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Polymorphisms of insulin receptor substrate 1 and β_3-adrenergic receptor genes in gestational diabetes and normal pregnancy

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Abstract

Gestational diabetes mellitus (GDM) is considered an important risk factor for the development of type 2 diabetes mellitus. We studied possible relations between GDM and both insulin receptor substrate 1 (IRS-1) (Gly972Arg) and β_3-adrenergic receptor (ADRB3 Trp64Arg, β_3-AR) gene mutations, considered potential modifying factors in the etiology of type 2 diabetes mellitus. We evaluated the 2 gene mutations in late gestation in 627 pregnant women, all studied using the glucose challenge test, followed (in positive tests) by the oral glucose tolerance test (100 g, Carpenter and Coustan [*J Obstet Gynecol*. 1982;144:768-773] criteria) We diagnosed 309 women with GDM, 41 with gestational impaired glucose tolerance and 277 normal pregnant women. Age, family history of diabetes, prepregnancy body mass index, weight gain during pregnancy, plasma glucose levels, hemoglobin A_{1c}, islet autoantibody levels, and insulin treatment during pregnancy were all evaluated. All pregnant women were genotyped for IRS-1 (Gly972Arg) and β_3-AR (ADRB3 Trp64Arg) polymorphisms. The frequency of IRS-1 gene polymorphism was significantly higher in women with GDM than in women with a normal glucose tolerance (NGT) ($P = .039$), and there was a significant trend ($P = .032$) in the increasing frequency of mutant allele Arg from NGT > gestational impaired glucose tolerance > GDM. The search for β_3-AR gene polymorphism showed no significant differences between women with GDM and women with NGT. The X-Arg genotype of IRS-1 was significantly associated with a positive family history of diabetes in NGT ($P = .006$) and neared significance in GDM ($P = .057$). Moreover, we found that NGT carriers of both polymorphisms had a higher prepregnancy body mass index than carriers of the IRS-1 variant alone ($P = .0034$), the β_3-AR variant alone ($P = .039$), or neither ($P = .048$), suggesting a possible synergistic effect of the 2 gene polymorphisms. These results suggest that the IRS-1 genetic polymorphism is involved in the occurrence of gestational diabetes, as well as type 2 diabetes mellitus.

1. Introduction

Pregnancy is characterized by peripheral insulin resistance, which is compensated by an increase in insulin secretion to maintain glucose homeostasis [1]. Gestational diabetes mellitus (GDM), which occurs in about 4% of pregnancies, develops if insulin secretion fails to overcome insulin resistance [2]. Therefore, GDM shares many features with type 2 diabetes mellitus, including not only glucose intolerance, insulin resistance, and impaired insulin secretion, but also association with similar risk factors, as well as obesity and family history of diabetes [3]. This is confirmed by the finding that women with a history of GDM are at significantly increased risk of developing type 2 diabetes mellitus in the future [4].

There is increasing evidence that genetic factors contribute significantly to the risk of type 2 diabetes mellitus and, in this context, some genetic polymorphisms, such as those of insulin receptor substrate 1 (IRS-1) and β_3-adrenergic receptor (β_3-AR), have recently been related to insulin resistance, obesity, and type 2 diabetes mellitus [5,6].

The gene product of insulin receptor substrate 1, the IRS-1 protein, is a cytoplasm molecule expressed in many insulin-sensitive tissues, which has an important role in regulating the cellular effect of insulin [7]. After the binding of insulin to its receptor, the β subunit of the receptor is activated (tyrosine kinase activity), leading to phosphorylation of

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specific tyrosine residues on IRS-1. When phosphorylated, IRS-1 binds to a series of cellular signal proteins, including phosphatidylinositol 3 kinase [8]. Genetic analysis of the IRS-1 gene has shown that a glycine-to-arginine substitution at codon 972 (Gly972Arg mutation), which occurs in about 6% of the general population, significantly impairs IRS-1 function and is associated with insulin resistance, lipid abnormalities [5], and type 2 diabetes mellitus [5,9].

