Roles for genomic imprinting and the zygotic genome in placental development

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The placenta contains several types of feto-maternal interfaces where zygote-derived cells interact with maternal cells or maternal blood for the promotion of fetal growth and viability. The genetic factors regulating the interactions between different cell types within feto-maternal interfaces and the relative contributions of the maternal and zygotic genomes are poorly understood. Genomic imprinting, the epigenetic process responsible for parental origin-dependent functional differences between homologous chromosomes, has been proposed to contribute to these events. Previous studies showed that mouse conceptuses with an absence of imprinted differences between the two copies of chromosome 12 (upon paternal inheritance of both copies) die late in gestation and have a variety of defects, including placentomegaly. Here we examined the role of chromosome 12 imprinting in these placentae in more detail. We show that the spatial interactions between different cell types within feto-maternal interfaces are defective and identify abnormal behaviors in both zygote-derived and maternal cells that are attributed to the genome of the zygote but not the mother. These include compromised invasion of the maternal decidualized endometrium and the central maternal artery situated within it by zygote-derived trophoblast, abnormalities in the wall of the central maternal artery, and defects within the zygote-derived cellular layer of the labyrinth, which is in direct contact with maternal blood. These findings demonstrate multiple roles for chromosome 12 imprinting in the placenta that have not previously been associated with imprinting effects. They provide insights into the function of imprinting in placental development and have evolutionary and clinical implications.

The placenta of eutherian mammals contains cells derived from two individuals: the conceptus and the mother. During placental development zygote-derived and maternal cells form various types of intimate anatomical associations with each other and as a result give rise to specialized regions known as feto-maternal interfaces (1, 2). It is due to interactions between cells within these interfaces that the placenta brings about the interactions between the fetus and the mother necessary for fetal growth and viability (1–5).

In mice, the genetic basis of early placental development is currently being unraveled (6), but the factors responsible for the regulation of cell behavior within feto-maternal interfaces later in gestation and the relative contributions of the zygotic and maternal genomes are ill-defined. Understanding of this feto-maternal relationship could be clinically relevant, inasmuch as human pregnancy complications of unknown genetic etiology such as unexplained miscarriage and preeclampsia are associated with defective feto-maternal interfaces of the placenta (7–9).

Genomic imprinting in mammals (10) has been proposed to have a role in regulating the interactions between different cell types within feto-maternal interfaces of the placenta (11). However, to date, there is no evidence that it does. Genomic imprinting is the process responsible for the generation of parental origin-dependent functional differences between homologous chromosomes that are manifest as gene expression differences between the two alleles of imprinted genes (10). Identification of all of the biological functions of imprinting is far from complete and could contribute to an understanding of why this process has evolved. In mice, information about the biological functions of imprinting/imprinted genes comes from a variety of experimental approaches. These include gene knock-out studies of imprinted genes (10), the generation of diploid conceptuses in which both parental chromosome sets are inherited from one parent (12), or the generation of chromosomally balanced conceptuses that inherit both copies of a given homologous chromosome pair from the same parent (13). These studies showed that genomic imprinting is involved in prenatal growth control and in the development and viability of both embryonic and extraembryonic tissues (12). In the placenta, apart from its function in growth regulation, imprinting is involved in the differentiation and proliferation of some of its zygote-derived cells (12, 14).

A recent study investigated the *in vivo* function of mouse chromosome 12 imprinting by generating conceptuses that inherited both copies of this chromosome from either their father (paternal uniparental disomy for chrmosome 12, pUPD12) or mother (mUPD12). mUPD12 animals die perinatally and exhibit embryonic and placental growth retardation. Examination of mUPD12 placentae did not reveal any morphological anomalies apart from a size reduction (15). On the other hand, pUPD12 conceptuses die late in gestation, have a small crown-rump length, and exhibit a variety of defects, including placentomegaly (15). Both types of UPD12 conceptuses abnormally express chromosome 12-linked imprinted genes of as yet unknown function (16, 17).

To investigate in more detail the function of genomic imprinting during pUPD12 placental development, we generated pUPD12 conceptuses and examined their placentae at various gestation stages. We show that during late gestation pUPD12 placentae exhibit a variety of defects in cell behavior within feto-maternal interfaces. These findings identify multiple functions for chromosome 12 imprinting in placental development. Because many of these roles have never been associated with imprinting effects, this study increases the list of placental processes regulated by genomic imprinting. This work also shows that a defective zygotic genome (pUPD12) is sufficient for abnormalities in the behavior of maternal cells of the placenta. The evolutionary and clinical implications of this work are discussed.

Materials and Methods

Generation and Identification of pUPD12 Conceptuses. pUPD12 conceptuses were generated by intercrossing mouse stocks, both of which were heterozygous for two balanced Robertsonian translocation chromosomes that share monobranchial homology for chromosome 12 as described (15). pUPD12 conceptuses were distinguished from normal and mUPD12 with a PCR-based assay as shown before (15). Pregnant females were dissected at various embryonic stages, and embryonic day 0.5 (E0.5) was defined as the noon of the day a vaginal plug was detected. Since ≈50% of pUPD12 embryos die between E15.5 and E18.5 whereas the rest

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Abbreviations: pUPD12, paternal uniparental disomy for chromosome 12; mUPD12, maternal uniparental disomy for chromosome 12; E*n*, embryonic day *n*; H&E, hematoxylin/eosin.

