Study of the Trp^{64}Arg Polymorphism of the β_3-Adrenergic Receptor in Greek Women With Gestational Diabetes

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OBJECTIVE — This study assessed whether the Trp^{64}Arg polymorphism of the β_3-adrenergic receptor (β_3-AR), which has been associated with obesity, insulin resistance, weight gain, and earlier onset of type 2 diabetes, is more frequent in women who develop gestational diabetes mellitus (GDM) or whether it is associated with weight gain during pregnancy.

RESEARCH DESIGN AND METHODS — A total of 311 Greek pregnant women (180 with GDM and 131 without GDM [control]) who underwent a 100-g oral glucose tolerance test (OGTT) in the third trimester of pregnancy were genotyped for the β_3-AR Arg^{64} polymorphism. Insulin levels were also determined during the OGTT.

RESULTS — The frequency of Trp^{64}Arg heterozygotes in this population was ~7% and was similar in the GDM and control groups (6.7 vs. 6.9%) as well as in the obese (BMI ≥27 kg/m^2) and the nonobese (6.3 vs. 6.8%) subgroups. In the GDM group, BMI, fasting insulin resistance index, and diastolic blood pressure were significantly higher in Trp^{64}Arg carriers; these differences were no longer observed when obesity was considered. In the 4 subgroups (control Trp^{64}Trp and Trp^{64}Arg and GDM Trp^{64}Trp and Trp^{64}Arg), a highly significant trend was evident of an increase in the percentage of subjects with shorter height.

CONCLUSIONS — The frequency of the Arg^{64} allele in Greek pregnant women is relatively rare compared with other ethnic groups and is probably not related to the development of GDM or obesity. The observed tendency for shorter body height in Arg^{64} carriers merits further evaluation in larger population samples.

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The β_3 subtype of the adrenergic receptors (β_3-AR) is expressed in brown adipose tissue as well as in white fat cells and is an important regulator of energy expenditure and lipolysis (1,2). A β_3-AR variant replacing the Tryptophan in codon 64 of the gene with Arginine has been recently described and is quite common in the population in its heterozygous form (3–5).

Several studies have examined the role of this gene variant on phenotypic features. In diabetic populations, the Trp^{64}Arg variant has been associated with earlier onset of the disease and weight gain in some (3,4,6,7) but not all (8–10) studies. In nondiabetic populations, the Trp^{64}Arg polymorphism has been associated with features of the insulin resistance syndrome such as increased visceral fat, abdominal obesity, increased diastolic blood pressure, and in vitro and in vivo measures of insulin resistance (4,7,11–13). These associations have not, however, been consistently found in all studies (8,14–16). The effect of the Trp^{64}Arg variant on obesity has also been examined in nondiabetic individuals; most studies have not shown strong associations with BMI (17–20). Some of the reports have shown that the effect of this variant on obesity is only detected in women and is probably related to age (21,22). However, most of the studies to date have concerned middle-aged populations, and data are often pooled for both men and women.

Both obesity and insulin resistance are associated with the development of gestational diabetes mellitus (GDM) (23,24). Therefore, this study examined whether the Trp^{64}Arg polymorphism may be more frequent in women who develop GDM and whether, in this group of women, its presence may be associated with a different course of GDM or increased weight gain during pregnancy.

RESEARCH DESIGN AND METHODS

All women participating in the study (n = 311) were of Greek ethnic origin. Our policy is to examine every pregnant woman with a 1-step approach that involves performing a diagnostic oral glucose tolerance test (OGTT) between 24 and 28 weeks of gestation independently of the presence of known risk factors. Two groups of pregnant women were studied. The GDM group consisted of 180 consecutive women (age 17–48 years, prepregnancy BMI 17.5–47.8 kg/m^2) who were diagnosed with GDM using the diagnostic criteria recently accepted by the American Diabetes Association (ADA) (25) (i.e., 2 or more abnormal values during a standard 100-g OGTT; normal glucose values were <95, <180, <155, and <140 mg/dl at 0, 60, 120, and 180 min, respectively). Dur-

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Abbreviations: ADA, American Diabetes Association; β_3-AR, β_3-adrenergic receptor; GDM, gestational diabetes mellitus; HOMA, homeostasis model assessment; NDDG, National Diabetes Data Group; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Anthropometric characteristics and biochemical parameters in the control and GDM groups*

