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Haldane's rule in the placenta: Sex-biased misregulation of the *Kcnq1* imprinting cluster in hybrid mice

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Hybrid phenotypes that contribute to postzygotic reproductive isolation often exhibit pronounced asymmetry, both between reciprocal crosses and between the sexes in accordance with Haldane's rule. Inviability in mammalian hybrids is associated with parent-of-origin placental growth abnormalities for which misregulation of imprinted gene (IGs) is the leading candidate mechanism. However, direct evidence for the involvement of IGs in hybrid growth dysplasia is limited. We used transcriptome and reduced representation bisulfite sequencing to conduct the first genome-scale assessment of the contribution of IGs to parent-of-origin placental growth dysplasia in the cross between the house mouse (*Mus musculus domesticus*) and the Algerian mouse (*Mus spretus*). IGs with transgressive expression and methylation were concentrated in the *Kcnq1* cluster, which contains causal genes for prenatal growth abnormalities in mice and humans. Hypermethylation of the cluster's imprinting control region, and consequent misexpression of the genes *Phlda2* and *Ascl2*, is a strong candidate mechanism for transgressive placental undergrowth. Transgressive placental and gene regulatory phenotypes, including expression and methylation in the *Kcnq1* cluster, were more extreme in hybrid males. Although consistent with Haldane's rule, male-biased defects are unexpected in rodent placenta because the X-chromosome is effectively hemizygous in both sexes. In search of an explanation, we found evidence of leaky imprinted (paternal) X-chromosome inactivation in hybrid female placenta, an epigenetic disturbance that may buffer females from the effects of X-linked incompatibilities to which males are fully exposed. Sex differences in chromatin structure on the X and sex-biased maternal effects are nonmutually exclusive alternative explanations for adherence to Haldane's rule in hybrid placenta. The results of this study contribute to understanding the genetic basis of hybrid inviability in mammals, and the role of IGs in speciation.

KEY WORDS: ***Ascl2*, imprinted genes, *Mus*, *Phlda2*, postzygotic reproductive isolation, speciation.**

The evolution of intrinsic postzygotic reproductive isolation in taxa with genetic sex determination is characterized by asymmetries whose direction is remarkably consistent. When one sex of hybrids is sterile or inviable, it is the heterogametic sex (Haldane 1922), and the contribution of the X (or Z) chromosome to these hybrid defects is often large relative to that of the autosomes (Coyne and Orr 1989; Tao et al. 2003; Masly and Presgraves 2007; Presgraves 2018). The study of these asymmetries has revealed others: hybrid sterility typically evolves before inviability and depends on cross direction during the early stages of speciation (Coyne and Orr 2004; Turelli and Moyle 2007; Turissini et al. 2018). Across taxa, the genetic architecture of hybrid defects is largely consistent with the Dobzhansky-Muller model for the evolution of intrinsic incompatibilities, in which independently evolving loci interact negatively when combined in hybrids (Bateson 1909; Dobzhansky 1937; Muller 1942; Coyne and Orr 2004). Strikingly, loci that cause or contribute to Dobzhansky-Muller incompatibilities (DMIs) are often

implicated in antagonistic coevolution within species (Presgraves 2010; McDermott and Noor 2010; Crespi and Nosil 2013; Patten 2018; Zanders and Unckless 2019), itself a consequence of asymmetries in the fitness optima of interacting partners (e.g., selfish genetic elements and host genomes, males and females, parasites and hosts).

In mammals, hybrid inviability is associated with another pronounced asymmetry: growth abnormalities that depend on cross direction are documented in four mammalian orders with the most detailed genetic studies conducted in rodents (Gray 1972; Zechner et al. 1996; Vrana et al. 1998; Brekke and Good 2014; Brekke et al. 2016). For example, the cross between a female oldfield mouse (*Peromyscus polionotus*) and a male deer mouse (*P. maniculatus*) produces hybrid conceptuses (the collective term for the embryo and the placenta) that are grossly over-sized and rarely survive to term. Offspring from the reciprocal cross are viable but significantly smaller than both parental species (Rogers and Dawson 1970; Vrana et al. 1998). Likewise, in the cross between the house mouse, *Mus musculus domesticus*, and the Algerian mouse, *M. spretus*, hybrid placentas are oversized (hyperplastic) when the paternal species is *M. m. domesticus* and undersized (hypoplastic) when *M. m. domesticus* is the maternal species and *M. spretus* is the father (Zechner et al. 1996). Similar patterns of hybrid over- and under-growth result from reciprocal crosses in dwarf hamsters (*Phodopus*) (Brekke and Good 2014).

Imprinted genes (IGs), a small but developmentally critical group of autosomal genes, are a priori candidates for these hybrid growth asymmetries. IGs are highly expressed in placenta and brain, and a subset are essential regulators of placental and embryonic growth (Ferguson-Smith 2011; Barlow and Bartolomei 2014). IG inheritance is Mendelian but expression is not. Paternally expressed IGs (Pegs) are expressed predominantly or exclusively from the paternally inherited allele. Maternally expressed IGs (Megs) have the opposite expression pattern. Thus, like the X chromosome in heterogametic males, IGs are effectively hemizygous, making them strong candidate contributors to DMIs in hybrids of both sexes (Vrana et al. 2000; Vrana 2007; Wolf et al. 2014).

The dominant hypothesis for the evolution of imprinted expression is based on parental conflict over maternal investment due to relatedness asymmetries between mothers and offspring (always 0.5) and fathers and offspring (0.5 or 0) in polyandrous mating systems (Moore and Haig 1991; Haig 2000). This adds to the appeal of IGs as speciation genes; hybrid inviability as a by-product of antagonistic coevolution within species would be consistent with the proposition that conflict is a common driver of reproductive isolation (Presgraves 2010; Crespi and Nosil 2013; Patten 2018).

The strongest empirical support for the role of IGs in speciation comes from the *Peromyscus* cross, in which severe hybrid conceptus overgrowth is accompanied by widespread loss of imprinting (biallelic expression) (Vrana et al. 2000). A mapping study identified the IG, *Peg3*, as a strong candidate for the autosomal locus in an X-autosome DMI for hybrid growth hyperplasia (Loschiavo et al. 2007). A similarly large effect of IG misexpression on hybrid overgrowth seems likely in *Phodopus* hybrids, in which loss of imprinting results in underexpression of growth restricting Megs (Brekke et al. 2016). In *Mus* hybrids, several IGs have biallelic expression and altered methylation in some adult tissues (Shi et al. 2005), but candidate gene studies did not establish a strong connection between IGs and placental growth phenotypes (Zechner et al. 2002; Zechner et al. 2004). Indeed, the genetic architecture of hybrid growth dysplasia in *Mus* exhibits the same fundamental asymmetries that characterize intrinsic postzygotic reproductive isolation in general: hybrid male placental phenotypes are more extreme than those of hybrid females and the effect of X chromosome genotype is larger in males (Zechner et al. 1996; Hemberger et al. 1999; Hemberger et al. 2001). Because the paternally inherited X chromosome is normally imprinted (silenced) in female mouse placenta (Tagaki and Sasaki 1975), both sexes are exposed to recessive-acting DMIs on the X. Adherence to Haldane's rule in the placenta is therefore unexpected.

We conducted the first genome-scale analysis of this long-standing example of parent-of-origin-dependent hybrid growth asymmetries. The data we present are from the cross direction in which hybrid placentas are undersized relative to the maternal species, *M. m. domesticus* (Zechner et al. 1996). We first compared sex-specific placental weight and histology between hybrids and both *M. m. domesticus* and *M. spretus*, and tested for elevated rates of inviability in hybrid litters. These analyses were essential to confirming that apparent abnormalities in hybrids cannot be explained by species differences in placental size and structure, and that hybrid males are more affected than females; data on embryonic viability in this cross are reported here for the first time. We then used placental transcriptome and reduced representation bisulfite sequencing to characterize genome-wide patterns of differential expression (DE) and methylation in hybrids relative to parental species, and to address three main questions. (1) Does sex-specific expression and methylation conform to Haldane's rule? Specifically, are there more genes with transgressive expression and/or methylation in hybrid males versus females? Here and throughout, we use the term transgressive to describe hybrid gene regulatory phenotypes that are outside of the range of both parental species (e.g., Barreto et al. 2014) and are therefore candidate effectors of placental growth and structural abnormalities. (2) Are IGs strong candidates for hybrid

placental abnormalities? We enumerated transgressively expressed and/or methylated IGs, and measured allele-specific expression in hybrids to test for evidence of loss of imprinting. We also used gene network analysis to test for functional associations between all transgressively expressed genes and IGs that retain strong allelic expression bias in hybrid placenta. (3) Are there sex differences in X-linked expression that could underlie the more extreme placental phenotype in hybrid males? We addressed this question in two ways. First, we tested the expectation that transgressive expression on the X chromosome would be male biased. Second, to evaluate the possibility that leaky imprinted X chromosome inactivation (XCI) buffers female hybrids from X-linked incompatibilities that are exposed in males, we identified X-linked genes with significant expression from the paternal allele and tested for functional associations between these XCI-escape genes and genes with transgressive expression unique to males. Placental transcriptome data were included in Arévalo and Campbell (2020) but sex differences in expression and analyses of allele specific expression are reported here for the first time. All other data and analyses are new to this study.

Methods

ANIMALS, TISSUE COLLECTION, AND PHENOTYPIC ANALYSES

Mice used in this study were maintained on a 12:12 light:dark cycle with lights on at 0900h and were provided with 5001 Rodent Diet (LabDiet, Brentwood, MO, USA) and water ad lib. All animal procedures were approved by the IACUC under protocol #141-AS. *Mus musculus domesticus* (hereafter, *Dom*) was represented by the wild-derived inbred strain WSB/EiJ (Jackson Laboratory) and *M. spretus* (hereafter, *Spret*) was represented by the wild-derived inbred strain SFM/Pas (Montpellier Wild Mice Genetic Repository). We conducted three crosses (female listed first): *Dom* × *Dom*, *Dom* × *Spret*, and *Spret* × *Spret*. The reciprocal cross (*Spret* × *Dom*) was attempted 25 times but was never successful. Although early-acting postzygotic incompatibilities likely contribute to previously reported low success rates for the *Spret* × *Dom* cross (e.g., Zechner et al. 1996), we think that the aggressive behavior of WSB/EiJ males paired with SFM/Pas females was a factor in our zero success rate.

