Gene expression patterns in human placenta

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The placenta is the principal metabolic, respiratory, excretory, and endocrine organ for the first 9 months of fetal life. Its role in fetal and maternal physiology is remarkably diverse. Because of the central role that the placenta has in fetal and maternal physiology and development, the possibility that variation in placental gene expression patterns might be linked to important abnormalities in maternal or fetal health, or even variations in later life, warrants investigation. As an initial step, we used DNA microarrays to analyze gene expression patterns in 72 samples of amnion, chorion, umbilical cord, and sections of villus parenchyma from 19 human placentas from successful full-term pregnancies. The umbilical cord, chorion, amnion, and villus parenchyma samples were readily distinguished by differences in their global gene-expression patterns, many of which seemed to be related to physiology and histology. Differentially expressed genes have roles that include placental trophoblast secretion, signal transduction, metabolism, immune regulation, cell adhesion, and structure. We found interindividual differences in expression patterns in villus parenchyma and systematic differences between the maternal, fetal, and intermediate layers. A group of genes that was expressed in both the maternal and fetal villus parenchyma sections of placenta included genes that may be associated with preeclampsia. We identified sets of genes whose expression in placenta was significantly correlated with the sex of the fetus. This study provides a rich and diverse picture of the molecular variation in the placenta from healthy pregnancies.

microarray | pregnancy | transcriptome | preeclampsia

The placenta is a temporary organ that performs the functions of several adult organs for the growing fetus. The placenta is designed uniquely for exchange of oxygen, nutrients, antibodies, hormones, and waste products between the mother and fetus and may carry valuable information about the pregnancy. Although a placenta after delivery is among the most easily accessible human tissues, it is usually discarded after a cursory evaluation (1). Several pregnancy disorders including preeclampsia (PE) and preterm labor are associated with placental pathology. Also, epidemiologic studies suggest that there are "fetal origins" that predispose adults to cardiovascular, metabolic, and endocrine diseases (2). Also, low-birth-weight fetuses associated with large placentas are associated with increased neonatal morbidity indicating abnormal placental activity in such scenarios (3, 4). The investigation of placenta may provide valuable insights into placental functions and help identify molecular mechanisms that have both immediate and long lasting effects on health of the fetus.

A schematic representation of the placenta is given in Fig. 1, with both the placental disk proper and the reflected membranes. The placenta is a composite organ of trophectoderm-derived cells and cells that are derived from the inner cell mass (ICM)/epiblast, and a minor component comes from the mother in the form of her blood. The ICM/epiblast-derived components are the amnion, the umbilical cord, and the mesodermal components of the chorion. The amnion and chorion that line the amniotic cavity are incompletely fused, and together, they are called "reflected membranes;" the amnion and chorion that line the placental disk proper are called chorionic plate. The amnion is embryologically continuous with the epithelium of the umbilical cord, where it firmly fuses during development and cannot be dislodged. The amnion is

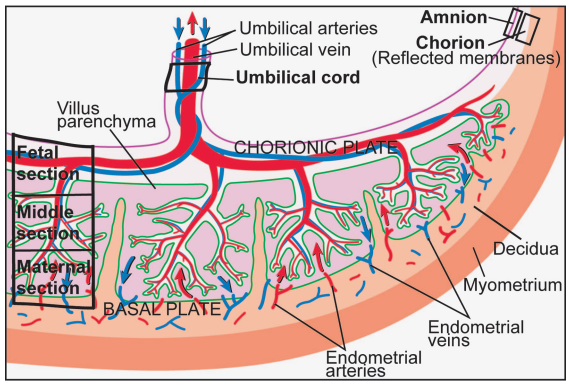

Fig. 1. Schematic representation of a human term placenta (44). Six parts of placenta from which mRNA was isolated [namely, amnion, chorion, umbilical cord, and three sections of villus parenchyma (fetal, middle, and maternal)] are shown in bold. The amnion and chorion were isolated by peeling them apart from the reflected membranes. The chorionic plate and the basal plate are also shown.

composed of a layer of epithelial cells resting on a basement membrane over a thin layer of connective tissue. The chorion is juxtaposed with chorionic connective tissue and at term includes atrophied remnants of villi and associated fetal blood vessels. The chorion is interdigitated with maternal decidua and its associated blood vessels. The umbilical cord consists of an amniotic epithelium, two arteries, and one vein embedded in a matrix called Wharton's Jelly. The villus parenchyma makes up most of the placenta and consists of 40–60 trophoblast villi. The trophoblastic villus is the functional unit of placenta where diffusion and active transport of nutrients and waste products takes place. The maternal side of villus parenchyma includes a thin basal plate corresponding to the maternofetal junction. The basal plate is made up of trophoblasts, interposed fibrinoid, and the endometrial components (stromal and fibroblast-like cells, as well as some macrophages, veins, and arteries).

