PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Arévalo L, Campbell P. 2020 Placental effects on the maternal brain revealed by disrupted placental gene expression in mouse hybrids. *Proc. R. Soc. B* **287**: 20192563.
http://dx.doi.org/10.1098/rspb.2019.2563

Received: 3 November 2019
Accepted: 11 December 2019

Subject Category:
Genetics and genomics

Subject Areas:
evolution, genomics, developmental biology

Keywords:
placenta, hybrid, maternal–fetal conflict, placental lactogens, paternal effects, genomic imprinting

Author for correspondence:
Lena Arévalo
e-mail: lena.lueke@gmail.com

†Present address: Department of Developmental Pathology, University Hospital, Bonn, Germany.
‡Present address: Department of Evolution, Ecology and Organismal Biology, University of California Riverside, Riverside, CA, USA.

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.4788795.

THE ROYAL SOCIETY PUBLISHING

Placental effects on the maternal brain revealed by disrupted placental gene expression in mouse hybrids

Lena Arévalo† and Polly Campbell‡

Department of Integrative Biology, Oklahoma State University, Stillwater, OK, USA

LA, 0000-0002-0681-0392; PC, 0000-0001-7660-9814

The mammalian placenta is both the physical interface between mother and fetus, and the source of endocrine signals that target the maternal hypothalamus, priming females for parturition, lactation and motherhood. Despite the importance of this connection, the effects of altered placental signalling on the maternal brain are insufficiently studied. Here, we show that placental dysfunction alters gene expression in the maternal brain, with the potential to affect maternal behaviour. Using a cross between the house mouse and the Algerian mouse, in which hybrid placental development is abnormal, we sequenced late-gestation placental and maternal medial preoptic area transcriptomes and quantified differential expression and placenta-maternal brain co-expression between normal and hybrid pregnancies. The expression of *Fmn1* and *Drd3* was significantly altered in the brains of females exposed to hybrid placentas. Most strikingly, expression patterns of placenta-specific gene families and *Drd3* in the brains of house mouse females carrying hybrid litters matched those of female Algerian mice, the paternal species in the cross. Our results indicate that the paternally derived placental genome can influence the expression of maternal–fetal communication genes, including placental hormones, suggesting an effect of the offspring's father on the mother's brain.

1. Background

The placenta is a unique, transient organ shared by two organisms. Placental morphology is surprisingly diverse across vertebrates and is subject to rapid evolutionary change and convergent evolution [1,2]. In most eutherian mammals, including mice and humans, successful blastocyst implantation relies on endometrial invasion by the embryonic trophoblast cells that give rise to the mature placenta [3]. As such, the placenta provides the closest physical and molecular link between mother and offspring seen in any animal [4]. This intimate connection promotes an array of maternal–fetal interactions, including bidirectional hormonal regulation and even the exchange of entire cells. These interactions are not spatially limited but extend to both the fetal and the maternal brain [5,6].

Throughout pregnancy, the placenta mediates the regulation of resource allocation, immune tolerance, fetal development and, importantly, hormonal priming of the maternal brain. A key subset of placenta-secreted molecules is involved in priming maternal physiology for parturition and lactation, and promoting the onset of maternal behaviours in late gestation [7,8]. In rodents, the medial preoptic area (MPoA) in the hypothalamus is thought to be the primary neural target of these placental molecules [5,9,10]. The MPoA has been characterized as the central hub of parenting behaviour [11]: receptors for key pregnancy hormones and neurotransmitters, including oestrogen, prolactin and dopamine, are highly expressed in this nucleus and interact with ligands of both maternal and placental origin [12].

Two classes of placental genes are of particular importance to the interaction between the placenta and maternal brain: imprinted genes (IGs) and placenta-specific gene families (PSFs). Several IGs and PSF genes are expressed in the

same placental compartment, especially in the placental endocrine compartment [13,14]. IGs are exclusively or predominantly expressed from one allele, and are highly expressed in placenta and brain. An allelic expression is determined by heritable epigenetic marks (imprints) in maternal and paternal germ cells, such that some IGs are maternally silenced and paternally expressed, whereas others are paternally silenced and maternally expressed [15]. During pregnancy, IGs are critical to placental development and function, maintaining the balance between maternal supply and embryonic demand, and regulating maternal–fetal exchange [14].

PSFs arose through lineage-specific gene duplication events during placental evolution [16]. In rodents, these are the prolactin gene family (placental lactogens, Prls), placental cathepsin proteases and their inhibitors, and pregnancy-specific glycoproteins [17–19]. PSF gene products are mainly expressed from the placental endocrine compartment and many are secreted into the maternal bloodstream; key functions include placental development, immunoregulation, and physiological and neurological priming of the mother [5,9,16]. Most notably, a subset of Prls binds prolactin receptors in the maternal MPoA, leading to the proposal that placental hormones directly affect maternal endocrine state and behaviour [5,10]. IGs are implicated in regulating PSF secretion via their effects on the structure and function of the placental endocrine compartment [20]. However, our current understanding of the role of IGs in PSF signalling is rudimentary, and the relationship between gene expression in placenta and the maternal MPoA is uncharted.

The majority of the placenta, including the endocrine compartment, is fetally derived. Placental representation of both parental genomes sets the stage for conflict (maternal–paternal and parent–offspring), and for coadaptation (mother–offspring), with IGs uniquely positioned to mediate both types of interactions [21–23]. However, while evolutionary models for IG expression abound [24], empirical studies of the interaction between paternally derived placental signals and signal reception in the maternal brain are rare [8,25,26].

Here, we use a natural hybrid system to explore the effects of placental dysregulation on gene expression in the maternal brain. Over- or under-growth that depends on the direction of the cross is a signature of disrupted imprinting in mammalian hybrids [27]. This pattern is documented in several orders, with the best-studied examples in the rodent genera *Peromyscus*, *Phodopus* and *Mus* [28–30]. Parent-of-origin growth effects in the cross between the house mouse, *Mus m. domesticus* (*Dom*) and the Algerian mouse, *M. spretus* (*Spret*), were first described over 20 years ago: placentas are undersized when the mother is *Dom* and the father is *Spret*, and severely oversized in the reciprocal cross, with more extreme size effects in both directions of the backcross [28]. Subsequent studies confirmed altered expression and methylation of candidate IGs, and disrupted placental organization [31,32]. Specifically, the placental endocrine compartment (or junctional zone) was shown to be reduced and disorganized [28,31]. However, the extent of placental misexpression has not been measured on a genomic scale, and this system's potential to uncover the maternal consequences of altered placental signalling has not been considered.

We recently showed that maternal responsiveness to pups is significantly altered in *Dom* females with newborn hybrid relative to conspecific litters [33]. These effects of litter genotype were only detected during the first 24 h postpartum, suggesting that altered behaviour in mothers of hybrid litters is the residual consequence of abnormal placental signalling rather than a response to phenotypic differences between hybrid and conspecific pups [33]. Here, by comparing MPoA expression between females of the same species that differ only in the type of pregnancy/placenta they experience (hybrid versus conspecific), we specifically isolate the effect of placental gene expression differences on the maternal brain (electronic supplementary material, figure S1). Characterization of altered gene expression at the maternal–fetal interface provides insight into the mechanisms of maternal–fetal communication, the contribution of the paternal genome to this interaction, and identifies promising candidate genes for future evolutionary and biomedical work.