Prior to pairing, females were placed in a cage with soiled conspecific male bedding for ∼48 h to induce receptivity to mating (Whitten 1956). Mice were paired between 1700h and 1800h, left undisturbed for two nights, and split on the morning of the second day. The second night was counted as embryonic day 0 (e0). Pregnant females ($n = 5$/cross) were euthanized by cervical dislocation between 1000h and 1100h on embryonic day 17-18 (e17.5). Placentas were separated from embryos, and the maternally derived decidual layer was removed as described in Qu et al. (2014). Embryos and placentas were weighed and transferred immediately to RNAlater (Thermo Fisher, USA), kept at 4°C overnight to allow RNAlater perfusion, and stored at −20°C until RNA extraction. Inviable conceptuses, when present, were also stored in RNAlater until DNA extraction for sexing. Because litter size can affect placenta and embryo size (Ishikawa et al. 2006), we used the number of viable conceptuses in each litter as a covariate in analyses of placental and embryonic weight. Inviable conceptuses were not counted because all were at a late stage of resorption (see Results) and therefore unlikely to affect the size of viable conceptuses in late gestation. Effects of litter genotype on litter size and on the relative number of inviable conceptuses were tested with a one-way ANOVA and with a generalized linear model, respectively.

Placentas used for histology were fresh frozen and stored at −80°C until cryosectioning. Frozen placenta was cryosectioned at 20 μm on a Leica CM 1950 cryostat and mounted on Fisher Superfrost Plus slides. Mounted sections were immediately stained with hematoxylin and eosin using the Thermo Scientific Shandon Rapid-Chrome H & E Frozen Section Staining Kit (Thermo Fisher). Stained sections were imaged at 10× with a Leica DM IL LED microscope, equipped with a QICAM Fast 1394 camera with QCapture software.

SAMPLE PREPARATION AND SEQUENCING

Embryo sex was determined by PCR for the Y-linked gene, *Zfy1*. Total RNA was extracted from one male and one female placenta per litter ($n = 5$ males/cross; $n = 4$ females [hybrid cross] and $n = 5$ females [conspecific cross]) using the AllPrep RNA/DNA Mini Kit (Qiagen), and was stored at −80°C until sequencing. Illumina library preparation was done at the sequencing facility (Novogene, Sacramento, CA) using the NEB Next Ultra RNA library prep kit for Illumina. Individual libraries were sequenced on the Illumina HiSeq 4000 platform, producing >40 million 150 bp paired-end reads per sample.

TRANSCRIPTOME DATA PROCESSING AND ANALYSIS

Quality control (QC), trimming of raw sequencing reads, filtering, and mapping were performed as described in Arévalo and Campbell (2020). To improve comparability, all samples (*Dom*, *Spret*, and hybrid) were mapped to a pseudo-hybrid genome, generated using the genome preparation tool of the program SNPsplit (Krueger and Andrews 2016). Briefly, SNPs from both *Dom* (WSB/EiJ) and *Spret* (SPRET/EiJ) relative to the mouse genome (GRCm38.89) available from the Ensembl FTP server (http://ftp.ensembl.org) were introduced into the mouse genome. SNPs between *Dom* and *Spret* were then N-masked to allow mapping of both *Dom*- and *Spret*-derived reads. We randomly downsampled all alignment files to the same number of reads

(∼40 million reads) using SAMtools 0.1.19 (Li et al. 2009). For analysis of allele specific expression (ASE) in hybrids, we used SNPsplit to split the hybrid alignment files, separating reads originating from the *Spret* and *Dom* alleles. Reads that were unassignable (did not overlap SNPs between WSB/EiJ and SPRET/EiJ) or contained conflicting or noninformative (polymorphic) SNPs were removed. On average, 69% of mapped RNAseq reads were assignable, 26% unassignable, and 5% conflicting.

Transcript quantification and annotation was done using StringTie 1.3.3 (Pertea et al. 2015). Mouse genome annotation information (GRCm38.89) was retrieved from the Ensembl FTP server (http://ftp.ensembl.org). We used the python script (preDE.py) included in the StringTie package to prepare gene-level count matrices for analysis of DE and ASE in DESeq2 1.16.1 (Love et al. 2014). Pseudogenes were removed from the count matrices based on "biotype" annotation information extracted from Biomart (R-package biomaRt; Durinck et al. 2005). Low counts were removed by the independent filtering process implemented in DESeq2 (Bourgon et al. 2010). The adjusted *P*-value (Benjamini-Hochberg method) cutoff was set at $\leq$0.05. Following the recommendations of Love et al. (2014) (see also Vallejos et al. 2016; Schurch et al. 2016), we chose a log2 fold change (LFC) cutoff of $\geq$0.5 (1.41 times higher or lower expression) for standard DE analysis. Gene expression in hybrids that was significantly higher or lower than both parental species was defined as transgressive.

We evaluated genome-wide ASE in hybrid placentas by testing for DE between alleles. We considered genes with LFC $\geq$ 1 (two times higher or lower expression from one allele compared to the other) and Benjamini-Hochberg-corrected $P \leq 0.05$ as showing significant ASE. We chose this stricter LFC cutoff for ASE to separate ASE from slightly allele-biased expression. In tests for loss of imprinting and biallelic X-chromosomal expression, genes with LFC ≤ 0.3 and Benjamini-Hochberg-corrected $P \geq 0.1$ were considered biallelically expressed. To avoid false positives in the test for loss of imprinting, only the subset of genes with robust evidence for imprinting in e17.5 mouse placenta ($n = 36$; Babak et al. 2015) were considered.

REDUCED REPRESENTATION BISULFITE SEQUENCING DATA PROCESSING AND ANALYSIS

We used genome-wide reduced representation bisulfite sequencing (RRBS) to identify differentially methylated regions in CpG islands. CpG islands are found in most mammalian gene promoters and their methylation status is one of the major mechanisms of transcriptional control in mammalian genomes. In general, the methylation levels of gene promoters correlate negatively with gene expression level (Cross and Bird 1995; Newell-Price et al. 2000). CpG enriching restriction digest, bisulfite treatment, size selection, library preparation, and sequencing were performed by the sequencing facility (NXT-Dx, Ghent, Belgium). Sequencing was performed on the Illumina HiSeq 4000 platform, producing >13 million 50 bp paired-end reads per sample.

QC of raw sequencing reads and trimming were performed in Trim Galore! 0.4.5 (Babraham Bioinformatics, http://www.bioinformatics.babraham.ac.uk/projects/trim_galore), using a Phred score cutoff of 20 and minimum sequence length of 20 after trimming. The pseudo-hybrid genome (described above) was bisulfite converted and indexed for mapping using the Bismark-genome-preparation tool. The RRBS reads were mapped to the converted pseudo-hybrid genome using the Bismark Bisulfite Aligner (Babraham Bioinformatics, http://www.bioinformatics.babraham.ac.uk/projects/bismark/). We filtered the resulting alignment files using SAMtools 0.1.19 (Li et al. 2009), retaining only high-quality (MAPQ score 40), uniquely mapped, paired reads for analysis. We randomly downsampled all alignment files to the same number of reads (∼10 million reads) using SAMtools 0.1.19 (Li et al. 2009). To analyze allele specific methylation (ASM) in hybrids, we split the hybrid alignment files with SNPsplit as described above for RNAseq alignments, but using the bisulfite option of the program. In this mode, SNPs involving C to T transitions are not considered for allele-specific sorting because these positions can either represent a SNP or a methylation state. On average, 53% of mapped reads were assignable to one of the two alleles, 45% were unassignable, and 1.25% conflicting.

CpG methylation information was extracted from the alignment files using the Bismark-methylation-extractor tool (Babraham Bioinformatics). Differential methylation (DM) and ASM were analyzed in SeqMonk (Babraham Bioinformatics, http://www.bioinformatics.babraham.ac.uk/projects/seqmonk/). CpG methylation percentage in CpG islands was calculated as per base percent methylation, averaged over the whole CpG island. DM and ASM were determined for CpG islands located in gene promoter regions using logistic regression with Benjamini-Hochberg correction. Log2 fold change between compared samples was calculated manually and cutoff was set to $\geq$0.5 for standard DM analysis and to $\geq$1 for ASM analysis. CpG islands in and around IGs, including imprinting control regions (ICRs), were tested for DM and ASM in the same manner.

GENE ONTOLOGY TERM AND PATHWAY OVERREPRESENTATION ANALYSIS

We performed gene ontology (GO) term and pathway overrepresentation analyses on relevant lists of genes from DE, ASE, and DM analyses using the PANTHER gene list analysis tool with Fisher's exact test and FDR correction (Mi et al. 2017). We tested for overrepresentation based on the GO annotation database (Biological Processes) (released 7 Jan 2017; Ashburner et al.

Table 1. Litter size and viability.

	n litters (embryos)[a]	Litters with inviable embryos	Total inviable (sex)	Mean proportion viable embryos (SD)	Mean litter size[b] (SD)
Dom × *Dom*	9 (47)	1	2 (f)	0.96 (0.13)	5.0 (1.1)
Spret × *Spret*	10 (53)	1	1 (m)	0.98 (0.05)	5.2 (1.6)
Dom × *Spret*	7 (41)	5	2 (f), 6 (m)	0.80 (0.16)	4.6 (1.0)

[a] All embryos, viable and inviable.
[b] Viable embryos only.

2000; The Gene Ontology Consortium 2017) and the Reactome pathway database (version 58; Fabregat et al. 2017).

IMPRINTED AND X-LINKED GENE NETWORK ANALYSES

We used String (version 11; Szklarczyk et al. 2019) to construct gene networks for sets of genes that are candidate effectors of hybrid placental abnormalities in one or both sexes: placental IGs that retain statistically significant ASE in hybrids and X-linked genes with significant expression from the paternal allele in hybrid females. Parameters for string were set to a maximum of 20 interactor genes in the first shell and a maximum of five interactor genes in the second shell, with a minimum required interaction score of 0.4. Results from text-mining, databases, experiments, and gene fusions were considered. We tested for overrepresentation of (1) genes with transgressive expression in either or both sexes in the IG gene networks and (2) genes with male-only transgressive expression in the X-linked gene networks.

Results

REDUCED VIABILITY IN HYBRID LITTERS

There was a significant effect of litter genotype on embryonic viability in late gestation that was due to a lower viability in hybrid litters (generalized linear model (GLM) [binominal] [$n = 26$]: Hybrid-*Dom*: $F = 1.85$, $P = 0.02$; Hybrid-*Spret*: $F = 2.68$, $P = 0.01$) (Table 1). Inviable conceptuses were consistently dark colored and highly condensed with no discernable distinction between embryonic and extraembryonic tissues, phenotypes indicative of late stage of resorption at e17.5 and thus early lethality (Pang et al. 2014). Litter size, counting only viable embryos, was slightly smaller for hybrid relative to *Dom* and *Spret* litters but the effect of genotype was not significant (one-way ANOVA: $F(2) = 0.51$, $P = 0.61$) (Table 1). There were numerically more inviable hybrid males ($n = 6$) than females ($n = 2$) but, for the five hybrid litters in which all embryos were sexed, within-litter sex ratios for viable embryos were also male biased (mean proportion males = 0.57 ± 0.27 SD).

