

Interactions Between ACE Deletion Allele and Obesity During Intervention With Lifestyle Modification in Mild Obese Japanese

Jianjun YANG^{1, 2}, Masayuki YAMASAKI¹, Paulin Beya wa Bitadi MUTOMBO¹, Mamiko IWAMOTO¹, Akiko NOGI³, Toru NABIKA⁴ and Kuninori SHIWAKU^{1§}

¹*Department of Environmental and Preventive Medicine, Shimane University Faculty of Medicine, Izumo 693-8501, Japan.*

²*School of Public Health, Ningxia Medical University, Yinchuan 750004, China.*

³*Department of Human Nutrition, Faculty of Nursing and Nutrition, Yamaguchi Prefectural University, Yamaguchi 753-8502, Japan.*

⁴*Department of Functional Pathology, Shimane University Faculty of Medicine, Izumo 693-8501, Japan.*

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As the gene-gene or gene-environment interactions on obesity are unresolved, we investigated the ACE I/D, UCP1 -3826 A/G, α_{2B} -ADR Glu¹²/Glu⁹, β_3 -ADR Trp64Arg, and PON1 Q192R genotypes at the baseline and after a lifestyle-modified intervention for body mass index (BMI). A total of 212 Japanese adults lowered their BMI $0.8 \pm 0.8\text{kg/m}^2$ over three months. The I/D + D/D genotypes of ACE had significantly higher BMI compared to the I/I genotype at the baseline, but there were no significant differences for the other four genetic polymorphisms and the addictive effects of these genetic polymorphisms. The I/D + D/D genotypes of ACE induced greater reduction of BMI. Greater weight-loss in the D allele group remained after adjustment for age, sex, BMI at the baseline, energy balance and the other four genetic polymorphisms. The I/D + D/D genotypes of ACE had significantly higher BMI at the baseline, but induced weight-loss through the lifestyle intervention, as compared to the I/I genotype.

Key words: BMI, ACE, UCP1, α_{2B} -ADR, β_3 -ADR, PON1, lifestyle modification

[§]Correspondence and reprint requests to: Kuninori Shiwaku
Department of Environmental and Preventive Medicine, Shimane University Faculty of Medicine 89-1 Enya-cho, Izumo City, Shimane 693-8501, Japan
Tel:+81-853-20-2167
Fax:+81-853-20-2165
E-mail:shiwaku@med.shimane-u.ac.jp

INTRODUCTION

Obesity has been recognized as one of the major risk factors for type 2 diabetes and atherosclerosis [1]. Although obesity occurs less frequently in Japanese compared to various other ethnic populations [2], recent changes in Japanese social environment and lifestyle have caused an increase in cardiovascular risk factors [3].

Lack of exercise and improper diet have been identified as modifiable risk factors for obesity [4]; however, not all individuals with such lifestyle become obese. It is clear that genetic heredity plays an important role in the development of obesity. An examination of plausible genetic and environmental interactions for obesity risk is, therefore, warranted.

Cross-sectional studies have shown the effects of the angiotensin converting enzyme (ACE) I/D (rs1799752) [5, 6], uncoupling protein1 (UCP1) -3826 A/G (rs1800592) [7, 8], α_{2B} -adrenergic receptor (α_{2B} -ADR) Glu¹²/Glu⁹ (rs76700079) [9], β_3 -adrenergic receptor (β_3 -ADR) Trp64Arg (rs4994) [10], and paraoxonase1 (PON1) Q192R (rs662) [11] genotypes on obesity. Interventional studies have also reported the relationship between the risk allele of these five gene polymorphisms and obesity [12, 13, 14, 15]. However, the associations between these genetic polymorphisms and obesity have been weak, and sometimes contradictory [16, 17, 18, 19, 20, 21, 22, 23]. A combination of genes, or a combination of genes with one or more environmental factors, may partially account for this missing heritability. The present study investigated through planned intervention the gene-gene or gene-

environment interactions thought to be of key importance in the etiology of obesity.

This study examined the crosstalk between ACE I/D, UCP1 -3826 A/G, α_{2B} -ADR Glu¹²/Glu⁹, β_3 -ADR Trp64Arg, and PON1 Q192R genotypes and lifestyle modification for body mass index (BMI) at the baseline and lifestyle-modified intervention. The strengths of the study were comprehensive and quantitative measurement of outcomes such as energy balance, including energy intake and expenditure, and BMI, to evaluate the subjects' responses.

MATERIALS AND METHODS

Study design and subjects

From 2001 to 2007, four hundred sixty-five Japanese adult volunteers (142 men of 55 ± 11 years and 323 women of 57 ± 8 years), were recruited in Izumo, Japan, through an advertisement in a local newspaper for participation in a lifestyle modification program for prevention of obesity. Information on each subject's lifestyle and healthy status was obtained using a self-reported questionnaire, including questions on smoking, alcohol consumption and use of prescription medications for obesity-related

diseases, such as cardiovascular disease (cerebral apoplexy, myocardial infarction and angina pectoris), diabetes, dyslipidemia and hypertension. Since most Japanese with BMI over 21.0 have a tendency toward obesity and show a higher prevalence of obesity-related diseases [24, 25], we excluded individuals with BMI < 21.0 or taking prescribed medications for obesity-related diseases; those subjects who dropped out of the three-month lifestyle-modified program were also excluded, as were subjects whose DNA sample extracts were damaged. A total of 212 subjects, 57 men (age 54 ± 10 years) and 155 women (age 57 ± 8 years), were eventually included in the present study (Fig. 1). Subjects underwent counseling programs twice during the three months intervention, including recommendation for diet recipes and promotion of exercise. No specific instructions were given, but subjects were rather given guidance and encouraged to decide which regimen best suited their lifestyle. Details of the program were previously described elsewhere [26].

