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OXFORD

High-throughput mRNA-seq atlas of human placenta shows vast transcriptome remodeling from first to third trimester[†]

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Abstract

The placenta, composed of chorionic villi, changes dramatically across gestation. Understanding differences in ongoing pregnancies are essential to identify the role of chorionic villi at specific times in gestation and develop biomarkers and prognostic indicators of maternal–fetal health. The normative mRNA profile is established using next-generation sequencing of 124 first trimester and 43 third trimester human placentas from ongoing healthy pregnancies. Stably expressed genes (SEGs) not different between trimesters and with low variability are identified. Differential expression analysis of first versus third trimester adjusted for fetal sex is performed, followed by a subanalysis with 23 matched pregnancies to control for subject variability using the same genetic and environmental background. Placenta expresses 14,979 polyadenylated genes above sequencing noise (transcripts per million > 0.66), with 10.7% SEGs across gestation. Differentially expressed genes (DEGs) account for 86.7% of genes in the full cohort [false discovery rate (FDR) < 0.05]. Fold changes highly correlate between the full cohort and subanalysis (Pearson = 0.98). At stricter thresholds (FDR < 0.001, fold change > 1.5), there remains 50.1% DEGs (3353 upregulated in first and 4155 upregulated in third trimester). This is the largest mRNA atlas of healthy human placenta across gestation, controlling for genetic and environmental factors, demonstrating substantial changes from first to third trimester in chorionic villi. Specific differences and SEGs may be used to understand the specific role of the chorionic villi throughout gestation and develop first trimester biomarkers of placental health that transpire across gestation, which can be used for future development of biomarkers for maternal–fetal health.

Summary Sentence

Comparison of first versus third trimester human placenta mRNA finds a subset of SEGs as well as significant changes in 86.7% of transcripts, highlighting the dynamic nature of placental function across gestation.

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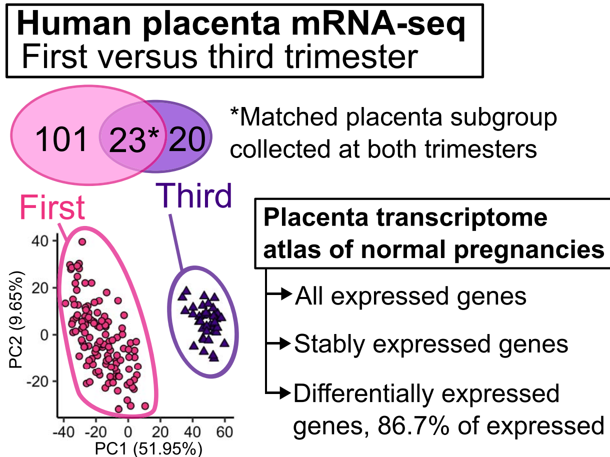
Graphical Abstract



Key words: mRNA, placenta, pregnancy, chorionic villi, gestational differences, human transcriptome

Introduction

The placenta consists of chorionic villi that are derived from the trophoblast cells of the embryo. Following implantation and maternal signaling, placentation is established in the late first trimester [1]. Throughout gestation, placenta serves a dynamic and critical role that impacts both maternal and fetal outcomes and provides communication between the fetal and maternal systems while providing the fetus with nutrients and removing waste [2]. In addition, the placenta has metabolic and endocrinologic functions which serve to regulate activity in pregnancy as well as to protect the fetus from exposures [1–3]. However, molecular features of the placenta in the late first trimester differ considerably from the third trimester placenta, despite a similar tissue architecture [2]. Gene expression changes likely correlate with functional changes at different gestational ages and suggest the placenta undergoes complex rearrangements to support fetal development, as suggested by microarray studies [4–8]. Understanding the normative placental transcriptome signature, including stably and differentially expressed genes (DEGs) throughout gestation, may aid in understanding healthy placental function and serve as a comparison for alterations that drive pathology [9–12]. Next-generation sequencing technologies, such as RNA sequencing (RNA-seq), allows for a more comprehensive view of the entire placental transcriptome including the identification of novel transcripts compared to older methods such as microarrays [13]. Although there are a few RNA-seq studies comparing the first trimester placenta to later gestational ages, the studies are small with only 5–8 subjects at first trimester and use chorionic villi collected from pregnancy terminations which may have abnormal placentation, not reflective of a healthy placenta in ongoing pregnancies [14, 15]. Furthermore, subject variability and underlying maternal and fetal conditions may contribute to differences that are not indicative of the normative state. To define the normative placenta in the first trimester, a large cohort of placenta from ongoing healthy pregnancies is needed. Furthermore, to control for environmental and genetic factors, and minimize subject variability, corresponding third trimester placenta for the same subjects are needed to define the normative human placenta transcriptome as pregnancy progresses.

We developed the largest mRNA-seq study that includes the most first trimester placentas to date ($n = 124$), including 23 subjects sequenced at both first and third trimester, which controls for genetic, demographics, and environmental variability among different subjects. Additionally, first trimester tissue was obtained from chorionic villus sampling, not terminations, allowing us to confirm normal placenta development leading to a healthy pregnancy and delivery. Our normative human placental transcriptome as gestation progresses, with differentially and stably expressed genes (SEGs) may be used to develop first trimester biomarkers of placental health that transpires across gestation, which will be the foundation for future development of biomarkers in maternal–fetal disease.

Materials and methods

Study population

The study population consisted of 144 singleton spontaneous pregnancies between 2009 and 2018, including 124 with first trimester (59 females, 65 males) and 43 with third trimester (18 females, 25 males) placentas available. Subjects with infertility, pre-existing diabetes, or hypertension were excluded. All protocols were performed with informed consent in accordance with the institutional review board's guidelines at the Cedars-Sinai Medical Center under IRB protocols Pro00006806 and Pro00008600. All pregnancies resulted in the delivery of a viable infant.

Analysis of demographic data

Demographics of pregnancies from the first versus third trimester placenta sequencing groups were compared. Specified pregnancy complications included pregnancy-induced hypertension, gestational diabetes, coagulation disorders, placenta previa, and placental abruption. Three patients classified as "placenta other" had either low-lying placenta, adherent placenta, or velamentous cord insertion. Means and standard deviations were reported for continuous variables. Proportions were reported as percentages. Student's t-test was used for normally distributed continuous variables, and the Wilcoxon rank-sum test was used for nonparametric data. Fisher's exact test was used when appropriate. For

comparison of categorical variables, the Chi-square test was used.

Collection of placenta samples

Placental research samples were collected from tissue which is normally discarded after clinical visits. First trimester placenta samples were collected between 70 and 100 days gestation after chorionic villus sampling procedures for prenatal diagnosis. Fetal-derived chorionic villi (first trimester placenta tissue) were cleaned and separated from any maternally derived decidua (nonplacenta tissue), and 5–25 mg were used for research. Third trimester placenta samples were collected between 254 and 290 days gestation, after delivery of a viable neonate. One centimeter cube of placental tissue samples were obtained immediately after delivery from the fetal side of the placenta near the site of cord insertion beneath the amnion. Tissue samples were kept on ice and submerged in RNA*later* RNA stabilization reagent (QIAGEN, Hilden, Germany) within 30 minutes of collection and stored at −80°C.

RNA extraction from first trimester placenta

RNA extraction was performed from the first trimester placenta samples utilizing a method optimized for delicate tissue [16–18]. Briefly, tissue samples were thawed on ice with 600 μl of RLT Plus lysis buffer (QIAGEN) and 1% β-mercaptoethanol added to each sample. Tissue was homogenized by passing at least 10 times through needles (22G, 25G, and 27G) attached to an RNase-free syringe. Homogenates were loaded onto AllPrep spin columns and further processing followed manufacturer instructions for the AllPrep DNA/RNA/miRNA Universal Kit (QIAGEN). RNA was eluted with 30–50 μl of RNase-free water at room temperature, and the elution was passed through the column twice to improve yields, as previously described [16–18]. For the 124 samples analyzed, the average RNA integrity number was 8.81.

RNA extraction from third trimester placenta

Third trimester placental tissue was thawed on ice, then a quarter of collected tissue was diced with single use blades coated in RNA*later* buffer. Tissue was sonicated on ice in lysis buffer (600 μl RLT Plus lysis buffer with 1% beta-mercaptoethanol buffer) using 5 s pulses on a low setting (#2 on the Branson Sonifier 150D, CT, USA) until tissue fragments were small enough to pass through needles and continue RNA isolation with the first trimester method. For the 43 samples analyzed, the average RNA integrity number was 8.82.

Library preparation and mRNA sequencing

Library construction was performed using the Illumina TruSeq Stranded mRNA library preparation kit (Illumina, San Diego, CA). Briefly, total RNA samples were assessed for concentration using a Qubit fluorometer (ThermoFisher Scientific, Waltham, MA) and quality using the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Up to 1 μg of total RNA per sample was used for poly-A mRNA selection, then cDNA was synthesized from enriched and fragmented RNA using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and random primers. The cDNA was further converted into double-stranded DNA (dsDNA), and the resulting dsDNA was enriched with PCR for library preparation. The PCR-amplified library was purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA). The concentration of the amplified library was measured with a Qubit fluorometer and an aliquot of the library was resolved on a Bioanalyzer. Sample libraries were multiplexed and sequenced on a NovaSeq 6000 platform (Illumina) using 75 bp single-end sequencing. On average, about 30 million reads were generated from each sample.

Data analysis of mRNAs

Raw sequencing data was demultiplexed and converted to fastq format by using *bcl2fastq* v2.20 (Illumina, San Diego, CA). Then reads were aligned to the transcriptome using STAR v2.6.1 [19] RSEM v1.2.28 [20] with default parameters, using a custom human GRCh38 transcriptome reference downloaded from http://www.gencodegenes.org, containing all protein coding and long noncoding RNA genes based on human GENCODE version 23 annotation. Principal components analysis (PCA) was performed with *DESeq2* Bioconductor package v1.36.0 using expression counts for each gene in all samples, normalized for sequencing depth, and transformed with a variance stabilizing transformation. The 500 most variable genes were input into R function *prcomp*, plotted with *ggplot2* v.3.3.6, and labeled with *ggrepel* 0.9.1.

