Interacting Networks Associated with Parent-of-Origin Effects on Metabolic Traits

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ABSTRACT

Parent-of-origin effects (POE) are unexpectedly common in complex traits, including metabolic and neurological diseases. POE can also be modified by the environment, but the architecture of these gene-by-environmental effects on phenotypes remains to be unraveled. Previously, quantitative trait loci (QTL) showing context-specific POE on metabolic traits were mapped in the F_{16} generation of an advanced intercross between LG/J and SM/J inbred mice. However, these QTL were not enriched for known imprinted genes, suggesting another mechanism is needed to explain these POE phenomena. Here, we use a simple yet powerful F_1 reciprocal cross model to test the hypothesis that non-imprinted genes can generate complex POE on metabolic traits through genetic interactions with imprinted genes. Male and female mice from a F_1 reciprocal cross of LG/J and SM/J strains were fed either high or low fat diets. We generated expression profiles from three metabolically-relevant tissues: hypothalamus, white adipose, and liver. We identified two classes of parent-of-origin expression biases: genes showing parent-of-origin-dependent allele-specific expression and biallelic genes that are differentially expressed by reciprocal cross. POE patterns of both gene classes are highly tissue- and context-specific, sometimes occurring only in one sex and/or diet cohort in a particular tissue. We then constructed tissue-specific interaction networks among genes from these two classes of POE. In some cases, the biallelic gene in an interacting pair falls within the support intervals of previously-identified F_{16} LG/J x SM/J metabolic POE QTL, potentially contributing to the POE observed. We highlight one such interaction in adipose, between *Nnat* and *Mogat1*, which associates with POE on multiple adiposity traits. Both genes localize to the endoplasmic reticulum of adipocytes and play a role in adipogenesis. The genes and networks we present here represent a set of actionable interacting candidates that can be probed to further identify the machinery driving POE on complex traits.

Parent-of-origin effects, where an allele's phenotypic effect depends on whether it is inherited maternally or paternally, are associated with a wide range of common complex traits and diseases[1]. Several mechanisms can cause parent-of-origin effects on phenotype including genomic imprinting, maternal/paternal effects, and sex-biased gene-specific trinucleotide expansions[2–4]. The best characterized parent-of-origin effect on phenotype is genomic imprinting, an epigenetic process in which either the maternally or paternally inherited allele is silenced. Diseases associated with parent-of-origin imprinted effects are often related to metabolism, neurological function, or both. Metabolic diseases include transient neonatal diabetes (ZAC1, HYMAI, ZFP57)[5–8], type-1 diabetes (DLK1/MEG3 cluster)[9], type-2 diabetes (GNAS, KLF14, GRB10)[10,11], some cancers (IGF2/H19, ZAC1)[12,13], metabolic syndrome (IGF2, PEG3, DLK1, SLC2A10, KCNK9) Beckwith-Wiedemann syndrome (IGF2/H19/KCNQ1 cluster), Wilm's tumors (H19/IGF2 cluster)[14], insulinomas (no known genes)[12], Silver–Russell syndrome (KLF4, GRB10)[15,16], and variants of Albright's hereditary osteodystrophy (GNAS)[17,18]. Neurological diseases include Alzheimers (no known genes)[19–21], myoclonus-dystonia syndrome (SGCE)[22], and Jervell and Lange-Nielsen syndrome (KCNQ1, KCNE1)[23,24]. Diseases that are both metabolic and neurological in nature include Prader-Willi syndrome (SNRPN, NECDIN, SNORD64, SNORD107, SNORD18, SNORD109, SNORD116, SNORD115) and Angelman syndrome (UBE3A)[25,26]. Parent-of-origin effects associated with disease can exhibit variance among individuals, and may be modified by the environment[1,27–29]. Unraveling the genetic architecture of these effects will improve efforts to predict phenotype from (epi)genotype. This can direct research aimed at developing novel therapeutic strategies for diseases associated with parent-of-origin imprinted effects[30].

The unexpected observation that parent-of-origin effects on complex traits and disease are fairly common, despite there being few known imprinted genes, suggests that canonical imprinting mechanisms are not sufficient to account for these phenomena. We hypothesize that interactions among imprinted genes and each other, as well as between imprinted genes and non-imprinted genes with equivalent expression of parental alleles (biallelic genes), may underlie some of these effects on phenotype (**Figure 1**).

Interactions among imprinted genes are likely. This is supported by their clustered nature and the fact that they are significantly co-expressed[31,32]. Analysis of interactions among imprinted genes indicates that these genes are particularly "interactive", and that they are enriched in complex networks that include both imprinted and non-imprinted genes[29,33,34]. Interactions between imprinted genes and biallelic genes may also underlie some of these effects. For example, a study of neural tumors in mice revealed that tumor development is influenced by epistatic interactions involving an imprinted locus near tumor suppressor genes[35]. In another example, there is evidence that the maternally expressed transcription factor KLF14 (kruppel-like factor 14) regulates biallelic adipose genes[10,16]. GWAS have identified variants (rs4731702 and rs972283) upstream of KLF14 that are associated with type-2 diabetes and cholesterol levels[36,37]. These variants have maternally-restricted *cis*-regulatory associations with KLF14 expression in adipose tissue[38]. eQTL analyses using human gene expression data found that rs4731702 is enriched for *trans*-associations in subcutaneous white adipose tissue, indicating that KLF14 may be an adipose master transcriptional regulator[10]. Furthermore, the top 46 associated genes for which rs4731702 is an eQTL show a significant enrichment for KLF family transcription factor binding sites. Together, this suggests that KLF14 propagates a parent-of-origin effect in biallelic genes that contributes to variation in expression and, subsequently, to variation in adiposity phenotypes. How many additional imprinted/biallelic gene pairs are similarly co-expressed and whether they are specific to certain tissue or environmental contexts is an open question. Interactions between imprinted and biallelic genes could explain some of the observed complex parent-of-origin effect patterns that are associated with regions lacking obvious candidate genes, as described in a recent survey of 97 traits measured in outbred mice[29].

In this study, we use F_1 reciprocal crosses of the LG/J and SM/J inbred mice (LxS and SxL) to test the hypothesis that non-imprinted genes can generate complex parent-of-origin effects on dietary-obesity phenotypes through genetic interactions with imprinted genes. LG/J and SM/J are frequently used in metabolic studies because these strains vary in their metabolic response to dietary fat[39]. Quantitative trait loci (QTL) showing parent-of-origin effects on dietary obesity traits have been mapped in the F_{16} generation of an advanced intercross between LG/J and SM/J[40–43]. Most of these QTL have additive

effects, but ≈60% of them also show parent-of-origin effects in some sex and/or dietary context. However, permutation analyses reveal that these parent-of-origin effect QTL are not enriched for known imprinted genes (**Supplemental Figure 1**).

We generated expression profiles from 20 week-old F_1 animals in order to match the age of the F_{16} LG/J x SM/J advanced intercross population in which dietary-obesity QTL having parent-of-origin effects were mapped. F_1 reciprocal cross (LxS and SxL) animals were subject to the same high and low fat diets and phenotyping protocols as the previously-studied F_{16} animals to keep environmental contexts consistent. We identify genes showing parent-of-origin-dependent allele-specific expression in three metabolically-relevant tissues: hypothalamus, white adipose (reproductive fatpad), and liver. Some of these genes are canonically imprinted, and most have the partial silencing of an allele that occurs with a parent-of-origin-dependent expression bias. We characterize interactions among these genes and biallelic genes that are differentially expressed by reciprocal cross under different sex and/or diet contexts. Finally, we integrate these interacting gene pairs with previously-mapped dietary-obesity QTL showing parent-of-origin effects in the F_{16} population and identify a set of actionable interacting candidates that can be probed to further identify the machinery driving POE on complex traits. We highlight one such pair of interacting candidates, *Nnat* and *Mogat1*, which associate with parent-of-origin effects on adiposity.

