Combined Effects of Genetic and Environmental Factors on Insulin Resistance Associated With Reduced Fetal Growth

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It has been suggested that the insulin resistance (IR) associated with reduced fetal growth results from interactions between genetic factors and an unfavorable fetal environment. In addition, the adipose tissue seems to play a key role in this association. We investigated whether polymorphisms in tumor necrosis factor (TNF)-α (G−308A), β3 adrenoreceptor (ADRB3)(G+250C), and peroxisome proliferator–activated receptor (PPAR)-γ2(Pro12Ala), key molecules of the adipose tissue, might affect the IR associated with reduced fetal growth. They were genotyped in 171 subjects who were born small for gestational age (SGA) and in 233 subjects who were born appropriate for gestational age (AGA) and underwent an oral glucose tolerance test (OGTT). The SGA group showed higher serum insulin concentrations than the AGA group at fasting (P = 0.03) and after stimulation (P = 0.0007), whereas no difference in serum glucose concentrations was observed. The frequencies of the alleles of these three polymorphisms were similar in both groups. In neither group did the polymorphisms affect glucose tolerance. In the SGA group, fasting insulin-to-glucose ratios were significantly higher in the TNF/−308A (P = 0.03), the PPAR/Ala12 (P = 0.01), and the ADRB3/+250G (P = 0.02) carriers than in the noncarriers. Results were comparable for fasting insulin concentration and insulin excursion under OGTT. No such amplification was observed in the AGA group. The effects of the PPAR/ProAla12 (P = 0.005) and the ADRB3/G+250G (P = 0.009) gene polymorphisms on IR indexes were significantly potentiated by BMI in the SGA group. In conclusion, our data exemplify the interaction between intrauterine environmental and genetic factors in the development of the IR associated with reduced fetal growth. They also point to the key role of adipose tissue in this association. *Diabetes* 51:3473–3478, 2002

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ADRB3, β3 adrenoreceptor; AGA, appropriate for gestational age; AUC, area under the curve; GLM, general linear model; IR, insulin resistance; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor; SGA, small for gestational age; TNF, tumor necrosis factor.

Reduced fetal growth is associated with an increased risk of type 2 diabetes and insulin resistance (IR) during adult life (1–6). In addition, infants who are born small for gestational age (SGA) are prone to excessive weight gain early in adult life (7–10). In a previous study, we had shown that insulin sensitivity was decreased at 20 years of age in subjects who were born SGA (6). Whereas glucose tolerance was maintained, both muscle and adipose tissue were affected by IR (5,6). Despite a strict definition of SGA status and of gestational age, the scatter of values of peripheral glucose uptake measurements in our subjects who were born SGA showed a larger interindividual variability of IR in this population than in subjects who were born appropriate for gestational age (AGA) (6).

Metabolic disorders such as the IR syndrome have multifactorial origins in which both genetic and environmental factors are thought to be involved (11). Hales et al. (1) first suggested that type 2 diabetes associated with low birth weight results form a detrimental fetal environment according to a programming process. The genetic contribution (12) and the unfavorable mother-child environment (13) have been later alternately privileged. However, it seems more likely that the increased susceptibility to IR of subjects who are born SGA results from the combination of both genetic factors and an unfavorable fetal environment. Several polymorphisms of genes involved in the development and in the metabolic function of adipose tissue have been shown to modulate the susceptibility to IR in humans (14–17). Because several lines of evidence point to a key role of adipose tissue in the IR associated with reduced fetal growth (6–10), it was tempting to hypothesize that the same polymorphisms modulate the predisposition of subjects who are born SGA to develop IR later in life.

