Primary research

Possible association of β_2- and β_3-adrenergic receptor gene polymorphisms with susceptibility to breast cancer

Xin-En Huang*†, Nobuyuki Hamajima*, Toshiko Saito*, Keitaro Matsuo*, Mitsuhiro Mizutani‡, Hiroji Iwata‡, Takuji Iwase‡, Shigeto Miura‡, Tsutomu Mizuno‡, Shinkan Tokudome† and Kazuo Tajima*

*Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan
†Department of Public Health, Medical School, Nagoya City University, Nagoya, Japan
‡Department of Breast Surgery, Department of Clinical Laboratories, Aichi Cancer Center Hospital, Nagoya, Japan

Correspondence: Dr Nobuyuki Hamajima, Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. Tel: +81 52 762 6111; fax: +81 52 763 5233; e-mail: nhamajim@aichi-cc.pref.aichi.jp

Received: 2 January 2001
Revisions requested: 6 February 2001
Revisions received: 2 March 2001
Accepted: 16 March 2001
Published: 26 April 2001

Breast Cancer Res 2001, **3**:264–269

(Print ISSN 1465-5411; Online ISSN 1465-542X)

Abstract

Background: The involvement of β_2-adrenergic receptor (ADRB2) and β_3-adrenergic receptor (ADRB3) in both adipocyte lipolysis and thermogenic activity suggests that polymorphisms in the encoding genes might be linked with interindividual variation in obesity, an important risk factor for postmenopausal breast cancer. In order to examine the hypothesis that genetic variations in ADRB2 and ADRB3 represent interindividual susceptibility factors for obesity and breast cancer, we conducted a hospital-based, case–control study in the Aichi Cancer Center, Japan.
Methods: A self-administered questionnaire was given to 200 breast cancer patients and 182 control individuals, and pertinent information on lifestyle, family history and reproduction was collected. ADRB2 and ADRB3 genotypes were determined by polymerase chain reaction (PCR) restriction fragment length polymorphism assessment.
Results: Twenty-five (12.4%) breast cancer patients and 32 (17.6%) control individuals were found to bear a glutamic acid (Glu) allele for the ADRB2 gene (odds ratio [OR] 0.67, 95% confidence interval [CI] 0.38–1.18), and 60 (30.0%) breast cancer patients and 61 (33.5%) control individuals were found to bear an Arg allele for the ADRB3 gene (OR 0.85, 95% CI 0.55–1.31). A significantly lower risk was observed in those who carried the Glu ADRB2 allele and who reported first childbirth when they were younger than 25 years (OR 0.35, 95% CI 0.13–0.99).
Conclusion: A potential association may exist between risk of breast cancer and polymorphisms in the ADRB2 and ADRB3 genes; further studies in larger samples and/or in different ethnic groups are warranted to investigate this potential association.

Keywords: β_2-adrenergic receptor gene, β_3-adrenergic receptor gene, breast cancer risk, polymorphisms, reproductive history

Introduction

The incidence of breast cancer has increased greatly in Japan over the past 2 decades [1], and it has been estimated that breast cancer would become the most common malignant disease in Japanese females by the year 2000 [2]. One of the main risk factors for breast cancer in menopausal women is obesity [3,4]. The increased amount of adipose tissue after menopause is considered to elevate estradiol production, which in turn increases the risk for breast cancer. Thus, genetic traits that are related to obesity may influence the risk of postmenopausal breast cancer in an indirect manner.

ADRB2 = β_2-adrenergic receptor; ADRB3 = β_3-adrenergic receptor; BMI = body mass index; CI = confidence interval; Glu = glutamic acid; OR = odds ratio; PCR = polymerase chain reaction.

It is now known that the adrenergic system plays a key role in regulating energy balance through both thermogenesis and lipid mobilization from brown or white adipose tissues [5,6], and that human fat cells are equipped with adrenergic receptors (adrenoceptors) β_1, β_2 (ADRB2) and β_3 (ADRB3). The degree of affinity for adrenaline (epinephrine) is $\beta_2 > \beta_1 > \beta_3$, and for noradrenaline (norephinephrine) it is $\beta_1 \geq \beta_2 > \beta_3$. The genes encoding ADRB2 and ADRB3, which have been cloned from humans [7–9], have therefore attracted much research attention with regard to their impact on obesity and obesity-related health problems.

