The evolving landscape of imprinted genes in humans and mice: Conflict among alleles, genes, tissues, and kin

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Three recent genome-wide studies in mice and humans have produced the most definitive map to date of genomic imprinting (gene expression that depends on parental origin) by incorporating multiple tissue types and developmental stages. Here, we explore the results of these studies in light of the kinship theory of genomic imprinting, which predicts that imprinting evolves due to differential genetic relatedness between maternal and paternal relatives. The studies produce a list of imprinted genes with around 120–180 in mice and ~100 in humans. The studies agree on broad patterns across mice and humans including the complex patterns of imprinted expression at loci like *Igf2* and *Grb10*. We discuss how the kinship theory provides a powerful framework for hypotheses that can explain these patterns. Finally, since imprinting is rare in the genome despite predictions from the kinship theory that it might be common, we discuss evolutionary factors that could favor biallelic expression.

Keywords:
genome-wide map; genomic imprinting; *Grb10*; *Igf2*; intragenomic conflict; kinship theory; RNA-seq

DOI 10.1002/bies.201500198

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Abbreviations:
ASE, allele specific expression; **DMR,** differentially methylated region; **IS,** imprinting score; **SNP,** single-nucleotide polymorphism.

Introduction

Genomic imprinting is the phenomenon where the expression of an allele depends on its parental origin [1, 2]. We tend to talk about imprinted genes in fairly simple terms. *This* gene is maternally expressed, while *that* gene is paternally expressed. But, typical of biology, the reality on the ground can be much more complicated. Individual "genes" often produce multiple transcripts, which can have different (imprinted and unimprinted/non-imprinted) expression patterns. Furthermore, these patterns can vary among cell lineages and developmental stages. While there has been increasing evidence of this complexity, a series of recent studies [3–5] substantially advances our understanding of this complexity by providing the most comprehensive characterization of tissue-specific and stage-specific imprinted gene expression to date in humans and mice on a genome-wide scale.

Crucially, these studies identify few novel imprinted genes, suggesting that the list of known imprinted genes in mammals may be nearly complete, and that most genes are not likely to have such expression. Evolutionary theories for genomic imprinting have long been evaluated on whether they can explain imprinted expression from particular genes [6]. However, with such extensive knowledge of which genes lack imprinted expression in which tissues, such theories must now also explain the lack of imprinted expression among these genes and tissues, imprinted expression through life and the transitions from imprinted expression early in life to unimprinted expression later in life and vice versa.

Genome-wide list of imprinted genes in humans and mice is stabilizing

In cases where experimental crosses can be performed, the typical method for identifying genomic imprinting genome-wide is to reciprocally cross two inbred strains and quantify allele-specific expression (ASE) in the F1 offspring, often by transcriptome (RNA) sequencing [7, 8]. Hybridizing different

inbred strains ensures the existence of SNPs in each gene, and the reciprocal cross is used to distinguish parent-of-origin effects from strain-specific effects on expression. Early studies using this method discovered relatively few new imprinted genes [7, 8] beyond those already known at the time [9]. One RNA-seq study of imprinting in mouse embryonic and adult brain identified more than 1300 novel imprinted loci [10], but a later analysis by DeVeale, van der Kooy, and Babak [11] using different statistical methods failed to replicate the result. DeVeale, et al. attributed this discrepancy to experimental noise that was unaccounted for in the original analysis [11].

Babak et al. [3] have now expanded their RNA-seq analysis by combining data from 26 new tissues and developmental stages with previously published data from seven tissues to produce a map of genomic imprinting in the mouse that spans 33 tissues in embryonic and adult stages. Babak et al. [3] identified 74 new candidate imprinted genes, 12 of which were validated by pyrosequencing (Fig. 1). In another new study of ASE in mouse, Crowley et al. [5] looked for imprinted gene expression by performing all pairwise reciprocal crosses among three inbred mouse strains, each derived from a different subspecies. Their RNA-seq analysis of adult brain tissue from these crosses revealed only 54 new imprinted genes (Fig. 1), many of which exhibit only a slight expression bias towards one parental allele [5]. There is no overlap between the two lists of new imprinted genes in Babak et al. [3] and Crowley et al. [5]. This lack of overlap could be due to the fact that Babak et al. [3] searches widely over many tissues and developmental stages whereas Crowley et al. [5] looks only in adult brain tissue but has more power to detect imprinting due to the combination of three reciprocal crosses.