RESULTS

Parent-of-origin-dependent allele-specific expression shows a high degree of tissue-specificity

We identified genes with significant parent-of-origin-dependent allele-specific expression in hypothalamus (n=108), white adipose (n=102), and liver (n=109) tissues across sex and dietary contexts (**Supplemental Table 1**). We calculated parent-of-origin effect (POE) scores for each expressed gene by subtracting the mean L_{bias} of the SxL (maternal SM/J, paternal LG/J) cross from the mean L_{bias} of the LxS (maternal LG/J, paternal SM/J) cross of all mice in a certain diet-by-sex context (high or low fat diets, equal representation of males and females). POE scores range from completely maternally-expressed (-

1), to biallelic (0), to completely paternally expressed (+1). A total of 298 unique genes show parent-of-origin-dependent allele-specific expression across these tissues. We also observe a relationship between sex and dietary context- and tissue-dependency among their expression profiles. If a gene shows parent-of-origin-dependent allele-specific expression across all diet-by-sex contexts in a given tissue (context-independent), then it tends to show parent-of-origin allele-specific expression across all contexts in the other two tissues (tissue-independent). However, if a gene shows varying degrees of parent-of-origin-dependent allele-specific expression across diet-by-sex contexts in a given tissue (context-dependency), then it also tends to have variable parent-of-origin-dependent allele-specific expression patterns across these contexts in the other two tissues (tissue-dependency) (**Figure 2**). Canonically imprinted genes are the most likely to be both context- and tissue-independent.

Parent-of-origin-dependent expression biases are context-specific

Parent-of-origin-dependent expression biases are highly context-specific in both genes showing allele-specific expression and biallelic genes that are differentially expressed by cross (**Figure 3**; **Supplemental Tables 1 and 2**). For allele-specific expression, context-specificity was assigned by the most complex model term for which a significant parent-of-origin-dependent expression bias was observed. For biallelic genes differentially expressed by cross, the significance of the nested terms (**see Methods**) was compared to identify the model term that produced the most significant differential expression. More biallelic genes that are differentially expressed by cross are context-specific than genes showing parent-of-origin-dependent allele-specific expression. However, the degree of context-specificity varies by tissue; hypothalamus has the lowest proportion of context-specific parent-of-origin-dependent allele-specific expression (hypothalamus = 44.78%; white adipose = 48.7%; liver = 49.54%) as well as the highest proportion of biallelic genes that are differentially expressed by cross (hypothalamus = 95.16%; white adipose = 92.25%; liver = 94.1%).

Genes showing parent-of-origin effects form highly interconnected networks

Networks were constructed in each tissue from genes showing parent-of-origin-dependent allele-specific expression biases (hypothalamus = 109; white adipose = 102; liver = 108) and biallelic genes that are differentially expressed by cross (hypothalamus = 123; white adipose = 427; liver = 203). Hypothalamus had more genes that demonstrate both classes of parent-of-origin biases, allele-specific expression and differential expression by cross, than either white adipose or liver. However, many of those genes did not pass our significance thresholds and thus were not included in these network analyses. Interacting gene pairs were predicted by modeling the expression of biallelic genes that are significantly differentially expressed by cross as a function of the expression of genes showing significant parent-of-origin-dependent allele-specific expression, their allelic bias (L_{bias}), diet, sex, and the diet-by-sex interaction. Genes showing parent-of-origin effects form highly interconnected networks in each tissue (**Figure 4**). Networks are comprised of 340 interacting gene pairs in hypothalamus, 2,570 in white adipose, and 1,171 in liver (**Supplemental Table 3**). These pairs represent both unique genes showing parent-of-origin-dependent allele-specific expression biases (hypothalamus = 83; white adipose = 82; liver = 86) and unique biallelic genes that are differentially expressed by cross (hypothalamus = 80; white adipose = 385; liver = 198) in some context (reciprocal cross, diet, sex, diet-by-sex).

Genes showing parent-of-origin-dependent allele-specific expression biases tend to cluster throughout the genome in a way that is comparable to how canonically imprinted genes are clustered. The degree of clustering varies by tissue, however, and hypothalamus shows the strongest clustering of genes. This is consistent with our finding that the hypothalamus has the highest proportion of genes showing context-independent parent-of-origin-dependent allele specific expression biases as well as the highest proportion of expressed canonically imprinted genes of the three tissues we assayed. Most significant interactions between genes showing parent-of-origin-dependent allele-specific expression biases and biallelic genes differentially expressed by cross are trans-chromosomal. This indicates that we are not identifying covariation among pairs that are controlled by the same regulatory element, as is often found among canonically imprinted genes that are controlled by the same *cis*-regulatory machinery[32,44].

Enrichment analyses of significantly interacting genes reveals over-representation of canonically imprinted genes in hypothalamus, over-representation of genes involved in regulatory activity and iron metabolism in liver, and over-representation of genes involved in multiple categories including immune function, extracellular matrix, and cell proliferation in white adipose (**Figure 5 and Supplemental Table 4**).

Interacting genes fall in dietary-obesity QTL showing parent-of-origin effects

A subset of the genes showing biallelic expression in our significant interaction networks fall within the support intervals of our previously-identified metabolic QTL showing parent-of-origin effects on phenotype. The numbers of unique biallelic genes were 7 in hypothalamus, 45 in white adipose, and 11 in liver. In hypothalamus, these genes are associated with 7 QTLs showing parent-of-origin effects, in white adipose, these genes are associated with 30 QTLs showing parent-of-origin effects, and in liver these genes are associated with 9 QTLs showing parent-of-origin effects (**Figure 6 and Supplemental Table 3**). Many of the parent-of-origin effects at these QTL are context dependent, and this is described in previous studies[40–43].

Interaction between Nnat and Mogat1 in white adipose tissue

Nnat (neuronatin) is a paternally-expressed imprinted gene encoding a lipoprotein involved in intracellular calcium signaling. In white adipose tissue, *Nnat* expression significantly covaries with *Mogat1* ($p = 3.8e^{-3}$), a biallelic gene showing significant differential expression by cross in high fat-fed females. *Mogat1* (monoacylglycerol O-acyltransferase 1) is a gene encoding an enzyme that catalyzes the synthesis of diacylglycerol from monoacylglycerol, a precursor of triacylglycerol and other important lipids. *Mogat1* falls within the support intervals of the F_{16} QTL *Dob1b* which shows significant diet-by-sex parent-of-origin effects on multiple adiposity traits, including weight at necropsy and reproductive, inguinal, and total fatpad weights (**Figure 7**) [40,43].

