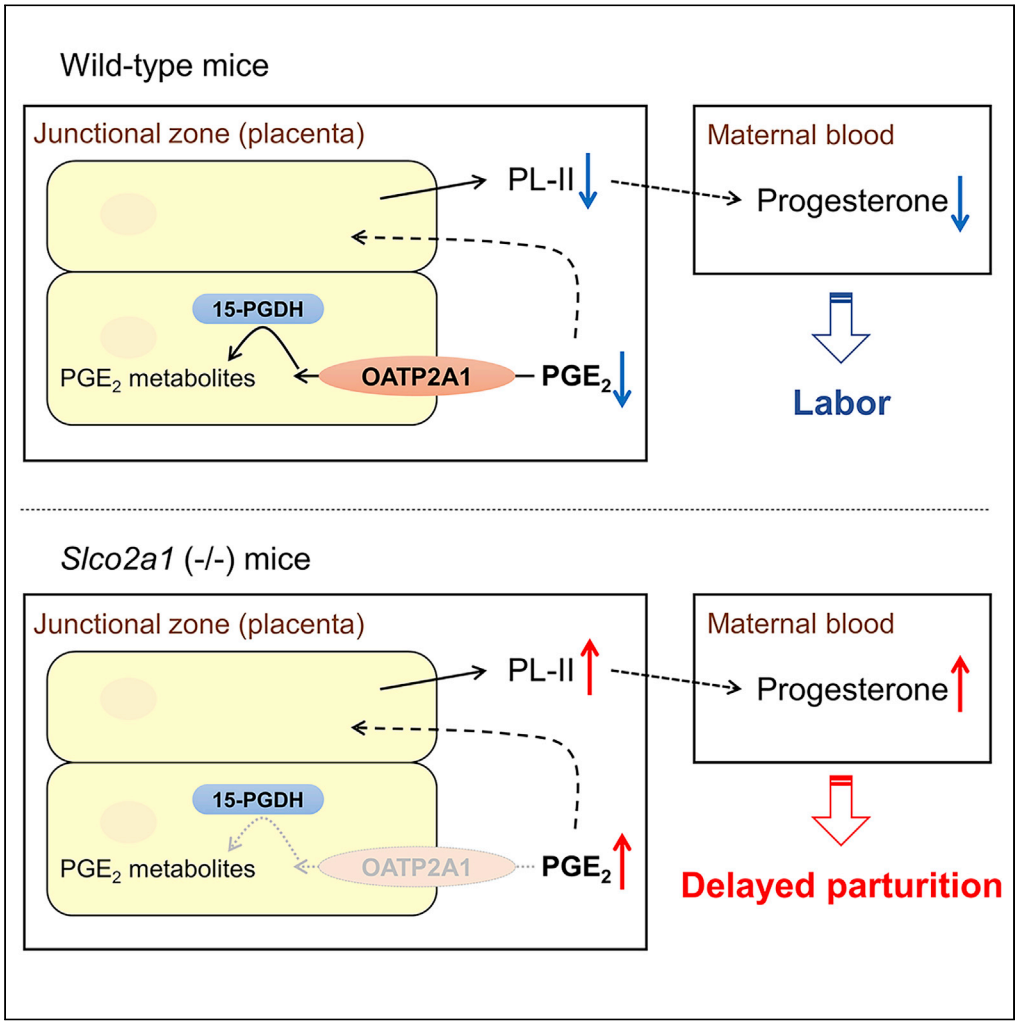
iScience

CellPress
OPEN ACCESS

Article

Contribution of Prostaglandin Transporter OATP2A1/*SLCO2A1* to Placenta-to-Maternal Hormone Signaling and Labor Induction



Mai Inagaki, Tomohiro Nishimura, Takeo Nakanishi, ..., Ikumi Tamai, Emi Nakashima, Masatoshi Tomi

tomi-ms@pha.keio.ac.jp

HIGHLIGHTS

Placental OATP2A1 promotes PGE_2 degradation by delivering PGE_2 to the cytoplasm

Placental PGE_2 stimulates the PL-II production

Extracellular PGE_2 decrease by placental OATP2A1 promotes progesterone withdrawal

Feto-placental OATP2A1 is associated with the initiation of parturition

Inagaki et al., iScience *23*, 101098
May 22, 2020
https://doi.org/10.1016/j.isci.2020.101098

iScience

CellPress
OPEN ACCESS

Article

Contribution of Prostaglandin Transporter OATP2A1/*SLCO2A1* to Placenta-to-Maternal Hormone Signaling and Labor Induction

Mai Inagaki,[1] Tomohiro Nishimura,[1] Takeo Nakanishi,[2,6] Hiroaki Shimada,[3] Saki Noguchi,[1] Shin-ichi Akanuma,[4] Masanori Tachikawa,[5] Ken-ichi Hosoya,[4] Ikumi Tamai,[6] Emi Nakashima,[1] and Masatoshi Tomi[1,7,*]

SUMMARY

We evaluated the contribution of organic anion transporting polypeptide 2A1 (OATP2A1/*SLCO2A1*), a high-affinity carrier for prostaglandins (PGs), to the parturition process. At gestational day (GD) 15.5, OATP2A1 is co-localized with 15-hydroxy-PG dehydrogenase in the mouse placental junctional zone and facilitates PG degradation by delivering PGs to the cytoplasm. *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males frequently showed elevated circulating progesterone at GD18.5 and delayed parturition. Progesterone receptor inhibition by RU486 treatment at GD18.5 blocked the delay of parturition. In the junctional zone, PGE_2 stimulated placental lactogen II (PL-II) production, resulting in higher expression of PL-II in *Slco2a1* (−/−) placenta at GD18.5. Indomethacin treatment at GD15.5 suppressed the PL-II overproduction at GD18.5 in *Slco2a1* (−/−) embryo-bearing dams, which promoted progesterone withdrawal and corrected the delayed parturition. These results suggest that extracellular PGE_2 reduction by OATP2A1 at mid-pregnancy would be associated with progesterone withdrawal by suppressing PL-II production, triggering parturition onset.

INTRODUCTION

Pre-term (<37 weeks of gestation) and post-term (>42 weeks of gestation) deliveries are leading causes of prenatal death and morbidity worldwide (Liu et al., 2015; Saigal and Doyle, 2008; Challis et al., 2000; Minakami and Sato, 1996). Prostaglandins (PGs), especially $PGF_{2\alpha}$ and PGE_2, have a uterotonic action in parturition and thus have been used clinically for induction of labor (Khan et al., 2008). In rodents, uterine $PGF_{2\alpha}$ production is induced via COX-1, and $PGF_{2\alpha}$ circulates to the ovary and binds to $PGF_{2\alpha}$ receptor to initiate corpus luteum regression, i.e., luteolysis (Sugimoto et al., 1997). Since progesterone is mainly produced by the corpus luteum, luteolysis reduces the circulating level of progesterone at late pregnancy (Malassine et al., 2003). Progesterone withdrawal, due to the decline of circulating progesterone levels, triggers COX-2 expression in the uterus, and COX-2-derived PGE_2 and $PGF_{2\alpha}$ promote uterine myometrial contraction and cervical ripening (Tsuboi et al., 2003), resulting in induction of labor. Therefore, autocrine/paracrine signaling by PGs is an important determinant of the timely onset of labor.

SLCO2A1, encoding organic anion transporting polypeptide (OATP) 2A1, also known as PG transporter, is a high-affinity carrier for prostanoids (Kanai et al., 1995) and is thus capable of regulating autocrine/paracrine signaling by PGs. In the lung, OATP2A1 mediates the transport of extracellular PGE_2 into alveolar epithelial cells, reducing extracellular PGE_2 levels (Chang et al., 2010; Nakanishi et al., 2015). PGE_2 *in utero* plays a critical role in maintaining patency of the fetal ductus arteriosus (Thorburn, 1992), and a fall in PGE_2 level after birth triggers its closure (Coggins et al., 2002). In *Slco2a1*-deficient neonates, the ductus arteriosus fails to close after birth owing to the lack of pulmonary uptake of PGE_2 (Chang et al., 2010). *In vitro* study has demonstrated that co-expression of OATP2A1 with 15-hydroxy-PG dehydrogenase (15-PGDH), a PG-degrading enzyme, accelerates the reduction of extracellular PG levels: PG inactivation involves active uptake into cells via OATP2A1 followed by cytoplasmic oxidation via 15-PGDH (Nomura et al., 2004). These findings indicate that OATP2A1 plays a central role in controlling extracellular PGE_2 concentration and thus in signaling via the PGE_2 receptor. In mice and humans, *SLCO2A1* mRNA is ubiquitously expressed across tissues but is most abundantly expressed in the placenta (Cheng et al., 2005; Kraft et al., 2010). The placenta has the ability to produce large amounts of PGE_2 (Okazaki et al., 1981; Helliwell et al.,

[1]Faculty of Pharmacy, Keio University, Minato-ku, Tokyo 105-8512, Japan

[2]Faculty of Pharmacy, Takasaki University of Health and Welfare, Takasaki, Gunma 370-0033, Japan

[3]Faculty of Pharmacy, Kindai University, Higashiosaka, Osaka 577-8502, Japan

[4]Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0194, Japan

[5]Graduate School of Biomedical Sciences, Tokushima University, Tokushima 770-8505, Japan

[6]Faculty of Pharmaceutical Sciences, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan

[7]Lead Contact

*Correspondence: tomi-ms@pha.keio.ac.jp

https://doi.org/10.1016/j.isci.2020.101098

2006; Inagaki et al., 2017), and secretion of placental PGE_2 into the fetal circulation has been proposed to maintain patency of the fetal ductus arteriosus (Thorburn, 1992). Nevertheless, the role of OATP2A1-mediated PG disposition in the placenta remains to be established.

In rat placenta, 15-PGDH is highly expressed in the junctional zone (Mark et al., 2013), which is positioned between the labyrinth and the maternal decidua. The junctional zone secretes placental lactogens (PLs) into the maternal circulation (Simmons et al., 2008). PL-I/*Prl3d* and PL-II/*Prl3b1* are considered to induce the production of progesterone in rodent luteal cells (Galosy and Talamantes, 1995; Thordarson et al., 1997; Zhong et al., 1997) and thus are proposed to be associated with the onset of parturition. This idea is supported by the fact that mice deficient in *Nrk*, a Ser/Thr kinase, show hyperproliferation of the junctional zone and delayed parturition of dams (Denda et al., 2011). Disruption of *Sirh7/Ldoc1*, a long terminal repeat retrotransposon, in mice likewise causes delayed parturition and overgrowth of the junctional zone with overproduction of PL-I (Naruse et al., 2014). It has been reported that the addition of PGE_2 and $PGF_{2\alpha}$ increases corticotropin-releasing hormone secretion from cultured human placental cells (Petraglia et al., 1987). Thus, we hypothesized that, assuming OATP2A1 is localized in the junctional zone of the placenta, the manipulation of PG disposition by OATP2A1 affects placental endocrine function and thereby influences the timing of parturition.

Our aims in this work are to investigate the role of OATP2A1 in placental PG disposition and to evaluate the effect of *Slco2a1* deficiency on placental endocrine function and the timing of parturition. The upregulation of COX-1 expression in the uterus, triggering progesterone withdrawal occurs at gestational day (GD) 16.5 in C57/BL6 mice (Tsuboi et al., 2000), and therefore, in this study, we mainly analyzed phenotypes in *Slco2a1*-deficient pregnant mice at GD15.5 (mid-pregnancy), the day before progesterone withdrawal, and at GD18.5 (late pregnancy), the day prior to parturition.

RESULTS

OATP2A1 Is Predominantly Expressed in Spongiotrophoblasts of Mouse Placenta

To investigate the distribution of *Slco2a1* mRNA in the placenta, sections obtained at GD 15.5 were hybridized with antisense RNA probe for *Slco2a1*. As shown in Figure 1A, *Slco2a1* is predominantly expressed in the junctional zone of fetal-derived placenta. In the junctional zone, which is composed of spongiotrophoblasts, glycogen trophoblast cells, and parietal trophoblast giant cells, staining of serial sections showed that the distribution of *Slco2a1*-positive cells (Figure 1A i and ii) coincides with the distribution of *Prl8a8* (Figure 1A iii and iv), a spongiotrophoblast marker (Simmons et al., 2008), strongly suggesting that *Slco2a1* mRNA is localized in spongiotrophoblast cells of the junctional zone. In addition, *Slco2a1* was weakly detected in parietal trophoblast giant cells (Figure 1A ii). Intense staining of OATP2A1 protein in the placenta at GD15.5 was selectively observed in the junctional zone (Figure 1B), in accordance with the distribution of *Slco2a1* mRNA (Figure 1A). Intense signals of 15-PGDH protein were detected in the maternal decidua and the junctional zone (Figure 1B). Double immunofluorescence staining showed that OATP2A1 (red) and 15-PGDH (green) were co-localized in spongiotrophoblasts of the junctional zone, and staining for OATP2A1 at the plasma membrane was diminished in placental sections prepared from *Slco2a1* (−/−) mice (Figure 1C). These results support the idea that 15-PGDH degrades PGE_2 after it has been taken up into spongiotrophoblasts via OATP2A1. Placentas of *Slco2a1* (−/−) mice were morphologically indistinguishable from those of wild-type littermates at both GD15.5 and GD18.5 (Figure S1). Moreover, there was essentially no difference in fetal or placental weight between wild-type and *Slco2a1* (−/−) littermates (Figure S2).