Among these adrenergic receptors, ADRB2 appears to be the most effective regarding the mobilization of lipids, especially from abdominal subcutaneous adipose tissues [10,11]. An epidemiological study [12] revealed a marked link between obesity and a polymorphism in codon 27 of the ADRB2 gene that features a replacement of glutamine by glutamic acid (Gln27→Glu). However, doubts have also been cast on the role of this polymorphism in obesity in German [13] and Japanese [14] women. A French study [15] further pointed out that only in those with a sedentary lifestyle is there an association.

A missense mutation in codon 64 of the ADRB3 gene that results in substitution of tryptophan by arginine (Trp64→Arg) in the first intracellular loop of the receptor protein has also been reported in various ethnic groups, including Japanese [16]. Increased body mass index (BMI), a broadly used index of obesity, has been demonstrated in Japanese Arg allele carriers [17]. A review [11] identified a link between obesity and the Trp64→Arg polymorphism in 13 studies, but not in 15. Therefore, it is not possible to draw firm conclusions.

To our knowledge, few studies have been conducted to investigate the combined effect of polymorphisms in codon 27 of ADRB2 and codon 64 of ADRB3 on female obesity and/or breast cancer. This combined effect more closely resembles the real physiological status. In the present study, associations with the Gln27→Glu polymorphism in the ADRB2 gene and the Trp64→Arg polymorphism in the ADRB3 gene were examined, both in premenopausal and in postmenopausal Japanese women. Although obesity is not a risk factor for premenopausal breast cancer, associations in premonopausal women were examined for comparison with findings in postmenopausal women. This exploratory analysis was expected to provide some clues for future investigations, and so the findings should be confirmed by studies with larger sample sizes and/or with samples from different ethnic groups.

Patients and methods

Study population

Between March 1999 and April 2000, staff from the Division of Epidemiology and Prevention interviewed 247 female patients, all of whom had had breast cancer confirmed by histopathology analysis at Aichi Cancer Center Hospital. Four patients expressed anxiety regarding genetic testing; the remaining 243 patients were enrolled. Another two patients refused to participate after enrollment. The participation rate was thus 98% (241/247). After written informed consent had been obtained, a self-administered questionnaire was given and 7 ml peripheral blood was taken. Three DNA samples were not available; one from a hepatitis C virus carrier whose blood sample was not stored, and two due to the failure of DNA extraction. After excluding two patients who were younger than 30 or older than 70 years, and 36 who had been diagnosed 5 years before enrollment, 200 patients were recruited.

The control individuals were 182 female noncancer outpatients who visited Aichi Cancer Center Hospital for an annual health check up, mainly at the Departments of Gastroenterology, Breast Surgery and Gynecology, during the same period. The study was approved by the Ethics Committee of Aichi Cancer Center Hospital (approval no 12-20).

Epidemiological investigation

In the self-administered questionnaire, information was requested on demography, past personal history of disease, history of disease in first-degree relatives, smoking and drinking habits, beverage and food intake, and reproductive history before the appearance of symptoms. An interviewer checked all written responses in order to ensure that there were no unanswered questions at the time of questionnaire collection.

Genetic analyses

Genomic DNA was isolated from the buffy-coat fraction of each blood sample, using a QIAamp DNA Mini Kit (Qiagen Incorporated, Valencia, CA, USA).

PCR amplification of the ADRB2 gene was conducted using the following primers [12]: 5′-GAA TGA GGC TTC CAG GCG TC-3′; and 5′-GGC CCA TGA CCA GAT CAG CA-3′. Aliquots of 30–100 ng genomic DNA were mixed with 25 µl reaction liquid containing 0.15 mmol/l dNTPs, 12.5 pmol of each primer, 0.5 units of AmpliTaq Gold, 2.5 µl GeneAmp 10 × PCR buffer with 15 mmol/l $MgCl_2$ (Perkin-Elmer Corporation, Foster City, CA, USA), and 1 µl glycerol. Amplification conditions were set as follows: a 10-min initial denaturation at 94°C, followed by 30 cycles at 95°C for 1 min, 63°C for 1 min and 72°C for 1 min; final extension was conducted at 72°C for 5 min. The amplified products were directly incubated with 1 unit of *Fnu*4HI (New England Biolab Incorporated, Beverly, MA, USA) for 3 h at 37°C, and digested fragments were visualized on 2.5% agarose gels with ethidium bromide staining. Genotyping was as follows: 174, 97, 55 and 27 bp for the Gln allele; and 229, 97 and 27 bp for the Glu allele.

