Genomic imprinting and parent-of-origin effects on complex traits

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Abstract | Parent-of-origin effects occur when the phenotypic effect of an allele depends on whether it is inherited from the mother or the father. Several phenomena can cause parent-of-origin effects, but the best characterized is parent-of-origin-dependent gene expression associated with genomic imprinting. The development of new mapping approaches applied to the growing abundance of genomic data has demonstrated that imprinted genes can be important contributors to complex trait variation. Therefore, to understand the genetic architecture and evolution of complex traits, including complex diseases and traits of agricultural importance, it is crucial to account for these parent-of-origin effects. Here, we discuss patterns of phenotypic variation associated with imprinting, evidence supporting its role in complex trait variation and approaches for identifying its molecular signatures.

Epigenetic
Pertaining to a difference in phenotype resulting from variations in DNA chemistry rather than DNA sequence. Epigenetic changes can be cell specific, can be modified by environmental factors, can affect gene expression and may underlie some parent-of-origin effects on complex traits.

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Parent-of-origin effects are epigenetic phenomena that appear as phenotypic differences between heterozygotes depending on the allelic parent of origin. Genomic imprinting (hereafter referred to as 'imprinting') results in two alleles at a locus being functionally non-equivalent and is considered to be the primary epigenetic phenomenon that can lead to the manifestation of parent-of-origin effects[1]. Imprinted loci show parent-of-origin-dependent gene expression and have been observed in mammals, flowering plants and insects[2]. However, the taxonomic distribution and the breadth of imprinting remain uncertain. Imprinting seems to play an important part in modulating sets of complex traits, notably in early development (including embryonic, placental and seed development) and behaviour (especially social behaviour)[1,3–5]. Much of our understanding of the phenotypic consequences of imprinting comes from gross genetic anomalies such as uniparental disomies, translocations, loss-of-function mutations and loss-of-imprinting epimutations, some of which are associated with complex disorders (for example, Prader–Willi, Beckwith–Weidemann and Angelman syndromes)[1]. Genes (or more generally, loci) associated with these disorders show the signature of imprinting manifested as parent-of-origin-dependent effects[6], with the anomalous phenotype depending on which parent the causal allele (or alleles) is inherited from, rather than an individual's diploid genotype.

Parent-of-origin effects are often considered synonymous with imprinting, but there are other scenarios that can lead to the appearance of a parent-of-origin effect in the absence of imprinting (see below). Here, we review recent developments in understanding the role of imprinting as a parent-of-origin effect underlying complex trait variation and provide a primer on approaches that can be used to identify and examine the contribution of imprinted loci to aspects of genetic architecture. Studies suggest that imprinted loci are important contributors to phenotypic variation[7–10], despite the fact that imprinting per se has been confirmed in a small proportion of all genes (<1% in humans or mice[11] and an even smaller percentage in plants[12]). However, most studies of complex traits have not implemented models that allow for the non-equivalence of parental alleles (that is, allow the identification of parent-of-origin effects), so the number and effects of imprinted genes remain important open questions. Studies that consider genetic and epigenetic variation at imprinted loci as a source of natural variation in complex traits can not only potentially identify additional imprinted genes but also reveal an important component of heritable variation that remains 'hidden' in traditional complex trait studies.

Other parent-of-origin effects

In this Review, we focus on imprinting, so first it is useful to consider other scenarios that can lead to the appearance of a parent-of-origin effect in the absence