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are dead by birth (15), only placentae from live conceptuses were used here.

Standard Histology, Immunocytochemistry, and Electron Microscopy. For standard histology and immunocytochemistry placentae were collected in PBS, fixed overnight in 4% paraformaldehyde at 4°C, dehydrated, and embedded in paraffin wax according to standard protocols. Seven-micrometer sections were either stained with hematoxylin/eosin (H&E) or subjected to immunocytochemical analysis for laminin $\alpha1$, p57^{KIP2}, α-smooth muscle actin, and proteins as described (5, 18, 19). Semithin (1 μm) sections for light or transmission electron microscopy were collected from placentae that were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer, processed, and stained according to standard methodology (20).

mRNA *in Situ* Hybridization. Placentae were collected in PBS, fixed overnight in 4% paraformaldehyde at 4°C, dehydrated, and embedded in paraffin wax according to standard protocols. Seven-micrometer sections were processed, hybridized with CTP[γ-^{35}S]-labeled RNA probes, autoradiographed, and stained with toluidine blue as described (21). For *Igf2* probes, a 2.3-kb mouse *Igf2* cDNA (*Bam*HI fragment of exon 6) cloned in pBluescript KS was used as a template for transcription of sense and antisense RNA probes with the use of T3 and T7 promoters, respectively (22). For *4311* probes, a 0.75-kb mouse *4311* cDNA (*Eco*RI/*Bam*HI fragment cloned in pGEM-1 plasmid) was used as a template for transcribing sense and antisense probes with the use of T7 and SP6 promoters, respectively.

Morphometric Analysis. Morphometric analysis for the estimation of the volume fraction of four labyrinthine parameters was carried out by point counting and expressed as percentages of the total number of points counted as indicated (23). Briefly, 64 randomly chosen labyrinthine areas from three different levels of sectioning, derived from two normal and two pUPD12 placentae, were used. Statistical significance was determined by the paired Student's t test. For this analysis methylene-blue-stained semithin sections were used (Fig. 2 *c* and *e*). We were able to distinguish fetal capillaries from maternal blood spaces because fetal erythrocytes tend to be larger and have polygonal-like shapes with sharp edges, whereas maternal erythrocytes tend to be smaller and have a smooth curvature. In addition, the wall of the fetal capillaries, unlike maternal blood spaces, is lined by endothelial cells that have distinct spindle-shaped nuclei (Fig. 2 *c* and *e*). Moreover, the transition from endothelium to fetal capillary lumen is sharper than that from trophoblast to maternal blood space (Fig. 2 *c* and *e*).

Results

The three major zones of the murine placenta are the labyrinth, the junctional zone, and the decidua basalis (1, 2, 24) (Fig. 1 *a* and *b*). These zones were examined in placentae from pUPD12 and normal littermate conceptuses with the use of standard histology, immunocytochemistry, mRNA *in situ* hybridization, and electron microscopy from E12.5 to near term (E18.5). Placentomegaly was evident by E18.5 (Fig. 1 *c* and *d*), consistent with the previously shown abnormally high pUPD12 placental mass (about 10%) at this stage (15). No defects were detected at E12.5, suggesting that early pUPD12 placental development is normal (data not shown). However, abnormalities were detected in all of the above zones later in gestation.

Anatomical Defects in the Labyrinth of pUPD12 Placentae. The labyrinth is the zone where fetal blood and maternal blood circulate within fetal capillaries and maternal blood spaces, respectively, for physiological exchange through zygote-derived feto-maternal interfaces (1, 2, 24–26). These labyrinthine interfaces consist of the endothelial cells of fetal capillaries, their associated basement membrane, and a trilaminar layer of trophoblast cells that directly lines the maternal blood spaces (25, 26) (Fig. 1*b*). In the E18.5 pUPD12 labyrinth, laminin $\alpha1$ immunostaining (a marker for fetal endothelium and its associated basement membrane) (5, 20) revealed that the fetal vasculature of the labyrinth is defective. The density of fetal capillaries is abnormally low, and their walls are irregularly shaped and at some points abnormally thickened, in contrast to their smooth, thin, and regular appearance in normal placentae (Fig. 2 *a* and *b*). A less severe manifestation of this defect was observed in pUPD12 placentae at E15.5 but not at E13.5 (data not shown). Acellular discontinuities within the feto-maternal interfaces are more numerous and more extensive in pUPD12 placentae (Fig. 2 *c* and *d*) and are located between the basement membrane and the innermost trophoblast layer (Fig. 2 *e–h*). Moreover, the middle trophoblast layer in pUPD12 placentae is abnormally thickened at many points, apparently as a result of more extensive invaginations of its maternal surface (Fig. 2 *e* and *f*). Taken together, these findings are consistent with morphometric analysis showing that in the pUPD12 labyrinth the volume fraction occupied by trilaminar trophoblast or acellular discontinuities within it is significantly increased, whereas that occupied by fetal capillaries or maternal blood spaces is significantly reduced (Fig. 3). These defects are associated with red blood cell clustering within the pUPD12 labyrinth, suggesting blood circulation defects (data not shown). These findings are also consistent with apparent differences in the expression of *Igf2* between normal and pUPD12 labyrinthine cells (Fig. 4 *c* and *d* and data not shown). *Igf2* is normally expressed in both the endothelium (strong) and trophoblast (weak) of the labyrinth