Postsequencing quality control and sample elimination

Initial mRNA-sequencing included 142 first trimester and 50 third trimester placenta samples, but PCA analysis showed outliers. Diagnostic heatmaps were created with R package *heatmaply* v1.1.1 to identify traces of maternal decidua contamination using genes from our sex differences analysis of first trimester placenta (NCBI GEO: GSE109082) [16] and decidua-upregulated genes [false discovery rate (FDR) $< 10^{-20}$, FC > 25, Fragments Per Kilobase of transcript per Million (FPKM) > 5] from our matched placenta versus decidua RNA-seq (NCBI GEO: GSE131874) [21]. Samples were removed if they had traces of decidua contamination, high chromosome Y signal in presumed 46,XX samples, or other outlier status. The final "full cohort" for analysis consisted of 124 first trimester and 43 third trimester samples.

Differential expression model

Each gene was fitted into a negative binomial generalized linear model adjusted for fetal sex, and the Wald test was applied to assess the differential expressions between first versus third trimester placenta by *DESeq2* [22, 23]. The Benjamini–Hochberg FDR procedure was applied to adjust *P*-values for multiple hypothesis testing. Differential expression analysis was performed for the full cohort (124 first versus 43 third trimester samples) and for a subanalysis of 46 matched samples (23 subjects).

Selection of thresholds

Expressed genes were defined "above sequencing noise" as determined by Y-linked gene signal from 46,XX female placenta, excluding pseudoautosomal regions. The maximum transcripts per million (TPM) from female samples was 4.01 on *PCDH11Y*, an outlier 6 orders of magnitude higher than the next gene, *RPS4Y1* (maximum TPM = 0.66). Although *PCDH11Y* is not in the canonical pseudoautosomal regions of chromosome Y, it is located in a recent human-specific transposition from Xq21.3 which retains very high X/Y homology

[24]. Therefore, *PCDH11Y* was ignored along with pseudoautosomal genes and a study-specific expression threshold of mean TPM > 0.66 was selected.

DEGs were defined using initial thresholds of FDR < 0.05 and mean TPM > 0.66 in either trimester. "Strict DEGs" were defined after the full cohort analysis (167 samples) and matched subanalysis (46 samples) were compared, leaving a robust set of "strict DEGs" with FDR < 0.001, absolute fold change (FC) >1.5, and mean TPM > 0.66 in either trimester.

SEGs were defined as genes with expression at both trimesters, excluding genes with possible differential expression and and/or high variability. Specifically, thresholds for SEGs were mean TPM > 0.66 in both groups, unadjusted *P*-value > 0.05, absolute FC < 1.5, and coefficient of variation<0.80 (calculated as standard deviation divided by mean TPM from combined first and third trimester sample data).

Pathway enrichment analysis

Genes of interest were analyzed for enrichment in canonical pathways using Ingenuity Pathway Analysis (IPA) software's "Core Analysis" feature (QIAGEN, Redwood City, CA, USA, www.qiagenbioinformatics.com/IPA) as previously described [16], run with the Fall 2023 release. For the top 15% expressed genes and SEGs inputs, the analysis was based on expression intensity and thus only enrichment *P*-values (Fisher's exact test) were calculated but not activation z-scores. For DEGs, the analysis was based on DESeq2-derived $\log_2$ fold changes and expression intensity (baseMeans) to calculate both enrichment *P*-values as well as activation z-scores to predict pathway directions. QIAGEN suggests a threshold of z-score > 2 either direction for significant activation or inactivation, with less reliable predictions if z-scores are 2 to −2.

Single-cell RNA-seq cell clusters

Unique molecular identifier (UMI) values were used from the Sun et al. single-cell RNA-seq dataset of $n = 6$ healthy first trimester placenta samples collected at time of chorionic villus sampling (NCBI GEO: GSE131696) [21, 25]. The cell cluster data was compiled into a dot plot for select SEGs and first trimester-upregulated strict DEGs using R package *ggplot2*.

Immunofluorescence

Two independent first and third trimester placenta tissue samples were collected from consenting subjects as described above. Tissues were embedded in Tissue-Tek O.C.T. Compound (Sakura #4583) and quickly frozen on dry ice, then stored at −80°C until further processing for immunofluorescence. See detailed methods and antibody information in Supplemental File S1.

Results

Cohort demographics

Of the 124 first trimester and 43 third trimester placenta samples analyzed, 23 subjects were overlapped. The demographics of first versus third trimester cohorts were compared without these 23 overlapping subjects (Table 1). There were significantly more Caucasian parents and fetuses in the first trimester only group. Race and ethnicity did not segregate in PCA of the mRNA transcriptome (Supplemental Figure S1). Maternal pre-pregnancy BMI (21.7 versus 24, $P = 0.03$) and thyroid disorders requiring thyroid replacement (3 versus 6, or 2.97% versus 30%, $P = 0.001$) were significantly greater in the third trimester only group. There were six pregnant women (30%) who developed hypertension in the third trimester only group (five required magnesium), compared to none in the first trimester placenta only group ($P < 0.001$) (Table 1).

The 23 subjects with matched first and third trimester placenta were included as a third group for the purposes of demographics analysis (Supplemental Table S1). Compared to the first or third trimester only cohorts, paternal age was lower in the matched group. The percentage of Caucasian mothers in the matched group was lower than in the first trimester only cohort. The percentage of Hispanic parents was higher than both cohorts. The percentage of Caucasian fetuses was lower than in the first trimester only cohort. There were no additional cases of thyroid disorders requiring medication. There were 3 (13%) pregnant women in the matched group who developed hypertension, which was greater than the first trimester only cohort; one required antihypertensive medication and two required magnesium sulfate prophylaxis.

All expressed genes (AEGs) in first and third trimester placenta

We analyzed high-throughput mRNA-sequencing of 167 placenta samples after quality control (Figure 1A, Supplemental Table S2) and observed clustering by trimester on PCA plots (Figure 1B, Supplemental Figure S1). Sequencing identified reads from 25,312 polyadenylated genes in human placenta, with 14,979 genes expressed above sequencing noise at TPM > 0.66 (Supplemental Figure S2, Supplemental Figure S3, Supplemental Table S3A). Placenta overall expressed (in either trimester) 14,132 protein coding genes and 847 long noncoding genes, although mRNA-seq only captured the portion of long coding genes with polyA tails. Third trimester placenta showed higher expression extremes in both biotypes (Figure 1C). The most highly expressed protein coding genes in both first and third trimesters were the placental lactogen encoding genes, *CSH1* and *CSH2*; however, their expression was an order of magnitude higher in third (TPM 182,551 and 82,060, respectively) compared to first trimester (TPM 17,936 and 13,339) and other genes (Figure 1D). Expression differences were evident but less drastic among the next most highly expressed genes: *KISS1, CGA, EEF1A1, TFPI2, ADAM12, HBG2,* and *TPT1* (Figure 1D). A shift in predominant gene families across gestation was seen in Chromosome 19, with higher first trimester expression of the chorionic gonadotropin subunits (*CGB5, CGB8, CGB*) and higher third trimester expression of pregnancy specific beta-1-glycoprotein genes (*PSG4, PSG1, PSG3*). *PAGE4* on chromosome X is highly expressed in first trimester with mean TPM = 4517, then reduced in third trimester to mean TPM = 880 (Figure 1D). Ribosomal protein subunit genes ranked higher within-chromosome and were more likely to be outliers of high expression in first trimester, compared to third trimester, suggesting that the placenta transcriptome focuses more on translation in early pregnancy than later.

Among long noncoding transcripts, the most highly expressed were three similar transcripts from chromosome 21 (*CH507-513H4.3/CH507-513H4.4/CH507-513H4.6*), followed by syncytin family member *ERVH48–1/SUPYN* (chr 21), active chromatin marker *NEAT1* (chr 11), and maternally

Table 1. Demographics and outcomes, without overlapping pregnancies.

	First trimester	Third trimester	*P* value
N	101	20	
Maternal age (SD)	37.8 (2.8)	37.1 (3.4)	0.73
Paternal age (SD)	39.6 (4.7)	39.8 (5.1)	0.59
Maternal race/ethnicity			
Caucasian (%)	99 (98.0%)	14 (70%)	<0.001
Non-Hispanic (%)	98 (97.0%)	19 (95.0%)	0.52
Paternal race/ethnicity			
Caucasian (%)	97 (96.0%)	15 (75%)	0.006
Non-Hispanic	99 (98.0%)	19 (95.0%)	0.42
Fetal race/ethnicity			
Caucasian (%)	97 (96.0%)	12 (60%)	<0.001
Non-Hispanic (%)	98 (97.0%)	19 (95%)	0.52
Fetal sex: female (%)	50 (49.5%)	9 (45%)	0.71
Maternal pre-pregnancy BMI, kg/m^2 (SD)	21.7 (3.6)	24.0 (5.1)	0.03
Maternal pre-existing medical conditions			
Hypertension	0	0	-
Diabetes	0	0	-
Thyroid disorder	3 (2.97%)	6 (30%)	0.001
Other	2 (1.98%)	2 (8.7%)	0.42
Hypertension management in pregnancy			
Antihypertensives	0 (0%)	3 (15%)	0.004
Any magnesium use (Ante- or postpartum)	0 (0%)	5 (25%)	<0.001
Pregnancy complications			
Hypertension (not pre-existing)	0 (0%)	6 (30%)	<0.001
Diabetes (not pre-existing)	4 (3.96%)	0 (0%)	1.0
Coagulation disorder	0	0	-
Placenta previa	0	0	-
Placental abruption	0	0	-
Placental other	0 (0%)	1 (5%)	0.17
Delivery			
Mode of delivery: cesarean section	28 (27.7%)	7 (35%)	0.51
Gestational age at delivery (SD), days	276.2 (7.0)	276.3 (8.7)	0.58
Birthweight (SD), grams	3420.8 (441.2)	3599.6 (515.6)	0.10

*Numbers are formatted as "mean (standard deviation)" or "count (percentage)". This table does not include the 23 subjects sampled at both trimesters, but they are included as a third group in Supplemental Table S1. SD = standard deviation.

imprinted gene *MEG3* (chr 14) (Figure 1E). *MALAT1*, a long noncoding partner for *NEAT1* [26], was also highly expressed in human placenta.