We next quantified the absolute expression levels of OATP2A1 in the plasma membrane-enriched fractions from the uterus and three major zones of placenta (i.e., maternal decidua, junctional zone, and labyrinth) by detecting peaks of OATP2A1-derived peptides using LC-MS/MS. The absolute expression level of OATP2A1 protein in the junctional zone was more than 10 fmol/μg protein at GD15.5, which far exceeds that in any other tissue examined, and was consistently about 10 fmol/μg protein or more from GD13.5 to GD18.5 (Figure 1D). No signal peak was observed in the plasma membrane-enriched fraction from the junctional zone of *Slco2a1* (−/−) mouse placenta (Figure S3).

OATP2A1 Is Involved in PG Degradation in Spongiotrophoblasts of Mouse Placenta

To examine the function of OATP2A1 in the junctional zone, isolated explants of each placental zone at GD15.5 and GD18.5 were prepared for [^{3}H]PGE_2 uptake study. In the junctional zone of *Slco2a1* (−/−) mice, the uptake of [^{3}H]PGE_2 was significantly lower than that of wild-type littermates at both GD15.5

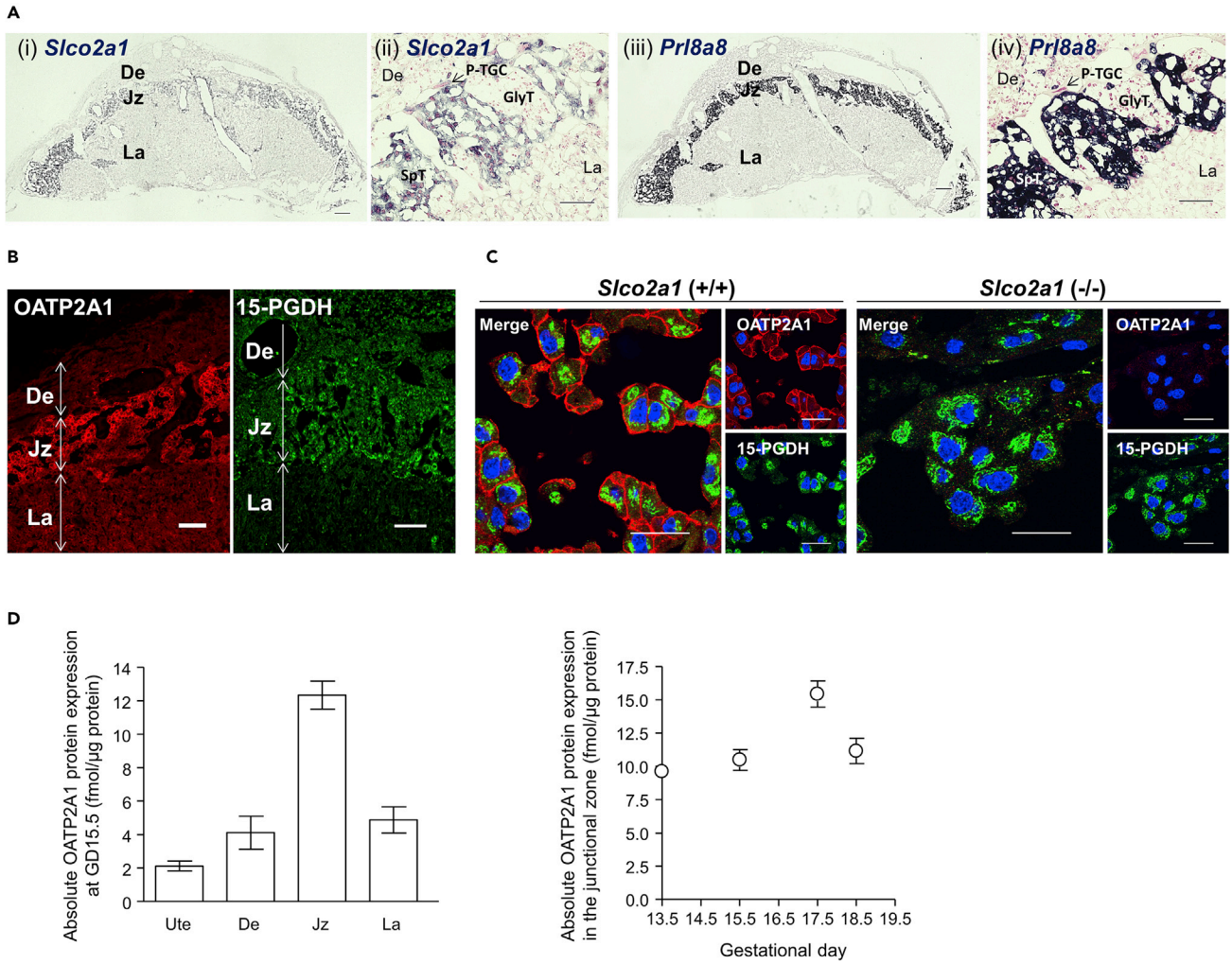


Figure 1. Placental Expression of OATP2A1

(A) *In situ* hybridization of *Slco2a1* (purple, i) and *Prl8a8* (purple, iii) in serial sections of GD15.5 mouse placenta. *Prl8a8* is used as a marker of spongiotrophoblasts. (ii) and (iv) are enlarged views of (i) and (iii), respectively. De, decidua; Jz, junctional zone; La, labyrinth; SpT, spongiotrophoblasts; GlyT, glycogen trophoblast cell; P-TGC, parietal trophoblast giant cell. Scale bars, 300 (i and iii) and 100 (ii and iv) µm.

(B) Immunofluorescence of OATP2A1 (red) and 15-PGDH (green) in the mouse placenta at GD15.5. Scale bars, 120 µm.

(C) Double immunofluorescence of OATP2A1 with 15-PGDH in the placental junctional zone of GD15.5 wild-type (left) and *Slco2a1* (−/−) placenta (right). Scale bars, 30 µm.

(D) (Left) The absolute expression levels of mouse OATP2A1 protein in the plasma membrane-rich fraction. (Right) Gestational changes of OATP2A1 protein expression in plasma membrane-rich fraction of the junctional zone, from GD13.5 to GD18.5. Data are expressed as the mean ± SEM. Ute, uterus; De, decidua; Jz, junctional zone; La, labyrinth.

(Figure 2A) and GD18.5 (Figure 2B), indicating that OATP2A1 mediates the uptake of extracellular PGE_2 into spongiotrophoblasts of the junctional zone. In the decidua and the labyrinth, there was no significant difference of [^{3}H]PGE_2 uptake between wild-type and *Slco2a1* (−/−) littermates (Figure 2).

We next measured endogenous concentrations of PGs in the homogenate of the junctional zone. PGE_2 and $PGF_{2\alpha}$ levels in *Slco2a1* (−/−) mice junctional zone at GD15.5 tended to be lower; this may reflect a decline in intracellular concentrations owing to the lack of uptake via OATP2A1. There was a more marked effect on the concentrations of 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto PGA_2, which are stable metabolites of PGE_2 generated by 15-PGDH (Figure 3A). Similarly, the level of 13,14-dihydro-15-keto $PGF_{2\alpha}$, a stable metabolite of $PGF_{2\alpha}$ generated by 15-PGDH, was also decreased in the junctional zone of *Slco2a1* (−/−) placenta (Figure 3A). These results suggest that the uptake of PGE_2 and $PGF_{2\alpha}$ via OATP2A1

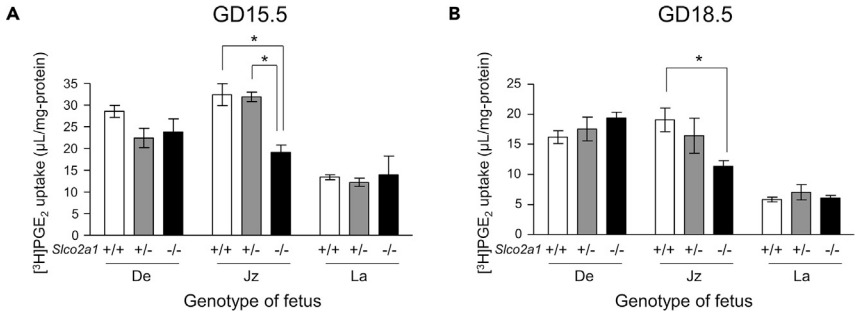


Figure 2. Effect of *Slco2a1* Deficiency on PGE_2 Uptake by Placental Explants

(A and B) Explants of the decidua (De), junctional zone (Jz), and labyrinth (La) at GD15.5 (A) and GD18.5 (B) were incubated for 20 min with [^{3}H]PGE_2 (5.6 nM) (n = 4–19). Data are expressed as the mean ± SEM. *p < 0.05, significant difference between groups (one-way ANOVA followed by Bonferroni's post hoc test).

facilitates their degradation by 15-PGDH and consequently results in a decline of extracellular PGE_2 and $PGF_{2\alpha}$ levels in the junctional zone. Conversely, the lack of PGE_2 uptake via OATP2A1 appears to result in accumulation of extracellular PGE_2. *Slco2a1* deficiency did not affect the expression levels of 15-PGDH- and PGE_2-synthesizing enzymes (i.e., COX-1, COX-2, and mPGES-1) (Figure S4), and this further supports the idea that OATP2A1 itself largely caused the change in PGE_2 level at GD15.5. In contrast to GD15.5, at GD18.5, there was no significant difference of endogenous PGE_2 and $PGF_{2\alpha}$ metabolite levels between wild-type and *Slco2a1* (−/−) littermates in the junctional zone (Figure 3B), suggesting that extracellular accumulation of placental PGE_2 and $PGF_{2\alpha}$ due to *Slco2a1* deficiency occurs at mid-pregnancy and not at late pregnancy.

Feto-Placental OATP2A1 Is Required for Parturition

In crosses of *Slco2a1* (−/−) mice, the mean length of gestation was comparable with that in wild-type counterparts, although 30% (2 of 7 cases) of dams exhibited post-term delivery (>GD20.0) and 30% (2 of 7 cases) of dams exhibited pre-term delivery (<GD19.0) (Figure 4A). To examine the requirement for placental OATP2A1 in parturition, we mated *Slco2a1* (+/−) females to wild-type or *Slco2a1* (−/−) males, giving rise to wild-type and *Slco2a1* (+/−) placentas/fetuses or *Slco2a1* (+/−) and *Slco2a1* (−/−) placentas/fetuses *in utero*, respectively. In crosses of *Slco2a1* (+/−) females to wild-type males, no difference in the timing of labor was evident compared with that in wild-type counterparts (GD19.6 ± 0.2; n = 24 versus GD19.5 ± 0.1; n = 12). Meanwhile, when *Slco2a1* (+/−) females were crossed with *Slco2a1* (−/−) males, more than 50% (16 of 29 cases) of dams gave birth at GD20.5 or later. Thus, a significant delay in parturition was observed in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males (GD20.4 ± 0.2; n = 29, $p < 0.05$) compared with that in the females mated with wild-type males. These results indicate that the initiation of parturition is delayed by the high proportion of *Slco2a1* (−/−) conceptuses *in utero* (Figure 4A). There was no significant difference of litter size (6.6 ± 0.3; n = 33 versus 6.8 ± 0.5; n = 26) or resorption rate (0.16 ± 0.02; n = 33 versus 0.15 ± 0.03; n = 26) *in utero* at GD18.5 between *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males and those mated with wild-type males (Figure 4B). Even at parturition, there was no difference in pup number between these two crosses (5.7 ± 0.3; n = 27 versus 5.9 ± 0.6; n = 14). On the other hand, in crosses of *Slco2a1* (+/−) females to *Slco2a1* (−/−) males, the average number of weaned pups was significantly lower than that of the females mated to wild-type males (3.6 ± 0.2; n = 40 versus 6.7 ± 0.5; n = 22, $p < 0.05$), and the average proportion of *Slco2a1* (−/−) pups in a litter at weaning was significantly decreased compared with that at GD18.5 (0.10 ± 0.03; n = 40 versus 0.48 ± 0.04; n = 33, $p < 0.05$). These findings indicate that *Slco2a1* deficiency does not lead to embryonic lethality and that more than 70% of *Slco2a1* (−/−) pups die after birth and before weaning, presumably due to patent ductus arteriosus (Chang et al., 2010).