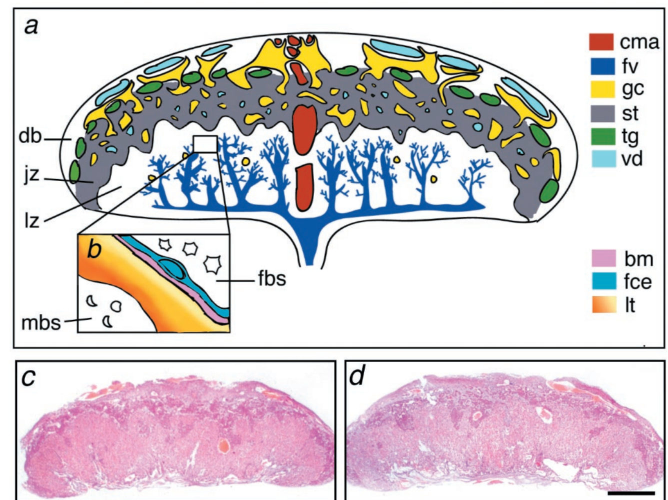

Fig. 1. The major regions and cell types of the mouse placenta at E15.5. (*a*) Schematic representation of a sagittal section of the mouse placenta. The placenta is oriented with its maternal side at the top and the fetal (flat) side at the bottom. The plane of sectioning is through the center of the placenta and perpendicular to its flat surface. All sections shown in this study were sectioned in this way. The major placental zones (db, decidua basalis; jz, junctional zone; lz, labyrinth zone) are shown; their constituent cell types are depicted by different colors. (*b*) Magnification of the boxed area in *a*, showing in more detail the labyrinthine feto-maternal interface (the zygote-derived tissue between fetal and maternal blood). The trilaminar nature of the labyrinthine trophoblast (orange) is only distinguishable with an electron microscope (26) (Fig. 2*e*). bm, basement membrane; cma, central maternal artery; fbs, fetal blood space; fce, fetal capillary endothelium; fv, fetal vasculature; gc, ''glycogen'' cell clusters; lt, labyrinthine trophoblast; mbs, maternal blood space; st, spongiotrophoblast cells; tg, trophoblast giant cells; vd, maternal venous drainage. (*c* and *d*) H&E staining of normal (*c*) and pUPD12 (*d*) E18.5 placentae at low power. (Scale bar = 1 mm.)

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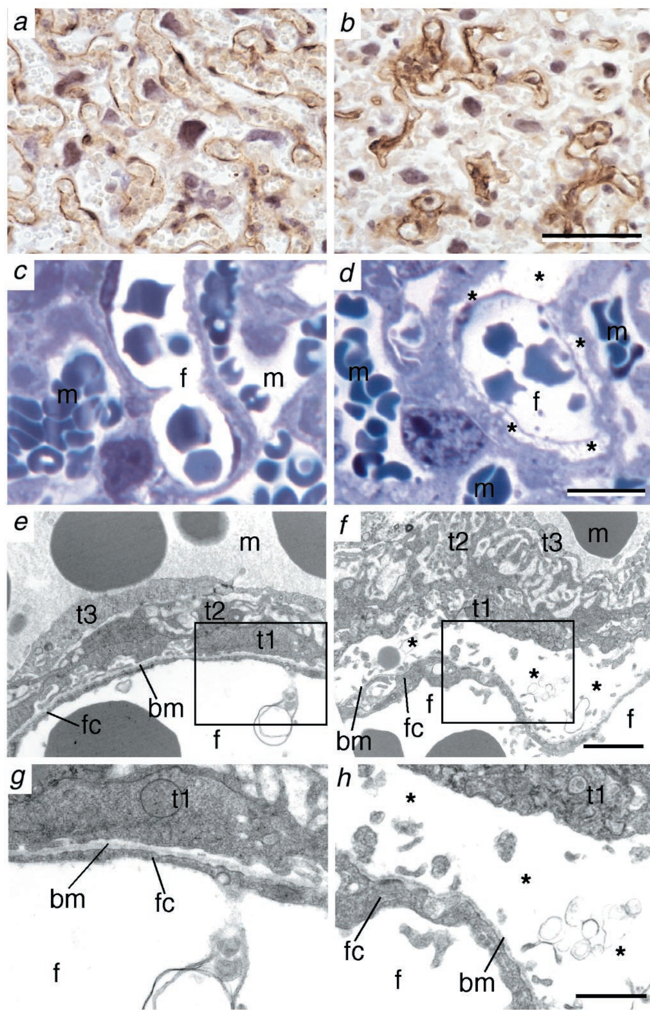