The aim of the present study was to address this hypothesis by testing whether genetic polymorphisms of key adipose tissue molecules might influence the degree of the IR associated with reduced fetal growth. For this purpose, we investigated the effect of three polymorphisms on IR assessed during oral glucose tolerance test (OGTT) in a case-control study comparing subjects who were born SGA with subjects who were born AGA. The three polymorphisms studied were the tumor necrosis factor (TNF)-α/G−308A, the β3 adrenoreceptor (ADRB3)/

TABLE 1
Clinical characteristics at birth and at the time of the study and glucose homeostasis parameters of the SGA and AGA groups

Characteristic	SGA (n = 171)	AGA (n = 233)	P value
At birth			
Gestational age (weeks)	40 (1)	40 (1)	0.69
Birth weight (g)	2500 (270)	3408 (220)	<0.0001
Birth length (cm)	46.9 (2.0)	50.4 (0.8)	<0.0001
Ponderal index ($g \cdot cm^3 \cdot 10^{-3}$)	24.5 (3.9)	26.6 (1.4)	<0.0001
At the time of the study			
Age (years)	20.5 (2.1)	20.7 (2.2)	0.36
Sex (F/M)	91/80	129/104	0.76
Weight (kg)	62.8 (13.6)	66.3 (13.9)	0.01
Height (cm)	166.9 (9.9)	171.9 (8.7)	<0.0001
BMI (kg/m^2)	22.4 (3.9)	22.3 (4.2)	0.76
Waist/hip ratio	0.83 (0.07)	0.82 (0.07)	0.21
Glucose homeostasis parameters			
Fasting plasma glucose (mmol/l)	4.79 (0.44)	4.68 (0.39)	0.10
120-min plasma glucose (mmol/l)	5.33 (1.04)	5.22 (0.93)	0.37
Fasting serum insulin (pmol/l)	35.4 (28.8)	29.8 (13.2)	0.03
Insulin AUC ($pmol \cdot min^{-1} \cdot 10^{-3}$)	33.7 (21.8)	27.1 (13.9)	0.0007
Fasting insulin/glucose ratio	7.3 (5.6)	6.3 (2.8)	0.02

Data are expressed as means (SD). Test were performed after adjustment for gestational or current age and sex, as well as for sex, age, BMI, family history of type 2 diabetes, smoking, and oral contraception. AUC = (fasting insulin + 4 × 30 min insulin + 3 × 120 min insulin)/8.

G+250C, and the peroxisome proliferator–activated receptor (PPAR)-γ2/Pro12Ala polymorphisms.

RESEARCH DESIGN AND METHODS

Subjects. The present study was performed on a cohort of young adults who were previously gathered to investigate the metabolic complications associated with reduced fetal growth (5). Briefly, subjects were identified from a population-based registry of the metropolitan area of the city of Haguenau in France that had recorded information about pregnancies, deliveries, and perinatal events. This registry covered >80% of the deliveries that occurred in this area (18). All subjects included in this cohort were singleton babies who were born full term (≥37 weeks of gestation) between 1971 and 1977. A total of 452 subjects who were born SGA, defined as having a birth weight below the third centile of the local sex-specific distribution for gestational age, were identified from the registry. Subjects who were born AGA were selected from the same registry as the first baby with birth sizes between the 25th and 75th centiles, born immediately after a baby who was born SGA without any attempt to match for gestational age or sex. The reasons for noninclusion were equally distributed between the two groups except for the number of deaths, which were significantly higher in the SGA group (15 vs. 5%; P = 0.002). Within each group, there was no significant difference between participants and nonparticipants in terms of birth weight or gestational age (5). Among the 517 subjects initially included, a blood sample suitable for DNA extraction was obtained in 404 subjects (171 subjects born SGA and 233 subjects born AGA). Clinical characteristics at birth of the two groups are shown in Table 1. As expected, subjects who were born SGA were significantly thinner and shorter at birth than subjects who were born AGA. Informed consent was obtained for each subject, and the study was approved by the local ethics committee of the University of Paris-Saint-Louis.

Study design. All participants underwent a medical visit. Information about medical history was recorded using a standardized questionnaire. Body weight was measured with a portable scale, and height was measured with a wall-mounted stadiometer. Weight for height was assessed by BMI (kg/m^2). Waist circumference was measured at the level of the umbilicus, and hip circumference was measured at the level of the greater trochanter. Blood samples were collected after an overnight fast for measurement of plasma glucose and serum insulin concentrations. All participants had a 75-g OGTT, and plasma glucose and serum insulin concentrations were measured 30 and 120 min after the glucose load. An additional sample was obtained for DNA extraction from peripheral blood cells.

