *BAR-3* and *ND2* genes were analyzed by the PCR-RFLP method according to Walston et al. [12] and the personal communication with Dr. Tanaka (Gifu International Institute of Biotechnology, Japan), respectively. Namely, PCR was carried out using the purified DNAs as a template, and the corresponding primers. PCR products were digested with *Bst*NI or *Alu*I. *Bst*NI fragments and *Alu*I fragments were loaded onto an agarose gel. Genotyping by RFLP was performed using a lumino-image analyzer LAS-1000 plus (Fujifilm).

Anthropometrics (stature and weight) and body fat measurement were conducted for 87 subjects. A food frequency questionnaire was conducted to the 87 same subjects using a commercial investigation kit (Jissun-Houshi, Dai-ichi Shuppan Publishing, Japan). It has been frequently used for the epidemiological research in Japan on a meal and adult-onset diseases, and since it is a self-entry formula, it is also available for individual nutrition diagnosis and instruction in the medical service area such as hospitals, etc. Body weight and body fat rate were measured with the electronic body fat analyzer TBF-310 (Tanita, Japan). Calculation of the amount of the intaken nutrients was performed by the software attached to the commercial investigation kit "Jissun Houshi." The items calculated were energy, total protein, animal protein, plant protein, total fat, animal fat, plant fat, carbohydrate, fatty acids, cholesterol, and vitamins. The statistical analyses were carried out by multiple comparisons with one-way ANOVA followed by Scheffe's test.

Results and discussion

Somatic characteristics and nutrient intake of the subjects enrolled are shown in Table 1. Height, weight, and body-mass index of the subjects were similar values to those of Japanese female standard (18–29 years old). The amount of nutrient intakes by the subjects was

Table 1
Characteristics of physical and nutrient intake in subjects

Items	Subjects ($n = 83$)	Anthropometric standard or recommended dietary allowances of female in Japan (Ministry of Health, Labour and Welfare)
Age	21 ± 0.15	18–29
Height (m)	1.575 ± 0.01	1.581
Weight (kg)	51.3 ± 0.7	51.2
Body-mass index	20.5 ± 0.3	20.5
Energy (kcal/day)	1521 ± 43	1550–2300
Total protein (g/day)	60 ± 2.0	55
Fat energy ratio (%/day)	34.6 ± 1.2	20–25
Carbohydrate energy ratio (%/day)	49 ± 1.6	>50
Cholesterol (mg/day)	431 ± 24	<300
Vitamin A (IU/day)	2301 ± 156	1800–5000
Vitamin B_1 (mg/day)	0.85 ± 0.02	0.8
Vitamin B_2 (mg/day)	1.55 ± 0.06	1.0
Vitamin C (mg/day)	105 ± 3.8	100
Vitamin E (mg (α-TE*)/day)	4.7 ± 0.2	8–600

*α-tocopherol equivalent.
Values are given as means ± SEM.

