Placental Expression of Peroxisome Proliferator-Activated Receptor γ (PPARγ): Relation to Placental and Fetal Growth

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Background and Objective: The nuclear receptor peroxisome proliferator activated receptor γ (PPARγ) contributes to placental development and thus to the maternofetal transfer of oxygen and nutrients that allow for prenatal growth. We tested the hypothesis that placental PPARγ expression relates to placental and fetal growth.

Design and Study Population: Placentas (n = 116) were collected at term delivery of singleton infants who were born small-for-gestational-age (SGA), appropriate-for-gestational-age (AGA), or large-for-gestational-age (LGA) (n = 32 SGA, 55 AGA, and 29 LGA). Placentas and newborns were weighed at birth. Real-time PCR was used to assess placental expression of PPARγ as compared to the housekeeping gene GAPDH.

Results: PPARγ expression in placentas from AGA and LGA infants was nearly 2-fold higher than in placentas from SGA infants. Placental PPARγ expression associated positively to placental and/or fetal weight at birth, particularly within the SGA subpopulation (P = 0.001).

Conclusion: PPARγ expression was found to be low in placentas of SGA fetuses and to associate positively to fetal and placental weights within this subpopulation. (J Clin Endocrinol Metab 97: E1468–E1472, 2012)

Peroxisome proliferator-activated receptor γ (PPARγ) is one of the PPAR proteins belonging to a family of ligand-activated nuclear hormone receptors that regulate metabolic, antiinflammatory, and developmental processes (1). PPARγ is present in numerous tissues but is particularly highly expressed in adipose tissue, where it has received much attention with regard to its role in adipogenesis and energy metabolism (2, 3). Specifically, PPARγ is essential for the initiation of adipogenesis and the subsequent development of both white and brown adipose tissue (4).

PPARγ is also expressed in the human placenta, particularly in trophoblasts, and appears to have a pivotal role in placental development (5). In addition to regulating trophoblast invasion, PPARγ plays a predominant role in differentiation of labyrinthine trophoblast lineages, which, along with fetal endothelium, form the vascular exchange interface with maternal blood (6). PPARγ is essential for regulation of fat accumulation in trophoblasts and transport of fatty acids from the placenta to the fetus, and it may play a role in modulating fetal membrane signals toward parturition (7).

Placental dysfunction, due to abnormalities in trophoblast differentiation and function, is associated with many pregnancy disorders. It has been described that PPAR are altered in pathologies of the human placenta (8), including...
gestational diabetes mellitus (9), preeclampsia (10), and intrauterine growth restriction (11). Specifically, in pregnancies complicated with intrauterine growth restriction, PPARγ mRNA expression has been reported to be either increased or unchanged (8, 11), whereas protein expression as well as DNA binding activity appear to be elevated (8). Some of these studies, however, were performed in animal models and/or preterm pregnancies and included a relatively small number of cases (8). In rats, maternal undernutrition during pregnancy has been shown to enhance placent al mitochondrial-dependent apoptosis, probably through PPARγ down-regulation (12).

Considering the pleiotropic function of PPARγ in the placenta, we hypothesized that placental PPARγ is reduced in pregnancies complicated by fetal growth restriction. In the present study, we examined the associations between placental PPARγ and fetoplacental growth in singleton term pregnant women delivering either appropriate- (AGA), small- (SGA), or large-for-gestational age (LGA) infants.

Subjects and Methods

Study population

The study cohort consists of 116 mother-placenta-newborn trios recruited between May 2006 and September 2009 (see flow chart in Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org). The specific inclusion criteria were:

1. Singleton pregnancy followed from the first trimester at Hospital Sant Joan de Déu, Barcelona; absence of maternal hypertension, gestational diabetes (O’Sullivan test in the second trimester), alcohol abuse, smoking, or drug addiction.