Analytical methods. Plasma glucose concentrations were measured with an enzymatic method. Serum insulin concentrations were measured using a double-antibody radioimmunoassay (ERIA Diagnostics Pasteur, Paris, France). Cross-reactivity with proinsulin and derived metabolites was <1%. Assay sensitivity was 1.2 pmol/l, and intra- and interassay coefficients of variations were 3.8 and 8% at 48 pmol/l and 2.4 and 4.8% at 300 pmol/l.

Genotyping. DNA was extracted from peripheral blood cells. Genotyping of the three polymorphisms was performed using allele-specific oligonucleotides (19). All information for genotyping (PCR primers, probes, conditions of amplification, and hybridization) can be found at genecanvas.idf.inserm.fr.

Statistical analyses. All analyses were performed using the SAS statistical software (SAS Institute, Meylan, France). The area under the curve (AUC) of insulin was calculated by the trapezoidal rule. A log transformation was applied to fasting insulin levels, insulin AUC, and insulin-to-glucose ratio to reduce the skewness of the distributions. Differences of biological and anthropometric variables between AGA and SGA subjects were tested by general linear model (GLM). Analyses were carried out on values adjusted for sex and age for anthropometric parameters and sex, age, BMI, smoking, oral contraception, and family history of type 2 diabetes for metabolic parameters. Allele frequencies were estimated by gene counting, and departure from Hardy-Weinberg equilibrium was tested using a χ^2 test with 1 degree of freedom. Allele frequencies were compared between AGA and SGA groups by a χ^2 test. Association of polymorphisms with the measured parameters was tested using a GLM procedure. The analyses were performed for each single anthropometric and metabolic parameters after adjustment for confounding factors (sex and age for anthropometric parameters and sex, age, BMI, smoking, oral contraception, and family history of type 2 diabetes for metabolic parameters). The homogeneity of genetic effects between AGA and SGA subjects was tested by introducing a genotype*group interaction term in each model. The effect of gene polymorphisms on these parameters was subsequently analyzed within each group using a GLM procedure after adjustment to the usual confounding factors. For the purpose of statistical analysis, individuals with at least one rare allele for each polymorphism were compared with subjects homozygous for the most common allele. $P < 0.05$ was considered to be significant.

RESULTS

The mean age of the two groups at examination was 20.5 years. Sex distribution did not differ significantly between the two groups. The proportion of smokers did not differ statistically between the two groups (39% vs. 36%; P = 0.53), neither did the proportion of women who were taking oral contraception (46% vs. 57%; P = 0.16). The percentage of family history of type 2 diabetes tended to be higher in the SGA group, although not significantly (6% vs. 2%; P = 0.06). As previously reported (5), current body size assessed by BMI and blood glucose level were comparable between the two groups, but the SGA group demonstrated significantly higher IR indexes (Table 1).

TABLE 2
Effect of the genetic polymorphisms on BMI and IR indices in the SGA and AGA groups