PCR amplification of the ADRB3 gene was conducted using the following primers [18]: 5′-CAA TAC CGC CAA CAC CAG TGG G-3′; and 5′-GGT CAT GGT CTG GAG TCT CG-3′. Each 25-µl reaction mixture contained 30–100 ng genomic DNA, 25 pmol of each primer, 15 mmol/l $MgCl_2$ (Perkin-Elmer Corporation), 0.15 mmol/l dNTPs, 0.5 units of Takara Taq, and 1 µl glycerol. The reactions were conducted with incubation at 95°C for 10 min, followed by 30 cycles at 95°C for 30 s, 60°C for 30 s and 72°C for 30 s. PCR products were directly digested by adding 15 units of *Msp*I enzyme (New England Biolab Incorporated) for 8 h at 37°C, and the fragments obtained were separated on a 4% agarose gel and visualized under ultraviolet light after staining with ethidium bromide. Genotypes were defined as follows: 99,54-bp for the Trp allele; and 70,54,29-bp for the Arg allele.

Statistical methods

The ORs and 95% CIs were calculated using an unconditional logistic regression model, using software from the SAS statistical program package [19]. Interaction terms between genotypes and risk factors were also examined by the logistic model. Differences between two means were assessed using Student's *t*-test. The null hypothesis was rejected at the 5% level ($P < 0.05$), and all reported *P* values were two-sided. The genotype frequencies in Japanese persons have been reported to be approximately 19% for 27Glu of ADRB2 [20] and approximately 30% for 64Arg of ADRB3 [21]. As estimated using the 'sampsi' command of the Stata statistical software, release 6 [22], with these genetic frequencies and with 200 cases and 182 control individuals, the statistical power to detect ORs 2 and 0.5 would be as follows: 80.7 and 52.8% for ADRB2, respectively; and 88.6 and 78.1% for ADRB3, respectively.

Results

Characteristics of all breast cancer patients and control individuals are summarized in Table 1. Premenopausal women constituted 54.2% of cases and 52.2% of control individuals. Participants with a positive family history of breast cancer, defined by breast cancer history in their mother and/or sister(s), made up 12.4% of the cases and 2.2% of the control individuals.

Table 2 shows genotypes and allele frequencies for Gln27→Glu and Trp64→Arg polymorphisms of ADRB2 and ADRB3, respectively. The Glu allele frequency was 6.7% for cases and 9.1% for control individuals, and homozygotes for Glu were rare in both groups; the Arg allele frequency was 16.0% for cases and 17.6% for control individuals. Because associations of Gln27→Glu and Trp64→Arg polymorphisms with obesity have recently been attracting research attention, the BMI for the control group was compared among genotypes of ADRB2 or ADRB3. No differences were found in mean BMI between those who harbored Gln/Gln alleles (BMI 22.2 ± 2.9 kg/m^2) and those with at least one Glu allele (BMI 22.3 ± 3.1 kg/m^2; $P = 0.426$ by *t*-test). BMIs for those with the Trp/Trp genotype and those with at least one Arg allele were 22.3 ± 3.0 kg/m^2 and 22.1 ± 2.8 kg/m^2, respectively ($P = 0.603$ by *t*-test).

Table 1

Characteristics of patients and control individuals

Characteristics	Patients (*n* [%])	Control individuals (*n* [%])
Age at diagnosis or check up (years)		
30–39	18 (9.0)	14 (7.7)
40–49	72 (36.3)	39 (21.4)
50–59	69 (34.3)	71 (39.0)
60–69	41 (20.4)	58 (31.9)
Age at menarche (years)		
–13	92 (46.0)	89 (48.9)
14–15	84 (42.0)	65 (35.7)
16+	23 (11.5)	28 (15.4)
No recall	1 (0.5)	0 (0.0)
Menopause		
Premenopausal	108 (54.2)	95 (52.2)
Postmenopausal	92 (45.8)	87 (47.8)
Body mass index		
<20	46 (23.0)	42 (23.1)
20–24	108 (54.0)	93 (51.1)
≥24	46 (23.0)	47 (25.8)
Breast cancer family history, in mother and/or sister(s)		
No	175 (87.6)	178 (97.8)
Yes	25 (12.4)	4 (2.2)

The total number of cases was 200, and that of control individuals was 182.