Assessing ASE and imprinting on a genome-wide scale in humans is more difficult because of the lack of controlled crosses. However, Babak et al. [3] and Baran et al. [4] have made great progress here by leveraging RNA-seq data derived from more than 40 tissues sampled from 178 post-mortem human donors (Genotype-Tissue Expression (GTEx) dataset; [12]). Baran et al. [4] included two additional RNA-seq datasets that added three tissues from 600 individuals. Babak et al. [3] and Baran et al. [4] detected imprinting in these RNA-seq data by looking for SNPs where one allele appeared to be monoallelically expressed in each individual, but where each SNP allele had an equal chance of being the expressed copy among all individuals. This method identifies loci with biased or monoallelic expression that is not due to cis regulatory variation, but it does not determine whether the candidate imprinted gene was maternally or paternally expressed. Parental origin was determined by using existing data on human imprinted genes and RNA-seq data from multigenerational families [3, 4]. Both studies found only a few new imprinted genes: 17 and 12 new candidates by Babak et al. [3] and Baran et al. [4], respectively (Fig. 1), that do not overlap.

Generally, Fig. 1 suggests that there are more imprinted genes in mice than in humans. Although the methods used to infer parental origin are necessarily different between the mouse and human studies (experimental crosses versus existing pedigrees), more mouse genes than human genes were known to be imprinted before the use of genome-wide techniques [9]. This suggests that the greater number of imprinted genes in the mouse may be a biologically robust pattern. Taken together, these genome-wide studies strongly suggest that our lists of imprinted genes are very nearly complete for both mouse and human.

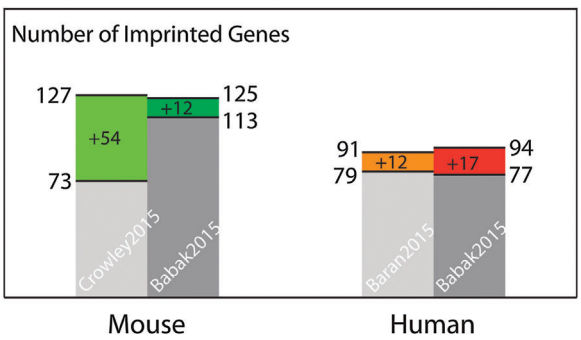

Figure 1. Number of imprinted genes in mouse and humans. This figure shows: the number of mouse imprinted genes known (in gray) and the new candidate imprinted genes (green) found in Crowley et al. [5] (light green) and in Babak et al. [3] (dark green); and the number of human imprinted genes known (in gray) and the new candidate imprinted genes (red) found in Baran et al. [4] (orange) and Babak et al. [3] (dark red). Known genes in each study are those with previous evidence of imprinting in the literature that also contained SNPs and were sufficiently expressed for evaluation.

Intragenomic conflict helps explain patterns in imprinted gene expression

These new results shed light on the evolutionary origins of genomic imprinting and how evolution modifies parent-of-origin marks following their establishment in gametogenesis [2, 13]. Although many other theories for the origin of imprinting have been discussed [6, 14], the data are consistent with an important role for intragenomic conflict, where maternally and paternally inherited alleles experience different inclusive fitness, and natural selection favors expression patterns that are conditional on parental origin. While this does not preclude the contribution of other factors to the evolution of imprinting [14, 15], it does affirm a central role for the forces captured by the formalism of the "kinship theory" [16–21]. The kinship theory predicts that if increased expression of a gene benefits the inclusive fitness of a paternally inherited allele more than the inclusive fitness of a maternally inherited allele, natural selection favors maternal silencing and paternal expression. On the contrary, if increased expression of a gene benefits the inclusive fitness of a maternally inherited allele more than the inclusive fitness of a paternally inherited allele, natural selection favors paternal silencing, and maternal expression [22, 23]. Differences between the inclusive fitness of maternally and paternally inherited alleles can result from many mechanisms that have sex-related effects on demography and life history [24], although the two most commonly discussed mechanisms are multiple paternity [22] and sex-biased dispersal [18–21].

