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Comparing human and macaque placental transcriptomes to disentangle preterm birth pathology from gestational age effects

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ABSTRACT

Introduction: A major issue in the transcriptomic study of spontaneous preterm birth (sPTB) in humans is the inability to collect healthy control tissue at the same gestational age (GA) to compare with pathologic preterm tissue. Thus, gene expression differences identified after the standard comparison of sPTB and term tissues necessarily reflect differences in both sPTB pathology *and* GA. One potential solution is to use GA-matched controls from a closely related species to tease apart genes that are dysregulated during sPTB from genes that are expressed differently as a result of GA effects.
Methods: To disentangle genes whose expression levels are associated with sPTB pathology from those linked to GA, we compared RNA sequencing data from human preterm placentas, human term placentas, and rhesus macaque placentas at 80% completed gestation (serving as healthy non-human primate GA-matched controls). We first compared sPTB and term human placental transcriptomes to identify significantly differentially expressed genes. We then overlaid the results of the comparison between human sPTB and macaque placental transcriptomes to identify sPTB-specific candidates. Finally, we overlaid the results of the comparison between human term and macaque placental transcriptomes to identify GA-specific candidates.
Results: Examination of relative expression for all human genes with macaque orthologs identified 267 candidate genes that were significantly differentially expressed between preterm and term human placentas. 29 genes were identified as sPTB-specific candidates and 37 as GA-specific candidates. Altogether, the 267 differentially expressed genes were significantly enriched for a variety of developmental, metabolic, reproductive, immune, and inflammatory functions. Although there were no notable differences between the functions of the 29 sPTB-specific and 37 GA-specific candidate genes, many of these candidates have been previously shown to be dysregulated in diverse pregnancy-associated pathologies.
Discussion: By comparing human sPTB and term transcriptomes with GA-matched control transcriptomes from a closely related species, this study disentangled the confounding effects of sPTB pathology and GA, leading to the identification of 29 promising sPTB-specific candidate genes and 37 genes potentially related to GA effects. The apparent similarity in functions of the sPTB and GA candidates may suggest that the effects of sPTB and GA do not correspond to biologically distinct processes. Alternatively,

Abbreviations: PTB, preterm birth; sPTB, spontaneous, idiopathic preterm birth; GA, gestational age; FPKM, fragments per kilobase per million base pairs.

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it may reflect the poor state of knowledge of the transcriptional landscape underlying placental development and disease.

1. Introduction

Preterm birth (PTB), or birth before 37 completed weeks of gestation in humans, is a global health issue affecting at least 15 million newborns every year [1–3]. This complex, multifactorial syndrome accounts for around 1 million neonatal deaths annually and surviving neonates often require lifelong care for common comorbidities including developmental, visual, and digestive problems [4,5]. 30% of PTB cases are indicated by medical conditions such as preeclampsia or intrauterine growth restriction, while the remaining 70% are caused by the spontaneous onset of labor either with (25%) or without (45%) premature membrane rupture [6,7].

Spontaneous, idiopathic preterm birth (sPTB), much like most other complex human genetic diseases, is augmented by environmental risk factors (e.g., stress, infection, and socioeconomic status) as well as by genetics. Several studies have shown that women are more likely to deliver preterm if a sister delivered preterm, if a previous child was born preterm, if they were born preterm themselves, or if they have African American ancestry [8–11]. In recent years, studies have also highlighted the importance of gene expression regulation in complex genetic diseases [12]. Thus, analysis of the genetic elements that are active or dysregulated in gestational tissues harbors great potential to identify candidate genes for sPTB and several genome-wide studies have already started to outline its genomic, transcriptomic, and methylomic architecture [13].

Nevertheless, a major obstacle in the transcriptomic study of sPTB in humans is the inability to collect gestational age-matched healthy control tissue to compare with pathologic preterm tissue. Without safe, non-invasive procedures to sample healthy preterm tissues destined for healthy term births, the most common approach is to use healthy term tissues as the control for pathologic preterm tissues [14–16]. This complicates downstream data analysis, though, because observed differences in gene expression reflect not only differences in pathology, but also differences in gestational age (GA).

One potential solution is to use GA-matched controls from a closely related species to distinguish genes dysregulated during sPTB from genes expressed differently at different points in pregnancy. The decoding of the rhesus macaque (*Macaca mulatta*) genome and subsequent comparison with that of human and chimpanzee revealed that these 3 primate species share about 93% of their DNA [17]. Thus, macaque is an ideal species for transcriptional comparison with humans not only because the two species share a close evolutionary affinity but also because of similarities with respect to key pregnancy-related traits. For example, even though placental morphology is highly variable across mammals, human and macaque placentas share the same discoid shape, hemochorial invasiveness, and villous interdigitation [18,19]. Similarly, the relationship between pelvis and fetal head size in humans is more akin to the relationship in macaques than it is to any other primates [20]. This is particularly important as it would alleviate any effects that cephalopelvic constraints might have on birth timing [21]. Finally, several human pregnancy pathologies have been recorded in macaques including stillbirth, PTB, placenta previa, and placental abruption [22].