Genome-scale transcriptional profiling has been used to study many human diseases and physiological processes, as well as to characterize gene-expression programs in diverse normal cells and tissues (5–7). A few studies have begun to explore interindividual variation in gene expression, revealing significant variation that is likely to provide insight into phenotypic variation (8–10). In addition to studies investigating individual genes or sets of specific genes (11), the genomewide gene-expression program during placenta development in mice has been described (12). Informative genome-scale studies using few or pooled human placenta samples have

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Abbreviations: PE, preeclampsia; IGF, insulin-like growth factor; PIGF, placental growth factor; HIF, hypoxia-inducible factor; P*n*, patient *n*; CCK, Cholecystokinin; NKB, neurokinin B; Flt1, Fms-like tyrosine kinase 1; FSTL3, follistatin-like 3.

Data deposition: The microarray data have been deposited in the Stanford MicroArray Database, http://smd.stanford.edu (accession no. GSE4421).

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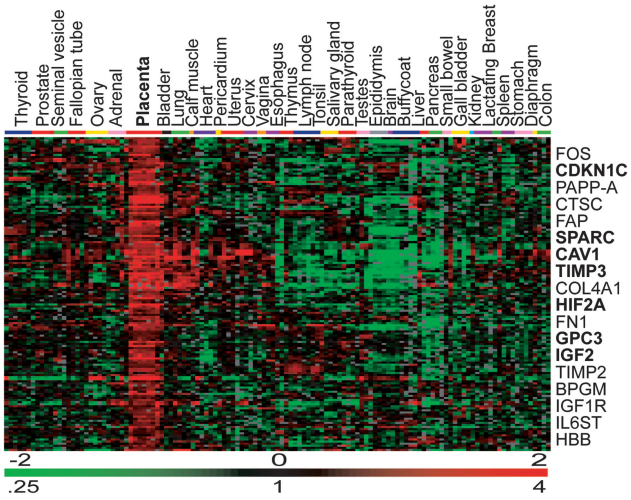

Fig. 2. Gene expression in villus parenchyma of placenta relative to other normal human tissues. We compared eight placental villus samples and 114 human tissue samples, representing 35 tissue types by using SAM (15). We chose 152 genes with a false-discovery rate of <0.06, based on differential expression between placental villus sections and other tissues, and the expression data for these genes are shown with the 35 tissues (columns; organized anatomically) and genes (rows; organized by hierarchical clustering). Expression levels are represented by a color tag, with red representing the highest levels and green representing the lowest levels of expression.

been done with a focus on pathological conditions rather than variation in normal placenta (13, 14).

Because a healthy placenta is essential for a successful pregnancy, we decided to begin with an investigation of the variation in gene expression in placentas of normal-term babies. The specific goal of this project was twofold. First, to identify the gene-expression patterns underlying the functional and histological specialization of discrete anatomical parts of nonpathological placentas. Second, begin to relate variation in gene expression to specific functional, clinical, anatomical, or genotypic differences among these placentas.