In early development, multiple paternity means that siblings are less likely to carry the same copy of the paternally inherited allele than they are to carry the same copy of the maternally inherited allele. Thus, alleles coding for higher demand of maternal resources at the expense of resources available to her future offspring (by enhancing growth, for example) increase the inclusive fitness of the paternally inherited allele more than that of the maternally inherited allele. Alleles expressed in an embryo coding for reduced demand on maternal resources (e.g., by restricting growth) increase the inclusive fitness of the maternally inherited allele more than that of the paternally inherited allele for the same reason [23]. The resulting pattern for genes expressed in early development is that paternally expressed genes enhance growth, while maternally expressed genes restrict it [23].

Female-biased dispersal (all else being equal) means that offspring born in a local community are less likely to carry the same copy of the maternally inherited allele than they are to carry the same copy of the paternally-inherited allele. The inclusive fitness of a paternally (maternally) inherited allele is the sum of the direct effect of allelic expression on an individual's fitness and the indirect effect of allelic expression on the fitness of a neighboring individual times the probability the neighbor shares the same copy of the paternally (maternally) inherited allele. If gene expression in adults causes those individuals to take a larger share of the community's resources (by increasing the provision of parental care to their offspring for example), then the direct fitness effect will be positive and the indirect effect on neighbors will be negative, since those neighbors will have fewer resources for themselves. Genes that enhance demand on community resources therefore have a more positive effect on the inclusive fitness of the maternally inherited allele compared to the paternally inherited allele under female-biased dispersal. Similarly, genes expressed in an adult that reduce the demand on community resources (e.g., by increasing provision of communal care) increase the inclusive fitness of the paternally inherited allele more than that of the maternally inherited allele, since the direct fitness effect becomes negative (less resources for self), and the indirect fitness effect becomes positive (more resources for neighbors and their offspring) [18–21]. Thus, under female-biased dispersal, it is predicted that paternally expressed genes will tend to enhance selfless behaviors, while maternally expressed genes enhance selfish ones [18, 20, 21]. Male-biased dispersal and other ecological factors that cause adults in a community to be more related to their neighbors through their mothers[1] reverse the predicted pattern of expression [18–21]. Thus, the kinship theory establishes clear links between allelic expression, phenotype, and social structure both early and later in life [25].

[1]E.g. male-biased adult mortality can cause increased genetic relatedness through maternally inherited alleles relative to paternally inherited alleles since it enhances female lifespan relative to male lifespan and leads to lower turnover of adult females relative to adult males.

Higher level conflicts revealed by genome-wide data in diverse tissue types

Discussion of the effects of intra-genomic conflict on the evolution of genomic imprinting has been limited mostly to intra-locus conflict and inter-locus conflict. Conflict among cell lineages has rarely been considered (except ref [18]). The studies of Babak et al. [3], Baran et al. [4], and Crowley et al. [5] buttress growing evidence in support of inter-locus conflict, where some genes with opposing phenotypic effects also have opposite patterns of imprinted expression. These studies also observe variation in imprinting within and between tissues, which opens up the possibility of two forms of inter–cell lineage conflict, namely: intra-tissue conflict and inter-tissue conflict.

Intra-locus conflict takes place when natural selection favors higher expression of one of the allelic copies and lower expression of the other (Fig. 2.1) and results in silencing of the copy in which lower expression is favored [22]. For example: *Igf2* in mouse placental tissue increases the ability of nutrients to passively diffuse across the placenta, resulting in enhanced embryonic growth [26]; natural selection favors higher expression of the paternally inherited copy and lower expression of the maternally inherited copy and may explain why *Igf2* is paternally expressed.