As the β_3-AR is mainly expressed in adipose tissue and plays an important part in the regulation of energy expenditure (lipid metabolism and thermogenesis), it is considered a candidate gene for obesity [10]. A mutation has recently been described in codon 64 of β_3-AR, determining the replacement of tryptophan by arginine in the receptor protein [11]. This polymorphism is also reportedly associated with increased body weight, insulin resistance, type 2 diabetes mellitus, and other features of metabolic syndrome [11-13]. A recent work by Perfetti et al [14] elucidates a novel site of activation for β_3-AR in islet beta cells and hypothesizes that subjects carrying the variant allele Arg64 have an insulin secretory defect characterized by the abnormal glucose-sensing activity of beta cells.

In this context, because of the scarcity of data [15-18], we believed it would be of interest to study these 2 polymorphisms in women with GDM and normal pregnant women selected at 2 specialized centers in Italy.

2. Materials and methods

2.1. Subjects

The study involved 627 white women, 309 of them with GDM, 41 with gestational impaired glucose tolerance (G-IGT) and 277 with a normal glucose tolerance (NGT).

All women were screened for GDM between 24 and 28 gestational weeks with the glucose challenge test; if the test was positive (plasma glucose 1 hour after ingestion of 50 g sugar >7.8 mmol/L) [19], the women underwent an oral glucose tolerance test with 100 g sugar, which was evaluated according to the Carpenter and Coustan [20] criteria. Gestational diabetes mellitus was diagnosed when 2 or more values were above normal (<5.3, <10, < 8.6, and <7.8 mmol/L at 0, 60, 120, and 180 minutes, respectively). Gestational impaired glucose tolerance was diagnosed when one plasma glucose value was above normal. The so-called normal (NGT) pregnant women were those with a negative glucose challenge test result.

Patients with GDM were prescribed a standard diet in accordance with the nutritional requirements of pregnancy calculating the appropriate energy level based on the patient's pregravid weight (125.58 kJ/kg/d in normal-weight women, 150.69-167.44 kJ/kg/d in underweight women, and 100.46 kJ/kg/d in overweight women) and with an energy distribution of 45% to 50% carbohydrates, 15% to 20% proteins, and 30% to 35% fats. They were also checked by evaluating their fasting and postprandial glucose levels. If plasma glucose levels were not maintained within an acceptable range (ie, fasting plasma glucose >5.3 mmol/L and/or 2 hours postprandial plasma glucose >6.7 mmol/L), insulin treatment was started after 2 weeks.

All women with GDM were monitored for metabolic and obstetric purposes until delivery. In general, women delivered at term unless additional obstetric complications set in.

Family history for diabetes (considering all and first-degree relatives), age, prepregnancy body mass index (BMI), and weight gain during pregnancy were recorded for all women.

In the third trimester of pregnancy (30-34 gestational weeks), a blood sample was drawn from all patients to determine fasting plasma glucose, islet autoantibodies (islet cell autoantibodies, glutamic acid decarboxylase), and genetic polymorphisms.

Informed written consent was obtained from all participants, and the local ethics committee's approval was granted.

2.2. Methods

2.2.1. Genetic studies

DNA was obtained from human leukocyte nuclei isolated from whole blood after protein kinase K digestion and phenol extraction. Insulin receptor substrate 1 (Gly972Arg) and β_3-AR (Trp64Arg) polymorphisms were genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods.