The ethics committee of the Shimane University Faculty of Medicine approved all study protocols, and all subjects provided written informed consent.

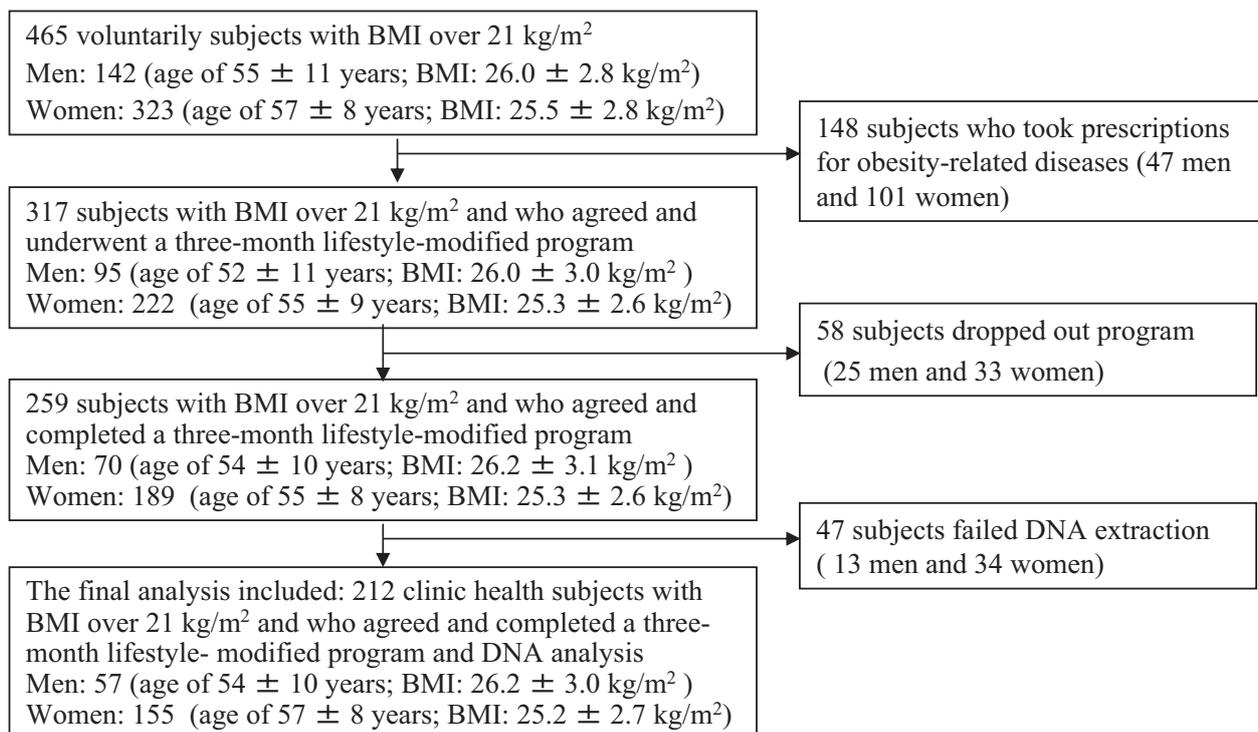


Fig. 1. Flow diagram for subjects included in the study according to including and excluding criteria

Diet

Information on a subject's daily diet was obtained by using an established self-administered quantitative food frequency questionnaire [27] prior to and after a three-month lifestyle-modified intervention. Trained nutritionists asked the subjects to report their weekly food consumption; the average amount and frequency of food intake for one month were estimated based on the Working Committee for Health Guidelines of the Japanese Ministry of Health and Welfare for Japanese people [28]. Average daily energy intake (kcal/day) for one month was calculated using the standard food composition tables for Japanese [29].

Physical activity:

Habitual physical activity was assessed before and after the intervention using the questionnaire, including physical exercise during leisure time as well as walking. Subjects were asked to submit pedometer readings for the one week before and during the intervention program. Daily energy expenditure (kcal/day) was calculated using metabolic equivalent units (MET) formula as follows: calories of physical activity = body weight (kg) × metabolic equivalent (MET) × time (h). The MET for sitting/resting was 1 and that of normal walking was 3. We used MET values for a variety of physical activities, based on the Exercise and Physical Activity Guide from the Ministry of Health, Labor and Welfare of Japan [30]. Subjects were encouraged to record pedometer readings daily (HJ-002, Omron Co. Ltd, Tokyo, Japan), and their body weight weekly.

Energy balance was calculated as follows: change

in intake - change in expenditure.

Anthropometric measurements

Data on demographic factors and lifestyles were obtained using a self-reported questionnaire. Before and after the intervention, following an overnight fast, the subjects underwent anthropometric evaluations. The subject's body weight with very light clothing was measured to an accuracy of ± 0.2 kg, and height was measured to an accuracy of -0.5 cm using a height bar. BMI was computed as weight (kg) divided by squared height (m^2). Resting energy expenditure (REE) was measured by indirect calorimetric using non-dispersive infrared analysis (VMB-002N, VINE, Tokyo, Japan).