DISCUSSION

Epistatic interactions involving parent-of-origin effects on complex traits occur when the genotypic effects of one gene depends on the parent-of-origin of alleles at another gene[1]. In this study, we examined interactions associated with parent-of-origin effects on dietary-obesity traits in three tissues that are central to metabolism: hypothalamus, white adipose, and liver. We quantified parent-of-origin-dependent allele-specific expression biases, which are consistent with imprinting mechanisms, but we cannot rule out that maternal and/or paternal effects also contribute to the phenomena we observe. In F_1 reciprocal crosses of the LG/J and SM/J inbred mouse strains, we identified genes showing significant parent-of-origin-dependent allele-specific expression biases and found that they are highly context-specific, sometimes occurring in only one sex and/or diet cohort. Further, the degree of context-specificity varies by tissue, with white adipose showing the highest degree of context-specific parent-of-origin effects on expression. This likely reflects adipose tissue's intrinsic responsiveness to sex and high degree of plasticity under different nutritional conditions[45–50]. Expression of canonically imprinted genes, such as *Peg10*, *Meg3*, *Grb10*, and *Snrpn*, show the lowest degree of context-specificity across all tissues. This likely reflects the fundamental role of these genes in growth and development[51–54].

We also identified biallelic genes that are significantly differentially expressed by cross (LxS or SxL) and found that they also show a high degree of context-specificity across all tissues. We then constructed interaction networks between these genes and those showing parent-of-origin-dependent allele-specific expression biases. Our proposed model is that genes showing parent-of-origin-dependent allele-specific expression biases affect the expression of biallelic genes differentially expressed by cross, which in turn contributes to parent-of-origin effects on phenotype. If environment affects the context-specificity of the parent-of-origin-dependent allele-specific expression biases, then the context-specificity would be propagated in the biallelic genes differentially expressed by cross. Indeed, we find that the subset of genes showing parent-of-origin-dependent allele-specific expression that significantly interact with biallelic genes that are differentially expressed by cross are enriched for those genes that are context-specific in white adipose ($p = 0.001$) and liver ($p < 0.001$). The proportion of context-specific

genes showing parent-of-origin-dependent allele-specific expression biases increases from 41.18% overall to 48.8% of genes in networks in white adipose, and from 41.28% to 51.8% in liver. Hypothalamus, which has the lowest amount of context-specificity in genes showing parent-of-origin-dependent allele-specific expression biases, shows a non-significant decrease in context-specificity of interacting gene pairs (31.48% overall to 31.33%; $p = 0.628$).

Our finding that genes showing parent-of-origin effects, both allele-specific expression biased and biallelic that are differentially expressed by cross, form highly interconnected networks of covarying expression is consistent with previous studies showing that imprinted genes are particularly interactive[29,32,33]. We find that genes showing parent-of-origin-dependent allele-specific biases tend to cluster in genomic regions, which is expected of genes that share *cis*-regulatory elements, as is the case in canonical imprinting, and is also consistent with other studies[31]. The degree of clustering varies among tissues; hypothalamus shows the highest degree of clustering compared to white adipose and liver. Along with the greater proportion of context-independent parent-of-origin-dependent allele-specific expression, this supports our finding that allele-specific expression in the hypothalamus is most consistent with canonical imprinting. This is further validated by the enrichment analyses showing that interacting pairs of genes in hypothalamus are over-represented in categories related to imprinting.

Enrichment analyses in liver reveal interacting gene pairs that are over-represented in DNA binding and iron metabolism categories. Variation in iron metabolism is associated with variation in diabetes-related traits. Recently, the LG/J and SM/J inbred strains have been shown to vary in their glucose parameters in response to dietary iron[55–57]. Gene pairs in white adipose are over-represented in categories involved in extra-cellular matrix and cell proliferation. This is an intriguing finding because the extra-cellular matrix is integral for cellular signaling, regulation of growth factor bioavailability, and tissue remodeling[58,59]. The adipose extra-cellular matrix undergoes constant remodeling to accommodate changes in adipocyte shape and function in response to nutritional cues[60]. Disruption of adipose extra-cellular matrix components is associated with inflammation and tissue fibrosis as well as obesity-induced insulin resistance[61,62]. If genes showing parent-of-origin-dependent allele-specific expression act as

intermediaries between environment (in our study: diet and/or sex) and cellular response, then interacting biallelic genes that are differentially expressed by cross may be a part of altered extra-cellular matrix or a product of that alteration. A previous study of interactions among imprinted genes in adipose also an found enrichment of genes involved in the extra-cellular matrix and cell proliferation[32]. Understanding the biological mechanisms and consequences of these interactions will improve our understanding of adipose biology and can direct research aimed at developing innovative therapeutic strategies for obesity-related metabolic disease.

We integrated our interacting gene pairs with previously identified dietary-obesity QTL showing parent-of-origin effects on metabolic phenotypes. These QTL show a high degree of context-dependency of parent-of-origin effects, which is consistent with our model and with the F_1 gene pairs we tested. We find more significant interactions between gene pairs identified in white adipose tissue than in either liver or hypothalamus. These interactions fall overwhelmingly in QTL associated with adiposity traits (instead of diabetic or lipid traits) and tend to be *trans*-interactions between genes on different chromosomes. This is consistent with the high proportion of *trans*-interactions seen in the overall interaction networks between genes showing parent-of-origin-dependent allele-specific expression and biallelic genes that are differentially expressed by cross.

Some of these genes may interact on a cellular or physiological level to affect adiposity. For example, we find the maternally-expressed imprinted gene *Grb10* (growth factor receptor-bound protein 10) interacts with *Wt1* (Wilms tumor 1), which falls in the support interval of the F_{16} parent-of-origin QTL, *Dob2d*, that is associated with variation in necropsy weight and reproductive and mesenteric fatpad weights. *Grb10* encodes an intracellular adaptor protein that interacts with several signaling molecules, including insulin[63]. Overexpression of *Grb10* inhibits growth. *Wt1* is a transcription factor that stimulates transcription of *Igfbp5* (insulin-like growth factor-binding protein 5), and *Igfpb5*-deficient mice have increased adiposity compared to littermate controls[64,65]. *Wt1* has been shown to be a determinant of visceral fat cell development[66,67]. It is plausible that physiological connections between *Grb10* and *Wt1* function to affect white adipose tissue growth. Many of the genes in our white adipose networks are

integral to adipose tissue functions, such as adipocyte differentiation (for example, *Mogat1*, *Bcl7a*, *Ndrg1*, *Emilin2*, *Nnat*, and *Smad7*)[68–73], and/or the synthesis and degradation of lipids (for example, *Plcd1*, *Mogat1*, *Mvd*, *Acly*, *Sorl1*, and *Dhcr7*)[69,74–78]. Others have been associated with variation in obesity, insulin, and glucose traits (for example, *Slc19a2*, *Chn1*, *Wt1*, *Cd44*, and *Grb10*)[63,66,79–81]. Of particular interest is the interaction between the paternally imprinted gene *Nnat* (neuronatin) and *Mogat1* (monoacylglycerol O-acyltransferase 1).

Mogat1 falls in a QTL, *Dob1b*, that shows significant diet-by-sex parent-of-origin effects on multiple adiposity traits: reproductive and inguinal fatpad weights, total fatpad weight, and weight at necropsy[40,43]. Both *Nnat* and *Mogat1* localize in the endoplasmic reticulum (ER) of white adipose tissue. *Nnat* is a diet-responsive proteolipid known to play a role in ER calcium efflux as a part of Ca^{2+} signaling[72]. Increased cytosolic Ca^{2+} levels lead to CREB activation, which in turn promotes adipogenesis. *Mogat1* expression is induced during adipogenesis. *Mogat1* plays an important role in adipocyte differentiation by contributing to the production and accumulation of triglycerides by catalyzing the conversion of mono-acylglycerides to di-acylglycerides, which are subsequently converted to tri-acylglycerides[68]. MOGAT1 is an eQTL in human subcutaneous adipose tissue ($p = 1.5e^{-5}$, GTEx), which is homologous to the mouse inguinal fatpad[82]. Thus, there is good evidence that both of these genes contribute to adiposity, that they may interact indirectly through CREB activity, and that this interaction contributes to the parent-of-origin effects on adiposity at this QTL.