In this study, we compare transcriptomes from human sPTB placentas, human term placentas, and macaque placentas at 80% completed gestation to distinguish between sPTB-specific and GA-specific candidate genes. Specifically, candidate genes that are differentially expressed between human sPTB and human term as well as between human sPTB and macaque are potentially sPTB-specific. In contrast, candidate genes that are differentially expressed between human sPTB and human term as well as between human term and macaque are potentially GA-specific. This novel comparative approach disentangles the confounding effects of sPTB and GA differences and allows for the educated prioritization of candidate genes for future studies of pregnancy and prematurity.

2. Methods

2.1. Tissue collection, RNA isolation, and RNA sequencing

Human placentas were collected immediately after delivery and the decidua basalis layer from a central cotyledon was dissected and discarded. Approximately 1 g of underlying villous tissue was then biopsied for further analysis. The 5 term (GA 38–39 weeks, mean 38 or 95% completed gestation) human placental tissue biopsies were all collected after cesarean delivery. Of the 5 preterm (GA 29–33 weeks, mean 32 or ~80% completed gestation) human placental tissue biopsies that were collected, 4 were collected after cesarean delivery and 1 after vaginal delivery. Each of the biopsies was flash frozen in liquid nitrogen and stored at −80 °C [23]. Total RNA was isolated using TRIzol and Illumina libraries were constructed using the TruSeq Stranded Total RNA Sample Prep Kit with Ribo-Zero Gold. RNA sequencing (RNA-seq) was performed on an Illumina HiSeq 2500 machine using HiSeq version 3 sequencing reagents. The samples were sequenced using a single-end approach with 50 bp reads, generating approximately 30 million reads per sample. Raw count data have been deposited in the NCBI Gene Expression Omnibus (GEO) database and are accessible through GEO Series accession number GSE73714 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73714).

Macaque placentas were collected immediately after delivery and full thickness biopsies (~2cmx2cm) free of clots and debris were taken midway between the attachment of the umbilical cord and the placenta edge. Both placental tissue samples were collected after cesarean delivery via hysterotomy (GA 128–131 days, mean 129.5 or ~80% completed gestation) [24]. Total RNA was isolated from 100 μg of frozen tissue using TRIzol and suspensions were stored at −80 °C. RNA-seq was performed on an Illumina HiSeq machine after passing initial quality control metrics. The two samples were sequenced using a paired-end approach with 50 bp reads, generating approximately 15 million paired reads per sample.

2.2. Data processing and differential expression analysis

RNA-seq data was analyzed from the 5 term and 5 preterm human placental tissue samples as well as the 2 macaque placental samples from 80% completed gestation. Raw sequence read files were first quality checked using FastQC and then trimmed for

quality and adapter sequences using Trimmomatic with the default parameters (LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36) [25]. Across all 10 human cDNA libraries, a total of 311.9 million reads were sequenced and subsequently mapped to the hg19 human reference genome using TopHat2 (Additional File 1, Additional File 2) [26]. Across the 2 macaque cDNA libraries, a total of 29.8 million read pairs were sequenced and subsequently mapped to the Mmul 1.0 macaque reference genome using TopHat2 (Additional File 1, Additional File 2).

Given our interspecies transcriptome comparisons, we restricted all analyses to 19,063 one-to-one (1:1) human-macaque orthologs obtained from Zimin et al. [27]. Specifically, only sequence reads that uniquely aligned to these 19,063 human or macaque genes were counted using HTSeq, resulting in 18,879 genes with expression values in both species (Additional File 3, Additional File 4). DESeq2 was used to quantify relative gene expression differences in terms of fold change (log2) and statistical significance (Benjamini Hochberg-corrected *p*-values), where positive fold change represents over-expression in human sPTB compared to human term (or human sPTB/term compared to macaque) and negative fold change represents under-expression in human sPTB compared to human term (or human sPTB/term compared to macaque) [28]. Genes were annotated as differentially expressed if the adjusted *p*-value was <0.1.

2.3. Functional enrichment analysis

GO Biological Process term enrichment was calculated using the Cytoscape plugin BiNGO [29]. Terms were considered significant if the adjusted *p*-value was <0.1 after the Benjamini-Hochberg multiple testing correction. FPKM for each gene was calculated across all human sPTB, human term, and macaque transcriptomes and associated RNA and protein expression data was extracted from Protein Atlas using GEneSTATION [30–32].