2. Material and methods

(a) Animals and tissue collection

Mice used in this study were maintained on a 12 : 12 light : dark cycle with lights on at 9.00, and were provided with 5001 Rodent Diet and water ad libitum. All animal procedures were approved by the Oklahoma State University IACUC under protocol 141-AS. *Mus m. domesticus* (*Dom*) was represented by the wild-derived inbred strain WSB/EiJ (Jackson Laboratory) and *Mus spretus* (*Spret*) was represented by the wild-derived inbred strain SFM/Pas (Montpellier Wild Mice Genetic Repository). We conducted three crosses (female shown first): *Dom* × *Dom* (*Dom* pregnancy), *Dom* × *Spret* (hybrid pregnancy) and *Spret* × *Spret* (*Spret* pregnancy). The reciprocal cross (*Spret* × *Dom*) was attempted 25 times but was never successful. Mice were paired between 17.00 and 18.00, left undisturbed for two nights and split on the morning of the second day. The second night was counted as embryonic day 0 (e0). Females were weighed after two weeks to confirm pregnancy but were otherwise left undisturbed. Pregnant females ($n = 5$/type of pregnancy) were euthanized by cervical dislocation between 10.00 and 11.00 on an embryonic day 17–18 (e17.5) and the maternal brain was extracted. We chose this late gestation time point based on prior work demonstrating that infusion of placental lactogen into the MPoA of near-term females promotes the onset of maternal care at parturition [5,9]. Embryos were separated from placentas, the maternally derived placental decidual layer was removed [34], and embryos and dissected placentas were weighed. All tissues were transferred to RNAlater, and stored at −20°C until microdissection and RNA extraction.

(b) Brain microdissection and RNA extraction

The maternal MPoA was localized using the mouse brain atlas (figures 26–33 in [35]), and microdissected by sectioning the RNAlater-perfused brain at 100 µm on a Leica CM 1950 cryostat, followed by dissection under a dissecting microscope in chilled PBS droplets for improved visibility of brain microstructure. DNA was extracted from embryonic tissue using the DNeasy Blood & Tissue Kit (Qiagen, USA) followed by PCR for the Y-linked gene, *Zfy1*, to determine sex. Placentas from one male and one female per litter were used for RNA extraction ($n = 5$ males per hybrid cross, $n = 5$ males per conspecific cross, $n = 4$ females per hybrid cross and $n = 5$ females per conspecific cross). RNA was extracted from all tissues immediately after microdissection using the RNeasy Plus Universal Mini Kit (Qiagen) for MPoA, and the AllPrep RNA/DNA Mini Kit (Qiagen) for placenta. RNA was stored at −80°C until sequencing.

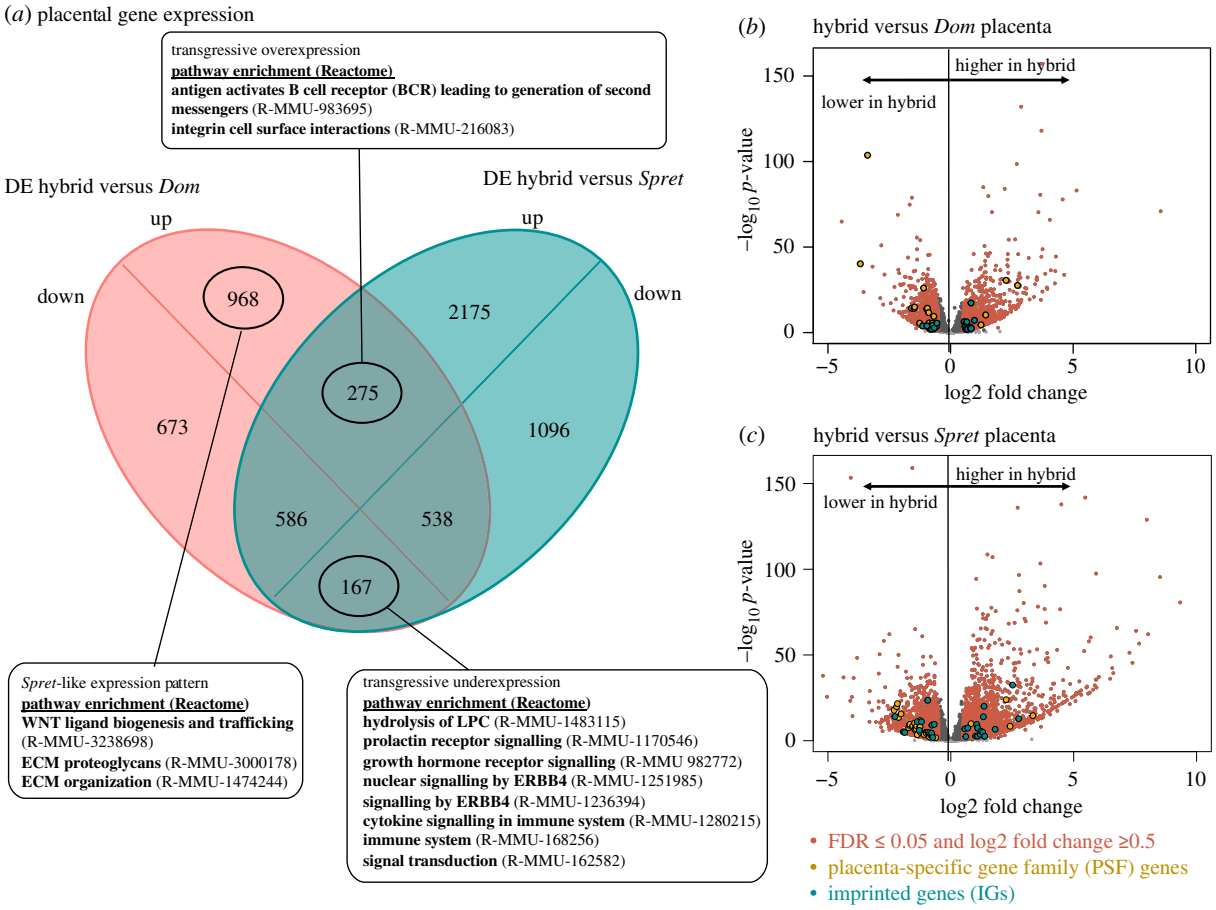

Figure 1. DE gene analysis between *Mus m. domesticus* (*Dom*), *M. spretus* (*Spret*) and hybrid placentas. (*a*) Overlap of DE genes between the comparisons hybrid versus *Dom* and hybrid versus *Spret*; up = genes expressed higher in hybrids compared to parental species, down = genes expressed lower in hybrids compared to parental species. Results of pathway overrepresentation in text boxes. Volcano plot of DE results of (*b*) hybrid versus *Dom* and (*c*) hybrid versus *Spret* placentas. (Online version in colour.)