Inter-locus conflict takes place when natural selection favors higher expression of the alleles at two loci with opposing phenotypic effects (Fig 2.2). This can result in

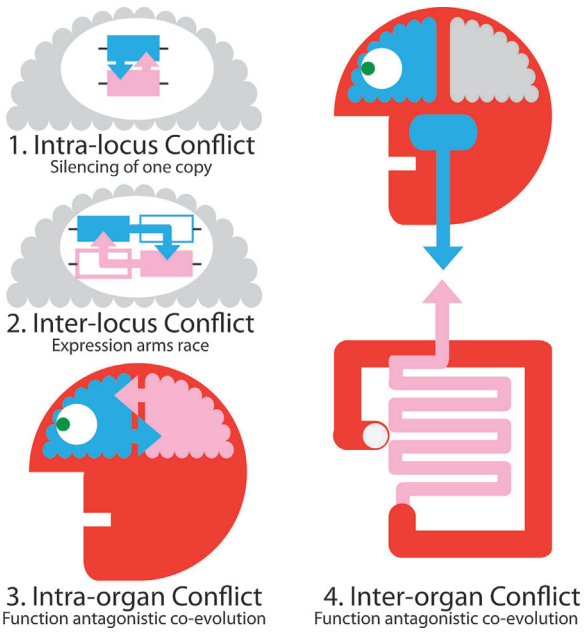

Figure 2. Types of conflict between imprinted genes. (1) Intra-locus conflict: conflict between genes at the same locus; (2) inter-locus conflict: conflict between genes at different loci. (3) Intra-organ conflict: conflict between genes expressed in two cell lineages of the same organ. (4) Inter-organ conflict: conflict between genes expressed in two cell lineages of different organs. Blue depicts either genes of paternal origin or cell lineages with paternally expressed genes. Pink depicts either genes of maternal origin or cell lineages with maternally expressed genes.

ever-increasing expression from one allelic copy at one locus and the opposite copy at the antagonistic locus [27, 28], which has the potential for pleiotropic fitness costs [29, 30]. For example: *Grb10* expression in mouse embryo inhibits its growth whereas *Dlk1* expression enhances the embryo's growth [31]; natural selection via inter-locus conflict can explain why *Grb10* is maternally expressed while *Dlk1* is paternally expressed and why alleles at these antagonistic loci might be over-expressed. In fact, Babak et al. [3] find general evidence of this sort of antagonistic expression: imprinted genes have higher expression than other genes, and there is an excess of maternal–paternal pairs among the most strongly co-expressed imprinted genes in mouse, patterns consistent with kinship theory's predictions of inter-locus conflict.

Inter-cell lineage conflict takes place when natural selection favors higher expression of the same loci in different cell lineages when expression in different cell lineages results in opposing phenotypic effect (Fig. 2.3 and 2.3). This can result in ever increasing expression of one allelic copy in one cell lineage and the opposite copy in the antagonistic cell lineage. There are at least two subtypes of inter-cell lineage conflict: (a) intra-organ conflict, when the two cell lineages are in the same organ (Fig 2.3); and (b) inter-organ conflict, when the two cell lineages are in different organs (Fig 2.4). For example: within the brain of mouse chimeras, cells with two maternal genomes concentrate in the frontal neocortex while cells with two paternal genomes concentrate in the hypothalamic and septal regions of the brain [32]; natural selection can explain why cells with greater expression of paternal genes would concentrate in regions of the brain controlling altruistic behaviors while cells with greater expression of maternal genes would concentrate in regions of the brain controlling egoistic behavior [18].