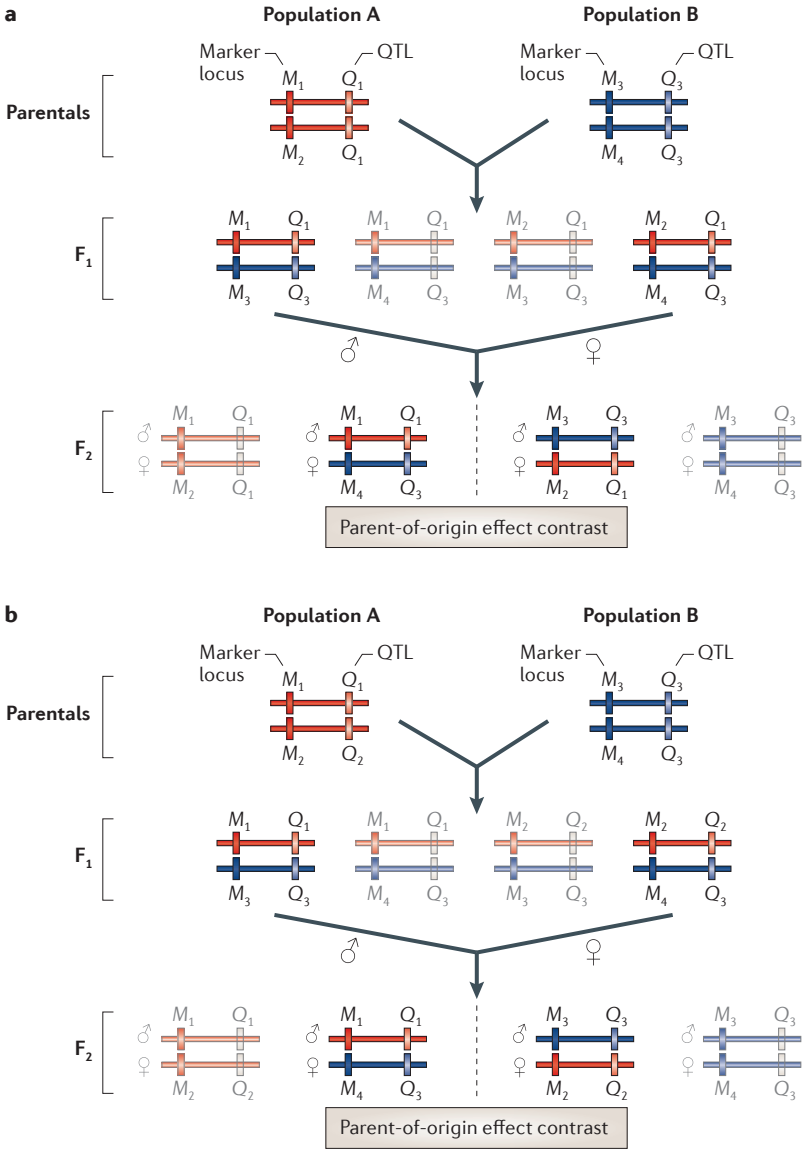


Figure 1 | **The line-cross design and the appearance of pseudo-imprinted loci.** Genetically variable individuals from two parental populations, A and B, are intercrossed to produce an F_2 population. Haplotypes are composed of a marker locus (*M*) and a linked quantitative trait locus (QTL) (*Q*). Haplotypes originating from population A are in red and those from population B are in blue. The marker locus has two alleles in each population, with markers M_1 and M_2 from population A and M_3 and M_4 from population B. The F_1 intercross contains four possible unordered genotypes. The F_2 population resulting from a random intercross of these F_1 genotypes would produce 16 possible ordered genotypes, but for simplicity only the cross between the M_1M_3 and M_2M_4 F_1 genotypes is illustrated. This cross produces four ordered F_2 genotypes, with the paternally inherited allele shown above the maternally inherited allele. The two genotypes that contain a marker allele from each of the A and B parental populations (M_1M_4 and M_3M_2) contribute to the parent-of-origin effect contrast. **a** | In the first scenario, the parental populations are fixed for alternative QTL alleles (Q_1 and Q_3). The parent-of-origin effect contrast therefore represents a comparison between the phenotypes of the Q_1Q_3 and Q_3Q_1 genotypes, which are genetically equivalent at the QTL but differ in the parent-of-origin of alleles. **b** | In the second scenario, population A has segregating variation at the QTL locus, with alleles Q_1 and Q_2, which are linked to markers M_1 and M_2, respectively. As a result, the parent-of-origin effect contrast represents a comparison between the phenotypes of the Q_1Q_3 and Q_3Q_2 genotypes, which are not genetically equivalent, and hence the contrast confounds the parent of origin of alleles at the marker locus and allelic differences at the QTL locus.

of imprinting. One scenario is that the reciprocal heterozygotes actually have a genetic difference. For example, gene-specific trinucleotide expansions can have sex-specific biases in occurrence and therefore transmission, and parent-of-origin effects resulting from such expansions have been associated with disorders such as myotonic dystrophy type 1 (REFS 13,14).

