Original Article

Leptin Gene and Leptin Receptor Gene Polymorphisms Are Associated with Sweet Preference and Obesity

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Leptin is an adipocyte-secreted hormone that regulates food intake and body weight, and that was recently reported to suppress sweet sensitivity in an animal model. We investigated the associations among sweet preference, obesity, and polymorphisms of the leptin gene (*LEP*) or leptin receptor gene (*LEPR*). A total of 3,653 residents randomly selected from among the citizens of Suita City, Osaka, Japan were enlisted as subjects, in whom we investigated sweet preference, clinical characteristics, including obesity and serum leptin level, and the polymorphisms of *LEP* and *LEPR* (G-2548A and A19G for *LEP*; R109K, R223Q, and rs3790439 for *LEPR*). We determined the associations among the parameters using logistic regression analysis, in order to consider potential confounding factors for sweet preference and/or obesity. The *LEP* A19G and *LEPR* R109K polymorphisms were associated with sweet preference, whereas the serum leptin level was not. Further, the *LEPR* 109KK genotype was found to be associated with obesity along with sweet preference. In conclusion, our results are the first to show associations of *LEP* and *LEPR* polymorphisms with sweet preference, and may provide useful information for diagnosis and treatment of lifestyle-related diseases. (*Hypertens Res* 2008; 31: 1069–1077)

Key Words: leptin, genetic polymorphism, obesity, taste

Introduction

Obesity is a risk factor for lifestyle-related and cardiovascular diseases (1), while leptin is an adipocyte-secreted hormone that regulates food intake, energy expenditure, and body weight (2, 3), and is well known to be related to obesity based on its ability to activate the leptin receptor (4). There have

been numerous studies examining the association between human leptin gene (*LEP*) or leptin receptor gene (*LEPR*) polymorphisms and obesity (5–10), with some of these studies reporting a positive correlation and some a negative correlation between the two.

Leptin has been shown to suppress sweet preference in animal models (11), and it suppressed neural and behavioral responses to sweet substances through its action on the leptin

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receptor in taste bud cells of mice (12). The mechanisms of this suppressive effect have been explained by the fact that leptin increases outward K^+ currents in taste bud cells *via* leptin receptors, leading to a reduction in excitability of the taste bud cells (13). The neural and behavioral sensitivities to sweet stimuli have been shown to be enhanced in db/db mice, an animal model for obesity, in the presence of a mutation of the *LEPR* (11).

Although it has been reported that genetic variations underlie specific eating patterns (14) and sweet preference is considered to be a risk factor for obesity, few reports have shown an association between them (15), and none have examined whether human *LEP* and *LEPR* polymorphisms have effects on sweet preference. However, there have been several reports indicating associations between *LEP/LEPR* polymorphisms and obesity (5). In the present study, we examined the associations of *LEP/LEPR* polymorphisms with sweet preference and attempted to determine whether these polymorphisms are associated with obesity because of their relationship to sweet preference.

Methods

Subjects

Between April 2002 and February 2004, we recruited 3,653 residents who had been participating in a public health physical checkup for citizens living in Suita City, located in the northern area of Osaka, Japan, since 1991. All subjects gave their written informed consent for the study, including genotyping. The study design was approved by the institutional review board and the ethics committee of the National Cardiovascular Center.

Measurements of Clinical Parameters

Blood pressure was determined after at least 10 min of rest in a sitting position. The final blood pressure value was the mean of 2 physician-obtained measurements (performed more than 3 min apart). Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or current use of antihypertensive agents. Diabetes mellitus (DM) was defined as fasting blood glucose ≥ 126 mg/dL, HbA1c $\geq 6.5\%$, or the current use of insulin or oral anti-diabetic agents. Hyperlipidemia was defined as total cholesterol ≥ 220 mg/dL, triglyceride ≥ 150 mg/dL, or the current use of anti-hyperlipidemia agents. Body mass index (BMI) was calculated by dividing weight (kg) by height (m) squared. For our analysis of obesity, we defined subjects in the top 25% of BMI results (above the third quartile) in each 5-year age group as obese, while those in the bottom 25% of BMI subjects were used as controls.