3. Results

3.1. RNA sequencing and transcriptome analysis

Analysis of 311.9 million reads from 5 term and 5 preterm human placental samples resulted in the successful mapping of 298.4 million reads (95%). 265.9 million (85%) of these mapped to a single position in the human genome and 96.9 million (32%) mapped to human genes that share 1:1 orthology with macaque genes. Analysis of 29.8 million reads from 2 macaque placental samples at 80% completed gestation resulted in the successful mapping of 28.7 million read pairs (96%). 26.6 million (89%) of these mapped to a single position in the macaque genome and 25.1 million (87%) mapped to macaque genes that share 1:1 orthology with human genes. Notably, the correlation coefficient between human sPTB samples ranges from 0.84 to 0.95, between human term samples ranges from 0.95 to 0.98, and between rhesus samples is 0.89 (Additional File 2). This degree of variation between samples is on par with the degree of variation reported between samples from other much more homogeneous tissues [33].

3.2. Distinguishing sPTB-specific and GA-specific candidate genes

To identify sPTB-specific and GA-specific candidate human genes, we performed three pairwise differential expression comparisons: human sPTB vs. human term, human sPTB vs. macaque (GA-matched control), and human term vs. macaque (healthy, early gestation comparison) (Fig. 1a). We identified 267 genes that were differentially expressed between human sPTB and term (149 over-expressed and 118 under-expressed), 12,379 genes that were differentially expressed between human sPTB and macaque (7193 over-expressed and 5186 under-expressed), and 12,566 genes that were differentially expressed between human term and macaque (7285 over-expressed and 5281 under-expressed) (Additional file 5).

Taken alone, differentially expressed genes from the human sPTB and human term comparison presumably represent expression differences that may be attributable to either sPTB pathology or GA. To further distinguish the 267 differentially expressed genes between those that represented sPTB-specific candidates or GA-specific candidates, we intersected the candidate gene results of all three differential expression experiments. This approach allowed for the identification of 29 sPTB-specific candidate genes and 37 GA-specific candidate genes (Fig. 1b, Table 1, Table 2, Additional file 6). 23/29 (70%) of the sPTB-specific genes were over-expressed in sPTB and only 6/29 (21%) were under-expressed. In contrast, 18/37 (49%) of the GA-specific genes were over-expressed in sPTB and 19/37 (51%) were under-expressed (Fig. 1b, Additional file 6).

3.3. Differentially expressed genes were enriched for developmental and metabolic functions

All together, these 267 genes were significantly enriched for involvement in a wide variety of developmental processes (e.g., GO:0005515) (Additional File 7). Furthermore, the gene set as a whole was enriched for function in metabolism, reproduction, immunity, inflammation, and cell signaling. Although statistical significance is limited due to small gene set size, the 29 sPTB-specific genes were involved in transcription factor activity and binding (e.g., GO:0008134 and GO:0005515) and the 37 GA-specific genes were involved in development and response to stimuli (e.g., GO:0007275 and GO:0009628).

3.4. Placental sPTB-specific and GA-specific candidate genes are heterogeneously expressed

Given the high heterogeneity of gene expression that has been previously reported in placental transcriptome studies, we evaluated the transcriptional profile of sPTB-specific and GA-specific candidate genes by comparing their relative expression to publicly available expression data from Protein Atlas [13,34]. 27 of the 29 sPTB-specific candidates and 34 of the 37 GA-specific candidates had been previously identified for placental gene expression at varying levels in Protein Atlas (Fig. 2, Additional File 8). Although mean expression levels of these candidate genes in human sPTB, human term, and macaque placentas were generally comparable to expression levels presented by Protein Atlas, our data also reflect the known variability in placental gene expression both within and between individuals [34]. For example, *HTRA4* was highly expressed across our sPTB transcriptomes (FPKM = 75.4) and lowly expressed across our term transcriptomes (FPKM = 11.7), but Protein Atlas reported an intermediate expression level (FPKM = 43.8).

4. Discussion

A major obstacle in the transcriptomic study of PTB in humans is the inability to collect healthy, GA-matched control tissue samples that facilitate the comparison of preterm diseased tissue to healthy tissue from a corresponding point in pregnancy. This complicates interpretation of results since candidate genes necessarily reflect both sPTB pathology as well as GA differences. Our comparative approach utilizing the transcriptomic profiles of rhesus macaque placental samples from 80% gestation overlaid with transcriptomic profiles of human preterm and term placental tissue allows for the