Genetic differences between reciprocal heterozygotes are particularly problematic for discovery research using a line-cross design[15] in which individuals from two variable parental populations are intercrossed to produce an experimental population[16,17], as illustrated in FIG. 1. In this scenario, spurious imprinting effects can arise when the assumption that the parental strains are fixed for quantitative trait locus (QTL) differences but have segregating marker variation is violated. Most problematically, the conditions that make a marker locus informative for detecting a parent-of-origin effect (segregating variation at marker loci in parental lines) are the same conditions that can lead to spurious results (segregating variation at linked QTLs in parental lines). The assumptions of the line-cross design are unlikely to be made in studies of natural variation (such as most human studies), and hence the problem of spurious results produced by this phenomenon is unlikely to apply to most approaches used to study parent-of-origin effects.

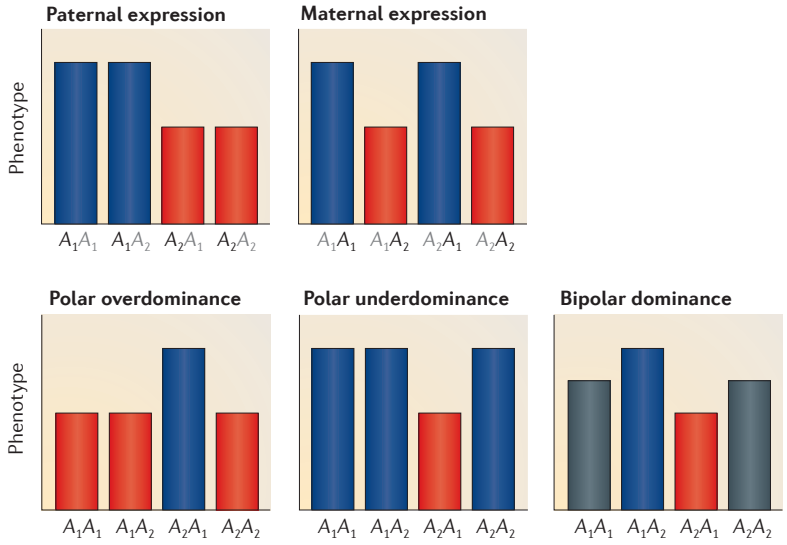
Another confounding factor is parental genetic effects[18]. In mammals, studies of parental effects have focused on maternal effects; however, paternal effects are equally plausible (but presumably less common). From a single-locus perspective, parental genetic effects occur when a locus expressed in mothers (or fathers) has some causal influence on the phenotype of her (or his) offspring[19]. For example, maternal genetic effects have been observed in a mouse model of anxiety, in which offspring that were born to mothers heterozygous for a knockout of 5-hydroxytryptamine (serotonin) receptor 1A (*Htr1a*) but that did not inherit the mutation themselves displayed an anxiety-like phenotype[20]. Maternal genetic effects can lead to the appearance of a parent-of-origin effect when mothers that are homozygous for different alleles have distinct phenotypic effects on their offspring. Because these homozygous mothers can each produce only one type of reciprocal heterozygote, such a maternal genetic effect is expected to lead to a difference in the average phenotypes of the reciprocal heterozygous offspring.

Other phenomena that can result in differences between reciprocal heterozygotes include random monoallelic expression and environmentally mediated epigenetic silencing. However, these processes are not expected to produce parent-of-origin-dependent patterns and are not considered here.

Identifying phenotypic signatures of imprinted loci

Our understanding of the genotype–phenotype relationship is largely conceptualized through the use of a single locus with two alleles. Within this framework, there are three genotype classes if the reciprocal heterozygotes are grouped into a single class because they are genetically equivalent. However, to understand the contribution of

Box 1 | **Classification of imprinting patterns**



We expect that parent-of-origin-dependent monoallelic expression of a single gene will produce a pattern of phenotypic variation in which the phenotypic effect of a locus is determined entirely by the single allele that is expressed (that is, by the paternally inherited allele for a paternally expressed locus and by the maternally inherited allele for a maternally expressed locus). Thus, monoallelic parent-of-origin-dependent expression leads to what has been called 'parental imprinting' (REFS 8,9), in which the canonical patterns of maternal versus paternal expression depend on whether genotypes group by the maternally versus the paternally inherited allele.

