Type 2 deiodinase Thr92Ala polymorphism is associated with disrupted placental activity but not with dysglycemia or adverse gestational outcomes: a genetic association study

José Miguel Dora, M.D., Ph.D., Simone M. Wajner, M.D., Ph.D., Juliano Dalla Costa, Rafaela Vanin Pinto Ribeiro, Leonardo Barbosa Leiria, Ph.D., Mariah G. Lopes, M.D., Aline Vitali da Silva, M.D., Daisy Crispim, Ph.D., and Ana Luiza Maia, M.D., Ph.D.
Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Objective: To study whether the D2 Thr92Ala polymorphism—a genetic marker that is associated with reduced thyroid type 2 deiodinase (D2) activity, increased insulin resistance, and risk for type 2 diabetes—is associated with disrupted placental D2 activity and with glycemic control and gestational outcomes.

Design: Cross-sectional study.

Setting: Tertiary hospital in Brazil.

Patient(s): Consecutive singleton-pregnancy patients, 18–45 years old.

Intervention(s): Clinical examination and genotyping of the D2 Thr92Ala polymorphism, with placental samples collected and assayed for D2 mRNA and activity.

Main Outcome Measure(s): Glucose homeostasis and gestational outcomes.

Result(s): A total of 294 patients were included in the study. The clinical and laboratory characteristics were similar among the D2 genotypes. No differences were observed in D2 placental mRNA levels, but D2 activity was decreased in patients with the Ala92Ala genotype (0.35 ± 0.15 vs. 1.96 ± 1.02 fmol/mg/min.). Newborn serum thyroid-stimulating hormone levels (TSHneo) did not differ according to maternal D2 Thr92Ala genotype. Also, maternal glucose control, insulin resistance evaluated by the homeostasis model assessment (HOMA-IR), and gestational outcomes did not differ across D2 genotypes.

Conclusion(s): The D2 Ala92Ala genotype is associated with reduced placental D2 activity but is not associated with dysglycemia, increased insulin resistance, or worse gestational outcomes. (Fertil Steril® 2014;101:833–9. ©2014 by American Society for Reproductive Medicine.)

Key Words: Glucose homeostasis, insulin resistance, iodothyronine deiodinase type II, pregnancy, Thr92Ala polymorphism

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Reprint requests: Jose Miguel Dora, M.D., Ph.D., Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Ramiro Barcelos 2350, 90035–003, Porto Alegre, RS, Brazil (E-mail: jmdora@gmail.com).

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of T3 derives from peripheral conversion of T4 to T3 \((1)\).

As thyroid hormone demand varies between different organs, the regulation of T3 production in peripheral tissues is under local control through activity of the deiodinases, a group of enzymes that activate or inactivate thyroid hormones. The type 1 (D1) and type 2 deiodinases (D2) catalyze activation of T4 to T3, whereas the type 3 deiodinase (D3) inactivates T4 and T3, to reverse T3 \(\text{rT3}\) and diiodothyronine \(\text{T2}\), respectively. D2 plays a critical role in maintenance of intracellular T3 levels \((2)\). Because of its location in the endoplasmic reticulum, the T3 produced by D2 is especially effective in entering the nucleus and binding to thyroid hormone receptors \((1, 3)\).