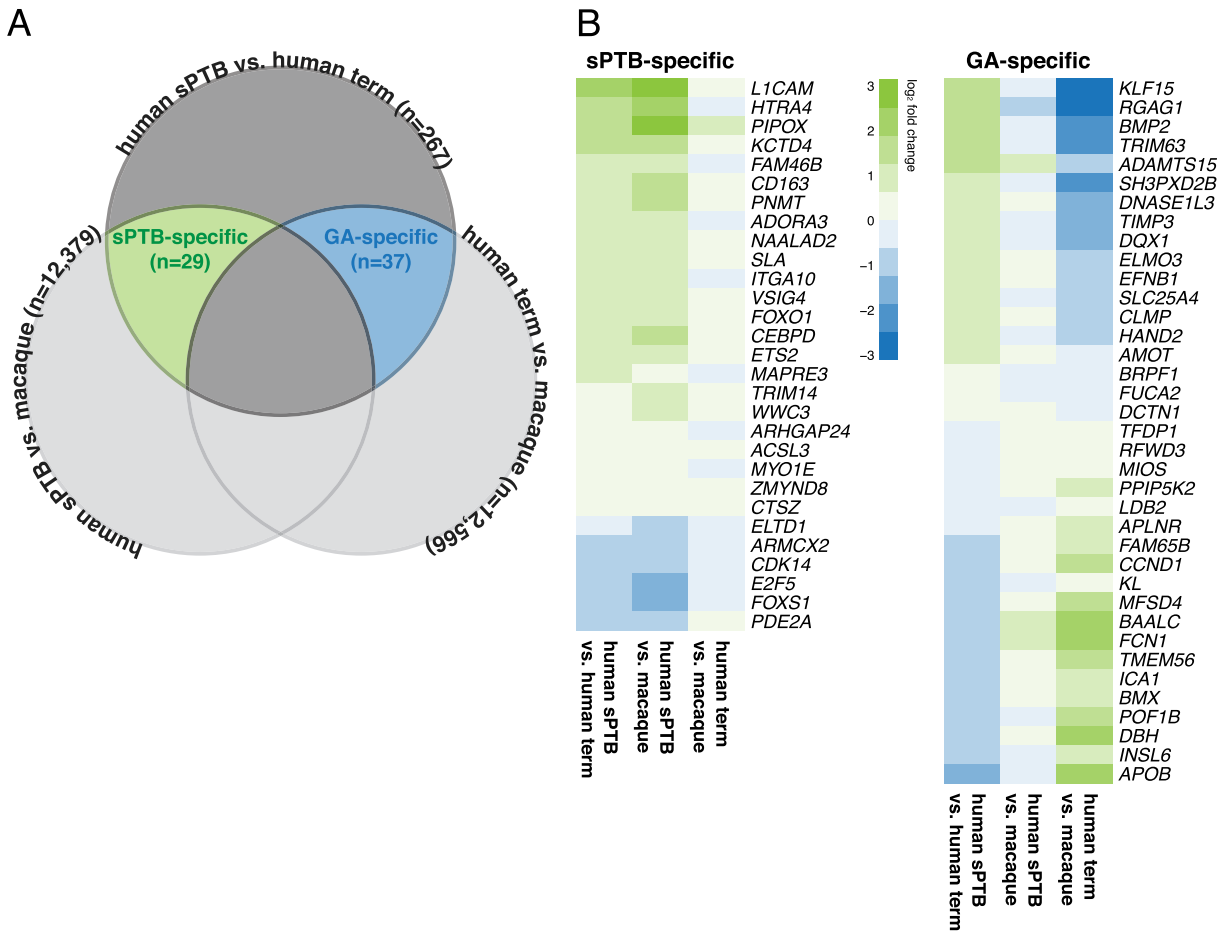

Fig. 1. A comparative approach to disentangling the effects of PTB and GA in human transcriptomic studies. (A) RNA-seq data from macaque placentas collected at 80% gestation serves as healthy nonhuman primate gestational age controls to disentangle the involvement of sPTB from that of gestational age when comparing human sPTB and term placentas. Differentially expressed genes were first identified between human sPTB and human term placentas and then intersected with differentially expressed genes from 2 other pairwise comparisons (human sPTB vs. macaque and human term vs. macaque). This intersection allowed for the categorization of the initially identified differentially expressed human sPTB vs. human term genes (dark grey, n = 267) as sPTB-specific (green, n = 29) or GA-specific (blue, n = 37). (B) For each of the 3 pairwise transcriptomic comparisons, $\log_2$ fold change values were plotted corresponding to each of the 29 sPTB-specific gene candidates as well as the 37 GA-specific gene candidates. For consistency, the macaque transcriptomes were used as controls in both inter-species comparisons and, therefore, the direction of fold changes in the human term vs. macaque comparison for GA-specific genes is reversed.

disentanglement of these variables and, thus, the identification of genes with roles specific to sPTB pathology or GA.