The chromosome frequencies of protein coding genes were similar between first and third trimester (Figure 1F). The highest numbers of expressed protein-coding genes came from chromosome 1 and chromosome 19, the longest and densest chromosomes, respectively [27]. Human placenta expresses a large proportion of protein-coding genes annotated in the human genome, with similar values for first and third trimester (average for autosomes 67.7% and 67%, respectively) (Figure 1F). Chromosomes 14 expressed the lowest proportion among autosomes (55.3%; 54.9%). Chromosome Y, only present in half the subjects, had an expected low percent expressed overall (26.4%; 29.2%). All 13 mitochondria-encoded protein-coding genes were in the top 15% of placental transcripts (Figure 1F, Supplemental Figure S4A).

Pathway enrichment analysis was performed to investigate the biological significance of the top 15% expressed genes in first and third trimester placenta. The most significant five pathways were the same for both trimesters: eukaryotic translation initiation ($P = 6.31 \times 10^{-93}$ first trimester, $P = 1.26 \times 10^{-84}$ third trimester), SRP-dependent cotranslational protein targeting to membrane ($P = 1.58 \times 10^{-89}$, $P = 1.00 \times 10^{-82}$), eukaryotic translation elongation ($P = 1.26 \times 10^{-82}$, $P = 6.31 \times 10^{-81}$), eukaryotic translation termination ($P = 1.58 \times 10^{-77}$, $P = 5.01 \times 10^{-76}$), and nonsense-mediated decay (NMD) ($P = 5.01 \times 10^{-76}$, $P = 1.00 \times 10^{-74}$) (Supplemental Table S4A, Supplemental Table S4B). Following the top five pathways, the next most significant pathways varied in order between first trimester and third trimester, but results were overall similar and included mitochondria-related pathways such as oxidative phosphorylation and mitochondrial dysfunction (Supplemental Figure 4).

Initial differential expression analysis demonstrated vast changes by gestational age

We performed differential expression analysis on the full cohort between first and third trimester placenta (adjusted for fetal sex) and found that the majority of polyadenylated RNAs significantly changed throughout gestation, with 12,986 DEGs at FDR < 0.05 and TPM > 0.66, representing 86.7% of all 14,979 expressed genes in placenta (Supplemental Table S3A). This was consistent for protein coding (86.7%; 12,253/14,132) and polyadenylated long noncoding genes (86.5%; 733/847). To increase confidence that these changes were due to gestational age differences, we performed a subanalysis using the 23 subjects with matched placenta samples collected at first and third trimester (Supplemental Table S3B). This subanalysis removes the variables of genetic, demographic, and environmental differences. The subanalysis

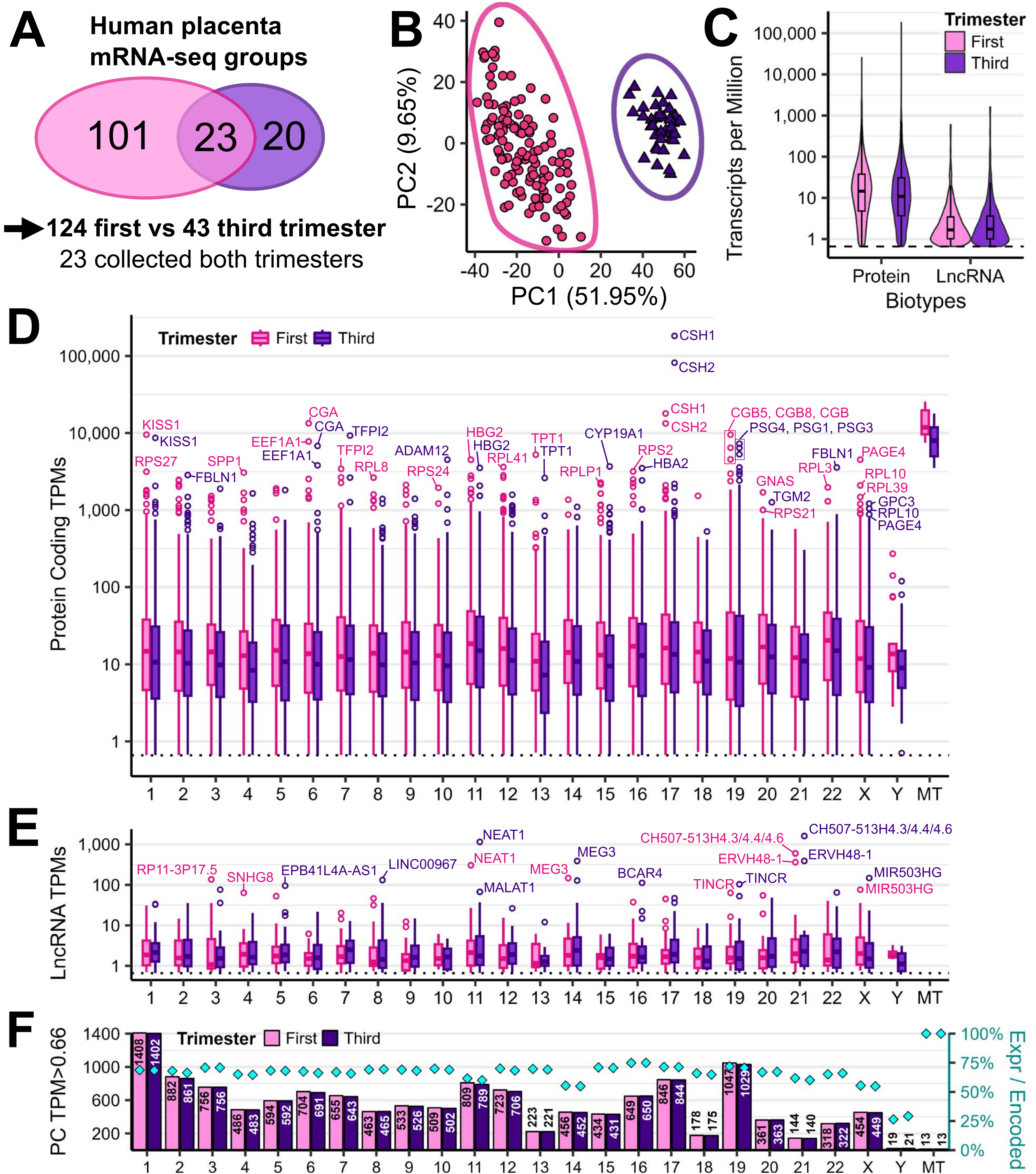


Figure 1. Expressed mRNAs in first and third trimester placenta. (A) The full cohort includes 124 first trimester and 43 third trimester placenta samples. (B) Principal components analysis shows separation by trimester with PC1. (C) Violin plots show the expression ranges of protein coding (Protein) and long noncoding RNAs (LncRNA). Box and whisker plots are super-imposed. The dotted line indicates the study-specific expression threshold of TPM = 0.66. Only genes TPM > 0.66 in either trimester are used in this figure. (D) Box and whisker plots of protein coding gene $\log_{10}$(TPM) expression values in first trimester and third trimester placenta, side-by-side per chromosome. Points are outliers more than 1.5 times outside the interquartile range. (E) Box and whisker plots of long noncoding gene $\log_{10}$(TPM) values, similar to D. (F) Bar plot of all expressed protein coding genes at each trimester, per chromosome. The diamond points and right *y*-axis indicate the proportion expressed in placenta out of all available protein-coding genes in the human reference genome ("Expr / Encoded"), per chromosome.

identified 11,608 DEGs (FDR < 0.05, TPM > 0.66), representing 77.0% of 15,071 expressed genes (TPM > 0.66) in the matched cohort (Supplemental Table S3B). The subanalysis ratios of DEGs/expressed genes were high for both protein coding genes (77.3%; 10,968/14,191) and polyadenylated long noncoding genes (72.7%; 640/880), showing that most placental mRNAs are also significantly changed from first to third trimester in a subanalysis controlled for external factors.