The patterns of phenotypic variation expected for paternal and maternal expression (see the figure) are illustrated in the top two graphs, which show the expected phenotypic value for the four possible ordered genotypes at the *A* locus (with the first allele listed being inherited from the father and the second from the mother). In both cases, the A_1 allele leads to a larger phenotypic value than the A_2 allele, and one allele is silenced (grey font). In each case, genotypes group phenotypically by allelic parent of origin, as indicated by their shading.

Although most studies have constrained their analysis to parental forms of imprinting, those that have not have generally identified loci showing 'dominance' imprinting patterns[8–10,74], in which the pattern of effect depends on the combination of alleles. Dominance imprinting occurs in the polar overdominance phenotype associated with the callipyge mutation in sheep[48] and has also been observed in humans[50] and mice[10,32,74]. Polar overdominance shows the signature of an imprinted locus manifested as a difference between the phenotypes of the reciprocal heterozygotes, but it lacks the expected difference between the two homozygotes that should occur under parent-of-origin-dependent monoallelic expression. With polar overdominance, the phenotype of a heterozygote is larger than that of the other genotypes, but there is 'polarity' because the dominance appears only in one of the two heterozygote configurations (see the figure). Similarly, it is also possible for a locus to show a pattern of polar underdominance, in which one of the heterozygotes has a smaller phenotypic value than the other genotypes (see the figure). For both polar over- and underdominance, the two homozygotes group phenotypically with one of the heterozygotes, but the phenomena differ in the pattern of the grouping.

Finally, it is possible for a locus to show both under- and overdominance at the same time, with one heterozygote having a phenotypic value larger than the two homozygotes and the other heterozygote having a value that is smaller (see the figure). This pattern of 'bipolar dominance' (REF. 8) reflects the opposing polarity of the heterozygotes.

imprinting to the genotype–phenotype relationship, we need to characterize the genetically equivalent, but potentially phenotypically non-equivalent, reciprocal heterozygotes as distinct genotype classes[7,21]. This increase in the number of genotype classes provides the critical extra degree of freedom required to test for the presence of imprinting. If a locus is imprinted, we expect these two classes to express different alleles (BOX 1). Imprinting will manifest as genotype classes that vary phenotypically according to allelic parent of origin, forming the foundation of studies aimed at identifying imprinting effects on complex traits (BOX 2).

Genomic imprinting
An epigenetic phenomenon in which the expression of a gene occurs in a parent-of-origin-dependent manner.

Assigning parent of origin to alleles

The crucial first step in analysing imprinting effects is assigning parent of origin to alleles. The earliest studies used the line-cross design[15], which is based on F_2 intercross populations in which non-inbred parental lines are crossed[7,17,21,22]. The parent of origin of marker alleles is assigned by identifying the grandparent of origin of an allele (which requires genotyping of founders). This approach has been used to identify imprinting effects on body composition in pigs, but it has been criticized because it can lead to the appearance of imprinting when there are QTLs segregating in the parental strains[16] (FIG. 1). Furthermore, this approach cannot be used to study imprinting effects using biallelic loci, so many genomic regions are uninformative[21].

Studies in mice have used a backcross design[23] in which F_1 heterozygotes from an inbred line cross (which have the unordered genotype A_1A_2) are backcrossed to either parental strain. The parent of origin of alleles in all heterozygotes produced in each backcross can be directly inferred. For example, if an A_1A_2 male is backcrossed to a female from the A_1A_1 parental line, then all heterozygous offspring will have received the A_1 allele from their mother and A_2 from their father. Such a design, although intuitive, fully confounds maternal genetic effects with imprinting effects[18] and restricts patterns of variation across the genome (as backcrossed populations are necessarily missing subsets of possible multilocus allelic combinations).

