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Short communication

Association of polymorphisms in the β2-adrenergic receptor gene with obesity, hypertriglyceridaemia, and diabetes mellitus

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Summary To assess the role of polymorphisms in the β2-adrenergic receptor gene in the development of obesity and obesity-related metabolic disorders, we analysed Arg16Gly, Gln27Glu, and Thr164Ile polymorphisms in 400 non-obese subjects (body mass index < 27 kg/m^2) and 108 obese subjects (body mass index ≥ 27 kg/m^2). The Gln27Glu substitution was twice as common in obese subjects as in non-obese subjects (0.14 vs 0.07, $p = 0.001$, odds ratio 2.14, 95 % confidence interval 1.35–3.41). The frequency of the Glu27 allele was also higher in patients with Type II (non-insulin-dependent) diabetes mellitus than non-diabetic subjects (0.14 vs 0.07, $p = 0.001$, odds ratio 2.13, 95 % confidence interval 1.34–3.41). Analysis of variance of multiple variables showed an association between 2-h post-load glucose concentrations and body mass index but not with the Glu27 variant, suggesting that the association with diabetes could be secondary to obesity. Obese subjects carrying the variant allele had higher concentrations of serum triglyceride than obese subjects homozygous for the wild type allele (2.68 ± 1.90 vs 1.18 ± 1.15 mmol/l, $p = 0.02$). Conversely, the frequency of Gly16 homozygotes was lower in obese women when compared with non-obese women (11 % vs 28 %, $p = 0.01$, odds ratio 0.30, 95 % confidence interval 0.12–0.75), although the association was not present in male subjects. Thr164Ile substitution was not detected in the subjects of this study. These observations suggest that the amino-terminal polymorphisms of the β2-adrenergic receptor gene could be involved in the molecular pathogenesis of obesity and hypertriglyceridaemia, and thereby the development of Type II diabetes mellitus. [Diabetologia (1999) 42: 98–101]

Keywords β2-adrenergic receptor gene, polymorphism, obesity, triglyceride, Type II diabetes.

Lipolysis in fat cells can be stimulated by catecholamines through subtypes of β-adrenergic receptors (β-ARs), and inhibited through α2-ARs. Associations of Trp64Arg substitution in the β3-AR gene with various anthropometric markers of obesity have been reported in several ethnic groups [1–3], although the role of the variant allele in traits related to obesity still remains controversial [4,5]. Human adipose cells express not only β3-ARs but considerable amounts of β2-ARs. Several polymorphisms have been found in the coding region of the β2-AR gene in humans [6]. Among them, amino acid substitutions at position 16, 27, and 164 alter receptor functions [7,8]. An association between the polymorphism at codon 27 of the β2-AR gene and obesity in white women has been reported [9]. To assess the role of the genetic variants of the β2-AR gene in susceptibility to obesity and obesity-related metabolic disorders, we analysed the polymorphisms in obese and non-obese subjects.

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Abbreviations: AR, adrenergic receptor; PCR, polymerase chain reaction, OR, odds ratio; CI, 95 % confidence interval, ANOVA, analysis of variance

Table 1. Codon 16 and codon 27 polymorphisms of the β2-AR gene and obesity

Codon 16 genotype	Arg/Arg	Arg/Gly	Gly/Gly	Gly 16 allele frequency
Non-obese	101 (25%)	200 (50%)	99 (25%)	0.50
Male	70 (24)	154 (52)	69 (24)	0.50
Female	31 (29)	46 (43)	30 (28)	0.50
Obese	30 (28)	56 (52)	22 (20)	0.46
Male	11 (22)	24 (47)	16 (31)	0.55
Female	19 (33)	32 (56)	6 (11)[a]	0.39
Codon 27 genotype	Gln/Gln	Gln/Glu	Glu/Glu	Glu 27 allele frequency
Non-obese	346 (86%)	52 (13%)	2 (1%)	0.07
Male	255 (87)	36 (12)	2 (1)	0.07
Female	91 (85)	16 (15)	0	0.07
Obese	81 (75)	24 (22)	3 (3)	0.14[b]
Male	40 (78)	9 (18)	2 (4)	0.13[c]
Female	41 (72)	15 (26)	1 (2)	0.15[d]