Semi-Quantification of Sweet Preference

A questionnaire designed to measure whether subjects had a "sweet tooth" was administered to each subject. The questionnaire consisted of a single question: "Do you like things that taste sweet?" to which the following responses were available: 1: No, I hate them; 2: No, I don't like them very much; 3: Neither yes or no; 4: Yes, I like them; and 5: Yes, I like them very much. The written answers were confirmed during an interview. To eliminate crossover, subjects who selected response number 5, above, were considered to have a sweet preference ($n=1,751$) and those that selected response numbers 1, 2, or 3 were defined as controls ($n=869$).

Polymorphism Study

Genomic DNA samples were collected from peripheral leukocytes using an NA-3000 (Kurabo Industries Ltd., Osaka, Japan) and initial polymorphism screening was performed using 24 DNA samples. All detected variations were further confirmed by a direct sequencing method using an ABI 3700 (Applied Biosystems, Foster City, USA). The genotypes were then determined using a TaqMan PCR system (ABI PRISM 7900HT; Applied Biosystems).

Statistical Analysis

The associations between sweet preference and obesity, the polymorphisms and sweet preference, and the polymorphisms and obesity were investigated using logistic regression analysis, with consideration of potential confounding factors (age, sex, smoking habit, drinking habit, and comorbidity of hypertension, diabetes, and hyperlipidemia). In addition, a Cochran-Armitage trend (CA) test was used to analyze the associations between the polymorphisms and sweet preference. Further, an analysis of covariance was used together with a path analysis, which is the statistical method commonly used to evaluate the relative importance of various causes (16), to investigate whether the polymorphisms were related to obesity based on their relationship to sweet preference. All analyses were performed using Statview software (SAS Institute Inc., Cary, USA). Values of $p < 0.05$ were considered to be statistically significant.

Results

LEP/LEPR Polymorphisms

Table 1 shows the characteristics and frequencies of the *LEP/LEPR* polymorphisms in 24 control subjects, along with the linkage disequilibrium (LD) patterns. Since the results indicated that *LEPR* N656K and rs3790439 had strong LD values, we further investigated rs3790439 instead of N656K. To examine the significant difference between the expected results and the raw ones in the genotype distribution, we used

Table 1. Characters of *LEP* and *LEPR* PolymorphismsA: Locations of *LEP* and *LEPR* polymorphisms

SNPs	db SNP ID	Region	Flanking sequence
<i>LEP</i>			
G-2548A	rs7799039	promoter	acagggttgc[g/a]ctgacctcc
A19G	rs2167270	5'-UTR	cgcagcgcca[a/g]cggttgcaag
<i>LEPR</i>			
R109K	rs1137100	exon 4	attgaaggaa[g/a]gacattgtt
R223Q	rs1137101	exon 6	gtaattttcc[g/a]gtcacctcta
N656K	rs8179183	exon 14	ctatgaaaaa[c/g]gagaaaaatg
rs3790439	rs3790439	intron 16	tcatgactag[g/a]taattagaag

B: Evaluation of patterns of linkage disequilibrium in *LEP* and *LEPR* polymorphisms

D'	r^2			
	G-2548A	A19G		
LEP				
G-2548A		0.745		
A19G	0.994			
D'	r^2			
	R109K	R223Q	N656K	rs3790439
$LEPR$				
R109K		0.636	0.206	0.206
R223Q	1.00		0.390	0.390
N656K	0.503	0.707		1.00
rs3790439	0.503	0.707	1.00	

C: Frequency of *LEP* and *LEPR* polymorphisms

	Allele 1 homo (<i>n</i>)	Hetero (<i>n</i>)	Allele 2 homo (<i>n</i>)
<i>LEP</i>			
G-2548A	GG 4.9% (179)	GA 34.2% (1,246)	AA 60.9% (2,222)
A19G	AA 3.0% (109)	AG 29.2% (1,065)	GG 67.9% (2,479)
<i>LEPR</i>			
R109K	GG 61.7% (2,243)	GA 33.8% (1,231)	AA 4.5% (163)
R223Q	GG 72.8% (2,646)	GA 24.9% (907)	AA 2.3% (83)
rs3790439	GG 1.0% (37)	GA 19.2% (701)	AA 79.8% (2,913)

SNP, single nucleotide polymorphism; *LEP*, leptin gene; *LEPR*, leptin receptor gene.

Hardy-Weinberg equilibrium. There were no significant differences in genotype frequencies of the polymorphisms between males and females (data not shown), which were nearly identical to the results in an Asian population reported by the National Center of Biotechnology Information (NCBI). Based on these results, we selected a total of 5 polymorphisms; G-2548A and A19G for *LEP*, and R109K, R223Q, and rs3790439 for *LEPR*.