Fig. 2. Abnormal cell behavior in pUPD12 labyrinthine feto-maternal interfaces. Images are sagittal sections through the labyrinth of E18.5 normal (*a, c, e,* and *g*) and pUPD12 placentae (*b, d, f,* and *h*) placentae, and *g* and *h* are magnifications of the boxed areas in *e* and *f*, respectively. (*a* and *b*) Immunostaining for laminin α1 (counterstained with hematoxylin), a marker of the fetal endothelium and its associated basement membrane. Note the abnormal shape and density of the fetal vasculature in pUPD12 (*b*). (*c* and *d*) Methylene blue-stained semithin sections showing feto-maternal interfaces situated between the blood of fetal capillaries (f) and maternal blood spaces (m). Note the extensive acellular spaces (asterisks) within the pUPD12 interfaces (*d*). (*e* and *g*) Transmission electron micrographs distinguishing all three trophoblast layers (t1–t3), the fetal capillary endothelium (fc), and its associated basement membrane (bm) in the normal feto-maternal interface (*e*) as shown previously (25, 26). The outermost trophoblast layer (t3), which is cytotrophoblastic (25), is loosely attached to the middle layer (t2), which is syncytial (25). Note that the t2 layer has many vacuole-like spaces. These were shown to be due to the irregular invaginations of its maternal surface and are in direct contact with maternal blood plasma via the pores present in the t3 layer (25). (*f* and *h*) Note that in the pUPD12 labyrinthine interfaces, the acellular discontinuity (asterisks) is between the innermost trophoblast layer (t1) and the basement membrane (bm). Also note the increased thickness of the middle trophoblast layer (t2) in pUPD12. [Scale bars = 50 μm (*a* and *b*), 10 μm (*c* and *d*), 2 μm (*e* and *f*), 1 μm (*g* and *h*).]

(27). In the pUPD12 labyrinth, the density of strong *Igf2* positivity appears to be reduced. This reduction is more likely to be associated with the anatomical disorganization of the zone rather than abnormalities in *Igf2* expression because Northern blot analysis of normal and pUPD12 placentae with *Igf2* probes did not reveal any quantitative differences (data not shown).

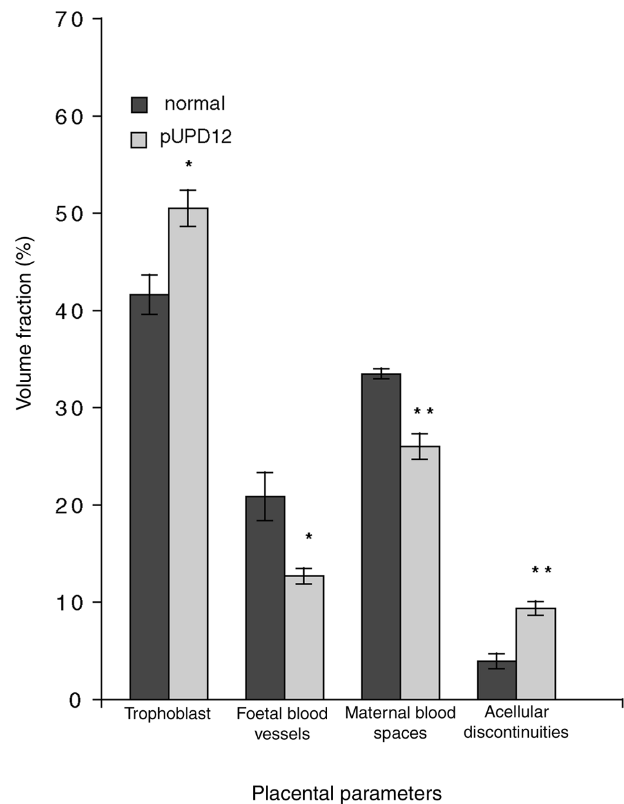

Fig. 3. Morphometric analysis showing the difference in the volume fraction (expressed as a percentage of total volume counted) of four labyrinthine parameters between normal and pUPD12 placentae at E18.5. Values are means; error bars representing the SEM. Statistical significance with $P \leq 0.01$ is indicated by one asterisk and that with $P \leq 0.001$ by two asterisks.