The support intervals for the F_{16} parent-of-origin QTL span large genomic regions and are not empowered to identify the specific genes that contribute to the parent-of-origin-dependent phenotypic variation. By leveraging the reciprocal F_1 hybrids, we are able to integrate parent-of-origin-dependent allele-specific expression and parent-of-origin-dependent differential expression with the mapped F_{16} phenotypes. Further, by incorporating multiple metabolically-relevant tissues as well as sex and dietary environments, we provide an authentic systems biology perspective on metabolic trait variation. By doing so, we identify plausible candidates for functional validation and describe discrete molecular networks that may contribute to the observed parent-of-origin effects on phenotypic variation. The genes and

networks we present here represent a set of actionable interacting candidates that can be probed to further identify the machinery driving these phenomena and make predictions informed by genomic sequence. We will not improve our ability to predict complex phenotypes from genomic sequence until we develop better models that explicitly include genetic, epigenetic, and environmental components in a rigorous way. In this study we focused on metabolic phenotypes, but the patterns we identified may translate to other complex phenotypes where parent-of-origin effects have been implicated.

METHODS

Mouse husbandry and phenotyping

LG/J and SM/J founders were obtained from The Jackson Laboratory (Bar Harbor, ME). F_1 reciprocal cross animals were generated by mating LG/J mothers with SM/J fathers (LxS) and the inverse (SxL). At three weeks of age, animals were weaned into same-sex cages and randomly placed on high-fat (42% kcal from fat; Teklad TD88137) or low-fat (15% kcal from fat; Research Diets D12284) isocaloric diets. Animals were weighed weekly until sacrifice. At 19 weeks of age, body composition was determined by MRI and a glucose tolerance test was performed after a 4 hour fast. At 20 weeks of age, animals were given an overdose of sodium pentobarbital after a 4 hour fast and blood was collected via cardiac puncture. Euthanasia was achieved by cardiac perfusion with phosphate-buffered saline. After cardiac perfusion, liver, reproductive fatpad and hypothalamus were harvested, flash frozen in liquid nitrogen, and stored at -80°C.

Genomes and annotations

LG/J and SM/J indels and SNVs were leveraged to construct strain-specific genomes using the GRC38.72-mm10 reference as a template[83]. This was done by replacing reference bases with alternative (LG/J | SM/J) bases using custom python scripts. Ensembl R72 annotations were adjusted for indel-induced indexing differences for both genomes.

RNA sequencing

Total RNA was isolated from adipose and hypothalamus tissues using the RNeasy Lipid Tissue Kit (QIAgen) and from liver using TRIzol (n = 32, 4 animals per sex/diet/cross cohort). RNA concentration was measured via NanoDrop and RNA quality/integrity was assessed with a BioAnalyzer (Agilent). RNA-Seq libraries were constructed using the RiboZero kit (Illumina) from total RNA samples with RIN scores >8.0. Libraries were checked for quality and concentration using the DNA 1000LabChip assay (Agilent) and quantitative PCR, according to manufacturer's protocol. Libraries were sequenced at 2x100 paired end reads on an Illumina HiSeq 4000. After sequencing, reads were de-multiplexed and assigned to individual samples.

Allele-specific expression

FASTQ files were filtered to remove low quality reads and aligned against both LG/J and SM/J pseudo-genomes simultaneously using STAR with multimapping disallowed[84]. Read counts were normalized via upper quartile normalization and a minimum normalized read depth of 20 was required. Alignment summaries are provided in **Supplemental Table 6 and Supplemental Figure 2**.

For each gene in each individual, allelic bias (L_{bias}) was calculated as the proportion of total reads for a given gene with the LG/J haplotype. Parent-of-origin-dependent allele-specific expression was detected by ANOVA using one of a number of models in which L_{bias} is responsive to cross and some combination of sex and diet:

$$Model \begin{cases} if\ each\ Cross\ context\ has \geq 2\ samples,\ Lbias \sim Cross \\ if\ each\ Cross{:}Sex\ context\ has \geq 2\ samples,\ Lbias \sim Cross + Cross{:}Sex \\ if\ each\ Cross{:}Diet\ context\ has \geq 2\ samples,\ Lbias \sim Cross + Cross{:}Diet \\ if\ each\ context\ has \geq 2\ samples,\ Lbias \sim Cross + Cross{:}Sex + Cross{:}Diet + Cross{:}Sex{:}Diet \end{cases}$$

Accurately estimating the significance of these effects is challenging for two reasons: 1) the complexity of the many environmental contexts, and 2) the correlation of allelic bias within and between imprinted domains breaks assumptions of independence. A permutation approach is an effective way to overcome these challenges. The context data was randomly shuffled and analyses were rerun in order

to generate a stable null distribution of F-statistics (**Supplemental Figure 3**). Significance thresholds were calculated from the empirical distribution of this null model and a p-value ≤ 0.05 was considered significant (**Supplemental Table 1**).

To determine the parental direction and size of expression biases, a parent-of-origin effect POE score was calculated as the difference in mean L_{bias} between reciprocal crosses (LxS or SxL). POE scores range from completely maternally-expressed (-1), to biallelic (0), to completely paternally-expressed (+1). POE score thresholds were calculated from a critical value of α = 0.01, determined from a null distribution created by permutation (**Supplemental Figure 4**). Genes with significant allele-specific expression and parent-of-origin scores beyond the critical value were considered to have significant parent-of-origin-dependent allele-specific expression.

Library complexity

Complexity was measured by fitting a beta-binomial distribution to the distribution of L_{bias} values using the VGAM package[85]. The shape parameters (α, β) of beta-binomial distributions were estimated and used to calculate dispersion (ρ). Dispersion values less than 0.05 indicate our libraries are sufficiently complex (**Supplemental Figure 5**).

$$\rho_s = \frac{1}{1 + \alpha_s + \beta_s}$$

Two libraries, one from white adipose and one from liver, were found to have insufficient complexity and were removed from the analyses.

Differential expression

Differential expression by reciprocal cross was determined by first aligning reads against the LG/J and SM/J genomes simultaneously with multimapping permitted. Reads were normalized by TMM and a minimum normalized read count of 10 was required. Generalized linear models accounting for diet, sex,

and diet-by-sex were fit in EdgeR[86]. Differential expression was detected by a likelihood ratio test. Significance was determined for four nested models for each gene:

$$1.\, Expression \sim Cross$$

$$2.\ \ Expression \sim Cross + Cross{:}Sex$$

$$3.\, Expression \sim Cross + Cross{:}Diet$$

$$4.\, Expression \sim Cross + Cross{:}Sex + Cross{:}Diet + Cross{:}Sex{:}Diet$$

To accurately estimate significance given the complexity of the many diet and sex cohorts, the context data was shuffled and the analyses rerun in order to generate an appropriate null distribution of likelihood ratio statistics for each model (**Supplemental Figure 6**). Stability of the permuted data was evaluated by calculating the total quantile deviation (TQD) at each iteration:

$$TQD = \sum_{i=2}^{I} \left\| \begin{bmatrix} \frac{\sum_{z=1}^{i-1} Q_{1\%,z}}{i-1} \\ \vdots \\ \frac{\sum_{z=1}^{i-1} Q_{100\%,z}}{i-1} \end{bmatrix} - \begin{bmatrix} Q_{1\%,i} \\ \vdots \\ Q_{100\%,i} \end{bmatrix} \right\|$$

Genes with a p-value of ≤ 0.05 and a $|log_2(fold\ change)| \geq 1$ were considered significantly differentially expressed by reciprocal cross (**Supplemental Figure 7 and Supplemental Table 2**).