(c) Transcriptome data processing and analysis

Maternal MPoA and placental transcriptomes were sequenced on the Illumina HiSeq 4000 platform, producing greater than 30 million, 150 bp paired-end reads per sample. To improve comparability, all placenta samples (*Dom*, *Spret* and hybrid) were mapped to a pseudo-hybrid genome, generated using the genome preparation tool in the programme SNPsplit (Babraham Bioinformatics [36]). MPoA samples were mapped to their corresponding genomes (WSB/EiJ_v1 for *Dom* MPoA, SPRET/EiJ_v1 for *Spret* MPoA [37]) using HISAT2 2.1 [38]. Post-processing of alignments (filtering and downsampling) was done using SAMtools 0.1.19 [39]. Transcript quantification and annotation were done using StringTie 1.3.3 [40] with gene annotation information retrieved from Ensembl (ftp://ftp.ensembl.org). To confirm equivalent expression from *Dom* and *Spret* alleles in the hybrid placenta we split the hybrid alignment files, separating reads originating from the *Spret* and *Dom* alleles. Splitting was performed using SNPsplit. Because the maternal brain is exposed to placental signals of both sexes simultaneously, male and female placental expression was analysed jointly. Differential expression was tested with DESeq2 1.16.1 [41]. Normalized read count tables produced by DESeq2 were used in subsequent co-expression analyses. GO-term and pathway overrepresentation analyses were performed using the PANTHER gene list analysis tool [42]. Additional information on RNAseq data processing and analysis is provided in electronic supplementary material, text S1.

(d) Evolutionary rates for selected genes

For evolutionary analysis of selected gene sequences, pairwise dN/dS (the per site ratio of non-synonymous to synonymous substitutions) between *Dom* and *Spret* was calculated using YN00 implemented in PAML 4.8 [43]. Further analysis of genes with dN/dS > 1 was performed with CodeML implemented in PAML 4.8 [43], including sequences from related *Mus* subspecies and species (*Mus m. musculus*, *Mus m. castaneus*, *Mus caroli* and *Mus pahari*). Detailed methods for evolutionary rates analyses are provided in electronic supplementary material, text S1.

3. Results

(a) Hybrid placental expression is globally closer to the maternal than the paternal species

As expected, large percentages of placental genes, including IGs, were differentially expressed (DE) in all three pairwise comparisons: hybrid versus *Dom*, 17.5% (3207/18 298 genes; 14/135 IGs), hybrid versus *Spret* 26.1% (4837/18 529 genes; 23/135 IGs), *Dom* versus *Spret*, 38.2% (7292/19 079; 32/135 IGs) (figure 1; electronic supplementary material, figures S2–S4, dataset S1). Consistent with less DE in the hybrid versus *Dom* than in the hybrid versus *Spret* comparison, hybrid samples clustered slightly closer to *Dom* in the diagnostic PCA plot (electronic supplementary material, figure S5). Notably, approximately twice as many genes in hybrid placentas were uniquely DE relative to *Spret* (3271) as opposed to *Dom* (1641) (figure 1*a*). Thus, the general expression pattern in hybrid placentas was more similar to the maternal species. *Dom*-like expressed genes were enriched for multiple immune related pathways, together

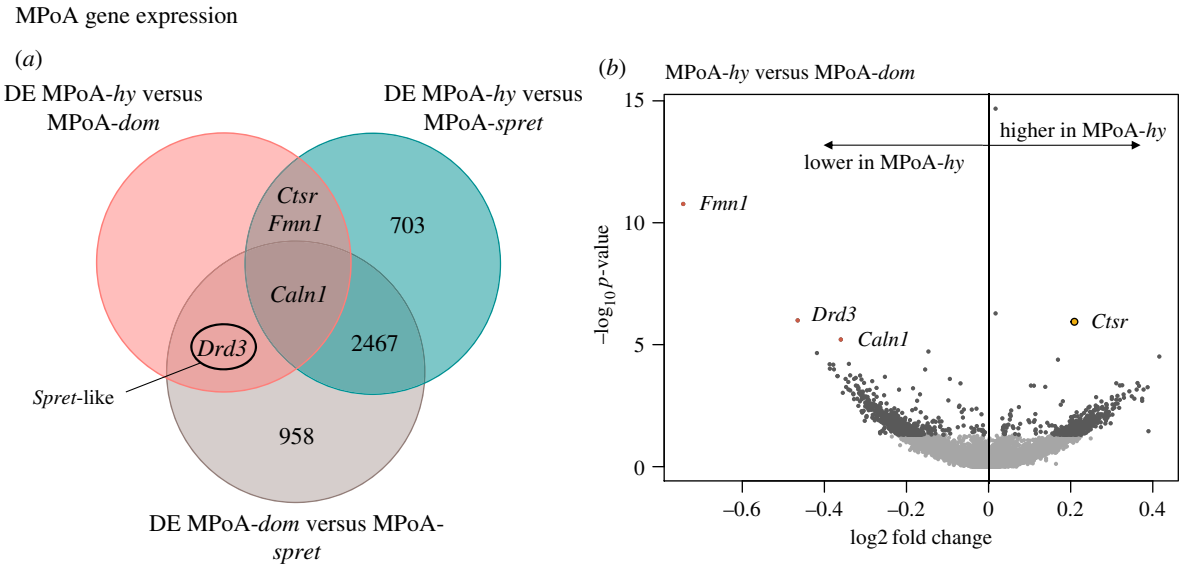

Figure 2. DE gene analysis between *Mus m. domesticus* MPoA during normal pregnancy (MPoA-*dom*), *Mus m. domesticus* MPoA during hybrid pregnancy (MPoA-*hy*) and *M. spretus* MPoA during normal pregnancy (MPoA-*spret*). (*a*) Overlap of DE genes between the three comparisons. (*b*) Volcano plot of DE results of MPoA-*hy* versus MPoA-*dom*. Significantly DE genes with FDR ≤ 0.05 and log2 fold change ≥ 0.2 are depicted in red and labelled with the gene symbol, PSF genes in yellow with a black border. (Online version in colour.)

with angiogenesis, vascular development and haemostasis related terms, among others (electronic supplementary material, dataset S1).

(b) Placenta-specific gene families and imprinted genes are enriched among differentially expressed genes in hybrid placentas

From the perspective of *M. m. domesticus* females carrying hybrid litters, abnormal placental expression constitutes all genes that are DE relative to *Dom* placentas. We divided these genes into three types. Transgressively expressed genes are significantly over- or underexpressed relative to both parental species; genes with intermediate expression are significantly overexpressed relative one parent but underexpressed relative to the other; genes with *Spret*-like expression are uniquely DE relative to *Dom* (figure 1*a*).

The 275 transgressively overexpressed genes were significantly enriched for B-cell receptor activation and integrin cell surface interaction pathways (figure 1*a*; electronic supplementary material, dataset S1). The 167 transgressively underexpressed genes were significantly enriched for pathways that involve Prls, including prolactin and growth hormone receptor signalling, epidermal growth factor (ERBB) signalling, and cytokine signalling (figure 1*a*; electronic supplementary material, dataset S1). Prls and other PSF genes were highly overrepresented among DE genes in hybrids compared to both *Dom* (Fisher's exact test: $p < 0.001$, odds ratio = 5.65) and *Spret* ($p < 0.001$, odds ratio = 3.41). Thirty-three PSF genes, including 14/22 Prls, were DE hybrid versus *Dom* placenta (electronic supplementary material, table S1). Of these, the majority (29/33) were underexpressed relative to *Dom* placenta.