Polymorphisms in the deiodinase genes might interfere in the expression of these enzymes, potentially altering the thyroid hormone metabolism \((1, 4–7)\). Notably, a single nucleotide polymorphism in D2, in which a threonine (Thr) change to alanine (Ala) at codon 92 \(\text{D2 Thr92Ala}\), is associated with decreased enzyme activity and greater insulin resistance in nondiabetic and in type 2 diabetes patients \((4, 8, 9)\). In skeletal muscle, intracellular T3 is known to up-regulate the expression of glucose transporter type 4 \(\text{GLUT4}\) and consequently to increase glucose uptake \((10)\). Thus, it is hypothesized that the D2 Ala92Ala genotype provides a lower level of intracellular D2-generated T3 in skeletal muscle, which results in a state of relative intracellular hypothyroidism, decreasing the expression of genes involved in energy use (such as GLUT4) and consequently increasing insulin resistance. Indeed, we have recently demonstrated that homozygosity for the Ala allele of the \(\text{D2 Thr92Ala}\) polymorphism is associated with an increased risk for type 2 diabetes \((11)\). Interestingly, the association of reduced D2 activity with insulin resistance has been recently replicated in an animal model with the disrupted \(\text{Dio2}\) gene \((\text{D2KO})\), which displayed increased fasting glucose and insulin resistance \((12)\). Of note, the \(\text{D2 Thr92Ala}\) polymorphism has also been associated with other conditions \((13–18)\). Intriguingly, most of these associations are independent of serum thyroid hormone levels, which highlights the importance of local regulation of thyroid hormones in peripheral tissues.

Pregnancy is characterized by a series of hormonal and metabolic changes that involve most maternal endocrine systems, marked by a state of increased insulin resistance resulting from augmented secretion of insulin counterregulatory hormones in the third trimester. With respect to maternal thyroid hormone metabolism, an increase in serum thyroxine-binding globulin \(\text{TBG}\) and increased production and turnover of T4 are observed \((19)\). It is noteworthy that, during this period, thyroid hormone transfer to the fetus is tightly regulated by the expression of D2 and D3, enzymes that are highly expressed in the placenta \((19)\).

As maternal physiology adaptation during pregnancy is marked by increased insulin resistance and enhanced thyroid hormone output, it is expected that any change in placental deiodinase activity could impact the fetal-maternal hormonal homeostasis. We evaluated whether the D2 Ala92Ala genotype is associated with disrupted placental enzyme activity and investigated its potential effects on maternal glucose homeostasis and gestational outcomes.
the Human TaqMan Genotyping Assay 40x (Applied Biosystems) were used for genotyping our samples. One allelic probe was labeled with VIC dye and the other was labeled with FAM dye. The reactions were conducted in a 96-well plate, in a total 5 μL reaction volume using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1x (Applied Biosystems), and Custom TaqMan Genotyping Assay 1x. The plates were then positioned in a real-time polymerase chain reaction (PCR) thermal cycler (7500 Fast Real PCR System; Applied Biosystems) and heated for 10 minutes at 95°C followed by 50 cycles of 95°C for 15 seconds and 63°C for 1 minute. Fluorescence data files from each plate were analyzed using automated allele-calling software (SDS 2.1; Applied Biosystems).

The patients were classified in groups of Ala/Ala, Thr/Ala, or Thr/Thr genotypes. All amplification reactions were performed twice. The genotyping success was more than 95%, with a calculated error rate based on PCR duplicates of 0.

RNA Isolation
Total RNA was isolated from 50–100 mg placental tissues using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The purity and integrity of total RNA were assessed by ultraviolet spectrophotometer (GeneQuant). Total RNA was isolated from 50–100 mg placental tissues using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The purity and integrity of total RNA were assessed by ultraviolet spectrophotometer (GeneQuant II; Amersham Biosciences) and agarose gel electrophoresis.

Real-time PCR
Total RNA was used to synthesize cDNA (SuperScript First-Strand Synthesis System for RT-PCR; Invitrogen). The generated cDNA was used in a real-time PCR with a SYBR Green PCR Master Mix (Applied Biosystems) in a 7500 Fast Real PCR System (Applied Biosystems). Standard curves representing 5-point serial dilution of cDNA were analyzed and used as calibrators of the relative quantification of product generated in the exponential phase of the amplification curve. The \( \text{r}^2 \) was greater than 0.99, and the amplification efficiency varied between 80% and 100%. The data generated by the ABI Prism 7500 system SDS software (Applied Biosystems) were then transferred to an Excel spreadsheet (Microsoft), and the experimental values corrected by that of the cyclophilin A standard. The following oligonucleotides were used: for human D2, 5’-ACTTCCCTGCTGGTCTACATGTG-3’ and 5’-CTTCTCCGTTGCTGGTTCTTCTC-3’; for human D3 5’-TCCAGAGCCAGCACATCT-3’ and 5’-ACGTGGGCTGGTACTTAGTG-3’; cyclophilin A (internal control), 5’-GTCACCCCCACCGTGTTTCTC-3’ and 5’-ACTTGCCACCCAGTCCATATG-3’.