Gene-gene interactions

Networks were constructed in each tissue by pairing genes showing parent-of-origin-dependent allele-specific expression with biallelic genes that are differentially expressed by cross. Pairs were predicted by modeling the expression of biallelic genes as a function of parent-of-origin-dependent allele-specific expression, L_{bias}, sex, diet, and sex-by-diet. The strength of a prediction was measured through model fit, which was estimated as a mean test error with 10-fold cross-validation employed to prevent overfitting. Given the complexity of contexts, the significance threshold was determined by permuting the context data to generate a stable null-distribution of mean test errors (**Supplemental Figure 8**). A p-

value ≤ 0.01 was considered significant (**Supplemental Table 3**). Genomic clusters of interacting genes were assigned by K-means clustering performed for each chromosome separately.

Functional enrichment analysis

Functional enrichment of interacting genes showing parent-of-origin-dependent allele-specific expression with biallelic genes that are differentially expressed by cross was tested by over-representation analysis in the WEB-based Gene Set Analysis Toolkit v2019[87]. We performed analyses of gene ontologies (biological process, cellular component, molecular function), pathway (KEGG), and phenotype (Mammalian Phenotype Ontology). For each tissue, the list of all unique interacting genes was analyzed against the background of all unique genes expressed in that tissue. A Benjamini-Hochberg FDR-corrected p-value ≤ 0.01 was considered significant (**Supplemental Table 4**).

Enrichment in QTL with genomic imprinting effects

Support intervals of quantitative trait loci (QTL) showing significant genomic imprinting effects were randomly shuffled throughout the genome to generate an empirical distribution of random QTL-sized regions. The following gene sets were intersected with these random regions and compared to their intersections in the mapped QTL: i) known imprinted genes; ii) genes differentially expressed by reciprocal cross; iii) genes showing parent-of-origin-dependent allele-specific expression; and iv) gene-gene interactions between genes differentially expressed by reciprocal cross and genes showing parent-of-origin-dependent allele-specific expression (**Supplemental Tables 1, 2, 3, and 6**). P-values ≤ 0.05 were considered significant and ≤ 0.1 considered suggestive.

DATA ACCESS

All data generated and/or analyzed during the current study are available in the Supplemental Materials, in referenced publications, and at lawsonlab.wustl.edu. RNAseq reads generated for this study have been submitted to the NCBI Gene Expression Omnibus. Code written to analyze data is available on GitHub (https://github.com/LawsonLab-WUSM/POE_Epistasis)

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AUTHOR CONTRIBUTIONS

HAL and JFM conceived of the study. HAL and JPW generated and collected data. LY, LS, and KF assisted in tissue harvesting. HAL, JFM, and CLS performed analyses. CFS provided resources. HAL, JFM, and CLS wrote the manuscript. All authors read and approved the submitted manuscript.

DISCLOSURE DECLARATION

All experiments were approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (WUSM) in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

COMPETING INTERESTS

None.

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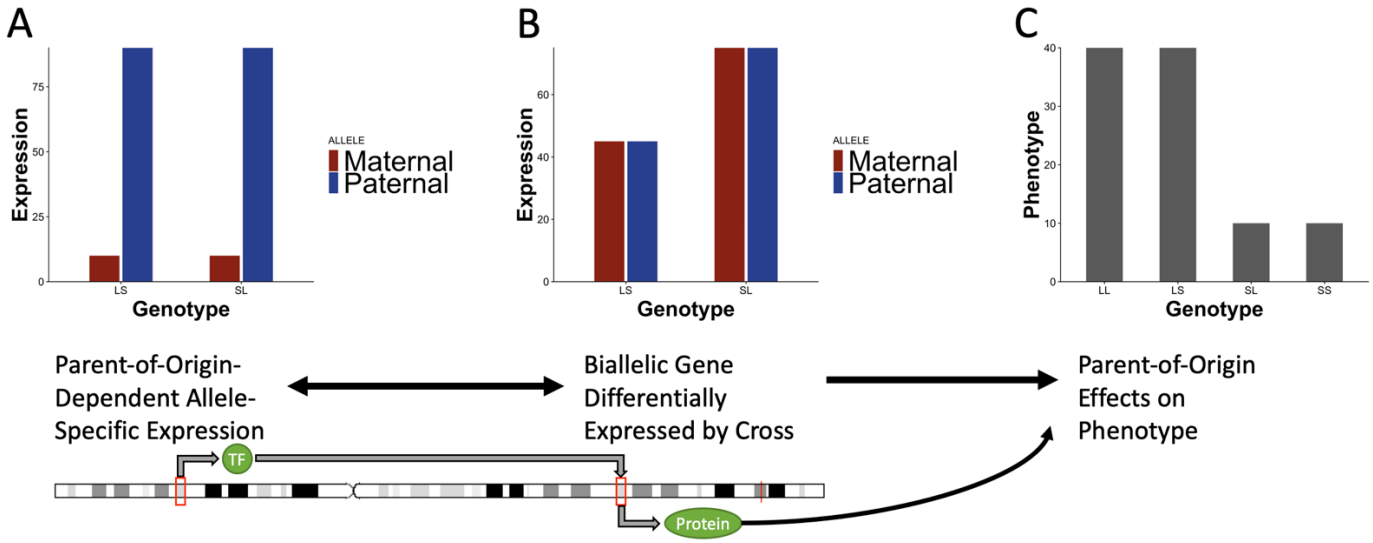

Figure 1. Proposed mechanism for how genes showing parent-of-origin-dependent allele -specific expression interacting with biallelic genes can lead to parent-of-origin effects on phenotype. A. A gene showing parent-of-origin-dependent allele-specific expression is a transcription factor that binds to the promotor of a biallelic gene. **B.** Variants in the promotor region effect binding efficiency leading to asymmetry in expression between reciprocal heterozygotes, **C.** resulting in a parent-of-origin effect on phenotype.