	SGA (n = 171)			AGA (n = 233)			P value*
TNF-α/G-308A	GG (n = 125)	A+ (n = 46)	P value†	GG (n = 172)	A+ (n = 61)	P value†	
BMI (kg/m^2)	22.1 (5.6)	22.0 (4.2)	0.20	22.0 (3.4)	23.2 (5.0)	0.07	0.74
Fasting insulin (pmol/l)	32.4 (26.4)	43.2 (31.2)	0.008	29.4 (13.2)	31.2 (13.8)	0.77	0.03
Insulin AUC (pmol · min^{-1} · 10^{-3})	31.8 (18.6)	40.2 (28.8)	0.09	26.4 (13.8)	27.6 (13.8)	0.50	0.44
Insulin/glucose ratio	6.1 (4.5)	8.2 (5.7)	0.03	5.8 (2.6)	5.9 (2.6)	0.67	0.01
ADRB3/G+250C	GG (n = 152)	C+ (n = 19)	P value†	GG (n = 207)	C+ (n = 26)	P value†	
BMI (kg/m^2)	22.4 (4.0)	22.4 (3.1)	0.99	22.4 (4.3)	21.4 (2.6)	0.34	0.42
Fasting insulin (pmol/l)	33.6 (24.0)	48.6 (50.4)	0.03	30.0 (13.2)	27.6 (12.6)	0.40	0.01
Insulin AUC (pmol · min^{-1} · 10^{-3})	31.8 (20.4)	45.6 (28.2)	0.07	27.6 (13.8)	22.2 (12.0)	0.05	0.005
Insulin/glucose ratio	6.4 (4.0)	9.2 (9.2)	0.02	5.9 (2.6)	5.6 (2.8)	0.58	0.01
PPAR-γ/Pro12Ala	Pro/Pro (n = 133)	Ala+ (n = 38)	P value†	Pro/Pro (n = 179)	Ala+ (n = 54)	P value†	
BMI (kg/m^2)	22.5 (4.0)	22.1 (3.2)	0.54	22.4 (4.2)	22.4 (3.7)	0.99	0.65
Fasting insulin (pmol/l)	33.6 (28.2)	39.0 (27.0)	0.12	30.0 (13.8)	28.8 (11.4)	0.77	0.03
Insulin AUC (pmol · min^{-1} · 10^{-3})	31.8 (21.6)	38.4 (22.8)	0.05	27.0 (13.2)	26.4 (15.6)	0.54	0.05
Insulin/glucose ratio	6.4 (4.9)	7.6 (5.1)	0.01	5.9 (2.8)	5.7 (2.2)	0.48	0.04

Data are expressed as means (SD). Tests were performed after adjustment for sex and age for BMI, and for sex, age, BMI, family history of type 2 diabetes, smoking, and oral contraception for IR indices. *For the tests of the statistical interaction between genetic effect (gene polymorphism) and size at birth (SGA versus AGA) for each parameter after adjustment for confounding factors. †Within each group for the tests of the statistical comparison of each parameter between the carriers and the noncarriers of the genetic polymorphisms.

Genotype distributions did not deviate from Hardy-Weinberg proportions for any of the polymorphisms investigated. The frequencies of the TNF/−308A, the ADRB3/+250C, and the PPAR-γ2/Ala12 alleles in the SGA and AGA groups were 0.27 vs. 0.26 (P = 0.74), 0.11 vs. 0.11 (P > 0.99), and 0.22 vs. 0.23 (P = 0.90), respectively.

The association of polymorphisms with glucose tolerance and IR indexes was investigated, and homogeneity of association between the two groups was tested. None of the polymorphisms had a significant effect on plasma glucose values, either at fasting or 2 h after the glucose load, either in the SGA or in the AGA group (data not shown).

By contrast, association of polymorphisms with IR parameters differed between the SGA and the AGA groups (Table 2). Whereas no association was detected in the AGA group for the TNF/G−308A polymorphism, carriers of the TNF/−308A allele had significantly higher fasting insulin levels and insulin-to-glucose ratios than noncarriers in the SGA group, and the same trend, although nonsignificant, was observed for the insulin AUC. A similar pattern was observed for carriers of the PPAR-γ2/Ala12 allele compared with noncarriers of these alleles within each group (Table 2). In the SGA group, fasting insulin and insulin-to-glucose ratios were significantly higher in the ADRB3/+250C carriers in comparison with noncarriers, and a similar trend was observed for the AUC. By contrast, in the AGA group, the AUC was significantly reduced in the carriers of the ADRB3/+250C in comparison with the noncarriers, although no significant effects were observed on the fasting insulin and insulin-to-glucose ratios (Table 2).

For nearly all parameters, there was a significant interaction between the genetic effect and size at birth (SGA versus AGA; Table 2). The differential effects of the ADRB3/+250C and the PPAR-γ2/Ala12 alleles are illustrated in Fig. 1, showing the insulin curves during OGTT according to genotypes in the two groups.

The TNF/−308A allele was associated with a significant increase in BMI (P = 0.04). Although this effect was more apparent in the AGA group, no significant heterogeneity was observed between the two groups (Table 2). Neither the ADRB3/G+250C nor the PPAR-γ2/Pro12Ala polymorphism had a significant effect on current body size (Table 2).