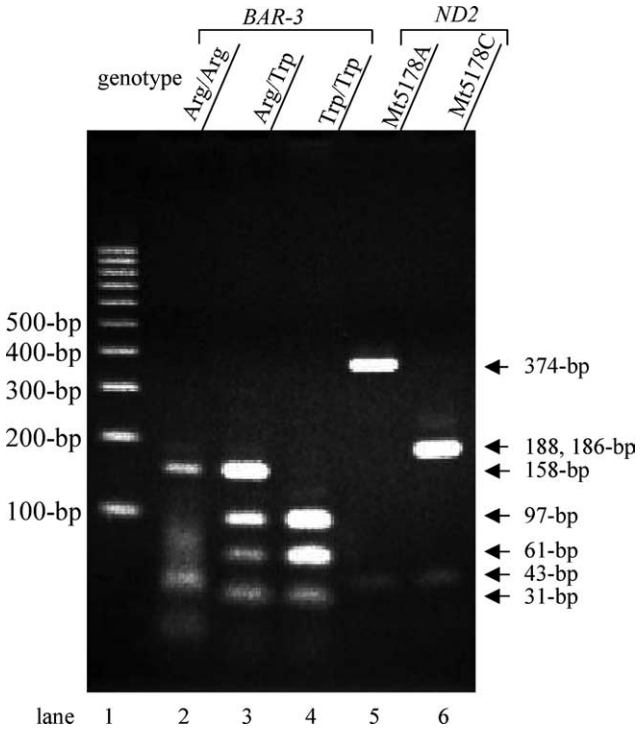

Fig. 1. Electrophoretic profiles of PCR-RFLP for polymorphism in β_3-adrenergic receptor gene and NADH dehydrogenase subunit-2 gene. Lane 1, DNA 100-bp ladder marker (BEXEL biotechnology); lane 2, *Bst*NI fragments from Arg/Arg; lane 3, fragments from Arg/Trp; lane 4, fragments from Trp/Trp; lane 5, *Alu*I fragments from Mt5178A; and lane 6, fragments from Mt5178C.

within the ranges of the recommended dietary allowances in Japanese adult female.

The PCR product (210 bp) with the primers for "Trp64Arg of *BAR-3*" was digested by *Bst*NI. The *BAR-3* variant was typed by a detection of a 158-bp fragment, although the 158-bp fragment was cleaved by *Bst*NI into two fragments (97 and 61 bp) in the *BAR-3* normal as shown in Fig. 1. The number of *Bst*NI fragments in each genotype was as follows. They were two main fragments (158 and 31 bp) of Arg/Arg, four (158, 97, 61, and 31 bp) of Trp/Arg, and three (97, 61, and 31 bp) of Trp/Trp. In the Mt5178C of *ND2*, the PCR product (417 bp) was cleaved into three *Alu*I fragments (188, 186, and 43 bp); whereas in Mt5178A of *ND2* it was cleaved into two fragments (374 and 43 bp) (Fig. 1).

Consistent with a report that the rate of the Arg-allele frequency of the *BAR-3* gene was 0.20 in the Japanese population [4], it was close to that, 0.26 (Arg/Arg, 6/87; Trp/Arg, 33/87) in the present study (Table 2). The frequency of M5178A was 0.44 (38/87) in the same range as its frequency of 0.45 reported earlier for Japanese people [10,11].

The combinations of SNPs were assigned to the following groups: Arg-allele carrier (Arg/Arg or Trp/Arg) + Mt5178A = [A + A] group, Trp/Trp + Mt5178A = [T + A] group, Arg-allele carrier + Mt5178C = [A + C] group, and Trp/Trp + Mt5178C = [T + C] group. The frequencies were as follows: [A + A] group, 19/87; [T + A] group, 19/87; [A + C] group, 20/87; and [T + C] group, 29/87.

As shown in Table 3, the body fat rate of the [A + A] group was significantly higher than those rates of the [T + A] and [T + C] groups ($p = 0.0388$, $p = 0.0485$). On the contrary, there was no significant difference in the body fat rate between the [A + C] group and the other three groups. With respect to the body-mass index, there was no significant difference among the four groups.

Although individuals with Mt5178C are more susceptible to adult-onset diseases than are those with Mt5178A [10,11], in the young women who were not obese, the body fat rate of the Mt5178A group was rather more influenced by the Arg-allele of the *BAR-3*

Table 2
Genotype frequency of the β_3-adrenergic receptor gene and NADH dehydrogenase subunit-2 gene in female students

β3-Adrenergic receptor gene genotype			NADH dehydrogenase subunit-2 gene genotype	
Arg/Arg	Trp/Arg	Trp/Trp	Mt5178A	Mt5178C
0.069	0.379	0.552	0.437	0.563
(6)	(33)	(48)	(38)	(49)

Allele frequency: Arg-allele 0.259; Trp-allele 0.741.
Numbers in parentheses indicate numbers from each group.

