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Ahlsson, F.; Åkerud, H.; Schijven, D.; Olivier, J.; Sundstrom-Poromaa, I.

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# Gene Expression in Placentas From Nondiabetic Women Giving Birth to Large for Gestational Age Infants

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F. Ahlsson, MD, PhD<sup>1</sup>, H. Åkerud, MD, PhD<sup>1</sup>, D. Schijven, MSc<sup>1</sup>,  
J. Olivier, PhD<sup>1,2,3</sup>, and I. Sundström-Poromaa, MD, PhD<sup>1</sup>

## Abstract

Gestational diabetes, obesity, and excessive weight gain are known independent risk factors for the birth of a large for gestational age (LGA) infant. However, only 1 of the 10 infants born LGA is born by mothers with diabetes or obesity. Thus, the aim of the present study was to compare placental gene expression between healthy, nondiabetic mothers ( $n = 22$ ) giving birth to LGA infants and body mass index-matched mothers ( $n = 24$ ) giving birth to appropriate for gestational age infants. In the whole gene expression analysis, only 29 genes were found to be differently expressed in LGA placentas. Top upregulated genes included insulin-like growth factor binding protein 1, aminolevulinic acid synthase 2, and prolactin, whereas top downregulated genes comprised leptin, gametocyte-specific factor 1, and collagen type XVII  $\alpha$  1. Two enriched gene networks were identified, namely, (1) lipid metabolism, small molecule biochemistry, and organismal development and (2) cellular development, cellular growth, proliferation, and tumor morphology.

## Keywords

large for gestational age, placental gene expression, microarray, leptin, and prolactin

## Introduction

The number of infants born large for gestational age (LGA) has increased during the last 2 decades.<sup>1</sup> Besides being at increased risk of later development of obesity, type 2 diabetes, cardiovascular disease, prostate cancer, and breast cancer, women who themselves were born LGA are also at increased risk of giving birth to LGA infants.<sup>2</sup>

Maternal pregestational diabetes, gestational diabetes, obesity, and excessive weight gain are all established risk factors for LGA.<sup>3,4</sup> The fetal hyperinsulinemic hypothesis<sup>5</sup> stipulates that increased maternal insulin resistance leads to enhanced maternal hepatic glucose production, which in turn results in increased glucose shunting to the fetus, ultimately causing fetal hyperinsulinemia and enhanced fetal growth.<sup>6</sup> However, only approximately 1 of the 10 infants born LGA is born by mothers with diabetes or obesity.<sup>2</sup>

Maternal overfeeding in rodents is associated with altered placental expression of several genes related to metabolic signaling, inflammation, fatty acid transporter proteins, and several growth regulatory imprinted genes.<sup>7,8</sup> Further, protein restriction during pregnancy results in downregulation of genes involved in nucleotide metabolism and upregulation of genes associated with the p53 pathway, apoptosis, negative regulators of cell growth, negative regulators of cell metabolism, and genes related to epigenetic control.<sup>9</sup> However, research on the

human placenta has thus far mostly addressed hypotheses related to growth restriction, small for gestational age (SGA) infants, and preeclampsia,<sup>10,11</sup> whereas studies of human placental gene expression in relation to excessive fetal growth are less frequent and mostly concerned with gestational diabetes. Among the few findings at hand, Diaz and colleagues suggested that the peroxisome proliferator-activated receptor  $\gamma$  (contributing to placental development) expression in placenta is positively associated with birth size.<sup>12</sup> Further, decreased messenger RNA and protein contents for insulin-like growth factor (IGF) I, IGF-II, and IGF-IR have been described in full-term placentas from LGA compared to appropriate for gestational age (AGA) and SGA newborns.<sup>13</sup>

In light of this, we hypothesized that genes in pathways involved in lipid and carbohydrate metabolism are differently

<sup>1</sup> Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

<sup>2</sup> Department of Behavioral Physiology, University of Groningen, Groningen, the Netherlands

<sup>3</sup> Centre for Gender Medicine, Karolinska Institutet, Stockholm, Sweden

## Corresponding Author:

F. Ahlsson, Department of Women's and Children's Health, Uppsala University, SE-751 85 Uppsala, Sweden.

Email: fredrik.ahlsson@kbh.uu.se

expressed in placentas from nondiabetic mothers giving birth to LGA infants. Hence, the aim of the present study was to compare placental gene expression in nondiabetic healthy mothers giving birth to LGA infants with placental gene expression of body mass index (BMI)-matched mothers giving birth to AGA infants.