Given the strong influence of BMI on IR, we further examined whether genotype would interact with BMI on IR parameters with respect to fetal growth. In the SGA group, for the three polymorphisms, the association between the insulin-to-glucose ratio was stronger in carriers of the rare allele than in subjects homozygous for the common allele, as attested by a steeper regression slope (Fig. 2). For example, the correlation coefficient between the insulin-to-glucose ratio and BMI was 0.35 in subjects homozygous for the PPAR-γ2/Pro12 allele, whereas it reached 0.63 in carriers of the Ala12 allele (P = 0.005 for interaction). A similar genotype*BMI interaction was observed on fasting insulin levels for the ADRB3/G+250C (P = 0.009). No interaction with BMI was observed in the AGA group for any of the three polymorphisms.

DISCUSSION

The present study illustrates the genetic contribution to IR associated with reduced fetal growth by showing that

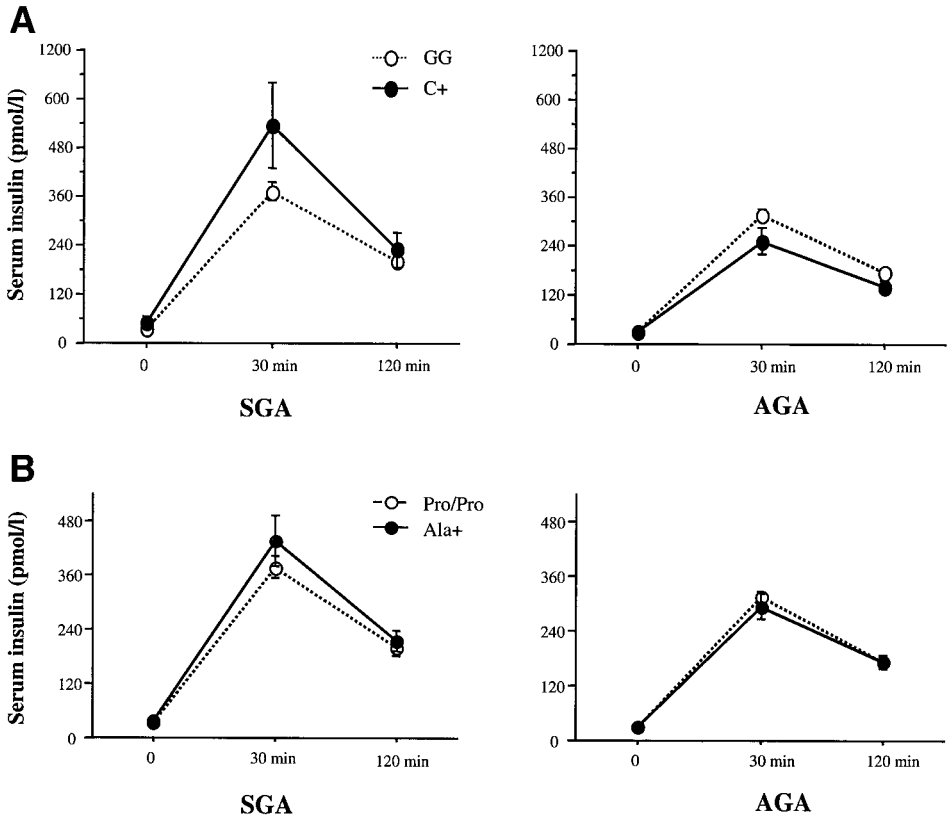

FIG. 1. Mean (SE) serum insulin levels during OGTT according to the ADRB3/G+250C polymorphism (*A*) and the PPAR-γ2/Pro12Ala polymorphism (*B*) in the SGA and AGA groups.

genetic polymorphisms modulate IR parameters in subjects who were born SGA. These findings support the hypothesis that IR in adulthood might be the consequence of interactions between detrimental environmental factors during fetal life and genetic susceptibility.