Results and Discussion

Gene Expression in Villus Parenchyma of Placenta Relative to Other Normal Human Tissues. As a step toward characterizing the placental transcriptome, we compared the gene-expression patterns in eight samples from the villus parenchyma portions of placenta with 114 normal human tissue samples representing 35 different tissue types (7). To define the genes whose patterns of expression were most distinctive in placenta, we used SAM (Significance Analysis of Microarrays) (15) to seek genes whose expression levels were consistently higher in placental villus parenchyma compared with the other 34 tissues. The 152 most significant differentially expressed genes (those with lowest q-value of $\leq$0.06), were hierarchically clustered and the samples were organized by tissue types (Fig. 2). Genes abundantly expressed in villus regions of placenta relative to other normal human tissues included insulin-like growth factor (IGF) 2 and pregnancy-associated plasma protein A (PAPP-A), which are known to be expressed in placenta (16, 17). In some cases, the extraplacental expression would suggest a potential biological role. The expression of hypoxia-inducible factor (HIF)2α was found to be at relatively higher levels in villus sections of placenta and in lung samples compared with all other normal tissues. In contrast, HIF1α, also a hypoxia-induced transcription factor, was expressed at relatively low levels in all tissues examined (including villus sections of placenta) except testes (data not shown). In response to hypoxia, the HIF transcription factors have been shown to induce transcription of genes with functions ranging from glycolysis, erythropoiesis to angiogenesis. The expression of HIF2α has been shown to be predominantly in vascular endothelial cells. The specificity in function and downstream targets of HIF transcription factors has begun to be investigated (18). Although there seems to be much redundancy, HIF1α but not HIF2α has been found to be associated with up-regulation of glycolytic genes (19). Consistent with this observation, within the placenta, HIF1α is expressed at the highest levels in the "branch 2" gene cluster (see Fig. 4*E*), in parallel with the elevated expression of enzymes involved in glucose metabolism including aldolase, enolase and GPI. Unlike other organs, both placenta and lung have primary roles in oxygen exchange (20), and the relatively high expression of HIF2α in these organs may be associated with a common response to hypoxia.

Several genes involved in growth and tissue remodeling were found to be expressed at relatively higher levels in the villus sections of placenta compared with other tissues. These genes include: GPC3, CDKN1C, and IGF2. GPC3, a heparin-sulfate proteoglycan, is mutated in patients with Simpson–Golabi–Behmel syndrome, a syndrome characterized by fetal–placental overgrowth and embryonal tumors. Another gene product that is associated with a fetal–placental overgrowth disease, Beckwith–Wiedemann syndrome is CDKN1C. In contrast, loss of IGF2, which is also an imprinted gene, is associated with fetal growth restriction in mice. During a short lifespan, the placenta undergoes rapid growth and an endometrial invasion that has been likened to tumor-like behavior. The relatively higher expression of genes that both promote and suppress growth suggests tight and local regulation of the pathways that control placental development.

Gene Expression in Different Parts of Placenta. We macroscopically dissected 19 singleton placentas that were obtained at delivery of full-term babies into amnion, chorion, umbilical cord, and three sections of villus parenchyma (Fig. 1) and processed for mRNA isolation. We analyzed 72 placental samples that included 7 amnion, 16 chorion, 5 cord, and 44 villus parenchyma sections. We focused on the $\approx$1,500 genes with the greatest variation in expression among the placental samples. To facilitate visualization and interpretation, the data were first organized by hierarchical clustering of both genes and samples based on overall similarity in expression pattern. We found striking differences reflected as distinct clustering of tissue samples into groups of similar anatomic origin, based on corresponding similarities in gene-expression patterns (Fig. 3*B*). The dendrogram (Fig. 3*A*) shows the major differences among amnion, chorion, and cord, as well as villus parenchyma. Among the villus parenchyma samples, sections from 9 of the 19 patients tended to cluster with other samples from the same patient, suggesting that consistent interindividual differences in gene-expression patterns are a significant component of the overall variation in gene expression. Therefore, the two major determinants of variation in the global expression patterns in villus parenchyma sections are the anatomic origin and the interindividual variation.

The amnion membrane has a unique physiological role and is a physical barrier between the fetal and external environment. Since 1910, the amnion has been used for a procedure called amniotic membrane transplantation for treatment of skin burns and certain ocular diseases because it seems to have antibacterial and antiadhesive properties (21). The amnion-expression profile shown in Fig. 4*A* provides some intriguing clues to the molecular basis of these properties. Note the high expression of a mucin protein (MUC1) in the amnion. MUC1 is a highly glycosylated transmembrane protein, expressed on mucosal surfaces of the stomach, lung, and amnion. *Muc1* knockout mice have been found to have chronic uterine infection caused by overgrowth of normal bacteria of the reproductive tract (22). The structure and expression patterns of mucin proteins suggest that they may protect the mucous membranes by sterically inhibiting bacterial access to the cell membrane. An association between high expression of MUC1 and aggressiveness