## Materials and Methods

### Patients

Placenta samples were collected from women delivering at the Department of Obstetrics, Uppsala University Hospital, as part of an ongoing longitudinal study on antenatal and postpartum depression (The BASIC study). Women were invited for participation in the BASIC study in gestational week 16 to 17. The mothers received written information about the study objectives and written consent for collection of placental tissue was obtained. At present the Biology, Affect, Stress, Imaging and Cognition in Pregnancy and the Puerperium (BASIC) biobank contains fresh frozen placental tissue from 913 pregnancies. Demographic data, clinical variables, maternal health care, delivery, and neonatal outcomes were retrieved from the medical records. This substudy to the BASIC project was approved by the regional Ethics Committee of the Medical Faculty of Uppsala University.

Inclusion criteria for cases and controls in this substudy were mothers of caucasian origin, normal pregnancies, and normal deliveries. Exclusion criteria were continued smoking or alcohol use during pregnancy, any severe chronic disease (the only exceptions being euthyroid women on levothyroxine treatment), daily use of any prescribed medication (with the exception of levothyroxine), prepregnancy type 1 or type 2 diabetes, gestational diabetes, preeclampsia, gestational hypertension, gestational age <35 weeks, other severe obstetric complications, and maternal age <18 or >42 years. Based on these criteria, 22 mothers who had given birth to an infant that was LGA were ascertained. LGA was defined as birth weight above the 90th percentile. Twenty-four randomly selected controls, matched on age, parity, first trimester BMI, and gestational age at birth, who had given birth to an infant that was AGA, defined in this study as  $\pm 0.8$  standard deviations (SDs) from the mean gestational weight, were included.

The exclusion of cases with gestational diabetes was ascertained by 5 random, nonfasting blood glucose measurements during pregnancy (gestational weeks 10–14, 25, 28–29, 33 and 37). If nonfasting blood glucose levels at any time exceeded 8.8 mmol/L, or glycosuria developed, or evidence of increased fetal growth was present (symphysis-fundal height deviation more than 5 cm from mean, fetal growth by ultrasound >22%, or polyhydramnios), an oral glucose tolerance test (OGTT) was performed. In addition, all women with at least 2 risk factors for gestational diabetes (family history of type 2 diabetes, BMI  $\geq 30.0$  kg/m<sup>2</sup> at first antenatal booking, previous history of LGA infant, or born outside of Europe) were subjected to 2 OGTTs during the pregnancy, in gestational weeks 12 to 14 and 28 to 30, respectively.

Placental tissue samples were obtained immediately following delivery. Two basal plate biopsy specimens of the maternal–fetal interface, approximately 2 cm in size, were excised from the central part of the placenta in a way that each sample contained the decidua basalis and villous placenta. Areas involving calcification or infarcts were avoided. The tissue samples were briefly washed in sterile phosphate-buffered saline and were immediately frozen and stored at  $-70^{\circ}\text{C}$ .

### Microarray Expression Analysis

Total RNA was prepared from frozen parts of the maternal side of the placenta. Total RNA was isolated using the miRNeasy mini kit (Qiagen, Hilden, Germany). Tissue was lysed with QIAzol reagent (Qiagen) using a rotor-stator homogenizer (up to 33,000 rpm; Ingenieurbüro CAT M Zipper GmbH, type x120, Staufen, Germany), and chloroform (Sigma Aldrich, St Louis, Missouri) was added for phase separation. The rest of the procedure was performed as described in the manufacturer's protocol. RNA concentration was measured with ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware), and RNA integrity number (RIN) value > 6.0 was confirmed by use of the Agilent 2100 Bioanalyzer system (Agilent Technologies Inc, Palo Alto, California). No difference in RNA concentrations between AGA and LGA placentas was found ( $439 \pm 281$  vs  $403 \pm 263$  ng/dl,  $P = .6$ ).