A 263–base pair (bp) fragment containing the Gly972Arg polymorphism was amplified with primers described by Almind et al [21]: IRSF (5′ CTTCTGTCAGGTGTCC ATCC3′) and IRSR (5′ TGGCGAGGTGTCCACG TAGC3′). Polymerase chain reactions were done with 0.4 μg of genomic DNA in a total volume of 35 μL, and the assay conditions were as follows: 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of the 4 deoxy ribonucleotide triphosphates (dNTPs), 0.4 μmol/L of each oligonucleotide primer, 1× GeneAmp Buffer II (Applied Biosystems), and 1.25 U Amplitaq Gold (Applied Biosystems, Foster City, CA). Polymerase chain reaction conditions were as follows: initial denaturation at 94°C for 11 minutes, 32 cycles of denaturation (94°C for 1 minute), annealing (60°C for 1 minute), and extension (72°C for 1 minute), with the final extension at 72°C for 12 minutes. The 263-bp PCR products were digested with 3 U of *Bst*NI (New England Biolabs, Ipswich, MA) for 2 hours at 60°C using the buffer recommended by the manufacturer. The fragments separated on 4.5% high-resolution agarose gel with ethidium bromide and visualized under ultraviolet light were of the following sizes: 159, 81, and 23 base pairs (bp) in Gly972 homozygotes; 159, 108, 81, 51, and 23 bp in Gly972/Arg972 heterozygotes; and 108, 81, 51, and 23 bp in Arg972 homozygotes.

The Trp64Arg polymorphism of β_3-AR gene was genotyped by PCR amplification as described previously [22], with the following changes: the PCR reactions were obtained in a total volume of 25 μL with 0.3 μg of genomic

Table 1
Subjects' clinical and metabolic parameters (mean ± SD and %; *t* test)

	GDM 309 (a)	G -IGT 41 (b)	NGT 277 (c)
Age (y)	34.1 ± 4.6*	34.5 ± 3.6	32.7 ± 4.3
Prepregnancy BMI (kg/m^2)	25.4 ± 5.0**	23.5 ± 4.2	23.8 ± 4.0
Weight gain (kg)	9.9 ± 5.1***,†††	9.8 ± 3.9	12.8 ± 6.3
FPG (mmol/L)	4.6 ± 0.7†,††	4.3 ± 0.5	4.4 ± 0.5
Insulin therapy (%)	29.4	–	–
FAMD+ (%, n)	60.8 (188)‡	26.8 (11)	28.5 (79)
FAMD+, first degree (%, n)	32.0 (101)‡‡,‡‡‡	9.7 (4)	13.3 (37)

FPG indicates fasting plasma glucose.
* $P = .0006$, a vs c.
** $P = .0002$, a vs c.
*** $P < .0001$, a vs c.
† $P = .039$, a vs b.
†† $P = .0029$, a vs c.
††† $P = .0002$, b vs c.
‡ $P < .0001$, a vs c and a vs b.
‡‡ $P = .0018$, a vs b.
‡‡‡ $P < .0001$, a vs c.

DNA, and the concentrations were $MgCl_2$ 1.5 mmol/L, 0.2 mmol/L of the 4 dNTPs, 0.4 μmol/L of each oligonucleotide primer, 4% formamide, 1× GeneAmp Buffer II (Applied Biosystems), and 0.8 U Amplitaq Gold (Applied Biosystems). Polymerase chain reaction conditions were as follows: initial denaturation at 94°C for 11 minutes, 35 cycles of denaturation (94°C for 1 minute), annealing (61°C for 1 minute), and extension (72°C for 1 minute), with the final extension at 72°C for 10 minutes. The 210-bp PCR products were digested with 5 U of *Bst*NI (New England Biolabs) for 2 hours at 60°C using the buffer recommended by the manufacturer. The fragments separated on 3% high-resolution agarose gel with ethidium bromide and visualized under ultraviolet light were of the following sizes: 99, 62, 30, 12, and 7 bp in Trp64 homozygotes; 161, 99, 62, 30, 12, and 7 bp in Trp64/Arg64 heterozygotes; and 161, 30, 12, and 7 bp in Arg64 homozygotes.