Sweet Preference and Clinical Characteristics

The distribution of answers to the sweet preference question

in the questionnaire was not a normal probability distribution, and response number 5 (like sweet-tasting things very much) was chosen with the highest frequency (response 1: 5.9%; 2: 13.8%; 3: 11.6%; 4: 5.9%; 5: 62.9%). Thus we used not covariance analysis but logistic regression analysis. There was a significant difference between males and females in the choice of response number 5, as females more frequently stated their preference for a sweet taste (males: 58.6%; females: 66.6%).

The clinical characteristics of the study subjects are shown in Table 2. When clinical characteristics were compared between the subjects with a sweet preference and the controls,

Table 2. Clinical Demography

Variables	A: Sweet tooth and control			B: With <i>LEPR</i> 109KK genotype and others			C: With <i>LEP</i> 19GG genotype and others		
	Sweet tooth (<i>n</i> =1,751)	Control (<i>n</i> =869)	<i>p</i> value	<i>LEPR</i> 109KK (<i>n</i> =163)	Others (<i>n</i> =3,474)	<i>p</i> value	<i>LEP</i> 19GG (<i>n</i> =2,479)	Others (<i>n</i> =1,174)	<i>p</i> value
Age (years old)	64.1±0.3	64.8±0.4	0.14	65.2±0.9	64.7±0.2	0.59	64.2±0.2	65.7±0.3	0.0002*
BMI (kg/m ²)	22.9±0.1	22.8±0.1	0.19	23.3±0.2	22.8±0.1	0.029*	22.9±0.1	22.7±0.1	0.29
SBP (mmHg)	128.1±0.5	130.5±0.6	0.023*	130.7±1.5	129.4±0.3	0.40	129.4±0.4	129.5±0.6	0.89
DBP (mmHg)	77.2±0.2	78.6±0.3	0.023*	78.0±0.7	77.8±0.2	0.79	77.9±0.2	77.5±0.3	0.33
T-cho (mg/dL)	209.5±0.9	206.0±1.2	0.38	207.4±2.7	208.0±0.6	0.83	208.1±0.7	207.6±1.0	0.66
TG (mg/dL)	103.3±1.6	109.7±2.7	0.16	105.0±5.1	106.2±1.2	0.83	106.5±1.5	105.3±2.0	0.66
LDL cholesterol (mg/dL)	128.4±0.8	123.0±1.1	0.016*	126.5±2.6	126.3±0.5	0.94	126.2±0.6	126.3±0.9	0.96
HDL cholesterol (mg/dL)	60.5±0.4	61.3±0.6	0.070	59.7±1.3	60.2±0.3	0.71	60.3±0.3	59.9±0.4	0.47
FBS (mg/dL)	95.7±0.4	97.0±0.6	0.45	96.0±1.2	96.0±0.3	0.99	96.1±0.3	95.9±0.4	0.81
HbA1c	5.35±0.01	5.39±0.02	0.19	5.34±0.04	5.37±0.01	0.54	5.37±0.01	5.36±0.01	0.92
f-Insulin (mg/dL)	5.7±0.1	5.6±0.2	0.41	5.5±0.3	5.5±0.1	0.98	5.6±0.1	5.3±0.1	0.051
HOMA-IR	1.38±0.03	1.42±0.07	0.54	1.36±0.11	1.35±0.02	0.92	1.38±0.03	1.28±0.03	0.039*
Hypertension (%)	29.2	32.9	0.055	31.3	31.1	0.97	31.2	31.3	0.92
Hyperlipidemia (%)	29.8	25.7	0.027*	23.9	29.3	0.14	28.2	30.8	0.10
Diabetes mellitus (%)	8.2	9.0	0.48	11.0	8.5	0.26	8.8	8.3	0.68
Current smoker (%)	14.4	21.3	<0.0001*	12.3	17.4	0.09	17.9	15.8	0.11
Alcohol consumer (%)	37.9	59.5	<0.0001*	46.0	45.8	0.96	47.0	43.5	0.052

LEP, leptin gene; *LEPR*, leptin receptor gene; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; T-cho, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FBS, fetal bovine serum; f-Insulin, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance. *Significant *p* values.