Total RNA of 250 nanograms of from each sample were used to generate amplified and biotinylated sense-strand complementary DNA (cDNA) from the entire expressed genome according to the Ambion WT Expression Kit (P/N 4425209 Rev C 09/2009) and Affymetrix GeneChip WT Terminal Labeling and Hybridization User Manual (P/N 702808 Rev. 5, Affymetrix Inc, Santa Clara, California). GeneChip ST Arrays (GeneChip Human Gene 1.0 ST Array) were hybridized for 16 hours in a  $45^{\circ}\text{C}$  incubator and rotated at 60 rpm. According to the GeneChip Expression Wash, Stain and Scan Manual (PN 702731 Rev 3, Affymetrix Inc) the arrays were then washed and stained using the Fluidics Station 450 (Affymetrix Inc) and finally scanned using the GeneChip Scanner 3000 7G (Affymetrix Inc).

### Microarray Data Analysis

The raw data was normalized in the free software Expression Console provided by Affymetrix (<http://www.affymetrix.com>) using the robust multiarray average method first suggested by Li and Wong in 2001.<sup>14,15</sup> Subsequent analyses of the gene expression data were carried out in the freely available statistical computing language R (<http://www.r-project.org>) using packages available from the Bioconductor project ([www.bioconductor.org](http://www.bioconductor.org)). In order to search for the differentially expressed genes between the LGA and the AGA groups, an empirical Bayes moderated *t* test with robust regression was then applied,<sup>16</sup> using the “limma” package.<sup>17</sup> To address the problem with multiple testing, the *P* values were adjusted using the method of Benjamini and Hochberg.<sup>18</sup> For this exploratory

study, a significance threshold ( $P < .1$ ) and a fold change of  $> \pm 0.5$  was used. The Genesis software, version 1.7.1 (<http://genome.tugraz.at/>), was used to produce hierarchical clustering (average linkage and correlation similarity) and to visualize differentially expressed genes by heatmaps.<sup>19</sup> The expression data were further analyzed using ingenuity pathway analysis (IPA) in order to determine significantly deregulated genes and pathways (Ingenuity Systems, Mountain View, California; [www.ingenuity.com](http://www.ingenuity.com)).

### Real-Time Quantitative Reverse Transcriptase Polymerase Chain Reaction Analysis

The validity of the microarray results was tested via quantitative real-time polymerase chain reaction (qRT-PCR) employing the StepOne Plus qPCR machine (Applied Biosystems, Life Technologies, Carlsbad, California). At the time of validation, 2 additional LGA cases were available. For validation we selected 5 genes (*LEP*, insulin-like growth factor binding protein 1 [*IGFBP-1*], *FOSB*, prolactin (*PRL*), and  $\alpha$ -hemoglobin-stabilizing protein [*AHSP*]) which showed a fold change  $>0.6$  in the microarray. Because commonly used housekeeping genes were differently expressed between cases and controls in the micro array, we chose *HPRT1* as reference gene for normalization.<sup>20</sup> The cDNA synthesis was performed by the use of SuperScriptIII First strand Synthesis kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. TaqMan Gene expression Assay primers, probes, and gene expression master mix (all Applied Biosystems, Life Technologies, Carlsbad, California) were used to run the qRT-PCR according to manufacturer's instructions. All samples were performed in triplicates and averaged for further calculations. Mean reaction efficiencies were calculated by LinReg. Gene expression values were calculated with the  $\Delta\Delta Cq$  method (ie,  $RQ = 2^{-\Delta\Delta Cq}$ ) to illustrate the differences between groups.<sup>21</sup>

### Statistics

Clinical characteristics of the pregnant women in the study were compared by means of independent  $t$  tests or chi-square tests. Differences in gene expression between subsets were calculated using independent  $t$  tests (validation study). Data were analyzed using the SPSS 20.0 software (SPSS Inc, Chicago, Illinois). Level of significance was set at  $P < .05$ . Data are presented as mean  $\pm$  standard deviation or median (range).