IGs were also significantly overrepresented among hybrid DE genes compared with both *Spret* (Fisher's exact test: $p = 0.02$, odds ratio = 0.59) and *Dom* ($p = 0.03$, odds ratio = 0.55). Three IGs (*Tnfrsf23*, *Phlda2* and *Klf14*) were transgressively overexpressed, two (*Ascl2* and *Sfmbt2*) were transgressively underexpressed, and two (*Tspan32* and *Th*) were significantly DE compared with both parental species but intermediate between the two (electronic supplementary material, table S2). Four of these DE IGs belong to the same imprinting cluster (IC2) on the distal part of mouse chromosome 7 (dist7) (MouseBook, https://www.mousebook.org, 22 March 2018), and are normally maternally expressed (electronic supplementary material, table S2 and dataset S1). We note that *Tnfrsf23* is expressed in decidual cells at the junction between the maternally derived decidua and the extraembryonic placenta [44]. The IGs with *Spret*-like expression were all overexpressed relative to *Dom* and 4/6 are normally paternally expressed. The latter pattern contrasts with other DE IGs, of which only 1/7 is normally paternally expressed (electronic supplementary material, table S2).

(c) Altered medial preoptic area expression in mothers exposed to hybrid placentas

To test for effects of abnormal placental expression on the maternal brain, we compared e17.5 MPoA expression of *Dom* females carrying hybrid litters (MPoA-*hy*) and *Dom* females carrying conspecific litters (MPoA-*dom*) (electronic supplementary material, figures S6–S8 and dataset S2). Several genes were DE between MPoA-*hy* and MPoA-*dom*: Dopamine receptor 3 (*Drd3*) (LFC = −0.47, padj < 0.001), Formin 1 (*Fmn1*) (LFC = −0.74, padj < 0.001) and Calneuron 1 (*Caln1*) (LFC = −0.36, padj = 0.02) had lower expression in MPoA-*hy*. Notably, *Drd3* had *Spret*-like expression in MPoA-*hy* (figure 2*a*). Interestingly, Cathepsin-R (*Ctsr*) (LFC = 0.21, padj < 0.001), a normally placenta-specific PSF gene was expressed at low levels in MPoA-*hy* but was not expressed in MPoA-*dom* (figure 2; electronic supplementary material, dataset S2 and table S3).

(d) Evidence for an effect of the placenta's paternal genome on maternal medial preoptic area

The MPoA is thought to be a primary target of placental lactogens [7] and the maternal hypothalamus is sensitive to altered placental expression of IG *Phlda2* [8]. Therefore,

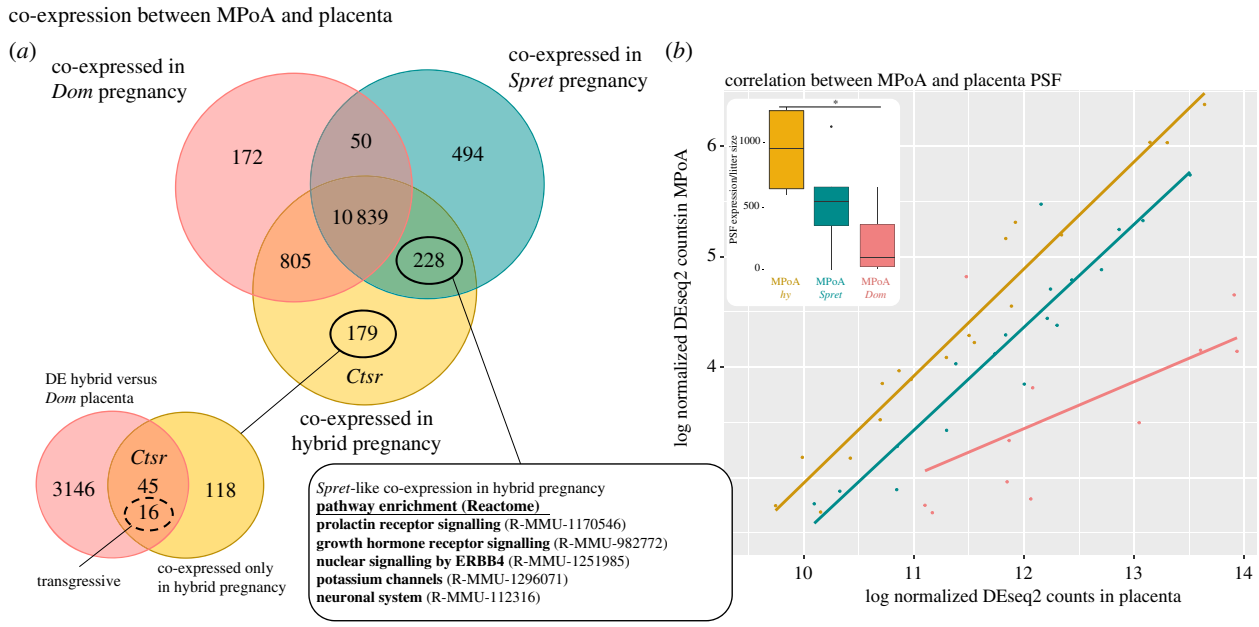

Figure 3. Co-expression between MPoA and placenta for *Mus m. domesticus* during normal pregnancy (*Dom* pregnancy), *Mus m. domesticus* during hybrid pregnancy (hybrid pregnancy) and *M. spretus* during normal pregnancy (*Spret* pregnancy). (*a*) Overlap of co-expressed genes between the three pregnancy types. A secondary Venn diagram shows overlap with DE genes in hybrid versus *Dom* placentas. (*b*) Correlation between PSF gene expression in placenta and MPoA: red = *Dom* pregnancy ($R^2_{adj} = 0.38$, $p = 0.01$), blue = *Spret* pregnancy ($R^2_{adj} = 0.9$, $p < 0.001$), yellow = *hybrid* pregnancy ($R^2_{adj} = 0.95$, $p < 0.001$). Inset boxplot shows total PSF gene expression in MPoA (sum of normalized PSF counts per litter size).

differences in placenta-MPoA co-expression between hybrid and *Dom* pregnancies inform MPoA response to abnormal placental expression. One hundred and seventy-six genes were uniquely co-expressed between placenta and MPoA in hybrid pregnancies (figure 3*a*; electronic supplementary material, dataset S3). These include 45 genes that were DE between hybrid and *Dom* placentas, 16 that were DE compared with both parental species' placentas, and *Ctsr*, which was uniquely co-expressed in hybrid pregnancies (figure 3; electronic supplementary material, dataset S3). Interestingly, whereas 50 co-expressed genes were common to *Dom* and *Spret* pregnancies, 228 (greater than 4.5× more) were common to hybrid and *Spret* pregnancies (figure 3*a*; electronic supplementary material, dataset S3). There was pathway overrepresentation overlap between this *Spret*-like gene set and genes with transgressive misexpression in hybrid placenta (figure 1*a*), including prolactin and growth hormone receptor signalling and ERBB signalling (figure 3; electronic supplementary material, dataset S3). Moreover, PSFs were significantly overrepresented among *Spret*-like co-expressed genes (Fisher's exact test: $p < 0.001$, odds ratio = 12.57).