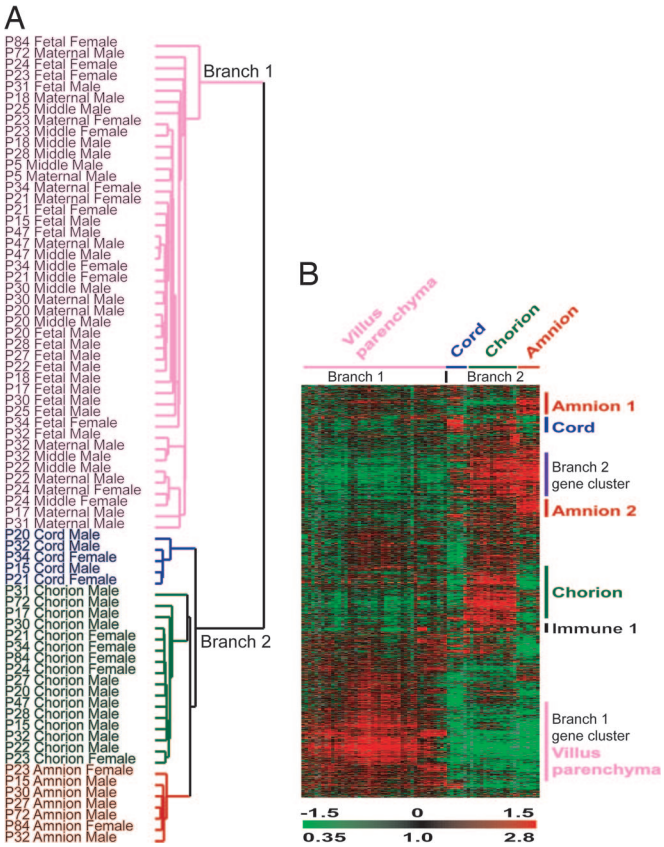

Fig. 3. Placental gene expression. (*A*) Unsupervised hierarchical clustering sorts placental samples based on their anatomical location. The 72 samples from 19 patients with successful pregnancies are designated according to the patient number (P*n*), part of the placenta, and fetal gender. Because of anatomic similarity in gene expression, the samples cluster to form shorter branches of amnion, chorion, umbilical cord, and villus parenchyma sections. Gene expression among sections of villus parenchyma forms one branch and varies significantly from the other branch that includes amnion, chorion, and umbilical cord samples. Among villus sections, the clustering relies on similarity in anatomical location and individual differences in gene expression. (*B*) Placental transcriptome. We selected ≈1,500 genes that were the most variably expressed among the samples by using a criterion of 3-fold change in level of expression in a minimum of two arrays. Each expression measurement represents the normalized ratio of fluorescence from the hybridized experimental material to a common internal reference. The prominent clusters of genes are shown on the right.

of some cancers has prompted speculation that this glycoprotein favors metastasis by inhibiting cell adhesion (23). Together, these observations suggest that expression of MUC1 may confer antibacterial and antiadhesive properties to amnion; its possible role in protecting against amniotic infections has not been determined.

The placenta is an immunologically privileged site. The regulation of complement system seems to be one of the mechanisms by which the allogeneic placenta evades the maternal immune defenses (24, 25). Three regulators of complement (namely, CD55, CD59, and MCP) are expressed at higher levels in placental villus sections compared with most other human tissues (Fig. 7, which is published as supporting information on the PNAS web site). Within the placenta, CD55 and CD59 are expressed at greatest levels in amnion, followed by the chorion and villus sections, whereas MCP is expressed at higher levels only in villus sections (Fig. 8, which is published as supporting information on the PNAS web site). Ref. 26 showed that these inhibitors of complement are expressed in syncytiotrophoblasts, which are the specialized placental cells lining the villi that are in direct contact with maternal blood and have been the focus of most immunological studies. Mice have a fourth complement inhibitor, Crry, which is similar to MCP and CD55. Deletion of *Crry* leads to death *in utero*, with C3 deposited on the placenta and marked invasion of inflammatory cells into the placenta (27). Also, the amnion compared with the chorion is remarkably nonimmunogenic: the amniotic membrane transplantation procedure does not require systemic immunosuppressives (21). Consistent with this observation, β_2-microglobulin and several MHC class I and II transcripts involved in antigen presentation are expressed at low levels or are absent in amnion and villus sections relative to the chorion (Fig. 4 *C* and *D*, Immune cluster). The immune properties of the amnion are intriguing because it is not in direct contact with maternal cells. The amnion may secrete the complement inhibitors themselves or in the form of protected exosomes (28) into the amniotic fluid or the neighboring maternofetal junction.