## Results

### Study Population

The characteristics of the mothers and the infants are presented in Table 1. Besides higher gestational weight gain among cases, no differences in clinical variables were found between cases and controls. Owing to high screening blood glucose values, 1 control was submitted to an OGTT, which was normal.

**Table 1.** Demographic Variables in the LGA Cases and Healthy Controls.<sup>a</sup>

	Controls (n = 24)	Cases (n = 22)	P Value
Age, years	31.7 $\pm$ 4.2	33.7 $\pm$ 4.5	.2
Parity, median (range)	1 (0-2)	1 (0-3)	.4
Prepregnancy smokers, n (%)	1 (4.2)	1 (4.5)	.6
First trimester weight, kg	72.9 $\pm$ 9.0	76.9 $\pm$ 13.2	.3
Weight gain during pregnancy, kg	12.6 $\pm$ 3.5	15.0 $\pm$ 4.0	.2
Height, cm	169 $\pm$ 6	170 $\pm$ 7	.3
BMI first trimester, kg/m <sup>2</sup>	25.7 $\pm$ 3.7	26.4 $\pm$ 4.4	.6
Gestational age, years	281 $\pm$ 4	284 $\pm$ 9	.2
Birth weight, g	3543 $\pm$ 263	4650 $\pm$ 484	.001
Birth weight, SDS	0.05 $\pm$ 0.51	2.31 $\pm$ 0.72	.001

Abbreviations: BMI, body mass index; LGA, large for gestational age; SD, standard deviation score.

<sup>a</sup>Data presented as mean  $\pm$  SD or median (range).

Three of the women who gave birth to LGA infants were treated for hypothyroidism, but they all had normal serum levels of thyroid-stimulating hormone during their pregnancy. Ten women were delivered by cesarean section (6 cases and 4 controls); only 4 of these were planned and the remaining 6 were emergency cesarean sections. In the control group, 13 of the infants were girls and in the LGA group 12 of the infants were girls. Two of the LGA infants were admitted to neonatal care. One of the control infants had Apgar score 6 at 5 minutes, and all of the other infants had Apgar score 8 or above at 5 minutes.

### Expression Profiling of Placenta Tissue From Women Giving Birth to LGA Infants and AGA Infants

Although a liberal threshold was used, only 29 up- or downregulated genes with a fold change greater than 0.5 were identified, Table 2. Top upregulated molecules were genes coding for *IGFBP1*, aminolevulinic acid synthase 2, and *PRL*, while the top downregulated molecules were genes coding for leptin (*LEP*), gametocyte-specific factor 1, and collagen, type XVII,  $\alpha$  1 (*COL17A1*). Some of the genes previously associated with increased fetal weight were also differently expressed in the micro array although with below threshold fold changes (peroxisome proliferator-activated receptor  $\gamma$  fold change 0.16,  $P < .01$ ; IGF-I fold change 0.35,  $P < .01$ ).