Blood samples and genotyping

Genomic DNA was prepared from blood leukocytes using a DNA Extractor WB Kit (Wako Pure Chemical, Japan).

The UCP1 -3826 A/G, β_3 -ADR Trp64Arg, and PON1 Q192R genotypes were determined using the restriction fragment length polymorphism polymerase chain reaction (PCR-RFLP); ACE I/D and α_{2B} -ADR Glu¹²/Glu⁹ genotypes were distinguished by gel electrophoresis and polymerase chain reaction (PCR) products. Genotyping was performed as described previously [15, 31, 32, 33] (Table 1).

Genetic polymorphisms selection

Previous studies have shown that five studied risk alleles (I/D + D/D genotypes of ACE [5, 6, 17, 23, 34, 35], -3826 A/G + G/G genotypes of UCP1 [7, 13, 14, 36], Glu¹²/Glu⁹ + Glu⁹/Glu⁹ genotypes

Table 1. Genotyping platforms with five genetic polymorphisms

Genetic polymorphism	Angiotensin-converting enzyme	Uncoupling protein1	α_{2B} -adrenergic receptor	β_3 -adrenergic receptor	Paraoxonase1
Gene abbreviation	ACE	UCP1	α_{2B} -ADR	β_3 -ADR	PON1
rs number	rs1799752	rs1800592	rs76700079	rs4994	rs662
Genetic polymorphism notation	I/D	-3826 A/G	Glu ¹² /Glu ⁹	Trp64Arg	Q192R
Analysis method	PCR-AFLP	PCR-RFLP	PCR-AFLP	PCR-RFLP	PCR-RFLP
PCR primers: forward	5' -CTGGAGACCAC TCCCATCCTTTCT-3'	5' -CTTGGGTAGT GACAAAGTAT--3'	5' -AGGGTGTTTGT GGGGCATCTCC-3'	5' -GCAACCTGC TGGTCATCGT-3'	5' -TATTGTGCTGT GGGACCTGAG-3'
: reverse	5' -GATGTGGCCATC ACATTCGTCAGAT-3'	5' -CCAAAGGGTC AGATTCTAC-3'	5' -CAAGCTGAGG CCGGAGACACTG-3'	5' -ACGAACACG TTGGTCATGGT-3'	5' -CACGCTAAACC CAAATACATCTC-3'
Annealing temperature	61°C	60°C	69°C	61°C	61°C
Restriction enzyme	—	BclI	—	MvuI	AlwI
Fragments	470bp, 190bp	470bp, 250bp, 220bp	112bp, 103bp	158bp, 97bp, 61bp	99bp, 69bp, 30bp
Genotype	I/I, I/D, D/D	A/A, A/G, G/G	Glu ⁹ /Glu ⁹ , Glu ¹² /Glu ⁹ , Glu ¹² /Glu ¹²	Trp/Trp, Trp/Arg, Arg/Arg	Q/Q, Q/R, R/R

of α_{2B} -ADR [9, 15, 20, 37], Trp64Arg + Arg64Arg genotypes of β_3 -ADR [12, 15, 18, 19, 38, 39, 40], and Q192R + R192R genotypes of PON1 [11, 41, 42]) are involved in adipose tissue and influence obesity. Subjects were divided into two groups based on genotype: subjects with risk allele group and subjects without risk allele group.

The ACE I/D genotype consisted of any ACE I/D + D/D versus ACE I/I; UCP1 -3826 A/G genotype consisted of any UCP1 A/G + G/G versus UCP1 A/A; α_{2B} -ADR genotype consisted of α_{2B} -ADR Glu¹²/Glu⁹ + Glu⁹/Glu⁹ versus α_{2B} -ADR Glu¹²/Glu¹²; β_3 -ADR Trp64Arg genotype consisted of any β_3 -ADR Trp64Arg + Arg64Arg versus β_3 -ADR Trp64Trp; and, PON1 Q192R genotype consisted of any PON1 Q/R + R/R versus PON1 Q/Q.

Statistical analysis

Data analysis was done with SPSS statistical analysis software (Version 12.0, SPSS Inc., Tokyo, Japan). Results are expressed as mean \pm S. D. or mean \pm S. E. The genotype distribution, allele frequency, and Hardy-Weinberg equilibrium were tested by chi-square (χ^2) analysis. Comparisons between two genotypes were by Student's *t*-test to assess the differences in BMI for five gene polymorphisms at the baseline and after the intervention. To assess changes in BMI, energy intake, energy expenditure, and physical activity during intervention, we used a paired Student's *t*-test. Univariate (GLM) multivariate analysis was used to assess differences in energy intake and energy expenditure, REE, REE/kg weight and physical activity adjusted for age and sex at the baseline or after the intervention or response to the intervention. Multiple linear regression analysis was conducted to investigate whether changes in BMI were independently related to the five gene polymorphisms and environmental factors. A nominal two-sided *P*-value of less than 0.05 was used to assess significance.

RESULTS

Characteristics of subjects at the baseline

The subjects' data at the baseline are summarized in Table 2. The BMI range of the participants at the baseline was 21.0 - 37.7 (average: 25.5 \pm 2.8).

Out of 212 participants, 91 subjects (42.9%) with 25.0 - 29.9 BMI were categorized as overweight, and 14 subjects (6.6%) with over 30.0 BMI were categorized as obese. The women had significantly lower BMI values, and less energy intake and expenditure than the men.