Type 2 Deiodinase Activity Assays
Deiodinase assays were performed as described previously elsewhere (21). In brief, placental tissue samples were individually homogenized on ice in PE buffer (0.1 M potassium phosphate and 1 mM EDTA) containing 10 mM dithiothreitol (DTT) and 0.25 M sucrose (pH 6.9). The protein concentration was determined using the Bradford assay using BSA as a standard. The D2 assays were performed using 100–300 μg tissue protein and 1 nM unlabeled \( \text{T}_4 \) in a total volume of 300 μL PE buffer containing 20 mM DTT, 1 mM propylthiouracil (PTU), and approximately 100.000 cpm \([^{125}\text{I}]\text{T}_4\) (Amersham Biosciences). The apparent \( K_m \) and \( V_{\text{max}} \) for D2 enzyme were determined using different amounts of unlabeled \( \text{T}_4 \) (0.25–6 nM). The \( V_{\text{max}} \) for D2 in placenta tissue is 18.41 fmol/min/mg protein and \( K_m \) \( \text{T}_4 \) 2.23 nM \( \text{T}_4 \). Based on these results, all measurements of D2 activity were performed using 4 nM of \( \text{T}_4 \) to correct for the specific activity reduction. The kinetic constants were calculated using double reciprocal plots. Incubations were performed at 37°C for 120 minutes. The reaction was terminated by adding 200 μL of horse serum and 100 μL of 50% trichloroacetic acid (TCA). We have previously demonstrated by high-performance liquid chromatography that the net iodide release in this system is specific and equivalent to \( \text{T}_3 \) production, the only exception being skeletal muscle (1, 21, 22). The results are presented as the mean values derived from at least two independent experiments.

Type 3 Deiodinase Activity Assays
D3 activity in cell sonicates was determined using paper chromatography as previously described elsewhere (detailed protocol kindly provided by Dr. Valerie Galton) (23–25). Cells were harvested and sonicated with 10 mM Tris–HCl, 0.25 sucrose buffer (pH 7.5). Tissue homogenates were incubated for an hour with 200,000 cpm \([^{125}\text{I}]\text{T}_4\) labeled \( \text{T}_3 \), 2 nM \( \text{T}_3 \), and 20 mM DTT. The reaction was stopped by adding 200 μL ethanol 95%, 50 μL NaOH 0.04 N, and 5 mg PTU. Deiodination was determined based on the amount of \([^{125}\text{I}]-3,3',5'-\text{T}_3\) produced after separation of reaction products by paper chromatography. The results are presented as the mean values derived from at least two independent experiments.

Statistical Analyses
Results are expressed as frequencies, mean ± standard deviation (SD), or median and percentile 25–75 (P25–75). To examine the main effect of the D2 Thr92Ala variant, the three genotypes (Thr/Thr, Thr/Ala, and Ala/Ala) were considered separately, followed by pooling the Thr/Thr and Thr/Ala groups. Allelic frequencies were determined by gene counting, and departures from the Hardy–Weinberg equilibrium were verified using chi-square tests. Clinical and laboratory data were compared using chi-square, unpaired Student’s \( t \)-test, Mann–Whitney \( U \) test, one-way analysis of variance (ANOVA) (followed by the Tukey test), and the Kruskal–Wallis \( H \) test. Sample size (142 patients) was calculated to show a difference of 10 mg/dL in the 2-hour glucose at the 75–g oral glucose tolerance test between groups (assuming a type I error of 0.05 and a study power of 0.80). Two-tailed \( P < 0.05 \) was considered statistically significant. All analyses were performed by SPSS version 17.0 (SPSS, Inc.). Graphics were elaborated using PRISM software (GraphPad Software).