Abnormal Behavior of Cells within the pUPD12 Junctional Zone. The junctional zone consists of two types of zygote-derived trophoblasts (spongiotrophoblasts and "glycogen" cells) and is directly adjacent to the labyrinth (1, 2, 24) (Fig. 1 *a*). It contains a centrally located arterial channel and many peripherally located venous channels through which, respectively, maternal blood enters and exits the labyrinth (1, 2, 24) (Fig. 1 *a*). "Glycogen" cells together with spongiotrophoblasts line the venous channels of this zone and thus are in direct contact with maternal blood exiting the labyrinth (Fig. 4 *a* and *b*). The function of "glycogen" cells within this zone is not known, but their access to maternal venous blood and their synthesis of secreted protein hormones such as insulin-like growth factor 2 (27, 28) could potentially influence feto-maternal interactions. Their close association with spongiotrophoblasts which also line maternal venous sinuses (Fig. 4 *a* and *b*) and the finding that "glycogen" cells express the secreted protein calcyclin (29) (an inducer of placental lactogen hormone secretion by trophoblasts) could also have a similar effect by influencing the secretory behavior of spongiotrophoblasts.

During normal development, the junctional zone initially consists exclusively of spongiotrophoblasts, and by E13 "glycogen" cells appear within it, increase in number, and almost disappear between E15.5 and E18.5 (2, 27). They can be distinguished from other cell types of the junctional zone by histology (2, 27, 30) (Fig. 4 *a*), protein markers such as $p57^{kip2}$ (Fig. 4 *a* and *b*), or gene expression markers such as *Igf2* (27, 28). With the use of all three assays, it was found that in the pUPD12 junctional zone, "glycogen" cells are more abundant than normal at E13.5 and E15.5 (data not shown), and their dramatic

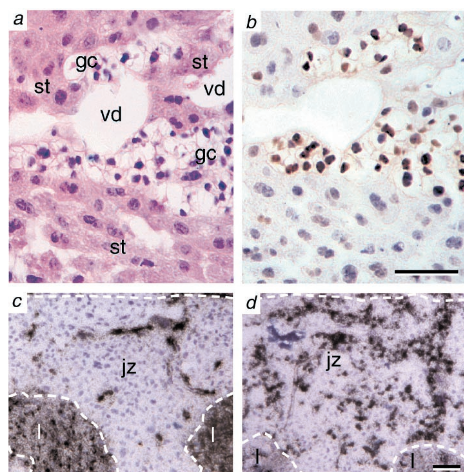

Fig. 4. Junctional zone ''glycogen'' cells in normal and pUPD12 placentae. (*a* and *b*) Adjacent sections of a region of the junctional zone in E15.5 normal placentae. H&E staining (*a*) shows the morphological distinctiveness of ''glycogen'' cells (gc) (clear cytoplasm with small, strongly stained nuclei) from spongiotrophoblast cells (st). Together with spongiotrophoblasts, they line venous drainage sinuses (vd) of the junctional zone. Note that strong immunostaining against p57KIP2 protein (*b*) (counterstaining with hematoxylin) or RNA *in situ* hybridization for *Igf2* gene transcripts (data not shown) marks ''glycogen'' cells as previously described (2, 27). (*c* and *d*) RNA *in situ* hybridization with an *Igf2* transcript-specific probe of E18.5 normal (*c*) and pUPD12 (*d*) junctional zone (jz) showing the abnormally high abundance of ''glycogen'' cells in pUPD12. The white lines in *c* and *d* depict the boundaries of the junctional zone from the decidua basalis/trophoblast giant cell layer junction and labyrinth zone (l) as judged from directly adjacent H&E sections. Note that *Igf2* is also expressed in the labyrinth as shown previously (27). [Scale bars = 50 μm (*a* and *b*) and 100 μm (*c* and *d*).]

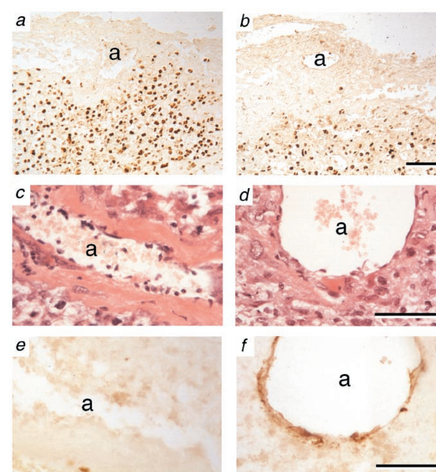

Fig. 5. Defects in the feto-maternal interfaces of the pUPD12 decidua basalis. (*a* and *b*) Sagittal sections immunostained with p57KIP2 antibody (without counterstaining) to show shallow invasion of the central decidua basalis by the p57KIP2-positive ''glycogen'' cells in pUPD12 placentae at E15.5 (*b*) when compared with normal E15.5 (*a*) placentae. The maternal surface of the decidua basalis is at the top of each picture. Note that in normal placentae, glycogen cells cluster around the wall of the central maternal artery (a). A similar defective spatial pattern of ''glycogen'' cells was also seen in pUPD12 placentae upon RNA *in situ* hybridization with the use of probes specific for *Igf2* or *4311* transcripts (data not shown), previously shown to mark ''glycogen'' cells situated within the decidua basalis (27, 30). (*c–f*) Sagittal sections of E18.5 normal (*c* and *e*) and pUPD12 (*d* and *f*) placentae through the central maternal artery situated within the decidua basalis, stained with H&E (*c* and *d*) or with an antibody against α smooth muscle actin (*e* and *f*) to detect its smooth muscle wall, as previously described (19). For both *e* and *f*, control arteries elsewhere in the body are stained positive (data not shown). Sections *c, e* and *d, f* are adjacent. Note that in contrast to normal material, the pUPD12 arterial wall lacks considerable acellular eosinophilic material (*d*) and failed to loose all of its positivity (*f*). [Scale bars = 100 μm (*a* and *b*) and 50 μm (*c–f*).]