Feto-Placental OATP2A1 Controls Progesterone Withdrawal

Progesterone is an absolute requirement for success of pregnancy, and withdrawal of progesterone signaling is critical for parturition (Cha et al., 2012). To examine whether progesterone withdrawal is impaired in *Slco2a1* (−/−) embryo-bearing dams, we measured circulating levels of maternal progesterone at GD18.5, when the circulating progesterone usually decreases in wild-type mice due to luteolysis. The progesterone levels in the maternal blood from *Slco2a1* (+/−) pregnancies produced by mating with *Slco2a1* (−/−) males were significantly higher than those produced by mating with wild-type males

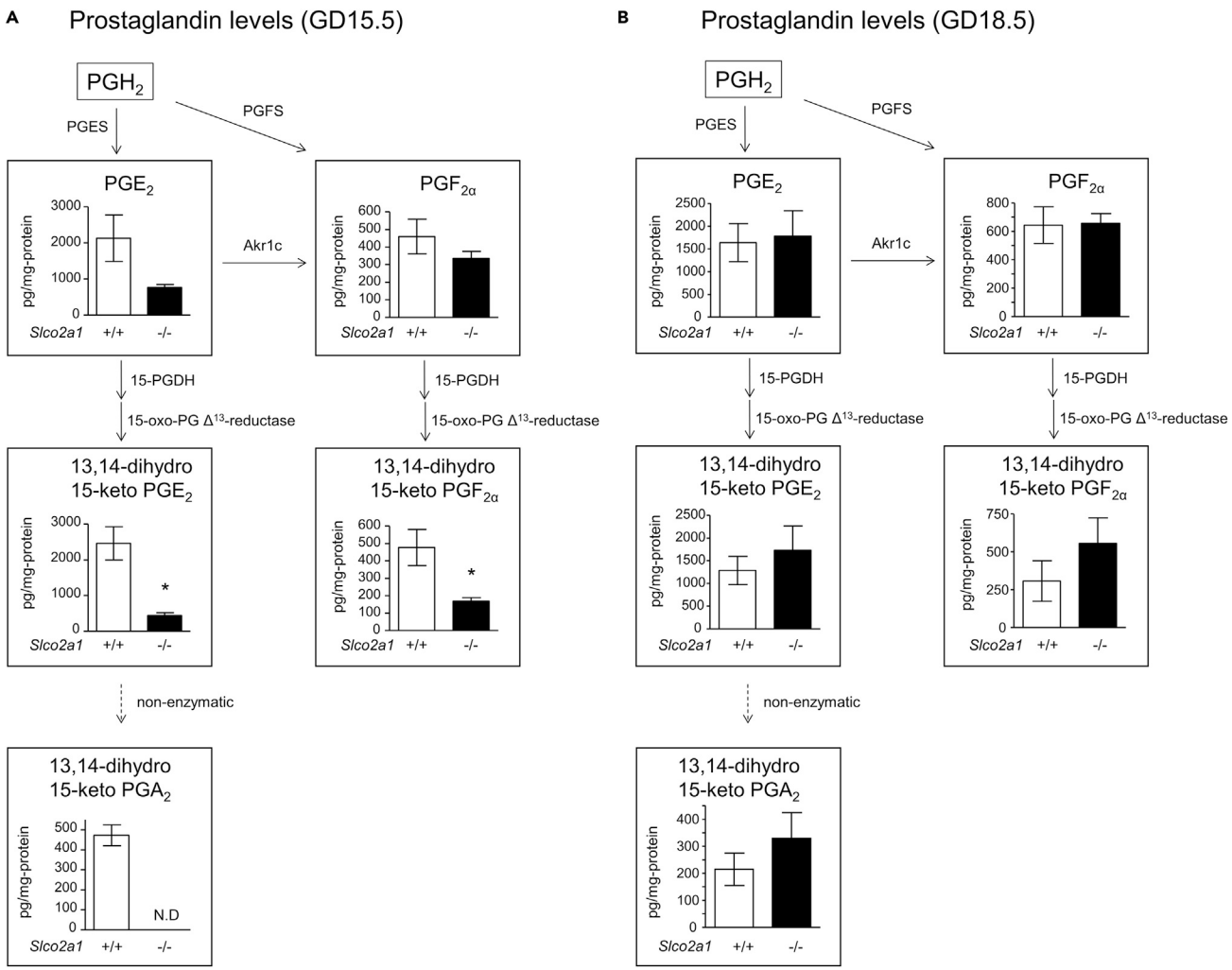


Figure 3. Effect of *Slco2a1* Deficiency on PG Levels in the Junctional Zone of *Slco2a1* (−/−) Mouse Placenta

PG levels at GD15.5 (A) and GD18.5 (B) in the junctional zone, normalized to total protein, for wild-type (n = 5–6) and *Slco2a1* (−/−) placenta (n = 6–8) from *Slco2a1* (+/−) females mated with *Slco2a1* (+/−) males. Data are expressed as the mean ± SEM. *p < 0.05, significantly different between groups (Student's two-tailed t test). N.D., not detected.

(Figure 4C). The expression level of *Akr1c18*, a progesterone-metabolizing enzyme, decreased to approximately half in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males, compared with that in the females mated with wild-type males (data not shown). These findings strongly suggest that the circulating progesterone levels maintain high levels owing to a decline in the metabolizing enzyme expression in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males.

In crosses of *Slco2a1* (+/−) females to *Slco2a1* (−/−) males, parturition was likely to be delayed (>GD20.0) in dams whose progesterone levels at GD18.5 had been high (Figure 4D). Moreover, when we plotted the proportion of *Slco2a1* (−/−) conceptuses for each of the crosses against circulating levels of maternal progesterone, we found that circulating progesterone levels at GD18.5 were positively correlated with the proportion of *Slco2a1* (−/−) conceptuses at GD18.5 in the corresponding litters (Figure 4E). These results suggest that impaired progesterone withdrawal is the major cause of delayed parturition in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males. To verify this, we injected RU486, a progesterone receptor antagonist, into *Slco2a1* (+/−) dams bearing *Slco2a1* (−/−) embryos. Administration of 150 μg RU486 at GD18.5 invariably induced delivery of pups within 24 h (Table 1). In contrast, vehicle treatment of *Slco2a1* (+/−) dams bearing *Slco2a1* (−/−) embryos at GD18.5 frequently resulted in delayed parturition (Table 1).

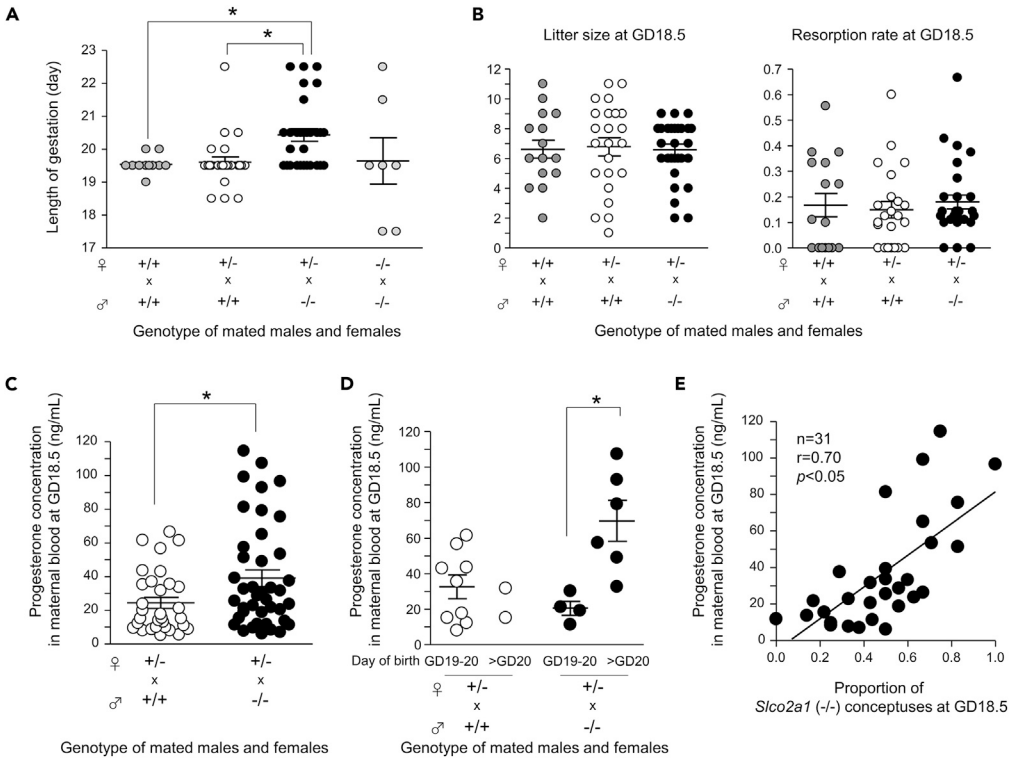


Figure 4. Prolonged Gestation of *Slco2a1* (+/−) Females Mated with *Slco2a1* (−/−) Males

(A) Gestation length of wild-type females mated with wild-type males (n = 12), *Slco2a1* (+/−) females mated with wild-type males (n = 24) or *Slco2a1* (−/−) males (n = 29), and *Slco2a1* (−/−) females mated with *Slco2a1* (−/−) males (n = 7).

(B) Number of pups (left) and resorption rate (right) at GD18.5 for wild-type females mated with wild-type males (n = 16), and *Slco2a1* (+/−) females mated with wild-type males (n = 17) or *Slco2a1* (−/−) males (n = 22).

(C) Progesterone concentration in maternal blood at GD18.5 from *Slco2a1* (+/−) females mated with wild-type males (n = 31) or *Slco2a1* (−/−) males (n = 41).

(D) Progesterone concentration in maternal blood at GD18.5 from *Slco2a1* (+/−) females showing term and post-term deliveries, mated with wild-type males (n = 11) or *Slco2a1* (−/−) males (n = 10).

(E) Progesterone concentration in maternal blood at GD18.5 from *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males is positively correlated with the proportion of *Slco2a1* (−/−) conceptuses at GD18.5. Data are expressed as the mean ± SEM. *p < 0.05, significant difference between groups (A and B, one-way ANOVA followed by Bonferroni's post hoc test; C and D, Student's two-tailed t test; E, Pearson's correlation test).

Overall, these findings suggest that feto-placental *Slco2a1* deficiency inhibits the drop in progesterone levels probably due to luteolysis and delays parturition.

Placental PG Regulates the Production of PL-II

In order to examine the effect of *Slco2a1* deficiency on the expression levels of placental hormones, we measured the absolute protein expression levels of PL-I and PL-II in the junctional zone at GD15.5 (Figure 5A) and GD18.5 (Figure 5B). At GD15.5, there was no significant difference in PL-II expression between wild-type and *Slco2a1* (−/−) mice junctional zone. In contrast, at GD18.5, PL-II expression level in the junctional zone of *Slco2a1* (−/−) placenta was significantly higher than that of wild-type placenta (Figure 5B). The expression level of PL-I was under the limit of quantification at both GD15.5 and GD18.5 (data not shown).

In the junctional zone of *Slco2a1* (−/−) placenta, extracellular PGE_2 appears to be increased owing to failure of PG degradation at GD15.5. Therefore, in order to examine the effect of PGE_2 on PL-II expression, we cultured wild-type placental explants of the junctional zone in medium containing PGE_2. PL-II expression in the junctional zone was significantly enhanced by PGE_2 treatment when compared with the vehicle-stimulated control measured at 24 h (Figure 5C). Consequently, it is possible that failure of PGE_2 degradation due to *Slco2a1* deficiency at mid-pregnancy resulted in the induction of PL-II expression at late pregnancy.

Genotype of Mother	Genotype of Father	RU486 (μg)	Rate of Delivery within 24 h
Slco2a1 (+/+)	*Slco2a1* (+/+)	150	100% (3/3)
Slco2a1 (+/−)	*Slco2a1* (+/+)	150	100% (3/3)
Slco2a1 (+/−)	*Slco2a1* (−/−)	0	43% (3/7)
Slco2a1 (+/−)	*Slco2a1* (−/−)	150	100% (8/8)

Table 1. Effect of RU486 Administration on Parturition Timing in *Slco2a1* (−/−) Females Mated with *Slco2a1* (−/−) Males

RU486 was administered on GD18.5 to *Slco2a1* (+/−) females mated with wild-type or *Slco2a1* (−/−) males.

It has been suggested that pulmonary surfactant protein A (SP-A), secreted from matured fetal lung, serves as a fetal signal to initiate parturition in dams by inducing progesterone withdrawal (Gao et al., 2015). However, we found no significant difference of expression level of SP-A between wild-type and *Slco2a1* (−/−) fetal lung at GD18.5 (Figure S5). The levels of PGE_2 and $PGF_{2\alpha}$ in uterus and ovary at GD18.5 were similar in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males and in those mated with wild-type males (Figure S6). Accordingly, feto-placental OATP2A1 locally influences PGE_2 and $PGF_{2\alpha}$ levels in the junctional zone of the placenta but does not affect those in maternal reproductive tissues, where PGs are involved in the induction of labor.

Treatment with Indomethacin at GD15.5 Rescues the Delayed-Labor Phenotype in *Slco2a1* (+/−) Females Mated with *Slco2a1* (−/−) Males

In order to address the relationship between high PGE_2 levels resulting from failure of PGE_2 degradation at mid-pregnancy and delayed parturition, *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males were treated with 1 mg/kg indomethacin at GD15.5. This corresponds to the time when degradation of PGE_2 was inhibited in the junctional zone of *Slco2a1* (−/−) placenta (Figure 3A). *Slco2a1* (+/−) pregnant mice injected with indomethacin delivered significantly earlier than vehicle-treated *Slco2a1* (+/−) pregnant mice (GD19.5 $\pm$ 0.1; n = 14 versus GD19.9 $\pm$ 0.1; n = 15, $p < 0.05$) (Figure 6A). Furthermore, we found that the increased expression of PL-II in the junctional zone of *Slco2a1* (−/−) placenta at GD18.5 was blocked by indomethacin (Figure 6B). In accordance with this, indomethacin treatment decreased the circulating progesterone levels (Figure 6C).