[a] $p = 0.01$ vs non-obese female subjects (OR 0.30, Cl 0.12–0.75), [b] $p = 0.001$ vs non-obese subjects (OR 2.14, Cl 1.35–3.41), [c] $p = 0.04$ vs non-obese men (OR 1.99, Cl 1.04–3.84), and [d] $p = 0.03$ vs non-obese women (OR 2.17, Cl 1.06–4.42)

Subjects and methods

Subjects. Subjects of this study consisted of two groups; 400 non-obese subjects with body mass index (BMI) less than 27 kg/m^2 randomly selected from patients who attended the Kumamoto Red Cross Health Care Centre for health screening, and 108 obese subjects (BMI > 27 kg/m^2) treated at the Kurume University Hospital. All subjects gave informed consent. The non-obese group (aged 50.8 ± 8.6 years, BMI 22.9 ± 2.0 kg/m^2) comprised 293 men and 107 women and the obese group (aged 48.4 ± 16.5 years, BMI 31.2 ± 3.5 kg/m^2) 51 men and 57 women. Diagnosis of diabetes mellitus was based on clinical findings and glucose tolerance evaluated by oral administration of 75 g glucose according to the World Health Organization criteria of 1980. Of the obese group 82 and of the non-obese group 22 patients were diagnosed as having diabetes.

Analysis of the polymorphisms. Genomic DNA was extracted from peripheral leucocytes by digestion with proteinase K followed by phenol/chloroform extraction. The amplification of β2-AR gene sequences was done by polymerase chain reaction (PCR) in a volume of 10 μl containing 0.1 U of AmpliTaq gold DNA polymerase (Takara, Otsu, Japan), 1.5 mmol/l of $MgCl_2$, 100μmol/l of dNTPs, 10 mmol/l of Tris-HCl pH 8.3, 50 mmol/l of KCl, with following oligonucleotide primers: 5′-CTTCTTGCTGGCACGCAAT–3′ and 5′-CCAGTGAAGTGATGAAGTAGTTGG-3′ for codon 16, 5′-GGCCCATGACCAGATCAGCA-3′ and 5′-GAATGAGGCTTCCAGGCGTC-3′ for codon 27, 5′-GGACTTTTGGCAACTTCTGG-3′ and 5′-ACGAAGACCATGATCACCAG-3′ for codon 164. Dimethylsulphoxide was added at the concentration of 10% when codon 27 sequence or codon 164 sequence was amplified. Anealing temperatures for codon 16, codon 27, and codon 164 were 56 °C, 60 °C, and 55 °C, respectively. After 35 cycles of amplification, aliquots (2 μl) of PCR products were analysed on 2% agarose gels to confirm the proper amplification. Then the amplified PCR products were digested with the addition of BsrD1 (codon 16), Ita1 (codon 27), or Mnl1 (codon 164). After an incubation for 2 h, the digested samples were separated by electrophoresis through 3% agarose gel and made visible by staining with ethidium bromide.

Statistical analysis. Values are given as means and SD. Differences between group means were estimated by the Student's unpaired *t* test. The chi-squared test was used to compare frequencies. A *p* value less than 0.05 was considered statistically significant. Relative risk was estimated by the odds ratios (ORs) and their 95% confidence intervals (CIs). The significance of individual values was ascertained by the analysis of variance (ANOVA) using GLM procedure of SAS (SAS Institute, Carey, N.C., USA).

Results

Codon 16 of the β2-AR gene was highly polymorphic in the Japanese subjects (Table 1). The incidence of Arg16Gly substitution was not significantly different between obese and non-obese subjects as a whole. In women, however, the Gly16 allele frequency tended to be lower in obese subjects when compared with non-obese subjects (0.39 vs 0.50, $p = 0.06$, OR 1.56, CI 0.98–2.48). Significant difference was obtained in the frequency of Gly16 homozygotes between obese women (11%) and non-obese women (28%, $p = 0.01$, OR 0.30, CI 0.12–0.75). The Glu27 allele frequency in obese subjects was twice as high as that in non-obese subjects (0.14 vs 0.07, $p = 0.001$, OR 2.14, CI 1.35–3.41). This association was significant both in male and female subjects ($p = 0.04$ and $p = 0.03$, respectively). Although BMI may be affected by diabetes and its treatment, there was no statistically significant difference in the duration of diabetes or the ratio of insulin treatment among Gln27 homozygotes, Gln27/Glu27 heterozygotes, and Glu27 homozygotes in the obese group. None of non-obese subjects was receiving insulin treatment. The Glu27 allele was detected only in 2 of 131 subjects homozygous for Arg16 allele, whereas 32 of 121 subjects homozygous for Gly16 allele carried the variant allele (Table 2) indicating that the Glu27 allele was in linkage disequilibrium to the Gly16 allele. All of the Glu27 homozygotes were homozygous for the Gly16 allele. Whereas, the homozygous Gly16/Gln27 genotype was obtained in 75 of 400 non-obese and 14 of 108 obese