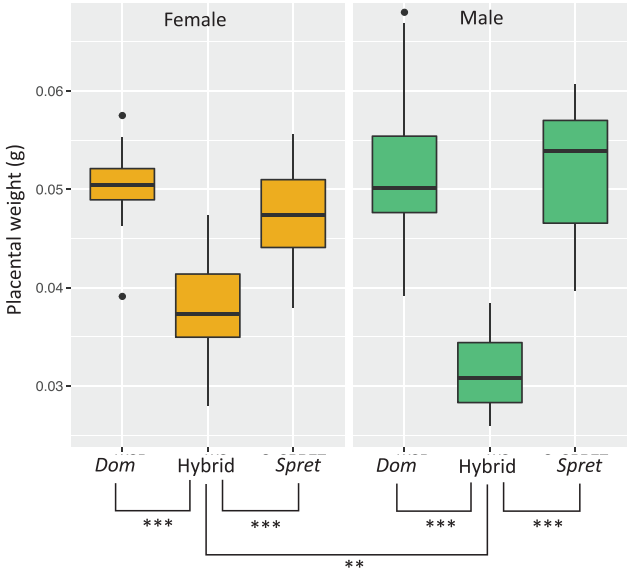

Figure 1. Sex-specific placental weight in hybrids and parental species, *Mus m. domesticus* (*Dom*) and *M. spretus* (*Spret*). Boxplots show placental weight for females (gold) and males (green). Asterisks indicate significance of one-way ANCOVA followed by Tukey HSD post hoc tests (**$P < 0.01$; ***$P < 0.001$). Sample sizes—females: *Dom* $n = 14$, Hybrid $n = 10$, *Spret* $n = 12$; males: *Dom* $n = 12$, Hybrid $n = 13$, *Spret* $n = 15$.

HYBRID PLACENTAL UNDERGROWTH IS MORE EXTREME IN MALES

Prior work on the *Dom* × *Spret* cross reported a hypoplastic placenta in F_1 hybrids relative to the maternal species (*Dom*) but did not test for hypoplasia relative to *Spret* (Zechner et al. 1996). We found that hybrid male placentas weighed significantly less than male placentas of both parental species with no significant differences between parental species (one-way ANCOVAs with post hoc Tukey tests [$n = 40$]: $F(2) = 48.25$, $P < 0.001$; Hybrid-*Dom* [$n = 25$], $P < 0.001$; Hybrid-*Spret* [$n = 28$], $P < 0.001$; *Dom*-*Spret* [$n = 27$], $P = 0.97$). The same was true for females (one-way ANCOVAs with post hoc Tukey tests [$n = 36$]: $F(2) = 20.17$, $P < 0.001$; Hybrid-*Dom* ($n = 24$), $P < 0.001$; Hybrid-*Spret* ($n = 22$), $P < 0.001$; *Dom*-*Spret* ($n = 26$), $P = 0.47$) (Fig. 1). When testing within genotype, hybrid male placentas weighed

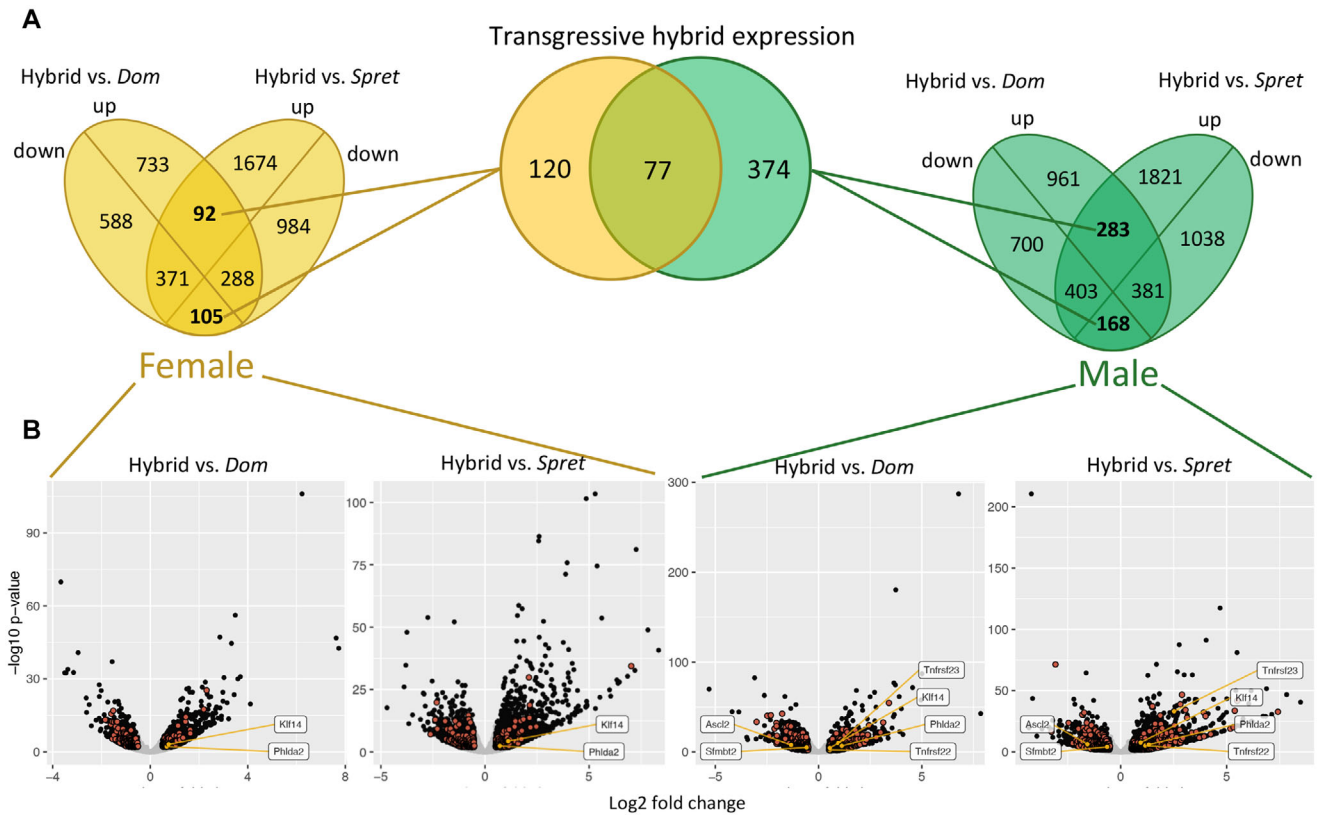

Figure 2. **Differential expression (DE) in female (gold) and male (green) hybrid placenta relative to *Mus m. domesticus* (*Dom*) and *M. spretus* (*Spret*). (A) The outer Venn diagrams show the overlap of DE genes between Hybrid vs. *Dom* and Hybrid vs. *Spret*; up = genes expressed higher and down = genes expressed lower in hybrids compared to parental species; transgressive expression is indicated in bold. The central Venn diagram shows the overlap of transgressively expressed genes between female and male hybrids. (B) Volcano plots of DE in Hybrid vs. *Dom* and Hybrid vs. *Spret* placentas for females and males. Significantly DE genes (FDR ≤ 0.05 and log2 fold change ≥ 0.5) are shown in black and transgressively expressed genes are shown in red; transgressively expressed IGs (orange) are labeled.**

significantly less than hybrid female placentas ($F(1) = 9.98$, $P = 0.004$, $n = 23$) (Fig. 1). There was no significant difference between sexes for *Dom* or *Spret*, and embryo weights did not differ significantly in any comparison (data not shown).

Placental hypoplasia in this cross was proposed to be due in part to a reduced spongiotrophoblast layer (Zechner et al. 1996), which lies at the maternal-fetal interface in the junctional zone (John 2013). While we did not differentiate placental cell types, placental histology confirmed that the junctional zone of the hybrid placenta was qualitatively narrower relative to that of both parental species (Fig. S1).

GENOME-WIDE TRANSGRESSIVE EXPRESSION AND METHYLATION IS MALE-BIASED IN HYBRID PLACENTA

Having found more extreme placental hypoplasia in male hybrids, we asked whether expression and methylation differences between hybrids and parental species were also greater in males. Consistent with prior analysis of DE in hybrid placenta (Arévalo and Campbell 2020), we found high levels of differential expression in both sexes of hybrids with >1800 DE genes in each comparison (Fig. 2; see Dataset S1 for complete numerical summaries and gene lists). Of the DE genes that met our criteria for transgressive expression, there were more than twice as many in hybrid males versus females (Fig. 2A), a difference that was highly significant (Fisher's exact test: $P < 0.001$, odds ratio = 2.36). Transgressively upregulated genes were highly enriched for GO terms related to developmental processes in both sexes, with enrichment for immune-related terms unique to males. Transgressively downregulated genes were enriched for prolactin receptor signaling in both sexes with additional enrichment in females (Dataset S1).

Patterns of transgressive methylation were similar, albeit less extreme. This is consistent with a previous study that showed that placental promoter and CpG island methylation is conserved between *M. m. musculus*, *M. m. domesticus*, and *M. spretus* (Decato et al. 2017). Although there was no genome-wide signal of transgressive methylation in either sex of hybrids (Fig. 3A; Dataset S3), there were significantly more transgressively methylated gene promoters in males than in females (Fisher's exact test:

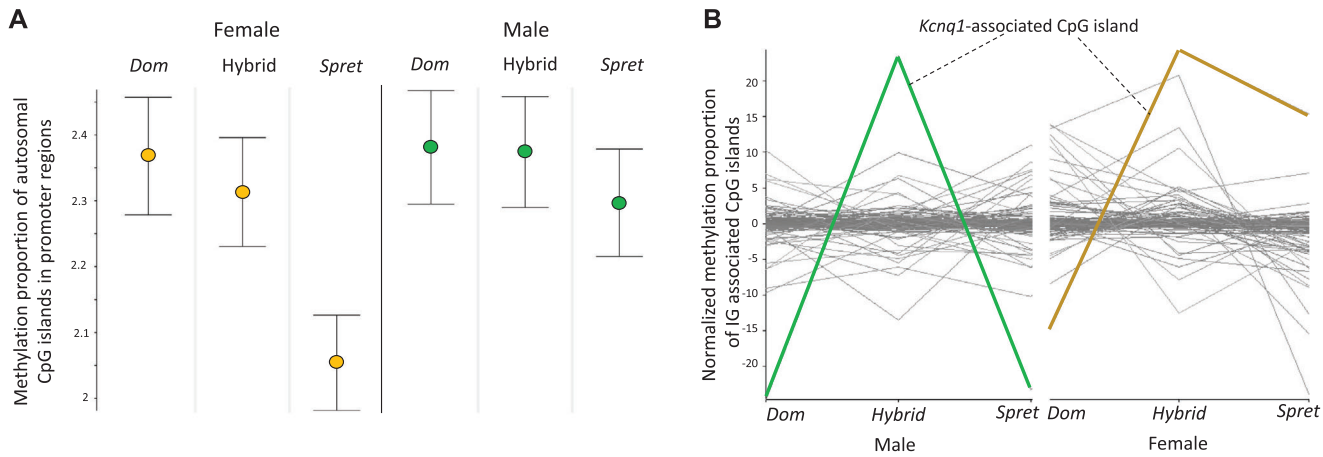

Figure 3. Methylation patterns of CpG islands in gene promoters and the *Kcnq1* imprinting control region (ICR). Male data depicted in green and female data in gold throughout the figure. (A) The mean proportions of methylated reads for CpG islands in autosomal promoter regions. (B) Line plots show the normalized proportions of methylated reads for all IG promoter and ICR-associated CpG islands, where each line represents a CpG island. The CpG island is indicated. Error bars in panel A, standard error.