Although expression levels were far lower in the MPoA (range = 10–589, mean = 100 normalized counts, LFC to placenta: range = −9.54 to −14.13, mean = −11.12) than in the placenta (range = 20–1 127 603, mean = 97 988 normalized counts), the apparent shift in *Dom* hybrid pregnancies towards *Spret*-like placenta–MPoA co-expression of PSFs was striking. To explore this relationship further, we tested for correlated expression of PSF genes between placenta and MPoA. We found very strong, positive correlations for hybrid ($R^2_{adj} = 0.95$, $p < 0.001$) and *Spret* pregnancies ($R^2_{adj} = 0.9$, $p < 0.001$) and a significant but, surprisingly, a weaker positive correlation for *Dom* pregnancies ($R^2_{adj} = 0.38$, $p = 0.01$; figure 3*b*). For all three pregnancy types, the R^2_{adj} estimates for the PSF correlations were significantly larger than expected by chance (300 random correlations: $R^2_{adj(meam)} = 0.244$; one-sample *t*-test, hybrid R^2_{adj}:$t_{299} = -60.94$, FDR < 0.001; *Dom* R^2_{adj}:$t_{299} = -11.94$, FDR < 0.001; *Spret* R^2_{adj}:$t_{299} = -56.8$, FDR < 0.001). Additionally, total PSF expression (sum of all PSF read counts divided by litter size) was significantly higher in MPoA-*hy* relative to MPoA-*dom* but statistically indistinguishable from MPoA-*spret* (one-way ANOVA: $F_{2,12} = 5.44$, $p = 0.02$, Tukey HSD: MPoA-*hy* versus MPoA-*dom*: $p = 0.016$, MPoA-*hy* versus MPoA-*spret*: $p = 0.18$, MPoA-*dom* versus MPoA-*spret*: $p = 0.36$; figure 3*b*). These patterns suggest that the placenta's paternally inherited genome (in this case from *M. spretus*) influences gene expression in the maternal MPoA.

(e) Evidence for positive selection on three placenta-specific gene family genes

PSF genes exhibit accelerated evolutionary rates, potentially driven by maternal–fetal conflict [45]. We therefore screened the top 10 co-expressed PSF genes with the highest expression in MPoA, together with *Ctsr*, for pairwise d*N*/d*S* > 1 between *Dom* and *Spret* (electronic supplementary material, table S4). Three genes, *Prl8a6*, *Tpbpb* and *Ctsr*, met this criterion (electronic supplementary material, figure S9 and table S5) and were tested for positive selection including one-to-one orthologues from four related *Mus* subspecies and species.

Codon site-based models detected evidence for positive selection on all three genes. We found significant positive selection on codon sites across the whole tree for *Ctsr* ($LRT_{(M1a–M2a)} = 9.4$, FDR = 0.03) and *Tpbpb* ($LRT_{(M1a–M2a)} = 10.6$, FDR = 0.02), but no selection specifically restricted to the *Dom* or *Spret* lineages (electronic supplementary material, table S5). *Prl8a6* did not show positive selection on codon sites across the whole tree ($LRT_{(M1a–M2a)} = 3.14$, FDR = 0.33) but we detected positive selection on one codon site within

the *Dom* lineage ($LRT_{(bsA1-bsA)} = 5.55$, FDR = 0.05; electronic supplementary material, table S5).

4. Discussion

Molecular communication between the placenta and the maternal brain is crucial for the expression of maternal behaviour in rodents [5,8,10]. In humans, altered regulation of placental IGs is associated with prenatal depression [46], a predictor of lower growth rate and higher disease risk in infants [47]. In this study, we used a hybrid mouse model to characterize the extent to which placental disruption influences gene expression in the maternal brain. Several maternally expressed IGs were transgressively misexpressed in the hybrid placenta. In *Mus m. domesticus* females carrying hybrid litters, we found altered PSF expression in the placenta, and in the maternal MPoA. Surprisingly, the expression of these genes was highly correlated between the two tissues, and was *M. spretus*-like in the MPoA. This suggests that paternally inherited alleles in the placenta exert substantial influence on expression in the maternal brain. Collectively, our results reveal the reciprocal effects of mothers on offspring and offspring on mothers, mediated in both cases by the placenta.

(a) Maternal effects on placental expression

Global patterns of expression in the placenta were strongly associated with maternal genotype. In hybrids, half as many genes were DE relative to normal *Dom* placentas as opposed to *Spret* placentas. Notably, the more than 1600 genes with *Dom*-like expression in the hybrid placenta were highly enriched for terms associated with immunity and regulation of blood flow, both of which are essential to placental mediation between mother and embryo [3]. Because maternal vasculature is incorporated into the placenta, whole placenta transcriptomes necessarily include some transcripts of maternal origin and detection of *Tnfsrs23* transcripts in all samples indicates that some cells from the maternal decidua remained following dissection. However, maternal blood flow within the placenta is under the direct control of placental cell lineages; trophoblast giant cells invade and replace maternal vascular endothelium, limiting maternally derived tissue to blood [48]. Likewise, *Tnfsrs23*-expressing cells are adjacent to fetal trophoblast giant cells at the junction between fetal and maternal tissues [44]. Therefore, while contamination from maternal transcripts may contribute to *Dom*-like expression in hybrid placenta, it is unlikely to bias the expression of such a large number of genes. The regulatory effects of maternal hormones, and of maternally inherited genes in the placenta, are non-mutually exclusive alternative explanations. For example, because paternal X chromosome inactivation is maintained in mouse placenta [49], maternally inherited X-linked genes are strong candidates for modulating autosomal expression in both sexes. While disentangling maternal effects (*sensu* [50]) from the effects of maternally inherited genes is a challenge for future studies, we note that the match between maternal genotype and placental expression of genes that modulate maternal immune tolerance and angiogenesis is consistent with the expectation of molecular coadaptation between mother and offspring [22,51], and the well-established effect of maternal environment on placental function [52].

(b) Altered placenta-specific gene family and imprinted gene expression in the placenta

Maternal adaptation to pregnancy relies to a great extent on placental signalling. Thus, altered expression of genes encoding or influencing placental signalling molecules can ultimately affect maternal physiological and behavioural response to pregnancy [5,8]. Misexpression in the hybrid placenta was substantial. However, the most striking pattern we found was the reduced expression of a large number of PSFs. In mice, most of these genes are expressed from the placental endocrine compartment and many are found in maternal plasma during pregnancy [15]. In this hybrid mouse model, the endocrine compartment is markedly reduced when the mother is *Dom* [28]. Thus, reduced abundance of PSF producing cell types probably contributes to an overall reduction in PSF expression.

IGs are thought to modulate PSF expression, primarily through effects on placental endocrine cell abundance, with maternally expressed genes (MEGs) repressing and paternally expressed genes (PEGs) promoting cell proliferation [19]. Two such MEGs, *Phlda2* and *Ascl2*, were transgressively misexpressed in hybrid placentas. Altered expression of either of these genes in laboratory mouse models results in an undersized endocrine compartment, altered glycogen energy stores and reduced PSF gene expression [53,54]. Indeed, *Phlda2* and *Ascl2* seem to be critical co-regulators of placental endocrine compartment development [19]. Collectively, our results are consistent with the proposed role of IGs in placental signalling [13,19,55], and identify MEG misexpression as a candidate mechanism for the undersized endocrine compartment and a consequent global reduction in PSF expression in the hybrid placenta.

(c) The effects of hybrid placental dysfunction on the maternal brain

Altered signalling in hybrid placentas has the potential to affect the maternal brain. We found subtle but significant differences in the expression of four genes in the MPoA of *Dom* females exposed to hybrid relative to conspecific placentas. Both *Fmn1* (Formin1) and *Caln1* (Calneuron1) were underexpressed. In the brain, *Fmn1* is involved in the formation of adherens junctions and in linear actin cable polymerization [56]. The formation of adherens junctions is important in the maintenance of the blood brain barrier (BBB) [57]. During pregnancy, the permeability of the BBB is increased by placenta-derived factors to which the maternal brain must respond in order to maintain this barrier [58]. Reduced expression of *Fmn1* therefore suggests alterations in BBB adaptation during hybrid pregnancies. *Caln1* encodes a neuron-specific protein with sequence similarities to calcium-binding calmodulins. While altered expression of a calcium-binding protein could indicate alterations in neuronal activity in the MPoA exposed to hybrid placentas, the functional effects such a small reduction in the expression are uncertain.