Table 2. Linkage of codon 16 polymorphism and codon 27 polymorphism in obese and non-obese subjects

	Gly16/ Gly16	Arg16/ Gly16	Arg16/ Arg16	Total
Obese				
Glu27/Glu27	3 (3%)	0	0	3
Gln27/Glu27	5 (5)	19 (18)	0	24
Gln27/Gln27	14 (13)	37 (34)	30 (28)	81
Total	22	56	30	108
Non-obese				
Glu27/Glu27	2 (0.5)	0	0	2
Gln27/Glu27	22 (6)	28 (7)	2 (0.5)	52
Gln27/Gln27	75 (19)	172 (43)	99 (25)	346
Total	99	200	101	400

$p = 0.00004$ for obese subjects, $p = 0.00005$ for non-obese subjects (chi squared test)

subjects. The wild type Gln27/Arg16 homozygous genotype was detected in 99 and 30 of non-obese and obese subjects, respectively. Thr164Ile substitution of the β2-AR gene was not observed in the subjects of this study.

Next we analysed the association of the polymorphisms in the β2-AR gene with glucose and lipid metabolism. The codon 16 polymorphism was not associated with glucose tolerance in either male or female subjects (Table 3). However, the codon 27 variant allele was more common in diabetic subjects than in non-diabetic subjects (0.14 vs 0.07, $p = 0.001$, OR 2.13, CI 1.34–3.41). The BMIs of diabetic and non-diabetic subjects were 29.0 ± 4.3, 23.5 ± 3.3, respectively ($p < 0.0001$). Analysis of the association between the Glu27 allele and diabetes within each group, showed there tended to be an association in the non-obese subjects (0.14 vs 0.07, $p = 0.08$) but not in the obese subjects (0.14 vs 0.13, NS). To assess whether the Glu27 allele was directly associated with diabetes in the lean group, correlations of the genotype and BMI with plasma glucose concentrations were estimated by ANOVA. We found 2-h post-load plasma glucose concentration was associated with BMI ($p = 0.002$) but not with the Glu27 allele. Basal plasma glucose concentrations were not associated with either BMI or Glu27 variants.

In the obese group, subjects having the Glu27 allele showed higher concentrations of fasting serum triglyceride (2.68 ± 1.90 mmol/l) than subjects without the variant (1.18 ± 1.15 mmol/l, $p = 0.02$), although there was no difference in age between the subjects with and those without the variant allele (49.5 ± 16.3 vs 47.9 ± 16.3 years old), BMI (31.3 ± 3.4 vs 31.1 ± 3.6), serum cholesterol (5.35 ± 0.99 vs 5.38 ± 1.02 mmol/l), glycohaemoglobin (7.1 ± 2.0 vs 7.1 ± 2.1%), systolic blood pressure (132.4 ± 19.1 vs 131.9 ± 19.9 mmHg), or diastolic blood pressure (77.1 ± 11.7 vs 76.5 ± 11.0 mmHg). Since hypertriglyceridaemia could have resulted from diabetes, correlations of the Glu27 allele and glycohaemoglobin concentrations with serum triglyceride values were analysed as independent variables with ANOVA. Both the genotype and glycohaemoglobin concentrations were associated with those of serum triglyceride in the obese subjects ($p = 0.004$ and $p = 0.026$, respectively). On the other hand, no significant association was seen in the non-obese group between the codon 27 polymorphism and BMI, serum lipids, glycohaemoglobin concentrations, or blood pressure. Whereas, the codon 16 polymorphism was not associated with any of the above variables either in obese or non-obese subjects.