Table 2. Differential expression of imprinted genes in male and female hybrids compared to both parental species.

			Hybrid vs. *Dom*		Hybrid vs. *Spret*				
	Gene symbol	Chr./Clust.	LFC	*P*-adj	LFC	*P*-adj	Expression in hybrid	Expressed allele	Associated DMR?
Male placenta	*Ascl2*	dist7-IC2	−1.33	0.00	−1.56	0.00	Transgressive down	Maternal	Yes
	Klf14	6	0.54	0.00	0.94	0.00	Transgressive up	Maternal	
	Phlda2	dist7-IC2	1.12	0.00	1.32	0.00	Transgressive up	Maternal	Yes
	Sfmbt2	2	−0.58	0.00	−0.64	0.00	Transgressive down	Paternal	
	Tnfrsf22	dist7-IC2	0.54	0.02	1.16	0.00	Transgressive up	Maternal	Yes
	Tnfrsf23	dist7-IC2	0.95	0.00	2.51	0.00	Transgressive up	Maternal	Yes
	Tspan32	dist7-IC2	−0.76	0.02	1.37	0.00	DE intermediate	Unknown	Yes
Female placenta	*Klf14*	6	0.75	0.01	1.11	0.00	Transgressive up	Maternal	
	Phlda2	dist7-IC2	0.67	0.04	0.71	0.02	Transgressive up	Maternal	Yes
	Th	7	0.95	0.04	−1.58	0.00	DE intermediate	Maternal	Yes

Chr. = chromosome; Clust. = imprinting cluster (https://www.mousebook.org [March 22, 2018]); LFC = log2 fold change in expression; *P*-adj = adjusted *P*-value (Benjamini-Hochberg); DE = differential expression, expressed allele according to https://www.mousebook.org; DMR = differentially methylated region.

$P = 0.011$, odds ratio = 1.96; Table S1). Transgressively hypermethylated genes were enriched for GO terms related to development in hybrid females only (Dataset S3).

LOCAL, NOT GLOBAL, MISREGULATION OF IGs IN HYBRID PLACENTA

We found that a localized subset of placental IGs was transgressively expressed in hybrids. As for autosomal genes in general, transgressive expression and methylation was male biased. In hybrid male placentas, four IGs (*Tnfrsf22*, *Tnfrsf23*, *Phlda2*, and *Klf14*) were transgressively upregulated and two (*Ascl2* and *Sfmbt2*) were transgressively downregulated. *Tspan32* was significantly DE compared to both parental species but intermediate between the two. Five of these misexpressed IGs belong to the *Kcnq1* imprinting cluster (IC2) on the distal part of mouse chromosome 7 (dist7) and are normally maternally expressed (Table 2; Dataset S1). Notably, *Phlda2* is a regulator of placental development that acts downstream of transcription factor, *Ascl2* (Tunster et al. 2016a, Tunster et al. 2016b; Oh-McGinnis et al. 2011). In hybrid female placentas, only two IGs (*Phlda2* and *Klf14*) were transgressively upregulated and *Th* (also in dist7-IC2) was significantly DE and intermediate between the parental species (Table 2; Dataset S1).

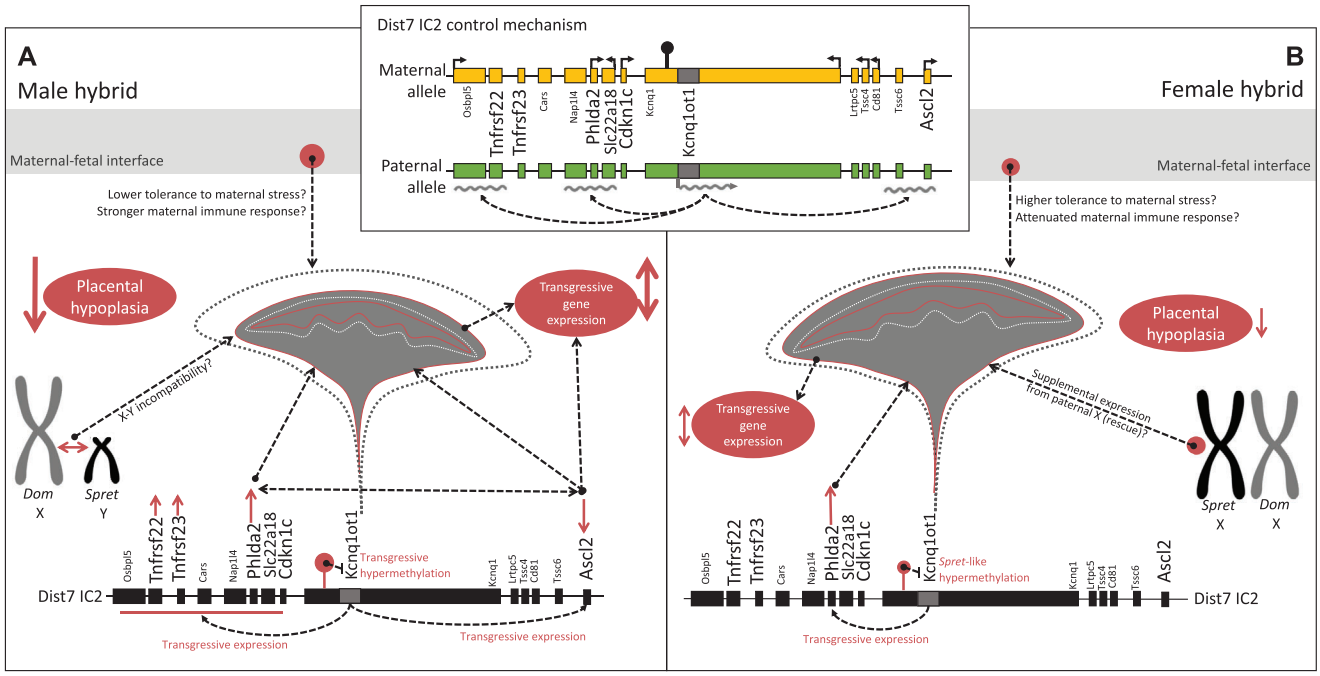

Figure 4. **Summary of results and proposed effects on (A) male and (B) female hybrid placentas. Aberrant phenotype, expression, methylation, or interactions are red. In the cartoons of the distal chromosome 7 imprinting cluster 2 (Dist7-IC2), transgressive/*Spret*-like methylation of *Kcnq1*-ICR (imprinting control region) is marked with a red circle. Transgressively expressed placental genes are marked with arrows indicating the direction of misexpression. Dashed arrows indicate the proposed effect on placental phenotype and expression patterns. When applicable, the proposed effect is labeled on the arrow. The inset shows a schematic of the proposed *Kcnq1ot1*-mediated imprinting control mechanism of the cluster (modified from Sanli and Feil 2015). Maternal methylation of the ICR inhibits the expression of lncRNA *Kcnq1ot1* from the maternal allele. *Kcnq1ot1* expressed from the paternal allele (wavy gray lines) represses paternal allelic expression in the cluster.**

IC2 is regulated in part by the paternally expressed long noncoding (lnc) RNA gene, *Kcnq1ot1*, which acts to repress the paternal alleles of the Megs in this cluster (Autuoro et al. 2014; Sanli and Feil 2015; Fig 4 inset). *Kcnq1ot1* was significantly downregulated in hybrid males compared to *Dom* males only. In addition, four IG-associated CpG islands were transgressively methylated in males: *Igf2r*, *Phlda2*, *Trmp5*, and *Kcnq1*. The latter three are located in the dist7-IC2 and include the CpG island in *Kcnq1* intron 10, the cluster's imprinting control region (ICR2) (Lee et al. 1999) (Table 3). ICR2, which is normally maternally methylated (Zhang et al. 2014), was strongly hypermethylated in hybrid males and, to a lesser extent, in hybrid females compared to all other IG promoter and ICR-associated CpGs for which methylation was measured (Fig. 3B). Together, these patterns of expression and methylation implicate the *Kcnq1* imprinting cluster as a candidate locus for the male-biased placental abnormalities in this direction of the *Dom*/*Spret* cross.

We also found transgressive hypermethylation associated with the IG, *Peg12*, in hybrid females (Table 3). However, like *Igf2r* in males, the gene was not transgressively or differentially expressed. Only three IGs met our criteria for loss of imprinting in hybrid placenta: *Copg2* and *Gnas* in both sexes, and *Zdbf2* in females only (Fig. S4; see Dataset S2 for complete numerical summaries and gene lists for ASE). None of these genes were transgressively expressed (Dataset S1).

Given that most placental IGs exhibited significant ASE in hybrid placenta (Fig. S4), we used gene network analysis to test for evidence of functional interactions between these effectively hemizygous genes and genes with transgressive expression in hybrids. Transgressively expressed genes were not significantly overrepresented among IG interactor genes ($n = 553$) extracted from the String database (Szklarczyk et al. 2019) (Fisher's exact test: Males: $P = 0.277$, odds ratio $= 0.542$; Females: $P = 0.412$, odds ratio $= 1.3$) (Dataset S2). We note that this coarse-grained analysis does not rule out negative epistatic interactions involving one or a subset of these IGs. In fact, 123 of 571 genes with transgressive expression in one or both sexes of hybrids are among the putative targets of the transcription factor, *Ascl2* (Casey et al. 2018).

TRANSGRESSIVE EXPRESSION AND PARTIAL LOSS OF IMPRINTING ON THE X CHROMOSOME

In contrast to the genome-wide sex differences in transgressive expression, transgressively expressed genes were enriched

Table 3. Transgressive imprinted gene-associated CpG island methylation in male and female hybrids.