DISCUSSION

In this study, we found that *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males, giving rise to *Slco2a1* (−/−) and *Slco2a1* (+/−) placentas/fetuses, frequently exhibited delayed parturition (Figure 4A). In contrast, *Slco2a1* (+/−) females mated with wild-type males, giving rise to wild-type and *Slco2a1* (+/−) placentas/fetuses, showed normal delivery (Figure 4A). These results indicate that the high proportion of *Slco2a1* (−/−) conceptuses *in utero* affects the initiation of parturition. In the junctional zone of *Slco2a1* (−/−) placentas, the PL-II level at late pregnancy was significantly increased compared with that in wild-type placentas (Figure 5B). PL-II is suggested to exert luteotropic effects, including suppression of the expression of 20α-HSD/*Akr1c18*, which encodes a progesterone-metabolizing enzyme, in luteal cells (Zhong et al., 1997), resulting in the induction of progesterone release from corpus luteum (Galosy and Talamantes, 1995; Thordarson et al., 1997). In our study, *Akr1c18* expression level in ovary is significantly lower in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males than that in the females mated with wild-type males (data not shown), and consistently the circulating progesterone levels were significantly higher in *Slco2a1* (−/−) females mated with *Slco2a1* (−/−) males (Figure 4C). Moreover, the dams with higher progesterone levels at GD18.5 exhibited delayed parturition in crosses of *Slco2a1* (+/−) females to *Slco2a1* (−/−) males (Figure 4D). These findings suggest that delayed progesterone withdrawal and subsequent parturition failure are caused by oversecretion of placenta-derived PL-II in *Slco2a1* (−/−) mice. More than 70% of *Slco2a1* (−/−) pups die soon after birth; therefore, we could not accurately determine the proportion of *Slco2a1* (−/−) pups in the litter, and so the correlation between their proportion and the length of gestation could not be directly determined. However, the contribution of placental PGE_2 disposition mediated by OATP2A1 to labor induction was further supported by the positive correlation between the proportion of *Slco2a1* (−/−) conceptuses in the litters and maternal progesterone levels at late pregnancy (Figure 4E).

PL-II is abundantly expressed in the junctional zone (Simmons et al., 2008) and PGE_2 stimulated PL-II production in cultured explants of the junctional zone (Figure 5C). The secretion of PL-II is reportedly stimulated by several

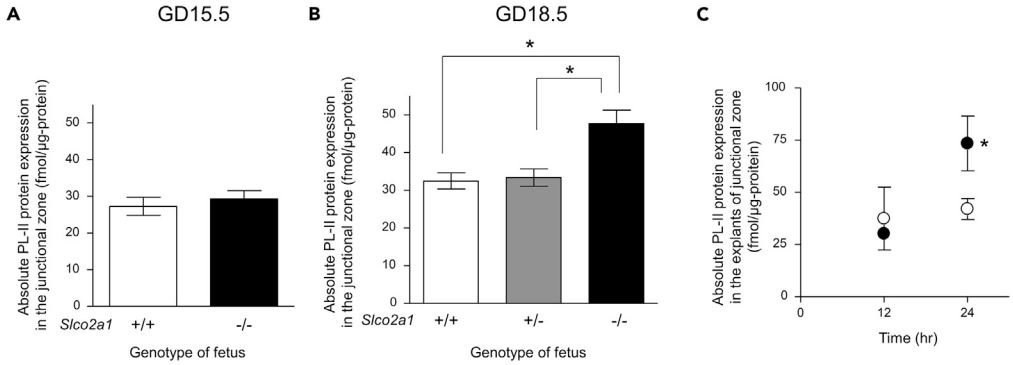


Figure 5. Overexpression of PL-II in the Junctional Zone of *Slco2a1* (−/−) Mouse Placenta

(A and B) The absolute protein expression of PL-II at GD15.5 (A) and GD18.5 (B) in the junctional zone of wild-type (n = 9–13), *Slco2a1* (+/−) (n = 23), and *Slco2a1* (−/−) mice placenta (n = 9–10).

(C) Absolute PL-II protein expression level in wild-type mouse explants of placental junctional zone cultured for 24 h in the presence (closed circle; n = 4–8) or absence (open circle; n = 4–8) of 1 μM PGE_2. Data are expressed as the mean ± SEM. *p < 0.05, significant difference between groups (A and C, Student's two-tailed t test; B, one-way ANOVA followed by Bonferroni's post hoc test).

factors, such as growth hormone-releasing hormone, dimeric inhibin A, dimeric inhibin B, calcyclin, EGAM1C, and TFAP2C (Kishi et al., 1993; Yamaguchi et al., 1995; Farnsworth and Talamantes, 1998; Saito et al., 2011; Ozturk et al., 2006). Among these factors, PGE_2 induces dimeric inhibin A production in cultured human granulosa-luteal cells (Eramaa and Ritvos, 1996) and inhibin subunits are expressed in mouse placental junctional zone at the mRNA level (Yamaguchi et al., 1995). Therefore, PGE_2 may stimulate PL-II production through the induction of dimeric inhibin A expression in the junctional zone. Here, we show for the first time that OATP2A1 is predominantly expressed in the placental junctional zone (Figure 1) and mediates uptake of extracellular PGs by spongiotrophoblasts (Figure 2). Therefore, the defect of PGE_2 uptake and the subsequent degradation of PGE_2 by 15-PGDH in the junctional zone of *Slco2a1* (−/−) placenta should increase PL-II expression via an increase of extracellular PGE_2 in the junctional zone. This possibility is supported by the observation that indomethacin treatment at GD15.5 in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males corrected PL-II overproduction in *Slco2a1* (−/−) placenta (Figure 6B). Moreover, indomethacin treatment decreased circulating progesterone levels at GD18.5 (Figure 6C) and corrected the parturition delay (Figure 6A). At GD15.5, COX-1, COX-2, and mPGES-1 are expressed in the placenta, including the junctional zone, although to a much lesser extent (Figures S4B–S4D) (Inagaki et al., 2017). These results raise the possibility that PGE_2 are synthesized in the junctional zone and/or other placental areas (i.e., labyrinth and decidua). These findings suggest that the uptake of extracellular PGE_2 by OATP2A1 and subsequent intracellular degradation in the placenta at mid-pregnancy would suppress the signaling for placental PL-II production in late pregnancy, which facilitates progesterone withdrawal for the onset of parturition.

Among fetal tissues, the lung also highly expresses OATP2A1, and OATP2A1 in the lung plays a critical role in the closure of the ductus arteriosus after birth by reducing the level of circulating PGE_2 (Chang et al., 2010). *Slco2a1* (−/−) neonates die within 24 h after birth because of patent ductus arteriosus, although the labor phenotype was not mentioned in the literature (Chang et al., 2010). Matured fetal lung is reported to be associated with the onset of parturition via secretion of SP-A, which serves as a hormonal signal (Gao et al., 2015). Thus, we have to consider the possibility that OATP2A1 in the fetal lung is involved in the onset of parturition by modulating the SP-A level. However, *Slco2a1* deficiency did not alter the expression level of SP-A in the fetal lung (Figure S5). Accordingly, it seems that OATP2A1 expressed in the fetal lung does not affect SP-A-mediated labor induction signals.

There is considerable evidence that PGs are universal mediators of parturition. At late pregnancy, elevation of PGE_2 and $PGF_{2\alpha}$ levels in the uterus and ovary promotes uterine myometrial contraction and cervical ripening, which trigger the onset of labor (Romero et al., 2014; Khan et al., 2008; Herington et al., 2018). Since OATP2A1 mediates the uptake of extracellular PGs for intracellular degradation, it is possible that feto-placental *Slco2a1* deficiency elevates PGE_2 and $PGF_{2\alpha}$ levels even in maternal tissues, including uterus and ovary, and thus causes pre-term delivery. However, in this study, *Slco2a1* (+/−) dams bearing *Slco2a1* (−/−) embryos exhibited delayed parturition (Figure 4A), and feto-placental *Slco2a1* deficiency did not affect the PGE_2 and $PGF_{2\alpha}$ levels in uterus or ovary at late pregnancy (Figure S6). Therefore, feto-placental OATP2A1 is likely to mediate the reduction of

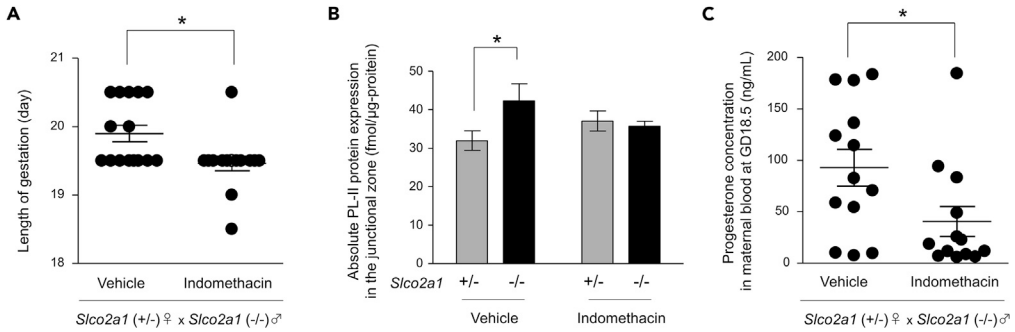


Figure 6. Indomethacin Treatment Rescues the Delayed-Labor Phenotype in *Slco2a1* (+/−) Females Mated with *Slco2a1* (−/−) Males

Administration of 1 mg/kg indomethacin on GD15.5 to *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males.

(A) Gestation length of dams treated with vehicle (n = 15) or indomethacin (n = 14).

(B) Absolute PL-II expression levels at GD18.5 in the junctional zone of *Slco2a1* (+/−) (n = 7–9) and *Slco2a1* (−/−) placenta (n = 6–10) from *Slco2a1* (+/−) pregnancies treated with vehicle or indomethacin.

(C) Circulating progesterone level in maternal blood at GD18.5 from *Slco2a1* (+/−) pregnancies treated with vehicle (n = 13) or indomethacin (n = 13). Data are expressed as the mean ± SEM. *p < 0.05, significant difference between groups (A and C, Student's two-tailed t test; B, two-way ANOVA followed by Bonferroni's post hoc test).

local PG levels in the placenta but may not affect the maternal level. Moreover, we found here that PGE_2 in the placenta has an opposite signaling function for the maintenance of pregnancy possibly by inducing placental PL-II production (Figures 5 and 6), in contrast to uterine PGE_2. This difference in the role of PGE_2 between uterus and placenta was further supported by the results of indomethacin treatment. Indomethacin delays preterm labor because of its tocolytic effect, reducing excessive PG levels in the uterus (Besinger et al., 1991; Morales and Madhav, 1993; Klauser et al., 2014), although there is concern about fetal adverse effects, such as premature closure of the fetal ductus arteriosus (Vermillion et al., 1997; Antonucci et al., 2012). In mice, indomethacin significantly decreased the incidence of preterm labor that was induced by lipopolysaccharide (Lee et al., 2003). On the other hand, in this study, a single administration of indomethacin at mid-pregnancy rescued the delayed-labor phenotype in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males (Figure 6A). Our findings provide insight into the role of PGE_2 in labor induction, indicating that excessive PGE_2 in the placenta at mid-pregnancy does not facilitate, but rather prevents, the initiation of parturition by modulating endocrine function in the placenta.

In crosses of *Slco2a1* (−/−) mice, 30% of dams exhibited post-term pregnancy, but 30% of dams exhibited preterm pregnancy (Figure 4A). In this mating, it is necessary to consider the effect of *Slco2a1* deficiency in dams as well as conceptuses. Based on our observations in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males, the high proportion of *Slco2a1* (−/−) conceptuses *in utero* causes delayed parturition. Thus, it is reasonable to consider that pre-term parturition is caused primarily by maternal *Slco2a1* deficiency. We confirmed that the uterus expresses OATP2A1 (Figure 1D); therefore, uterine OATP2A1 is likely to be involved in the maintenance of pregnancy by reducing extracellular PG levels in the uterus. Moreover, in *Slco2a1* (−/−) pregnant mice, uterine PG levels might be elevated through an increase in circulating PGs due to the lack of PG degradation in maternal lung. This is consistent with a report showing that, in *Slco2a1* (−/−) male mice, the plasma PGE_2 level was 3.7-fold higher than in wild-type mice at 1 h after the induction of PGE_2 by lipopolysaccharide (Nakamura et al., 2018). Moreover, in mice treated with T26A, a specific OATP2A1 inhibitor, for 3 weeks, the plasma PGE_2 level was 2-fold higher than that in the vehicle control (Chi et al., 2015). Accordingly, maternal *Slco2a1* deficiency is likely to favor preterm delivery owing to the elevated levels of uterine PGs.

At both GD15.5 and GD18.5, [^{3}H]PGE_2 uptake was significantly lower in the junctional zone of *Slco2a1* (−/−) placenta compared with that of wild-type littermates, indicating that OATP2A1 acts as an influx transporter of PGs at GD18.5 as well as GD15.5 (Figure 2). However, the levels of stable metabolites of PGE_2 and $PGF_{2\alpha}$ in the junctional zone at GD18.5 were similar in both wild-type and *Slco2a1* (−/−) littermates (Figure 3B), although a substantial decrease in PG metabolite levels was observed in *Slco2a1* (−/−) littermates at GD15.5 (Figure 3A). In the case of *Slco2a1* (−/−) mice, the levels of PG metabolites in the junctional zone at GD18.5 were even higher than those at GD15.5, raising the possibility that 15-PGDH and/or 15-oxo-PG Δ^{13}-reductase, PG-degrading enzymes, are upregulated to compensate for *Slco2a1* deficiency. In the case of wild-type mice junctional zone, PG metabolite levels at GD18.5 were decreased to approximately half compared with those at GD15.5 (Figure 3),

which is consistent with the finding that the placental activity of 15-PGDH at GD18.25 and GD19.0 was decreased from that at GD17.5, becoming similar to that in 15-PGDH hypomorphic mice placenta (Roizen et al., 2008). Accordingly, the uptake of PGs via OATP2A1 at mid-pregnancy is the rate-controlling step for PG degradation, whereas, at late pregnancy, OATP2A1-mediated uptake has little effect on the PG degradation, probably due to the low activity of 15-PGDH.