	Control group		GDM group	
	β_3-AR Trp^{64}/Trp	β_3-AR Trp^{64}/Arg	β_3-AR Trp^{64}/Trp	β_3-AR Trp^{64}/Arg
n	122	9	168	12
Age (years)	30.6 ± 5.3	32.2 ± 7	33.4 ± 5.4	34.3 ± 5.1
Height (cm)	161.1 ± 6.2	159.6 ± 5.6	158.5 ± 6.2	156.1 ± 3.7*
Prepregnancy BMI (kg/m^2)	25.6 ± 5.2	23 ± 2	27.2 ± 5.2	31.6 ± 8.1*
Weight gain (g/day)	50.4 ± 21	56.1 ± 13.7	51.5 ± 23	56.3 ± 19.7
Systolic blood pressure (mmHg)	111 ± 12	101 ± 7*	115 ± 12	121 ± 20
Diastolic blood pressure (mmHg)	68 ± 10	62 ± 6	70 ± 10	78 ± 9*
HbA_{1c} (%)	4.0 ± 0.4	4.2 ± 0.4	4.3 ± 0.5	4.4 ± 0.5
Glucose (mmol/l)				
0 min	4.7 ± 0.4	5.1 ± 0.6	5.4 ± 1.1	6.1 ± 0.9*
60 min	8.6 ± 1.4	7.9 ± 1.5	11.9 ± 1.8	12.1 ± 2.3
120 min	7.0 ± 1.3	6.7 ± 1.3	10.4 ± 2.3	10.1 ± 2.2
180 min	5.7 ± 1.3	5.4 ± 0.9	8.0 ± 2.2	7.7 ± 2.6
Insulin (pmol/l)				
0 min	85 ± 38	79 ± 22	97 ± 42	118 ± 23†
60 min	533 ± 233	392 ± 136	532 ± 280	544 ± 275
120 min	517 ± 263	373 ± 115	677 ± 316	734 ± 210
180 min	368 ± 235	219 ± 55*	533 ± 280	566 ± 245
Insulin resistance (HOMA)	3.0 ± 1.5	3.0 ± 1.50	4.0 ± 2.3	5.4 ± 1.5†

Data are *n* or means ± SD. *$P < 0.05$; †$P < 0.01$ for the difference from the wild-type allele in the same group.

ing the same period, the first 131 consecutive pregnant women who underwent the routine OGTT for GDM and had a normal OGTT were recruited as the control group (age 18–45 years, prepregnancy BMI 16.5–50.8 kg/m^2). In the GDM group, 94 women were subsequently treated with diet alone, and 86 women were treated additionally with insulin.

The study was performed between weeks 28 and 36 of pregnancy before initiation of treatment in the GDM group. All women gave informed consent to participate in the study. In all pregnant women, age, blood pressure, height, current weight, and prepregnancy weight were recorded, and the respective BMIs were calculated. Weight gain during the current pregnancy was estimated in grams per day as follows: current weight – reported prepregnancy weight/days of gestation. The birth weight of the women when known (125 cases) was also recorded. The birth weight of neonates was also available for 157 women. The subjects' characteristics are shown in Table 1.

In all women, HbA_{1c} and insulin levels during the OGTT were also measured. Plasma glucose concentrations were measured with an autoanalyzer using the hexokinase method. Serum insulin immunoreactivity was measured by enzyme-linked immunosorbent assay (Boehringer Mannheim, Mannheim, Germany); the intra- and interassay coefficients of variation were 7.8 and 6.4%, respectively, at 100 μU/ml. To evaluate basal insulin resistance, we used the fasting insulin resistance index derived from the homeostasis model assessment (HOMA) model (26) by applying the following: insulin resistance = fasting insulin × fasting glucose/22.5, where fasting insulin is expressed in microunits per milliliter and fasting glucose is expressed in millimoles per liter.

β_3-AR genotyping

DNA was extracted from peripheral lymphocytes using standard methods. A region of 202 base pairs corresponding with the first exon of the β_3-AR gene was amplified by polymerase chain reaction (PCR). The sequence-specific primers used were 1F:CCAATACCGCCAACACCAGT and 2R:AGGAGTCCCATCACCAGGTC (11). A total of 50–100 ng of DNA was amplified in 100-μl reactions containing 10 mmol/l Tris-HCl, pH 9.0, 50 mmol/l KCl, 0.75 mmol/l $MgCl_2$, 100 μmol/l dNTPs, and 1 U Taq DNA polymerase with 1 pmol each of the 1F and 2R primers. The amplification protocol consisted of 40 cycles of 30 s at 95°C, 30 s at 55°C, 90 s at 72°C, and a 10-min final extension at 72°C.

The PCR products were checked for correct size by electrophoresis in a 2.0% agarose gel using DNA markers. The PCR product was subsequently cleaned with phenol/chloroform extraction and was tested for the presence of the Trp^{64}Arg mutation by digestion with the enzyme *Bst*NI (12–18 h incubation at 60°C in manufacturer's buffer), which recognizes the normal sequence at the site of the mutation.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (SPSS, Chicago). The χ^2 criterion and the nonparametric Mann-Whitney *U* test (or *t* test for normally distributed values) were applied as appropriate. Multiple linear regression analysis was used to investigate the correlation between insulin resistance features and anthropometric variables.