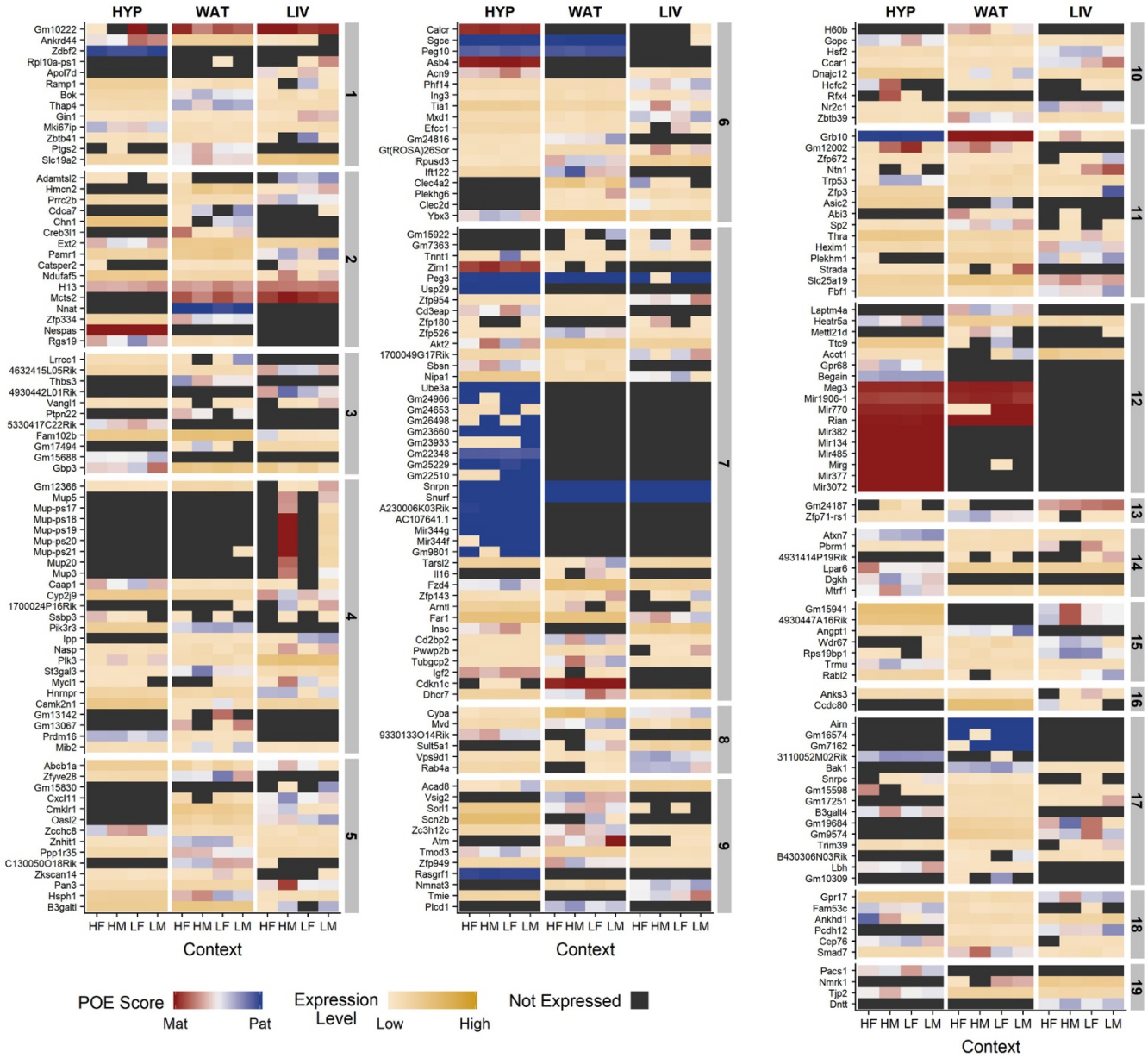

Figure 2. Genes show parent-of-origin dependent allele-specific expression in both context-dependent and context-independent manners. An expression profile heat map of genes showing significant parent-of-origin dependent allele-specific expression in at least one diet-by-sex context across three metabolically-relevant tissues. Expression levels range from white (lowly expressed) to dark goldenrod (highly expressed). If a gene is not expressed in a certain tissue and diet-by-sex context, then it is shown as black. Parent-of-origin (POE) scores range from completely maternally-expressed (-1, Mat, dark red), to biallelic (0, white), to

completely paternally-expressed (+1, Pat, dark blue). The x-axis denotes diet-by-sex context (HF = high fat-fed females; HM = high fat-fed males; LF = low fat-fed females; LM = low fat-fed males) across three tissues (HYP = hypothalamus; WAT = white adipose; LIV = liver). The y-axis denotes genes with significant parent-of-origin dependent allele-specific expression ordered by genomic position across the chromosomes (vertical grey panels). Canonically imprinted genes are indicated by *.

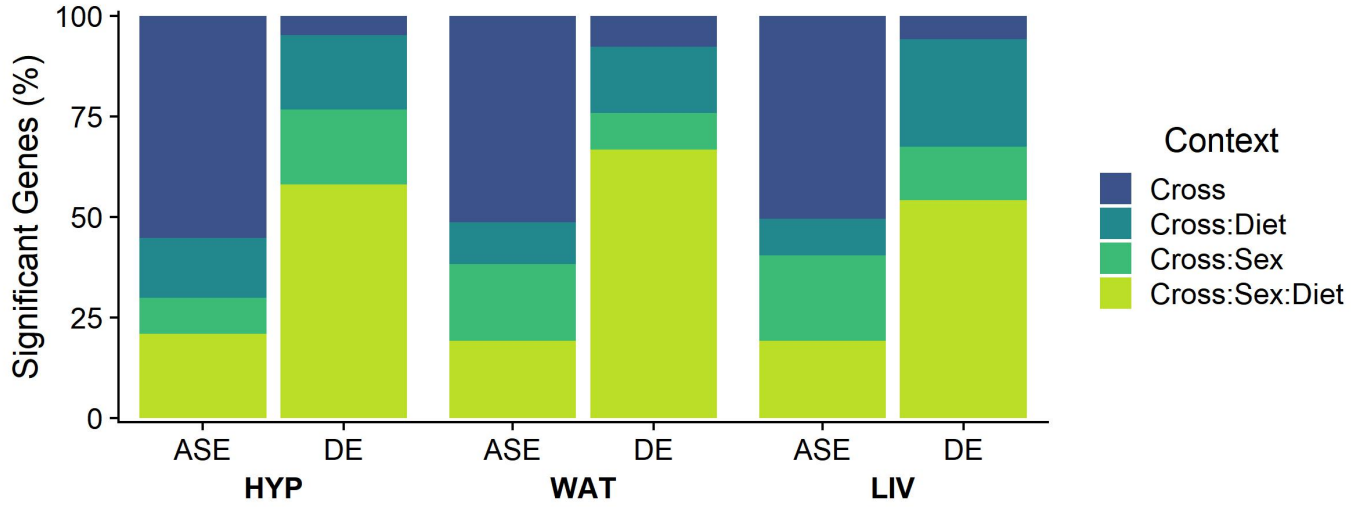

Figure 3. Parent-of-origin-dependent expression biases are context-specific. Parent-of-origin expression biases are considered significant if any of the Cross (Blue), Cross:Sex (Green), Cross:Diet (Teal), or Cross:Sex:Diet (lime green) terms were significant in the models tested. Parent-of-origin dependent biases show a high degree of context-specificity in both genes showing parent-of-origin-dependent expression biases and biallelic genes that are differentially expressed by cross. This context-specificity is also tissue-dependent. ASE = parent-of-origin-dependent allele-specific expression biases; DE = biallelic genes differentially expressed by cross (LxS or SxL); HYP = hypothalamus; WAT = white adipose; LIV = liver.