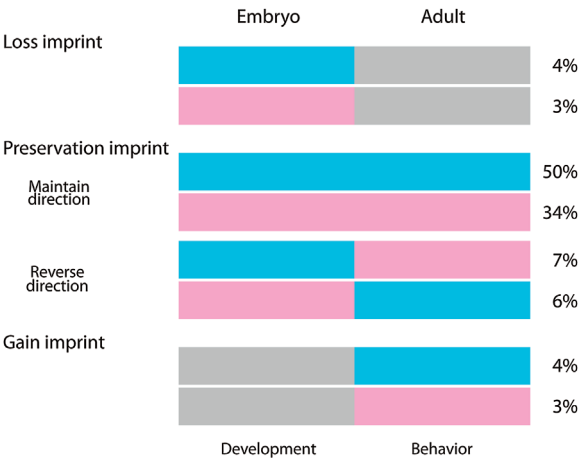

Figure 3. Temporal pattern of expression of imprinted genes in the mouse using the data from Babak et al. [3]. Loss of imprint: when a paternally or maternally expressed gene in the embryo is biallelically expressed in the adult; preservation of imprint: when an imprinted gene in the embryo continues to be imprinted in the adult; maintaining the direction: when a paternally or maternally expressed gene in the embryo continues to be paternally or maternally expressed in the adult; reversing the direction: when a paternally or maternally expressed gene in the embryo switches to be maternally or paternally expressed in the adult; gain of imprint: when a biallelically expressed gene in the embryo is monoallelically expressed in the adult from the maternally or paternally derived allele. The percentage of imprinted genes falling into each of these categories is indicated to the right of each category. Note that these percentages add up to more than 100; this is because several genes expressed in multiple tissues belong to more than one category (e.g., some genes both "maintain direction" and "reverse direction" depending on the tissue considered).

Imprinting status may vary across development time

Many genes are imprinted in embryo and adult

Most genes that exhibit imprinted expression in the embryo also exhibit imprinted expression in adults. In particular, over 83% of all imprinted genes found by Babak et al. [3] fall into this category (see Fig. 3).[2] Within this category there are two possibilities: (i) that genes paternally or maternally expressed in the embryo are paternally and maternally expressed in the adult tissue, respectively (maintenance of the expression pattern), and (ii) that genes paternally or maternally expressed in the embryo are maternally or paternally expressed in the adult tissue, respectively (reversal of the expression pattern).

While maintenance of the embryonic expression pattern in adults may or may not be driven by selection, reversal of the expression pattern is most likely indicative of selection. Natural selection may favor the expression of the opposite allelic copy in embryo and adult tissue in mammals when offspring transition after birth from maternal care only to a combination of maternal and paternal care [33]. It may also happen in the transition from infant to adult when individuals switch from interacting with their nuclear family to interacting within a social structure in which individuals are more patrilineally related – which may happen for example when there is a female-biased dispersal pattern [18, 24].

Mouse data from Babak et al. [3] show that only 11% of imprinted genes (with IS $>$ 2) reverse their expression patterns between embryonic and adult life stages (e.g., show paternal expression in one life stage and maternal expression in the other stage). Two of these genes, *Igf2* and *Grb10*, have been the focus of considerable research. *Igf2* was one of the first imprinted genes discovered and is expressed from the paternal allele in embryonic tissues [34, 35]. Disruption of paternally expressed *Igf2* results in prenatal undergrowth [34, 36]. This finding was a key piece of evidence in establishing the kinship theory, which predicts that fetal growth-enhancing genes will be paternally expressed. More recently, some work has suggested that *Igf2* may be expressed from the maternal allele in adult brain tissue [10, 37]. All three genome-wide studies reviewed here confirm this expression pattern (paternal in embryos, but maternal in adult brain) (Refs [3, 5] in mice; Ref. [4] in humans). Work in mice suggests that *Igf2* expression in adult brain has a role in neurogenesis in the hippocampus [38, 39], but it is still unclear how this

[2]There are 103 genes that received an imprinting score (IS) greater than two in Babak et al. [3]. See Ref. [3] for methodological details on IS.

neurogenesis affects behaviors that might be under natural selection.