decline by E18.5 fails to occur in pUPD12 (Fig. 4 *c* and *d*). No anatomical abnormalities were detected in spongiotrophoblasts and trophoblast giant cell layer (Fig. 1 *a*), as judged by histology and *4311* gene expression, a marker for both "glycogen" cells and spongiotrophoblasts (30) (data not shown).

Defective Behavior of Zygote-Derived and Maternal Cells in the pUPD12 Decidua Basalis. The decidua basalis (Fig. 1 *a*) contains maternal decidualized endometrium stromal cells, and after E13 it is progressively invaded by the junctional zone-derived "glycogen" cells (2, 27, 30). These are the only zygote-derived trophoblasts found beyond the trophoblast giant cell layer after E13 (2, 27, 30). Importantly, this region also contains a centrally located maternal artery that is continuous with the central arterial channel of the junctional zone and supplies the labyrinth with maternal blood (1, 2, 24) (Fig. 1 *a*). As gestation proceeds, "glycogen" cells are found in close proximity to the maternal cells that make up the wall of the central maternal artery, because they tend to cluster around it as they invade (2, 27, 30) (Fig. 5*a*). In normal decidua basalis, "glycogen" cell invasion is relatively advanced by E15.5 (2, 27, 30) (Fig. 5 *a*), and by E18.5 the wall of the central maternal artery has undergone a transformation that includes extensive deposition of eosinophilic acellular material and loss of smooth muscle contractability (Fig. 5 *c* and *e*). This change also occurs in humans and other mammals and is thought to free the artery from the vasomotor influences of the mother, thus allowing undisturbed maternal blood flow to the labyrinth (1, 31, 32). This physiological change is believed to be essential for the maintenance of a healthy pregnancy because its absence in humans is associated with fetal death and other pregnancy complications (7–9). The genetic basis of this maternal vasculature transformation and the role of the zygotic genome in this process are unknown. The function of "glycogen" cells in the decidua basalis and the factors controlling their invasion are also unclear.

Importantly, in the pUPD12 placentae, "glycogen" cell invasion of the decidua basalis is shallow in some and almost absent in others at both E13.5 and E15.5 (Fig. 5 *a* and *b*), but by E18.5 "glycogen" cells are evident throughout this zone in most placentae examined. This delayed and/or shallow invasion is associated with failure of complete transformation of the wall of the central maternal artery (Fig. 5 *c–f*). These data are consistent with the progressive lethality of pUPD12 fetuses after E15.5 (15). This model of defective trophoblast invasion and arterial transformation has not been described in a nonhuman mammalian system.

Discussion

The eutherian placenta functions in the promotion of fetal growth and viability by mediating interactions between the mother and the fetus through its feto-maternal interfaces (1–5). The present study identifies hitherto unknown functions for mouse chromosome 12-linked imprinted genes in the control of cell behavior within feto-maternal interfaces of the placenta and demonstrates that absence and/or overexpression of these genes results in defective placental development. Because many of these functions have not previously been associated with imprinting effects, our study provides insights into the function of genomic imprinting in placental development. Specifically, it is shown that genomic imprinting regulates the spatial interactions between different cell types within several types of feto-maternal interfaces. This regulation is evidenced by (*i*) the failure of the

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fetal capillary wall to retain its close association with the innermost trophoblast layer in many areas of the pUPD12 labyrinth, (*ii*) the abnormally prolonged access of "glycogen" cells to maternal venous blood and spongiotrophoblasts within the pUPD12 junctional zone after E15.5, and (*iii*) the shallow and/or delayed invasion of the maternal decidualized stromal cells and the wall of the central maternal artery by "glycogen" cells in the pUPD12 decidua basalis. Moreover, genomic imprinting is shown to have a role in the control of behavior of maternal cells within feto-maternal interfaces. This role is indicated by the failure of the wall of the pUPD12 central maternal artery to lose its α-smooth muscle actin positivity and to exhibit large quantities of acellular eosinophilic material. This finding also demonstrates that the inheritance of an abnormal zygotic genome (pUPD12) is sufficient for the abnormal behavior of maternal cells (central maternal arterial vasculature) of the placenta.