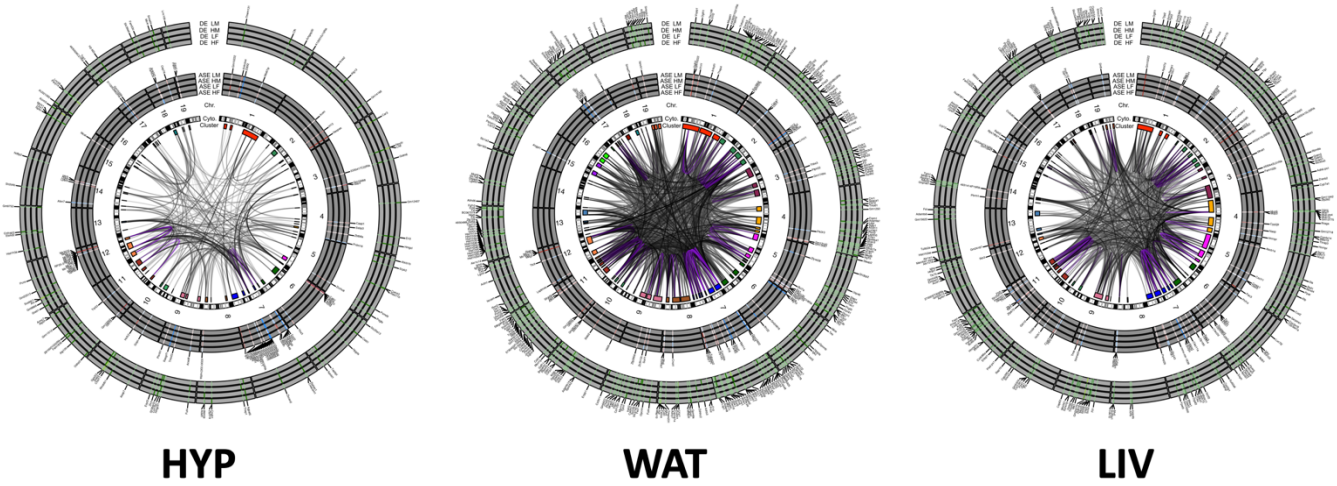

Figure 4. Genes showing parent-of-origin effects form highly interconnected networks. Networks are comprised of two classes of parent-of-origin effect: parent-of-origin-dependent allele-specific expression (middle tracks) and biallelic genes showing differential expression by cross, LxS or SxL (outer tracks). Genes showing parent-of-origin-dependent allele-specific expression are colored coded by their parent-of-origin effect score, with blue indicating a paternal bias and red indicating a maternal bias. Biallelic genes showing differential expression between crosses are colored by their absolute $\log_2$ fold change on a green gradient. Darker green indicates a larger fold change magnitude. The inner-most track represents the chromosome and the boxes represent clusters of genes. The clusters are color-coded by chromosome for ease of visualization. Significant interactions are denoted with a line connecting their genomic positions, with *cis*-interactions colored purple and *trans*-interactions colored black. HYP = hypothalamus; WAT = white adipose; LIV = liver.

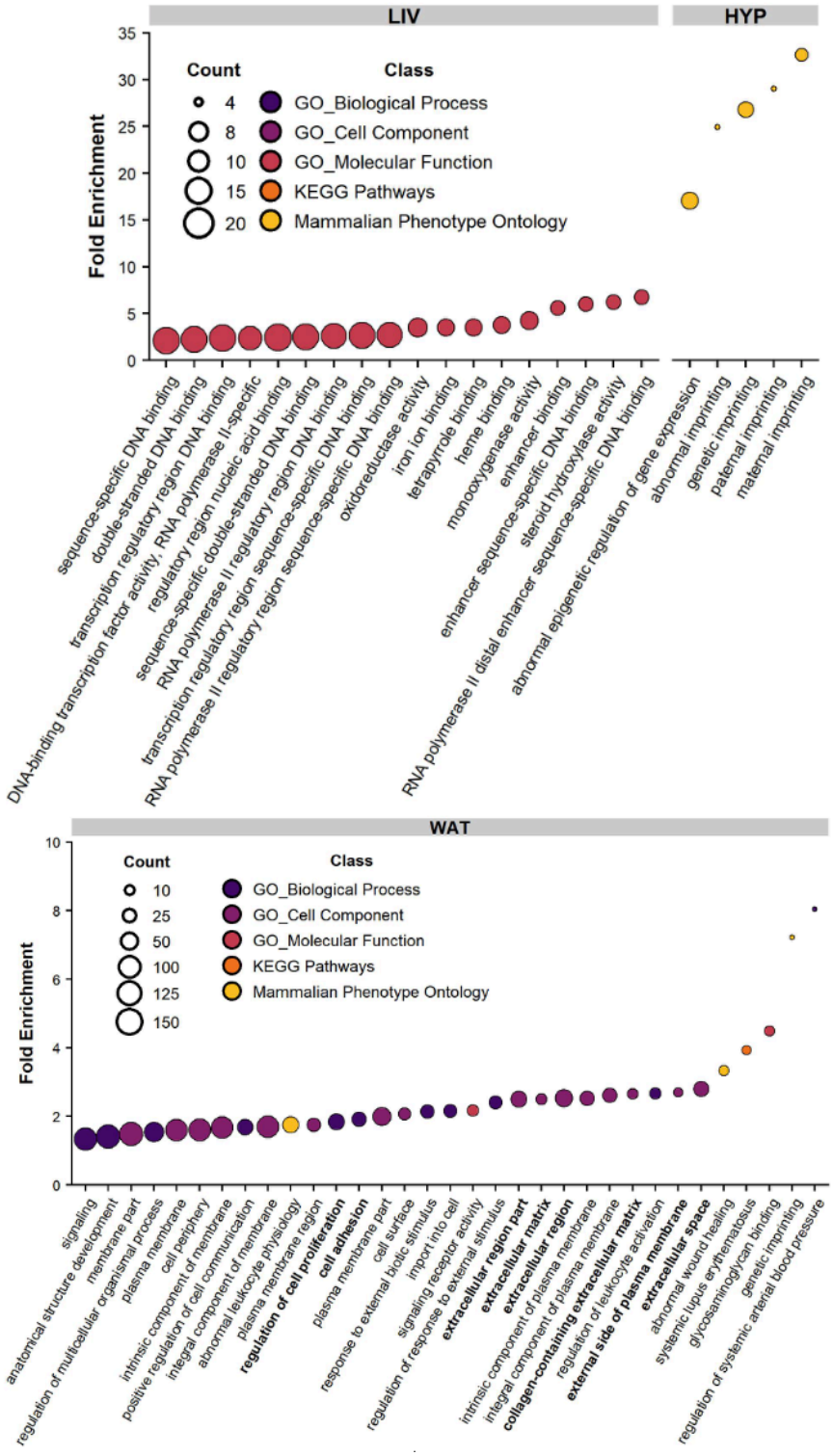

Figure 5. Enrichment analysis of interacting genes showing parent-of-origin effects. Over-representation of gene ontologies, KEGG pathways and Mammalian Phenotype ontologies in hypothalamus (HYP), white adipose (WAT) and liver (LIV).