RESULTS — The distribution of the Trp^{64}/Trp and Trp^{64}/Arg genotypes in the 2 groups of pregnant women is shown in Table 2. The frequency of the Trp^{64}/Arg genotype was similar in both groups of pregnant women (6.9% in the control group and 6.7% in the GDM group). We found no patients or control subjects who were homozygous for the mutation (Table 2). The allele frequency of Arg^{64} in the total population was 0.034. Furthermore, we analyzed the data in the control group by considering whether all 4 glucose values were normal or whether 1 value was abnormal. In the group with 4 normal values, 6 women (6.9%) had the Trp^{64}/Arg genotype, and 81 women (93.1%) had the Trp^{64}/Trp genotype; in the group with 1 abnormal value, 3 women (6.8%) had the Trp^{64}/Arg genotype, and 41 women (93.2%) had the Trp^{64}/Trp genotype (NS).

In addition, we analyzed our data using other proposed criteria for GDM such as the National Diabetes Data Group (NDDG) criteria (normal glucose values <105, <190, <165, and <145 mg/dl at 0, 60, 120, and 180 min after a glucose load, respectively). Again, no difference was observed in the fre-

Table 2—*Genotype frequencies of the Trp^{64}/Arg β_3-AR polymorphism in the 2 groups of Greek pregnant women*

Group	β_3-AR Trp^{64}/Trp	β_3-AR Trp^{64}/Arg	β_3–AR Arg^{64}/Arg
GDM			
ADA criteria	168 (93.3)	12 (6.7)	0
NDDG criteria	138 (93.2)	10 (6.8)	0
Control			
ADA criteria	122 (93.1)	9 (6.9)	0
NDDG criteria	152 (93.3)	11 (6.7)	0

Data are *n* (%).

quency of the Trp^{64}/Arg genotype between GDM and control subjects (Table 2).

No significant difference was evident according to mode of treatment in the GDM group. A total of 4 of 94 patients who were treated with diet alone and 8 of 86 patients who took insulin were Arg^{64} carriers (NS).

To examine whether the presence of the Arg allele was related to the presence of obesity, we divided the cohort of pregnant women into 2 groups according to prepregnancy BMI: nonobese group (BMI $<$27 kg/m^2 [*n* = 191]) and obese group (BMI $\geq$27 kg/m^2 [*n* = 111]). The allele was present in 6.8 and 6.3% of individuals in the 2 groups, respectively (NS). Similar results were obtained when BMI $<$25 or $\geq$25 kg/m^2 was considered.

The demographic and biochemical characteristics of the study groups according to the presence of the variant are shown in Table 1. The pregnancy week at the time when the study was performed did not differ between subgroups (control: Trp^{64}/Trp 27.6 ± 4.4 weeks, Trp^{64}/Arg 29 ± 2.2 weeks; GDM: Trp^{64}/Trp 27 ± 7.6 weeks, Trp^{64}/Arg 30.7 ± 3.5 weeks; NS). Birth weight of neonates of Arg^{64}carriers did not differ from that of the neonates of noncarriers in either group of women (GDM: 3,362 ± 432 g [*n* = 10] vs. 3,277 ± 369 g [*n* = 105]; control: 3,383 ± 126 g [*n* = 3] vs. 3,245 ± 368 g [*n* = 39], for carriers and noncarriers, respectively).

In the control group, women carrying the mutation were less obese (NS), had marginally lower systolic blood pressure levels, and had lower 180-min insulin levels during the OGTT. In the GDM group, women carrying the Arg^{64} allele were significantly more obese, had higher diastolic blood pressure levels, had higher fasting glucose and insulin levels, and had a higher calculated basal insulin resistance index (HOMA) (Table 1). Multivariate analysis was performed to see whether the differences in the GDM group were due to an independent effect of the Arg^{64} allele or whether they could be attributed to differences in obesity. The effect of the Arg^{64} allele on insulin resistance was no longer observed in the GDM group when the prepregnancy BMI was considered (for the effect of BMI, $P < 0.001$; for the effect of the Arg^{64} allele, $P = 0.143$). Similarly, when BMI was considered, blood pressure was no longer influenced by the presence of the Arg^{64} allele (for the effect of BMI, $P < 0.001$; for the effect of the Arg^{64} allele, $P = 0.477$).