Stricter thresholds identify the most robust changes across gestation

Subsequently, DEGs (FDR < 0.05, TPM > 0.66) from the full cohort analysis and matched subjects subanalysis were compared for congruency to identify strict DEG thresholds that serve to control for any genetic, demographic, and environmental factors (Supplemental Table S3C). Out of 13,617 total pooled DEGs, most were significantly upregulated in the same direction in both analyses (10,977 genes; 80.6%) or trended in the same direction but only reached significance in one analysis (2201 genes; 16.2%). There were 447 genes (3.3%) that changed direction in the subset analysis compared to the full cohort analysis, though only eight of these mismatched genes were significant at FDR < 0.05 in both analyses (only 0.059%), and all were at lower fold changes. Of these eight, the highest expressed gene was *RHOC* (TPM = 276 full cohort, TPM = 308 matched cohort), though fold-change was very low (FC = 1.06 third/first full cohort, FC = 1.12 first/third matched cohort). All direction-switching genes were FC < 1.16 except *SLPI* (FC = 3.16 first/third full cohort, FC = 7.38 third/first matched cohort) but *SLPI* had low expression (TPM = 6 full cohort, TPM = 1 matched cohort). A linear model shows that the full cohort and subanalysis $\log_2$FC values are highly correlated, with a Pearson correlation coefficient of *Rho* = 0.978 and *P*-value = 2.2×10^{-16} (Supplemental Figure S5A). To select genes with more robust differences between first and third trimester placenta, we filtered to "strict DEGs" using thresholds of FDR < 0.001, FC > 1.5, and TPM > 0.66 (either trimester) which resulted in a small increase in correlation (*Rho* = 0.982, *P*-value = 2.2×10^{-16}, Pearson correlation) (Supplemental Figure S5B). There were total 8274 strict DEGs significant in at least one of the two analyses, with 3836 (46.4%) upregulated in first trimester and 4422 (53.4%) upregulated in third trimester placenta, and 16 (0.1%) of inconsistent direction only significant in one analysis, and none of inconsistent direction significant in both analyses. Box and whisker plots for each analysis show more genes upregulated in third trimester than first trimester with either set of thresholds but this was more pronounced with the stricter thresholds (Supplemental Figure S5). All subsequent analyses were performed with strict DEGs thresholds in the full cohort analysis, resulting in 7508 strict DEGs or 50.1% of all expressed protein coding genes.

SEGs throughout gestation

We identify SEGs in placenta across gestation, meaning genes which are expressed in both first and third trimester placenta, not statistically different, and less variable between samples. Scatter plots of the coefficients of variation for the full cohort analysis versus matched subset analysis were color-coded by different variables. High *P*-values did not correlate with low variability, demonstrating that high *P*-value alone is insufficient to define similar expression between trimesters (Figure 2A). However, low variability genes did trend towards low absolute FC values (Figure 2B). Overall expression of each gene was highly correlated between analyses, with a Pearson coefficient of *Rho* = 0.997 and linear model *P*-value = 2.2×10^{-16} for mean TPM per trimester (Figure 2C). Based on these analyses, thresholds were selected to remove highly variable genes and better define SEGs across gestation in the full cohort: TPM > 0.66 in both trimesters, *P*-value > 0.05, FC < 1.5, and a coefficient of variation < 0.80. Of AEGs, 10.7% are SEGs (1610/14,979).

Pathway enrichment analysis of SEGs in placenta showed significant enrichment for protein sorting signaling pathway ($P = 2.04 \times 10^{-8}$), generic transcription pathway ($P = 3.09 \times 10^{-8}$), mitochondrial dysfunction ($P = 6.31 \times 10^{-7}$), protein kinase A signaling ($P = 6.46 \times 10^{-6}$), and Rho GTPase cycle ($P = 8.91 \times 10^{-6}$) pathways (Figure 2D, Supplemental Table S4C).

The chromosome distributions of SEGs were similar to AEGs, with most genes from chromosome 1 (141 SEGs), then chromosome 19 (135 SEGs) (Figure 2E). The fewest SEGs were from chromosome Y (1 SEG), the mitochondrial genome (6 SEGs), and chromosome 21 (25 SEGs).

Strict DEGs in the full cohort

The full cohort analysis identified 7508 strict DEGs (FDR < 0.001, FC > 1.5, TPM > 0.66), composed of 3353 upregulated in first trimester and 4155 upregulated in third trimester placenta. The volcano plot shows more third trimester upregulated DEGs with high FC, compared to first trimester placenta (Figure 3A). The most highly expressed DEGs were chorionic somatomammotropin hormones 1 and 2, which encode human placental lactogen: *CSH1* with TPM increase from 17,936 to 182,551 in first to third trimester (14.0-fold, FDR 1.24×10^{-40}) and *CSH2* with TPM increase from 66,113 to 165,274 in first to third trimester (8.3-fold, FDR 5.36×10^{-24}) (Figure 3B). There is significantly higher first trimester expression for five of the six genes encoding the beta subunit of chorionic gonadotropin, with greatest significance and highest expression from type 2 (*CGB/CGB5/CGB8*), followed by type 1 (*CGB7)* and variant *CGB1*, and only missing variant *CGB2* with FDR = NA. Conversely, the pregnancy-specific glycoprotein (PSG) gene cluster is significantly higher in third trimester placenta. The median fold changes were higher in third trimester upregulated DEGs across all chromosomes, except chromosomes 12 and 18 which were higher in first trimester (Figure 3C). The DEGs with greatest fold increase in the first trimester includes *ANGPT4* with TPM decrease from 10.55 to 0.03 from first to third trimester (219.0-fold, FDR = 5.17×10^{-83}) and *MMP12* with TPM decrease from 33.7 to 0.11 from first to third trimester (186.3-fold, FDR = 2.78×10^{-75}) (Figure 3C). The four DEGs with greatest fold increase in the third trimester placenta are *IDO2* with TPM increase from 0.04 to 11.39 in first to third trimester (463.7-fold, FDR = 4.31×10^{-288}), *ALPP* with TPM increase from 9.39 to 2850 from first to third trimester (434.3-fold, FDR = 4.27×10^{-297}), *IL1R1* with TPM increase from 0.27 to 50 from first to third trimester (223.3-fold, FDR = 1.19×10^{-227}), and *FABP4* with TPM increase from 1.01 to 134 in first to third trimester (198.0-fold, FDR = 0). All chromosomes were represented, with DEGs most represented on chromosome 1 (811 genes; 10.8%) and chromosome 19 (559 genes; 7.4%) (Figure 3D).

Biological functions of the strict DEGs were classified using pathway enrichment analysis. Genes upregulated in first trimester placenta were most enriched for cell cycle checkpoints ($P = 2.51 \times 10^{-44}$), eukaryotic translation elongation ($P = 7.94 \times 10^{-43}$), eukaryotic translation termination ($P = 5.01 \times 10^{-42}$), selenoamino acid metabolism ($P = 1.00 \times 10^{-40}$), and response to EIF2AK4 (GCN2) to

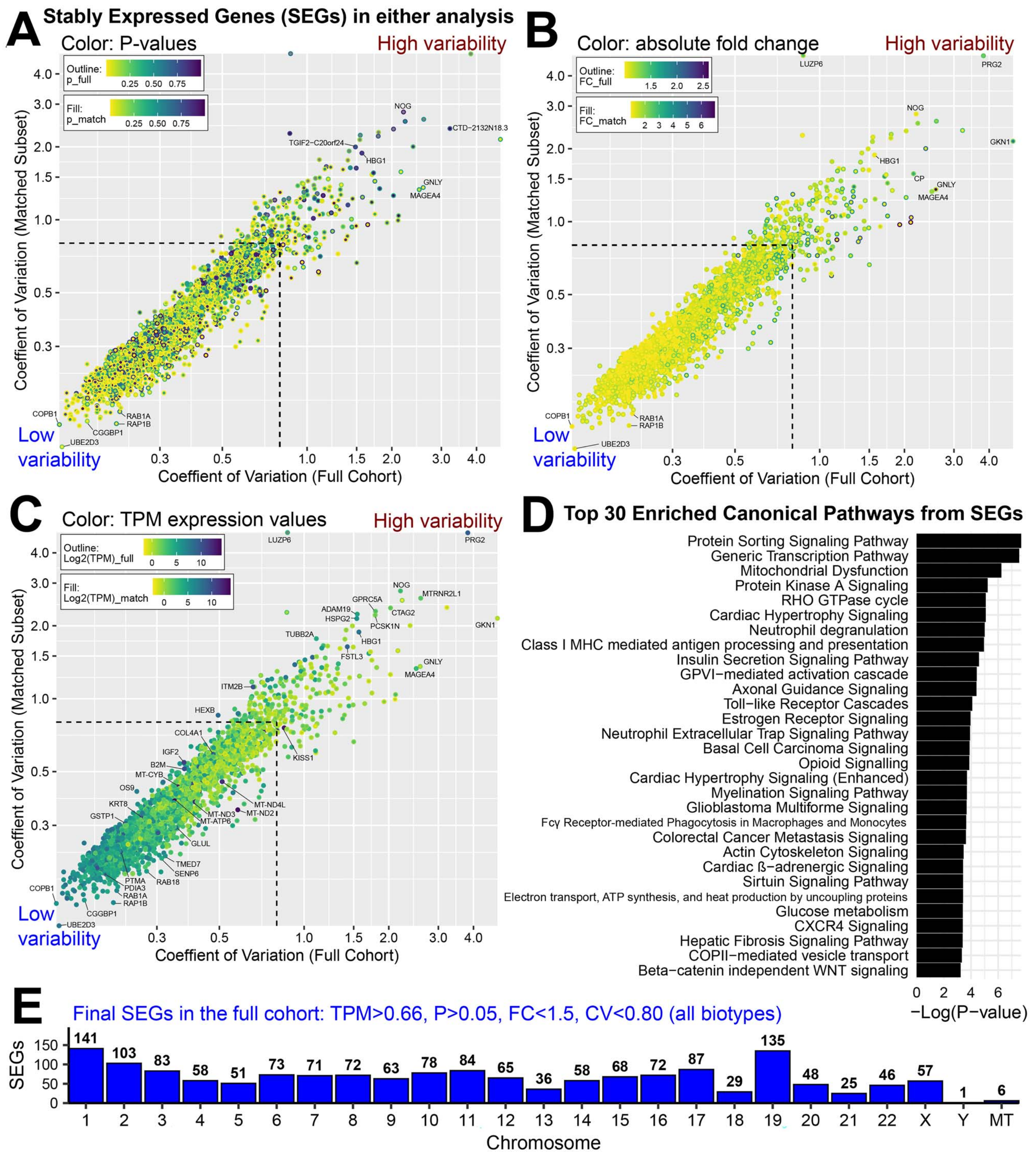


Figure 2. Variability and selection of stably expressed genes (SEGs). Comparison of the full cohort analysis (124 first versus 43 third trimester samples) with a subanalysis of only matched first and third trimester samples (first versus third trimester from the same 23 pregnancies). (A–C) Scatter plots comparing the coefficients of variation (CV) from each analysis, per gene. Genes are plotted if TPM > 0.66 (both trimesters), *P*-value>0.05, and FC < 1.5 in either analysis. SEGs are defined by those three filters and also CV < 0.80. Dashed lines show CV = 0.80. Points are color-coded by different variables to identify differences between the full cohort value (outline color) and subanalysis value (fill color). Axes are log2-transformed to better visualize low variability genes. Color coding based on (A) Wald P-value, (B) fold change, (C) $\log_2$(TPM) expression, (D) Top 30 enriched canonical pathways among SEGs from the full cohort, and (E) Bar plot of SEGs from the full cohort.

amino acid deficiency ($P = 3.98 \times 10^{-40}$) (Figure 4A, Supplemental Table S4D). Genes upregulated in third trimester placenta were most enriched for cardiac hypertrophy signaling (enhanced) ($P = 3.16 \times 10^{-11}$); hepatic fibrosis/hepatic stellate cell activation ($P = 2.09 \times 10^{-10}$); molecular mechanisms of cancer ($P = 2.24 \times 10^{-10}$); Rho GTPase cycle ($P = 2.82 \times 10^{-10}$); and IL-10 signaling ($P = 1.10 \times 10^{-9}$) pathways (Figure 4B, Supplemental Table S4E).