Generally, most genes in the sPTB-specific and GA-specific categories have previously been annotated for placental tissue expression in Protein Atlas, sometimes uniquely so, although the level of expression often differed, in line with previous work on the high heterogeneity of placental gene expression (Fig. 2) [34]. Moreover, the 29 genes in the sPTB-specific category included several previously identified for involvement in pregnancy pathologies. For example, *HTRA4*, a serine peptidase, is over-expressed during early-onset preeclampsia [35]. *CD163*, a hemoglobin scavenger receptor expressed exclusively in macrophages, is over-expressed in the preterm preeclamptic decidua and has also been identified as a predictor of preterm birth in maternal serum [36,37]. The presence of *CD163* among differentially expressed genes in our data may indicate that some maternal decidual tissue (and thus, macrophages) was inadvertently captured during placental biopsy. *ADORA3*, an adenosine receptor, is over-expressed in preeclamptic trophoblasts and also modulates secretion of matrix metalloproteinases that serve as important components in PPROM signaling pathways [38]. *VSIG4* encodes a protein with an immunoglobulin domain that has been characterized as a maternal biomarker of preeclampsia [39,40]. *PDE2A* is a phosphodiesterase and the gene contains a SNP associated with idiopathic recurrent miscarriage [41]. Finally, *NAALAD2* has been shown to be under-expressed in decidua during preeclampsia [42]. Our results indicate that pathways previously identified as involved in other pregnancy pathologies may also be involved in sPTB pathogenesis.

The 37 genes in the GA-specific category also included several previously identified for involvement in pregnancy pathologies. For example, *BMP2*, an extracellular growth factor, is over-expressed in fetal membranes during spontaneous term labor and preterm labor with chorioamnionitis [43,44]. *TIMP3*, a matrix metallopeptidase inhibitor, is expressed in fetal membranes during labor and has also been shown to be hypomethylated during preeclampsia [45–47]. *APLNR*, a G-protein coupled receptor, is a key receptor of apelin, a gene that is under-expressed during term and preterm labor in amnion as well as during preeclampsia in placenta [48,49]. *KL*, or klotho, is under-expressed in pregnancies where the neonate is small-for-gestational-age [50]. *INSL6* is a member of the relaxin family of peptide hormones and, although little is known about *INSL6* specifically, relaxin expression at the fetomaternal interface has been linked to PPROM pathogenesis and serum relaxin concentration has been identified as a potential PTB biomarker [51,52].

Table 1
sPTB-specific genes.

Symbol	Description	Fold change (log2)[a]	Adjusted *p*-value[a]
L1CAM	L1 cell adhesion molecule	2.00	5.63E-05
PNMT	phenylethanolamine N-methyltransferase	1.02	3.58E-04
MYO1E	myosin 1E	0.39	5.42E-04
ARMCX2	armadillo repeat containing, X-linked 2	−0.66	1.24E-03
PDE2A	phosphodiesterase 2A, cGMP-stimulated	−1.03	1.44E-03
MAPRE3	microtubule-associated protein, RP/EB family, member 3	0.65	2.47E-03
CD163	CD163 molecule	1.13	5.98E-03
ETS2	v-ets avian erythroblastosis virus E26 oncogene homolog 2	0.70	1.22E-02
CTSZ	cathepsin Z	0.32	1.22E-02
CDK14	cyclin-dependent kinase 14	−0.76	1.22E-02
VSIG4	V-set and immunoglobin domain containing 4	0.81	1.50E-02
NAALAD2	N-acetylated alpha-linked acidic dipeptidase 2	0.89	1.75E-02
E2F5	E2F transcription factor 5, p130-binding	−0.92	2.44E-02
HTRA4	HtrA serine peptidase 4	1.50	2.63E-02
KCTD4	potassium channel tetramerization domain containing 4	1.26	2.83E-02
TRIM14	tripartite motif-containing 14	0.60	3.19E-02
ZMYND8	zinc finger, MYND-type containing 8	0.38	3.72E-02
ARHGAP24	Rho GTPase activating protein 24	0.54	3.79E-02
FOXO1	forkhead box 01	0.80	4.06E-02
FOXS1	forkhead box S1	−1.01	4.43E-02
ITGA10	integrin, alpha 10	0.83	4.93E-02
SLA	Src-like-adaptor	0.85	4.97E-02
ACSL3	acyl-CoA synthetase long-chain family member 3	0.41	4.97E-02
FAM46B	family with sequence similarity 46, member B	1.18	6.46E-02
ELTD1	adhesion G protein-coupled receptor L4	−0.57	6.51E-02
WWC3	WWC family member 3	0.55	6.53E-02
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	0.80	7.03E-02
ADORA3	adenosine A3 receptor	1.01	7.09E-02
PIPOX	pipecolic acid oxidase	1.27	7.40E-02

[a] Values from human sPTB vs. human term comparison.