Genetic polymorphisms and BMI at the baseline

The wild type, heterozygous and mutant homozygous genotype frequencies of the present studied genes are shown in Table 3 and are in agreement with those predicted by the Hardy-Weinberg equilibrium (Table 3). For ACE I/I, I/D and D/D: 37.7%, 47.6% and 14.6%; for UCP1 A/A, A/G and G/G: 27.4%, 46.7% and 25.9%; for α_{2B} -ADR Glu¹²/Glu¹², Glu¹²/Glu⁹ and Glu⁹/Glu⁹: 43.9%, 46.2% and 9.9%; for β_3 -ADR Trp/Trp, Trp/Arg and Arg /Arg: 58.5%, 35.4% and 6.1%; for PON1 Q/Q, Q/R and R/R: 42.5%, 44.3% and 13.2%, respectively.

However, despite a total of 212 subjects, the number of mutant homozygous genotype carriers was still insufficient for reliable statistical analyses. Genotype studies have shown that homogenous for risk allele + heterogenous are analyzed by comparing to homogenous for wild allele and report significant results. Therefore, in our present study, subjects were divided into two groups: those with risk allele and those without risk allele.

As there were no significant differences between men and women for the risk allele carriers percentage of ACE, UCP1, α_{2B} -ADR, β_3 -ADR, and PON1 genes (data not shown), the men and women subjects were jointly studied.

At the baseline, subjects with I/D + D/D genotypes of ACE had significantly higher BMI compared with the I/I genotype ($P = 0.004$) (Fig. 2 A), even after adjusting for age and sex (Fig. 2C). There was a significant increase in energy expenditure and REE in subjects with I/D + D/D genotypes of ACE compared to the I/I genotype at the baseline, but the significant differences between the two groups disappeared after adjustment for body weight (Table 4). The I/D + D/D genotypes of ACE had a significant difference in BMI, even after adjusting for the other gene polymorphisms, age, and sex ($\beta = 0.219$, $P = 0.001$, Table 4). No other gene polymorphisms showed any significant association with

Table 2. Characteristics of subjects at the baseline and response to the intervention

Variants	At the baseline			After the intervention			Response to the intervention		
	Men	Women	All subjects	Men	Women	All subjects	Men	Women	All subjects
Number	57	155	212						
Age (years)	54 ± 10	57 ± 8	57 ± 8						
Body weight (kg)	74.3 ± 9.9	60.1 ± 8.1*	63.9 ± 10.6	71.8 ± 9.6	58.4 ± 7.5*	62.0 ± 10.0	-2.5 ± 2.5 [#]	-1.7 ± 2.0 ^{#S}	-1.9 ± 2.1 [#]
BMI (kg/m ²)	26.2 ± 3.0	25.2 ± 2.7*	25.5 ± 2.8	25.4 ± 2.9	24.4 ± 2.5*	24.7 ± 2.7	-0.9 ± 0.9 [#]	-0.7 ± 0.8 [#]	-0.8 ± 0.8 [#]
Energy intake (kcal/day)	2308 ± 446	1974 ± 408*	2064 ± 444	1855 ± 440	1671 ± 345*	1720 ± 381	-453 ± 438 [#]	-303 ± 381 ^{#S}	-343 ± 402 [#]
Protein (g/day)	77.2 ± 18.0	69.8 ± 17.8*	71.8 ± 18.1	66.5 ± 15.3	63.9 ± 13.9	64.6 ± 14.3	-10.7 ± 17.4 [#]	-5.9 ± 15.8 [#]	-7.2 ± 16.3 [#]
Lipid (g/day)	62.5 ± 19.3	57.9 ± 18.1	59.1 ± 18.5	45.6 ± 13.0	45.7 ± 13.5	45.7 ± 13.3	-16.8 ± 17.0 [#]	-12.2 ± 17.4 [#]	-13.5 ± 17.4 [#]
Carbohydrate (g/day)	302.8 ± 72.5	288.0 ± 70.2	292.0 ± 71.0	256.7 ± 71.7	249.3 ± 63.1	251.3 ± 65.4	-46.0 ± 63.6 [#]	-38.7 ± 64.3 [#]	-40.6 ± 64.1 [#]
Energy expenditure (kcal/day)	2492 ± 661	1891 ± 464*	2053 ± 587	2685 ± 705	2035 ± 496*	2210 ± 628	193 ± 153 [#]	142 ± 117 ^{#S}	157 ± 131 [#]
REE (kcal/day)	1869 ± 434	1394 ± 317*	1534 ± 430	1756 ± 361	1436 ± 321*	1531 ± 363	-116 ± 332 [#]	44 ± 277 ^{#S}	-3 ± 302
Physical activity (kcal/day)	—	—	—	117 ± 92	104 ± 91	108 ± 91	117 ± 92	104 ± 91	108 ± 91

BMI: Body mass index. REE: resting energy expenditure. —: we did not evaluate physical activity at the baseline. Data are means ± S.D. All *P* values are two-sided and *P* < 0.05 is significant. **P* values were calculated between men and women at the baseline or after the intervention by Student's *t*-test. [#]*P* values were calculated between baseline and after the intervention in men or women or all subjects by paired *t*-test. ^S*P* values were calculated the variant changes between men and women by Student's *t*-test.