Additionally, we used our previous single cell RNA-seq of first trimester placenta to identify if SEGs or first

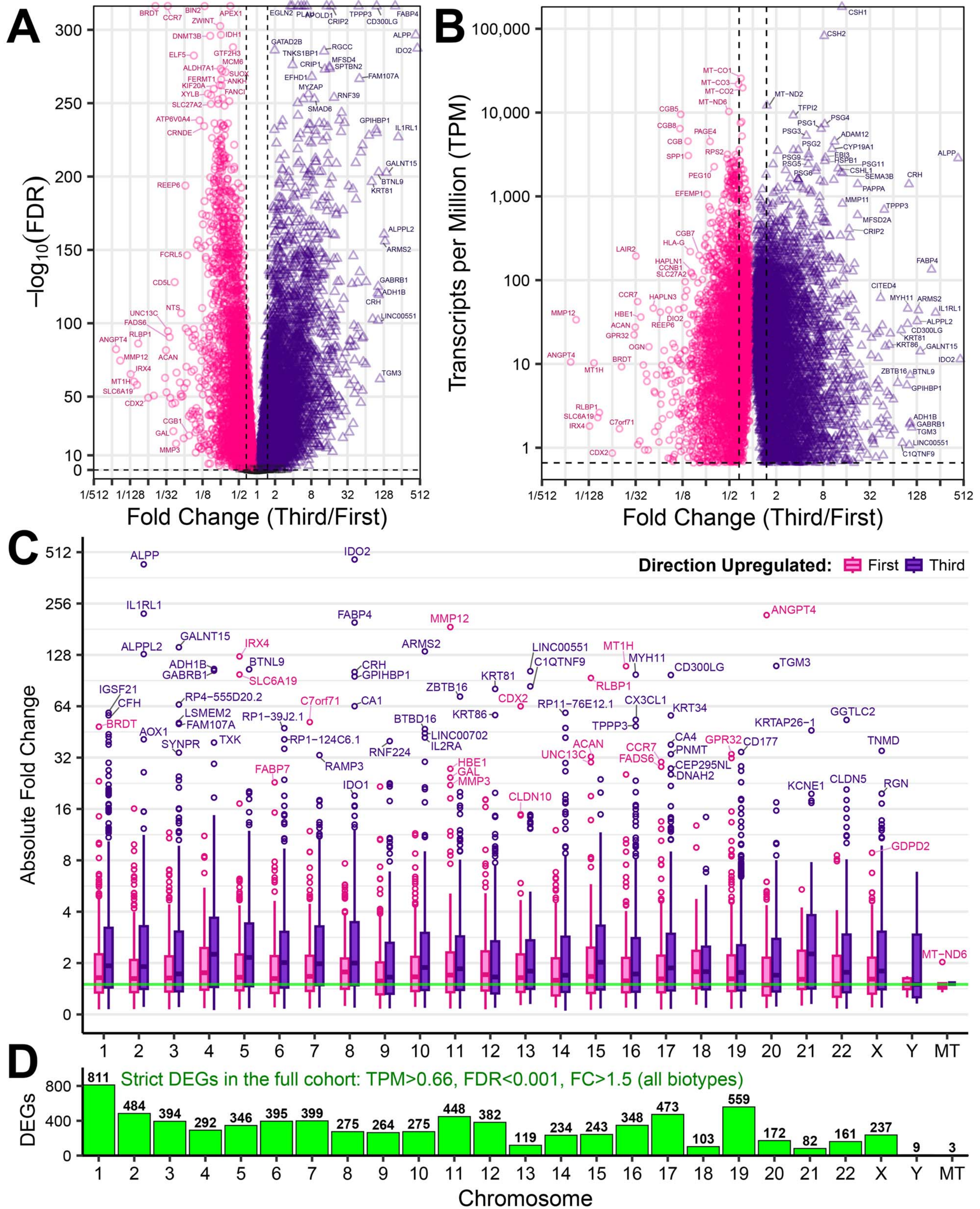


Figure 3. Differentially expressed protein coding genes between first and third trimester placenta (full cohort). (A) Volcano plot of all expressed genes (TPM >0.66). (B) Expression versus FC for DEGs. The vertical dotted lines represent FC = 1.5. (C) Box and whisker plots of absolute FC distribution across chromosomes for all genes at FDR < 0.001 and TPM > 0.66 (any FC). Solid bold green line is FC = 1.5. (D) Chromosome frequency of all "strict DEGs" at FDR < 0.001, FC > 1.5, and TPM > 0.66 either trimester.

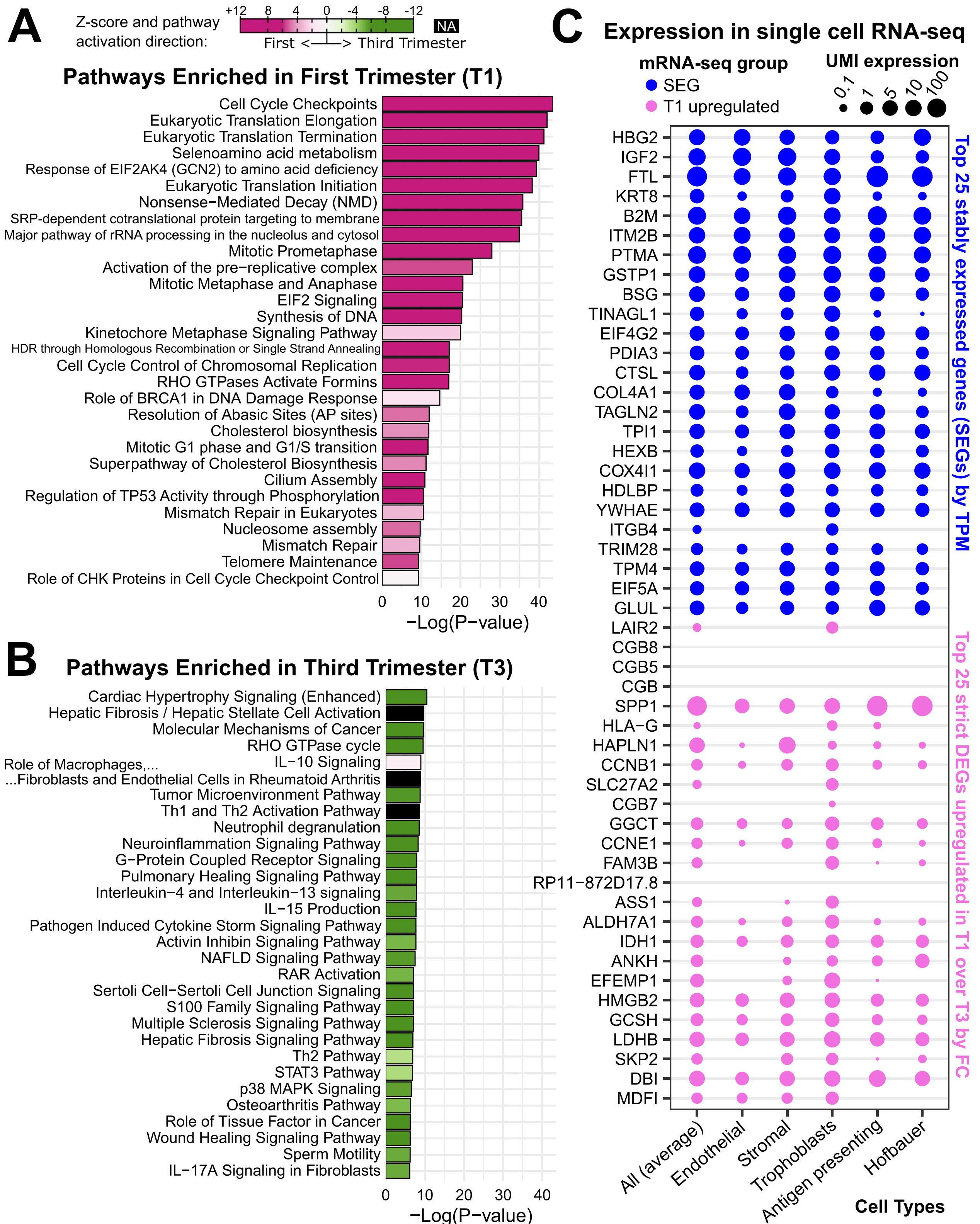


Figure 4. Top 30 enriched canonical pathways. Z-scores of magnitude >2 in either direction indicate significant predicted pathway activation or inactivation. Pathway predicted activation color coding: pink = activated in first trimester, green = activated in third trimester, black = not available ("NA"). Enriched pathways among strict DEGs upregulated in (A) first trimester, (B) third trimester. (C) Dot plot with Sun et al. 2020 [21] first trimester placenta single cell RNA-seq expression values (UMI), showing select TPM > 100 SEGs and first trimester-upregulated strict DEGs. Blue: top 25 nonmitochondrial SEGs sorted by highest expression. Pink: top 25 first trimester-upregulated genes sorted by first/third trimester FC, starting with LAIR2. Single cell RNA-seq values were plotted if average UMI > 0.05.