EFNB1 may play a role in cell adhesion and has been shown to be differentially expressed during preeclampsia [53]. *ADAMTS15* is a member of a disintegrin and metalloproteinase with thrombospondin motifs protein family and has been previously identified as dysregulated during PPROM [54]. *KLF15* has been shown to be under-expressed in the preeclamptic decidua [42]. Finally, *APOB* is a low density lipoproteins and variation in the gene sequence has been associated with preterm delivery [55]. Our identification of these genes as GA candidates raises the hypothesis that they might be differentially expressed due to differences in gestational age of the tissues being compared rather than due to the underlying pathology. Alternatively, these genes might be involved in both disease *and* development.

In addition to involvement in pregnancy pathologies, some sPTB-specific candidate genes have been annotated for involvement in more general biological processes related to healthy pregnancy, labor, and placentation. For example, *FOXO1*, is a potential modulator of inflammatory events in the myometrium during labor and its expression in endometrium is regulated by progesterone [56,57]. *CEBPD* is a transcription factor and, through its interaction with *CEBPA*, is involved in the regulation of immune and inflammatory responses in various gestational tissues as well as in the development of fetal lungs [58,59]. *ETS2* has been shown to mediate matrix metalloproteinase activity and trophoblast invasion [60]. *ARHGAP24* is a RHO GTPase activator involved in myometrium contractility and shows increased mRNA expression in myometrium during labor [61]. *E2F5* is a member of the *E2f* transcription factor family and has been shown to help coordinate placental development in mice [62]. *FAM46B* has been shown to be under-expressed in myometrium and cervix during labor [58]. Finally, *PNMT* is an enzyme known to be expressed in myometrium and fetal membranes that shows decreased enzymatic activity in myometrium during labor [63–65]. GA-specific candidate genes have similarly been annotated for involvement in biological processes related to healthy pregnancy and labor. For example, *HAND2* is a progesterone-regulated transcription factor that is expressed in myometrium and essential for embryo implantation and *FCN1* is involved in innate immune defense and over-expressed in myometrium and cervix during labor (50,58).

Comparison of the functions of sPTB-specific candidate genes with those of GA-specific candidate genes, however, does not identify any notable differences. For example, both sPTB-specific and GA-specific candidate genes include developmental genes (GO:0032502) (e.g., *E2F5*, *ARHGAP24*, *ETS2*, *ACSL3*, *MYO1E*, *PDE2A*, *FOXS1*, *CTSZ*, *L1CAM*, and *FOXO1* for sPTB, and *CLMP*, *FAM65B*, *CCND1*, *KL*, *KLF15*, *BMX*, *INSL6*, *LDB2*, *EFNB1*, *HAND2*, *APOB*, *BMP2*, *DNASE1L3*, *SH3PXD2B*, *TIMP3*, *APLNR*, *AMOT*, and *DCTN1* for GA, respectively), genes involved in immunity-related functions (GO:0002376) (e.g., *L1CAM*, *TRIM14*, *FOXO1*, and *VSIG4* for sPTB, and *KL*, *DBH*, *BMX*, *APOB*, *DCTN1*, and *FCN1* for GA, respectively), and genes involved in stress response (GO:0006950) (e.g., *TRIM14*, *CD163*, *FOXO1*, and *VSIG4* for sPTB, and *FCN1*, *CCND1*, *BMP2*, *KL*, *DBH*, *KLF15*, *BMX*, *MIOS*, *RFWD3*, and *DCTN1* for GA, respectively).

Similarity between the functions of sPTB-specific and GA-specific candidates may genuinely reflect the idea that dysregulation during sPTB pathology is not a biologically separate and distinct process from GA. Alternatively, the overlap of functions may reflect our poor state of knowledge of the transcriptional landscape underlying placental development and disease. For example, the vast majority of transcriptomic studies on placental tissues have traditionally focused on identifying the impact of environmental factors or known clinical subtypes in placental gene expression, heavily biasing any knowledge of the functions of placental genes toward disease (as opposed to developmental or physiological) phenotypes [13]. Furthermore, only a handful of studies have mapped the transcriptional changes occurring during placental development and, given the inability to collect healthy

Table 2
GA-specific genes.