The other well studied gene with a pattern of reversal, *Grb10*, is expressed from the maternally derived allele in fetal and extra-embryonic tissues, and knockouts of maternal expression lead to increased growth of the embryo and placenta [40, 41]. In adults, *Grb10* is expressed from the paternal allele in the brain, and knockouts of paternal expression result in increased displays of certain measures of social dominance [42]. Babak et al. [3] and Cowley et al. [5] both confirm paternal expression in the brain in mice, and Baran et al. [4] shows opposing expression patterns in human brain versus other tissues. This concordance between human and mouse for the reciprocal imprinting of *Grb10* suggests that paternal *GRB10* expression in humans may have interesting effects on social behavior. The reversal of imprinted expression for *Grb10*/*GRB10* is also important on a mechanistic level as it reveals how stage or tissue-specific patterns of genomic imprinting are established at the molecular level. In both mice and humans, *Grb10*/*GRB10* expression from the maternal allele arises from a major promoter upstream of the differentially methylated region (DMR) that is necessary for imprinting of the gene [43, 44]. Expression from the paternal allele in brain tissue derives from alternative promoters downstream of the major promoter and located near the DMR [43, 44]. Thus, stage or tissue-specific patterns of imprinting do not require the reversal of epigenetic imprints established in the gametes.

Some genes are imprinted in embryo and unimprinted in adult

Some genes that exhibit imprinted expression in embryo lose their imprinting at some point in development and exhibit biallelic expression in adults. About 7% of all imprinted genes (with IS >2) found by Babak et al. [3] fall into this category, including the newly discovered maternally expressed gene *Dact2*. Even in the absence of selective pressure to maintain imprinted expression in the adult tissue, the kinship theory does not necessarily predict the loss of imprinted expression [45]. However, the consistent absence of imprinted expression in a specific tissues or at specific developmental stages could be the outcome of natural selection for biallelic expression via other mechanisms [14].

Other genes are unimprinted in embryo and imprinted in adult

Babak et al. [3] find only a handful of genes that exhibit biallelic expression in embryonic tissues and imprinted expression in adult tissues (6%), including the genes *Tnk1* (paternally expressed in bone marrow) and *Edn3* (maternally expressed in the adrenal gland), both of which were not previously known to be imprinted.

Consensus exists for a global paternal bias in imprinted gene expression

Finally, all three genome-wide studies find that paternally expressed genes outnumber maternally expressed ones. In Babak et al. [3], 34% of imprinted genes were expressed from the maternal allele when averaged across all tissues. This fraction changes slightly when looking at specific tissue categories (Table 1). Embryonic (i.e., fetal) tissues show the highest fraction of maternally expressed genes, 40%, whereas extra-embryonic tissues show the lowest, 27%. Adult tissues have fewer maternally expressed genes than embryonic tissues: 33% are expressed maternally in non-neural adult tissues and 37% in adult neural tissues. Embryonic neural tissues have a maternally expressed fraction, 38%, that is similar to adult neural tissues. Crowley et al. [5] looked only at brain tissue only in adult mice,where they found that 41% of imprinted genes are expressed maternally, similar to the fraction reported by Babak et al. [3]. Baran et al. [4] had a more limited ability to detect maternal and paternal imprinting specifically, but did find that maternally expressed genes are imprinted in fewer tissues than paternally expressed genes.

The kinship theory predicts a bias in favor of paternal expression at imprinted loci resulting from the genome-wide epigenetic reprogramming that follows fertilization. Maternal-store proteins carried by the egg have an inclusive fitness that is different from either maternally or paternally inherited alleles in the offspring. This creates a three-way conflict in early development [46], in which the maternal-store proteins

Table 1. The mean fraction of imprinted genes that are maternally expressed and the mean number of genes imprinted are calculated across tissues in each tissue category in the mouse data from Babak et al. [3]

Tissue category	Number of tissues	Fraction maternal	Mean # genes imprinted
Extra-embryonic	3	0.27	33
Non-neural	23	0.33	22
Non-neural adult	19	0.33	20
All tissues	33	0.34	32
Non-embryonic	27	0.34	29
Neural adult	8	0.37	51
Neural embryonic	2	0.38	70
Embryonic	3	0.40	57

See Fig. 1 of Babak et al. [3] for a list of the tissue types and imprinted genes and the full gene-by-tissue pattern of imprinted and biallelic expression. In obtaining the above numbers, a threshold of 2 was used for the minimum imprinting score from Babak et al. [3]. These numbers depend additionally on other thresholds and filters in the bioinformatic pipeline (T. Babak, pers. comm.), and thus are more reliably interpreted relative to one another than as absolute magnitudes.

are selected to reverse certain paternal epigenetic marks [47], making direct paternal silencing evolutionarily less stable than direct maternal silencing, and potentially driving asymmetries in the way the two sets of genes are silenced [48].