Table 3
Influence of nuclear and mitochondrial SNP combination on selected phenotypes

Items	[T + A] group ($n = 19$)	[T + C] group ($n = 29$)	[A + A] group ($n = 19$)	[A + C] group ($n = 20$)
Height (m)	1.579 ± 0.014	1.565 ± 0.010	1.568 ± 0.009	1.592 ± 0.013
Weight (kg)	51.2 ± 1.5	49.2 ± 1.3	52.6 ± 1.1	51.7 ± 1.5
Body fat rate (%)	22.2 ± 0.8^{b}	22.6 ± 0.7^{b}	26.2 ± 0.9^{a}	24.2 ± 1.1^{ab}
Body-mass index	20.6 ± 0.4	19.9 ± 0.4	21.6 ± 0.5	20.5 ± 0.6

Values are given as means ± SEM. Different letters show significant difference ($p < 0.05$).

Table 4
Daily amounts of energy and nutrient intakes in four genotype groups

Items	[T + A] group ($n = 19$)	[T + C] group ($n = 28$)	[A + A] group ($n = 16$)	[A + C] group ($n = 20$)
Energy (kcal/day)	1357 ± 71	1633 ± 74	1568 ± 89	1454 ± 83
Total protein (g/day)	50.3 ± 2.8	64.5 ± 3.7	61.5 ± 3.0	60.2 ± 4.4
Animal protein (g/day)	29.0 ± 1.5^{A}	39.0 ± 2.5^{B}	40.2 ± 2.2^{B}	38.7 ± 2.6^{B}
Total fat (g/day)	48.7 ± 3.8	61.9 ± 4.1	61.0 ± 4.6	55.6 ± 3.6
Saturated fatty acid (g/day)	15.8 ± 1.7	20.6 ± 1.4	19.9 ± 1.7	18.1 ± 1.4
Monoenoic fatty acid (g/day)	18.2 ± 1.4	23.9 ± 1.5	23.2 ± 1.9	21.0 ± 1.3
Polyenoic fatty acid (g/day)	10.4 ± 0.8	13.0 ± 0.8	12.4 ± 1.0	11.5 ± 0.9
Animal fat (g/day)	22.0 ± 2.3	29.6 ± 2.4	27.8 ± 2.0	27.8 ± 2.5
Carbohydrate (g/day)	200.2 ± 11.6	182.2 ± 11.8	183.7 ± 14.1	171.7 ± 10.0
(energy ratio (%)	60.2 ± 3.4^{a}	44.6 ± 2.5^{b}	46.6 ± 2.3^{b}	48.3 ± 2.6^{b})
Cholesterol (mg/day)	326 ± 30^{a}	486 ± 45^{b}	479 ± 46^{ab}	409 ± 39^{ab}
Vitamin A (IU/day)	1842 ± 152	2514 ± 275	2610 ± 590	2202 ± 219
Vitamin B_1 (mg/day)	0.71 ± 0.02^{a}	0.96 ± 0.04^{b}	0.90 ± 0.06^{ab}	0.81 ± 0.05^{ab}
Vitamin B_2 (mg/day)	1.15 ± 0.06^{a}	1.83 ± 0.10^{bc}	1.53 ± 0.09^{abc}	1.56 ± 0.11^{c}
Vitamin C (mg/day)	103 ± 6	112 ± 9	106 ± 6	107 ± 8
Vitamin E (mg (α-TE*)/day)	4.0 ± 0.3	5.1 ± 0.4	4.8 ± 0.4	4.7 ± 0.4

*α-tocopherol equivalent.
Values are given as means ± SEM. Different letters show significant difference ($p < 0.05$).

gene compared with the Mt5178C group (Table 3). Namely, even if an individual has the longevity-related gene Mt5178A, the Arg-allele of the *BAR-3* gene may dominantly affect body fat rate. In sharp contrast, individuals with Mt5178C cannot be apparently influenced by the Arg-allele. In regard to the interaction of the Arg-allele of *BAR-3* gene with other genes, Shihara et al. [13] reported an interesting research about the synergistic inhibitory effect of uncoupling protein 1 SNP and Arg-allele of the *BAR-3* gene on autonomic nervous system activity.