			% Reads methylated in CpG island						
	Chr.	Closest IG	*Dom*	Hybrid	*Spret*	LFC hybrid vs. *Dom*	LFC hybrid vs. *Spret*	FDR hybrid vs. *Dom*	FDR hybrid vs. *Spret*
Male placenta	**17**	***Igf2r***	**40.33**	**27.35**	**45.07**	**–0.56**	**–0.72**	**0.015**	**0.001**
	7	***Phlda2***	**5.68**	**3.22**	**5.41**	**–0.82**	**–0.75**	**<0.001**	**0.011**
	7	***Trpm5***	**18.07**	**10.09**	**24.28**	**–0.84**	**–1.27**	**<0.001**	**<0.001**
	7	***Kcnq1***	**21.40**	**69.62**	**22.78**	**1.70**	**1.61**	**<0.001**	**<0.001**
Female placenta	6	*Peg10*	52.79	28.23	38.15	–0.90	–0.43	<0.001	0.001
	12	*Begain*	48.25	33.72	42.68	–0.52	–0.34	<0.001	<0.001
	7	***Peg12***	**5.70**	**8.91**	**4.78**	**0.64**	**0.90**	**<0.001**	**<0.001**
	17	*Igf2r*	53.46	36.33	42.35	–0.56	–0.22	<0.001	<0.001
	7	*Kcnq1*	30.98	70.44	61.44	1.18	0.20	0.017	0.002

Chr. = Chromosome; IG = imprinted gene; LFC = log2 fold change in expression. Transgressively methylated CpG islands in bold; FDR = false discovery rate (Benjamini-Hochberg).

on the X chromosome in both sexes of hybrids (Fisher's exact test: Males: $P = 0.005$, odds ratio = 1.79; Females: $P = 0.001$, odds ratio = 2.40; Fig. S2; Dataset S1). Although there was more transgressive X-linked expression in males (29 genes) versus females (17 genes), enrichment relative to the autosomes was higher in females (Fig. S2; Dataset S1). There was no GO term enrichment among transgressively expressed X chromosome genes in either sex.

In males, mean methylation levels on the *Dom* X chromosome were higher in hybrid relative to *Dom* placentas (6.99 vs. 6.19) and the mean LFC in methylation was significantly higher on the X versus the autosomes (mean $LFC_X = 0.36$, mean $LFC_{Autosomes} = 0.10$; one-sample *t*-test: $t(172) = -2.17$, $P = 0.03$). However, 62.1% (44/71) of the DE X-linked genes in the Hybrid-*Dom* comparison were upregulated in hybrid males, a pattern counter to the generally repressive effects of promoter methylation on gene expression. As expected for imprinted X inactivation, the mean methylation level of the maternal X chromosome in female hybrids was lower compared to the paternal X ($X_{dom} = 12.03$, $X_{spret} = 28.92$) (Dataset S3).

In hybrid female placenta, 578 X-linked genes contained informative SNPs for analysis of ASE. Consistent with imprinted X inactivation and the high level of methylation on the paternal X, X chromosome-wide allelic expression was highly skewed toward the *Dom* allele (Mean: 0.783, Skewness: −1.449, one-sample *t*-test: $t(486) = 26.477$, $P < 0.001$) (Fig. S3; Dataset S2). Despite this global pattern, only 65.1% (376/578) of these X-linked genes had significantly higher expression from the *Dom* allele. After excluding the 47 genes known to escape X inactivation in normal mouse placenta (Andergassen et al. 2017), 29 genes met our cutoff criteria (see Methods and Dataset S2). Of these, 11 had biallelic expression, whereas 18 had significantly higher expression from the paternal *Spret* allele (Table S2). As for IGs with biallelic expression, none of the X-linked genes with biallelic or paternally biased expression were transgressively expressed in females.

Genes with transgressive expression unique to hybrid males were not enriched among interactor genes ($n = 1037$) for the X-linked genes with significant expression from the paternal allele in hybrid females (Fisher's exact test: $P = 0.12$, odds ratio = 0.67) (Dataset S2). Thus, there was no evidence that leaky imprinted X inactivation buffers females from X-linked incompatibilities that underlie the genome-wide excess of transgressive expression in males. However, *Smarca1*, an X-linked gene with biallelic expression in hybrid females, was transgressively overexpressed in hybrid males (Table S2; Dataset S2).

Discussion

IGs are critical regulators of prenatal growth and development in mammals (Ferguson-Smith 2011). This function, together with characteristic features of regulation and expression, makes IGs compelling candidates for asymmetric growth abnormalities in mammalian hybrids. We used transcriptome and reduced representation bisulfite sequencing to evaluate the contribution of IGs to a long-standing example of parent-of-origin placental undergrowth in hybrid mice. Consistent with Haldane's rule, we found more extreme undergrowth and more transgressive expression and methylation in male versus female hybrid placentas. Transgressive expression and methylation of IGs was concentrated in

the *Kcnq1* cluster, which includes strong candidate loci for hybrid placental growth dysplasia. Although transgressive expression was moderately enriched on the X chromosome in both sexes of hybrids, we found an excess of genes that escape imprinted XCI in hybrid females. We discuss these results in light of prior work on this and other cases of hybrid growth dysplasia, and address the perplexing question of why hybrid placental phenotypes are more extreme in the heterogametic sex.

PLACENTAL DEFECTS AND INVIABILITY IN HYBRID MICE

The first description of hybrid developmental abnormalities in the cross between female *M. m. domesticus* and male *M. spretus* (*Dom* × *Spret*) reported placental undergrowth relative to *M. m. domesticus* (Zechner et al. 1996) but did not make comparisons to the smaller parental species, *M. spretus*. Here, we show that hybrid placental weight is significantly reduced relative to both parental species, with more extreme undergrowth in males. Placental histology is also consistent with the original report, in which the spongiotrophoblast layer (the placenta's endocrine compartment) was reduced in hybrid relative to *M. m. domesticus* placentas (Zechner et al. 1996). We find that the junctional zone, of which the spongiotrophoblast layer is the main component (Coan et al. 2006; John 2013), is qualitatively narrower in hybrid placenta relative to both parental species. Notably, we find a moderate but statistically significant reduction in the viability of hybrid conceptuses, providing the first quantitative evidence that developmental abnormalities contribute to postzygotic reproductive isolation in this direction of the cross.

THE CONTRIBUTION OF IGs TO HYBRID PLACENTAL GROWTH DYSPLASIA

Prior analysis of allelic expression and DNA methylation for 18 candidate IGs in various adult F_1 hybrid tissues found patterns suggestive of partial loss of imprinting in both directions of the *M. m. domesticus*/*M. spretus* cross (Shi et al. 2005). In the placenta, however, tests for the contribution of two paternally expressed IGs to hybrid growth dysplasia were inconclusive (Zechner et al. 2002; Zechner et al. 2004). Excess Igf2 protein was observed in overgrown placentas but the overgrowth phenotype persisted in the absence of functional Igf2 (Zechner et al. 2002); *Peg3* retained normal expression from the paternal allele in hybrid placenta and the locus was not associated with placental abnormalities in a backcross mapping panel (Zechner et al. 2004).

Using whole transcriptome data, we show that the typical parent-of-origin expression patterns of most placental IGs are retained in *Dom* × *Spret* F_1 hybrids. Moreover, the expression of three IGs with evidence of biallelic expression in hybrid placenta is within the range of both parental species. Thus, loss of imprinting and associated altered dosage of IGs cannot explain transgressive placental undergrowth in *Dom* × *Spret* hybrids. These results are consistent with work in *Peromyscus* and *Phodopus* hybrids, in which loss of imprinting is only evident in overgrown placentas (Wiley et al. 2008; Brekke et al. 2016). Similarly, we did not find widespread transgressive expression of IGs, nor did functional associations with canonically expressed (i.e., monoallelic) IGs explain a significant proportion of all transgressive expression. Instead, transgressively expressed IGs were concentrated in the *Kcnq1* cluster on distal chromosome 7 in association with hypermethylation of the cluster's imprinting control region, ICR2, and downregulation of the regulatory lncRNA, *Kcnq1ot1*, in males (Fig. 4). In humans, loss of methylation on ICR2, and consequent upregulation of *KCNQ1OT1* and repression of maternally expressed genes in the *KCNQ1* cluster, is a common cause of Beckwith-Wiedemann syndrome, an imprinting disorder characterized by fetal and placental overgrowth (Lee et al. 1999; Cooper et al. 2017). This association between ICR2 hypomethylation and conceptus overgrowth implicates ICR2 hypermethylation as an effector of the placental undergrowth phenotype studied here.

Importantly, functional studies in lab mice indicate that misexpression of just two maternally expressed genes in the *Kcnq1* cluster, *Phlda2* (overexpressed in hybrids of both sexes) and *Ascl2* (underexpressed in males), could underlie placental undergrowth in hybrids, and the more extreme male phenotype. Overexpression of *Phlda2* in transgenic mice results in both fetal and placental growth restriction, with placentas characterized by a reduced junctional zone and deficits in trophoblast glycogen cells (Tunster et al. 2016a), a putative fetal energy source in late gestation (Coan et al. 2006). Although *Ascl2* (formerly *Mash2*) knockouts die in midgestation (e10) due to complete failure of spongiotrophoblast layer formation (Guillemot et al. 1994; Tanaka et al. 1999), underexpression of *Ascl2* causes nonlethal placental undergrowth characterized by reduced spongiotrophoblast and a lack of trophoblast glycogen cells together with an expanded giant cell layer and disorganization of vasculature critical to fetomaternal exchange (Oh-McGinnis et al. 2011). This placental phenotype bears histological resemblance to that in *Dom* × *Spret* hybrids (Fig. S1; Zechner et al. 1996). Notably, reduction in *Ascl2* expression in the transgenic model also causes upregulation of *Phlda2*, suggesting an upstream regulatory effect of *Ascl2* on *Phlda2* (Oh-McGinnis et al. 2011).

Collectively, these and other functional studies of *Phlda2* and *Ascl2* indicate that the essential role of *Ascl2* in placental development includes modulation of the suppressive effects of *Phlda2* on spongiotrophoblast expansion (Tunster et al. 2016b). Application of this model to placental undergrowth in hybrids suggests that moderate *Phlda2* overexpression is sufficient to cause observed placental growth restriction in females, whereas the addition of *Ascl2* underexpression in males increases the severity of the growth phenotype in association with more

extreme upregulation of *Phlda2* (Fig. 4). Given the availability of transgenic mouse models for both *Ascl2* and *Phlda2* (e.g., Tunster et al. 2016a,b; Creeth et al. 2018), it may be possible to test the proposed effects of altered dosage on hybrid placenta using crosses between transgenic females and unmanipulated *M. spretus* males.