Drd3 (Dopamine receptor D3) was also underexpressed compared to *Dom* mothers, but not to *Spret* mothers, in the hybrid pregnancy MPoA. DRD3, a D2-like receptor with a generally inhibitory role, is implicated in treatment-resistant major depression [59] and *Drd3* knock-out mice exhibit a suite of anxiety- and depressive-like behaviours with similar

but milder effects in heterozygous knock-outs [59,60]. Given that the action of dopamine in the MPoA is critical for the expression of maternal behaviour in rats [61], and hypothalamic dopamine is altered in a mouse model for postpartum depression [62], reduced *Drd3* expression in the MPoA might cause deficits in maternal behaviour. Notably, we found that *Dom* mothers of hybrid relative to conspecific litters are slower to retrieve their newborn pups to the nest and spend less time in the nest on the first night after parturition [33]. Whether reduced dopamine–Drd3 binding in the prepartum MPoA contributes to this reduction in maternal responsiveness is an intriguing question for future study.

Ctsr (Cathepsin R), a placenta-specific cathepsin, was the only gene that was upregulated in the MPoA exposed to hybrid placentas. The difference in expression, although significant, was so small that the biological relevance is questionable. However, unlike other PSF genes, expression of *Ctsr* in the maternal brain was unique to the hybrid pregnancy. Interestingly, loss of the IG *Peg3* leads to de-repression of several PSF members, including *Ctsr*, in the fetal and adult brain [63]. Although *Peg3* was not misexpressed in the hybrid placenta, the transgressively overexpressed MEG, *Phlda2*, was recently shown to perturb maternal behaviour and neural gene expression when its dosage was altered in mouse placenta [8]. In particular, overexpression of placental *Phlda2* reduced postpartum nurturing behaviour [8], an effect of a single gene manipulation that is strikingly similar to early postpartum deficits in maternal behaviour in *Dom* mothers of hybrid litters [33]. Since *Phlda2* and *Ascl2* jointly regulate the development of the endocrine compartment [19], it is likely that transgressive misexpression of both genes in hybrid placenta impacts the maternal brain via effects on placental hormone expression.

(d) Paternal effects on the maternal brain

The hybrid placenta expresses both maternally derived (*Dom*) and paternally derived (*Spret*) alleles. Thus, females pregnant with hybrids are exposed to gene products from a foreign paternal genome. In the MPoA of *Dom* females exposed to hybrid placentas, we found a substantial subset of genes, including PSF genes and *Drd3*, with expression patterns that differed from *Dom* mothers with conspecific litters, but closely matched those of *Spret* mothers. A surprisingly large number of genes were co-expressed between placenta and MPoA in hybrid and *Spret* pregnancies but not in *Dom* pregnancies. In particular, placental and MPoA PSF gene expression were highly correlated in hybrid pregnancies and in *Spret* pregnancies, while *Dom* pregnancies showed a weaker correlation. Likewise, total MPoA PSF gene expression was *Spret*-like in hybrid pregnancies.

Together, these results suggest that PSF expression levels in the maternal MPoA are driven by placental expression levels of the same genes and that PSF, and potentially *Drd3*, expression in the MPoA is influenced by paternally inherited alleles in the placenta. Because PEGs are, by definition, expressed from the paternal allele, PEGs with *Spret*-like expression in the hybrid placenta are candidates for these paternal effects on maternal MPoA expression. For example, while *Igf2* is best known for promoting placental cell proliferation and invasion, it also influences placental endocrine function with downstream effects on maternal physiology [64].

Pregnancy requires substantial investment from the mother, which is offset by costs to her capacity to invest in future offspring [65]. However, when offspring are sired by multiple males, selection favours fathers who extract maximal maternal resources for their own offspring [65]. Haig and colleagues proposed that these asymmetries in the reproductive interests of males and females, and the coefficients of relatedness between mothers and offspring (always 0.5) versus fathers and offspring (0.5 or 0), should promote parental antagonism, played out at the molecular level between maternally and paternally expressed IGs in the placenta [20,66]. Because placental endocrine signals promote maternal investment in current offspring, placental hormones are also proposed players in both parental and mother–offspring conflicts [55,67]. Consistent with a history of antagonistic coevolution, PSFs, in general, are the fastest evolving genes in the rodent placenta [45]. We report a similar signature of selection on three PSF genes that are co-expressed in the hybrid placenta and the maternal MPoA.

Trivers [67] described placental hormones as the molecular equivalent of begging calls. Here, we show for the first time that the expression of Prls and other PSFs is highly correlated between the placenta and maternal brain. While the function of PSFs in the brain is undefined, placental genotype-dependent differences between *Dom* females in the strength of the correlation and the number of co-expressed genes indicate that the relationship is driven by the placenta not the mother. Moreover, the *Spret*-like co-expression patterns of PSF genes in mothers of litters sired by *Spret* males implicate the paternally inherited genome as the driver of these placental begging calls, which are echoed in the maternal brain.

5. Conclusion

Evolutionary theoreticians have modelled mammalian pregnancy as both intimate cooperation and antagonistic struggle between two genetically distinct organisms [21,65,68]. Whether driven by conflict or coadaptation, it is clear that the placenta is the mediator of these complex interactions between mother and offspring. Here, we concentrated on placental effects on the maternal brain during the final stages of pregnancy, when it is believed to be a critical source of signal molecules that prime female physiology and behaviour for motherhood [7,10]. We found both hybrid placental misexpression with the potential to disrupt maternal–fetal communication and altered expression in the brains of mothers exposed to hybrid placentas. Global expression in the hybrid placenta seems to be dominated by the maternally derived genome and/or driven by maternal effects. However, maternal–placental communication genes co-expressed in maternal brain and placenta show elevated evolutionary rates, consistent with antagonistic coevolutionary processes. The expression of a proportion of transcripts of these genes from a foreign paternal genome in the placenta has the potential to affect the maternal brain and explain postpartum effects on maternal behaviour [33]. In addition to the effects of placental disruption on the maternal brain, expression differences between the parental species in this hybrid system reveal an unanticipated influence of the placenta's paternal genome on the maternal brain. These paternal effects on the maternal brain could play a major role in the expression of maternal behaviour

and the quality of maternal care, and open novel avenues of research in both evolutionary and biomedical fields.

Ethics. All animal procedures were approved by the Oklahoma State University IACUC under protocol 141-AS.

Data accessibility. RNA-seq data from this study have been submitted to the NCBI Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession no. GSE126469.

Authors' contributions. P.C. conceived of the study. P.C. and L.A. designed the study. L.A. generated and analysed the data, and wrote the manuscript with input from P.C.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by the National Science Foundation (grant no. NSF-IOS714 1558109 to P.C).

Acknowledgements. We thank M. Nachman and members of the Campbell laboratory for useful discussion, B. Horn and S. Windle for assistance with mouse husbandry, and M. Nightengale for assistance in the laboratory. P.C. thanks K. Mack for generously sharing the ideas that catalysed this project and J. Good for the gift of the SFM/Pas strain. The manuscript was improved by the thoughtful comments of two anonymous reviewers and R. John.