A prominent feature of the umbilical cord gene cluster (Fig. 4*B*) is the relatively elevated expression of genes involved in ECM synthesis (including types III, V, and VI collagens, along with LOXL2). Ultrastructure studies and immunohistochemistry of umbilical cord have shown that Wharton's Jelly contains microfibrils of collagen V and VI. The greater expression of follistatin in umbilical cord relative to other parts of placenta is unexpected because most follistatin was believed to be synthesized in placental villi and membranes (29). Follistatin is a direct inhibitor of activin and bone morphogenetic proteins (BMPs) that have been shown to regulate differentiation of progenitor cell types, including hematopoietic cells; its expression may have a role in regulating stem cell renewal vs. differentiation in the umbilical cord.

Many of the genes that are expressed at relatively higher levels in chorion have roles in signal transduction, cell differentiation, and immunity (Fig. 4*D*). The gene-expression profile of chorion may reflect the contrasting immunological milieus, because this fetal membrane was processed along with adhering atrophied trophoblast villi, remnant fetal blood vessels, and the interdigitated decidua. The chorion gene cluster includes tissue remodeling genes, such as lysosomal cysteine proteases, cathepsins, matrix metalloproteinases, and several genes that are induced by interferons (including *IFIT1*, *MX1*, and *JAK1*). Many genes that are known to have roles in immunity were expressed at especially high levels in chorion specimens, and in all other placental sites from patient 32 (P32) (Fig. 4 *C* and *D*, Immune 1 and 2). The first immune cluster comprises MHC type II molecules, and the second immune cluster has MHC type I molecules, β_2-microglobulin, CD68, and STAT1. The high level of expression of MHC genes in all samples from P32, including amnion, cord, and villus sections, suggests an inflammatory process in this placenta, although, clinically it was considered to be healthy and free of infection. Studies have found subclinical chorioamnionitis, which was discovered incidentally during histopathology of placenta from clinically noninfected women (30). Unfortunately, no samples of the P32 placenta were available for histological analysis.

A transcriptional profile that is common to the branch 2 cluster of genes, which were especially highly expressed in amnion, chorion, and some umbilical cord samples, is shown in Fig. 4*E*. A notable feature of this cluster of genes is the relatively high level of expression of HIF1α in these tissues. Given the known range in downstream targets of HIF1α (18, 19), we suspect it accounts for the coexpression of a set of hypoxia-induced genes (including VEGF, aldolase A, enolase, and TPI) in this gene cluster. In Fig. 4*F*, the "villus sections" cluster prominently features many secreted placental cell-signaling proteins [including human chorionic gonadotropin (HCG), placental growth factor (PIGF), activin A, and PAPP-A].

Genes That Define Interindividual Variation in Villus Sections of Placenta. This study investigates interindividual variation in placental villus parenchyma gene expression. This interindividual varia-