trimester-upregulated strict DEGs were enriched in specific cell types (Figure 4C) [21]. The SEGs with high TPM values were expressed in most placental cell types. For example, *cytokeratin 8* (*KRT8*) had highest expression in trophoblast cells but some expression in other cell types. Growth regulator *TINAGL1* was also higher in trophoblast cell types compared to others, with very low signal from fetal-derived macrophages (Hofbauer cells). Collagenase gene *COL4A1* was expressed in all cell types with reduced expression in immune cells (antigen presenting cells and Hofbauer cells). Of the 25 highest expressed SEGs, only *integrin subunit beta 4* (*ITGB4*) showed strong cell type specificity, expressed in trophoblast cells. Conversely, genes with high fold-upregulation in first versus third trimester placenta showed stronger cell type specificity. *LAIR2, SLC27A2*, and *CGB7* were exclusively expressed by trophoblast cells. The extravillous trophoblast marker, *HLA-G,* was primarily expressed in the trophoblast cell cluster as expected, with minor expression from antigen presenting cells. The other CGB family genes were not represented in single cell RNA-seq at our expression threshold. *Secreted phosphoprotein 1* (*SPP1)* was highly expressed in all cell types.

Immunofluorescence

Three strict DEGs protein products were validated with immunofluorescence using independently collected first and third trimester placenta samples (Supplemental File 1). *HAPLN1* (6.2-fold higher in first versus third trimester, TPM drops from 124 to 14.9) encodes hyaluronan and proteoglycan link protein 1. HAPLN1 protein was observed in first trimester syncytiotrophoblasts with lighter diffuse expression in surrounding stroma. No HAPLN1 protein expression was observed in third trimester placenta. *TPPP3* (48.8-fold higher in third trimester, TPM rises from 20.8 to 692.9) encodes tubulin polymerization promoting protein family member 3. TPPP3 protein was not observed in first trimester, but third trimester placenta showed low to moderate expression in syncytiotrophoblasts and stroma, consistent with previous results. Finally, *CGB/CGB5/CGB8* (6.8 to 8.6-fold higher in first trimester) drop expression from first to third trimester by an order of magnitude, but they remain in the top 15% of expressed genes in both trimesters. CGB family genes were expected in syncytiotrophoblasts [28], the multinucleated and largest cell type of placenta which tends to be underrepresented in single cell RNA-seq. Immunofluorescence showed moderate-to-bright CGB protein expression in first trimester placenta, primarily in syncytiotrophoblasts with some expression in cytotrophoblasts. Third trimester placenta showed similar localization with reduced expression intensity.

Discussion

The main component of the placenta, the chorionic villi, changes significantly over the course of gestation to meet its changing roles for the growing fetus. These differences are seen here with high-throughput transcriptomics of the normative human placenta, with 86.7% of expressed genes significantly different between first and third trimester using the typical significance threshold for RNA-seq (FDR < 0.05) and over half the transcriptome (50.1%) remains different at stricter thresholds. These differences provide insight into placental function changes across gestation. We also identified SEGs (10.7% of the placental transcriptome) which sustain core placental functions. This is the largest sample size of first and third trimester placentas of female and male singletons in ongoing and completed healthy pregnancies conceived without fertility treatments, constituting the normative human placenta atlas.

At both first and third trimester, human placenta devotes the top 15% of expressed genes to regulation of translation at all stages (initiation, elongation, termination), destruction of RNAs with premature termination codons (NMD), and metabolic resource sensing and response (including amino acid deficiency and oxidation pathways). This enrichment profile highlights the growth environment of placenta. Enrichment of NMD could indicate error correction from rapid RNA synthesis or perhaps alternative splicing regulation of protein synthesis [29, 30]. The pathways enriched among SEGs show more diverse roles and many signaling cascades, including cancer-like pathways which contribute to maternal immune tolerance of placenta [31]. One of the most highly expressed SEGs across gestation was *glutamate-ammonia ligase* (*GLUL*), a gene previously suggested as a housekeeping gene for placenta qPCR using term placenta data [32], which we confirm here is also stably expressed across gestation and expressed in all placental cell types.

The high expression of the mitochondrial genes reflects their vital role in placental utilization of oxygen to generate energy via oxidative phosphorylation [33, 34] and is consistent with the described adaptation of mitochondria to the changing oxygen demand over gestation [35]. Mitochondrial dysfunction is implicated in oxidative stress gestational disorders including intrauterine growth restriction (IUGR), preeclampsia, gestational diabetes, and intrahepatic cholestasis of pregnancy [33, 34, 36, 37]. Studies profiling the mitochondrial genes associated with these disorders are sparse. *MT-ND6* (2.03-fold upregulated in first trimester, FDR $= 9.67 \times 10^{-31}$) as well as *MT-ATP6* (a SEG in our healthy cohort) were negatively associated with IUGR in a case control study [38]. Of the four mitochondrial genes associated with change in regulation in intrahepatic cholestasis of pregnancy described in the literature [37], three were stably expressed across gestation in this study: *MT-ND4L*, *MT-CYB*, *MT-ND4*. An increased copy number of *MT-ND1* (a SEG) was found in early onset preeclampsia (<34 weeks), suggesting an adaptative response to oxidative stress [39]. Further characterization of the mitochondrial biomarkers involved in oxidative stress pathologies of gestational disorders is important in aiding future clinical interventions.

Human placenta gene expression differences between first and third trimester show how placenta adapts to different challenges as gestation progresses. The two strict DEGs most upregulated in first trimester highlight the environmental challenges of early placenta development in low oxidative states: angiopoietin 4 (*ANGPT4*, 218-fold) and matrix metallopeptidase 12 (*MMP12*, 186-fold). *ANGPT4* is a secreted angiogenic factor expressed in a variety of tissue, but there is little information on its expression in placenta. *MMP12* plays a role in low oxygen states and invasive trophoblast and may play a critical role early in placentation [40, 41]. The two strict DEGs most upregulated in the third trimester include an isoform of indoleamine 2,3-dioxygenase (*IDO2*, 463-fold) and placental alkaline phosphatase (*ALPP*, 434-fold). Indoleamine 2,3-dioxygenase is an enzyme involved

in tryptophan catabolism which contributes to immunotolerance of the fetus and other foreign bodies, with isoforms *IDO* and *IDO2* possibly having distinct functions in cancer and placenta [42, 43]. Placental alkaline phosphatase (ALPP) is a marker of syncytiotrophoblasts at term but not first trimester [21, 44–46] and is associated with regulation of fetal growth later in pregnancy [47]. Abnormal levels of ALPP have been implicated in intrauterine growth restricted pregnancies and preterm deliveries [48]. The expression pattern of ALPP is well established, and this study supports other evidence that it increases throughout gestation [49, 50]. Two other genes that increase dramatically in third trimester are the human placental lactogen genes (*CSH1* and *CSH2*), which had expression an order of magnitude higher than the next most highly expressed genes. Human placental lactogen promotes production of insulin-like growth factor, thus modulating fetal growth and metabolism [51, 52]. The high expression of *CSH1* and *CSH2* in third trimester reflects the increasing metabolic demands of the placenta and fetus as gestation progresses. CGB family genes were higher in first trimester placenta, consistent with the role of human chorionic gonadotropin (hCG) as the pioneer molecule of pregnancy, the first hormone that placenta expresses to begin implantation and placentation, which peaks at weeks 10–12 of gestation [28]. The pregnancy-specific β1-glycoproteins (PSG) family genes were higher in third trimester placenta and may be involved in the regulation of cell invasion, angiogenesis, and cytokine expression to promote maternal tolerance [53, 54]. Dysregulation of PSG genes during pregnancy is associated with preeclampsia and IUGR, and aberrant expression in other tissues is associated with cancer [53].

Comparing the pathway enrichment results for DEGs shows that early human placenta focuses more on cell division-related pathways (e.g. cell cycle checkpoints, various mitosis and DNA repair pathways), DNA replication and protein synthesis regulation, and apoptosis, and self-renewal control (e.g. regulation of TP53 activity through phosphorylation, telomere maintenance). The fourth most significantly enriched pathway in first trimester is "selenoamino acid metabolism" which involves selenium-containing proteins important for placenta's response to oxidative stress and prevention of preeclampsia [55]. By third trimester at delivery, the placenta's growth is complete and its transcriptional profile focuses on maintaining oxygen and homeostasis (e.g. cardiac hypertrophy signaling, molecular mechanisms of cancer, tumor microenvironment) [31], maintaining maternal immune tolerance via T-cell modulation (e.g. IL-10 signaling, Th1 and Th2 activation) [56], stress and wound responses (e.g. inflammation signaling, hepatic fibrosis signaling), and IL-15 production which is highest during labor [57].