Table 2
χ^2 Test results for genotype and allele variations at 2 exonic polymorphisms (IRS-1 Gly/Arg972 and β_3-AR Trp/Arg64) in 627 pregnant women

		Total N = 627	GDM n = 309 (a)	G-IGT n = 41 (b)	NGT n = 277 (c)
IRS-1 Gly972Arg					
Genotype	Gly/Gly	89.8 (563)	87.7 (271)	90.2 (37)	92.1 (255)
	Gly/Arg	9.6 (60)	11.0 (34)	9.8 (4)	7.9 (22)
	Arg/Arg	0.6 (4)	1.3 (4)	0.0 (0)	0.0 (0)
	X/Arg	10.2 (64)	12.3 (38)*	9.8 (4)	7.9 (22)
Allele	Gly	94.6	93.2	95.1	96.1
	Arg	5.4	6.8†	4.9	3.9‡
β_3-AR Trp64Arg					
Genotype	Trp/Trp	88.8 (557)	88.1 (309)	87.8 (36)	89.5 (248)
	Trp/Arg	11.0 (69)	11.3 (35)	12.2 (5)	10.5 (29)
	Arg/Arg	0.3 (2)	0.6 (2)	0.0 (0)	0.0 (0)
	X-Arg	11.3 (71)	11.9 (37)	12.2 (5)	10.5 (29)
Allele	Trp	94.2	93.7	94	94.8
	Arg	5.8	6.3	6.0	5.2

* $P = .054$ (χ^2), a vs c.
† $P = .039$ (χ^2), a vs c.
‡ $P = .032$ (χ^2 for trend), a, b, and c.

2.2.2. Metabolic and autoimmune parameters

Plasma glucose was measured using a glucose oxidase method [23].

Islet cell autoantibodies were detected in all patients using the classic indirect immunofluorescence technique on normal unfixed human pancreas of blood group O, as previously reported [24]. Glutamic acid decarboxylase antibodies were measured by the radioimmunoassay technique using a commercial kit (Biochem Immunosystems, Milan, Italy), according to the procedure recommended by the International Committee for Glutamic Acid Decarboxylase Antibody Standardization [25].

2.2.3. Statistical analysis

Data are expressed as means ± SD. Student *t* test for unpaired data was used. Nonparametric data were expressed as percentage and evaluated by the χ^2 test and

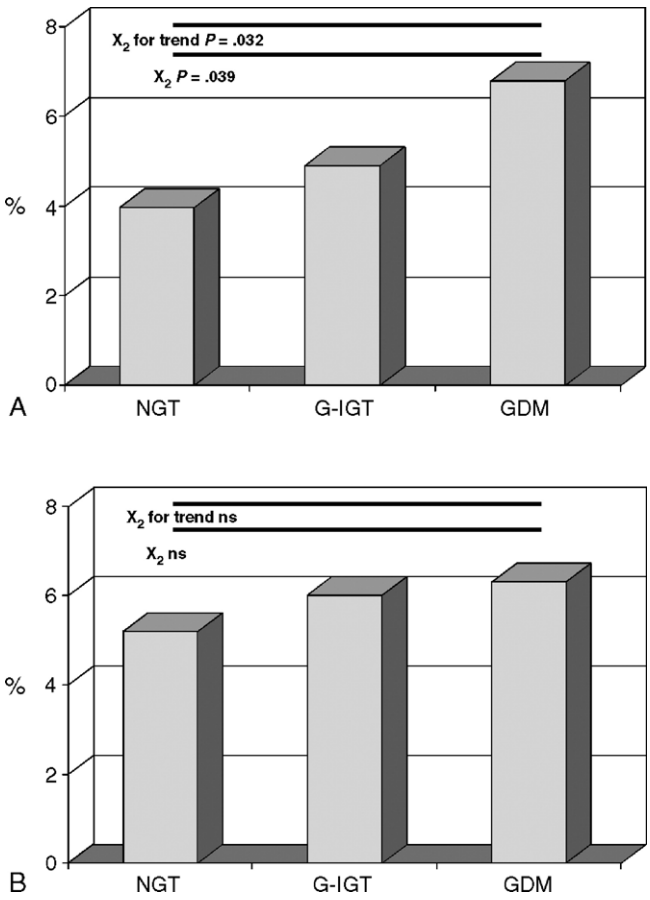

Fig. 1. A, Frequencies (%) of allelic variant 972 Arg of IRS-1 evaluated in 277 patients with NGT, 41 patients with G-IGT, and 309 patients with GDM. B, Frequencies (%) of allelic variant 64 Arg of β_3-AR evaluated in 277 patients with NGT, 41 patients with G-IGT, and 309 patients with GDM.