The outcome of this asymmetric three-way conflict can be seen in the fact that that the primary methylation marks required for imprinting are predominantly found in DMRs in female gametes, whereas only a few DMRs are methylated in male gametes [49, 50]. Thus, genes that are paternally silenced are more likely to rely on indirect mechanisms for silencing rather than direct silencing through methylation of the promoter or other cis-regulatory elements. It is possible that such indirect mechanisms maybe more difficult to evolve, or their molecular machinery maybe more difficult to maintain.

Consistent with this prediction, Babak et al. [3] find that new, species-specific imprinted genes are more likely to be paternally silenced, while genes where imprinting is older are predominantly maternally silenced. This same logic would seem to predict that the bias in favor of paternal expression would be more pronounced at early developmental stages. However, comparison across tissues reveals a more complex pattern in the relative abundances of maternally and paternally expressed imprinted genes (Table 1). The fact that the bias toward maternal silencing is more pronounced in extra-embryonic tissues than in adults seems consistent with an early bias that becomes attenuated via developmental reprogramming. However, the fact that embryonic tissues show less bias than either extra-embryonic or adult tissues is not an obvious consequence of either the kinship theory or known mechanistic constraints. This complex pattern of spatio-temporal variation in expression bias from imprinted loci deserves further study.

Discussion

One of the interesting results of the Babak et al. [3] study is the relative absence of imprinting in adult tissues outside of the central nervous system. Negative results tend not to get much attention in science, but this one settles a long-standing question. It has been unclear whether the domination of the imprinting literature by genes involved in development and cognition was reflective of reality or simply due to ascertainment bias. This result favors the interpretation as a real effect and suggests that we should focus future efforts on these two domains.

Evolution of imprinting related to growth effects should be qualitatively similar across mammals: while rates of multiple paternity vary across species, it is always the mother who gets pregnant. For these effects, the standard molecular-biology approach of focusing on a few model species will tell us most of the story. Imprinted genes affecting cognition and behavior may be more variable because of the greater diversity in social structures among mammals. A number of cognitive and behavioral traits have been connected to imprinted genes [51], but models in this domain are sensitive to a number of factors, including dispersal, patterns of parental care and alloparenting, and the nature and importance of social interaction [18–21, 24]. Understanding imprinting in the brain will require a much more taxonomically extensive comparative approach.

Patterns in tissue-specific imprinting reveal the importance of both evolutionary and mechanistic hypotheses

The patterns of tissue-specific imprinting pose a set of interdependent mechanistic and evolutionary questions. The first question is what aspects of tissue-specific imprinting require an evolutionary explanation at all? To answer that question, we need to understand what we would expect those tissue-specific patterns to look like in the absence of selection for or against imprinted expression in adult tissues. We know that the molecular mechanisms of imprinting are largely the same as the mechanisms involved in tissue differentiation. The same machinery propagates epigenetic marks like cytosine methylation and histone modifications at imprinted and unimprinted loci alike.

One possible prediction suggested by this shared machinery is that imprinted expression established in early development should be passively inherited by all adult tissues. So, we would expect a gene that is maternally silenced due to an early-development growth effect to be maternally silenced in all tissues. In that case, any tissue in which the gene was biallelically expressed would beg an evolutionary explanation. We would want to identify the selective pressure that favored biallelic expression in those tissues and recruited the machinery required for epigenetic reprogramming at specific points in development.

But another possible prediction is that biallelic expression could arise in a particular tissue as a side-effect of the epigenetic changes underlying tissue differentiation. Many of the epigenetic changes occurring throughout development are shared across broadly divergent taxa, making them both ancient relative to mammalian imprinting and subject to strong purifying selection and canalization. In cases where normal development disrupts imprinting, our evolutionary question would be reversed. We would need to identify the selective pressures responsible for maintaining imprinted expression in specific adult tissues.