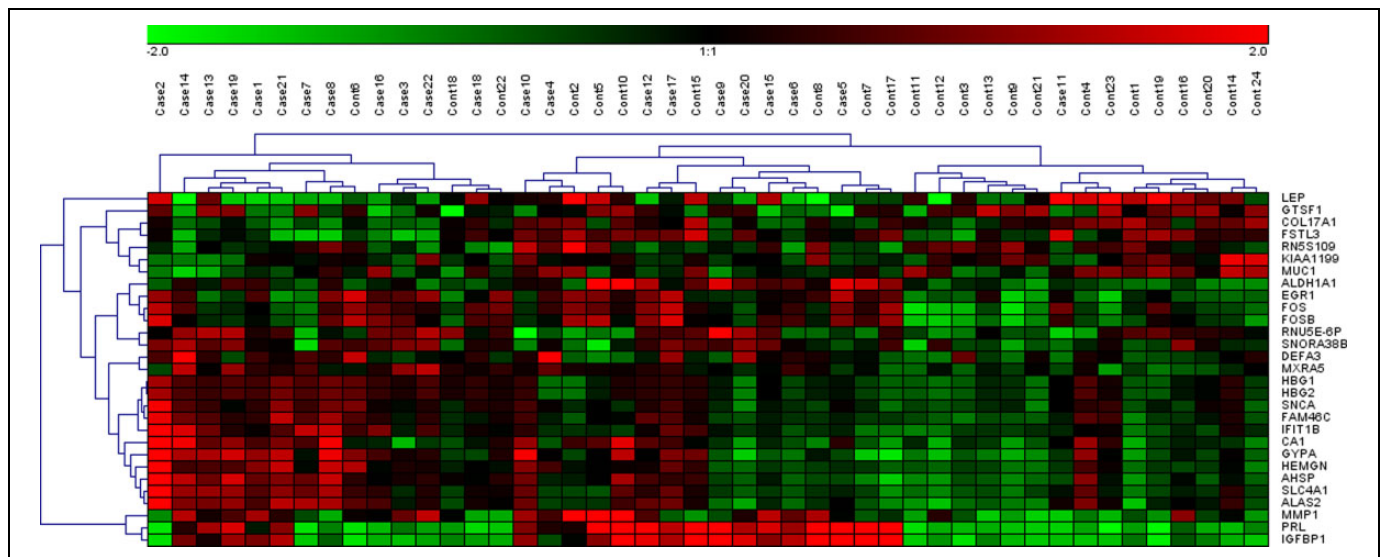
We then visualized the differentially expressed genes using hierarchical clustering of genes in LGA versus AGA placentas (Figure 1).

In order to determine the biological relevance, IPA, focusing on genes that differed in expression between placentas from LGA pregnancies and AGA pregnancies, was performed. We identified 2 gene networks that were significantly enriched and classified as follows: (1) lipid metabolism, small molecule biochemistry, and organismal development with an IPA score of 21 and (2) cellular development, cellular growth, proliferation, and tumor morphology with an IPA score of 21 (Table 3).

**Table 2.** Pathway Analysis of the Differentially Regulated Placental Genes in LGA Cases.

Gene Name	Symbol	Fold Change Log <sup>2</sup>	P Value
LEP	Leptin	-0.93	.033
GTSF1	Gametocyte-specific factor 1	-0.72	.015
COL17A1	Collagen, type XVII, $\alpha$ 1	-0.68	.006
FSTL3	Follistatin-like 3	-0.56	.064
RNS5109	RNA 5S ribosomal 109	-0.54	.002
KIAA1199	KIAA1199	-0.52	<.001
MUC1	Mucin 1 cell surface associated	-0.50	.073
EGR1	Early growth response 1	0.50	.061
SNCA	Synuclein $\alpha$	0.50	<.001
RNU5E-6P	RNA U5E small nuclear 6 pseudogene	0.50	.003
DEFA3	Defensin $\alpha$ 3 neutrophil specific	0.53	.005
MXRA5	Matrix-remodeling associated 5	0.53	.002
ALDH1A1	Aldehyde dehydrogenase 1 family member A1	0.53	.027
FOS	FBJ murine osteosarcoma viral oncogene homolog	0.56	.032
HBG1	Hemoglobin $\gamma$ A	0.58	<.001
HBG2	Hemoglobin $\gamma$ G	0.58	<.001
FAM46C	Family with sequence similarity 46 member C	0.58	<.001
MMP1	Matrix metalloproteinase 1	0.60	.073
HEMGN	Hemogen	0.64	<.001
FOSB	FBJ murine osteosarcoma viral oncogene homolog B	0.64	.021
CAI	Carbonic anhydrase 1	0.65	.024
IFIT1B	Interferon-induced protein with tetratricopeptide repeats 1B	0.67	<.001
SNORA38B	Small nucleolar RNA H/ACA box 38B	0.71	<.001
SLC4A1	Solute carrier family 4 anion exchanger member 1	0.75	.002
AHSP	$\alpha$ Hemoglobin stabilizing protein	0.78	.003
GYPA	Glycophorin A	0.87	.011
PRL	Prolactin	0.89	.097
ALAS2	Aminolevulinic acid synthase 2	0.89	<.001
IGFBP1	Insulin-like growth factor binding protein 1	0.91	.053

Abbreviation: LGA, large for gestational age.