Interestingly, in the cross between female *Peromyscus polionotus* and male *P. maniculatus*, *Kcnq1* is one of three major IG clusters that are dysregulated in overgrown placentas (Duselis and Vrana 2007; Wiley et al. 2008). In particular, *Phlda2* is downregulated (Duselis and Vrana 2007) in association with loss of methylation on ICR2 (Wiley et al. 2008). Given that opposite patterns of altered methylation at this locus are associated with opposite placental growth dysplasia phenotypes in hybrids from distantly related rodent genera, we predict that hypomethylation of *Kcnq1* ICR2 will emerge as a major determinant of hybrid placental overgrowth in the reciprocal *Spret* × *Dom* cross. More generally, the association between altered methylation on *Kcnq1* ICR2 and prenatal growth dysplasia in both rodent hybrids and humans suggests that, whether due to intrinsic incompatibilities in hybrids or de novo epimutations, the locus is particularly vulnerable to epigenetic perturbation.

HALDANE'S RULE AND THE LARGE X EFFECT IN HYBRID PLACENTA

In agreement with prior studies of the *M. m. domesticus*/*M. spretus* cross (e.g., Zechner et al. 1996; Hemberger et al. 2001), we found that hybrid placental phenotypes obey Haldane's rule: *Dom* × *Spret* hybrid male placentas are significantly smaller than those of their already growth-restricted sisters. Moreover, there were more than twice as many transgressively expressed genes in hybrid males and, even within the *Kcnq1* cluster, transgressive expression and methylation were greater in hybrid males. Importantly, although male-limited underexpression of *Ascl2* may be the direct cause of the more extreme male growth phenotype, and may account for some of the excess transgressive expression in males, sex-specific dysregulation of autosomal genes requires additional explanation.

Of the genetic hypotheses for Haldane's rule, all that apply to hybrid inviability involve large negative effects of X- (or Z-) linked incompatibilities in the heterogametic sex, with the predominant explanation being that most DMI loci act as recessives or partial recessives in hybrids and are therefore uniquely exposed on the X chromosome in the heterogametic sex (Turelli and Orr 1995; Orr and Turelli 1996; Coyne and Orr 2004; Delph and Demuth 2016). This "large X effect" (Coyne and Orr 1989) is further increased if DMIs accumulate preferentially on the X chromosome relative to the autosomes (Charlesworth et al. 1987), as is the case for loci involved in hybrid male sterility in both *Drosophila* and mice (Masly and Presgraves 2007; Good et al. 2008; Larson et al. 2017).

Consistent with a large X effect on hybrid placental phenotypes in the *M. m. domesticus*/*M. spretus* cross, backcross mapping studies found a large contribution of the X chromosome to placental growth dysplasia, with undergrowth associated with *M. m. domesticus* genotypes and overgrowth with *M. spretus* genotypes (Zechner et al. 1996; Hemberger et al. 1999, Hemberger et al. 2001). However, because imprinted XCI in normal (i.e., non-hybrid) rodent placenta results in functional hemizygosity for the maternal X chromosome in both sexes, adherence to Haldane's rule in this tissue is unexpected. We consider four potential explanations: XCI escape in females, X-Y interactions in males, *trans*-acting effects of altered chromatin structure on the X chromosome in males, and sexually dimorphic maternal effects.

First, partial dysregulation of imprinted XCI, resulting in biallelic expression of some X-linked genes, could buffer females from the effects of X-linked incompatibilities that are fully exposed in males. This explanation for hybrid male-biased placental growth dysplasia was proposed in the original description of the phenotype (Zechner et al. 1996) but later dismissed when three X-linked markers showed the normal pattern of maternal expression/paternal silencing in hybrid female placenta (Hemberger et al. 2001). We found that, although allelic expression for the majority of X-linked genes retains strong maternal bias, 29 genes that are not known to escape XCI in mouse placenta met our criteria for having biallelic or paternally biased expression in hybrid female placenta. None of these genes are obvious candidates for direct effects on placental growth and development. Moreover, genes with transgressive expression in male placenta were not globally enriched among functional interactors for the X-linked genes with significant expression from the paternal allele in hybrid females. However, among the genes with biallelic expression in females, *Smarca1* is a candidate for regulatory effects on hybrid males. *Smarca1* encodes a chromatin remodeling enzyme that suppresses cell proliferation and attenuates Wnt-signaling (Eckey et al. 2012), processes crucial for embryogenesis and placental development (Parr et al. 2001; Logan and Nusse 2004). Members of the Smarca gene family promote insulator protein CTCF binding with downstream effects on CTCF-regulated genes (Wiechens et al. 2016), which include several IG clusters (Lléres et al. 2019). Given that *Smarca1* was transgressively overexpressed in hybrid male placenta, the potential contribution of this gene to the difference between hybrid male and female phenotypes is worthy of further study.

Second, the opportunity for negative epistatic interactions between the X and Y chromosomes is unique to hybrid males. In the absence of support for alternative explanations, Hemberger and colleagues proposed that X-Y interactions account for adherence to Haldane's rule in the *M. m. domesticus*/*M. spretus* cross

(Hemberger et al. 2001). The current data do not address this hypothesis; a test would require generation of Y-introgression lines for crosses in which X and Y chromosomes derive from the same parent species in hybrid males. However, we think it is unlikely to explain male-biased placental deficits. Although negative X-Y interactions are important in a handful of cases of Haldane's rule for hybrid male sterility (Coyne et al. 2004; Mishra and Singh 2007; Cocquet et al. 2012), there are, to our knowledge, no published examples of this genetic architecture underlying preferential inviability of the heterogametic sex in animals. In *Dom* × *Spret* hybrids, transgressive placental phenotypes are in the same direction in both sexes, albeit more extreme in males, and X chromosome genotype has a large effect on growth dysplasia in both sexes (Zechner et al. 1996; Hemberger et al. 1999, Hemberger et al. 2001). Therefore, a male-specific genetic architecture for which there is minimal precedent in other systems does not provide a parsimonious explanation for the observed data.

Third, sex differences in chromatin structure on the X could have *trans*-acting effects on autosomal expression that differ between male and female hybrids (Hemberger et al. 1999). Sex chromosome complement explains sex differences in autosomal gene expression that are independent of gonadal sex and associated hormonal differences between mammalian males and females (Bermejo-Alvarez et al. 2010; Raznahan et al. 2018). Moreover, the parental origin of the single X chromosome in humans with Turner syndrome (45,X) impacts prenatal survival and the severity of postnatal phenotypes, indicating parent-of-origin epigenetic effects (Sagi et al. 2007; Grande et al. 2019). Circumstantial evidence supporting the hypothesis that aberrant epigenetic effects of the X chromosome contribute to male-biased placental growth dysplasia includes our finding of elevated mean LFC in methylation levels on the *M. m. domesticus* X chromosome in hybrid versus *Dom* males and the fact that, in crosses using subcongenic mice with intervals of the *M. spretus* X on an *M. m. domesticus* autosomal background, the degree of placental overgrowth increases with the size, rather than the location, of the *M. spretus*-derived interval (Hemberger et al. 1999).

Fourth, we would expect male-biased sensitivity to any negative effect of a hybrid pregnancy on the maternal intrauterine environment. Maternal stress during pregnancy, whether physiological or psychological, negatively impacts both sexes (Davis and Pfaff 2014). However, the immediate consequences of an adverse intrauterine environment are generally more severe in males, with more pronounced placental pathology and intrauterine growth restriction, and a higher rate of mortality for male fetuses (Cooperstock and Campbell 1996; Walker et al. 2012; Sandman et al. 2013; Davis and Pfaff 2014). Female *M. m. domesticus* carrying hybrid litters are potentially exposed to altered placental endocrine signaling due to deficits in spongiotrophoblast cells in hybrid placentas (Zechner et al. 1996), and exhibit altered neural gene expression at e17.5 (Arévalo and Campbell 2020) and reduced maternal behavior immediately postpartum (Gardner et al. 2019). These observations suggest that hybrid pregnancy disrupts maternal homeostasis and, in this sense, is a physiological stressor. Moreover, maternal immunotolerance of the conceptus is mediated by the placenta and maternal immune response to male fetuses may be greater than that to female fetuses (Kahn and Baltimore 2010; Bogaert et al. 2018). Maternal immune response to males could be elevated by heterospecific Y-linked gene expression in placenta in hybrid pregnancies.

We emphasize that none of the mechanisms proposed here to explain Haldane's rule in hybrid placenta are mutually exclusive. For example, intrinsic negative effects of hybrid male sex chromosome complement on autosomal gene expression, whether epigenetic or genetic, could be amplified by male-biased sensitivity to an adverse maternal environment and/or reduced maternal immunotolerance of hybrid male conceptuses (Fig. 4). Finally, it is noteworthy that 18 X-linked genes have effectively hemizygous expression from the paternal (*M. spretus*) allele in hybrid female placenta. Given the large contribution of the *M. spretus* X chromosome to overgrowth phenotypes in the reciprocal cross (Zechner et al. 1996; Hemberger et al. 1999, Hemberger et al. 2001), it is possible that one or more of these paternally expressed genes are involved in a growth-promoting DMI, resulting in less extreme placental undergrowth in hybrid females relative to males.

Conclusions

The results presented here provide new insight into the genetic basis of hybrid developmental abnormalities and add to prior evidence that placental IGs play a key role in growth dysplasia and associated inviability in mammalian hybrids (Vrana et al. 2000; Brekke et al. 2016). In particular, we find hypermethylation of the *Kcnq1* ICR2 in hybrid placenta, in conjunction with transgressive expression of several maternally expressed IGs in this cluster. Importantly, the direction of *Phlda2* and *Ascl2* misexpression, and of placental growth phenotypes, parallels that in transgenic mouse models for the same genes. This suggests that epigenetic dysregulation of few IGs can have large negative effects on hybrid placental development. Whether misregulation of the *Kcnq1* cluster contributes to the extreme overgrowth phenotypes in the reciprocal direction of the cross remains to be determined.

Given that there is also a large X effect on placental abnormalities in the *M. m. domesticus*/*M. spretus* cross (Zechner et al. 1996; Hemberger et al. 1999), it is tempting to infer a simple genetic architecture in which IGs are the autosomal interaction partners in X-linked incompatibilities. However, transgressive expression of *Phlda2* and *Ascl2* is likely a direct consequence of excess methylation on the cluster's imprinting control region.