Symbol	Description	Fold change (log2)[a]	Adjusted *p*-value[a]
ICA1	islet cell autoantigen 1, 69 kDa	−1.08	2.49E-04
EFNB1	ephrin-B1	0.78	3.58E-04
TMEM56	transmembrane protein 56	−1.05	3.58E-04
KL	klotho	−0.83	1.62E-03
BMX	BMX non-receptor tyrosine kinase	−1.09	2.47E-03
BMP2	bone morphogenetic protein 2	1.53	2.49E-03
SH3PXD2B	SH3 and PX domains 2B	1.19	2.87E-03
KLF15	Kruppel-like factor 15	1.76	3.05E-03
TIMP3	TIMP metallopeptidase inhibitor 3	0.93	5.69E-03
DCTN1	dynactin 1	0.25	5.83E-03
PPIP5K2	diphosphoinositol pentakisphosphate kinase 2	−0.46	5.98E-03
RGAG1	retrotransposon gag domain containing 1	1.54	8.67E-03
ADAMTS15	ADAM metallopeptidase with thrombospondin type 1 motif, 15	1.29	1.29E-02
ELMO3	engulfment and cell motility 3	0.81	1.29E-02
INSL6	insulin-like 6	−1.26	1.33E-02
APOB	apolipoprotein B	−1.55	1.75E-02
LDB2	LIM domain binding 2	−0.57	2.02E-02
APLNR	apelin receptor	−0.62	2.41E-02
POF1B	premature ovarian failure, 1B	−1.12	2.66E-02
MIOS	missing oocyte, meiosis regulator, homolog (Drosophila)	−0.38	2.97E-02
MFSD4	major facilitator superfamily domain containing 4	−0.84	3.83E-02
RFWD3	ring finger and WD repeat domain 3	−0.37	4.01E-02
AMOT	angiomotin	0.69	4.30E-02
DQX1	DEAQ box RNA-dependent ATPase 1	0.92	4.32E-02
DBH	dopamine beta-hydroxylase (dopamine beta-monooxygenase)	−1.26	4.37E-02
TRIM63	tripartite motif containing 63, E3 ubiquitin protein ligase	1.40	4.43E-02
SLC25A4	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	0.78	4.63E-02
BRPF1	bromodomain and PHD finger containing, 1	0.31	5.22E-02
FCN1	ficolin (collagen/fibrinogen domain containing) 1	−0.99	5.50E-02
DNASE1L3	deoxyribonuclease I-like 3	1.14	6.31E-02
BAALC	brain and acute leukemia, cytoplasmic	−0.92	6.70E-02
CCND1	cyclin D1	−0.74	7.40E-02
TFDP1	transcription factor Dp-1	−0.29	8.20E-02
HAND2	hand and neural crest derivatives expressed 2	0.71	8.61E-02
CLMP	CXADR-like membrane protein	0.74	8.69E-02
FAM65B	family with sequence similarity 65, member B	−0.71	8.69E-02
FUCA2	fucosidase, alpha-L-2, plasma	0.25	9.45E-02

[a] Values from human sPTB vs. human term comparison.

tissue from multiple time points, these studies are typically limited to comparing the transcriptional landscapes of first and third-trimester placentas [66–68]. Interestingly, only 2 of the 37 GA-specific genes in this study (*LDB2* and *BMP2*) have been previously annotated as differentially expressed between early and late pregnancy [66,67].

Although our comparative analysis allowed for the more detailed categorization of otherwise general candidate genes, comparison of transcriptomes across species has several important caveats. For example, biological and analytical differences such as those stemming from alternative splicing, annotation heterogeneity, and genetic variation are potential sources of 'noise' [69–71]. The development and increasingly wide-spread use of RNA-seq, however, has facilitated a more straightforward inter-species transcriptome comparison due to the breadth and depth of expression data generated by this approach [33,71–73]. Like previous inter-species transcriptomics experiments, our comparative analysis was limited only to 1:1 orthologs in order to facilitate sensible comparison and, even further, the human-macaque transcriptome comparisons were overlaid with a direct human-human comparison as an attempt to filter out potentially large amounts of expected gene expression differences. Still, the results of inter-species transcriptome comparisons must be carefully interpreted due to the many inherent differences in gene expression and its regulation across species.

Additionally, this study would have benefited from the inclusion of term macaque placental tissue samples. Although unavailable at the time of analysis, these samples would allow for an understanding of gene expression in term macaque placentas that could then be compared with the other groups in our analysis. Furthermore, gestation length and the timing of some early developmental events differ between human and macaque, although little is known about comparative placentation towards the end of pregnancy [22]. Therefore, the use of placenta collected at 80% completed gestation in macaque may not be the ideal time point for comparison with human sPTB.

These caveats notwithstanding, our results demarcate how a comparative transcriptomics approach to the study of human sPTB allows for the identification and prioritization of candidate genes and pathways involved specifically in sPTB pathogenesis and GA changes during pregnancy. Although a handful of recent studies have analyzed genome-wide gene expression in the human term (and occasionally preterm) placenta, ethical issues prevent comparisons of sPTB tissue to a healthy tissue at the same GA in humans. Our novel, comparative approach is the first to utilize GA-matched control placental tissue from a closely related species for comparison with human term and preterm placental tissue samples. Despite the challenges inherent in inter-species transcriptome comparisons, the use of RNA-seq data and well-annotated reference genomes makes possible the human-macaque comparison and ultimate prioritization of otherwise convoluted differentially expressed gene sets as promising sPTB-specific candidates.