**Figure 1.** Messenger RNA (mRNA) profiles of samples from large for gestational age placentas ( $n = 22$ ) and appropriate for gestational age placentas ( $n = 24$ ; average linkage, correlation similarity). Samples are in columns and mRNA in rows.

Further, the significant canonical pathways identified by IPA ( $P < .01$ ) are shown in Table 4, along with the included genes and  $P$  values. These included interleukin 17A signaling

in fibroblasts, retinoid acid receptor activation, pregnane X receptor/retinoid X receptor activation, prolactin signaling, and glucocorticoid receptor signaling.

**Table 3.** Enriched Ingenuity Pathway Analysis (IPA) Categories Including Differentially Expressed Genes in LGA Cases.

IPA Network	Gene	P Value
Lipid metabolism, small molecule biochemistry, organismal development (IPA score 21)	AHSP	.003
	EGR1	.061
	FOS	.032
	FOSB	.021
	IGFBP1	.053
	LEP	.033
	MMP1	.073
	MUC1	.073
	PRL	.097
	SNCA	<.001
Cellular development, cellular growth, proliferation, and tumor morphology (IPA score 21)	ALAS2	<.001
	ALDH1A1	.027
	COL17A1	.006
	FSTL3	.064
	GYP A	.011
	IGFBP1	.053
	KIAA1199	<.001
	LEP	.033
	MUC1	.073
	SLC4A1	.002

Abbreviations: ALAS2, aminolevulinate δ synthase 2; COL17A1, collagen, type XVII, α 1; IGFBP1, insulin-like growth factor binding protein 1; LEP, leptin; LGA, large for gestational age.

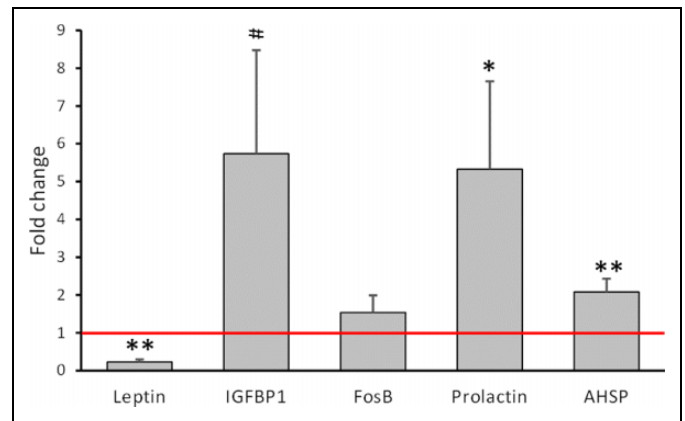
**Table 4.** Canonical Pathway Analysis.

Canonical Pathway	Genes	P Value IPA
IL-17A signaling in fibroblasts	FOS, MMP1	1.53 E <sup>-03</sup>
RAR activation	FOS, MMP1, ALDH1A1	2.56 E <sup>-03</sup>
PXR/RXR activation	ALDH1A1, IBFBP1	4.45 E <sup>-03</sup>
Prolactin signaling	PRL, FOS	6.52 E <sup>-03</sup>
Glucocorticoid receptor signaling	FOS, MMP1, PRL	7.13 E <sup>-03</sup>

Abbreviations: IL-17A, interleukin 17A; IPA, ingenuity pathway analysis; PXR, pregnane X receptor; RAR, retinoid acid receptor; RXR, retinoid X receptor.

### Confirmation of Array Data Using Real-Time Quantitative Polymerase Chain Reaction Analysis

To confirm the array data, we selected 5 differentially expressed genes in functionally relevant pathways. *LEP*, coding for leptin, which plays a major role in the regulation of body weight, food intake, and adipose mass both in the pregnant and in the nonpregnant state. *IGFBP-1*, a member of the insulin-like growth factor binding proteins, which regulates the amount of free circulating IGF-1. *FOSB* involved in forming transcription factor complex AP-1; as such, the FOS proteins have been implicated as a regulator of cell proliferation, differentiation, and transformation. *PRL*, encoding for the anterior pituitary hormone prolactin, which is a growth regulator for many tissues, including cells of the immune system. Finally,