Table 3. Association of Sweet Preference with Obesity

	Obesity (+) (%) (<i>n</i>)	Obesity (−) (%) (<i>n</i>)	Odds ratio (95% CI)	<i>p</i> value
Total				
Sweet tooth	53.1 (483)	46.2 (195)	1.36 (1.06–1.75)	0.017*
Control	46.9 (427)	53.8 (227)		
Male				
Sweet tooth	54.9 (208)	45.1 (171)	1.57 (1.09–2.26)	0.016*
Control	43.5 (101)	56.5 (131)		
Female				
Sweet tooth	51.8 (275)	48.2 (256)	1.16 (0.82–1.65)	0.39
Control	49.5 (94)	50.5 (96)		

CI, confidence interval. Potential confounding factors were age, sex, smoking habit, drinking habit, and prevalence of hypertension, diabetes, and hyperlipidemia. *Significant *p* values.

the results indicated that low-density lipoprotein (LDL) cholesterol values were significantly higher in the subjects with a sweet preference and that SBP and DBP were lower in those groups (*p* values: LDL cholesterol, 0.016; SBP, 0.023; DBP, 0.023) (Table 2A). In addition, the ratios for smoking and alcohol habits were significantly lower (*p* values: current smoker, <0.0001; alcohol drinker, <0.0001) and the ratio for hyperlipidemia was higher (*p* values, 0.027) in those with a sweet preference. Also, Table 2B and C show the clinical

characteristics of the subjects with and without the specific genotype *LEPR* 109KK or *LEP* 19GG.

With regard to the potentially confounding factors of age, sex, smoking habit, drinking habit, and comorbidity of hypertension, diabetes or hyperlipidemia, the subjects with a sweet preference tended toward obesity (odds ratio [OR], 1.36; 95% confidence interval [95% CI], 1.06–1.75; *p* value, 0.017) and the tendency was stronger in males than in females (*p* value, 0.016) (Table 3).

Table 4. Association of *LEP* and *LEPR* Polymorphisms with Sweet PreferenceA: Association of *LEP* polymorphisms with sweet preference

<i>LEP</i>	Total		Male		Female	
	Sweet tooth	Control	Sweet tooth	Control	Sweet tooth	Control
G-2548A						
GG	79 (4.5)	42 (4.8)	41 (5.4)	24 (5.2)	38 (3.8)	18 (4.4)
GA	599 (34.3)	307 (35.4)	248 (32.8)	160 (34.7)	351 (35.5)	147 (36.2)
AA	1,069 (61.2)	518 (59.7)	468 (61.8)	277 (60.1)	601 (60.7)	241 (59.4)
OR (95% CI)	0.91 (0.77–1.09)		0.87 (0.68–1.11)		0.83 (0.47–1.49)	
<i>p</i> value	0.30 (dom)		0.26 (dom)		0.54 (rec)	
A19G						
AA	45 (2.6)	26 (3.0)	22 (3.0)	14 (2.9)	23 (2.3)	12 (2.9)
AG	499 (28.5)	273 (31.5)	216 (31.9)	147 (28.5)	283 (28.6)	126 (31.0)
GG	1,206 (68.9)	569 (65.6)	521 (65.1)	300 (68.6)	685 (69.1)	269 (66.1)
OR (95% CI)	0.82 (0.69–0.98)		0.78 (0.60–1.01)		0.87 (0.70–1.09)	
<i>p</i> value	0.032* (dom)		0.056 (dom)		0.23 (add)	