We also performed immunofluorescence on first and third trimester placenta to validate protein expression and localization of three gene products. *HAPLN1* (upregulated in first trimester) encodes hyaluronan and proteoglycan link protein 1, a secreted protein involved in cell migration and invasion in gastric cancer [58]. *HAPLN1* expression is observed early in gestation in day 5 trophectoderm [59] and is not yet well characterized in placenta but is likely involved in cell invasion critical for implantation. Our previous single cell RNA-seq of first trimester chorionic villi found *HAPLN1* as a stromal cell marker for first trimester with minor expression in trophoblast cells, and another study found it expressed generally in villous fibroblasts (the most common stromal cell type) [21, 60, 61]. Here, HAPLN1 protein was observed in first trimester, but not third trimester, syncytiotrophoblasts and stroma. *TPPP3* (third trimester upregulated) is a protein which is more studied in the endometrium than placenta, associated with endometrial receptivity and implantation [62]. We previously identified *TPPP3* as a syncytiotrophoblasts marker in first trimester placenta [21, 45] and other researchers have found *TPPP3* gene expression in decidual stromal cells [63]. Here, we observed TPPP3 protein expression in syncytiotrophoblasts and stroma from third trimester placenta tissue facing the fetal surface, showing TPPP3 is also expressed in third trimester placenta. Lastly, *CGB/CGB5/CGB8* are highly expressed throughout gestation and encode the beta 3 subunit of hCG, a secreted protein essential for maternal-fetal communication. Consistent with previous studies indicating that CGB is primarily produced by syncytiotrophoblasts and peaks in first trimester [64], we observed protein expression in syncytiotrophoblasts and cytotrophoblasts at both trimesters but higher expression in first trimester.

The major strengths of this study are the use of first and third trimester tissue from healthy pregnancies that continued until delivery, the largest cohort sizes to date, and the use of high-throughput sequencing. Uniquely, this study contains chorionic villi from pregnancies sampled at both first and third trimester, which minimizes subject variability in the full cohort and reduces the effect of genetic and environmental differences, with full cohort results highly correlated to the subanalysis of only matched subjects (coefficient of 0.98). These strengths allow for a more precise evaluation of human placental transcriptomes across gestation.

The limitations of this study include some demographic differences between the first and third trimester groups, including race and ethnicity differences. However, the overall differences were minimal and PCA plots did not demonstrate clustering based on race and ethnicity. We also identified small differences in maternal BMI and thyroid disorders, but PCA plots did not show clustering or outliers. Although there was a higher rate of pregnancy complications, specifically hypertension, subjects were enrolled in the first trimester prior to development of pregnancy complications. Furthermore, the subanalysis with only overlapping subjects shows that these demographic differences have a minimal effect on results, and differences seen are driven by gestational age.

Overall, this study demonstrates that although the early and late placenta is composed of a similar structure, the chorionic villi, these villi change dramatically to support the pregnancy throughout gestation. As the largest study, and the only study with matched subjects at both collection times, this work provides a normative mRNA atlas of first and third trimester human placenta. This work serves as a reference for future studies aimed at understanding gestational age-specific functional mechanisms and development of gestation-specific biomarkers.

Author contributions

Conceptualization: TLG, SW, AEF, CAJ, KL, YZ, HRT, SAK, MDP.
Data curation: TLG, YW, ELC.
Formal analysis: TLG, SW, YW, JLC.
Funding acquisition: HRT, MDP.
Investigation: TLG, SW, AEF, CS, ELC, NVJ, RD.
Methodology: TLG, YW, CS, JLC.
Project administration: TLG, MDP.
Resources: JW.

Software: TLG, YW.
Supervision: MDP.
Visualization: TLG, YW.
Writing—original draft preparation: TLG, SW, MDP.
Writing—review and editing: TLG, MDP.

Supplementary Material

Supplementary Material is available at *BIOLRE* online.

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Conflict of Interest: The authors declare no competing interests.

Data availability

The mRNA-sequencing data has been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) under accession GSE247382 available at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE247382>.

References

1. Turco MY, Moffett A. Development of the human placenta. *Development* 2019; **146**:dev163428.
2. Burton GJ, Jauniaux E. The human placenta: new perspectives on its formation and function during early pregnancy. *Proc Biol Sci* 2023; **290**:20230191.
3. Hemberger M, Hanna CW, Dean W. Mechanisms of early placental development in mouse and humans. *Nat Rev Genet* 2020; **21**: 27–43.
4. Tsui NBY, Chim SS, Chiu RW, Lau TK, Ng EK, Leung TN, Tong YK, Chan KC, Lo YM. Systematic micro-array based identification of placental mRNA in maternal plasma: towards non-invasive prenatal gene expression profiling. *J Med Genet* 2004; **41**:461–467.
5. Mikheev AM, Nabekura T, Kaddoumi A, Bammler TK, Govindarajan R, Hebert MF, Unadkat JD. Profiling gene expression in human placentae of different gestational ages: an OPRU network and UW SCOR study. *Reprod Sci* 2008; **15**:866–877.
6. Sitras V, Fenton C, Paulssen R, Vårtun Å, Acharya G. Differences in gene expression between first and third trimester human placenta: a microarray study. *PloS One* 2012; **7**:e33294.
7. Leavey K, Benton SJ, Grynspan D, Bainbridge SA, Morgen EK, Cox BJ. Gene markers of normal villous maturation and their expression in placentas with maturational pathology. *Placenta* 2017; **58**:52–59.
8. Soncin F, Khater M, To C, Pizzo D, Farah O, Wakeland A, Arul Nambi Rajan K, Nelson KK, Chang C-W, Moretto-Zita M, Natale DR, Laurent LC, *et al.* Comparative analysis of mouse and human placentae across gestation reveals species-specific regulators of placental development. *Development* 2018; **145**:dev156273.
9. Sõber S, Reiman M, Kikas T, Rull K, Inno R, Vaas P, Teesalu P, Marti JML, Mattila P, Laan M, Kleinrouweler CE, Rull K, *et al.* Extensive shift in placental transcriptome profile in preeclampsia and placental origin of adverse pregnancy outcomes. *Sci Rep* 2015; **5**:13336–13336.
10. Singh K, Williams J, Brown J, Wang ET, Lee B, Gonzalez TL, Cui J, Goodarzi MO, Pisarska MD. Up-regulation of microRNA-202-3p in first trimester placenta of pregnancies destined to develop severe preeclampsia, a pilot study. *Pregn Hypertens* 2017; **10**:7–9.
11. Myatt L. Placental adaptive responses and fetal programming. *J Physiol* 2006; **572**:25–30.
12. Aplin JD, Myers JE, Timms K, Westwood M. Tracking placental development in health and disease. *Nat Rev Endocrinol* 2020; **16**: 479–494.
13. Han Y, Gao S, Muegge K, Zhang W, Zhou B. Advanced applications of RNA sequencing and challenges. *Bioinform Biol Insights* 2015; **9s1**:BBI.S28991–BBI.S28946.
14. Suryawanshi H, Max K, Bogardus KA, Sopeyin A, Chang MS, Morozov P, Castano PM, Tuschl T, Williams Z. Dynamic genome-wide gene expression and immune cell composition in the developing human placenta. *J Reprod Immunol* 2022; **151**:103624.
15. Prater M, Hamilton RS, Wa Yung H, Sharkey AM, Robson P, Abd Hamid NE, Jauniaux E, Charnock-Jones DS, Burton GJ, Cindrova-Davies T. RNA-Seq reveals changes in human placental metabolism, transport and endocrinology across the first-second trimester transition. *Biol Open* 2021; **10**:10.
16. Gonzalez TL, Sun T, Koeppel AF, Lee B, Wang ET, Farber CR, Rich SS, Sundheimer LW, Buttle RA, Chen Y-DI, Rotter JI, Turner SD, *et al.* Sex differences in the late first trimester human placenta transcriptome. *Biol Sex Differ* 2018; **9**:4.
17. Pisarska MD, Akhlaghpour M, Lee B, Barlow GM, Xu N, Wang ET, Mackey AJ, Farber CR, Rich SS, Rotter JI, Y-dI C, Goodarzi MO, *et al.* Optimization of techniques for multiple platform testing in small, precious samples such as human chorionic villus sampling. *Prenat Diagn* 2016; **36**:1061–1070.
18. Gonzalez TL, Eisman LE, Joshi NV, Flowers AE, Wu D, Wang Y, Santiskulvong C, Tang J, Buttle RA, Sauro E, Clark EL, DiPentino R, *et al.* High-throughput miRNA sequencing of the human placenta: expression throughout gestation. *Epigenomics* 2021; **13**: 995–1012.
19. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013; **29**:15–21.
20. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform* 2011; **12**:323.
21. Sun T, Gonzalez TL, Deng N, DiPentino R, Clark EL, Lee B, Tang J, Wang Y, Stripp BR, Yao C, Tseng HR, Karumanchi SA, *et al.* Sexually dimorphic crosstalk at the maternal-Fetal Interface. *J Clin Endocrinol Metab* 2020; **105**:e4831–e4847.
22. Anders S, Huber W. Differential expression analysis for sequence count data. *Genome Biol* 2010; **11**:R106–R106.
23. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; **15**:550–550.
24. Blanco P, Sargent CA, Boucher CA, Mitchell M, Affara NA. Conservation of PCDHX in mammals; expression of human X/Y genes predominantly in brain. *Mamm Genome* 2000; **11**: 906–914.
25. Sun T, Gonzalez TL, Deng N, DiPentino R, Clark EL, Lee B, Tang J, Wang Y, Stripp BR, Yao C, Tseng H-R, Karumanchi SA, Koeppel AF, *et al.* Table S1. Patient demographics and pregnancy outcomes. *FigShare*. 2020. https://doi.org/10.6084/m9.figshare.10314425.v1.
26. West JA, Davis CP, Sunwoo H, Simon MD, Sadreyev RI, Wang PI, Tolstorukov MY, Kingston RE. The long noncoding RNAs NEAT1 and MALAT1 bind active chromatin sites. *Mol Cell* 2014; **55**: 791–802.
27. Grimwood J, Gordon LA, Olsen A, Terry A, Schmutz J, Lamerdin J, Hellsten U, Goodstein D, Couronne O, Tran-Gyamfi M, Aerts A, Altherr M, *et al.* The DNA sequence and biology of human chromosome 19. *Nature* 2004; **428**:529–535.
28. Fournier T, Guibourdenche J, Evain-Brion D. Review: hCGs: different sources of production, different glycoforms and functions. *Placenta* 2015; **36**:S60–S65.
29. Lareau LF, Brooks AN, Soergel DAW, Meng Q, Brenner SE. The coupling of alternative splicing and nonsense-mediated mRNA decay. *Adv Exp Med Biol* 2007; **623**:190–211.