Based on the observation that, in wild-type mice, placental 15-PGDH activity becomes negligible prior to parturition (Roizen et al., 2008), an increase in extracellular PGE_2 has the potential to induce PL-II production and subsequent inhibition of progesterone withdrawal. However, prior to parturition, 15-PGDH activity also becomes negligible in the uterus (Roizen et al., 2008), and crossing of 15-PGDH hypomorphic mice resulted in increased levels of PGE_2 in ovary and $PGF_{2\alpha}$ in uterus and ovary at GD17.5 and GD18.25, leading to pre-term delivery (Roizen et al., 2008). Therefore, it can be considered that, prior to parturition, the luteolytic effect, inducing progesterone withdrawal, of $PGF_{2\alpha}$ in uterus predominates over the luteotropic effect, inducing progesterone production, of PGE_2 in the placenta through the production of PL-II. On the other hand, in *Slco2a1* (+/−) females mated with *Slco2a1* (−/−) males, owing to overproduction of PL-II, the luteotropic effect of placental PGE_2 seems to overcome the luteolytic effect of uterine $PGF_{2\alpha}$.

Term labor and preterm labor in humans and rodents are both mediated by elevated levels of PGE_2 and $PGF_{2\alpha}$ in the uterus, and these PGs induce uterine myometrial contraction and cervical ripening (Herington et al., 2018; Romero et al., 2014). In rodent ovary, PGE_2 exerts a luteotropic effect, whereas $PGF_{2\alpha}$ is a luteolytic factor (Sugimoto et al., 1997; Henderson et al., 1977; Tamura et al., 2016). In the present study, feto-placental *Slco2a1* deficiency in mice disrupted the timing of parturition by dysregulating the PGE_2 concentration in the placenta at mid-pregnancy without affecting the PGE_2 and $PGF_{2\alpha}$ levels in the uterus and ovary at late pregnancy (Figure S6). Whole-exome sequencing has indicated the pathophysiological significance of the *SLCO2A1* gene in human; loss-of-function mutations in *SLCO2A1* are related to primary hypertrophic osteoarthropathy and pachydermoperiostosis (Nakanishi and Tamai, 2017). Although the labor phenotype in humans deficient for OATP2A1 function has not been described so far, *SLCO2A1* is most abundantly expressed in the placenta among human tissues (Kraft et al., 2010). The mechanism of labor induction in humans is considered to be complex; luteolysis does not play a role in human parturition and the circulating progesterone level does not decrease antepartum (Mitchell and Taggart, 2009; Malassine et al., 2003). Nevertheless, it has been reported that, in women with premature uterine contractility, the circulating level of human placental lactogen (hPL) was significantly lower than in women with uncompleted pregnancy between 23 and 28 weeks of gestation (Wojcicka-Jagodzinska et al., 1998), implying that hPL affects the initiation of labor in humans.

In conclusion, we have demonstrated that OATP2A1 is predominantly expressed in spongiotrophoblasts of the junctional zone and is responsible, together with 15-PGDH, for the degradation of PGs in the junctional zone of the placenta at mid-pregnancy. Since PGE_2 signaling is shown to contribute to the secretion of PL-II, a luteotropic hormone, in the junctional zone, it is possible to consider that PG disposition via OATP2A1 suppresses the secretion of PL-II to induce the production of ovarian progesterone. These results indicate that feto-placental OATP2A1 contributes to the withdrawal of circulating progesterone at late pregnancy, possibly by suppressing placental PL-II production, for the initiation of parturition.

Limitations of the Study

In our experiments, we demonstrate that the presence of *Slco2a1* (−/−) conceptuses results in delayed parturition of dams by inhibiting progesterone withdrawal and that the expression level of PL-II in *Slco2a1* (−/−) junctional zone is higher than that in the wild-type. PL-II is suggested to exert a luteotropic hormone, but we did not directly demonstrate the causal relationship between the overproduction of PL-II in the junctional zone and delayed parturition. Moreover, we did not clarify the reason why the time lag effect of PGE_2 signaling on PL-II production occurs. Further experiments will be needed to clarify the mechanism of PL-II production via PGE_2 signaling and the contribution of PL-II to parturition timing.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Prof. Masatochi Tomi (tomi-ms@pha.keio.ac.jp).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The data that support the findings of this study are available from the authors on reasonable request.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101098.

ACKNOWLEDGMENTS

This study was partly supported by JSPS KAKENHI (grant numbers: 18J12895 to M.I. and 18H03183 to M. Tomi). It was also partially founded by the Smoking Research Foundation (grant number: 055 to T. Nakanishi), Keio University Doctorate Student Grant-in-Aid Program, and Ono Medical Research Foundation.

AUTHOR CONTRIBUTIONS

M.I., T. Nakanishi, E.N., K.H., and M. Tomi designed the research. M.I., and S.N. performed the research. T. Nakanishi, H.S., S.A., M. Tachikawa, K.H., and I.T. contributed reagents/analytic tools. M.I., T. Ni., S.A., M. Tachikawa, I.T., and M. Tomi analyzed data. M.I., T. Ni., T. Nakanishi, H.S., S.N., E.N., and M. Tomi discussed and edited the manuscript. M.I., T. Ni., S.N., and M. Tomi wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

Received: January 15, 2020
Revised: March 22, 2020
Accepted: April 21, 2020
Published: May 22, 2020

REFERENCES

Antonucci, R., Zaffanello, M., Puxeddu, E., Porcella, A., Cuzzolin, L., Pilloni, M.D., and Fanos, V. (2012). Use of non-steroidal anti-inflammatory drugs in pregnancy: impact on the fetus and newborn. Curr. Drug Metab. *13*, 474–490.

Besinger, R.E., Niebyl, J.R., Keyes, W.G., and Johnson, T.R. (1991). Randomized comparative trial of indomethacin and ritodrine for the long-term treatment of preterm labor. Am. J. Obstet. Gynecol. *164*, 981–986, discussion 986-8.

Cha, J., Sun, X., and Dey, S.K. (2012). Mechanisms of implantation: strategies for successful pregnancy. Nat. Med. *18*, 1754–1767.

Challis, J.R.G., Matthews, S.G., Gibb, W., and Lye, S.J. (2000). Endocrine and paracrine regulation of birth at term and preterm. Endocr. Rev. *21*, 514–550.

Chang, H.Y., Locker, J., Lu, R., and Schuster, V.L. (2010). Failure of postnatal ductus arteriosus closure in prostaglandin transporter-deficient mice. Circulation *121*, 529–536.

Cheng, X., Maher, J., Chen, C., and Klaassen, C.D. (2005). Tissue distribution and ontogeny of mouse organic anion transporting polypeptides (Oatps). Drug Metab. Dispos. *33*, 1062–1073.

Chi, Y., Jasmin, J.F., Seki, Y., Lisanti, M.P., Charron, M.J., Lefer, D.J., and Schuster, V.L. (2015). Inhibition of the prostaglandin transporter PGT lowers blood pressure in hypertensive rats and mice. PLoS One *10*, e0131735.

Coggins, K.G., Latour, A., Nguyen, M.S., Audoly, L., Coffman, T.M., and Koller, B.H. (2002). Metabolism of PGE2 by prostaglandin dehydrogenase is essential for remodeling the ductus arteriosus. Nat.Med. *8*, 91–92.

Denda, K., Nakao-Wakabayashi, K., Okamoto, N., Kitamura, N., Ryu, J.Y., Tagawa, Y., Ichisaka, T., Yamanaka, S., and Komada, M. (2011). Nrk, an X-linked protein kinase in the germinal center kinase family, is required for placental development and fetoplacental induction of labor. J. Biol. Chem. *286*, 28802–28810.

Eramaa, M., and Ritvos, O. (1996). Prostaglandin E2 induces inhibin alpha- and beta A-subunit mRNA and secretion of dimeric inhibin A in cultured human granulosa-luteal cells. Mol. Hum. Reprod. *2*, 815–822.

Farnsworth, R.L., and Talamantes, F. (1998). Calcyclin in the mouse decidua: expression and effects on placental lactogen secretion. Biol. Reprod. *59*, 546–552.

Galosy, S.S., and Talamantes, F. (1995). Luteotropic actions of placental lactogens at midpregnancy in the mouse. Endocrinology *136*, 3993–4003.

Gao, L., Rabbitt, E.H., Condon, J.C., Renthal, N.E., Johnston, J.M., Mitsche, M.A., Chambon, P., Xu, J., O'Malley, B.W., and Mendelson, C.R. (2015). Steroid receptor coactivators 1 and 2 mediate fetal-to-maternal signaling that initiates parturition. J. Clin. Invest. *125*, 2808–2824.

Helliwell, R.J., Keelan, J.A., Marvin, K.W., Adams, L., Chang, M.C., Anand, A., Sato, T.A., O'Carroll, S., Chaiworapongsa, T., Romero, R.J., and Mitchell, M.D. (2006). Gestational age-dependent up-regulation of prostaglandin D synthase (PGDS) and production of PGDS-derived antiinflammatory prostaglandins in human placenta. J. Clin. Endocrinol. Metab. *91*, 597–606.

Henderson, K.M., Scaramuzzi, R.J., and Baird, D.T. (1977). Simultaneous infusion of prostaglandin E2 antagonizes the luteolytic action of prostaglandin F2alpha in vivo. J. Endocrinol. *72*, 379–383.

Herington, J.L., O'Brien, C., Robuck, M.F., Lei, W., Brown, N., Slaughter, J.C., Paria, B.C.,

Mahadevan-Jansen, A., and Reese, J. (2018). Prostaglandin-endoperoxide synthase 1 mediates the timing of parturition in mice despite unhindered uterine contractility. Endocrinology *159*, 490–505.

Inagaki, M., Nishimura, T., Akanuma, S.I., Nakanishi, T., Tachikawa, M., Tamai, I., Hosoya, K.I., Nakashima, E., and Tomi, M. (2017). Co-localization of microsomal prostaglandin E synthase-1 with cyclooxygenase-1 in layer II of murine placental syncytiotrophoblasts. Placenta *53*, 76–82.

Kanai, N., Lu, R., Satriano, J.A., Bao, Y., Wolkoff, A.W., and Schuster, V.L. (1995). Identification and characterization of a prostaglandin transporter. Science *268*, 866–869.

Khan, A.H., Carson, R.J., and Nelson, S.M. (2008). Prostaglandins in labor–a translational approach. Front. Biosci. *13*, 5794–5809.

Kishi, K., Itoh, M., Kanamori, S., Hirashiba, M., and Kawai, M. (1993). Stimulation of rat placental lactogen-II (rPL-II) secretion by cultured trophoblasts by insulin: development of a rat placental cell culture system and effects of peptide hormones on rPL-II secretion in vitro. J. Reprod. Fertil. *99*, 519–527.

Klauser, C.K., Briery, C.M., Martin, R.W., Langston, L., Magann, E.F., and Morrison, J.C. (2014). A comparison of three tocolytics for preterm labor: a randomized clinical trial. J. Matern. Fetal. Neonatal. Med. *27*, 801–806.

Kraft, M.E., Glaeser, H., Mandery, K., Konig, J., Auge, D., Fromm, M.F., Schlotzer-Schrehardt, U., Welge-Lussen, U., Kruse, F.E., and Zolk, O. (2010). The prostaglandin transporter OATP2A1 is expressed in human ocular tissues and transports the antiglaucoma prostanoid latanoprost. Invest. Ophthalmol. Vis. Sci. *51*, 2504–2511.

Lee, P.R., Kim, S.R., Jung, B.K., Kim, K.R., Chung, J.Y., Won, H.S., and Kim, A. (2003). Therapeutic effect of cyclo-oxygenase inhibitors with different isoform selectivity in lipopolysaccharide-induced preterm birth in mice. Am. J. Obstet. Gynecol. *189*, 261–266.

Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J.E., Cousens, S., Mathers, C., and Black, R.E. (2015). Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet *385*, 430–440.

Malassine, A., Frendo, J.L., and Evain-Brion, D. (2003). A comparison of placental development and endocrine functions between the human and mouse model. Hum. Reprod. Update. *9*, 531–539.

Mark, P.J., Lewis, J.L., Jones, M.L., Keelan, J.A., and Waddell, B.J. (2013). The inflammatory state of the rat placenta increases in late gestation and is further enhanced by glucocorticoids in the labyrinth zone. Placenta *34*, 559–566.

Minakami, H., and Sato, I. (1996). Reestimating date of delivery in multifetal pregnancies. JAMA *275*, 1432–1434.

Mitchell, B.F., and Taggart, M.J. (2009). Are animal models relevant to key aspects of human parturition? Am. J. Physiol. Regul. Integr. Comp. Physiol. *297*, R525–R545.

Morales, W.J., and Madhav, H. (1993). Efficacy and safety of indomethacin compared with magnesium sulfate in the management of preterm labor: a randomized study. Am. J. Obstet. Gynecol. *169*, 97–102.

Nakamura, Y., Nakanishi, T., Shimada, H., Shimizu, J., Aotani, R., Maruyama, S., Higuchi, K., Okura, T., Deguchi, Y., and Tamai, I. (2018). Prostaglandin transporter OATP2A1/SLCO2A1 is essential for body temperature regulation during fever. J. Neurosci. *38*, 5584–5595.

Nakanishi, T., Hasegawa, Y., Mimura, R., Wakayama, T., Uetoko, Y., Komori, H., Akanuma, S., Hosoya, K., and Tamai, I. (2015). Prostaglandin transporter (PGT/SLCO2A1) protects the lung from bleomycin-induced fibrosis. PLoS One *10*, e0123895.

Nakanishi, T., and Tamai, I. (2017). Roles of organic anion transporting polypeptide 2A1 (OATP2A1/SLCO2A1) in regulating the pathophysiological actions of prostaglandins. AAPS J. *20*, 13.