Table 3 shows the crude and age-adjusted ORs for the genotypes of ADRB2 and ADRB3. Regarding the Gln27→Glu polymorphism of the ADRB2 gene, those with a Glu allele had a decreased risk for breast cancer as compared with those with a Gln/Gln genotype (crude OR 0.67, 95% CI 0.38–1.18; age-adjusted OR 0.65, 95% CI 0.37–1.16). Because this is a case–control study with prevalent cases, the OR was also examined separately according to the period between diagnosis and enrollment. The OR for those who had been enrolled for less than 3 years was 0.68 (95% CI 0.35–1.35), and for those who had been enrolled within 3–4 years the OR was 0.65 (95% CI 0.31–1.36). Statistically significant differences in crude and age-adjusted ORs were not observed between premenopausal and postmenopausal women. Accordingly, further subgroup analyses were conducted, regardless of menopausal status. No significant differences in ORs were found for age at menarche, BMI and breast cancer family history. However, crude and age-adjusted ORs were significantly lower in those who reported first childbirth at younger than 25 years than in those who

Table 2

Genotypes and allele frequencies for the ADRB2 and ADRB3 genes among all study subjects

Genotypes and allele frequencies	Patients (*n* [%])	Control individuals (*n* [%])	Mean ± SD BMI (kg/m^2) of subgrouped control individuals
Genotypes of ADRB2			
Gln/Gln	175 (87.6)	150 (82.4)	22.2 ± 2.9*
Gln/Glu	23 (11.4)	31 (17.0)	
Glu/Glu	2 (1.0)	1 (0.6)	
Gln/Glu and Glu/Glu			22.3 ± 3.1*
Allele frequency of ADRB2			
Gln	373 (93.3)	331 (90.9)	–
Glu	27 (6.7)	33 (9.1)	–
Genotypes of ADRB3			
Trp/Trp	140 (70.0)	121 (66.5)	22.3 ± 3.0†
Trp/Arg	56 (28.0)	58 (31.9)	
Arg/Arg	4 (2.0)	3 (1.6)	
Trp/Arg and Arg/Arg			22.1 ± 2.8†
Allele frequency of ADRB3			
Trp	336 (84.0)	300 (82.4)	–
Arg	64 (16.0)	64 (17.6)	–

The total number of cases was 200, and that of control individuals was 182. *$P = 0.426$, †$P = 0.603$ (by *t*-test).

Table 3

ORs and 95% CIs for ADRB2 and ADRB3 genes

			ADRB2 gene		ADRB3 gene	
Subjects	Patients	Control individuals	/Glu versus Gln/Gln OR (95% CI)	/Glu versus Gln/Gln OR (95% CI) adjusted for age	/Arg versus Trp/Trp OR (95% CI)	/Arg versus Trp/Trp OR (95% CI) adjusted for age
All subjects	200	182	0.67 (0.38–1.18)	0.65 (0.37–1.16)	0.85 (0.55–1.31)	0.83 (0.54–1.29)
Interval after diagnosis						
<3 years	110	182	0.68 (0.35–1.35)	0.67 (0.34–1.33)	0.81 (0.49–1.36)	0.80 (0.48–1.34)
3–4 years	90	182	0.65 (0.31–1.36)	0.65 (0.31–1.38)	0.90 (0.52–1.54)	0.89 (0.51–1.55)
Menopause						
Premenopausal	109	95	0.72 (0.32–1.65)	0.65 (0.28–1.51)	0.75 (0.42–1.35)	0.82 (0.45–1.48)
Postmenopausal	91	87	0.63 (0.29–1.38)	0.58 (0.26–1.28)	0.98 (0.51–1.87)	0.93 (0.48–1.80)
Body mass index						
<22	103	94	0.60 (0.27–1.33)	0.54 (0.24–1.25)	0.97 (0.53–1.77)	0.92 (0.49–1.72)
≥22	97	88	0.75 (0.34–1.69)	0.76 (0.34–1.69)	0.75 (0.40–1.38)	0.74 (0.40–1.38)
Menarche						
<14 years	92	89	0.79 (0.34–1.88)	0.78 (0.33–1.86)	0.82 (0.44–1.53)	0.83 (0.44–1.57)
≥14 years*	108	93	0.59 (0.28–1.25)	0.54 (0.25–1.18)	0.89 (0.49–1.62)	0.81 (0.44–1.50)
First birth						
<25 years	84	67	0.35 (0.13–0.99)	0.34 (0.12–0.97)	0.92 (0.46–1.83)	0.95 (0.47–1.93)
≥25 years or no birth	116	115	0.93 (0.47–1.85)	0.93 (0.46–1.85)	0.81 (0.46–1.41)	0.78 (0.44–1.36)
Family history of breast cancer, in mother or sister(s)						
No	175	178	0.67 (0.37–1.23)	0.65 (0.36–1.2)	0.77 (0.49–1.20)	0.75 (0.47–1.18)
Yes	25	4	0.19 (0.02–1.78)	0.16 (0.01–1.78)	2.36 (0.21–25.9)	2.30 (0.20–26.6)