**Figure 2.** For validation of the microarray results, 5 genes, which showed a fold change >0.6 in the microarray, were selected. RQ-PCR validation of leptin (*LEP*), insulin-like growth factor binding protein 1 (*IGFBP1*), *FOSB*, prolactin (*PRL*), and α-hemoglobin-stabilizing protein 1 (*AHSP*) genes in large for gestational age (n = 24) and appropriate for gestational age (n = 24) placentas. Gene expression values are presented as mean ± standard deviation (SD). \*P < .05, \*\*P < .01, and #P = .074.

*AHSP*, coding for α-hemoglobin-stabilizing protein, which provides protection against damaging oxidative reactions. The microarray analysis was partially confirmed, as placental expression of *LEP*,  $t(1, 46) = -2.82, P < .01$ , *PRL*,  $t(1,46) = 2.13, P < .05$ , and *AHSP*,  $t(1,46) = 2.71, P < .01$ , was significantly different in the LGA group, whereas *IGFBP-1*,  $t(1,46) = 1.83, P = .074$ , and *FOSB*,  $t(1,46) = 0.961$ , not significant, did not reach statistical significance in the RT-Q-PCR analysis (Figure 2).

### Discussion

In this case-control study, we show with genome wide microarray technique that there are surprisingly few differences in global gene expression pattern between placentas from nondiabetic healthy women giving birth to LGA infants and placentas from BMI-matched women giving birth to AGA infants. Possibly, the relatively low number of differently expressed genes is due to the fact that we specifically focused on healthy women giving birth to LGA infants and that matching for other known risk factors such as BMI and weight gain was successful. Nevertheless, the findings we obtained indicate that genes involved in lipid metabolism pathways, including leptin and prolactin, seem to be differently expressed in placentas from women giving birth to LGA infants.

The leptin gene was downregulated in placentas from women giving birth to LGA infants. Leptin is of interest, given its involvement in satiety control, regulation of body fatness, insulin resistance, and energy expenditure. During pregnancy, serum concentrations of leptin increase by the action of pregnancy-specific hormones and as a consequence of central leptin resistance.<sup>22</sup> However, the regulation of maternal leptin during pregnancy is complex, and production and regulation by



adipose tissue as well as the placenta are thought to contribute to the pregnancy-induced increase.<sup>23</sup> In relation to our results, it is well known that leptin levels in cord blood are related to infant size.<sup>24</sup> Further, although the relationship between maternal leptin levels and fetal weight has been difficult to establish,<sup>25</sup> a number of recent studies have suggested increased leptin to be a strong negative predictor for birth weight and LGA infant, even after adjustment for important covariates such as prepregnancy BMI and weight gain during pregnancy.<sup>26,27</sup> Our finding is also in line with studies suggesting that placentas from mothers with gestational diabetes (unless treated with insulin<sup>28,29</sup>) releases less leptin compared to mothers with normal pregnancies,<sup>30</sup> and findings from our group suggesting that also women with polycystic ovary syndrome have downregulated placental leptin and leptin receptor gene expression.<sup>31</sup> Finally, our finding is also in agreement with studies on placentas from intrauterine growth restriction (IUGR) pregnancies where leptin gene expression was increased, although the mechanism in IUGR may be compensatory due to hypoxia and thus different from the placental milieu of the LGA infant.<sup>32</sup>

Further, we showed that the gene expression of prolactin was increased in the placentas from the LGA pregnancies. Prolactin is important for fetal growth and serum levels in the pregnant woman increases throughout gestation.<sup>33</sup> Prolactin maintains and increases maternal food intake in the later stage of pregnancy by induction of central leptin resistance and promotes maternal (and fetal) beta-cell expansion and insulin production to defend against the development of gestational diabetes.<sup>34</sup> For this reason, it is surprising that only 1 study has evaluated prolactin in LGA infants, suggesting an inverse correlation between maternal prolactin levels in gestational week 27 and birth weight.<sup>35</sup> However, our findings are also in line with a prior study showing that the expression profile of placental growth hormone (*GH*)/chorionic somatomammotropin hormone genes, which all interact with the *GH/PRL*/cytokine receptors, are altered in placentas from pregnancies accompanied by SGA and LGA.<sup>36</sup> Clearly, our finding of increased *PRL* expression in the LGA placentas suggest that prolactin plays a role, although it is unclear if it is primary or compensatory, and further studies in LGA infants are warranted.