Naruse, M., Ono, R., Irie, M., Nakamura, K., Furuse, T., Hino, T., Oda, K., Kashimura, M., Yamada, I., Wakana, S., et al. (2014). Sirh7/Ldoc1 knockout mice exhibit placental P4 overproduction and delayed parturition. Development *141*, 4763–4771.

Nomura, T., Lu, R., Pucci, M.L., and Schuster, V.L. (2004). The two-step model of prostaglandin signal termination: in vitro reconstitution with the prostaglandin transporter and prostaglandin 15 dehydrogenase. Mol. Pharmacol. *65*, 973–978.

Okazaki, T., Casey, M.L., Okita, J.R., MacDonald, P.C., and Johnston, J.M. (1981). Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. Am. J. Obstet. Gynecol. *139*, 373–381.

Ozturk, A., Donald, L.J., Li, L., Duckworth, H.W., and Duckworth, M.L. (2006). Proteomic identification of AP2 gamma as a rat placental lactogen II trophoblast cell-specific enhancer binding protein. Endocrinology *147*, 4319–4329.

Petraglia, F., Sawchenko, P.E., Rivier, J., and Vale, W. (1987). Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. Nature *328*, 717–719.

Roizen, J.D., Asada, M., Tong, M., Tai, H.H., and Muglia, L.J. (2008). Preterm birth without progesterone withdrawal in 15-hydroxyprostaglandin dehydrogenase hypomorphic mice. Mol. Endocrinol. *22*, 105–112.

Romero, R., Dey, S.K., and Fisher, S.J. (2014). Preterm labor: one syndrome, many causes. Science *345*, 760–765.

Saigal, S., and Doyle, L.W. (2008). An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet *371*, 261–269.

Saito, K., Ogawa, A., Toyofuku, K., Hosoi, Y., Soma, M., Iha, M., Kasuga, K., Kojima, I., and Kobayashi, M. (2011). Relationships between homeoprotein EGAM1C and the expression of the placental prolactin gene family in mouse placentae and trophoblast stem cells. Reproduction *141*, 259–268.

Simmons, D.G., Rawn, S., Davies, A., Hughes, M., and Cross, J.C. (2008). Spatial and temporal expression of the 23 murine Prolactin/Placental Lactogen-related genes is not associated with their position in the locus. BMC Genomics *9*, 352.

Sugimoto, Y., Yamasaki, A., Segi, E., Tsuboi, K., Aze, Y., Nishimura, T., Oida, H., Yoshida, N., Tanaka, T., Katsuyama, M., et al. (1997). Failure of parturition in mice lacking the prostaglandin F receptor. Science *277*, 681–683.

Tamura, K., Naraba, H., Hara, T., Nakamura, K., Yoshie, M., Kogo, H., and Tachikawa, E. (2016). A positive feedback loop between progesterone and microsomal prostaglandin E synthase-1-mediated PGE2 promotes production of both in mouse granulosa cells. Prostaglandins Other Lipid Mediat. *123*, 56–62.

Thorburn, G.D. (1992). The placenta, PGE2 and parturition. Early Hum. Dev. *29*, 63–73.

Thordarson, G., Galosy, S., Gudmundsson, G.O., Newcomer, B., Sridaran, R., and Talamantes, F. (1997). Interaction of mouse placental lactogens and androgens in regulating progesterone release in cultured mouse luteal cells. Endocrinology *138*, 3236–3241.

Tsuboi, K., Iwane, A., Nakazawa, S., Sugimoto, Y., and Ichikawa, A. (2003). Role of prostaglandin H2 synthase 2 in murine parturition: study on ovariectomy-induced parturition in prostaglandin F receptor-deficient mice. Biol. Reprod. *69*, 195–201.

Tsuboi, K., Sugimoto, Y., Iwane, A., Yamamoto, K., Yamamoto, S., and Ichikawa, A. (2000). Uterine expression of prostaglandin H2 synthase in late pregnancy and during parturition in prostaglandin F receptor-deficient mice1. Endocrinology *141*, 315–324.

Vermillion, S.T., Scardo, J.A., Lashus, A.G., and Wiles, H.B. (1997). The effect of indomethacin tocolysis on fetal ductus arteriosus constriction with advancing gestational age. Am. J. Obstet. Gynecol. *177*, 256–259, discussion 259-61.

Wojcicka-Jagodzinska, J., Romejko, E., Piekarski, P., Czajkowski, K., Smolarczyk, R., and Lipinski, T. (1998). Second trimester calcium-phosphorus-magnesium homeostasis in women with threatened preterm delivery. Int. J. Gynaecol. Obstet. *61*, 121–125.

Yamaguchi, M., Tasaka, K., Ogura, K., Sakata, M., Mizuki, J., and Miyake, A. (1995). Activin inhibits but inhibin activates mouse placental lactogen-II secretion. J. Endocrinol. *146*, 469–474.

Zhong, L., Parmer, T.G., Robertson, M.C., and Gibori, G. (1997). Prolactin-mediated inhibition of 20alpha-hydroxysteroid dehydrogenase gene expression and the tyrosine kinase system. Biochem. Biophys. Res. Commun. *235*, 587–592.

iScience, Volume *23*

Supplemental Information

Contribution of Prostaglandin Transporter OATP2A1/*SLCO2A1* to Placenta-to-Maternal Hormone Signaling and Labor Induction

Mai Inagaki, Tomohiro Nishimura, Takeo Nakanishi, Hiroaki Shimada, Saki Noguchi, Shin-ichi Akanuma, Masanori Tachikawa, Ken-ichi Hosoya, Ikumi Tamai, Emi Nakashima, and Masatoshi Tomi

Supplemental figures

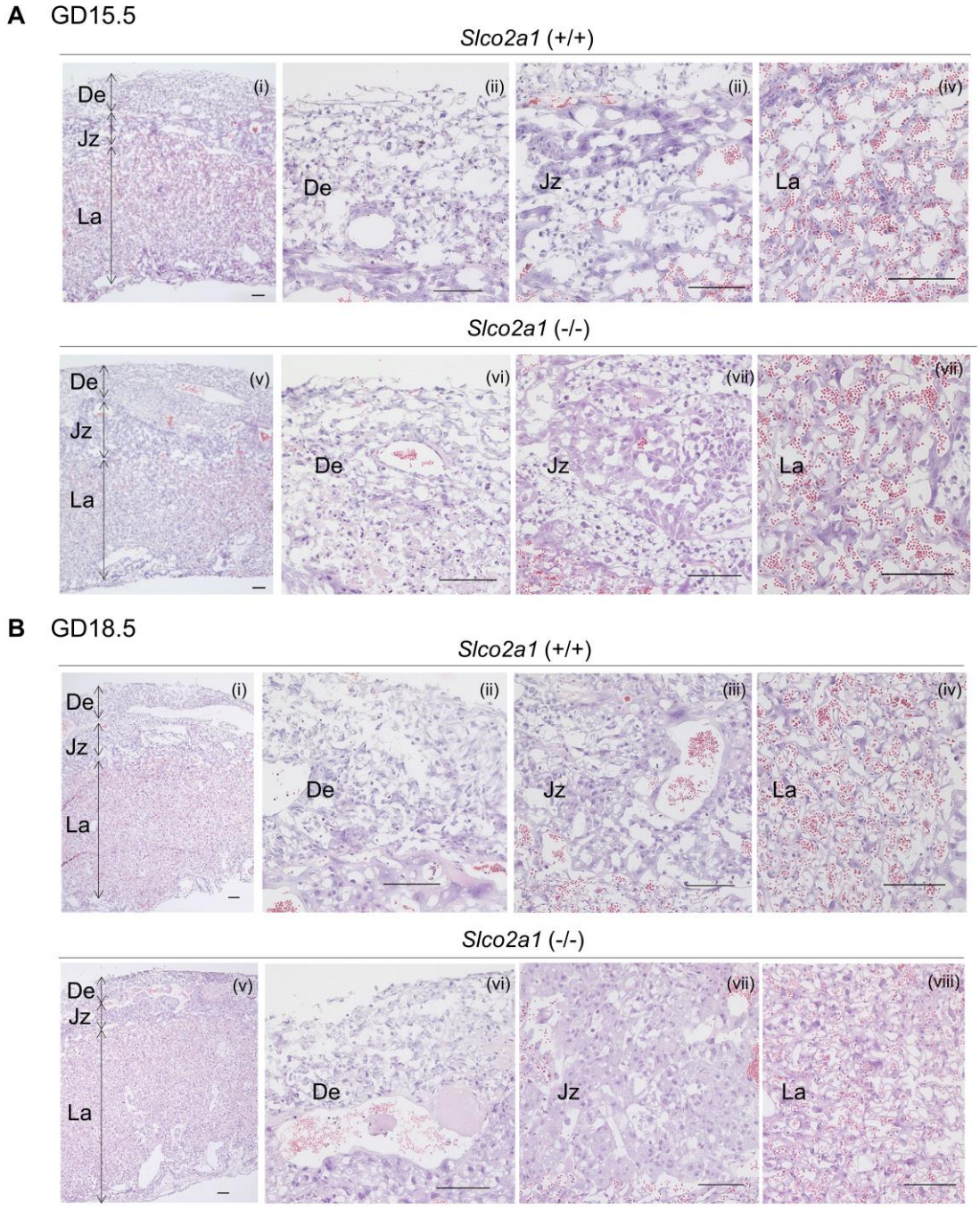


Figure S1: H&E staining of wild-type and *Slco2a1* (-/-) placentas at GD15.5 (A) and GD18.5 (B), Related to Figure 1.

Low (i and v) and high (ii, iii, iv, vi, vii, and viii) magnification. De, decidua; Jz, junctional zone; La, labyrinth. Scale bars = 100 μm.

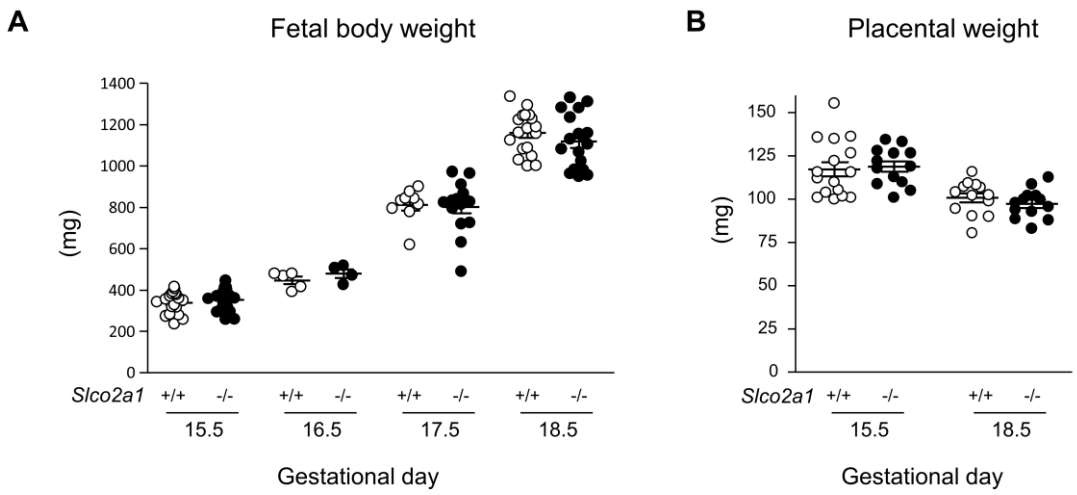


Figure S2: Fetal and placental weights during pregnancy, Related to Figure 1.

(A and B) Weight of wild-type and *Slco2a1* (-/-) fetuses (A) and placentas (B) from GD15.5 to 18.5. Data are expressed as the mean ± SEM (n = 4-22).

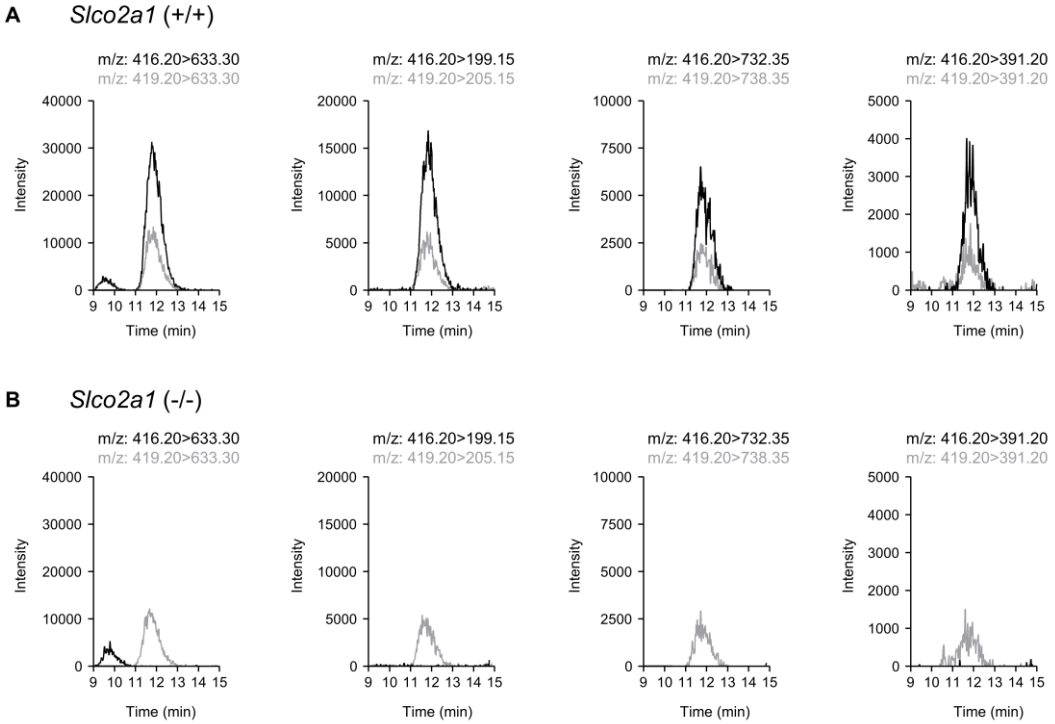


Figure S3: Peak chromatogram of an OATP2A1-specific peptide, VVNQDEK, Related to Figure 1.