Table 3
Clinical and metabolic data (mean ± SD and %) of women with GDM and pregnant women with NGT according to IRS-1 Gly/Arg972 and β_3-AR Trp/Arg64

	IRS-1		β_3-AR	
	X-Arg	Gly/Gly	X-Arg	Trp/Trp
GDM				
n	38	271	37	272
FAMD+ (%, n)	73.7 (28)	59 (160)*	64.9 (24)	60.3 (164)
FAMD+, first degree, (%, n)	31.6 (12)	32.8 (89)	32.4 (12)	32.7 (89)
Prepregnancy BMI (kg/m^2)	25.1 ± 4.2	25.5 ± 5.1	25.8 ± 3.7	25.4 ± 5.1
FPG (mmol/L)	4.5 ± 0.6	4.6 ± 0.7	4.7 ± 0.6	4.6 ± 0.7
Insulin therapy (n)	11	80	12	79
NGT				
n	22	255	29	248
FAMD+ (%, n)	54.5 (12)	25.9 (66)**	34.5 (10)	27.4 (68)
FAMD+, first degree, (%, n)	22.7 (5)	12.5 (32)	17.2 (5)	12.9 (32)
Prepregnancy BMI (kg/m^2)	23.6 ± 3.9	23.8 ± 4.0	24.1 ± 4.5	23.8 ± 4.0
FPG (mmol/L)	4.3 ± 0.4	4.4 ± 0.5	4.3 ± 0.6	4.4 ± 0.5

* $P = .057$ (χ^2).
** $P = .006$.

χ^2 for trend. A P value of less than .05 was considered statistically significant.

3. Results

3.1. Clinical and metabolic parameters

The clinical and metabolic parameters of the patients are listed in Table 1. A significantly larger number of patients with GDM than patients with NGT or G-IGT had a positive family history of diabetes (FAMD+) (60.8% GDM vs 26.8% G-IGT and 28.5% NGT; and first-degree relatives only: 32% GDM vs 9.7% G-IGT and 13.3% NGT). Mean age and prepregnancy BMI were significantly higher in women with GDM than in women with NGT (34.1 vs 32.7 years and BMI of 25.4 vs 23.8). Weight gain during pregnancy was significantly lower in patients with GDM and G-IGT than in those with NGT (9.9 and 9.8 vs 12.8 kg). Good metabolic control was achieved in patients with GDM, as evidenced by the mean fasting plasma glucose levels (4.6 mmol/L).

Insulin therapy was required for 29.4% of patients with GDM: the mean gestational week of starting therapy was 28.9 ± 3 and the mean insulin dosage was 15 ± 2 U/d. Thirty percent of patients required 2 insulin injections (rapid-acting insulin at lunch and dinner); 30% of patients required 3 insulin injections (rapid-acting insulin at breakfast, lunch, and dinner); and 40% of patients requested 4 insulin injections (rapid-acting insulin at breakfast, lunch, and dinner, plus an injection of isophane insulin before dinner or at bedtime, but no later than 11:30 PM).

As regards autoimmunity, islet cell autoantibodies were found in 4 women with GDM, 1 woman with G-IGT, and 3 women with NGT; all except 1 patient with NGT carried no IRS-1 972 Arg or β_3-AR 64 Arg polymorphic allele. Glutamic acid decarboxylase antibodies were present in 5 women with GDM, 1 woman with G-IGT, and 4 women with NGT; all but 1 patient with NGT carried no IRS-1 972 Arg or β_3-AR 64 Arg. The investigation of autoimmune markers may exclude a higher prevalence of type 1 diabetes mellitus in women with GDM than in nondiabetic pregnant women.

3.2. Genetic study

The frequency of the Arg allele of IRS-1 was 5.4%, and the genotype distribution (563 Gly/Gly, 60 Gly/Arg, 4 Arg/Arg) was in Hardy-Weinberg equilibrium (Table 2).