Table 3. Comparison of genetic polymorphism distributions and allele frequencies

Genetic polymorphisms	Genotype frequency			Allele frequency		Hardy-Weinberg equilibrium	
	I/I	I/D	D/D	I	D	χ^2	<i>P</i> values
ACE I/D	80 (37.7 %)	101 (47.6 %)	31 (14.6 %)	0.61	0.39	0.022	NS
UCP1 -3826A/G	58 (27.4 %)	99 (46.7 %)	55 (25.9 %)	0.51	0.49	0.470	NS
α_{2B} -ADR Glu ¹² /Glu ⁹	93 (43.9 %)	98 (46.2 %)	21 (9.9 %)	0.67	0.33	0.196	NS
β_3 -ADR Trp64Arg	124 (58.5 %)	75 (35.4 %)	13 (6.1 %)	0.76	0.24	0.043	NS
PON1 Q192R	90 (42.5 %)	94 (44.3 %)	28 (13.2 %)	0.65	0.35	0.127	NS

ACE: Angiotensin converting enzyme. UCP1: Uncoupling protein 1. α_{2B} -ADR: α_{2B} -adrenergic receptor. β_3 -ADR: β_3 -adrenergic receptor. PON1: Paraoxonase 1. *P* values were calculated between genotypes by Chi-square tests. NS: nonsignificant (*P* ≥ 0.05)

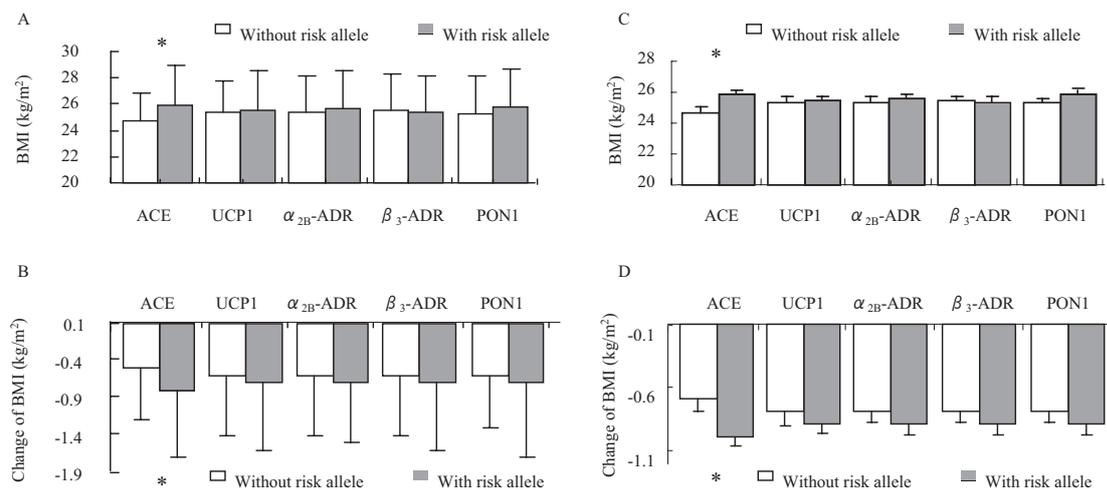


Fig. 2. BMI between subjects without risk allele and with risk allele at the baseline and changes in BMI response to the intervention

Bars are means ± S.D. (A, B) or S.E. (C, D). White bars express values of subjects without risk allele. Gray bars express values of subjects with risk allele. BMIs with risk allele or without risk allele at the baseline (A) and response to the intervention (B) are shown, and were analyzed by Student's *t*-test. Estimated BMIs with risk allele and without risk allele at the baseline (C) and response to the intervention (D) are shown, and were analyzed by univariate (GLM) multivariate analyses with adjustment for age and sex. **P* < 0.05 compared with without risk allele. Risk alleles include I/D + D/D genotypes of ACE I/D, A/G + G/G genotypes of UCP1 -3826 A/G, Glu⁹/Glu⁹ + Glu¹²/Glu⁹ genotypes of α_{2B} -ADR Glu¹²/Glu⁹, Trp/Arg + Arg/Arg genotypes of β_3 -ADR Trp64Arg, and Q/R + R/R genotypes of PON1 Q192R genes, respectively.