Plasma membrane-rich fraction of the placental junctional zone was obtained from wild-type (A) and *Slco2a1* (-/-) (B) pregnancies at GD15.5. Tryptic digests of placental sample spiked with internal standard (IS) peptides were subjected to LC-MS/MS. Peak chromatograms of four SRM transitions for a target peptide (black) and the corresponding IS peptide (gray).

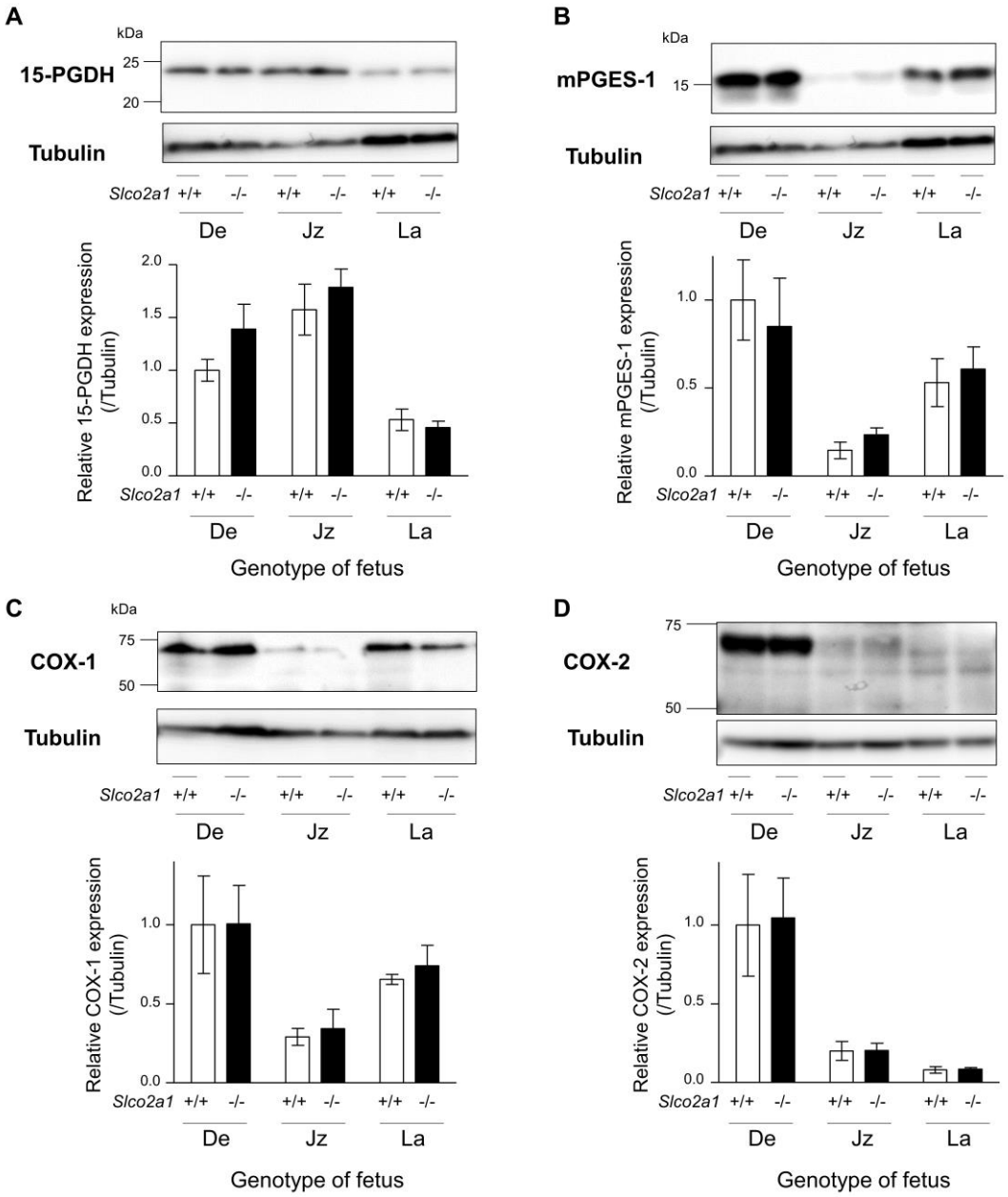


Figure S4: Western blot analysis of 15-PGDH (A), mPGES-1 (B), COX-1 (C), and COX-2 (D) in three placental zones at GD15.5, Related to Figure 3.

Western blot analysis using placental homogenates prepared from wild-type and *Slco2a1* (-/-) mice. Representative images (top) and densitometric analyses of the blots (bottom) from 3 to 4 independent experiments with different placentas are shown. Data are expressed as the mean ± SEM.

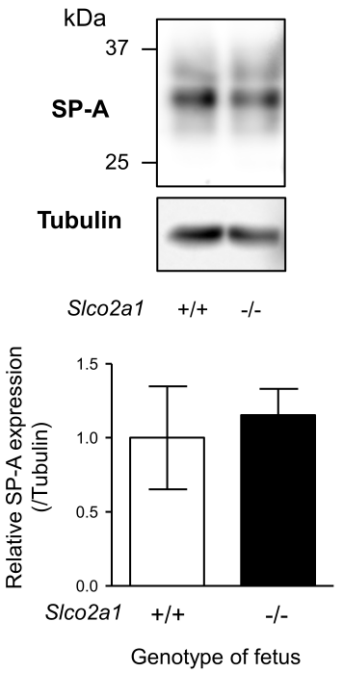


Figure S5: Western blot analysis of surfactant protein A (SP-A) in fetal lung at GD18.5, Related to Figure 4.

Western blot analysis of fetal lung homogenates prepared from wild-type and *Slco2a1* (-/-) mice. Representative images (top) and densitometric analyses of the blots (bottom) from 5 to 7 independent experiments with different placentas are shown. Data are expressed as the mean ± SEM.

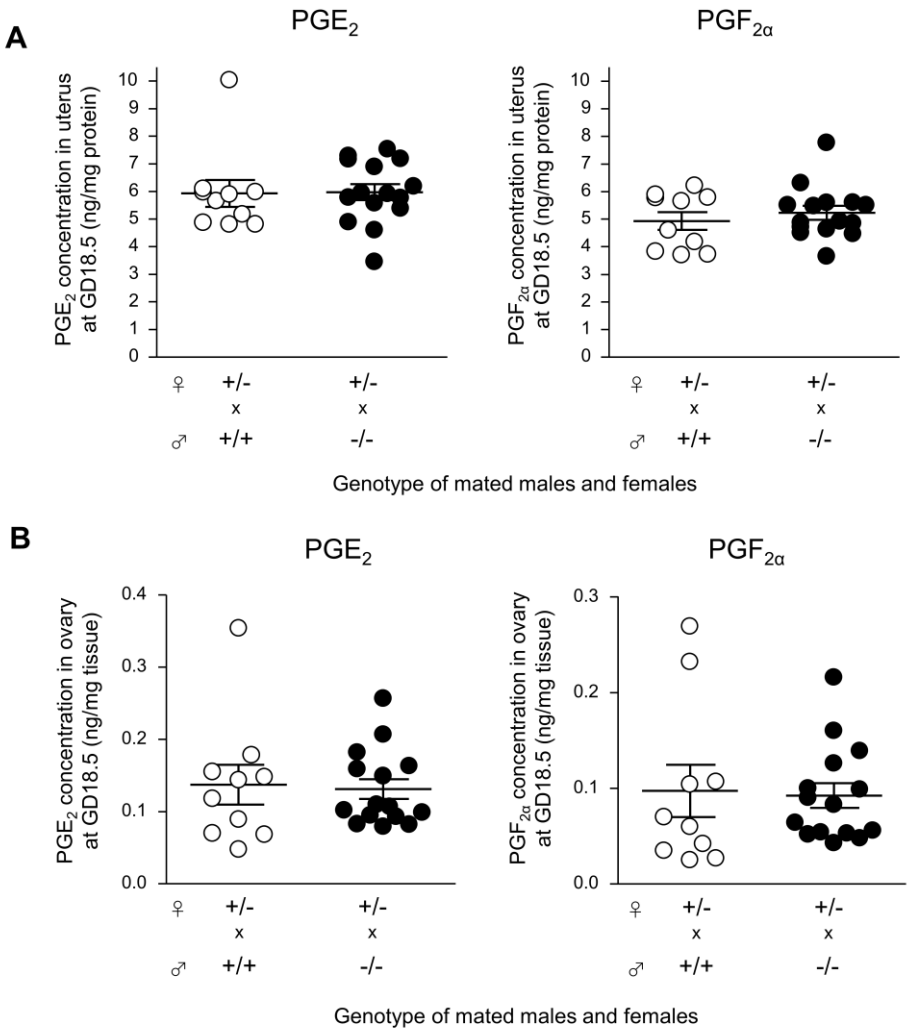


Figure S6: PG levels in ovary, myometrium, and fetal membrane at GD18.5, Related to Figure 4.

PGE_2 and $PGF_{2\alpha}$ levels in uterus (A) and ovary (B) from *Slco2a1* (+/-) females mated with wild-type males (n=10) or *Slco2a1* (-/-) males (n = 15). Data are expressed as the mean ± SEM.

Supplemental table

Gene symbol	ST/IS	Probe sequence	SRM/MRM transition (m/z)				
			Q1	Q3-1	Q3-2	Q3-3	Q3-4
mOATP2A1	ST	VVNQDEK	416.20	633.30	199.15	732.35	391.20
	IS	V**V***NQDEK	419.20	633.30	205.15	738.35	391.20
mPL-I	ST	EFDLDFFDK	588.25	392.15	671.30	556.30	262.15
	IS	EFD**L***DFFDK	591.80	392.15	671.30	556.30	262.15
mPL-II	ST	LPTESLYQR	553.80	211.14	795.40	896.45	666.35
	IS	L**P***TESLYQR	556.80	217.15	795.40	896.45	666.35

Table S1: Target peptide transitions in LC-MS/MS analysis, Related to Figure 1 and 5.

ST and IS mean standard peptides and internal standard peptides, respectively. The stable-isotope-labeled amino acids are indicated with an asterisk.

Transparent Methods

Animals.

All mice were maintained under a 12-hour light/12-hour dark cycle at 25°C with free access to water until use. Animal experiments were approved by the Institutional Animal Care Committee and complied with the standards set out in the Guideline for the Care and Use of Laboratory Animals in Keio University. The presence of a vaginal plug was designated as GD0.5. Parturition was monitored from GD16.5 by observing mice twice daily, in the morning (8:00-9:00 AM) and evening (6:00-8:00 PM). Parturition timing was defined as the observation of the first pup. To ensure that parturition timing was analyzed in mice of the same physiological status, only data on first pregnancies were included.

Slco2a1 (-/-) mice.

To produce *Slco2a1* (flox/+) mice, in vitro fertilization of eggs from C57BL/6 females with sperm cells from *Slco2a1* (flox/flox) males (Nakanishi et al., 2015) was performed and the blastocysts were transferred into pseudopregnant C57BL/6 females. To disrupt the *Slco2a1* gene, *Slco2a1* (flox/+) offspring were interbred with CAG-Cre transgenic mice (RIKEN BRC) (Matsumura et al., 2004), which express Cre recombinase at early stages of development. Mice were genotyped by PCR analysis of genomic DNA from mouse tissue (ear or tail snips) using the forward primers 5'-AGGCTCTCGTGGGGAGTAAT-3' for wild-type mice and 5'-AGGACCTGATAGGCAGCCAA-3' for *Slco2a1* deficient mice, with the reverse primer 5'-CACAGCAGAGACCCAACAGA-3'. Under deep anesthesia with isoflurane, the ovaries, uterus, fetal lungs, and maternal blood were collected from pregnant *Slco2a1* (+/-) female mice mated with wild-type or *Slco2a1* (-/-) males at GD18.5. Blood was collected from the tail vein and plasma was prepared by centrifugation at 10,000 x g for 10 min at 4°C. Placentas were collected at GD15.5 and GD18.5 from pregnant *Slco2a1* (+/-) female mice mated with *Slco2a1* (+/-) male mice. Isolated placentas were dissected into maternal decidua (whitish color), junctional zone (yellowish color), and labyrinth (dark red color) using tweezers (Inagaki et al., 2017). We analyzed qRT-PCR using mRNA gene expression markers (decidua; *Desmin*, junctional zone; *Prl8a8*, Labyrinth; *Syncytin-A*) to validate each layer enrichment.

Histological analysis.

Isolated placentas were fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C and immersed in 20% sucrose before being embedded in OCT compound. Frozen sections were cut at 4 μm and mounted on silanized glass slides. Sections were stained with Mayer's hematoxylin solution (Fujifilm Wako, Osaka, Japan) and Eosin Y (Fujifilm Wako) for

general morphology.