RESULTS
A total of 294 patients were enrolled in this study. According to the D2 genotype distribution, 107 (36.4%) individuals were homozygous for the Thr allele, 133 (45.2%) were heterozygous (Thr/Ala), and 54 (18.4%) were homozygous for the Ala allele. The frequency of the minor Ala allele was...
and the genotypes were in Hardy–Weinberg equilibrium ($P= .94$).

The cohort characteristics were as follows: the mean age was $26.7\pm 6.5$ years, $73.5\%$ (n = 216) were Caucasians, the mean body mass index was $30.8\pm 5.6$ kg/m$^2$, the frequency of smokers was $20.4\%$ (n = 60), the mean number of previous gestations was $1.89\pm 1.65$, the serum TSH was $1.91\pm [1.32–3.06] \mu$L/L, free $T_d$ was $1.12\pm 0.41$ ng/dL, and $T_3$ was $178\pm 41$ ng/dL. The clinical and laboratory baseline characteristics were similar among the D2 genotypes (Table 1).

**Glucose Homeostasis**

Assuming a recessive model for the Ala/Ala vs. Thr/Ala-Thr/Thr genotypes, respectively, there were no differences in maternal fasting glucose in the first ($81.3\pm 12.4$ vs. $82.4\pm 11.0$ mg/dL; $P= .65$), second ($80.8\pm 11.3$ vs. $80.1\pm 10.2$ mg/dL; $P= .79$), or third ($77.6\pm 11.8$ vs. $80.6\pm 13.6$ mg/dL; $P= .20$) trimesters of pregnancy (Fig. 1). Similarly, the 2-hour glucose in the oral glucose tolerance test with 75-g glucose (2-hour OGTT 75 g: $111.1\pm 11.3$ vs. $109.4\pm 33.1$ mg/dL; $P= .50$) were not different between the groups (see Fig. 1).

We also accessed HOMA-IR in the third trimester of pregnancy in a representative subgroup of patients (n = 33) who did not differ from the whole group of 294 patients in terms of clinical or laboratory features (Supplemental Table 1, available online). No differences were observed between the genotype groups ($2.4\pm [2.1–3.8]$ vs. $3.2\pm [2.1–4.6]$; $P= .36$).

**Gestational Outcomes**

When we compared the D2 Ala–Ala with the Thr-Ala/Thr-Thr group, we found no differences in the percentage of patients with a previous miscarriage ($23.0\%$ vs. $27.8\%$; $P= .68$) or in the rate of miscarriages per patient ($0.30$ vs. $0.33$; $P= .85$). There were no differences regarding the rates of preeclampsia ($5.6\%$ vs. $5.8\%$; $P= .79$), cesarean delivery ($27.8\%$ vs. $35.0\%$; $P= .38$), neonatal birth weight ($3,299\pm 470$ vs. $3,350\pm 476$ g; $P= .53$), or crown–heel length ($49.2\pm 2.1$ vs. $49.2\pm 1.9$ cm; $P= .97$) for the Ala/Ala vs. Thr/Ala-Thr/Thr genotypes, respectively.

**Newborn Thyroid Function**

Results of TSHneo were available for a subset of 45 patients. The clinical and laboratory features of this subgroup were similar to the whole group studied (see Supplemental Table 1). We found no differences in TSHneo levels according to the maternal D2 Ala/Ala versus Thr/Ala-Thr/Thr genotypes, respectively ($0.92\pm [0.46–0.98]$ vs. $0.65\pm [0.38–1.52] \mu$L/L; $P= .97$).