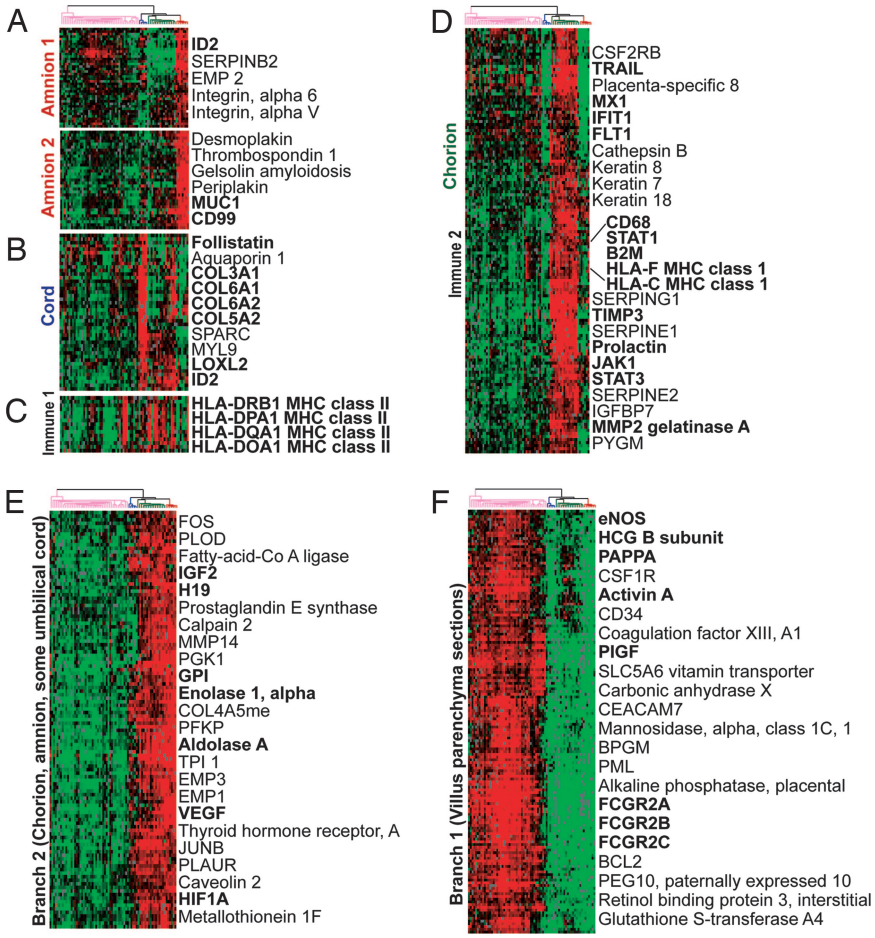

Fig. 4. Gene expression in different parts of placenta. Genes have been selectively shown from each of the prominent gene clusters in Fig. 3*B* to represent the range of diversity in placental gene expression. The clusters showing genes correlated with anatomy or immune function are amnion (*A*), umbilical cord (*B*), chorion (*D*), branch 2 (amnion, chorion, and umbilical cord) gene cluster (*E*), and branch 1 (villus parenchyma) (*F*). Also, a cluster of immune-function-related genes (*C*) expressed in most chorion samples (but, intriguingly, also in villus sections and amnion from P32) are shown. The visualization format is the same as in Fig. 3.

tion may be associated with the many facets of diversity in a population as well as variables of pregnancy, including race, other genetic variation, maternal health, maternal diet, age, fetal gender, fetal health, gestational age, variables of labor and delivery, and subsequent handling of placenta. For work on placental gene expression in disorders of pregnancy, pregnancy disorder-related gene-expression patterns and normal interindividual variations need to be distinguished. To identify genes whose expression differs more between individual placentas than among multiple samples of the same placenta, we calculated an "intrinsic score" for ≈10,000 genes from this primary data set. The intrinsic score was the ratio of the mean squared pairwise difference in the transcript level of that gene between placentas to the mean squared pairwise difference in the transcript levels of the gene between samples from the same placenta. We chose 303 genes that had an intrinsic score of >1.5 SD from the mean. When the 44 placenta villus section samples were clustered based on the expression of these "intrinsic" genes, all samples from the same placenta clustered together in the dendrogram, except for a single sample that was split from the remaining two samples from the same placenta (Fig. 5). This result clearly shows the molecular individuality of each placenta and by inference, the potential for individual variation in the placenta to lead to differences in fetal environment and, possibly, short- and long-term effects.

Genes that best distinguish individual placentas have roles in various pathways. Differences in expression of genes on sex chromosomes formed a significant component of the intrinsic gene cluster in placenta. Immune-related genes, including MHC class II, FPR1, SELL, and complement factors C2 and DF also vary in expression between placentas. The expression of sex chromosome genes was quite similar among sections of the same placenta including the "maternal" section, indicating that we did not see significant gene expression from the maternal cells. The Cholecystokinin (CCK) gene, encoding a neuropeptide with an important role in regulating satiety, also showed significant interindividual variation in placental expression. A large-scale study of global gene expression in normal human tissues show that *CCK* is expressed at highest levels in brain and placenta, but its role in the placenta has not been investigated (6). Many polymorphisms have been reported in the *CCK* gene (31), and the association of *CCK* polymorphisms with appetite and satiety remains to be determined (32). It would be interesting to determine whether there is a correlation between placental CCK expression and diet intake, satiety, and/or weight gain during pregnancy.

Genes That Distinguish Fetal, Maternal, and Middle Sections of the Placenta. We used a supervised approach to search for genes with consistent differences in expression in the maternal, fetal, and middle sections of the placenta (Fig. 6). We used SAM to choose 230 genes that have a q-value of <0.6. We found three sets of genes that are differentially expressed among the fetal, middle, and maternal sections and the gene clusters are named