Mean height was significantly shorter in Arg^{64} carriers in the GDM group. In the control group, mean height in Arg^{64} carriers was also lower (by ~1.5 cm); however, this difference did not reach statistical significance (Table 1). Nevertheless, a tendency was evident of shorter height in the control group because 67% of Arg^{64} carriers in the control group (versus 71.5% of wild-type homozygotes and 91% of Arg^{64} carriers in the GDM group) had a shorter height than the mean height observed in the wild-type homozygotes in the control group (Table 3). The χ^2 test for trend considering the 4 levels (control Trp/Trp to GDM Trp/Arg) was highly significant ($\chi^2 = 12.56$, $P < 0.001$).

No significant difference was evident in weight gain during pregnancy in either of the 2 groups (Table 1). Birth weights did not differ between those with the Arg^{64} allele and those without it (3,442 ± 739 vs. 3,494 ± 767 g).

CONCLUSIONS

The frequency of the Arg^{64} β_3-AR allele in our southern European Mediterranean population was 0.04. This allele frequency is the same as that reported for Sweden (0.04); lower than that reported for German, French, British, and other European populations (0.08–0.15); and much more rare than the 0.31–0.37 reported for the Pima Indian and Japanese populations (3–5,10,15, 20,27). This study did not reveal any difference in the frequency of this allele between the GDM and control groups. No association was found with weight gain during the current pregnancy in either the GDM group or the control group. Finally, no effect on the clinical severity of GDM was observed, as assessed by the need for insulin administration. Furthermore, obesity was not associated with a higher frequency of the Arg^{64} allele in the total population studied.

The findings in these relatively young women argue against a major role of the Trp^{64}/Arg β_3-AR variant in the development of GDM or obesity in our ethnic group, although the rather rare frequency of this allele in our population should be noted. In this respect, our findings agree with most reports in the literature in which little or no effect on obesity has been observed (17–20).

While this article was in preparation, a report appeared in the literature that showed that the presence of the β_3-AR variant confers a very high relative risk of the development of GDM and increased weight gain during pregnancy (28). However, these authors defined GDM using uncommon criteria based on relatively low cutoff points (28). Furthermore, they also pointed out that when the conventional criteria of the NDDG were used, no differences were observed between the GDM and control groups, which agrees with our present findings. Power estimates showed that our sample had >80% power to detect a difference in the reported order (i.e., 2.5 times higher) (28), although we would need a sample of several thousand women with GDM to detect more subtle differences in the order of a 50% increase in risk.

Despite the fact that results in the literature do not support a major role of the

Table 3—*Percentage of women with height lower than the mean height of Trp^{64}/Trp control subjects in the 4 subgroups of the study*

Group	*n*	Women with less-than-average height
Control		
Trp^{64}/Trp	121	53.7 (65)
Trp^{64}/Arg	9	66.7 (6)
GDM		
Trp^{64}/Trp	165	71.5 (118)
Trp^{64}/Arg	11	90.9 (10)

Data are *n* or % (*n*). χ^2 for trend = 12.56; $P = 0.0004$.

Arg^{64} allele in human disease (10,14,16,29), reports (although inconsistent) have shown a mild effect on obesity (18,19,21,22). Our results showed an association of obesity with the Arg allele in the GDM group only. In the control group, the opposite effect was observed because Arg^{64} carriers were slightly less obese. In view of this finding, one cannot exclude the possibility that the presence of the Arg allele predisposes women to GDM if obesity is superimposed.

In a substantial number of reports in the literature, the Trp^{64}/Arg β_3-AR variant has been associated with various traits that are all related, albeit weakly, to the metabolic syndrome (4,7,11,12,17,22); one study using even more sophisticated methods to evaluate insulin resistance in subjects properly matched for obesity showed an independent association of the Trp^{64}/Arg variant with insulin resistance (13). In our study, we found a significant increase in fasting insulin resistance index in the GDM group; however, this association was related to the degree of obesity. One should bear in mind, however, that the method we used provides only a rough estimate of insulin resistance.

Finally, an unexpected finding of this study was the association of the Arg^{64} allele with shorter body height in the GDM group. A similar tendency was also present in the control group of pregnant women (Table 3). Although we cannot exclude the possibility that the difference in height may represent a chance phenomenon because of the small number of Arg carriers, we should point out that this phenotypic trait has previously been shown to be associated with glucose intolerance, GDM, and insulin resistance (30–32). This association of the Trp^{64}/Arg β_3-AR polymorphism with height has not been reported before, and addressing this point in the studies where data are available for larger population samples of young women would be interesting.

In conclusion, our data show that the frequency of the Arg allele in the Greek population is relatively rare compared with other ethnic groups and is probably not related to the development of GDM or obesity. The tendency for decreased body height in Arg carriers is very interesting and merits further investigation in larger population samples.

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