2. Placenta collected at delivery for research purposes; the inclusion rate was limited by logistic restraints (particularly for nighttime collection and sample preparation) and also by ethical restraints (placentas were not studied unless timely obtained, written, informed, maternal consent was available; see Statistics and ethics).

3. Infants born at Hospital Sant Joan de Déu, Barcelona, at term (37–42 wk); birth weight between 2.9 and 3.8 kg for AGA (range thus between approximately 1.1 and +1.1 SD), between 1.9 and 2.6 kg for SGA (below –2 SD), or between 4.0 and 4.8 kg for LGA infants (above +2 SD).

4. Written, informed consent in Spanish/Catalan language, obtained in the third trimester before parturition.

Exclusion criteria were complications at birth (need for resuscitation or parenteral nutrition) and congenital malformations.

Maternal age at conception, parity, and height, as well as pregestational weight and body mass index (BMI), weight (kilograms)/height (meters)², blood pressure, and smoking habits were retrieved from the mothers’ clinical records along with placental weight. Gestational age was calculated by last menses and confirmed by first-trimester ultrasound (~10 wk).

Weight and length of the newborn were measured in the delivery room. Delivery rate by cesarean section was 27%. Of the 116 newborns, 55 were born AGA (30 girls, 25 boys), 32 were born SGA (12 girls, 20 boys), and 29 were born LGA (12 girls, 17 boys). All SGA infants were classified as having asymmetric growth restriction (head growth spared).

Placenta collection

Placentas were weighed after delivery, and placental tissue was then obtained from the maternal side. Each placenta was sectioned transversally (over approximately 5 mm), and three fragments of about 1 cm³ of tissue near the umbilical cord’s insertion were biopsied after removal of the amniotic and chorionic layers. Each sample was rinsed three times in saline; samples were frozen in liquid nitrogen and stored at −80°C until RNA extraction.

RNA extraction, reverse transcription, and quantitative PCR

Between 100 and 125 mg of frozen placenta was homogenized using a Polytron benchtop homogenizer (Kinematica Ag, Littau-Luzern, Switzerland). Total RNA was isolated using Trizol reagent (Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s instructions. The quantity of isolated RNA was determined by a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). To test the integrity of total RNA, in addition to the A260 nm/A280 nm ratio, 1 µg of RNA was run on a 1.0% agarose gel using ethidium bromide staining to visualize 28S and 18S RNA bands. Samples with partially degraded RNA were rejected.

Total RNA (2 µg) was reverse-transcribed to cDNA using TaqMan Reverse Transcription reagents (Applied Biosystems, Foster City, CA), and subsequently diluted with nuclease-free water (Sigma, St. Louis, MO) to 20 ng/µl cDNA. The gene amplifications were assessed using specific pairs of primers: PPARγ forward, 5’-GCTGTGCAGGAGATCAC-3’, and reverse, 5’-GGGCTCCATAGAGTCAACCA-3’; and GAPDH forward, 5’-GTCAGTGGTGACCTGACCT-3’, and reverse, 5’-GCTGCGAATAATGCTGG-3’, using GADPH as endogenous control. The PCR amplification mixtures (20 µl) contained 40 ng template cDNA, 2X SYBR Green 1 Master Mix buffer (14 µl) (Applied Biosystems), and 250 nM forward and reverse primer. Reactions were run on an ABI PRISM 7500 Sequence Detector (Applied Biosystems). The cycling conditions comprised 10-min polymerase activation at 95°C and 40 cycles at 95°C for 15 sec and 60°C for 60 sec. Fluorescence intensity was recorded with ABI software (Applied Biosystems), and the results were plotted vs. cycle number. Relative expression was calculated according to the 2-ΔCt (where Ct is cycle threshold) method. ΔCt value was determined by subtracting the Ct value for PPARγ from the Ct value for the GAPDH housekeeping gene obtained in the same sample and same conditions.