So a part of the next challenge facing us will be determining which genes (in which species and which cell types) require which sort of evolutionary explanation. In certain cases (e.g., the reversal of the direction of imprinting of *Grb10* and *Igf2* in the brain), the complexity of the pattern of expression is sufficient to suggest the need for a specific evolutionary explanation. In other cases, framing the evolutionary questions will require careful analysis in the context of the pattern of epigenetic reprogramming in normal development and comparison among a larger number of taxa.

In cases where the transition from monoallelic to biallelic expression in specific tissues is the result of natural selection, there are at least two potential sources of that selective pressure. One possibility is that imprinting could be lost as a result of a reversal of the selection asymmetry acting on the gene [52]. That is, a gene might be paternally expressed early in development because natural selection favored higher expression from alleles when they were paternally inherited. If

that same gene exhibited a pleiotropic effect in some adult tissue, where higher expression was favored by maternally inherited alleles, this could lead to reactivation of the silenced allele in that tissue.

Another possibility is that there may be a selective advantage of having two active alleles because it would provide a defense against deleterious recessive somatic mutations. Protection against inherited deleterious recessive mutations is unlikely to provide significant selection against imprinting with the reasons being both the weakness of selection [53] and the fact that deleterious loss-of-function mutations at genes that evolve imprinting are unlikely to be recessive. However, depending on the nature of the gene and its mechanisms of regulation and action in a specific tissue, protection against somatic mutation could provide a significant selective force favoring biallelic expression.

Why so few imprinted genes?

The other question raised by our near-complete list of imprinted genes is, why are there so few of them? The logic of the kinship theory applies to any gene where the overall level of expression that maximizes inclusive fitness differs between maternally and paternally inherited alleles. If we imagine selection to be all-powerful, we might expect imprinting to be common, or even universal, as there is probably no gene in any species where the inclusive fitness effects of changes in gene expression are *exactly* identical for maternally and paternally inherited alleles.

The fact that imprinted expression appears to be limited to fewer than 1% of the genes in mouse and human suggests that there must, in fact, be significant factors favoring biallelic expression. Part of the explanation may simply be that imprinted gene expression is complicated, and the selection asymmetry on most genes is weak. As with any other evolved trait, selection must be sufficiently strong and persistent to overcome the effects of mutation and drift. Another part of the explanation may be that most genes are not that dosage sensitive, or have regulatory feedback loops that buffer against environmental or allelic variation in expression. The fact that most deleterious loss-of-function mutations are recessive does not provide strong selection against imprinting in and of itself. However, it does indicate that most genes can suffer a 50% reduction in expression without substantial effects on individual fitness. At such a locus, the differential effect on matrilineal and patrilineal inclusive fitness resulting from a 50% reduction in gene expression is likely to be vanishingly small.

Conclusions and outlook

RNA-seq has now successfully defined the scope of genomic imprinting in mammals, and other tools from molecular genetics have gone a long way towards elucidating the mechanisms through which imprinted gene expression is achieved. These advances set the stage for us to pursue a more thorough and detailed understanding of the causes and consequences of imprinting, particularly in the context of its relationship to cognition and behavior. For example, imprinted genes, like *Grb10*, with effects on a social behavior, like social dominance, interact with other imprinted and unimprinted genes that also affect that behavior. Genome-wide data on imprinted expression allow the construction of more detailed hypotheses of how interactions among these genes result in effects on social behavior and in selection for imprinted expression at some loci but not others. However, properly testing this next set of hypotheses is going to require a more profoundly comparative approach that looks at a broader set of species. Comparative data among diverse species provide variation in demographic parameters, such as sex-specific dispersal, that drive selection for imprinted gene expression in the kinship theory. Testing these hypotheses will also require integration of knowledge from evolutionary biology, behavioral ecology, and neurobiology to understand the complex interplay of natural selection and development within the context of ecological and mechanistic constraints.

Conflict of interest

The authors have declared no conflict of interest.

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