As to the food frequency questionnaire, which was acceptably completed by all but four of the participants, the results, analyzed by ANOVA followed by Scheffe's test, were as follows (Table 4): as shown in Fig. 2, the [T + A] group had the significantly higher carbohydrate energy ratio ([T + A] group, 60.2 ± 3.4 vs. [T + C] group, 44.6 ± 2.5 ($p = 0.0011$); vs. [A + A] group, 46.6 ± 2.3 ($p = 0.0194$); vs. [A + C] group, 48.3 ± 2.6 ($p = 0.034$)), and the amount of animal protein intake by the [T + A] group was markedly lower than those by the other three groups ([T + A] group, 29.0 ± 1.5 vs. [T + C] group, 39.0 ± 2.5 ($p = 0.0233$); vs. [A + A] group, 40.2 ± 2.2 ($p = 0.025$); vs. [A + C] group, 38.7 ± 2.6 ($p = 0.0477$)). Although the [T + A] group preferred carbohydrate, there was no correlation between the carbohydrate energy ratio and body fat rate or body-mass index in this group (data not shown), indicating that their preference for carbohydrate did not become a critical factor that raised their body fat rate or body-mass index. This [T + A] group also had few amounts of vitamin B1, B2, and cholesterol intake (vitamin B1: [T + A] group, 0.71 ± 0.02 vs. [T + C] group, 0.96 ± 0.04 ($p = 0.014$); vitamin B2: [T + A] group, 1.153 ± 0.06 vs. [T + C] group, 1.835 ± 0.10 ($p < 0.0001$) vs. [A + C] group, 1.564 ± 0.11 ($p = 0.0357$); cholesterol; [T + A] group, 326 ± 30 vs. [T + C] group, 486 ± 45 ($p = 0.0461$)).

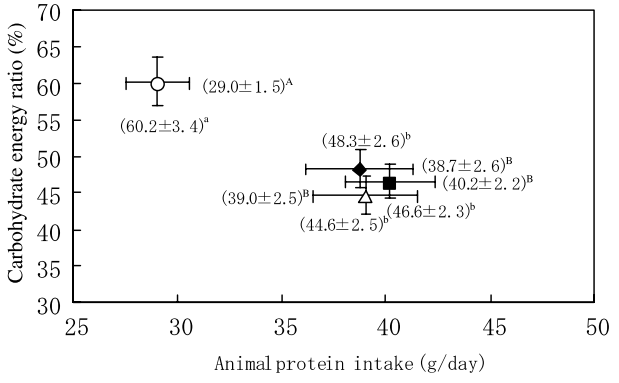

Fig. 2. Correlation between gene variations and nutrient intake profile ○ [T + A] group ($n = 19$), ■ [A + A] group ($n = 19$), △ [T + C] group ($n = 29$), and ◆ [A + C] group ($n = 20$). Vertical lines and horizontal lines indicate ± SEM of carbohydrate energy ratio and amount of animal protein, respectively. Different letters show significant difference ($p < 0.05$).

Interestingly, it was recently reported that RNA interference of any one of three mitochondrial genes of *C. elegans,* i.e., *nuo-2* encoding NADH-ubiquinone oxidoreductase; *cyc-1* encoding cytochrome *c* reductase; or *cco-1* encoding cytochrome *c* oxidase, affected the eating behavior and longevity of the worms [14]. Moreover, the relation between Amish people's eating behavior with the three-factor eating questionnaire and the analysis of a heritability was analyzed by whole-genome wide linkage studies [15]. They showed a linkage of specific chromosomal regions to energy intake [15].

It is, therefore, noteworthy to point out in the present study that eating behavior of the [T + A] group was unique or quite different from that of the other three

groups. This group is reasonably considered most invulnerable to adult-onset diseases, because they carry a SNP combination of the nuclear thriftless (or lavish) gene and the mitochondrial longevity gene. They prominently preferred much carbohydrate and less animal protein as compared with the other three groups. Their food preference is in good agreement with the balance of the traditional Japanese meal, and so further study may be required to determine whether this SNP combination is more frequent in the Japanese centenarians.

In conclusion, the present results strongly indicate that certain combinations of nuclear and mitochondrial SNPs can determine some phenotypes including eating behavior, which may result in prevention of life-style-related diseases.

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