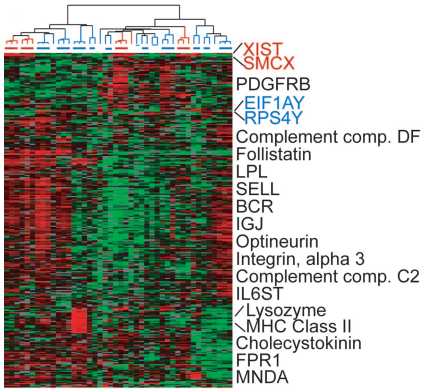

Fig. 5. Interindividual differences in placental villus parenchyma gene-expression patterns. We calculated an intrinsic score for ≈10,000 genes from this primary data set of villus sections. The intrinsic score was the ratio of the mean squared pairwise difference in the transcript level of that gene between villus sections of different placentas to the mean squared pairwise difference in the transcript levels of the gene between villus sections of the same placenta. We chose 303 genes that had an intrinsic score >1.5 SD from the mean to define genes that are intrinsic to villus parenchyma of each individual placenta. By using these genes, all except one sample clustered together with other villus samples of the same placenta. Genes located on the X and Y chromosome whose expression is associated with placentas of female or male fetuses (colored branches) are shown in red and blue, respectively. The visualization format is the same as in Fig. 3.

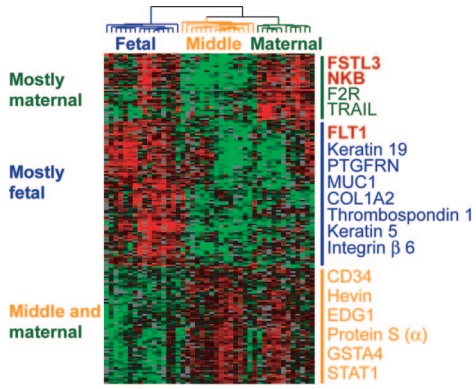

Fig. 6. Genes uniquely expressed in different layers of villus sections. SAM algorithm was used to select 230 genes whose expression varied significantly among fetal, middle, and maternal sections. These genes, which were selected with a delta of 0.2 to get a maximum false-discovery rate (*q*-value) of 0.6, were then used to hierarchically cluster the samples to visualize the expression profile within the villus parenchyma. The three gene clusters were assigned names (shown on the left) based on the sections that show a relatively greater expression of the genes (right). Genes whose names are shown in red are potentially associated with a hypertensive pregnancy disorder, PE, and are expressed mostly in maternal and/or fetal sections. The visualization format is the same as Fig. 3.

according to the sections that express them. The differentially expressed genes included two genes that have been reported to be associated with PE: the vasodilator neurokinin B (NKB) and the VEGF receptor Fms-like tyrosine kinase 1 (Flt1) (33, 34). Also associated with PE is follistatin-like 3 (FSTL3), which is an activin inhibitor. In our preliminary studies, FSTL3 is expressed at higher levels in placenta from PE (data not shown), which is consistent with refs. 13 and 14. Of these genes, NKB and FSTL3 were expressed at relatively elevated levels in most maternal and some fetal sections, whereas Flt1 was expressed at higher levels in most fetal and some maternal sections. Placental NKB has been measured in both maternal and cord blood (35), and our data suggest local expression at the maternal and fetal sections. FSTL3 is an inhibitor of activin A, which is important for differentiation of trophoblasts (36). Some articles suggest abnormal levels of activin A in maternal serum in PE, but FSTL3 sera levels have not yet been measured (37). Soluble Flt1 (sFlt1), which is encoded by an alternatively spliced transcript of Flt1, is an antagonist of VEGF and PIGF. Levels of sFlt1 in maternal blood have been shown to be elevated in PE patients (33). The anatomic expression of NKB, Flt1, and FSTL3 in maternal and fetal but not middle sections (all encoding potentially secreted proteins with hemodynamic effects) suggests that they may be part of a system for regulating blood flow, which is perturbed in PE.

Genes Differentially Expressed in Male and Female Placentas. We used SAM to determine genes whose expression differs between the placentas of male and female fetuses. Villus sections were used for the analyses, to enable greater sample size in each of the classes used for significance testing. We selected genes that met a criterion of a false-discovery rate of <6.2%, and we found that a greater proportion of these genes were expressed at higher levels in villus samples associated with female rather than male fetuses (see Table 1, which is published as supporting information on the PNAS web site, for gene names and chromosomal loci). Although many of these gender-correlated genes are located on sex chromosomes, some of these genes were autosomal, suggesting that they might be differentially expressed because of underlying differences between male and female physiology. Male babies are generally heavier and larger at term compared with female babies. Also, one study (38) shows that male gender is a predisposing factor toward prolongation of pregnancy. Genes expressed at higher levels in female placentas included those with roles in immune regulation like JAK1, IL2RB, Clusterin, LTBP, CXCL1, and IL1RL1. Females tend to have a greater immune response to a variety of stimuli, including immunization, infection, and autoantigens (39). JAK1 is an essential component of IFN signaling pathway, and, in *Drosophila*, the homologous JAK–STAT pathway has been implicated in the additional role of sex determination and sexual identity (40). The gender differences that we describe in gene expression in placenta suggest some interesting candidates for the pathways that might be responsible for gender differences observed in fetal development and physiology. We also found differentially expressed genes correlated with delivery method and birth weight (Tables 2 and 3, respectively, which are published as supporting information on the PNAS web site).