B: Association of *LEPR* polymorphisms with sweet preference

<i>LEPR</i>	Total		Male		Female	
	Sweet tooth	Control	Sweet tooth	Control	Sweet tooth	Control
R109K						
GG	1,075 (61.6)	553 (63.9)	468 (62.1)	302 (65.7)	607 (61.3)	251 (61.8)
GA	576 (33.0)	283 (32.7)	246 (32.6)	140 (30.4)	330 (33.3)	143 (35.2)
AA	93 (5.3)	30 (3.5)	40 (5.3)	18 (3.9)	53 (5.4)	12 (3.0)
OR (95% CI)	0.64 (0.42–0.98)		0.83 (0.67–1.02)		0.59 (0.31–1.13)	
<i>p</i> value	0.042* (dom)		0.081 (add)		0.11 (dom)	
R223Q						
GG	1,270 (72.9)	639 (73.9)	548 (72.5)	346 (75.2)	722 (73.2)	293 (72.3)
GA	429 (24.6)	211 (24.4)	189 (25.0)	104 (22.6)	240 (24.3)	107 (26.4)
AA	44 (2.5)	15 (1.7)	19 (2.5)	10 (2.2)	25 (2.5)	5 (1.2)
OR (95% CI)	0.66 (0.36–1.22)		0.84 (0.66–1.07)		0.54 (0.20–1.42)	
<i>p</i> value	0.18 (dom)		0.17 (add)		0.21 (dom)	
rs3790439						
AA	19 (1.1)	8 (0.9)	8 (1.1)	6 (1.3)	11 (1.1)	2 (0.5)
AG	332 (19.0)	157 (18.1)	138 (18.2)	73 (15.8)	194 (19.6)	84 (20.6)
GG	1,397 (79.9)	703 (79.9)	611 (80.7)	382 (82.9)	786 (79.3)	321 (78.9)
OR (95% CI)	1.37 (0.58–3.20)		1.16 (0.85–1.58)		2.13 (0.47–9.71)	
<i>p</i> value	0.47 (rec)		0.36 (dom)		0.33 (rec)	

LEP, leptin gene; *LEPR*, leptin receptor gene; OR, odds ratio; CI, confidence interval; add, additive; dom, dominant; rec, recessive. *Significant *p* values.

Associations of *LEP* and *LEPR* Polymorphisms with Sweet Preference and Obesity

The associations of *LEP* and *LEPR* polymorphisms with sweet preference are depicted in Table 4A and B, respectively. The results indicated that there was a significant association between the *LEP* A19G and *LEPR* R109K polymorphisms and sweet preference (*LEP* A19G, $p=0.032$ [dominant model]; *LEPR* R109K, $p=0.042$ [dominant model]). We also examined the associations by χ^2 tests, and

the results indicated the same tendency (data not shown). Thus, subjects with *LEP* 19GG or *LEPR* 109KK tended to be subjects with a sweet preference. However, no correlation between *LEP* A19G and *LEPR* R109K was observed. In the Cochran-Armitage trend (CA) test, associations between sweet preference and *LEP* A19G and *LEPR* R109K were shown much more clearly (Fig. 1). Those results indicated that there was a significant association between sweet preference and *LEP* 19GG (trend p value =0.026) and *LEPR* 109KK (trend p value =0.025). Further, the ratios of *LEP*

Fig. 1. Distribution of answers to the sweet preference question by subjects with the *LEP* 19GG (A) and *LEPR* 109KK (B) polymorphisms. The ratios of *LEP* 19GG and *LEPR* 109KK occurrence gradually increased with increases in the degree of sweet preference, and there was a significant association between sweet preference and *LEP* 19GG (trend p value = 0.026) and *LEPR* 109KK (trend p value = 0.025).

19GG and *LEPR* 109KK occurrence gradually increased with higher levels of sweet preference.

Next, we investigated the association of *LEP* and *LEPR* polymorphisms with serum leptin levels, since some reports have noted that the *LEP* G-2548A and *LEP* A19G polymorphisms are associated with serum leptin levels. (10, 17–19) We indeed found that both *LEP* G-2548A (total, male, and female) and *LEP* A19G (total and male) were significantly associated with serum leptin levels in our subjects (data not shown). However, we concluded that *LEP* A19G does not affect sweet preference through its change of serum leptin levels, since no correlation was found between sweet preference and serum leptin level (data not shown). We also studied the association of these polymorphisms with obesity (Table 5), and the results indicated that there was a strong association between the *LEPR* polymorphisms (R109K and R223Q) and obesity (*LEPR* R109K, $p=0.0076$ [dominant]; *LEPR* R223Q, $p=0.025$ [dominant]). We also evaluated the mean value of BMI among each genotype subject, and the results showed the same tendency (data not shown). Subjects with *LEPR* 109KK or 223QQ tended toward obesity and the tendency with *LEPR* 109KK was stronger in males.

Based on our results, *LEPR* 109KK was considered to be associated with both sweet preference and obesity. Then, we

investigated whether *LEPR* 109KK had an effect on obesity by means of its relationship to sweet preference. First we confirmed that there were no interactions between sweet preference and the genotypes affecting obesity. We further utilized a path analysis to consider several potential confounding factors—age, sex, smoking habit, drinking habit, and comorbidity of hypertension, diabetes or hyperlipidemia—and we used obesity as an induced variable, and both *LEPR* 109KK presence and sweet preference as objective variables. We found that there was a significant association of both *LEPR* 109KK and sweet preference with obesity, and that *LEPR* 109KK had an effect on obesity *via* its induction of sweet preference (Fig. 2). The contribution of sweet preference to obesity was calculated to be about 2.8%, so we speculated that *LEPR* 109KK might also affect obesity through other means.