Other studies have used an F_2 generation of intercrosses between inbred strains, in which individuals are produced by genetically identical F_1 parents (and hence the pedigree contains no information about allelic parent of origin)[23]. Allelic parent of origin in such a population can be inferred if there are sex differences in recombination rates and sufficient marker information to determine the number of recombination events on each chromosomal haplotype. This approach has been used in mice, relying on the fact that females have higher recombination rates than males, which is common in mammals[24]. However, this approach can only be implemented in systems in which there is a large sex difference in recombination rates and in which it is possible to accurately determine the number of recombination events present on each haplotype. Sex differences in recombination rates can be small, and this approach therefore lacks power owing to high error rates.

In samples in which parents are genetically variable (such as an advanced intercross), one can simply genotype parents and their offspring and then directly infer allelic inheritance. Allelic parent of origin can be determined for all heterozygous offspring produced by all matings between a heterozygote and a homozygote parent or between two opposite homozygote parents. In

Complex traits
Quantitative traits that are influenced by many genetic, epigenetic and environmental factors and their interactions.

a population with genotypes in Hardy–Weinberg proportions, this approach can be used to assign the parent of origin to alleles in at least three-quarters of all heterozygotes (the proportion of uninformative heterozygotes under random mating is approximately pq, where p and q are the frequencies of the two alleles at a locus). The only families in which the parent of origin of alleles cannot be directly inferred are those families in which both parents are heterozygotes at the locus in question. Studies in human populations have used family-based genotype information (parent–offspring trios) to assign the parent of origin to offspring alleles, and transmission disequilibrium tests (TDTs) can be used to identify biased transmission of the parental alleles[25,26]. TDT methods are robust to the confounding effects of population admixture and stratification; however, they are generally underpowered because they do not account for between-family variation in human samples[27].

Although matings between two heterozygous parents do not allow direct inference of the parent of origin of alleles, it is possible to use linkage information to infer allelic parent of origin in some or all cases (depending on marker density). That is, if allelic parent of origin at a locus cannot be determined directly, but the locus is linked to informative loci, the linked marker information can be used to infer allelic parent of origin at the ambiguous locus (or assign a conditional probability that each allele came from each parent[7,17]). This process can be efficiently achieved through haplotype reconstruction approaches, wherein entire chromosomal haplotypes are assigned a parent of origin on the basis of algorithms that determine the most likely haplotype configuration in a population[8,28], or through approaches that more generally use linkage information to assign parent-of-origin probabilities to alleles. Recently, extended pedigree information was used to assign the parent of origin of haplotypes using a likelihood-based framework in >38,000 Icelanders[29].

Box 2 | Genetic effects and mapping models

There are several different statistical frameworks used to identify imprinting effects, but the vast majority are built on the approach pioneered by Knott *et al.*[21] (which was formalized by Mantey *et al.*[22] and refined by others[7,8]). This framework is an extension of the single-locus two-allele model underlying most mapping studies. Using unordered genotypes, the simplest mapping model is a regression model built on the classic quantitative genetics model with additive and dominance effects. The additive effect (a) corresponds to a contrast between the two homozygotes, whereas the dominance effect (d) measures the deviation of the heterozygote from the midpoint (unweighted average) of the two homozygotes[75]. This model can be expressed as a linear equation wherein the mean phenotypes of the three genotypes at a locus (indicated by the genotype ID with the overbar)[22] are decomposed into additive and dominance effects:

$$\begin{bmatrix}\overline{A_1A_1}\\ \overline{A_1A_2}\\ \overline{A_2A_2}\end{bmatrix} = \begin{bmatrix}1 & 1 & 0\\ 1 & 0 & 1\\ 1 & -1 & 0\end{bmatrix}\begin{bmatrix}r\\ a\\ d\end{bmatrix}$$

in which r is the reference point (intercept) for the model. In this case, r is the midpoint between the two homozygotes:

$$r = \frac{\left[\overline{A_1A_1} + \overline{A_2A_2}\right]}{2}.$$

Under this model, the estimated additive effect corresponds to half the difference between the means of the two homozygotes:

$$a = \frac{\left[\overline{A_1A_1} - \overline{A_2A_2}\right]}{2},$$

whereas the dominance effect corresponds to the deviation of the mean heterozygote phenotype from the midpoint between the two homozygotes:

$$d = \frac{\overline{A_1A_2} - \left[\overline{A_1A_1} + \overline{A_2A_2}\right]}{2}.$$