In situ hybridization analyses were performed as described previously (Akashi et al., 2016). Antisense and sense RNA probes for mouse *Slco2a1* (GeneBank accession number NM_03314.4) and *Prl8a8* (NM_001311125.1) mRNAs (nucleotide residues 803-1946 and 56-852, respectively) were labeled with digoxigenin (DIG) or fluorescein-conjugated UTP (Roche, Basel, Switzerland). *Prl8a8* was used as a marker of spongiotrophoblasts (Simmons et al., 2008). Frozen sections were treated with 1 μg/mL proteinase K for 37°C at 30 min, acetylated in 0.25% acetic anhydride/0.1 M triethanolamine for 10 min, and then hybridized overnight with DIG or the fluorescent probe at 60°C. After blocking with blocking regent (Roche), sections were incubated overnight with alkaline phosphate-conjugated anti-DIG antibody (1:2,000) (Roche) or anti-fluorescein antibody (1:2,000) (Roche) at 4°C, and then incubated with nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate at room temperature for color development. Sections were counterstained with Nuclear Fast Red, dehydrated in ethanol, cleared in xylene, and mounted in VectaMount Permanent Mounting Medium (Vector Laboratories).

Frozen sections were incubated with HistoVT One solution (Nacalai Tesque, Kyoto, Japan) for 20 min at 70°C for antigen retrieval. After blocking, sections were incubated with guinea-pig polyclonal OATP2A1 antibody (2 μg/mL) (Tachikawa, Tsuji et al., 2012) and rabbit polyclonal 15-PGDH antibody (1:100) (160615; Cayman Chemical, Ann Arbor, MI) at 4°C overnight, then incubated with species-specific Alexa Fluoro 594-conjugated and Alexa Fluoro 488-conjugated secondary antibodies (Thermo Fisher Scientific, Waltham, MA) at room temperature for 1 hour, and mounted in Vectashield Hard Set Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA).

Immunoblot analysis.

The placentas and fetal lungs were homogenized in cell lysis buffer (Cell Signaling Technology, Danvers, MA). Proteins were separated by SDS–polyacrylamide gel electrophoresis, and electroblotted onto a polyvinylidene difluoride membrane. After blocking, the membrane was incubated at 4°C overnight with rabbit polyclonal anti-15-PGDH antibody (1:2,000), rabbit polyclonal anti-COX-1 antibody (1:500) (160109; Cayman Chemical), rabbit polyclonal anti-COX-2 antibody (1:500) (160126; Cayman Chemical), guinea-pig polyclonal anti-mPGES-1 antibody (0.3 μg/mL) (Tachikawa, Ozeki et al., 2012), rabbit polyclonal anti-SP-A antibody (1:1,000) (ab115791; abcam, Cambridge, UK), or rat monoclonal anti-tubulin antibody (1:5,000) (ab6160; abcam), and subsequently with species-specific horseradish peroxidase-conjugated secondary antibodies. The bands were visualized with ECL Prime Western Blotting Detection reagent (GE Healthcare, Buckinghamshire, UK) and

quantitated by densitometric analysis using a CS Analyzer 2.0 (ATTO, Tokyo, Japan).

[^{3}H]Prostaglandin E_2 uptake.

Explants of each placental zone were pre-incubated with extracellular fluid (ECF) buffer (122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 2 mM $MgSO_4$, 0.4 mM K_2HPO_4, 10 mM D-glucose, 10 mM HEPES, pH 7.4) at 37°C for 10 min. After pre-incubation, explants of decidua, junctional zone, and labyrinth were incubated for 20 min in ECF buffer supplemented with 5.6 nM [^{3}H]PGE_2 (prostaglandin E_2 [5,6,8,11,12,14,15-3H(N)]; PerkinElmer, Boston, MA). The uptake was terminated by removal of the buffer and the explants were immediately washed with ice-cold ECF buffer. The explants were homogenized in saline, scintillation cocktail was added, and the radioactivity was measured with a liquid scintillation counter.

Mouse placental explant culture.

Placenta was cut in half, placed in 48-well plates with 400 μL of sterile culture medium (45% DMEM/45% Ham's F12/10% FBS supplemented with penicillin and streptomycin), and incubated at 37°C in a humidified incubator under 20% O_2 and 5% CO_2. After preincubation for 1 hour, all explants were left in culture media containing 0.04% DMSO in the absence or presence of 1 μM PGE_2. The culture medium was changed every 12 hours, and after 24 hours the explants were collected and stored at -80°C until use.

Preparation of plasma membrane-enriched fraction.

Plasma membrane-enriched fractions were prepared as described previously with some modifications (Nishimura et al., 2019). Murine tissues were homogenized in Tris-sucrose buffer (250 mM sucrose, 10 mM Tris-HCl, 1 μM pepstatin A, 10 μM leupeptin, 100 μM phenylmethanesulfonyl fluoride, pH 7.4). The homogenate was centrifuged at 5,800 x g for 15 min at 4°C, and the supernatant was centrifuged again at 10,000 x g for 15 min at 4°C. The resulting supernatant was centrifuged at 100,000 x g for 30 min at 4°C, and the pellet was resuspended in Tris-sucrose buffer. The suspension was overlaid on 38% (w/v) sucrose solution and centrifuged at 100,000 x g for 40 min at 4°C with a swing-out rotor. The turbid layer at the interface was collected and diluted with 10 mM Tris-HCl buffer (pH 7.4). The resulting suspension was centrifuged at 100,000 x g for 40 min at 4°C to afford the plasma membrane-enriched fraction as a pellet.

LC-MS/MS-based targeted protein quantification analysis.

Absolute protein expression levels were determined by quantifying the absolute amounts of specific peptides produced by trypsin digestion of OATP2A1 and PLs using LC-MS/MS as

described previously, with some modifications (Nishimura et al., 2019, Uchida et al., 2013). Fifty micrograms of protein was reduced with dithiothreitol (Fujifilm Wako) and S-carbamoylmethylated with iodoacetamide (Fujifilm Wako). The S-carbamoylmethylated samples were precipitated with a mixture of methanol and chloroform. The precipitates were dissolved in 6 M urea, diluted 5-fold with 100 mM Tris-HCl (pH 8.5), and treated at room temperature for 3 hours with proteaseMAX^{TM} surfactant (Promega, Madison, WI) and lysyl endopeptidase (Wako Pure Chemical Industries) at a final concentration of 0.05% and a final enzyme/substrate ratio of 1:100, respectively. The samples were then digested with sequence-grade modified trypsin (Promega) at an enzyme/substrate ratio 1:100 at 37°C for 16 hours. The resulting peptides were spiked with internal standard peptides, which are stable isotope (^{13}C and ^{15}N)-labeled peptides having the same amino acid sequences as the analyte peptides, and acidified with formic acid.

The LC-MS/MS system consisted of an HPLC instrument (Shimadzu, Kyoto, Japan) and an electrospray ionization triple quadrupole mass spectrometer (LCMS-8050, Shimadzu). Mobile phases A and B consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. Chromatographic separation was performed on an XBridge Peptide BEH C18 column (3.5 μm, 1 mm × 150 mm, Waters, Milford, MA) at 40°C with a linear gradient of mobile phase B as follows: 1% for 0-5 min, 1% to 50% for 5-25 min, 100% for 25-30 min, and 1% for 30-60 min. MS spectrometric detection was performed by multiple reaction monitoring in the electrospray ionization mode. Peak data were extracted by using 4 sets of SRM transitions (m/z) of the precursor ion (Q1) and the product ion (Q3) per peptide, as listed in Supplemental Table 1, with the dwell time of 10 msec per transition. The amount of the peptide in the sample was determined for each transition using the peak area ratio (analyte peptide/corresponding internal standard peptide) of the positive peak and a calibration curve obtained by using known concentrations of synthetic peptides, and expressed as the average of 4 positive peaks from different transitions.

Measurement of prostaglandins and progesterone.

Murine tissues were homogenized with a sonicator in the presence of d4-PGE_2 (Cayman Chemical) as an internal standard. The homogenates were deproteinized with the same volume of methanol. The supernatant was obtained by centrifugation at 15,000 rpm for 5 min at 4°C and applied to a hydrophilized C18 solid-phase extraction cartridge. The eluate was evaporated in a vacuum centrifuge and the pellets were reconstituted in 50% acetonitrile.

The amounts of prostaglandins and progesterone extracted from tissue homogenates were determined by LC-MS/MS. Mobile phases A and B consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. Chromatographic separation was performed on a

Capcell Pak C18 UG120 column (5 μm, 2.0 mm × 150 mm, Shiseido, Tokyo, Japan) at 40°C with gradients of mobile phase B as follows: 10% to 25% for 0-5 min, 25% to 35% for 5-10 min, 35% to 75% for 10-20 min, 100% for 20-25 min, and 10% for 25-30 min for measurement of prostaglandins; 30% for 0-5 min, 30% to 55% for 5-20 min, 100% for 20-25 min, and 30% for 25-30 min for measurement of progesterone. Mass spectrometric detection was performed by multiple reaction monitoring in the electrospray ionization mode, using m/z 351.50→271.40 for PGE_2, 351.50→175.25 for 13,14-dihydro 15-keto PGE_2, 333.50→175.20 for 13,14-dihydro 15-keto PGA_2, 353.50→193.50 for $PGF_{2\alpha}$, 353.50→113.10 for 13,14-dihydro 15-keto $PGF_{2\alpha}$, 355.50→275.10 for d4-PGE_2, and 315.25→96.95 for progesterone.

RU486 and indomethacin treatment.

The progesterone receptor antagonist RU486 (mifepristone, Wako Pure Chemical Industries) was administered by subcutaneous injection (150 μg in ethanol/mineral oil) to pregnant mice at 4:00 PM on GD18.5, following the method used in a previous study (Dudley et al., 1996). Indomethacin was administered by intraperitoneal injection (1 mg/kg animal in PBS) to pregnant mice at 10:00 AM on GD15.5. Control mice received the vehicle.

Statistics.

Results are presented as mean ± S.E.M. Statistical analysis was performed by means of one-way ANOVA or two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons or Student's 2-tailed *t* test for comparison between two groups. Linear regression analysis was performed using GraphPad Prism4 (GraphPad Software, San Diego, CA). *P* values of less than 0.05 were considered statistically significant.

Supplemental References

Akashi, T., Nishimura, T., Takaki, Y., Takahashi, M., Shin, B.C., Tomi, M., and Nakashima, E. (2016). Layer II of placental syncytiotrophoblasts expresses MDR1 and BCRP at the apical membrane in rodents. Reprod.Toxicol. *65*, 375-381.

Dudley, D.J., Branch, D.W., Edwin, S.S., and Mitchell, M.D. (1996). Induction of preterm birth in mice by RU486. Biol.Reprod. *55*, 992-995.

Inagaki, M., Nishimura, T., Akanuma, S.I., Nakanishi, T., Tachikawa, M., Tamai, I., Hosoya, K.I., Nakashima, E., and Tomi, M. (2017). Co-localization of microsomal prostaglandin E synthase-1 with cyclooxygenase-1 in layer II of murine placental syncytiotrophoblasts. Placenta. *53*, 76-82.

Matsumura, H., Hasuwa, H., Inoue, N., Ikawa, M., and Okabe, M. (2004). Lineage-specific cell disruption in living mice by Cre-mediated expression of diphtheria toxin A chain. Biochem.Biophys.Res.Commun. *321*, 275-279.

Nakanishi, T., Hasegawa, Y., Mimura, R., Wakayama, T., Uetoko, Y., Komori, H., Akanuma, S., Hosoya, K., and Tamai, I. (2015). Prostaglandin Transporter (PGT/SLCO2A1) Protects the Lung from Bleomycin-Induced Fibrosis. PLoS One. *10*, e0123895.

Nishimura, T., Sano, Y., Takahashi, Y., Noguchi, S., Uchida, Y., Takagi, A., Tanaka, T., Katakura, S., Nakashima, E., Tachikawa, M., Maruyama, T., Terasaki, T., and Tomi, M. (2019). Quantification of ENT1 and ENT2 Proteins at the Placental Barrier and Contribution of These Transporters to Ribavirin Uptake. J.Pharm.Sci.

Simmons, D.G., Rawn, S., Davies, A., Hughes, M., and Cross, J.C. (2008). Spatial and temporal expression of the 23 murine Prolactin/Placental Lactogen-related genes is not associated with their position in the locus. BMC Genomics. *9*, 352-2164-9-352.

Tachikawa, M., Ozeki, G., Higuchi, T., Akanuma, S., Tsuji, K., and Hosoya, K. (2012). Role of the blood-cerebrospinal fluid barrier transporter as a cerebral clearance system for prostaglandin E(2) produced in the brain. J.Neurochem. *123*, 750-760.

Tachikawa, M., Tsuji, K., Yokoyama, R., Higuchi, T., Ozeki, G., Yashiki, A., Akanuma, S., Hayashi, K., Nishiura, A., and Hosoya, K. (2012). A clearance system for prostaglandin D2, a

sleep-promoting factor, in cerebrospinal fluid: role of the blood-cerebrospinal barrier transporters. J.Pharmacol.Exp.Ther. *343*, 608-616.

Uchida, Y., Tachikawa, M., Obuchi, W., Hoshi, Y., Tomioka, Y., Ohtsuki, S., and Terasaki, T. (2013). A study protocol for quantitative targeted absolute proteomics (QTAP) by LC-MS/MS: application for inter-strain differences in protein expression levels of transporters, receptors, claudin-5, and marker proteins at the blood-brain barrier in ddY, FVB, and C57BL/6J mice. Fluids Barriers CNS. *10*, 21-8118-10-21.