Discussion

In the present study, sweet preference was shown to be associated with obesity, and the *LEP* A19G and *LEPR* R109K polymorphisms were significantly associated with sweet preference. It was also revealed that *LEPR* 109KK had an effect on obesity *via* its induction of sweet preference. *LEPR* 223QQ was also shown to be associated with obesity but its

Table 5. Association of *LEP* and *LEPR* Polymorphisms with ObesityA: Association of *LEP* polymorphisms with obesity

<i>LEP</i>	Total		Male		Female	
	Obesity	Control	Obesity	Control	Obesity	Control
G-2548A						
GG	43 (13.9)	42 (12.1)	27 (13.9)	20 (12.1)	16 (3.3)	22 (4.5)
GA	300 (46.5)	305 (42.7)	133 (47.9)	147 (40.9)	167 (34.3)	158 (32.6)
AA	570 (39.6)	562 (45.2)	266 (38.1)	258 (47.0)	304 (62.4)	304 (62.8)
OR (95% CI)	1.10 (0.70–1.75)		1.55 (0.82–2.93)		0.72 (0.36–1.44)	
<i>p</i> value	0.68 (rec)		0.17 (rec)		0.35 (rec)	
A19G						
AA	30 (3.3)	26 (2.9)	15 (3.5)	14 (3.3)	15 (3.1)	12 (2.5)
AG	239 (26.2)	265 (29.1)	111 (26.1)	125 (29.3)	128 (26.3)	140 (28.8)
GG	644 (70.5)	621 (68.1)	300 (70.4)	287 (67.4)	344 (70.6)	334 (68.7)
OR (95% CI)	0.89 (0.72–1.10)		0.88 (0.64–1.20)		0.89 (0.67–1.18)	
<i>p</i> value	0.27 (dom)		0.41 (dom)		0.40 (dom)	

B: Association of *LEPR* polymorphisms with obesity

<i>LEPR</i>	Total		Male		Female	
	Obesity	Control	Obesity	Control	Obesity	Control
R109K						
GG	559 (61.4)	571 (62.9)	269 (63.6)	274 (64.6)	290 (59.4)	297 (61.4)
GA	301 (33.0)	309 (34.0)	129 (30.5)	141 (33.3)	172 (35.2)	168 (34.7)
AA	51 (5.6)	28 (3.1)	25 (5.9)	9 (2.1)	26 (5.3)	19 (3.9)
OR (95% CI)	0.51 (0.32–0.84)		0.28 (1.13–0.63)		0.73 (0.39–1.37)	
<i>p</i> value	0.0076* (dom)		0.0022* (dom)		0.33 (dom)	
R223Q						
GG	684 (74.9)	664 (73.1)	322 (75.8)	321 (75.4)	362 (74.2)	343 (71.2)
GA	201 (22.0)	229 (25.2)	92 (21.6)	98 (23.0)	109 (22.3)	131 (27.2)
AA	28 (3.1)	15 (1.7)	11 (2.6)	7 (1.6)	17 (3.5)	8 (1.7)
OR (95% CI)	0.47 (0.24–0.91)		0.46 (0.17–1.27)		0.45 (0.18–1.09)	
<i>p</i> value	0.025* (dom)		0.13 (dom)		0.077 (dom)	
rs3790439						
AA	8 (0.9)	9 (1.0)	4 (0.9)	4 (0.9)	4 (0.8)	5 (1.0)
AG	170 (18.6)	189 (20.7)	71 (16.7)	79 (18.5)	99 (20.3)	110 (22.6)
GG	736 (80.5)	714 (78.3)	351 (82.4)	343 (80.5)	385 (78.9)	371 (76.3)
OR (95% CI)	0.84 (0.66–1.07)		1.34 (0.30–6.05)		0.79 (0.57–1.08)	
<i>p</i> value	0.15 (dom)		0.70 (rec)		0.13 (dom)	

LEP, leptin gene; *LEPR*, leptin receptor gene; OR, odds ratio; CI, confidence interval; dom, dominant; rec, recessive. *Significant *p* values.

association was found to be weaker than that of *LEPR* 109KK.