Whether this local epigenetic perturbation is a consequence of X-linked or other genic incompatibilities, or a byproduct of epigenetic instability in hybrid genomes, is currently unclear. Genome-wide QTL and eQTL mapping in both directions of the *M. m. domesticus*/*M. spretus* cross will be a critical first step in reconciling the large role of the X, adherence to Haldane's rule, and the contribution of IGs to hybrid placental growth dysplasia.

AUTHOR CONTRIBUTIONS

PC conceived and designed the study with input from LA. SG collected histological data. LA collected and analyzed all other data. PC and LA wrote the article with input from SG.

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DATA ARCHIVING

RNA-seq data from this study are available in the NCBI Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE126469. RRBS data are available under accession number GSE161051.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

LITERATURE CITED

Arévalo, L., and P. Campbell. 2020. Placental effects on the maternal brain revealed by disrupted placental gene expression in mouse hybrids. Proc. R. Soc. B 287:20192563.

Andergassen, D., C. P. Dotter, D. Wenzel, V. Sigl, P. C. Bammer, M. Muckenhuber, D. Mayer, T. M. Kulinski, H. C. Theussl, J. M. Penninger, et al. 2017. Mapping the mouse allelome reveals tissue-specific regulation of allelic expression. Elife 6:e25125.

Ashburner, M., C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, et al. 2000. Gene ontology: tool for the unification of biology. Nat. Genet. 25:25–29.

Autuoro, J. M., S. P. Pirnie, and G. G. Carmichael, 2014. Long noncoding RNAs in imprinting and X chromosome inactivation. Biomolecules 4:76-100.

Babak, T., B. DeVeale, E. K. Tsang, Y. Zhou, X. Li, K. S. Smith, K. R. Kukurba, R. Zhang, J. B. Li, D. van der Kooy, et al. 2015. Genetic conflict reflected in tissue-specific maps of genomic imprinting human and mouse. Nat. Genet. 47:544–549.

Barlow, D. P., and M. S. Bartolomei. 2014. Genomic imprinting in mammals. Cold Spring Harb. Perspect. Biol. 6:a018382.

Barreto, F. S., R. J. Pereira, and R. S. Burton. 2014. Hybrid dysfunction and physiological compensation in gene expression. Mol. Biol. Evol. 32:613–622.

Bateson, W. 1909. Heredity and variation in modern lights. Pp. 85–101 in A. C. Seward, ed. Darwin and modern science. Cambridge Univ. Press, Cambridge, U.K.

Bermejo-Alvarez, P., D. Rizos, D. Rath, P. Lonergan, and A. Gutierrez-Adan. 2010. Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. Proc. Natl. Acad. Sci. USA 107:3394–3399.

Bogaert, A. F., M. N. Skorska, C. Wang, J. Gabrie, A. J. MacNeil, M. R. Hoffarth, D. P. Vanderlaan, K. J. Zucker, and R. Blanchard. 2018. Male homosexuality and maternal immune responsivity to the Y-linked protein NLGN4Y. Proc. Natl. Acad. Sci. USA 115:302–306.

Bourgon, R., R. Gentleman, and W. Huber. 2010. Independent filtering is a generic approach that needs domain specific adaptation. Proc. Natl. Acad. Sci. 107:E175–E175.

Brekke, T. D., and J. M. Good. 2014. Parent-of-origin growth effects and the evolution of hybrid inviability in dwarf hamsters. Evolution 68:3134–3148.

Brekke, T. D., L. A. Henry, and J. M. Good. 2016. Genomic imprinting, disrupted placental expression, and speciation. Evolution 70:2690–2703.

Casey, B. H., R. K. Kollipara, K. Pozo, and J. E. Johnson. 2018. Intrinsic DNA binding properties demonstrated for lineage-specifying basic helix-loop-helix transcription factors. Genome Res. 28:484–496.

Charlesworth, B., J. A. Coyne, and N. Barton. 1987. The relative rates of evolution of sex chromosomes. Am. Nat. 130:113–146.

Coan, P. M., N. Conroy, G. J. Burton, and A. C. Ferguson-Smith. 2006. Origin and characteristics of glycogen cells in the developing murine placenta. Dev. Dyn. 235:3280–3294.

Cocquet, J., P. J. Ellis, S. K. Mahadevaiah, N. A. Affara, D. Vaiman, and P. S. Burgoyne. 2012. A genetic basis for a postmeiotic X versus Y chromosome intragenomic conflict in the mouse. PLos Genet. 8:e1002900.

Cooper, W. M., A. Luharia, G. A. Evans, H. Raza, A. C. Haire, R. Grundy, S. C. Bowdin, A. Riccio, G. Sebastio, J. Bliek, et al. 2017. Molecular subtypes and phenotypic expression of Beckwith-Wiedemann syndrome. Eur. J. Hum. Genet. 25:S85–S90.

Cooperstock, M., and J. Campbell. 1996. Excess males in preterm birth: interactions with gestational age, race, and multiple birth. Obstet. Gynecol. 88:189–193.

Coyne, J. A., and H. A. Orr. 1989. Two rules of speciation. Pp. 180–207 in D. Otte and J. Endler, eds. Speciation and its consequences. Sinauer Associates, Sunderland, MA.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Coyne, J. A., S. Elwyn, S. Y. Kim, and A. Llopart. 2004. Genetic studies of two sister species in the *Drosophila melanogaster* subgroup, *D. yakuba* and *D. Santomea*. Genet. Res. 84:11–26.

Creeth, H. D. J., G. I. McNamara, S. J. Tunster, R. Boque-Sastre, B. Allen, L. Sumption, J. B. Eddy, A. R. Isles, and R. M. John. 2018. Maternal care boosted by paternal imprinting in mammals. PLoS Biol. 16:e2006599.

Crespi, B., and P. Nosil. 2013. Conflictual speciation: species formation via genomic conflict. Trends Ecol. Evol. 28:48–55.

Cross, S. H., and A. P. Bird. 1995. CpG islands and genes. Curr. Opin. Genet. Dev. 5:309-314.

Davis, E. P., and D. Pfaff. 2014. Sexually dimorphic responses to early adversity: implications for affective problems and autism spectrum disorder. Psychoneuroendocrinology 49:11–25.

Decato, B. E., J. Lopez-Tello, A. N. Sferruzzi-Perri, A. D. Smith, and M. D. Dean. 2017. DNA methylation divergence and tissue specialization in the developing mouse placenta. Mol. Biol. Evol. 34:1702-1712.

Delph, L. F., and J. P. Demuth. 2016. Haldane's rule: genetic bases and their empirical support. J. Hered. 107:383–391.

Dobzhansky, T. 1937. Genetics and the origin of species. Columbia Univ. Press, New York, NY.

Durinck, S., Y. Moreau, A. Kasprzyk, S. Davis, B. De Moor, A. Brazma, and W. Huber. 2005. BioMart and Bioconductor: a powerful link between

biological databases and microarray data analysis. Bioinformatics 21:3439–3440.

Duselis, A. R., and P. B. Vrana. 2007. Assessment and disease comparisons of hybrid developmental defects. Hum. Mol. Genet. 16:808–819.

Eckey, M., S. Kuphal, T. Straub, P. Rümmele, E. Kremmer, A. K. Bosserhoff, and P. B. Becker. 2012. Nucleosome remodeler SNF2L suppresses cell proliferation and migration and attenuates Wnt signaling. Mol. Cell. Biol. 32:2359–2371.

Fabregat, A., K. Sidiropoulos, G. Viteri, O. Forner, P. Marin-Garcia, V. Arnau, P. D'Eustachio, L. Stein, and H. Hermjakob. 2017. Reactome pathway analysis: a high-performance in-memory approach. BMC Bioinformatics 18:142.

Ferguson-Smith, A. C. 2011. Genomic imprinting: the emergence of an epigenetic paradigm. Nat. Rev. Genet. 12:565–575.

Fountain, M. D., H. Tao, C. A. Chen, J. Yin, and C. P. Schaaf. 2017. Magel2 knockout mice manifest altered social phenotypes and a deficit in preference for social novelty. Genes Brain Behav. 16:592-600.

Gardner, S., J. L. Grindstaff, and P. Campbell. 2019. Placental genotype affects early postpartum maternal behaviour. R. Soc. Open Sci. 6:190732.

Good, J. M., M. D. Dean, and M. W. Nachman. 2008. A complex genetic basis to X-linked hybrid male sterility between two species of house mice. Genetics 179:2213–2228.

Grande, M., I. Stergiotou, M. Pauta, B. Marques, C. Badenas, A. Soler, Y. Yarn, and A. Borrell. 2019. Parental origin of the retained X chromosome in monosomy X miscarriages and ongoing pregnancies. Fetal Diagn. Ther. 45:118–124.

Gray, A. P. 1972. Mammalian hybrids. 2nd ed. Commonwealth Agricultural Bureau, Slough, U.K.

Guillemot, F., A. Nagy, A. Auerbach, J. Rossant, and A. L. Joyner. 1994. Essential role of Mash-2 in extraembryonic development. Nature 371:333–336.

Haig, D. 2000. The kinship theory of genomic imprinting. Annu. Rev. Ecol. Syst. 31:9–32.

Haldane, J. B. S. 1922. Sex ratio and unisexual sterility in animal hybrids. J. Genet. 12:101–109.

Hemberger, M. C., R. S. Pearsall, U. Zechner, A. Orth, S. Otto, F. Rüschendorf, R. Fundele, and R. Elliott. 1999. Genetic dissection of X-linked interspecific hybrid placental dysplasia in congenic mouse strains. Genetics 153:383–390.

Hemberger, M., H. Kurz, A. Orth, S. Otto, A. Lüttges, R. Elliott, A. Nagy, S.-S. Tan, P. Tam, U. Zechner, et al. 2001. Genetic and developmental analysis of X-inactivation in interspecific hybrid mice suggests a role for the Y chromosome in placental dysplasia. Genetics 157:341–348.

Ishikawa, H., R. Seki, S. Yokonishi, T. Yamauchi, and K. Yokoyama. 2006. Relationship between fetal weight, placental growth and litter size in mice from mid-to late-gestation. Reprod. Toxicol. 3:267-270.

John, R. M. 2013. Epigenetic regulation of placental endocrine lineages and complications of pregnancy. Biochem. Soc. Trans. 41:701–709.

Kahn, D. A., and D. Baltimore. 2010. Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. Proc. Natl. Acad. Sci. USA 107:9299–9304.

Krueger, F., and S. R. Andrews. 2016. SNPsplit: allele-specific splitting of alignments between genomes with known SNP genotypes. F1000Res 5:1479.

Larson, E. L., S. Keeble, D. Vanderpool, M. D. Dean, and J. M. Good. 2017. The composite regulatory basis of the large X-effect in mouse speciation. Mol. Biol. Evol. 34:282–295.