Conclusion

We have systematically characterized the gene-expression profile of human placentas from successful term pregnancies. We identified genes differentially expressed in anatomically different parts of the placenta that contribute to the physiology of this organ. Identification of genes expressed in placenta suggests features of placental physiology or roles for the differentially expressed genes, such as gender-specific functions of placenta. Also, we have identified genes whose expression levels vary among individuals and linked the variation in some of these genes to potential causes and consequences. This study is limited by the 72 samples from 19 placentas that we were able to investigate, and it by no means addresses all of the causes of variation that may underlie all changes in placental gene expression. However, these results should provide a valuable resource for investigations into pregnancy disorders that involve placental defect and, perhaps, even for diseases of later life that may have fetal origins.

Materials and Methods

Specimen Collection and Processing. Human placenta samples that were obtained after delivery with Institutional Review Board approval were chilled on ice and dissected into 1-g tissue samples.

The umbilical cord sample was cut ≈4 cm away from its site of insertion at the placental disk. The villus parenchyma sections were obtained by dissecting a 1.5-cm square-shaped segment through the entire ≈2.5-cm thickness of the placental disk (≈5 cm away from site of cord insertion) and then splitting it into the following three equal parts: maternal (includes thin basal plate), middle, and fetal (includes the chorionic plate). We isolated the amnion and chorion from the reflected membranes by peeling apart the incompletely fused membranes, as described in ref. 41. We chose an area of the membranes at least 4 cm away from the junction with placenta disk and from the site of rupture of the amniotic bag. The peeled chorion was processed along with its atrophied villi and interdigitated decidua. The tissues were snap frozen and stored at −80°C. Total RNA was isolated by using the protocols provided with Trizol reagent (Life Technologies, Rockville, MD), and mRNA was isolated by using the FastTrack kit (Invitrogen).

Microarray Procedure. We implemented microarray analysis by using a two-color approach, with the same reference sample as a common internal standard in every hybridization experiment. We prepared a reference RNA specifically for analysis of placenta. RNA was isolated from amnion, chorion, umbilical cord, and villus portions of seven normal and two preeclamptic placentas. These mRNA samples were pooled together and mixed with equal amounts of the described (42) common reference (CR) mRNA, derived from 11 cell lines. The modified placenta reference (PR) allowed measurement of expression levels of ≈300 additional genes that were not represented adequately in the CR alone. We reasoned that these ≈300 transcripts represented only in placenta samples might be particularly important for studies of specialized functions of placenta in analysis of placentas from pathological pregnancies. Retrospectively, the use of a placenta reference was justified, because it allowed reliable measurements of several additional transcripts including NKB and PIGF, whose differential expression in placenta was revealed in this study and are of possible interest in association with pregnancy disorders (33, 34). Samples were labeled and hybridized to the Human cDNA microarrays (Stanford Functional Genomics Facility, Stanford, CA) by using the protocols described in ref. 7. The microarray procedure for comparing gene expression among normal human tissue was the same, except that the CR was used as the internal reference.

Data Analysis. The data were extracted from the Stanford MicroArray Database, and we restricted our analysis to array elements for which the regression correlation between the signal intensities in the two channels across all pixels was >0.5 and for which the median hybridization signal intensity divided by the median background intensity was >1.2 in both the sample and reference channel for at least 85% of the analyzed samples (*Supporting Methods*, which is published as supporting information on the PNAS web site). To focus this initial analysis on the genes with the greatest variation in expression, we selected genes whose expression (in at least two samples) differed by ≥3-fold from their average expression across the entire set of samples. We then performed unsupervised hierarchical clustering (43) by using average-linkage clustering to group the genes and the tissue samples based on similarity in expression patterns. The data were further analyzed by using SAM to identify genes whose expression was significantly correlated with selected classes of samples (15).

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