Statistics and ethics

Statistical analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL). Skewed data were log-transformed before analyses. One-way ANOVA with post hoc comparisons and Bonferroni correction was used to study differences in placental PPARγ expression among groups (SGA vs. AGA and LGA vs. AGA). Correlation and multiple regression analyses were used to study the associations between placental PPARγ expression and...
outcomes of interest. A $P$ value $\leq 0.01$ (the conventional $P$ value of 0.05 divided by five groups of variables tested at a time) was deemed significant in correlation and multivariate analyses to correct for multiple comparisons.

The study had an 80% power to detect clinically significant differences in placental PPARγ gene expression of a least 0.75 SD among the study subpopulations. The study was similarly powered to detect in bilateral tests significant associations between placental PPARγ and the outcomes of interest with a Pearson $r$ coefficient of at least 0.26 in the studied subjects and of at least 0.48 in any of the studied subpopulations.

The study was approved by the Institutional Review Board of Barcelona University, Hospital of Sant Joan de Déu; written informed consent for placental collection was obtained before delivery and was an inclusion criterion (see Study population).

**Results**

Table 1 shows the results by birth weight subgroups. PPARγ expression in placentas from AGA and LGA infants was nearly 2-fold higher than in those from SGA infants. This difference in gene expression remained essentially unchanged after controlling for gestational age.

Table 2 shows that placental PPARγ expression associated to fetal and/or placental weight at birth. This was highly significant in the SGA group (Table 2), but not in the AGA and LGA subpopulations (data not shown).

In multivariate analysis adjusting for maternal BMI, parity, and gestational age (which are variables commonly associated with birth weight and/or placental weight; Supplemental Table 1), birth weight SD score (SDS) was independently explained by maternal BMI ($\beta = 0.493; P < 0.0001; r^2 = 0.201$), PPARγ ($\beta = 0.218; P = 0.01; r^2 = 0.039$), and parity ($\beta = 0.354; P < 0.0001; r^2 = 0.095$). PPARγ expression was the only variable independently associated with birth weight SDS in the SGA subpopulation ($\beta = 0.419; P = 0.01; r^2 = 0.144$). Similar results were found in multivariate analysis with placental weight as dependent variables (Supplemental Table 1).

**Discussion**

SGA fetuses were found to have a lower placental PPARγ expression, compared with AGA or LGA fetuses. Placental PPARγ expression associated positively with placental and fetal weight at birth only in the SGA subpopulation.

**Table 1.** Maternal, placental, and neonatal data by birth weight subgroup

<table>
<thead>
<tr>
<th></th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>32</td>
<td>55</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>30±1</td>
<td>29±1</td>
<td>31±1</td>
<td>ns</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55±1</td>
<td>62±1</td>
<td>74±2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pregnancy weight gain (kg)</td>
<td>11.3±0.6</td>
<td>11.6±0.5</td>
<td>11.8±0.9</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.7±0.6</td>
<td>23.7±0.8</td>
<td>28.7±0.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Primiparous</td>
<td>62%</td>
<td>54%</td>
<td>28%</td>
<td>0.01 ns</td>
</tr>
<tr>
<td>Placenta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARγ expression*</td>
<td>0.12±0.01</td>
<td>0.21±0.03</td>
<td>0.23±0.03</td>
<td>0.007</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>462±13</td>
<td>633±14</td>
<td>910±22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Newborn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>41</td>
<td>54</td>
<td>41</td>
<td>ns</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>38.2±0.2</td>
<td>39.5±0.1</td>
<td>39.8±0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2220±55</td>
<td>3202±41</td>
<td>4395±38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>−2.3±0.1</td>
<td>−0.2±0.1</td>
<td>2.6±0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>45.1±0.3</td>
<td>49.7±0.3</td>
<td>52.7±0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Length SDS</td>
<td>−2.0±0.1</td>
<td>−0.1±0.1</td>
<td>1.3±0.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. $P$ values are from linear-trend ANOVA, and those for comparisons among subgroups are from post hoc analyses with Bonferroni correction. ns, Not significant.