30. Gonzalez TL, Liang Y, Nguyen BN, Staskawicz BJ, Loqué D, Hammond MC. Tight regulation of plant immune responses by combining promoter and suicide exon elements. *Nucleic Acids Res* 2015; **43**:7152–7161.
31. Pang H, Lei D, Guo Y, Yu Y, Liu T, Liu Y, Chen T, Fan C. Three categories of similarities between the placenta and cancer that can aid cancer treatment: cells, the microenvironment, and metabolites. *Front Oncol* 2022; **12**:977618.
32. Cleal JK, Day PL, Hanson MA, Lewis RM. Sex differences in the mRNA levels of housekeeping genes in human placenta. *Placenta* 2010; **31**:556–557.
33. Fisher JJ, Bartho LA, Perkins AV, Holland OJ. Placental mitochondria and reactive oxygen species in the physiology and pathophysiology of pregnancy. *Clin Exp Pharmacol Physiol* 2020; **47**: 176–184.
34. Yung HW, Colleoni F, Dommett E, Cindrova-Davies T, Kingdom J, Murray AJ, Burton GJ. Noncanonical mitochondrial unfolded protein response impairs placental oxidative phosphorylation in early-onset preeclampsia. *Proc Natl Acad Sci* 2019; **116**:18109–18118.
35. Holland OJ, Hickey AJR, Alvsaker A, Moran S, Hedges C, Chamley LW, Perkins AV. Changes in mitochondrial respiration in the human placenta over gestation. *Placenta* 2017; **57**:102–112.
36. Holland O, Dekker Nitert M, Gallo LA, Vejzovic M, Fisher JJ, Perkins AV. Review: placental mitochondrial function and structure in gestational disorders. *Placenta* 2017; **54**:2–9.
37. Mella MT, Kohari K, Jones R, Peña J, Ferrara L, Stone J, Lambertini L. Mitochondrial gene expression profiles are associated with intrahepatic cholestasis of pregnancy. *Placenta* 2016; **45**:16–23.
38. Jones R, Peña J, Mystal E, Marsit C, Lee MJ, Stone J, Lambertini L. Mitochondrial and glycolysis-regulatory gene expression profiles are associated with intrauterine growth restriction. *J Matern Fetal Neonatal Med* 2020; **33**:1336–1345.
39. Pandey D, Yevale A, Naha R, Kuthethur R, Chakrabarty S, Satyamoorthy K. Mitochondrial DNA copy number variation - a potential biomarker for early onset preeclampsia. *Pregn Hypertens* 2021; **23**:1–4.
40. Hiden U, Eyth CP, Majali-Martinez A, Desoye G, Tam-Amersdorfer C, Huppertz B, Ghaffari T-WN. Expression of matrix metalloproteinase 12 is highly specific for non-proliferating invasive trophoblasts in the first trimester and temporally regulated by oxygen-dependent mechanisms including HIF-1A. *Histochem Cell Biol* 2018; **149**:31–42.
41. Chakraborty D, Cui W, Rosario GX, Scott RL, Dhakal P, Renaud SJ, Tachibana M, Rumi MAK, Mason CW, Krieg AJ, Soares MJ. HIF-KDM3A-MMP12 regulatory circuit ensures trophoblast plasticity and placental adaptations to hypoxia. *Proc Natl Acad Sci* 2016; **113**:E7212–E7221.
42. Metz R, DuHadaway JB, Kamasani U, Laury-Kleintop L, Muller AJ, Prendergast GC. Novel tryptophan catabolic enzyme IDO2 is the preferred biochemical target of the antitumor Indoleamine 2,3-dioxygenase inhibitory compound d-1-methyl-tryptophan. *Cancer Res* 2007; **67**:7082–7087.
43. Macchiarulo A, Camaioni E, Nuti R, Pellicciari R. Highlights at the gate of tryptophan catabolism: a review on the mechanisms of activation and regulation of indoleamine 2,3-dioxygenase (IDO), a novel target in cancer disease. *Amino Acids* 2009; **37**: 219–229.
44. Leitner K, Szlauer R, Ellinger I, Ellinger A, Zimmer KP, Fuchs R. Placental alkaline phosphatase expression at the apical and basal plasma membrane in term villous trophoblasts. *J Histochem Cytochem* 2001; **49**:1155–1164.
45. Sun T, Gonzalez TL, Deng N, DiPentino R, Clark EL, Lee B, Tang J, Wang Y, Stripp BR, Yao C, Tseng H-R, Karumanchi SA, Koeppel AF, *et al*. Table S5. Trophoblast subcluster markers in human first trimester. *FigShare* 2020. https://doi.org/10.6084/m9.figshare.10314419.v2.
46. Yabe S, Alexenko AP, Amita M, Yang Y, Schust DJ, Sadovsky Y, Ezashi T, Roberts RM. Comparison of syncytiotrophoblast generated from human embryonic stem cells and from term placentas. *Proc Natl Acad Sci U S A* 2016; **113**:E2598–E2607.
47. She QB, Mukherjee JJ, Chung T, Kiss Z. Placental alkaline phosphatase, insulin, and adenine nucleotides or adenosine synergistically promote long-term survival of serum-starved mouse embryo and human fetus fibroblasts. *Cell Signal* 2000; **12**:659–665.
48. Miranda J, Paules C, Nair S, Lai A, Palma C, Scholz-Romero K, Rice GE, Gratacos E, Crispi F, Salomon C. Placental exosomes profile in maternal and fetal circulation in intrauterine growth restriction - liquid biopsies to monitoring fetal growth. *Placenta* 2018; **64**:34–43.
49. Okamoto T, Seo H, Mano H, Furuhashi M, Goto S, Tomoda Y, Matsui N. Expression of human placenta alkaline phosphatase in placenta during pregnancy. *Placenta* 1990; **11**:319–327.
50. Valenzuela GJ, Munson LA, Tarbaux NM, Farley JR. Time-dependent changes in bone, placental, intestinal, and hepatic alkaline phosphatase activities in serum during human pregnancy. *Clin Chem* 1987; **33**:1801–1806.
51. Handwerger S, Freemark M. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *J Pediatr Endocrinol Metab* 2000; **13**: 343–356.
52. Männik J, Vaas P, Rull K, Teesalu P, Rebane T, Laan M. Differential expression profile of growth hormone/chorionic somatomammotropin genes in placenta of small- and large-for-gestational-age newborns. *J Clin Endocrinol Metabol* 2010; **95**:2433–2442.
53. Moore T, Williams JM, Becerra-Rodriguez MA, Dunne M, Kammerer R, Dveksler G. Pregnancy-specific glycoproteins: evolution, expression, functions and disease associations. *Reproduction* 2022; **163**:R11–r23.
54. Snyder SK, Wessner DH, Wessells JL, Waterhouse RM, Wahl LM, Zimmermann W, Dveksler GS. Pregnancy-specific glycoproteins function as immunomodulators by inducing secretion of IL-10, IL-6 and TGF-beta1 by human monocytes. *Am J Reprod Immunol* 2001; **45**:205–216.
55. Hogan C, Perkins AV. Selenoproteins in the human placenta: how essential is selenium to a healthy start to life? *Nutrients* 2022; **14**:628.
56. Cheng SB, Sharma S. Interleukin-10: a pleiotropic regulator in pregnancy. *Am J Reprod Immunol* 2015; **73**:487–500.
57. Gordon SM. Interleukin-15 in outcomes of pregnancy. *Int J Mol Sci* 2021; **22**:11094.
58. Zhang T, Li X, He Y, Wang Y, Shen J, Wang S, You Q, Zhai J, Shen L. Cancer-associated fibroblasts-derived HAPLN1 promotes tumour invasion through extracellular matrix remodeling in gastric cancer. *Gastric Cancer* 2022; **25**:346–359.
59. Haouzi D, Dechaud H, Assou S, Monzo C, de Vos J, Hamamah S. Transcriptome analysis reveals dialogues between human trophectoderm and endometrial cells during the implantation period. *Hum Reprod* 2011; **26**:1440–1449.
60. Sun T, Gonzalez TL, Deng N, DiPentino R, Clark EL, Lee B, Tang J, Wang Y, Stripp BR, Yao C, Tseng H-R, Karumanchi SA, Koeppel AF, *et al*. Table S2. Placental cell markers from single cell RNA sequencing. *FigShare* 2020. https://doi.org/10.6084/m9.figshare.10314404.v1
61. Suryawanshi H, Morozov P, Straus A, Sahasrabudhe N, Max KEA, Garzia A, Kustagi M, Tuschl T, Williams Z. A single-cell survey of the human first-trimester placenta and decidua. *Sci Adv* 2018; **4**:eaau4788.
62. Yang J, Wang L, Ma J, Diao L, Chen J, Cheng Y, Yang J, Li L. Endometrial proteomic profile of patients with repeated implantation failure. *Front Endocrinol (Lausanne)* 2023; **14**:1144393.
63. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, Park JE, Stephenson E, Polanski K, Goncalves A, Gardner L, Holmqvist S, *et al*. Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature* 2018; **563**:347–353.
64. Cocquebert M, Berndt S, Segond N, Guibourdenche J, Murthi P, Aldaz-Carroll L, Evain-Brion D, Fournier T. Comparative expression of hCG β-genes in human trophoblast from early and late first-trimester placentas. *Am J Physiol Endocrinol Metab* 2012; **303**:E950–E958.