In addition to leptin and prolactin, validation also secured a role for the *AHSP* in LGA. During hemoglobin synthesis, the *AHSP* specifically binds free alpha-hemoglobin subunits, thus limiting the production of the reactive oxygen species. While the role of *AHSP* is unclear in LGA, previous studies have pointed to its relevance for placental function in that low expression levels have been associated with recurrent miscarriage,<sup>37</sup> severe preeclampsia and intrauterine fetal death.<sup>38</sup>

Expression of *IGFBP1* in LGA placenta was increased, although the difference did not reach statistical significance. Previous studies on placental *IGFBP1* have suggested increased placental expression in intrauterine growth restriction.<sup>32,39</sup> Again, the findings in LGA placenta may not be readily comparable with intrauterine growth restriction, the latter also being characterized by placental insufficiency and reduced availability of oxygen.<sup>40</sup>

In the IPA analysis, lipid metabolism came out as 1 important biological pathway in the placentas from women giving birth to LGA infants, exemplified for instance by *EGR1*, which controls lipolysis in adipocytes,<sup>41</sup> and *MUC* which directly regulates expression of a number of metabolic genes.<sup>42</sup> Previous studies have suggested altered placental expression of genes involved in lipid metabolism in obese mothers and in mothers with insulin-dependent diabetes mellitus (IDDM) or gestational diabetes.<sup>43-45</sup> For instance, Dube and colleagues reported an increase in placental lipoprotein lipase (LPL) activity and a decrease in solute carrier family 27 member 4 (*SLC27A4*) expression level in the placenta from obese women.<sup>46</sup> However, these changes in placental gene expression were not associated with altered birth weight of the infants. Further, Magnusson et al<sup>47</sup> reported an increased LPL activity in placentas of IDDM pregnancies by 39% which also corresponded to an increased birth weight of the infants in the IDDM group. Thus, our findings strengthen the hypothesis that lipid metabolism and transport is important not only for the fetal brain and eye development, the regulation of the fetal blood pressure, smooth muscle contraction, and blood clotting, but also that lipids may contribute as a source of energy to promote fetal growth. In addition, the similarities noted between our healthy patient population and previous studies in GDM underlines the fact that maternal hyperglycemia, although not fulfilling criteria for GDM, may be associated with increased risk of giving birth to an LGA infant.<sup>4</sup>

The cases included in this study were carefully chosen from a large bio bank, which made it possible to exclude those with possible confounders and investigate placentas from exposed and unexposed women with very similar characteristics. The randomly selected controls were matched on age, parity, first-trimester BMI, and gestational age at birth, and those cases and controls who had chronic diseases and/or daily medication were excluded. However, as the screening for GDM was based on clinical routine, and as the best screening strategy or glucose targets for GDM remains to be established,<sup>48</sup> it is however possible that undiagnosed cases with GDM may have been hidden among the cases. Additional limitations include the lack of blood samples from these women at the time of birth, why levels of leptin and prolactin at the time of birth could not be established.

## Conclusion

The *LEP* gene coding for leptin is downregulated and the *PRL* gene coding for prolactin is upregulated in placentas from women giving birth to LGA infants. The downregulation of leptin is consistent with findings in patients with gestational diabetes and polycystic ovary syndrome and with findings suggesting maternal leptin serum concentrations to be a negative predictor of LGA. The upregulation of the *PRL* gene is in line with the fact that prolactin is involved in appetite regulation via induction of central leptin resistance. Clearly, further studies on mechanisms leading to LGA are warranted in order to find

appropriate interventions in the short- and long-term perspective for these children and their mothers.

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### Authors' Note

F. Ahlsson and H. Åkerud contributed equally to this work.

### Declaration of Conflicting Interests

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