There is now accumulating evidence that most of the susceptibility genes to multifactorial diseases do not have a primary causative role in predisposition to disease but rather act as response modifiers to triggering factors such as diet, physical exercise, and pathological condition. It is interesting that genetic polymorphisms investigated in the present study seem to modulate the predisposition to IR associated with reduced fetal growth. The association between reduced fetal growth and IR has been clearly demonstrated in numerous study populations and using various methods (3,4,20,21). However, subjects who were born SGA show a variable susceptibility to IR, and the wide range of peripheral glucose uptake values observed under euglycemic-hyperinsulinemic clamps strongly reflects this variability (6). Family history of type 2 diabetes is known to potentiate the risk for IR associated with reduced fetal growth, thereby suggesting a genetic contribution (22,23). The present data provide an additional example of this genetic contribution that can explain at least in part the variable susceptibility to IR observed in this population.

Among the risk factors that potentiate the IR associated with reduced fetal growth, obesity is known to play a key role (1,3,6,12). Indeed, we demonstrate here that increased BMI amplifies the genetic effects. This observation clearly demonstrates the role of adiposity in the magnification of genetic contribution in the IR associated with reduced fetal growth. Taken together, our data suggest that IR in this particular clinical situation, as in other clinical conditions, is multifactorial, resulting from both genetic and environmental factors. Reduced fetal growth should therefore be considered as an additional risk factor for metabolic and cardiovascular diseases as proposed by Valdez et al. (13).

We did not observe any significant difference in the frequency of the TNF/308A allele between subjects who were born SGA and AGA. This observation is in keeping with a previous study that failed to find a significant association between birth weight and this genetic polymorphism in young, healthy adults (24). However, these authors did not study the effect of this polymorphism according to size at birth on IR parameters (25). According to previous studies that did not observe any association between the TNF/G−308A gene polymorphism and IR indexes in healthy, lean adults (25–27), no significant effect of this polymorphism was observed on IR parameters in subjects who were born AGA in the present study. In contrast, subjects who were born SGA and carried the TNF/308A allele seemed to be less insulin sensitive than the noncarriers. It should be noted that this polymorphism does not seem to modulate the IR susceptibility in subjects with other risk factors, such as family history of type 2 diabetes or obesity (28–30). This discrepancy suggests that in such multifactorial diseases, the effect of the susceptibility genes could vary from one risk factor to another. Data on PPAR-γ2/Pro12Ala gene polymorphisms are in keeping with this hypothesis. The PPAR-γ2/Ala12