Table 4. Energy intake and energy expenditure by five gene polymorphisms

Variable	At the baseline			After the intervention			Response to the intervention		
	Without risk allele	With risk allele	<i>P</i> value	Without risk allele	With risk allele	<i>P</i> value	Without risk allele	With risk allele	<i>P</i> value
ACE I/D									
Number (men/women)	80 (18/62)	132 (39/93)	NS	80 (18/62)	132 (39/93)	NS	80 (18/62)	132 (39/93)	NS
BMI (kg/m ²)	24.7 ± 0.3	25.9 ± 0.2	0.002	24.1 ± 0.3	25.0 ± 0.2	0.016	-0.6 ± 0.1	-0.9 ± 0.1	0.008
Energy intake (kcal/day)	2040 ± 47	2078 ± 36	NS	1664 ± 45	1717 ± 34	NS	-323 ± 45	-356 ± 34	NS
Energy expenditure (kcal/day)	1916 ± 58	2134 ± 45	0.003	2074 ± 62	2291 ± 48	0.006	155 ± 14	156 ± 11	NS
REE (kcal/day)	1453 ± 39	1563 ± 30	0.026	1469 ± 40	1565 ± 30	NS	2 ± 36	-6 ± 27	NS
REE/kg weight (kcal)	24 ± 1	24 ± 0	NS	25 ± 1	25 ± 0	NS	-1 ± 1	-1 ± 0	NS
Physical activity (kcal/day)	—	—	—	118 ± 10	102 ± 8	NS	118 ± 10	102 ± 8	NS
UCP1 -3826 A/G									
Number (men/women)	58 (11/47)	154 (46/108)	NS	58 (11/47)	154 (46/108)	NS	58 (11/47)	154 (46/108)	NS
BMI (kg/m ²)	25.5 ± 0.4	25.4 ± 0.2	NS	24.8 ± 0.3	24.6 ± 0.2	NS	-0.7 ± 0.1	-0.8 ± 0.1	NS
Energy intake (kcal/day)	2039 ± 55	2073 ± 34	NS	1698 ± 49	1729 ± 30	NS	-341 ± 52	-344 ± 32	NS
Energy expenditure (kcal/day)	2089 ± 69	2039 ± 42	NS	2242 ± 73	2197 ± 45	NS	154 ± 17	156 ± 10	NS
REE (kcal/day)	1539 ± 46	1515 ± 28	NS	1586 ± 45	1509 ± 29	NS	52 ± 40	-25 ± 25	NS
REE/kg weight (kcal)	24 ± 1	24 ± 0	NS	26 ± 1	24 ± 0	NS	-2 ± 1	-1 ± 0	NS
Physical activity (kcal/day)	—	—	—	113 ± 12	106 ± 7	NS	113 ± 12	106 ± 7	NS
α_{2B}-ADR Glu¹²/Glu⁹									
Number (men/women)	93 (19/74)	119 (38/81)	NS	93 (19/74)	119 (38/81)	NS	93 (19/74)	119 (38/81)	NS
BMI (kg/m ²)	25.4 ± 0.3	25.5 ± 0.3	NS	24.7 ± 0.3	24.7 ± 0.2	NS	-0.7 ± 0.1	-0.8 ± 0.1	NS
Energy intake (kcal/day)	2069 ± 43	2060 ± 38	NS	1764 ± 39	1687 ± 34	NS	-305 ± 41	-373 ± 36	NS
Energy expenditure (kcal/day)	2046 ± 55	2058 ± 48	NS	2211 ± 58	2209 ± 52	NS	165 ± 13	148 ± 12	NS
REE (kcal/day)	1510 ± 36	1531 ± 32	NS	1527 ± 37	1534 ± 32	NS	6 ± 33	-10 ± 29	NS
REE/kg weight (kcal)	24 ± 1	24 ± 0	NS	25 ± 1	25 ± 1	NS	-1 ± 1	-1 ± 0	NS
Physical activity (kcal/day)	—	—	—	106 ± 9	109 ± 8	NS	106 ± 9	109 ± 8	NS
β₃-ADR Trp64Arg									
Number (men/women)	124 (29/95)	88 (28/60)	NS	124 (29/95)	88 (28/60)	NS	124 (29/95)	88 (28/60)	NS
BMI (kg/m ²)	25.6 ± 0.3	25.3 ± 0.3	NS	24.8 ± 0.2	24.5 ± 0.3	NS	-0.7 ± 0.1	-0.8 ± 0.1	NS
Energy intake (kcal/day)	2068 ± 38	2058 ± 45	NS	1709 ± 34	1737 ± 40	NS	-359 ± 36	-321 ± 42	NS
Energy expenditure (kcal/day)	2065 ± 47	2036 ± 56	NS	2211 ± 51	2208 ± 60	NS	144 ± 11	172 ± 13	NS
REE (kcal/day)	1523 ± 31	1519 ± 37	NS	1518 ± 32	1550 ± 38	NS	-21 ± 28	23 ± 34	NS
REE/kg weight (kcal)	24 ± 0	24 ± 1	NS	25 ± 1	25 ± 1	NS	-1 ± 0	-1 ± 1	NS
Physical activity (kcal/day)	—	—	—	103 ± 8	114 ± 10	NS	103 ± 8	114 ± 10	NS
PON1 Q192R									
Number (men/women)	90 (30/60)	122 (27/95)	NS	90 (30/60)	122 (27/95)	NS	90 (30/60)	122 (27/95)	NS
BMI (kg/m ²)	25.3 ± 0.3	25.6 ± 0.3	NS	24.6 ± 0.2	24.9 ± 0.3	NS	-0.8 ± 0.1	-0.8 ± 0.1	NS
Energy intake (kcal/day)	2079 ± 38	2042 ± 44	NS	1738 ± 34	1696 ± 40	NS	-341 ± 36	-346 ± 42	NS
Energy expenditure (kcal/day)	2025 ± 48	2091 ± 56	NS	2178 ± 51	2253 ± 59	NS	153 ± 12	159 ± 13	NS
REE (kcal/day)	1502 ± 32	1548 ± 37	NS	1527 ± 32	1537 ± 37	NS	17 ± 29	-31 ± 33	NS
REE/kg weight (kcal)	24 ± 0	24 ± 1	NS	25 ± 1	25 ± 1	NS	-1 ± 0	-1 ± 1	NS
Physical activity (kcal/day)	—	—	—	114 ± 8	99 ± 10	NS	114 ± 8	99 ± 10	NS

BMI: Body mass index. REE: resting energy expenditure. —: we did not evaluate physical activity at the baseline. Data are means ± S.E. Univariate (GLM) multivariate analysis was used to assess differences in BMI, energy intake, energy expenditure, REE, REE/kg weight, and physical activity adjusted for age and sex at the baseline or after the intervention or response to the intervention. All *P* values are two-sided. NS, nonsignificant. Risk alleles include I/D + D/D genotypes of ACE I/D, A/G + G/G genotypes of UCP1 -3826 A/G, Glu⁹/Glu⁹ + Glu¹²/Glu⁹ genotypes of α_{2B}-ADR Glu¹²/Glu⁹, Trp/Arg + Arg/Arg genotypes of β₃-ADR Trp64Arg, and Q/R + R/R genotypes of PON1 Q192R genes, respectively.