The frequency of the Arg allele of β_3-AR was 5.8%, and the genotype distribution (557 Trp/Trp, 69 Trp/Arg, 2 Arg/Arg) was in Hardy-Weinberg equilibrium (Table 2).

For statistical purposes, heterozygous and homozygous carriers of the polymorphic allele are treated together and referred to as IRS-1 X/Arg or β_3-AR X/Arg, respectively.

The frequency of the IRS-1 Arg allele was significantly higher in women with GDM than in women with NGT (6.8% vs 3.9% $P = .039$), also showing a significant trend (χ^2 $P = .032$) from NGT of 3.9%, to G-IGT of 4.9%, to GDM of 6.8% (Table 2, Fig. 1A).

Table 4
Clinical and metabolic parameters (mean ± SD and %) of women with GDM and NGT women according to combined genotype of IRS-1 Gly/Arg972 and β_3-AR Trp/Arg64

IRS-1	X/Arg	X/Arg	Gly/Gly	Trp/Trp
β_3-AR	X/Arg	Trp/Trp	X/Arg	Trp/Trp
	A	B	C	D
GDM				
n	9	29	28	243
FAMD+ (%, n)	88.8 (8)	69 (20)	57.1 (16)	59.2 (144)*
FAMD+, first degree, (%, n)	33.3 (3)	31 (9)	32.1 89)	32.9 (80)
Prepregnancy BMI (kg/m^2)	25.8 ± 3.4	25.1 ± 4.7	26.3 ± 4.0	25.4 ± 5.2
FPG (mmol/L)	4.6 ± 0.6	4.5 ± 0.6	4.7 ± 0.7	4.6 ± 0.7
Insulin therapy (%, n)	44.4 (4)	24.1 (7)	28.6 (8)	29.6 (72)
NGT				
n	3	19	26	229
FAMD+ (%, n)	66.6 (2)	58.8 (10)	30.7 (8)	25.3 (58)***
FAMD+, first degree, (%, n)	66.6 (2)	15.8 (3)	11.5 (3)	12.7 (29)**
Prepregnancy BMI (kg/m^2)	29.8 ± 4.9	21.8 ± 2.2†††	23.5 ± 4.0†	23.8 ± 4.8††
FPG (mmol/L)	4.5 ± 0.3	4.2 ± 0.3	4.2 ± 0.6	4.4 ± 0.5

* $P = .049$ (χ^2 for trend), A, B, and D.
*** $P = .003$ (χ^2 for trend), A, B, and D.
** $P = .048$ (χ^2 for trend), A, B, and D.
† $P = .048$ (t test), A vs C.
†† $P = .039$ (t test), A vs D.
††† $P = .003$ (t test), A vs B.

Similar results were obtained for β_3-AR gene polymorphism, but the differences were not significant (NGT, 5.2%; G-IGT, 6.0%; GDM, 6.3%) (Table 2, Fig. 1B).

Association analysis of IRS-1 revealed that the frequency of an FAMD+ was higher in subjects with than in those without the Arg variant (significantly so in NGT [54.0% vs 25.9%, $P = .006$] and nearing significance in GDM [73.7% vs 59.0%, $P = .057$]). In addition, no correlation was detected between prepregnancy BMI and IRS-1 gene polymorphism (Table 3).

We next evaluated the effects of both variants together on the same parameters (FAMD+ and BMI), defining 4 combinations of subjects for each group (GDM and NGT) depending on whether subjects had a variant in both genes (a), in IRS-1 alone (b), in β_3-AR alone (c), or in neither (d).

The frequency of FAMD+ showed a significant trend in 3 groups, a, b, and d (χ^2 for trend: a = 88.8%, b = 69.0%, d = 59.2%; $P = .049$) in GDM, and even more so in NGT (χ^2 for trend: a = 66.6%, b = 58.8%, d = 25.3%; $P = .0033$ (Table 4).

Moreover, we found that NGT carriers of both polymorphisms had higher prepregnancy BMI than subjects who carried the IRS-1 variant alone ($P = .003$), the β_3-AR variant alone ($P = .039$), or neither polymorphism ($P = .048$) (Table 4).