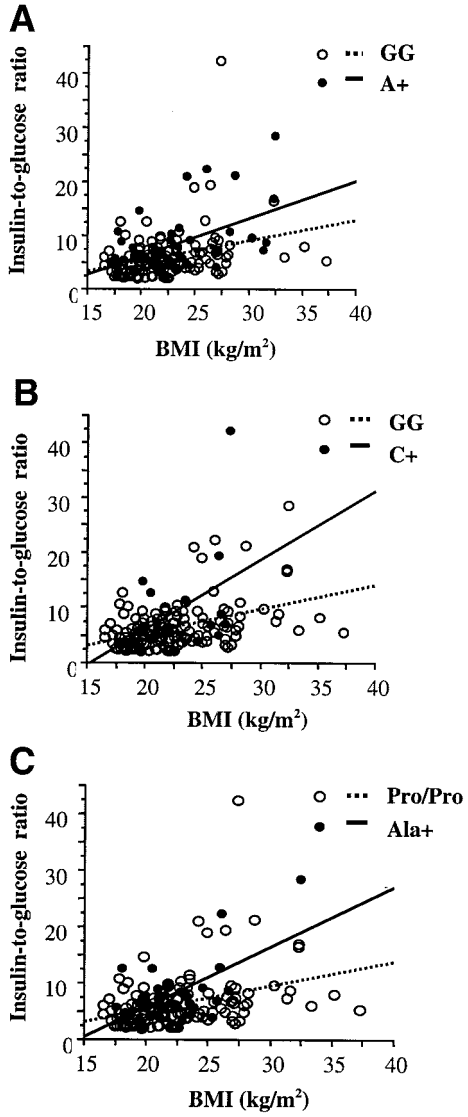

FIG. 2. Effect of BMI on the insulin-to-glucose ratio in the SGA group according to the three polymorphisms. The effect of BMI on the insulin-to-glucose ration was higher in the TNF-α/−308A carriers than in the subjects homozygous for the common allele (r = 0.44 [P < 0.0001] vs. r = 0.42 [P = 0.05]; interaction P = 0.07). A comparable but significant amplification of the effect of BMI on the insulin-to-glucose ratio was also observed in carriers of the rare alleles of the ADRB3/G+250C gene polymorphism (C+ r = 0.52 [P = 0.0002] vs. G/G r = 0.31 [P = 0.0004]; interaction P = 0.009; *B*) and of the PPAR-γ2/Pro12Ala gene polymorphism (Ala+ r = 0.63 [P < 0.0001] vs. Pro/Pro r = 0.35 [P < 0.0001]; interaction P = 0.005; *C*).

allele has been shown to be associated with an increased insulin sensitivity in various clinical situations, such as type 2 diabetes (subjects and their offspring) or obesity (17,31–33). It is surprising that when carried by subjects who were born SGA, the PPAR-γ2/Ala12 allele had an opposite effect, increasing significantly the insulin excursion under OGTT and the fasting insulin-to-glucose ratio. A comparable interaction was reported recently between this polymorphism and birth weight as a quantitative trait (34). The ADRB3/G+250C gene polymorphism is known to be in complete association with the ADRB3/Trp64Arg gene polymorphism (35) that has been shown to favor IR and type 2 diabetes when associated with other genetic polymorphisms or risk factors such as BMI or age (15,36–38). A similar effect on insulin sensitivity was observed in subjects who were born SGA and carried the ADRB3/+250C allele. It should be noted that although there was no significant effect on fasting insulin and insulin-to-glucose ratio, the ADRB3/+250C allele was associated with a significantly decreased insulin excursion under OGTT in the AGA group. Taken together, these data suggest that risk factors for IR can amplify the spontaneous effect of genetic polymorphisms but can also change the effect itself. They also point to the complexity of the interactions between genetic and environmental factors in such diseases. It would be particularly helpful to understand these discrepancies on a molecular basis between one risk factor and another to better understand the pathophysiological mechanisms of IR.

Regarding the reduced fetal growth situation, we previously gathered strong evidence of an active contribution of adipose tissue to the IR (6,7,10). In subjects who were born SGA, IR is strongly associated with a decreased antilipolytic action of insulin (6). The PPAR-γ2/Pro12Ala gene polymorphism has been shown to modulate the antilipolytic insulin sensitivity (33). In addition, the ADRB3 plays a key role in the lipolytic action of catecholamines (14). Therefore, our data strengthen the hypothesis of the role of adipose tissue in this association and underline the putative involvement of lipolysis regulation in the IR associated with reduced fetal growth. Indeed, the functional consequences of these genetic polymorphisms on metabolic functions of the adipose tissue in subjects who were born SGA remain to be clarified to better understand their intrinsic contribution in the IR observed in these subjects.

We propose that such genetic polymorphisms that are able to alter lipolysis regulation might interact with reduced fetal growth to promote IR later in life. Reduced fetal growth, which often results from an unfavorable mother-child environment, is corrected in a large majority of cases by a postnatal catch-up growth involving adiposity. It can be speculated, therefore, that the effect of these genetic polymorphisms conferring an individual susceptibility to a dysregulated lipolysis might be sharply amplified by the particular adipose tissue development observed in subjects who were born SGA.

In conclusion, our data provide a strong example of the involvement of genetic factors in the IR associated with reduced fetal growth and strengthen the hypothesis that this association could be the consequence of interactions between detrimental environmental factors during fetal life and a genetic susceptibility. Moreover, they support the hypothesis of an active contribution of the adipose tissue in the pathophysiology of the IR observed in this clinical situation. Our results also point to the complexity of the interaction between genetic polymorphisms and environmental factors that could modulate either the strength or the nature of their effects.

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