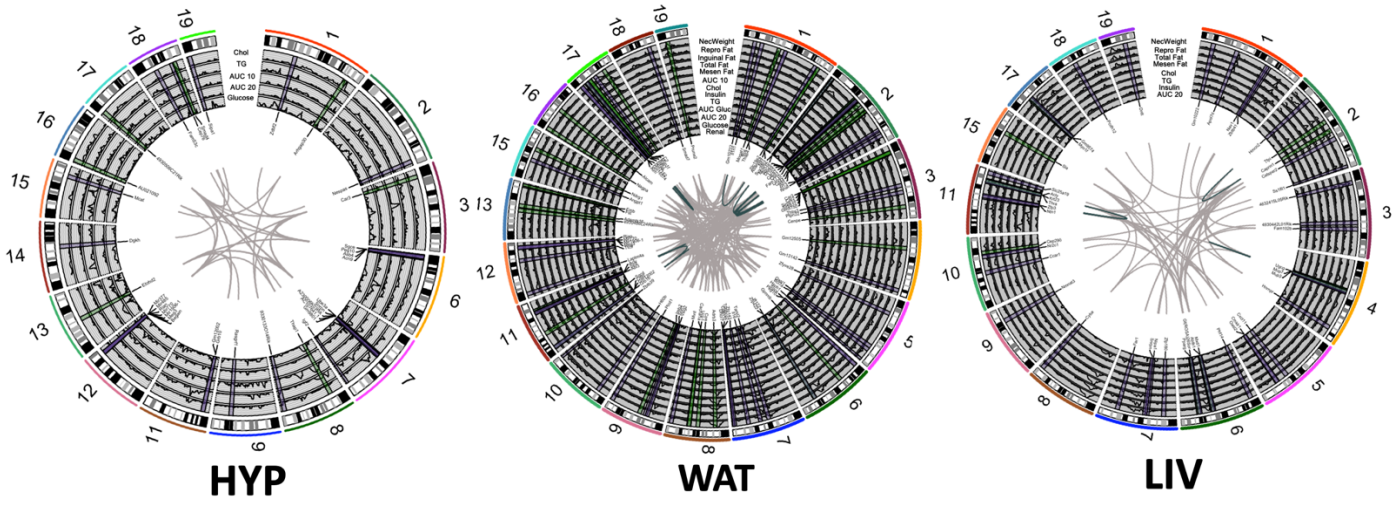

Figure 6. Networks of genes showing parent-of-origin effects in QTL showing parent-of-origin effects on dietary-obesity traits. Networks are comprised of two classes of parent-of-origin effect: parent-of-origin-dependent allele-specific expression (purple text and shading) and biallelic genes showing differential expression by cross, LxS or SxL (green text and shading). Inner tracks depict dietary-obesity QTL mapped in an F_{16} advanced intercross of the LG/J and SM/J inbred mouse strains. The outer track represents chromosomal position. Interacting gene pairs are connected by lines with *cis*-interactions colored purple and *trans*-interactions colored black. HYP = hypothalamus; WAT = white adipose; LIV = liver. AUC 10 = area under the curve calculated from a glucose tolerance test at 10 weeks of age; AUC 20 = area under the curve calculated from a glucose tolerance test at 20 weeks of age; AUC Glc = basal glucose level before glucose tolerance test; Chol = serum cholesterol; Glucose = serum glucose; Insulin = serum insulin; TG = serum triglycerides; Inguinal Fat = inguinal fatpad weight; Mesen Fat = mesenteric fatpad weight; Nec Weight = weight at necropsy; Repro Fat = reproductive fatpad weight; Total Fat = total fatpad weights.

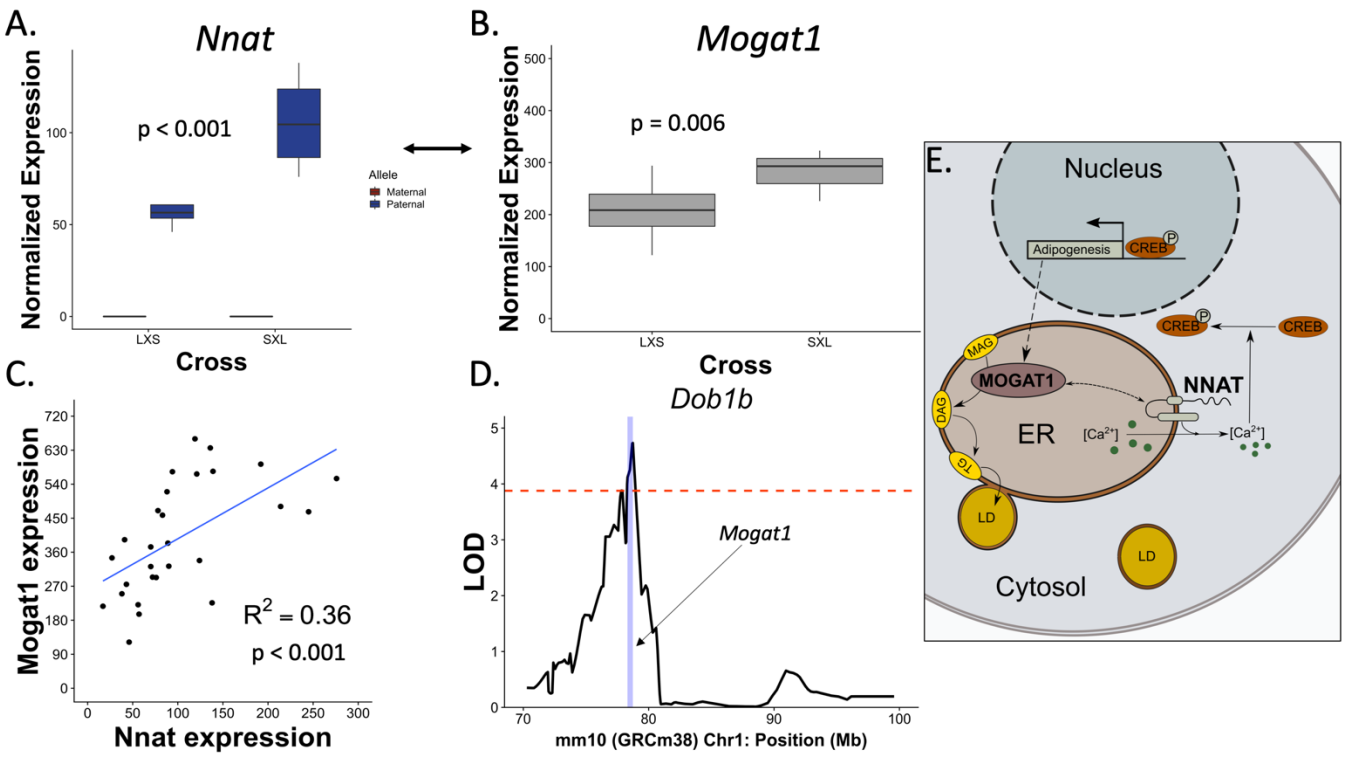

Figure 7. Interaction in white adipose between a gene showing parent-of-origin dependent allele-specific expression and a biallelic gene differentially expressed by cross. **A**. We identified *Nnat* as an imprinted gene showing paternal expression. **B**. *Mogat1* was identified as a biallelic gene showing differential expression between reciprocal crosses (LxS versus SxL), and **C**. found to significantly covary with *Nnat*. **D**. *Mogat1* falls in a QTL for multiple adiposity traits identified in an F_{16} LGxSM advanced intercross. **E**. *Nnat* and *Mogat1* both localize to the endoplasmic reticulum in adipocytes and contribute to adipose function.

Supplemental Materials:

Supplemental Figure 1: Metabolic QTL showing parent-of-origin effects are not enriched for imprinted genes.

Supplemental Figure 2: Number of reads mapped to LG/J x SM/J pseudo-genome.

Supplemental Figure 3: Stable null permutation plots for allele-specific expression.

Supplemental Figure 4: Permutation plots for parent-of-origin effect scores.

Supplemental Figure 5: RNAseq libraries are sufficiently complex to detect allele specific expression.

Supplemental Figure 6: Stable null permutation plots for differential expression by cross.

Supplemental Figure 7: Volcano plots of differentially expressed genes

Supplemental Figure 8: Stable null permutation plots for network pairs.

SupplementalTable1.xlsx: Allele-specific expression

SupplementalTable2.xlsx: Biallelic genes differentially expressed by cross

SupplementalTable3.xlsx: Networks of genes showing parent-of-origin allele-specific expression interacting with biallelic genes that are differentially expressed by cross

SupplementalTable4.xlsx: Over-representation input/output

SupplementalTable5.xlsx: Alignment summaries

SupplementalTable6.xlsx: List of imprinted genes queried

SupplementalTable7.xlsx: QTL traits, positions, and genes falling within support intervals