**D2 Expression in Placental Tissue**

To determine whether homozygosity for the D2 92Ala allele affects D2 expression, we obtained 30 fresh human placental samples and processed them for D2 mRNA and activity. All subjects had normal serum TSH levels at the time the tissues were collected. The D2 activity displayed statistically significantly lower velocity in the Ala92Ala genotype subjects ($0.35\pm 0.15$ vs. $1.96\pm 1.02$ fmol/mg protein/min; $P< .001$). To verify whether the changes in D2 mRNA concentrations in tissues with the Ala92Ala genotype would explain the lower D2 activity, we estimated the D2 mRNA levels by real-time PCR in 30 placental tissue samples. No differences in D2 mRNA levels were found between the D2 genotypes ($3.65\pm 0.66$ vs. $3.58\pm 0.80$ arbitrary units [AU]; $P= .88$) (Fig. 2).

**D3 Expression in Placental Tissue**

Given the reduced D2 activity in Ala92Ala placental tissues and considering the possibility of a compensatory decrease in D3 activity, we next evaluated D3 placental mRNA and

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**TABLE 1**

Clinical and laboratory characteristics of patients grouped according to type 2 deiodinase (D2) Thr92Ala genotype.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ala/Ala (n = 54)</th>
<th>Thr/Ala-Thr/Thr (n = 240)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>26.6 ± 6.7</td>
<td>26.8 ± 6.5</td>
<td>.89</td>
</tr>
<tr>
<td>Caucasians (n, %)</td>
<td>42 (78)</td>
<td>174 (73)</td>
<td>.56</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>30.3 ± 5.2</td>
<td>30.9 ± 5.7</td>
<td>.51</td>
</tr>
<tr>
<td>Current smoking (n, %)</td>
<td>6 (11)</td>
<td>54 (23)</td>
<td>.08</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>1.8 ± 1.5</td>
<td>1.9 ± 1.7</td>
<td>.55</td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>2.40 (1.29–3.13)</td>
<td>1.80 (1.35–3.02)</td>
<td>.70</td>
</tr>
<tr>
<td>Free $T_d$ (ng/dL)</td>
<td>1.15 ± 0.30</td>
<td>1.14 ± 0.67</td>
<td>.96</td>
</tr>
<tr>
<td>$T_d$ (ng/dL)</td>
<td>189 ± 47</td>
<td>173 ± 35</td>
<td>.10</td>
</tr>
<tr>
<td><strong>Gestational outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean delivery (n, %)</td>
<td>15 (28)</td>
<td>84 (35)</td>
<td>.38</td>
</tr>
<tr>
<td>Preeclampsia (n, %)</td>
<td>3 (5.8)</td>
<td>14 (5.6)</td>
<td>.79</td>
</tr>
<tr>
<td>Gestational age at birth (wk)</td>
<td>38.5 ± 1.7</td>
<td>38.8 ± 1.7</td>
<td>.15</td>
</tr>
<tr>
<td>Neonatal birth weight (g)</td>
<td>3299 ± 471</td>
<td>3350 ± 477</td>
<td>.53</td>
</tr>
<tr>
<td>Neonatal crown–heel length (cm)</td>
<td>49.2 ± 2.1</td>
<td>49.2 ± 1.9</td>
<td>.97</td>
</tr>
<tr>
<td>TSHneo (mU/L)</td>
<td>0.92 (0.46–0.98)</td>
<td>0.65 (0.38–1.52)</td>
<td>.97</td>
</tr>
</tbody>
</table>

Note: Data are presented as number of patients (percentages), mean ± standard deviation or median (percentiles 25–75). BMI = body mass index; $T_d$ = triiodothyronine; $T_3$ = thyroxine; TSH = thyroid-stimulating hormone; TSHneo = newborn TSH. The reference ranges for laboratory values are: TSH, 0.4–4.5 mU/L; free $T_d$, 0.93–1.70 ng/dL; $T_3$, 60–200 ng/dL, and TSHneo, <0.10 mU/L. To convert free $T_d$ values to ng/dL, divide by 12.87. To convert $T_3$ values to ng/dL, divide by 0.01536.