Lastly, in patients with GDM, the presence of genetic polymorphisms was not related to metabolic control or insulin therapy (Table 4).

4. Discussion

The results of our study revealed that the variant allele Arg972 of the IRS-1gene is significantly associated with gestational diabetes and impaired glucose tolerance. The frequency of IRS-1 mutation in our normal pregnant women was not significantly different from the one in healthy subjects from various ethnic groups (7.9% vs 6.8%) [26].

Both groups (GDM and NGT) with FAMD+ also had a higher frequency of this polymorphism, consistent with its role as a risk factor for type 2 diabetes mellitus. The greater prevalence of the IRS-1 polymorphism found in women with GDM parallels the known situation in type 2 diabetes mellitus, and the mechanisms of insulin resistance in the 2 conditions are probably similar. Studies regarding the possible association of IRS-1 in GDM are lacking. Catalano et al [18], in a very elegant article, showed a decrease in IRS-1 protein levels in adipose tissue of women with GDM, which may contribute to the reduced insulin suppression of lipolysis with advancing gestation.

A series of studies have shown that patients with GDM show postpartum signs of metabolic syndrome [27] with changes in lipid levels, correlated with cardiovascular risk factors [28]. These observations indicate that the Arg972 variant in the IRS-1 gene in patients with GDM may be a useful genetic marker of an increased risk of metabolic syndrome. In this context, a follow-up study of patients carrying the genetic polymorphism would be useful.

Few studies to date have examined whether the β_3-AR Arg variant could be related to the development of GDM. Festa et al [15] reported an association between the Trp64 Arg variant of the β_3-AR and mild GDM, whereas conflicting results have been reported by Alevizaki et al [17] in Greek women. More recently, Tsai et al [29] found that genotype distribution and allele frequency of β_3-AR did not differ between GDM and NGT, although subjects with GDM with the Arg64 variant had higher fasting and postload (oral glucose tolerance test) insulin levels, suggesting a possible role of β_3-AR polymorphism in insulin resistance in GDM. In our study, we found only a slight increase in this variant in patients with GDM. Furthermore, its frequency in normal pregnant women is generally comparable with the one found in nonpregnant, nondiabetic white women (7.5%-12.5%) [30].

There are no studies on IRS-1 and β_3-AR polymorphisms in women with GDM. In our study, the combined presence of both polymorphisms was found associated with a stronger positive family history of diabetes mellitus in GDM, as well as in NGT, and with a higher prepregnancy BMI in NGT. Even if the positive association is due mainly to the IRS-1 genotype, this observation would suggest that some clinical (as well as metabolic) features could be the outcome of more than one genetic factors: because of genetic interactions, gene defects having little or no effect when expressed alone may well influence the phenotype when present in combination, in addition to intra- and extrauterine environmental factors.

The latest observations suggest that some genetic polymorphisms (such as that of IRS-1) are related mainly to a family history of diabetes mellitus, which in turn characterizes GDM. The synergistic effect of genetic polymorphisms with intra- and extrauterine environmental factors may justify the greater prevalence of IRS-1 polymorphism in our women with GDM. Our finding in a little-studied GDM population could have a relevant impact in obstetric practice, where the physician may need to delve more carefully into a pregnant woman's family history of diabetes.

Our patients with GDM carrying 1 or 2 genetic polymorphisms were also all autoantibody-negative, indicating even more clearly that GDM and type 2 diabetes mellitus overlap both clinically, due to their metabolic behavior, and genetically.

To our knowledge, no data have been reported until now on the frequency of IRS-1 polymorphism in pregnant women, and in GDM in particular. On the other hand, the association of this polymorphism with type 2 diabetes mellitus and insulin resistance is well known [5,9]. Thus, the results of the present study, made in a large number of women, show that IRS-1 genetic abnormality in GDM overlap that reported in type 2 diabetes mellitus, further supporting the theory that GDM and type 2 diabetes mellitus are 2 facets of the same complex disorder [31].

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