*One missing value in the case group. /Arg, a combination of Trp/Arg and Arg/Arg; /Glu, a combination of Gln/Glu and Glu/Glu.

reported first childbirth when they were older than 25 years (crude OR 0.35, 95% CI 0.13–0.99; age-adjusted OR 0.34, 95% CI 0.12–0.97). The interaction term between the genotype and age at first childbirth estimated by a case-only study [23] was 0.40 (95% CI 0.15–1.04), with marginal significance ($P = 0.06$).

Table 4

ORs and 95% CIs for combinations of ADRB2 and ADRB3 genetic polymorphisms

Combined genotypes		Patients (*n*)	Control individuals (*n*)	ORs for breast cancer (95% CI)	Age-adjusted ORs for breast cancer (95% CI)	Mean BMI of control individuals ± SD
ADRB2	ADRB3					
Gln/Gln	Trp/Trp	119	98	1.00	1.00	22.3 ± 3.0
Gln/Gln	/Arg	56	52	0.89 (0.56–1.41)	0.88 (0.55–1.41)	22.1 ± 2.8
/Glu	Trp/Trp	21	23	0.75 (0.39–1.44)	0.76 (0.39–1.46)	22.4 ± 3.1
/Glu	/Arg	4	9	0.37 (0.11–1.23)	0.32 (0.10–1.10)	22.2 ± 2.9

The total number of cases was 200, and that of control individuals was 182. /Arg, a combination of Trp/Arg and Arg/Arg; /Glu, a combination of Gln/Glu and Glu/Glu.

With regard to the Trp64→Arg polymorphism in the ADRB3 gene, those who harboured at least one Arg allele had a crude OR of 0.85 (95% CI 0.55–1.31) relative to those who were homozygous for Trp. The OR for postmenopausal women was similar to that for their premenopausal counterparts. Substantial differences in ORs were also not observed for variations in the period between diagnosis and enrollment, as well as BMI, first menarche, childbirth and family history of breast cancer.

The ORs for combined impact of gene polymorphisms in ADRB2 and ADRB3 on risk of breast cancer, together with the mean BMI of control individuals, are presented in Table 4. Relative to individuals who were homozygous for the Gln allele of ADRB2 and for the Trp allele of ADRB3, the other three groups demonstrated a decreased risk for breast cancer; those who simultaneously carried the ADRB2 Glu and ADRB3 Arg alleles had the most markedly reduced risk (OR 0.37, 95% CI 0.11–1.23), but none of these decreases were statistically significant. It was also found that the average BMIs among the four genotype groups were very similar (all around 22.2 kg/m^2), with standard deviations of approximately 3.0 kg/m^2.

Discussion

Before the results are interpreted, several limitations of the present study should be addressed. First, the study was a case–control study with hospital control individuals, and not a population-based one. However, we performed a validation study in order to evaluate the impact of this, and confirmed that lifestyle differences between Aichi Cancer Center Hospital noncancer outpatients and the general population in Nagoya were small [24]. The majority of the control individuals visited Aichi Cancer Center Hospital only for regular health check-ups. The allele frequencies for both polymorphisms were similar to those reported in other population-based Japanese studies [17,21,25]. The average BMI of the control individuals was 22.3 kg/m^2, which is consistent with the BMI in other population-based control groups [26]. Furthermore, the genotypes were apparently not reasons for visiting the Aichi Cancer Center Hospital. Thus, the assessment of the control group in the present study is unlikely to have biased the results. The second concern is recall bias. In the present study all questionnaires were completed before genotyping; therefore, the information from the questionnaires and data from genetic analyses were independent. Third, the breast cancer patients were under treatment and/or follow up (ie prevalent cases). However, the participation rate of patients was 98%; the OR for those diagnosed within the past 3 years was similar to the OR in those who had been diagnosed more than 3 years previously, indicating that the influence on prognosis was small.