*Data. D2 Thr92Ala polymorphism and pregnancy. Fertil Steril 2014.*
activity levels. Thirty samples of human placental tissue were analyzed for D3 mRNA and activity. Neither D3 activity (43.70 ± 3.83 vs. 40.85 ± 2.08 fmol/mg protein/min; P = .35) nor D3 mRNA expression (1.47 ± 0.11 vs. 1.36 ± 0.21 AU; P = .15) differed between the D2 genotypes (Fig. 3).

**DISCUSSION**

In our present study, we demonstrated that placental D2 enzyme activity is markedly reduced in patients harboring the D2 Ala92Ala genotype. Notwithstanding, the D2 Thr92Ala polymorphism was not associated with disrupted glycemic homeostasis during pregnancy nor with adverse gestational outcomes.

Thyroid metabolism changes substantially along pregnancy. As a result of elevated TBG concentrations, placental degradation of thyroid hormones, and transfer of thyroid hormones to the fetus, the requirements of maternal thyroid hormone synthesis increase 30% to 50% during pregnancy. Moreover, maternal metabolic modifications take place to ensure the adequate provision of fuel and nutrients to the fetus. The placenta secretes hormones that counterregulate insulin action (growth hormone, cortisol, placental lactogen, and progesterone), and maternal adipose deposition increases. These metabolic changes lead to a state of insulin resistance and hyperinsulinemia, which is more pronounced in the third trimester when the nutrient requirements of the fetus increase.

In this context, if pancreatic insulin secretion is not sufficient to overcome the insulin resistance that follows, dysglycemia may occur. Given the detrimental effects of dysglycemia on the fetus, understanding the predisposing factors to glucose homeostasis disruption is of particular interest. Thus, as gestation is a state of increased thyroid hormone demand and insulin resistance, it would be reasonable to speculate that the D2 Ala92Ala genotype could contribute to a dysglycemic state during pregnancy [4, 9, 11].

We have evaluated the impact of D2 Thr92Ala polymorphism on glycemic homeostasis and gestational outcomes. Fasting glucose and 2-hour OGTT 75 g are the laboratory tests that guide glycemic-oriented management of gestational patients, and favorable gestational outcomes are the ultimate goal of glycemic surveillance during this period. We have shown that the presence of the Ala92Ala genotype is not associated with disrupted glucose homeostasis, increased insulin resistance accessed by the HOMA-IR, or adverse fetal and maternal gestational outcomes.

Interestingly, however, D2 activity is markedly reduced (~82%) in the placenta of patients homozygous for the D2 Ala92Ala polymorphism.
92Ala allele. These findings were in consonance with our previous reports of reduced D2 activity in the thyroid tissue of patients with the D2 Ala92Ala genotype, further demonstrating that homozygosis for the D2 92Ala allele is associated with reduced D2 activity in peripheral tissues (4). The mechanism that explains the decreased D2 activity associated with the D2 Ala92Ala genotype is still a matter of debate, but it probably occurs at the posttranscriptional level, as no changes in the mRNA levels have been observed. Given that no significant changes in the biochemical properties of the mutant enzyme have been identified, this variant seems to be a marker for abnormal D2 expression (4).

During fetal development, thyroid hormone levels are tightly regulated to be kept in a narrow range. D2 and D3 are expressed at the placental-uterine barrier, activating and inactivating T4 to meet the fetal T3 demand (26, 27). Thus, it is reasonable to suppose that the reduction of D2 activity and consequent decrease in intradecidual levels of thyroid hormones in the placenta of patients homozygous for the D2 92Ala allele could result in down-regulation of D3 activity. However, we found no changes in placental D3 transcripts or activity levels in D2 Ala92Ala patients.

Taken together, our findings suggest that the reduction in placental D2 activity of patients with the Ala92Ala genotype does not result in glycemic metabolic disarrangement in euthyroid pregnant patients. However, dysglycemia is a multifactorial condition with environmental and genetic factors involved, in which the contribution of a single polymorphism to phenotype is determined by its prevalence and effect size. Thus, despite including 294 participants, we cannot rule out a type 2 (β) error for a small gene-variant-induced phenotype effect. Moreover, one should consider that compensatory mechanisms might take place, particularly considering the short-term duration of pregnancy. In this context, a genetic predisposing factor may not be clinically relevant to phenotype expression.