Table 5. Multivariable linear regression with BMI at the baseline and response to the intervention

Variables	At the baseline				Response to the intervention			
	R ²	β	<i>t</i>	<i>P</i> values	R ²	β	<i>t</i>	<i>P</i> values
	0.082			0.014	0.338			<0.001
Age (years)		-0.034	-0.497	0.620		-0.079	-1.347	0.180
Sex (Men 1, Women 2)		-0.140	-1.998	0.047		-0.047	-0.771	0.441
ACE I/D (I/I: 1, D/D + I/D: 2)		0.219	3.224	0.001		-0.141	-2.340	0.020
UCP1 -3826 A/G (A/A: 1, A/G + G/G: 2)		-0.014	-0.206	0.837		-0.065	-1.114	0.267
α _{2B} -ADR Glu ¹² /Glu ⁹ (Glu ¹² /Glu ¹² : 1, Glu ¹² /Glu ⁹ + Glu ⁹ /Glu ⁹ : 2)		0.008	0.112	0.911		0.002	0.033	0.974
β ₃ -ADR Trp64Arg (Trp/Trp: 1, Trp/Arg + Arg/Arg: 2)		-0.044	-0.646	0.519		-0.051	-0.864	0.388
PON1 Q192R (Q/Q: 1, Q/R + R/R: 2)		0.090	1.308	0.192		-0.017	-0.284	0.777
BMI at the baseline (kg/m ²)						-0.175	-2.760	0.006
Change in energy intake (kcal/day)						0.461	7.516	<0.001
Increase in physical activity (kcal/day)						-0.120	-2.029	0.044

BMI: body mass index. *P* values of variants associated with BMI changes are expressed. *P* < 0.05 is significant. β: regression coefficient for the predictor is the difference in response per unit difference in the predictor.

BMI or other parameters (Fig. 2A and 2C, and Table 5).

Response of BMI to the intervention

The response to the intervention by gender is summarized in Table 2. Eighty-two percent of the subjects reduced their body weight, with significant weight-loss of 1.9 ± 2.1kg, and 0.8 ± 0.8 kg/m² of BMI, and -500 ± 441 kcal of energy balance. In-

tervention resulted in a decrease of 16.7% (*P* < 0.001) in daily energy intake, and an increase of 7.6% (*P* < 0.001) in daily energy expenditure. The daily energy intake decrease in men was more significant than in the women (19.6% vs. 15.3%, *P* < 0.05, respectively), as was the increase in daily energy expenditure (7.7% vs. 7.5%, *P* < 0.05, respectively). There was a significant difference in weight loss changes between men and women, but not in BMI.

Response of BMI by gene polymorphisms to the intervention

Intervention yielded a greater decrease in BMI for subjects with I/D + D/D genotypes of ACE than those with I/I genotype ($-0.9 \pm 0.9 \text{ kg/m}^2$ vs. $-0.6 \pm 0.7 \text{ kg/m}^2$, $P = 0.008$, Fig. 2 B); the significant difference remained after adjustment for age and sex (Fig. 2D and Table 4). There was a significant increase in energy expenditure and REE in the I/D + D/D genotypes of ACE compared to the I/I genotype, but the significant difference disappeared following adjustment for body weight (REE/kg weight) (Table 4). The risk allele of the UCP1, α_{2B} -ADR, β_3 -ADR, and PON1 showed no significant difference in BMI response, energy intake, energy expenditure, or REE by the intervention compared to those without the risk allele (Fig. 2 B, D and Table 4).

We evaluated the gene-gene-environment effects on BMI at the baseline and to the intervention, using multiple linear regression analysis. The I/D + D/D genotypes of ACE independently reduced BMI after adjustment for age, sex, and BMI at the baseline. The women had lower BMI than the men, independently, but gender had less effect on BMI compared with the ACE genotype at the baseline. In BMI response to the intervention, the standard partial regression coefficients (β) were 0.461 for changes of energy intake, -0.175 for BMI at the baseline, -0.120 for increases of physical activity and -0.141 for I/D + D/D genotypes of ACE (Table 5). Gene-environment interaction during the intervention was evidenced by reduced BMI brought about by the environmental factor, energy balance (decrease in energy intake and increase in physical activity) and the genetic factor, I/D + D/D genotypes of ACE. However gene-gene interactions among the candidate genetic polymorphisms were not observed at the baseline or during the intervention (Table 5).

DISCUSSION

Common obesity is polygenic, involving complex gene-gene and gene-environment interactions. Candidate gene variants for polygenic obesity appear to disrupt pathways involved in the regulation of ener-

gy intake and expenditure. Therefore, this prospective study was undertaken to investigate gene-gene or gene-environment interactions for obesity. The results indicate that the interactions of ACE I/D + D/D genotypes, diet, and physical activity influence BMI at baseline as well as response to intervention.