In the present study, the major finding was that presence of 27Glu in ADRB2 or 64Arg in ADRB3 was associated with a decreased risk for breast cancer, especially when combined. This decrease in risk was statistically significant for those who simultaneously harboured 27Glu ADRB2 and had given birth early in life, but was not significant for the following subgroups: premenopausal women, postmenopausal women and women with a small BMI. There did not appear to be any association of these two polymorphisms, separate or combined, with BMI.

Since the present study did not support our hypothesis that the alleles related to obesity increase postmenopausal breast cancer risk, other possible explanations should be considered. First, the process of fat metabolism might be far more complicated than can be accounted for by genetic polymorphisms. Obesity is a combined consequence of environment and host factors. The latter includes not only genetic variations, but also psychological and pathophysiological aspects that may cause interindividual differences in energy intake, absorption, transportation, storage and metabolism. All of these modifiers should be considered when interpreting data regarding associations with genetic polymorphisms. Second, a previous study [27] showed that a full lipolytic response of fat cells can be obtained when only a fraction of the β_1 and β_2 receptors are occupied. Thus, the process of β-adrenergic receptor-mediated lipolysis should also be further investigated.

Other findings were as follows. The preventive effect of the 27Glu allele for ADRB2 was stronger than that of 64Arg for ADRB3, which is consistent with their affinities for adrenaline and noradrenaline [28]. Furthermore, on the basis of the estimated OR (0.75 for ADRB2 and 0.89 for ARDB3) the effect was synergistic (OR = 0.37, which is smaller than $0.75 \times 0.89 = 0.67$), and is biologically plausible. The preventive effect of 27Glu ADRB2 was larger in low-risk women, such as those with late menarche, early childbirth, or low BMI. All of these features are characteristic of Asian females. Thus, the present results indicate that the mechanisms that underlie the preventive effects of 27Glu in ADRB2 and 64Arg in ADRB3 may not be related to lipolytic and/or thermogenic activity, and other aspects of these polymorphisms should be elucidated.

Conclusion

The present exploratory analysis suggests that an association may exist between risk of breast cancer and polymorphisms in codon 27 of ADRB2 and 64 of ADRB3 genes; further studies in larger samples and/or in different ethnic groups are warranted to investigate this potential association.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (grant no 12670383) from the Ministry of Education, Science, Sports and Culture of Japan. X-E Huang was supported by a scholarship and research grant from Gakujutsu Shinkou Association of Japan. The authors are grateful to Ms H Fujikura and Ms M Tani for their technical assistance.