This study also identifies roles for chromosome 12 imprinting in the control of additional cell behaviors within the labyrinth. These roles are shown by the association of the pUPD12 labyrinth with increased volume fraction and thickness of the trophoblast layer, a reduced volume fraction and density, and the abnormal shape of the fetal capillaries. Similar defects have been reported in the labyrinth of placentae from a subset of mouse conceptuses lacking the maternally expressed imprinted gene *p57*^{KIP2} and were proposed to account for the late gestation lethality observed in some of these mutants (14, 33). Interestingly, in the absence of both *p57*^{KIP2} and the paternally expressed imprinted gene *Igf2,* the placental dysplasia observed in *p57*^{KIP2} single mutants is completely ameliorated (34). One interpretation of this observation is that in the absence of *Igf2*, *p57*^{KIP2} has no functional significance in placental development. It would be interesting to determine whether such a relationship also exists between *Igf2* and the pUPD12 labyrinthine phenotype. The two known chromosome 12-linked imprinted genes *Gtl2* and *Dlk* (16, 17) show absence and double expression, respectively, in pUPD12 conceptuses (16), but their contribution to the UPD12 phenotype is not yet known.

The failure of a complete transformation of the wall of the central maternal artery situated within the pUPD12 decidua basalis indicates that genomic imprinting is required in this process. The functional significance of this transformation during normal development is not known. It has been suggested that it is necessary for embryo viability by ensuring undisturbed flow of maternal blood to the labyrinth due to the inability of the arterial wall to contract in response to vasomotor signals from the mother (1, 32). This conclusion is supported by the findings that (*i*) experimentally induced temporal restriction of maternal blood through the rat central maternal artery on the fourth day before birth is associated with fetal death (35) and (*ii*) the absence of this arterial transformation in human placentae is associated with fetal death and other complications (7–9). The progressive lethality of pUPD12 embryos after E15.5 in the absence of any obvious embryonic defects that could be compatible with intrauterine death (15) is consistent with the theory that the absence of arterial wall transformation leads to embryonic lethality. Although this is still a theory, the pUPD12 placentae represent an experimentally induced mammalian system to examine this connection.

The compromised invasion of the central pUPD12 decidua basalis by "glycogen" cells indicates that imprinting is required for their correct invasion, a process hitherto of unknown genetic etiology. Even though the function of this type of invasive trophoblast is not known, it is interesting that their shallow and/or delayed invasion in pUPD12 is associated with failure of the wall of the central maternal artery to undergo a complete transformation. Importantly, there is strong circumstantial evidence in humans that absence or delay of the invasion of the proposed human analogues of mouse "glycogen" cells (see below) causes failure of arterial wall transformation (2, 7, 27, 28, 31).

The "conflict" theory concerned with the evolution of imprinting presupposes interactions between the fetus and the mother and has predicted the involvement of imprinting in the control of cell behavior within feto-maternal interfaces of the placenta (11). This theory states that the parental origin-specific functional differences between homologous chromosomes (imprinted differences) of the conceptus evolved as a consequence of a conflict between the paternally and maternally inherited genomes. Paternal genome function is said to have been selected to drain maternal resources for the benefit of the conceptus, whereas that of the maternal genome evolved to counteract this drain for the benefit of the mother and her subsequent pregnancies. In the absence of a maternal copy of chromosome 12, one would expect the function of the paternal copies to prevail. Our findings are consistent with the theory in the sense that imprinting does function in the control of cell behavior within placental feto-maternal interfaces. However, apart from the pUPD12 placentomegaly (15), it is not clear whether the other abnormalities of pUPD12 placentae and the death of pUPD12 fetuses (15) fit this model.

Our findings may have clinical implications because similar defects have also been seen in the analogous cell types/regions in human placentopathies of unknown genetic etiology. For example, in human placentae the compromised invasion of the placental bed (analogous to murine decidua basalis) (1) by extravillous interstitial invasive cytotrophoblasts (proposed analogues of mouse "glycogen" cells) (2, 7, 27, 28, 31), their abnormally prolonged presence within the basal plate (analogous to the mouse junctional zone) (1, 7), and the concomitant failure of complete arterial wall transformation of spiral arteries (analogous to the murine central maternal artery) (1) are thought to be causative factors in unexplained miscarriage and preeclampsia (7–9). Interestingly, one attempt to understand the complex inheritance pattern of human preeclampsia, a disease of unknown genetic basis associated with both fetal and maternal morbidity and mortality, suggested the absence of a maternally expressed imprinted gene in the conceptus, but not the mother, as a cause (36). This conclusion is consistent with the present findings and the death of pUPD12 fetuses (15). However, the identification of such a gene and whether mouse mothers respond to the pUPD12 placentopathy remain to be investigated.