References

1. Roberts RM, Green JA, Schulz LC. 2016 The evolution of the placenta. *Reproduction* **152**, R179–R189. (doi:10.1530/REP-16-0325)
2. Armstrong DL *et al.* 2017 The core transcriptome of mammalian placentas and the divergence of expression with placental shape. *Placenta* **57**, 71–78. (doi:10.1016/j.placenta.2017.04.015)
3. Cross JC, Werb Z, Fisher SJ. 1994 Implantation and the placenta: key pieces of the development puzzle. *Science* **266**, 1508–1518. (doi:10.1126/science.7985020)
4. Wagner GP, Kin K, Muglia L, Pavličev M. 2014 Evolution of mammalian pregnancy and the origin of the decidual stromal cell. *Int. J. Dev. Biol.* **58**, 117–126. (doi:10.1387/ijdb.130335gw)
5. Bridges RS, Robertson MC, Shiu RP, Friesen HG, Stuer AM, Mann PE. 1996 Endocrine communication between conceptus and mother: placental lactogen stimulation of maternal behavior. *Neuroendocrinology* **64**, 57–64. (doi:10.1159/000127098)
6. Boddy AM, Fortunato A, Wilson Sayres M, Aktipis A. 2015 Fetal microchimerism and maternal health: a review and evolutionary analysis of cooperation and conflict beyond the womb. *Bioessays* **37**, 1106–1118. (doi:10.1002/bies.201500059)
7. Bridges RS. 2015 Neuroendocrine regulation of maternal behavior. *Front. Neuroendocrinol.* **36**, 178–196. (doi:10.1016/j.yfrne.2014.11.007)
8. Creeth HD, McNamara GI, Tunster SJ, Boque-Sastre R, Allen B, Sumption L, Eddy JB, Isles AR, John RM. 2018 Maternal care boosted by paternal imprinting in mammals. *PLoS Biol.* **16**, e2006599. (doi:10.1371/journal.pbio.2006599)
9. Mann PE, Bridges RS. 2001 Lactogenic hormone regulation of maternal behavior. *Prog. Brain Res.* **133**, 251–262. (doi:10.1016/S0079-6123(01)33019-4)
10. Larsen CM, Grattan DR. 2012 Prolactin, neurogenesis, and maternal behaviors. *Brain Behav. Immun.* **26**, 201–209. (doi:10.1016/j.bbi.2011.07.233)
11. Kohl J, Dulac C. 2018 Neural control of parental behaviors. *Curr. Opin Neurobiol.* **49**, 116–122. (doi:10.1016/j.conb.2018.02.002)
12. Numan M, Insel TR. 2003 *The neurobiology of parental behavior*. New York, NY: Springer.
13. Simmons DG, Rawn S, Davies A, Hughes M, Cross JC. 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. (doi:10.1186/1471-2164-9-352)
14. Tunster SJ, Jensen AB, John RM. 2013 Imprinted genes in mouse placental development and the regulation of fetal energy stores. *Reproduction* **145**, R117–R137. (doi:10.1530/REP-12-0511)
15. Ferguson-Smith AC. 2011 Genomic imprinting: the emergence of an epigenetic paradigm. *Nat. Rev. Genet.* **12**, 565–575. (doi:10.1038/nrg3032)
16. Rawn SM, Cross JC. 2008 The evolution, regulation, and function of placenta-specific genes. *Annu. Rev. Cell Dev. Biol.* **24**, 159–181. (doi:10.1146/annurev.cellbio.24.110707.175418)
17. Zebhauser R, Kammerer R, Eisenried A, McLellan A, Moore T, Zimmermann W. 2005 Identification of a novel group of evolutionarily conserved members within the rapidly diverging murine Cea family. *Genomics* **86**, 566–580. (doi:10.1016/j.ygeno.2005.07.008)
18. Soares MJ, Konno T, Alam SMK 2007 The prolactin family: effectors of pregnancy-dependent adaptations. *Trends Endocrinol. Metab.* **18**, 114–121. (doi:10.1016/j.tem.2007.02.005)
19. Mason RW. 2008 Emerging functions of placental cathepsins. *Placenta* **29**, 385–390. (doi:10.1016/j.placenta.2008.02.006)
20. John RM. 2017 Imprinted genes and the regulation of placental endocrine function: pregnancy and beyond. *Placenta* **56**, 86–90. (doi:10.1016/j.placenta.2017.01.099)
21. Moore T, Haig D. 1991 Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet.* **7**, 45–49. (doi:10.1016/0168-9525(91)90040-W)
22. Wolf JB, Hager R. 2006 A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biol.* **4**, 2238–2243. (doi:10.1371/journal.pbio.0040380)
23. Keverne EB, Curley JP. 2008 Epigenetics, brain evolution and behaviour. *Front. Neuroendocrinol.* **29**, 398–412. (doi:10.1016/j.yfrne.2008.03.001)
24. Patten MM, Ross L, Curley JP, Queller DC, Bonduriansky R, Wolf JB. 2014 The evolution of genomic imprinting: theories, predictions and empirical tests. *Heredity* **113**, 119–128. (doi:10.1038/hdy.2014.29)
25. Janssen AB, Capron LE, O'Donnell K, Tunster SJ, Ramchandani PG, Heazell AE, Glover V, John RM. 2016 Maternal prenatal depression is associated with decreased placental expression of the imprinted gene PEG3. *Psychol. Med.* **46**, 2999–3011. (doi:10.1017/S0033291716001598)
26. McNamara GI, Creeth HD, Harrison DJ, Tansey KE, Andrews RM, Isles AR, John RM. 2018 Loss of offspring Peg3 reduces neonatal ultrasonic vocalizations and increases maternal anxiety in wild-type mothers. *Hum. Mol. Genet.* **27**, 440–450. (doi:10.1093/hmg/ddx412)
27. Vrana PB. 2007 Genomic imprinting as a mechanism of reproductive isolation in mammals. *J. Mammal.* **88**, 5–23. (doi:10.1644/06-MAMM-S-013R1.1)
28. Zechner U, Reule M, Orth A, Bonhomme F, Strack B, Guénet JL, Hameister H, Fundele R. 1996 An X-chromosome linked locus contributes to abnormal placental development in mouse interspecific hybrids. *Nat. Genet.* **12**, 398–403. (doi:10.1038/ng0496-398)
29. Vrana PB, Guan XJ, Ingram RS, Tilghman SM. 1998 Genomic imprinting is disrupted in interspecific Peromyscus hybrids. *Nat. Genet.* **20**, 362–365. (doi:10.1038/3833)
30. Brekke TD, Good JM. 2014 Parent-of-origin growth effects and the evolution of hybrid inviability in dwarf hamsters. *Evolution* **68**, 3134–3148. (doi:10.1111/evo.12500)
31. Kurz H, Zechner U, Orth A, Fundele R. 1999 Lack of correlation between placenta and offspring size in mouse interspecific crosses. *Anat. Embryol. (Berl.)* **200**, 335–343. (doi:10.1007/s004290050284)
32. Shi W, Krella A, Orth A, Yu Y, Fundele R. 2005 Widespread disruption of genomic imprinting in adult interspecies mouse. *Mus. hybrids. Genesis* **43**, 100–108. (doi:10.1002/gene.20161)
33. Gardner S, Grindstaff JL, Campbell P. 2019. Placental genotype affects early postpartum maternal behavior. *R. Soc. open sci.* **6**, 190732. (doi:10.1098/rsos.190732).
34. Qu D, McDonald A, Whiteley KJ, Bainbridge SA, Adamson SL. 2014 Layer-enriched tissue dissection of the mouse placenta in late gestation. In *The guide to investigation of mouse pregnancy* (eds B Anne Croy, A Yamada, F DeMayo, SL Adamson), pp. 529–535. Amsterdam, The Netherlands: Elsevier.
35. Paxinos G, Franklin K. 2013 *The mouse brain in stereotaxic coordinates*. Amsterdam, The Netherlands: Elsevier.

