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Increased glucocerebrosidase expression and activity in preeclamptic placenta

J.M. Jebbink [a, b, *, 1], R.G. Boot [c, 1], R. Keijser [b], P.D. Moerland [d], J. Aten [e], G.J.M. Veenboer [b], M. van Wely [f], M. Buimer [a], E. Ver Loren van Themaat [d], J.M.F.G. Aerts [c], J.A.M. van der Post [a], G.B. Afink [b], C. Ris-Stalpers [a, b]

[a] Department of Obstetrics, Academic Medical Center, Amsterdam, The Netherlands
[b] Reproductive Biology Laboratory, Academic Medical Center, Amsterdam, The Netherlands
[c] Department of Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands
[d] Bioinformatics Laboratory, Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, The Netherlands
[e] Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands
[f] Center for Reproductive Medicine, Academic Medical Center, Amsterdam, The Netherlands

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ABSTRACT

Introduction: Lysosomal glucosidase beta acid (GBA) deficiency is inherent to Gaucher disease, Parkinsonism and Lewy-body dementia. Increased GBA expression has never been associated with human disease. We describe increased GBA expression and activity in placenta from preeclamptic pregnancies.
Methods: 112 placenta biopsies were available for qPCR, analysis of GBA gene expression and activity. Microanalysis was performed on 20 placenta samples. Alternatively spliced placental GBA transcripts were cloned, expressed in HEK293 cells and analyzed by Western blot and activity assay.
Results: GBA is expressed in the syncytiotrophoblast layer of human placenta already at 5 weeks of gestation. We identified five novel GBA transcripts in placenta that enzymatically inactive when expressed in HEK293 cells. Both GBA RNA expression and enzymatic activity are upregulated in preeclamptic placenta. Microarray analysis of 20 placenta tissues identified 158 genes co-regulating with GBA expression and gene enrichment analysis highlights lysosomal function. In our micro-array data GBA expression does not correlate with FLT1 expression, currently the most powerful marker for preeclampsia. There are 89 transcripts that are negatively correlated with *GBA* expression of which *BMP4* and *TFEB* are interesting as they are essential to early placenta function.
Discussion: Although very speculative, we hypothesize that increased GBA expression might relate to placentation through decreased *BMP4* signaling or vascularization through downregulation of *TFEB*. Ceramide, the product of hydrolysis of glucosylceramide by GBA and involved in the regulation of cell differentiation, survival and apoptosis, is another putative candidate linking increased GBA activity to preeclampsia. Both pathways merit further investigation.

1. Introduction

Normal placenta development is a prerequisite for successful pregnancy outcome. In about 10% of all pregnancies placentation is defective and maternal and fetal health are severely threatened by diseases as preeclampsia (de novo hypertension and proteinuria after 20 weeks of gestation), its rare variant Hemolysis Elevated Liver enzymes Low Platelets (HELLP) syndrome and intra uterine growth restriction. The only curative treatment for these complications is (often preterm) delivery [1].

Remodeling of the muscular wall of uterine arteries by invading extravillous trophoblasts during the first and early second trimester of pregnancy is essential. This process results in adequate maternal blood flow to the placenta that in turn ensures sufficient supply of nutrients and oxygen to the developing fetus. Preeclampsia is strongly associated with insufficient remodeling of the maternal spiral arteries [2,3]. Although many gene expression-based studies

* Corresponding author. Department of Obstetrics, Academic Medical Center, Amsterdam, The Netherlands.
E-mail address: j.m.jebbink@amc.uva.nl (J.M. Jebbink).
[1] Contributed equally to the work.

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comparing normotensive to preeclamptic placenta have identified the important role of anti-angiogenic proteins in preeclampsia [4,5], our understanding of the complete molecular sequence from aberrant invasion of extravillous trophoblasts to severe maternal clinical disease still contains many gaps.

In the current quest to identify a molecular placental preeclamptic signature we identified glucosidase beta acid (*GBA*) as a gene that is differentially over-expressed in preeclamptic versus normotensive placentas. The GBA gene encodes the enzyme glucocerebrosidase, involved in the penultimate lysosomal degradation step of glycosphingolipids and hydrolyzes glucosylceramide to free glucose and ceramide [6]. Glucosylceramide and ceramide are important structural components of the cell membrane. Moreover, ceramide is known to be a signaling molecule involved in the regulation of cell differentiation, survival and apoptosis [7–9].

GBA deficiency results in Gaucher disease [10], a lysosomal storage disorder with a characteristic accumulation of glucosylceramide in lysosomes of macrophages. More recently it has been recognized that reduced glucocerebrosidase activity is associated with an increased risk for Parkinsonism and Lewy-body dementia [9]. Pathological conditions associated with overexpression of glucocerebrosidase have not been described before. It has been long recognized that the placenta is remarkably rich in *GBA* content as it was initially used as a source to isolate GBA for the treatment of Gaucher patients with enzyme replacement therapy before the recombinant protein became available [10].

The current paper reports on the increased GBA mRNA expression and enzymatic activity in preeclamptic placenta, the detection of novel GBA transcripts in human placenta and the identification of genes and pathways correlating with GBA expression.

2. Results

2.1. GBA as novel candidate transcript separating normotensive from preeclamptic placenta

Based on further analysis (Supplementary Fig. 1) of previously reported placental SAGE libraries [11] a set of transcripts was selected for downstream expression analysis using quantitative Reverse Transcription PCR (RT-qPCR). The expression analysis was performed on placenta tissue of 17 normotensive and 14 preeclamptic pregnancies collected in RNAlater. Patient characteristics of both groups (Table 1A and Supplementary Table 1) are comparable, with the exception of parameters relating to preeclampsia (highest maternal diastolic blood pressure and neonatal weight).