References

1. Parkin DM, Pisani P, Ferlay J: **Estimates of the world incidence of 25 major cancers in 1990.** *Int J Cancer* 1999, **80**:827–841.
2. Kuroishi T, Nishikawa Y, Tominaga S, Aoki K: **Cancer mortality statistics in 33 countries (1953–1992).** *Gann Monogr Cancer Res* 1999, **47**:153–217.
3. Hirose K, Tajima K, Hamajima N, Takezaki T, Inoue M, Kuroishi T, Miura S, Tokudome S: **Effect of body size on breast-cancer risk among Japanese women.** *Int J Cancer* 1999, **80**:349–355.
4. Vatten LJ, Kvinnsland S: **Body mass index and risk of breast cancer. A prospective study of 23,826 Norwegian women.** *Int J Cancer* 1990, **45**:440–444.
5. Lafontan M, Berlan M: **Fat cell adrenergic receptors and the control of white and brown fat cell function.** *J Lipid Res* 1993, **34**:1057–1091.
6. Arch JRS, Kaumann AJ: **β3 and atypical adrenoceptors.** *Med Res Rev* 1993, **13**:663–729.
7. Emorine LJ, Marullo SM, Briend-Sutren M, Patey G, Tate K, Delavier-Klutchko C, Strosberg D: **Molecular characterization of the human β3-adrenergic receptor.** *Science* 1989, **245**:1118–1121.
8. Granneman JG, Lahners KN, Chaudry A: **Characterization of the human β3-adrenergic receptor gene.** *Mol Pharmacol* 1993, **44**: 264–270.
9. Lelias JM, Kaghad M, Rodriguez M, Chalon P, Bonnin J, Dupré I, Delpech B, Bensaid M, LeFur G, Ferrara P, Caput D: **Molecular cloning of a human β3-adrenergic receptor.** *FEBS Lett* 1993, **324**:127–130.
10. Enocksson S, Shimizu M, Lonnqvist F, Nordenstrom J, Arner P: **Demonstration of an in vivo functional β3-adrenoceptor in man.** *J Clin Invest* 1995, **95**:2239–2245.
11. Arner P, Hoffstedt J: **Adrenoceptor genes in human obesity.** *J Intern Med* 1999, **245**:667–672.
12. Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lanfeld L, Arner P: **Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function.** *J Clin Invest* 1997, **100**:3005–3013.
13. Kortner B, Wolf A, Wendt D, Beisiegel U, Evans D: **Lack of association between a human β-2 adrenoceptor gene polymorphism (gln27glu) and morbid obesity.** *Int J Obes Relat Metab Disord* 1999, **23**:1099–1100.
14. Sakane N, Yoshida T, Umekawa T, Kogure A, Kondo M: **Beta2-adrenoceptor gene polymorphism and obesity [letter].** *Lancet* 1999, **353**:1976.
15. Meirhaeghe A, Helbecque N, Cottel D, Amouyel P: **Beta2-adrenoceptor gene polymorphism, body weight, and physical activity [letter].** *Lancet* 1999, **353**:896.
16. Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, Walston J, Yoshinaga H, Kosaka K, Yamada N: **A mutation in the β3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects.** *Biochem Biophys Res Commun* 1995, **215**:555–560.
17. Yoshioka K, Yoshida T, Sakane N, Umekawa T, Takahashi T, Sakai Y, Kondo M: **Association of Trp64Arg mutation of the beta 3-adrenergic receptor gene with NIDDM, current and maximal body mass index.** *Diabetologia* 1996, **39**:1410–1411.
18. Gagnon J, Mauriege P, Roy S, Sjostrom D, Chagnon YC, Dionne FT, Oppert JM, Perusse L, Sjostrom L, Bouchard C: **The Trp64Arg mutation of the beta 3 adrenergic receptor gene has no effect on obesity phenotypes in the Quebec Family Study and Swedish Obese Subjects cohorts.** *J Clin Invest* 1996, **98**:2086–2093.
19. SAS Institute: *SAS/STAT User's guide*, ver 6, 4th ed. Cary, NC: SAS Institute Inc; 1990.
20. Sato R: **The role of beta2-adrenoceptor gene polymorphism in asthma.** *Hokkaido J Med Sci* 2000, **75**:81–94.
21. Fujisawa T, Ikegami H, Yamato E, Takekawa K, Nakagawa Y, Hamada Y, Oga T, Ueda H, Shintani M, Fukuda M, Ogihara T: **Association of Trp64Arg mutation of the beta3-adrenergic-receptor with NIDDM and body weight gain.** *Diabetologia* 1996, **39**:349–352.
22. Stata Corp: *Stata Statistical Software*, release 6. College Station, TX: Stata Corporation; 1999.
23. Hamajima N, Yuasa H, Matsuo K, Kurobe Y: **Detection of gene-environment interaction by case-only studies.** *Jpn J Clin Oncol* 1999, **29**:490–493.
24. Inoue M, Tajima K., Hirose K, Hamajima N, Takezaki T, Kuroishi T, Tominaga S: **Epidemiological features of first-visit outpatients in Japan: comparison with general population and variation by sex, age, and season.** *J Clin Epidemiol* 1997, **50**:69–77.
25. Ishiyama-Shigemoto S, Yamada K, Yuan X, Ichikawa F, Nonaka K: **Association of polymorphisms in the beta2-adrenergic receptor gene with obesity, hypertriglyceridaemia, and diabetes mellitus.** *Diabetologia* 1996, **42**:98–101.
26. Kyogoku S, Hirohata T, Takeshita S, Hirota Y, Shigematsu T: **Anthropometric indicators of breast cancer risk in Japanese women in Fukuoka.** *Jpn J Cancer Res* 1990, **81**:731–737.
27. Arner P: **Adrenergic receptor function in fat cells.** *Am J Clin Nutr* 1989, **55(suppl)**:S228–S236.
28. Lafontan M, Barbe P, Galitzky J, Tavernier G, Langin D, Carpene C, Bousquet-Melou A, Berlan M: **Adrenergic regulation of adipocyte metabolism.** *Hum Reprod* 1997, 1**2(suppl 1)**:6–20.