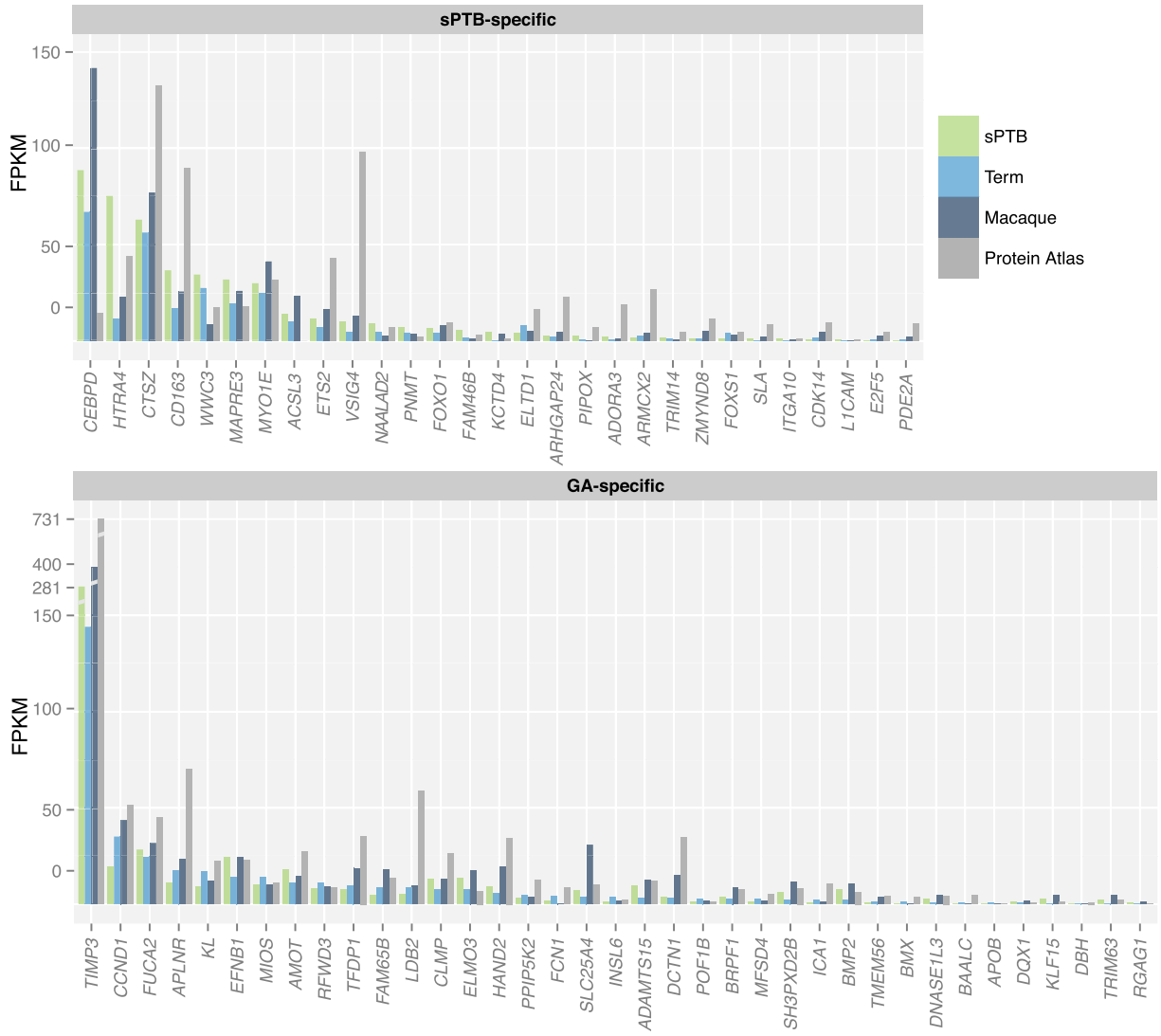

Fig. 2. sPTB-specific and GA-specific candidate genes show heterogeneous expression patterns in placenta. For sPTB-specific (top panel) and GA-specific (bottom panel) candidate genes, mean mRNA expression in Fragments Per Kilobase of transcript per Million mapped reads (FPKM) was compared between the 5 human sPTB, 5 human term, and 2 macaque placental samples as well as to data from Protein Atlas.

Author contributions

HRE and AR designed the study. WEA, IAB, and CSB provided human RNA-seq data and MP, CDF, SGK and LJM provided macaque RNA-seq data. HRE performed the analysis with assistance from DCR. HRE drafted the manuscript with contributions and revisions from all other authors. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2016.03.006.

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