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1. Pijnenborg, R., Robertson, W. B., Brosens, I. & Dixon, G. (1981) *Placenta* **2,** 71–92.
2. Redline, R. W. & Lu, C. Y. (1989) *Lab. Invest.* **61,** 27–36.
3. Bladt, F., Rietmacher, D., Isenmann, S., Aguzzi, A. & Birchmeier, C. (1995) *Nature (London)* **376,** 768–771.
4. Dietrich, S., Abou-Rebyeh, F., Brohmann, H., Bladt, F., Sonnerberg-Riethmacher, E., Yamaai, T., Lumsden, A., Brand-Saber, B. & Birchmeier C. (1999) *Development (Cambridge, U.K.)* **126,** 1621–1629.
5. Li, Y. & Behringer, R. R. (1998) *Nat. Genet.* **20,** 309–311.
6. Cross, J. C. (2000) *Semin. Cell. Dev. Biol.* **11,** 105–113.
7. Zhou, Y., Damsky, C. H., Chiu, K., Roberts, J. M. & Fisher, S. J. (1993) *J. Clin. Invest.* **91,** 950–960.
8. Khong, T. Y., De Wolf, F., Robertson, W. B. & Brosens, I. (1986) *Br. J. Obstet. Gynaecol.* **93,** 1049–1059.
9. Hustin, J., Jauniaux, E. & Schaaps, J. P. (1990) *Placenta* **11,** 477–486.

10. Joyce, J. A. & Ferguson-Smith, A. C. (1999) in *Development: Genetics, Epigenetics and Environmental Regulation*, eds. Russo, V., Cove, D., Edgar, L., Jaenisch, R. & Salamini, F. (Springer, Berlin), pp. 421–434.
11. Haig, D. (1996) *Am. J. Reprod. Immunol.* **35,** 226–232.
12. Fundele, R., Surani, M. A. & Allen, N. D. (1997) in *Genomic Imprinting*, eds. Reik, W. & Surani, M. A. (IRL, Oxford), pp. 98–117.
13. Cattanach, B. M. & Beechey, C. V. (1997) in *Genomic Imprinting*, eds. Reik, W. & Surani, M. A. (IRL, Oxford), pp. 118–145.
14. Zhang, P., Wong, C., DePinho, A., Harper, J. W. & Elledge, S. J. (1998) *Genes Dev.* **12,** 3162–3167.
15. Georgiades, P., Watkins, M., Surani, M. A. & Ferguson-Smith A. C. (2000) *Development (Cambridge, U.K.)* **127,** 4719–4728.
16. Takada, S., Tevendale, M., Baker, J., Georgiades, P., Campbell, E., Freeman, T., Johnson, M. H., Paulsen, M. & Ferguson-Smith, A. C. (2000) *Curr. Biol.* **10,** 1135–1138.
17. Schmidt, J. V., Matteson, P. G., Jones, B. K., Guan, X.-J. & Tilghman, S. M. (2000) *Genes Dev.* **14,** 1997–2002.
18. Yan, Y., Frisen, J., Lee, M.-H., Massague, J. & Barbacid, M. (1997) *Genes Dev.* **11,** 973–983.
19. Hellstrom, M., Kalen, M., Lindahl, P., Abramsson, A. & Betsholtz, C. (1999) *Development (Cambridge, U.K.)* **126,** 3047–3055.
20. Miner, J. H., Cunningham, J. & Sanes, J. R. (1998) *J. Cell Biol.* **143,** 1713–1723.
21. Georgiades, P. & Brickell, P. M. (1997) *Dev. Dyn.* **210,** 227–235.
22. Ferguson-Smith, A. C., Cattanach, B. M., Barton, S. C., Beechey, C. V. & Surani, M. A. (1991) *Nature (London)* **35,** 667–670.
23. Mayhew, T. M. & Burton, G. J. (1988) *Placenta* **9,** 565–581.
24. Muntener, M. & Hsu, Y.-C. (1977) *Acta Anat.* **98,** 241–252.
25. Enders, A. C. (1965) *Am. J. Anat.* **116,** 29–68.
26. Kirby, D. R. S. & Bradbury, S. (1965) *Anat. Rec.* **152,**279–281.
27. Redline, R. W., Chernicky, C. L., Tan, H.-Q., Ilan, J. & Ilan, J. (1993) *Mol. Reprod. Dev.* **36,** 121–129.
28. Han, V. K. M. & Carter, A. M. (2000) *Placenta* **21,** 289–305.
29. Farnsworth, R. L. & Talamantes, F. (1998) *Biol. Reprod.* **59,** 546–552.
30. Teesalu, T., Blasi, F. & Talarico, D. (1998) *Dev. Dyn.* **213,** 27–38.
31. Kam, E. P. Y., Gardner, L., Loke, Y. W. W. & King, A. (1999) *Hum. Reprod.* **14,** 2131–2138.
32. Nanaev, A. K. Chwalisz, K., Frank, H. G., Kohnen, G., Hegele-Hartung, C. & Kaufmann, P. (1995) *Cell Tissue Res.* **282,** 53–64.
33. Zhang, P., Wong, C., Liu, D., Finegold, M., Harper, J. W. & Elledge, S. J. (1999) *Genes Dev.* **13,** 213–224.
34. Caspary, T., Cleary, M. A., Perlman, E. J., Zhang, P., Elledge, S. J. & Tilghman, S. M. (1999) *Genes Dev.* **13,** 3115–3124.
35. Bruce, N. W. (1977) *Teratology* **16,** 327–332.
36. Graves, J. A. M. (1998) *Reprod. Fertil. Dev.* **10,** 23–29.