The Gly972Arg Polymorphism in Insulin Receptor Substrate-1 Is Associated With Decreased Birth Weight in a Population-Based Sample of Brazilian Newborns

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OBJECTIVE — We studied the association between the Gly972Arg polymorphism in insulin receptor substrate-1 (IRS-1) and birth weight in a population-based sample of Brazilian newborns.

RESEARCH DESIGN AND METHODS — We studied 194 newborn children with adequate gestational age to identify the association between the Gly972Arg polymorphism and birth weight using PCR–restriction fragment length polymorphism analysis.

RESULTS — The data showed that the birth weight was lower in the newborns with the Gly972Arg polymorphism in IRS-1 compared with control subjects (3,141 ± 31.8 vs. 3,373 ± 80.3 g, P < 0.008). The results also showed that the frequency of this polymorphism was increased in newborns with a birth weight <3,000 g (P = 0.041).

CONCLUSIONS — These results suggest that the genotype Gly972Arg may influence birth weight, reinforcing the hypothesis that genetically determined insulin resistance and/or reduced insulin secretion can result in impaired insulin-mediated growth in the fetus.

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Low birth weight is associated with insulin resistance and type 2 diabetes (1,2). However, it is not known whether this association is explained by unfavorable intrauterine environment or by specific susceptibility genotypes predisposing for reduced fetal growth, insulin resistance, and type 2 diabetes. The same genetic factors that caused impaired insulin secretion and/or insulin resistance may alter both intrauterine growth and glucose tolerance in adulthood, thereby providing a link between them (3). Recently, it was observed that mutations in the glucokinase gene associated with the type 2 form of maturity-onset diabetes of the young result in a reduced birth weight, most likely caused by changes in fetal insulin secretion, thus suggesting that genetic factors that modify insulin secretion might be involved in intrauterine growth retardation (4).

Low birth weight can also be associated with insulin resistance resulting from genetic defects that influence insulin action during embryogenesis (5). Insulin receptor substrate-1 (IRS-1) is the major substrate of insulin receptor tyrosine kinase. Of 11 known polymorphisms in the IRS-1 gene, the most common variant, a Gly→Arg substitution at codon 972 (Arg972–IRS-1), is more prevalent among subjects who have features of insulin resistance syndrome, associated or not with type 2 diabetes (6–11). Moreover, in vitro studies show that this variant may impair insulin signaling (12,13). The purpose of this study was to analyze whether this polymorphism is associated with decreased birth weight in a sample of Brazilian newborns.

RESEARCH DESIGN AND METHODS — In the present study, we analyzed whether the IRS-1 Gly972Arg genotype is associated with decreased birth weight in 194 newborn children with adequate gestational age, whose mothers had no disorders (gestational diabetes and hypertension were also excluded) during pregnancy and were not cigarette smokers. The mothers were screened for gestational diabetes between the gestational ages of 24 and 28 weeks by using fasting plasma glucose levels with a threshold of 85 mg/dl (14). Mothers with fasting plasma glucose levels >85 mg/dl were submitted to a standardized 2-h 75-g oral glucose tolerance test (15). The study was retrospective, and the birth weight, length, and head circumference used were from hospital records. The genomic DNA was extracted from umbilical cord blood leukocytes by standard techniques using phenol/chloroform. The Gly972Arg genotype was obtained by polymerase chain reaction using the primers forward 5'−CTT CTG TCA GGT GTC CAT CC−3' and reverse 3'−CGA TGC ACC TGT GGA GCG GT−5' (7). The product of amplification (263 bp) was subsequently digested with the restriction enzyme MvaI, and the fragments were run in 3.5% agarose gel stained with ethidium bromide. The study was approved by the Ethical Committee of the University Campinas Hospital and was performed in accordance with the principles of the Declaration of
Helsinki. We obtained informed consent from parents.

Differences in categorical variables were tested with χ² test, and differences in continuous variables between groups were tested by nonpaired Mann-Whitney U test. The SAS system for Windows (version 6.1) was used for statistical analysis. P < 0.05 was considered significant.

RESULTS — Genotype frequencies were Gly/Gly 86.6% (168 of 194), Gly/Arg 12.9% (25 of 194), and Arg/Arg 0.5% (1 of 194), which are in Hardy-Weinberg equilibrium. These are similar to genotype frequencies previously reported in normal control populations from the U.K. (10,16), Denmark (17), Finland (9), or the U.S. (18).

The data showed that the birth weight was lower in the newborn with the IRS-1 Gly972Arg polymorphism compared with control subjects (arginine: 3,141 ± 80.3 g vs. glycine: 3,373 ± 31.8 g, P < 0.008) (Table 1). The gestational ages were similar in both groups (mothers with newborns Gly/Gly: 40 ± 0.1 weeks vs. mothers with newborns Gly/Arg: 39.4 ± 0.3 weeks, NS) (Table 1), showing no influence of gestational age on the difference in birth weight between the groups. The head circumference (arginine: 33.5 ± 0.3 cm vs. glycine: 34.4 ± 0.1 cm, P = 0.005) (Table 1) and the birth length (arginine: 48.9 ± 0.4 cm vs. glycine: 49.7 ± 0.1 cm, P = 0.04) (Table 1) were lower in the newborn with the IRS-1 polymorphism compared with control subjects.

The results also showed that the frequency of this polymorphism was increased in newborns with a birth weight <3,000 g (P = 0.041, χ² test for association) (Table 2). When we adjusted the birth weight for the gestational age (percentile of intrauterine growth for weight) using standard charts from Lubchenco et al. (19), we observed that the newborns with the Gly972Arg polymorphism had a significantly lower percentile than the control group (P59 ± 2 vs. p48 ± 5, P = 0.034).