* Expression relative to the expression of a housekeeping gene (see Subjects and Methods).
PPARγ plays a key role in placental development and trophoblast differentiation (7). The administration of PPARγ antagonists to pregnant mice is known to reduce fetal weight gain (13, 14), possibly by raising the circulating levels of soluble fms-like tyrosine kinase 1 (10, 15). PPARγ null placentas exhibit a malformed labyrinth zone, with no permeation of fetal blood vessels and dilatation and rupture of maternal blood sinuses; in addition, labyrinthine trophoblasts fail to differentiate properly (16). Poor differentiation can occur in labyrinthine trophoblast cells of fetuses with low placental PPARγ, which can interfere with the maternal-to-fetal blood transport and impair placental and fetal growth. PPARγ also plays a major role in placental fatty acid trafficking (7). It has been shown that null placentas exhibit reduced lipid droplets in trophoblasts (4), whereas PPARγ and its heterodimeric nuclear receptor partner retinoid X receptor enhance trophoblast fatty acid uptake and accumulation in vitro (17). Moreover, several proteins that modulate fatty acid transport and accumulation are expressed in placenta and are increased with the activation of PPARγ and/or retinoid X receptor in primary human trophoblasts (17, 18).

Taken together, these data suggest that basal PPARγ expression is required for proper placental development and for regulation in the uptake and accumulation of fatty acids, which, in turn, appear to be necessary for PPARγ activation (7, 18). Target genes for PPARγ include GLUT4 (glucose transporter type 4), PI3K (phosphoinositide 3-kinase), PEPCK (phosphoenolpyruvate carboxykinase), and adiponectin, which are known to be pivotal in the regulation of glucose and lipid metabolism. In addition, PPARγ is a key player in the transcriptional cascade initiating and regulating adipocyte differentiation.

The down-regulation in placental PPARγ expression in the SGA subpopulation and the similar PPARγ expression in the AGA and LGA newborns could be partly explained by epigenetic mechanisms because PPARγ is capable of promoting DNA methylation and chromatin changes and, in turn, being regulated through histone modification (19). The placental expression of genes favoring methylation appears to be increased in case of adverse intrauterine environment (19). Accordingly, it is tempting to speculate that increased placental genome methylation in the SGA population would result in a decreased expression of PPARγ and possibly of other coregulators. These mechanisms would be less operational in AGA and LGA pregnancies that evolve in a less adverse intrauterine environment.

An alternative mechanism could involve the serine/threonine kinase mTOR—a mammalian target of rapamycin—that acts by regulating the expression and activation status of PPARγ. Inhibition of mTOR down-regulates PPARγ mRNA and protein level (20). The mTOR signaling pathway could be impaired in SGA placentas and account for a decrease in PPARγ expression and thus in adipogenesis.

The present study appears to be the first that assessed placental PPARγ expression in a relatively large population containing only term, singleton, and uncomplicated pregnancies. One of the limitations is that we did not assess placental expression of other PPAR isoforms and did not perform protein expression and DNA binding studies, which might have provided additional information on the mechanisms underpinning prenatal growth restriction. Longitudinal assessment of the methylation patterns of specific genes, including of PPAR in placenta and in peripheral blood of infants, may contribute to disclosing the role of epigenetic changes in fetal growth and in long-term metabolic risk.

In conclusion, PPARγ expression was found to be low in placentas of SGA fetuses and to associate positively to fetal and placental weights within this subpopulation.

Acknowledgments

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Disclosure Summary: M.D., J.B., A.L.-B., M.D.G.-R., F.d.Z., and L.I. have nothing to declare.

References

1. Desvergne B, Michalik L, Wahli W 2004 Be fit or be sick: peroxisome proliferator-activated receptors are down the road. Mol Endocrinol 18:1321–1332