36. Krueger F, Andrews SR. 2016 SNPsplit: Allele-specific splitting of alignments between genomes with known SNP genotypes. *F1000Research* **5**, 1479. (doi:10.12688/f1000research.9037.1)
37. Keane TM *et al.* 2011 Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature* **477**, 289–294. (doi:10.1038/nature10413)
38. Kim D, Langmead B, Salzberg SL. 2015 HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods* **12**, 357–360. (doi:10.1038/nmeth.3317)
39. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009 The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079. (doi:10.1093/bioinformatics/btp352)
40. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. 2015 StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat. Biotechnol.* **33**, 290–295. (doi:10.1038/nbt.3122)
41. Bourgon R, Gentleman R, Huber W. 2010 Independent filtering is a generic approach that needs domain specific adaptation. *Proc. Natl Acad. Sci. USA* **107**, E175. (doi:10.1073/pnas.1011698107)
42. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, Thomas PD. 2017 PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Res.* **45**, D183–D189. (doi:10.1093/nar/gkw1138)
43. Yang Z. 2007 PAML 4: Phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* **24**, 1586–1591. (doi:10.1093/molbev/msm088)
44. Clark L, Wei M, Cattoretti G, Mendelsohn C, Tycko B. 2002 The Tnfrh1 (Tnfrsf23) gene is weakly imprinted in several organs and expressed at the trophoblast-decidua interface. *BMC Genet.* **3**, 37. (doi:10.1186/1471-2156-3-37)
45. Chuong EB, Tong W, Hoekstra HE. 2010 Maternal–fetal conflict: rapidly evolving proteins in the rodent placenta. *Mol. Biol. Evol.* **27**, 1221–1225. (doi:10.1093/molbev/msq034)
46. Janssen AB, Kertes DA, McNamara GI, Braithwaite EC, Creeth HDL, Glover VI, John RM. 2016 A role for the placenta in programming maternal mood and childhood behavioural disorders. *J. Neuroendocrinol.* **28**. (doi:10.1111/jne.12373)
47. Rahman A, Iqbal Z, Bunn J, Lovel H, Harrington R. 2004 The impact of maternal depression on infant nutritional status and illness—a cohort study. *Arch. Gen. Psychiatry* **61**, 946–952. (doi:10.1001/archpsyc.61.9.946)
48. Rai A, Cross JC. 2014 Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. *Dev. Biol.* **387**, 131–141. (doi:10.1016/j.ydbio.2014.01.015)
49. Takagi N, Sasaki M. 1975 Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. *Nature* **256**, 640–642. (doi:10.1038/256640a0)
50. Wolf JB, Wade MJ. 2009 What are maternal effects and what are they not? *Phil. Trans. R. Soc. B* **364**, 1107–1115. (doi:10.1098/rstb.2008.0238)
51. Wolf JB, Brodie ED. 1998 The coadaptation of parental and offspring characters. *Evolution* **52**, 299–308. (doi:10.1111/j.1558-5646.1998.tb01632.x)
52. Monk C, Spicer J, Champagne FA. 2012 Linking prenatal maternal adversity to developmental outcomes in infants: the role of epigenetic pathways. *Dev. Psychopathol.* **24**, 1361–1376. (doi:10.1017/S0954579412000764)
53. Tunster SJ, Creeth HD, John RM. 2016 The imprinted Phlda2 gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. *Dev. Biol.* **409**, 251–260. (doi:10.1016/j.ydbio.2015.10.015)
54. Tunster SJ, McNamara GI, Creeth HDJ, John RM. 2016*b* Increased dosage of the imprinted *Ascl2* gene restrains two key endocrine lineages of the mouse Placenta. *Dev. Biol.* **418**, 55–65. (doi:10.1016/j.ydbio.2016.08.014)
55. Haig D. 1996 Placental hormones, genomic imprinting, and maternal–fetal communication. *J. Evol. Biol.* **9**, 357–380. (doi:10.1046/j.1420-9101.1996.9030357.x)
56. Kobielak A, Pasolli HA, Fuchs E. 2004 Mammalian formin-1 participates in adherens junctions and polymerization of linear actin cables. *Nat. Cell Biol.* **6**, 21–30. (doi:10.1038/ncb1075)
57. Stamatovic SM, Keep RF, Andjelkovic AV. 2008 Brain endothelial cell-cell junctions: how to 'open' the blood brain barrier. *Curr. Neuropharmacol.* **6**, 179–192. (doi:10.2174/157015908785777210)
58. Schreurs MPH, Houston EM, May V, Cipolla MJ. 2012 The adaptation of the blood-brain barrier to vascular endothelial growth factor and placental growth factor during pregnancy. *FASEB J.* **26**, 355–362. (doi:10.1096/fj.11-191916)
59. Lattanzi L *et al.* 2002 Pramipexole in treatment-resistant depression: a 16-week naturalistic study. *Bipolar Disord.* **4**, 307–314. (doi:10.1034/j.1399-5618.2002.01171.x)
60. Moraga-Amaro R, Gonzalez H, Pacheco R, Stehberg J. 2014 Dopamine receptor D3 deficiency results in chronic depression and anxiety. *Behav. Brain Res.* **274**, 186–193. (doi:10.1016/j.bbr.2014.07.055)
61. Numan M, Stolzenberg DS. 2009 Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. *Front. Neuroendocrinol.* **30**, 46–64. (doi:10.1016/j.yfrne.2008.10.002)
62. Avraham Y, Hants Y, Vorobeiv L, Staum M, Ahmad WA, Mankuta D, Galun E, Arbel-Alon S. 2017 Brain neurotransmitters in an animal model with postpartum depressive-like behavior. *Behav. Brain Res.* **326**, 307–321. (doi:10.1016/j.bbr.2017.01.013)
63. Kim J *et al.* 2013 Peg3 mutational effects on reproduction and placenta-specific gene families. *PLoS ONE* **8**, e83359. (doi:10.1371/journal.pone.0083359)
64. Sferruzzi-Perri AN, Sandovici I, Constancia M, Fowden AL. 2017 Placental phenotype and the insulin-like growth factors: resource allocation to fetal growth. *J. Physiol.* **1**, 5057–5093. (doi:10.1113/JP273330)
65. Trivers RL. 1974 Parent–offspring conflict. *Integr. Comp. Biol.* **14**, 249–264.
66. Haig D. 2000 The kinship theory of genomic imprinting. *Annu. Rev. Ecol. Syst.* **31**, 9–32. (doi:10.1146/annurev.ecolsys.31.1.9)
67. Trivers RL. 1985 *Social evolution*. Menlo Park, CA: Benjamin-Cummings Pub Co.
68. Haig D. 1993 Genetic conflicts in human pregnancy. *Q. Rev. Biol.* **68**, 495–532. (doi:10.1086/418300)