The minimal transcript set that separates normotensive from preeclamptic placenta was determined by fitting 500 classification trees using repeated random sampling with training sets of 21 samples and test sets of 10 samples. Thirteen transcripts were included in at least 1 of the 500 signatures (Fig. 1A). Average classification accuracy of the 500 classification trees is 82% (95% CI: 60–100%), as illustrated by unsupervised hierarchical clustering of the expression levels of these 13 transcripts (Fig. 1B). *GBA*, *EBI3* (Epstein–Barr virus induced 3, a subunit to the interleukins IL-27 and IL-35) [12], and *TFPI* (tissue factor pathway inhibitor, a protease inhibitor regulating the tissue factor dependent pathway of blood coagulation) [13] were identified as the transcripts that optimally separate normotensive from preeclamptic placenta (Fig. 1A). Of the other 3 transcripts included in at least 4 signatures (*BCAR3*, *TIMP3*, *PLIN2*) only the expression of *TIMP3* (TIMP metallopeptidase inhibitor 3, inhibits peptidases involved in the degradation of the extracellular matrix) [14] does not show a strong correlation with *GBA*, *EBI3*, and *TFPI* (data not shown) and was therefore also selected for further experiments. To validate results obtained in the first tissue cohort, expression levels of *GBA*, *EBI3*, *TFPI* and *TIMP3* were determined in additional placenta samples from 47 normotensive and 34 preeclamptic pregnancies. Patient characteristics of both groups (Table 1B and Supplementary Table 1) are comparable with the exception of parameters relating to preeclampsia (highest maternal diastolic blood pressure, occurrence of HELLP syndrome, gestational age at delivery and neonatal weight). Using repeated random sampling with training sets of 55 samples and test sets of 26 samples, the *GBA* transcript is included in 429 out of the total 500 classification tree signatures (Fig. 1C). This identifies GBA as the prime transcript that without additional support from the other 3 transcripts can best distinguish normotensive from preeclamptic placenta. Average classification accuracy of the 500 classification trees is 65% (95% CI: 46–81%).

Table 1
Clinical Characteristics Summary of the first (A) and second (B) patient cohort for RT-q-PCR validation of SAGE results. Data are represented as median (range) or numbers (%). *P*-values were calculated by Mann–Whitney *U* test*, Chi-square# or Fisher's exact test§. Abbreviations: n.a. not applicable, n.d. not determined, n.s. not significant, P Birth percentile <10 below refers to the neonatal sex and gestational age specific Dutch neonatal weight charts available at www.perinatreg.nl. ^ Within cohort A one case of preexisting hypertension with superimposed preeclampsia and 1 case of pregnancy induced hypertension in the absence of proteinuria at the second measurement; within cohort B one case with single high blood pressure measurement while all other blood pressure measurements were within the normal range.

A: Clinical parameter	Normotensive $n = 17$	Preeclamptic $n = 14$	*p*-value
Highest diastolic BP (mmHg)	75 (60–85)	105 (95–120)	$p < 0.0001$*
Urinary protein (g/24 h)	n.d.	3.18 (0.15–17.6)	n.a.
HELLP	0 (0%)	3 (21%)	n.s.§
Gestational age at delivery (weeks)	31^{+0} (27^{+0} –38^{+4})	32^{+3} (28^{+5} –38^{+2})	n.s.*
Female neonates	9 (53%)	8 (57%)	n.s. #
Neonatal weight (g)	1740 (980–3900)	1195 (660–2725)	$p < 0.05$*
Birth percentile <10	0 (0%)	4 (29%)	$p = 0.03$ §
B: Clinical parameter	**Normotensive $n = 47$**	**Preeclamptic $n = 34$**	***p*-value**
Highest diastolic BP (mmHg)	75 (60–94^)	110 (95–185)	$p < 0.0001$*
Urinary protein (g/24 h)	n.d.	3.30 (0.3–13.95)	n.a.
HELLP	0 (0%)	14 (41%)	$p < 0.0001$§
Gestational age at delivery (weeks)	37^{+5} (26^{+2} –42^{+0})	34^{+4} (27^{+0} –41^{+0})	$p < 0.01$*
Female neonates	21 (45%)	15 (50%)	n.s.#
Neonatal weight (g)	2710 (710–4225)	1830 (600–3990)	$p < 0.01$*
Birth percentile <10P	7 (15%)	8 (24%)	n.s. #

2.2. GBA mRNA expression and enzyme activity is increased in preeclamptic placenta

GBA mRNA expression in preeclamptic placenta ($n = 48$) as determined by quantitative real time RT-PCR is increased compared to normotensive placenta ($n = 64$) (Fig. 2A). GBA mRNA expression does not correlate ($p = 0.38$) with gestational age in normotensive placenta ($n = 64$). In preeclamptic placenta ($n = 48$) there is a negative correlation ($p = 0.031$) between GBA expression and gestational age (data not shown).

From 33 of the 48 preeclamptic and 45 of the 64 normotensive placenta tissues, sufficient material was available for the determination of GBA enzyme activity using 4-MU-β-glucoside as substrate [15]. In line with mRNA expression levels, GBA enzymatic activity is increased in preeclamptic placenta ($p = 0.017$; Fig. 2B). The correlation between GBA mRNA expression and enzyme activity in