Lee, M. P., M. R. DeBaun, K. Mitsuya, H. L. Galonek, S. Brandenburg, M. Oshimura, and A. P. Feinberg. 1999. Loss of imprinting of a paternally expressed transcript, with antisense orientation to KVLQT1, occurs frequently in Beckwith-Wiedemann syndrome and is independent of insulin-like growth factor II imprinting. Proc. Natl. Acad. Sci. USA 96:5203–5208.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079.

Lléres, D., B. Moindrot, R. Pathak, V. Piras, M. Matelot, B. Pignard, A. Marchand, M. Poncelet, A. Perrin, V. Tellier, et al. 2019. CTCT modulates allele-specific sub-TAD organization and imprinted gene activity at the mouse *Dlk1-Dio3* and *Igf2-H19* domains. Genome Biol. 20:272.

Logan, C. Y., and R. Nusse. 2004. The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. 20:781–810.

Loschiavo, M., Q. K. Nguyen, A. R. Duselis, and P. B. Vrana. 2007. Mapping and identification of candidate loci responsible for *Peromyscus* hybrid overgrowth. Mamm. Genome 18:75–85.

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:1–21.

Masly, J. P., and D. C. Presgraves. 2007. High-resolution genome-wide dissection of the two rules of speciation in Drosophila. PLoS Biol. 5: e243.

McDermott, S. R., and M. A. Noor. 2010. The role of meiotic drive in hybrid male sterility. Philos. Trans. R Soc. Lond. B 365:1265–1272.

Mi, H., X. Huang, A. Muruganujan, H. Tang, C. Mills, D. Kang, and P. D. Thomas. 2017. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res. 45:D183–D189.

Mishra, P. N., and B. N. Singh. 2007. Assessing the putative roles of X-autosome and X-Y interactions in hybrid male sterility of the Drosophila bipectinata species complex. Genome 50:653–659.

Moore, T., and D. Haig. 1991. Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet. 7:45–49.

Muller, H. J. 1942. Isolating mechanisms, evolution and temperature. Biol. Symposia 6:71–125.

Newell-Price, J., A. J. Clark, and P. King. 2000. DNA methylation and silencing of gene expression. Trends Endocrinol. Metab. 11:142-148.

Oh-McGinnis, R., A. B. Bogutz, and L. Lefebvre. 2011. Partial loss of Ascl2 function affects all three layers of the mature placenta and causes intrauterine growth restriction. Dev. Biol. 351:277–286.

Orr, H. A., and M. Turelli. 1996. Dominance and Haldane's rule. Genetics 143:613–616.

Pang, S. C., J. Janzen-Pang, M. Yat Tse, B. A. Croy, and P. D. A. Lima. 2014. The cycling and pregnant mouse: gross anatomy. Pp. 3–19 in B. A. Croy, A. T. Yamada, F. J. DeMayo, and S. L. Adamson, eds. The guide to investigation of mouse pregnancy. Academic Press, San Diego, CA.

Parr, B. A., V. A. Cornish, M. I. Cybulsky, and A. P. McMahon. 2001. Wnt7b regulates placental development in mice. Dev. Biol. 237:324–332.

Patten, M. M. 2018. Selfish X chromosomes and speciation. Mol. Ecol. 27:3772–3782.

Pertea, M., G. M. Pertea, C. M. Antonescu, T. C. Chang, J. T. Mendell, and S. L. Salzberg 2015. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat. Biotechnol. 33:290–295.

Presgraves, D. C. 2010. The molecular evolutionary basis of species formation. Nat. Rev. Genet. 11:175–180.

Presgraves, D. C. 2018. Evaluating genomic signatures of "the large X-effect" during complex speciation. Mol. Ecol. 27:3822–3830.

Qu, D., A. McDonald, K. J. Whiteley, S. A. Bainbridge, and S. L. Adamson. 2014. Layer-enriched tissue dissection of the mouse placenta in late gestation. Pp. 529–535. in B. A. Croy, A. T. Yamada, F. J. DeMayo, and S. L. Adamson, eds. The guide to investigation of mouse pregnancy. Academic Press, San Diego, CA.

Raznahan, A., N. N. Parikshak, V. Chandran, J. D. Blumenthal, L. S. Clasen, A. F. Alexander-Bloch, A. R. Zinn, D. Wangsa, J. Wise, D. G. M. Murphy, et al. 2018. Sex-chromosome dosage effects on gene expression in humans. Proc. Natl. Acad. Sci. USA 115:7398–7403.

Rogers, J. F., and W. D. Dawson. 1970. Foetal and placental size in a *Peromyscus* species cross. J. Reprod. Fertil. 21:255–262.

Sagi, L., N. Zuckerman-Levin, A. Gawlik, L. Ghizzoni, A. Buyukgebiz, Y. Rakover, T. Bistritzer, O. Admoni, A. Vottero, O. Baruch, et al. 2007. Clinical significance of the parental origin of the X chromosome in Turner syndrome. J. Clin. Endocrinol. Metab. 92:846–852.

Sandman, C. A., L. M. Glynn, and E. P. Davis. 2013. Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. J. Psychosom. Res. 75:327–335.

Sanli, I., and R. Feil. 2015. Chromatin mechanisms in the developmental control of imprinted gene expression. Int. J. Biochem. Cell Biol. 67:139-147.

Schurch, N. J., P. Schofield, M. Gierliński, C. Cole, A. Sherstnev, V. Singh, N. Wrobel, K. Gharbi, G. G. Simpson, T. Owen-Hughes, et al. 2016. How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? RNA 22:839-851.

Shi, W., A. Krella, A. Orth, Y. Yu, and R. Fundele. 2005. Widespread disruption of genomic imprinting in adult interspecies mouse (*Mus*) hybrids. Genesis 43:100–108.

Szklarczyk, D., A. L. Gable, D. Lyon, A. Junge, S. Wyder, J. Huerta-Cepas, M. Simonovic, N. T. Doncheva, J. H. Morris, P. Bork, et al. 2019. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 47:D607–D613.

Takagi, N., and M. Sasaki. 1975. Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. Nature 256:640–642.

Tanaka, M., M. Puchyr, M. Gertsenstein, K. Harpal, R. Jaenisch, J. Rossant, and A. Nagy. 1999. Parental origin-specific expression of *Mash2* is established at the time of implantation with its imprinting mechanism highly resistant to genome-wide demethylation. Mech. Dev. 87:129–142.

Tao, Y., S. Chen, D. L. Hartl, and C. C. Laurie. 2003. Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *Drosophila mauritiana*. I. Differential accumulation of hybrid male sterility effects on the X and autosomes. Genetics 164:1383–1397.

The Gene Ontology Consortium. 2017. Expansion of the gene ontology knowledgebase and resources: the gene ontology consortium. Nucleic Acids Res. 45:D331–D338.

Tunster, S. J., H. D. Creeth, and R. M. John. 2016a. The imprinted Phlda2 gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. Dev. Biol. 409:251–260.

Tunster, S. J., G. I. McNamara, H. D. Creeth, and R. M. John. 2016b. Increased dosage of the imprinted Ascl2 gene restrains two key endocrine lineages of the mouse Placenta. Dev. Biol. 418:55–65.

Turelli, M., and L. C. Moyle. 2007. Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. Genetics 176:1059–1088.

Turelli, M., and H. A. Orr. 1995. The dominance theory of Haldane's rule. Genetics 140:389–402.

Turissini, D. A., J. A. McGirr, S. S. Patel, J. R. David, and D. R. Matute. 2018. The rate of evolution of postmating-prezygotic reproductive isolation in *Drosophila*. Mol. Biol. Evol. 35:312–334.

Vallejos, C. A., S. Richardson, and J. C. Marioni. 2016. Beyond comparisons of means: understanding changes in gene expression at the single-cell level. Genome Biol. 17:1-14.

Vrana, P. B. 2007. Genomic imprinting as a mechanism of reproductive isolation in mammals. J. Mammal. 88:5–23.

Vrana, P. B., X. J. Guan, R. S. Ingram, and S. M. Tilghman. 1998. Genomic imprinting is disrupted in interspecific *Peromyscus* hybrids. Nat. Genet. 20:362–365.

Vrana, P. B., J. A. Fossella, P. Matteson, T. del Rio, M. J. O'Neil, and S. M. Tilghman. 2000. Genetic and epigenetic incompatibilities underlie hybrid dysgenesis in *Peromyscus*. Nat. Genet. 25:120–124.

Walker, M. G., B. Fitzgerald, S. Keating, J. G. Ray, R. Windrim, and J. C. P. Kingdom. 2012. Sex-specific basis of severe placental dysfunction leading to extreme preterm delivery. Placenta 33:568–571.

Wiechens, N., V. Singh, T. Gkikopoulos, P. Schofield, S. Rocha, and T. Owen-Hughes. 2016. The chromatin remodeling enzymes SNF2H and SNF2L position nucleosomes adjacent to CTCF and other transcription factors. PLoS Genet. 12:e1005940.

Whitten, W. K. 1956. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. J. Endocrinol. 13:399–404.

Wiley, C. D., H. H. Matundan, A. R. Duselis, A. T. Isaacs, and P. B. Vrana. 2008. Patterns of hybrid loss of imprinting reveal tissue- and cluster-specific regulation. PLoS One 3:e3572.

Wolf, J. B., R. J. Oakey, and R. Feil. 2014. Imprinted gene expression in hybrids: perturbed mechanisms and evolutionary implications. Heredity 113:167-175.

Zanders, S. E., and R. L. Unckless. 2019. Fertility costs of meiotic drivers. Curr. Biol. 29:R512–520.

Zechner, U., M. Reule, A. Orth, F. Bonhomme, B. Strack, J.-L. Guénet, H. Hameister, and R. Fundelle. 1996. An X-chromosome linked locus contributes to abnormal placental development in mouse interspecific hybrids. Nat. Genet. 12:398–403.

Zechner, U., M. Hemberger, M. Constância, A. Orth, I. Dragatsis, A. Lüttges, H. Hameister, and R. Fundelle. 2002. Proliferation and growth factor expression in abnormally enlarged placentas of mouse interspecific hybrids. Dev. Dyn. 224:125–134.

Zechner, U., W. Shi, M. Hemberger, H. Himmelbauer, S. Otto, A. Orth, V. Kalscheuer, U. Fischer, R. Elango, A. Reis, et al. 2004. Divergent genetic and epigenetic post-zygotic isolation mechanisms in Mus and Peromyscus. J. Evol. Biol. 17:453–460.

Zhang, H., M. J. Zeitz, H. Wang, B. Niu, S. Ge, W. Li, J. Cui, G. Wang, G. Qian, M. J. Higgins, et al. 2014. Long noncoding RNA-mediated intrachromosomal interactions promote imprinting at the Kcnq1 locus. J. Cell Biol. 204:61–75.

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