Nearly half of the subjects had a BMI equal to or greater than 25.0, thus were a fair representation of obesity in Japanese [2].

Among our subjects, the risk allele frequency was 0.39 for ACE, 0.49 for UCP1, 0.33 for α_{2B} -ADR, 0.24 for β_3 -ADR, and 0.35 for PON1 genes, which is similar to risk allele frequencies ranges of ACE I/D (0.30 - 0.40) [43], UCP1 -3826A/G (0.46 - 0.48) [44], β_3 -ADR Trp64Arg (0.17 - 0.25) [45, 46], and PON1 Q192R (0.33 - 0.40) [47] genes reported in other studies among Japanese. The risk allele frequencies of these five genes were considered to be more common, although Glu⁹ allele frequency of α_{2B} -ADR Glu¹²/Glu⁹ is not mentioned in any relevant reports on Japanese populations.

At the baseline (natural environments), results herein showed that subjects with the I/D + D/D genotypes of ACE had significantly increased BMI compared to the I/I genotype, independent of age and sex. This finding is in accord with those found in adult Italian men and three black populations [5, 6], but most studies of Asian populations have failed to observe any association [17, 23], due to small sample size or severely obese subjects non-representative of general Asian populations.

Accordingly, there is sufficient experimental and clinical evidence showing that subjects with the I/D + D/D genotypes may have enhanced ACE protein expression, which results in higher levels of angiotension II (Ang II), and subsequent increased adipogenesis via angiotension II type 1 receptor (AT₁R) on the fat cell [48, 49].

However, results here did not indicate an association of the polymorphic variants of the UCP1, α_{2B} -ADR, β_3 -ADR, and PON1 genes with obesity. The relationships between obesity and these gene polymorphisms are not constant by ethnicity or degree of obesity [7, 9, 10, 11, 13, 50]. Such results may stem from the weak effect of these genetic polymorphisms base on obesity in the present environment [51].

The intervention for 3 months yielded 1.9kg of weight loss, -0.8 ± 0.8 of BMI, and 82.1% of the subjects reduced their body weight. The National Heart, Lung and Blood Institute in the USA recommends the initial goal of weight-loss therapy be a 10% reduction in body weight from baseline through 6 months [52]. Participants in this study were encouraged to achieve a 3.0 kg weight loss. While not all subjects accomplished this, there was a sufficient number of subjects to conduct a study of the gene-gene or gene-environment interactions on obesity.

The results showed that the I/D + D/D genotypes of ACE induced greater reduction of BMI through food intake restriction and physical activity increase. Furthermore, greater reduction of BMI in the I/D + D/D genotypes remained after adjustment for age, sex, BMI at the baseline, energy balance and the other four gene polymorphisms. The effect of ACE genotype on greater BMI reduction may be mediated by two mechanisms. The local skeletal-muscle renin-angiotensin system may modify the use of substrate [53]. Skeletal muscle cells contain a kallikrein-kinin system, and release kinins locally. Exercise intervention may lead to an increase of concentrations of kinins, increasing insulin sensitivity, and improving glucose tolerance and increasing fatty acid oxidation in skeletal muscle [54]. Second, diet restriction may reduce ACE synthesis and activity and make it more responsive to oxidative stress when linked to I/D + D/D genotypes [55, 56]. The results of this study provide for the first time firm evidence of the effects of lifestyle-modifying intervention on carriers of the I/D + D/D genotypes for changes in BMI in mild obese Japanese, although the mechanisms of such results are not yet clearly identified.

Numerous investigators have reported the gene-environment interaction influence on dyslipidemia [57] and hypertension [58, 59, 60]. While genetic factors for obesity have been extensively studied in Caucasians [61], few studies have investigated the gene-environment interaction of obesity in Asians, who have a different genetic background and food lifestyle [62]. The present study found such interactions between ACE polymorphism and environmental factors, and that I/D + D/D genotypes of

ACE played an important role in weight loss.

However, these findings suggest no association between the polymorphic variants of the UCP1, α_{2B} -ADR, β_3 -ADR, and PON1 genetic polymorphisms and energy intake and energy expenditure from physical activity on BMI by the intervention, although such association has been previously reported. The α_{2B} -ADR Glu⁹, β_3 -ADR Arg64, and β_2 -ADR Gln27 gene variants and their gene-gene interactions have reportedly enhanced fat loss with physical activity [20]. It is possible this inconsistency may result from comparisons of individuals with contrasting lifestyles [10], or from the comparison of the same individuals on separate occasions where the environment has changed [26]. Therefore, genetic associations with obesity frequently fail to replicate, and the relationship between genomic variation and obesity risk is sometimes inconsistent across environmentally diverse populations [12, 13, 14].

This study has some limitations. The sample size, though adequate for gene-environment interaction assessment, was not large enough to capture potential effects of gene-gene interaction. Thus, a large-scale, prospective study with quantitative information for lifestyle and dietary intake is required to identify gene-gene-environmental interactions.

In summary, the results herein support the evidence that I/D polymorphism of ACE plays an important role in the development of obesity, and that the I/D plus D/D genotypes of ACE is associated with easy weight loss in a lifestyle-modifying program. The results also suggest that the gene polymorphisms studied here play an important role, but are likely acting as effect modifiers to environmental factors such as diet or physical activity. Therefore, greater understanding is needed of the interaction between such genes and diet/lifestyle to help development of personalized strategies for preventing obesity.

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