We also found no differences in TSHneo according to the maternal D2 genotype, a finding that suggests that major thyroid function abnormalities are not induced by the reduced D2 activity in the maternal environment. Notwithstanding, given the detrimental effects of maternal hypothyroidism for the neurodevelopment of the offspring, the long-term consequences of reduced placental activation of thyroid hormones during fetal development (an issue not addressed in our study) remain to be clarified (28, 29).

In conclusion, placental D2 activity is reduced in euthyroid pregnant patients with the D2 Ala92Ala genotype. However, the D2 Thr92Ala polymorphism was not associated with dysglycemia, increased insulin resistance, or adverse gestational outcomes.

REFERENCES


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## SUPPLEMENTAL TABLE 1

Clinical and laboratory features of the whole population and of the 33 patients evaluated by HOMA-IR and the 45 patients with newborn thyroid function evaluation by neonate TSH.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All study participants (n = 294)</th>
<th>HOMA-IR subgroup (n = 33)</th>
<th>TSHneo subgroup (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala/Ala genotype (n, %)</td>
<td>54 (18)</td>
<td>7 (21)</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>26.7 ± 6.5</td>
<td>27.7 ± 8.3</td>
<td>26.7 ± 6.3</td>
</tr>
<tr>
<td>Caucasians (n, %)</td>
<td>216 (74)</td>
<td>26 (79)</td>
<td>37 (82)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.8 ± 5.6</td>
<td>31.4 ± 6.0</td>
<td>31.2 ± 5.4</td>
</tr>
<tr>
<td>Current smoking (n, %)</td>
<td>60 (20)</td>
<td>6 (18)</td>
<td>11 (24)</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>1.9 ± 1.6</td>
<td>1.6 ± 1.9</td>
<td>1.1 ± 1.6</td>
</tr>
<tr>
<td><strong>Gestational outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean delivery (n, %)</td>
<td>99 (34)</td>
<td>16 (35)</td>
<td>13 (39)</td>
</tr>
<tr>
<td>Preeclampsia (n, %)</td>
<td>17 (5.8)</td>
<td>2 (6.1)</td>
<td>3 (6.7)</td>
</tr>
<tr>
<td>Gestational age at birth (wk)</td>
<td>38.7 ± 1.7</td>
<td>38.6 ± 1.7</td>
<td>38.4 ± 1.2</td>
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<tr>
<td>Neonatal birth weight (g)</td>
<td>3339 ± 474</td>
<td>3271 ± 444</td>
<td>3200 ± 450</td>
</tr>
<tr>
<td>Neonatal crown-heel length (cm)</td>
<td>49.2 ± 2.0</td>
<td>48.9 ± 1.7</td>
<td>48.8 ± 1.9</td>
</tr>
<tr>
<td><strong>Glycemic control</strong></td>
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<tr>
<td>Fasting first trimester</td>
<td>82.2 ± 11.3</td>
<td>80.5 ± 12.7</td>
<td>77.9 ± 10.4</td>
</tr>
<tr>
<td>Fasting second trimester</td>
<td>80.2 ± 10.3</td>
<td>89.9 ± 11.8</td>
<td>83.5 ± 14.2</td>
</tr>
<tr>
<td>Fasting third trimester</td>
<td>80.0 ± 13.3</td>
<td>80.9 ± 10.7</td>
<td>81.3 ± 13.7</td>
</tr>
<tr>
<td>2-hour OGTT third trimester</td>
<td>108.1 ± 32.1</td>
<td>114.5 ± 38.2</td>
<td>113.4 ± 40.1</td>
</tr>
</tbody>
</table>

Note: All comparisons were P > .05. TSHneo = newborn serum thyroid-stimulating hormone levels.