We also performed genotyping in 23 mothers of newborns with the polymorphism and detected the polymorphism in 11. The birth weight of the newborns from mothers with the polymorphism was similar to that of mothers without the polymorphism.

CONCLUSIONS — Analysis of the gene encoding IRS-1 has revealed several mutations resulting in amino acid substitutions. The most prevalent amino acid substitution in IRS-1 is a glycine-to-arginine change at codon 972. The occurrence of this amino acid polymorphism has been examined in type 2 diabetic patients and control subjects from different ethnic groups (7–11,20). The Gly972Arg polymorphism in IRS-1 was found to have a higher prevalence in type 2 diabetic subjects than in control subjects (7–11), suggesting that mutation of the IRS-1 gene may act as a risk factor predisposing to type 2 diabetes. Expression of the Gly972Arg polymorphism in 32D cells is associated with a significant impairment of insulin signaling (12,13). Recently, it was demonstrated that the IRS-1 Gly972Arg variant in pancreatic β-cells results in a reduction in insulin secretion stimulated by glucose (21). In subjects with this polymorphism, there is also a decrease in insulin secretion, whether induced by glucose or arginine (22). Thus, in humans, the Gly972Arg polymorphism may contribute to both the peripheral tissue insulin resistance and impaired insulin secretion.

IRS-1 is considered the major insulin receptor substrate in most insulin-sensitive tissues. IRS-1 is required for the insulin-mediated cellular effects, including activation of glycogen synthesis and glucose transport, and for cellular events leading to mitogenesis (23,24). The importance of IRS-1 in vivo in insulin signaling has been most directly demonstrated in mice made deficient in IRS-1 using targeted gene knockout by homologous recombination (23). IRS-1-deficient mice have impaired glucose tolerance, a decrease in insulin- and IGF-1-stimulated glucose uptake, and a 50% reduction in intrauterine growth. The results of the present study, showing that the birth weight, birth length, and head circumference of the newborns with the Gly972Arg polymorphism are lower compared with control subjects, suggest that reduced IRS-1 function may alter fetal growth. Because IRS-1 is also a substrate of IGF-1 receptor, a reduction in IGF-1/insulin signaling and also in insulin secretion may contribute to the reduced birth weight observed in newborns with the Gly972Arg polymorphism. Recently, studies by Rasmussen et al. (3) in young Danish Caucasians and Mason et al. (16) in English children found no significant differences in birth weight between the Arg 972 variant of IRS-1 gene and the control group. It is important to note that although the growth of a fetus is obviously influenced by its genes, the relative

**Table 1—Sizes at birth of randomly newborn healthy Brazilians classified in accordance with the genotype of polymorphism of the IRS-1 gene**

<table>
<thead>
<tr>
<th>IRS-1 genotype</th>
<th>Gly/Gly</th>
<th>Gly/Arg or Arg/Arg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>168</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>82/86</td>
<td>10/16</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3.373 ± 31.8</td>
<td>3.141 ± 80.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Birth weight centile</td>
<td>59 ± 2</td>
<td>48 ± 5</td>
<td>0.034</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>49.7 ± 0.1</td>
<td>48.9 ± 0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>34.4 ± 0.1</td>
<td>33.5 ± 0.3</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Data are means ± SEM. The Mann-Whitney U test was used to determine the significance of any difference between the genotypes at P = 0.05.

**Table 2—Distribution frequency of the Gly972Arg polymorphism of the IRS-1 gene in different birth weight categories**

<table>
<thead>
<tr>
<th>Birth weight category (g)</th>
<th>2,500–3,000</th>
<th>3,000–3,500</th>
<th>3,500–4,000</th>
<th>&gt;4,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly/Gly</td>
<td>73.7 (28/38)</td>
<td>87.1 (81/93)</td>
<td>94.1 (48/51)</td>
<td>91.7 (11/12)</td>
</tr>
<tr>
<td>Gly/Arg or Arg/Arg</td>
<td>26.3 (10/38)*</td>
<td>12.9 (12/93)</td>
<td>5.9 (3/51)</td>
<td>8.3 (1/12)</td>
</tr>
</tbody>
</table>

Data are % (n). *P = 0.04, χ² test.
contributions of genes to the variation in weight at birth are unknown, and several non-genetic maternal and fetal factors also contribute to the determination of size and weight at birth. In this regard, we excluded newborns from mothers with diabetes, hypertension, or who smoked during pregnancy, and these variables were not mentioned in these recent studies. In addition, genetic factors from the mothers that might have substantial effects on birth weight of their children were not considered in the two previous studies (3,16). Hattersley and Tooke (4) showed that a defect in the sensing of glucose by the pancreas, caused by a heterozygous mutation in the glucokinase gene, resulted in a mean reduction of birth weight of 533 g. However, maternal hyperglycemia due to a glucokinase mutation resulted in a mean increase in birth weight of 601 g. This study demonstrates that for glucokinase mutations, both maternal and fetal genotypes interact to influence birth weight. In our study, the mother’s genotypes did not influence the birth weight of the newborn. Because we excluded mothers with diabetes or gestational diabetes, we might have excluded possible mothers with this polymorphism and hyperglycemia during the pregnancy that could have influenced the birth weight of their newborns. In conclusion, our results suggest that the genotype Gly972Arg may influence the birth weight, reinforcing the hypothesis that genetically determined insulin resistance and/or reduced insulin secretion can result in impaired insulin-mediated growth in the fetus.

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References