It has been reported that sweeteners activate transduction cascades in sweet-responsive cells, with one involving cAMP and the other involving inositol trisphosphate (IP₃) in taste bud cells (20). Sugar increases intracellular cAMP, which activates protein kinase A (PKA) and closes K⁺ channels mediated by phosphorylation, which in turn leads to influx of Ca²⁺ through voltage-gated Ca²⁺ channels. In contrast, artificial sweeteners such as saccharin elevate IP₃ levels, which

induces Ca²⁺ release from intracellular stores and an elevation of intracellular Ca²⁺ concentration. The IP₃ pathway is proposed to result in closure of K⁺ channels *via* protein kinase C (PKC)-mediated phosphorylation activated by concomitantly produced diacylglycerol (DAG) (21). Therefore, both second messenger pathways may converge on the same K⁺ channels in taste bud cells. The recent studies (11, 13) noted that leptin increased the K⁺ conductance of taste cells, leading to hyperpolarization and reduction of cell excitability, and leptin suppressed behavioral responses to sweet substances. Thus we

Fig. 2. Correlations among *LEPR* 109KK, sweet preference, and obesity. Each value shown represents the coefficient of covariance analysis. There was a significant association of both *LEPR* 109KK and sweet preference with obesity, and *LEPR* 109KK had an affect on obesity via its induction of sweet preference. The contribution of sweet preference to obesity was calculated to be about 2.8%.

considered that the *LEPR* R109K or the *LEP* A19G polymorphism might affect the molecular mechanisms of leptin activation of K^+ currents like the currents in pancreatic β cells (22) and hypothalamic neurons (23).

The *LEPR* R109K polymorphism is located in exon 4 of the gene, and changes an arginine to a lysine. *LEPR* R109K is not located in the intracellular domain, which is essential for the JAK-STAT signal transduction pathway (24, 25) of this peptide, nor in the leptin-binding site (26). The functional relevance of the polymorphism has not been clarified, though previous reports have found that it is associated with obesity, including in Asians (8). Thus, our results may provide useful information for the treatment and prevention of obesity, while they also suggest a new biological pathway for the role of *LEPR* R109K in sweet preference, which might induce obesity. In addition, our findings may inform future clinical attempts to apply genetic information to the treatment of obesity, though additional investigations are needed.

Another intriguing polymorphism, *LEP* A19G, is located in the 5' untranslated region (5'-UTR) of the *LEP* gene. It was previously reported that obese individuals with the A19 allele had significantly higher leptin concentrations as compared to subjects with 19GG (19), which is supported by our results. Although both the *LEP* G-2548A and A19G polymorphisms are associated with serum leptin levels, only A19G had a significant association with sweet preference. Since the rs1349419 polymorphism in the 5'-flanking region of *LEP* is considered to modify transcription factor-binding sites (13, 24), it is intriguing that A19G showed a strong LD with rs1349419. Thus, it could be possible that A19G has an effect on sweet preference through functional changes of the rs1349419 polymorphism. In addition, the *LEPR* R109K polymorphism might also influence conformational changes of *LEPR*, thus affecting sweet preference, as it has been

reported that the leptin receptor exists as a multimeric complex and leptin activates the receptor by inducing a conformational change (26).

In clinical practice, obesity is closely associated with increased morbidity and mortality caused by cardiovascular diseases, diabetes, and hypertension (1, 27–30). Therefore, obese patients should be given dietary instruction, though many with obesity or lifestyle-related diseases are dissatisfied when sweets are restricted. Our results indicate that individuals with the *LEPR* 109KK allele tend to prefer sweets naturally, and therefore dietary therapy for those individuals may have to be well thought out. We consider that our data regarding the *LEPR* R109K polymorphism could lead to its use as a marker for personalized medical intervention when treating obesity and lifestyle-related diseases. Additionally, in this study, there was a gender difference in the distribution of answers in regard to sweet preference and the association between the polymorphisms and sweet preference. However, it was difficult to accurately determine the reason for this gender difference, because sweet preference is also influenced by various unquantifiable factors, such as cultures and customs.

In conclusion, we found associations among sweet preference, obesity and *LEPR* polymorphisms. Further, the role of *LEPR* R109K in sweet preference in the present subjects was found to be a possible cause of obesity. This is the first report showing a gene for sweet preference in humans, and we consider that our findings will contribute to better treatment of obesity and lifestyle-related diseases.

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