Discussion

In Swedish women obesity was found to be associated with the codon 27 polymorphism, but not with the codon 16 polymorphism [9]. In this study, the prevalence of Gly16 homozygotes was lower in obese women than in non-obese women ($p = 0.01$, OR 0.30, CI 0.12–0.75). This discrepancy could be explained by differing environmental influence or genetic background. Our observation accorded, however, with adipocytes from subjects homozygous for the Gly16 allele having fivefold higher sensitivity to β2-selective agonist terbutaline than those from subjects homozygous for the Arg16 allele [9]. Thus the Gly16 allele could be a resistant gene of obesity in female subjects.

In the Swedish study [9] obesity was associated only with homozygosity for Glu27. Although

Table 3. Association between polymorphisms in the β2-AR gene and diabetes mellitus

Glucose tolerance	Codon 16				Codon 27			
	Arg/Arg	Arg/Gly	Gly/Gly	Gly16 allele frequency	Gln/Gln	Gln/Glu	Glu/Glu	Glu27 allele frequency
Non-diabetic	103	201	100	0.50	349	53	2	0.07
Non-obese	94	186	98	0.51	330	46	2	0.07
Obese	9	15	2	0.37	19	7	0	0.13
Diabetic	28	55	21	0.47	78	23	3	0.14[a]
Non-obese	7	14	1	0.39	16	6	0	0.14
Obese	21	41	20	0.49	62	17	3	0.14

[a] $p = 0.001$ vs Glu27 frequency in non-diabetic subjects (OR 2.13, Cl 1.34–3.41)

Glu27 homozygotes were rare in our Japanese subjects, an association was seen between the heterozygous Gln27/Glu27 genotype and obesity. The association between the Glu27 allele and obesity was observed in both male and female subjects. All of the Glu27 homozygotes were homozygous for the Gly16 allele, suggesting that the Gly16/Glu27 haplotype could be associated with obesity. Although the Gly16 variant and the Glu27 variant were in linkage disequilibrium, the incidence of Glu27 was much lower than that of Gly16. The frequency of Gly16/Gln27 homozygotes was higher than that of Gly16/Glu27 homozygotes both in non-obese (19% vs 0.5%) and obese subjects (13% vs 3%). This could be the reason why obesity was associated with Glu27, but not with Gly16, despite the linkage disequilibrium. Thus the haplotype that was associated with leanness in women could be Gly16/Gln27.

The frequency of the variant Glu27 allele of the β2-AR gene was twofold higher in diabetic subjects ($p = 0.001$). The association between the Glu27 allele and diabetes could be explained by the high frequency of obesity in the subjects with diabetes. On the other hand, obese subjects carrying the Glu27 allele of the β2-AR gene showed higher concentrations of serum triglyceride than obese subjects without the variant allele despite equivalent concentrations of glycohaemoglobin. The ANOVA showed that both the Glu27 allele and glycohaemoglobin concentrations were independently associated with hypertriglyceridaemia in the obese group. The association was not observed in non-obese subjects, probably because relative contribution of lipolysis to triglyceride concentrations was small in lean subjects. In a recombinant expression system using fibroblasts as host cells it has been shown that the Glu27 variant receptor is resistant to agonist-promoted down-regulation as compared with wild type [7, 8]. The absence of down-regulation might augment the release of non-esterified fatty acid and thereby result in hypertriglyceridaemia and insulin resistance, although the mechanism by which the Gln27Glu substitution induces fat accumulation is not known. These observations contrasted with a report that the Arg64 allele of the β3-AR gene is associated with decreased serum triglyceride despite its association with visceral obesity, probably through the low lipolytic activity [10]. The incidence of Trp64Arg substitution was not increased in patients with diabetes [1, 2]. It should be noted that β2-ARs are expressed not only in adipose tissue but in a variety of tissues including artery of skeletal muscles and pancreatic beta-cells, which could affect insulin sensitivity and secretion. There is a possibility that the association of the polymorphism with obesity could be attributable to β2-ARs in tissue other than adipose tissue.

In conclusion, the amino-terminal polymorphisms in the β2-AR gene were associated with obesity and hypertriglyceridaemia in Japanese subjects. Furthermore, the Glu27 variant could be involved in the development of Type II diabetes through fat accumulation and insulin resistance.

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