To estimate imprinting effects, this model uses ordered genotypes[21], allowing the estimation of an additional parameter, the imprinting effect (i)[8,22]:

$$\begin{bmatrix}\overline{A_1A_1}\\ \overline{A_1A_2}\\ \overline{A_2A_1}\\ \overline{A_2A_2}\end{bmatrix} = \begin{bmatrix}1 & 1 & 0 & 0\\ 1 & 0 & 1 & 1\\ 1 & 0 & 1 & -1\\ 1 & -1 & 0 & 0\end{bmatrix}\begin{bmatrix}r\\ a\\ d\\ i\end{bmatrix}.$$

This model has the same reference point (intercept) and yields the same definitions of the additive and dominance effects as the unordered genotype model, except that the mean heterozygote in the unordered model is replaced by the mean of the reciprocal heterozygotes:

$$\frac{\left[\overline{A_1A_2} + \overline{A_2A_1}\right]}{2}.$$

Under this model, the imprinting effect is defined as half the difference in the mean phenotypes of the reciprocal heterozygotes:

$$i = \frac{\left[\overline{A_1A_2} - \overline{A_2A_1}\right]}{2}.$$

If there is complete silencing of an allele, we expect a locus showing paternal expression to have $a = i$, whereas maternal expression would correspond to $a = -i$.

Imprinting effects on complex traits

Do candidate imprinted QTLs map to known imprinted regions? Studies assigning the parent of origin to alleles and subsequently mapping QTLs with parent-of-origin-dependent effects in model organisms have had mixed success in linking loci to known imprinted regions. For example, one of the first analyses of imprinted QTLs (using a porcine intercross for body composition[7]) found that three of the four candidate imprinted QTLs identified fell outside known imprinted regions. Another study[8] mapping body weight and growth in a mouse intercross found little overlap between known imprinted genes and candidate imprinted QTLs (only two of ten loci overlapped confirmed imprinted genes), but all candidate imprinted QTLs contained multiple genes that were predicted to be imprinted by bioinformatic approaches (discussed below)[30]. Similar patterns, where most of these QTLs map to regions that do not contain known imprinted genes but do contain bioinformatically predicted imprinted genes, were found in a study of bovine growth and body composition[31].

As discussed above, most known imprinted genes are associated with gross genetic anomalies, but QTL studies identify genomic regions that are associated with normally distributed phenotypic variation. Although some candidate imprinted QTLs may result from other parent-of-origin effects (see above), these mapping results suggest that there are more imprinted genes than have been characterized to date and that imprinted genes are likely to be associated with normal phenotypic variation. Indeed, recent studies on an F_{16} generation of

a randomly mated advanced intercross of the LG/J and SM/J inbred mouse lines found evidence that imprinted genetic effects are almost as prevalent as additive genetic effects for multiple metabolic traits: 40 QTLs were found to be associated with variation in adiposity[32], 64 with variation in diabetes-related traits[10] and 23 with variation in serum lipid levels[33]. Almost all of these QTLs had additive effects, and about 60% had imprinted effects. Although these candidate imprinted QTLs have yet to be validated, simulation studies in an earlier generation of this intercross indicate that the distribution of false positives for imprinting effects is the same as that for additive and/or dominance effects[8] (that is, there is no bias for the appearance of parent-of-origin effects). Thus, as with all QTLs, loci showing apparent imprinted genetic effects should be treated as candidates that require validation. Such caution is especially crucial for QTL mapping to regions with no known imprinted genes.

Studies analysing known imprinted genes for association with phenotypic variation. When studies have specifically targeted known imprinted genes for association with normal variation rather than with gross genetic anomalies, results indicate that these genes have important roles in complex traits. For example, a study in cattle[34] targeted a series of single-nucleotide polymorphisms (SNPs) in eight candidate imprinted genes (calcitonin receptor (*CALCR*), growth factor receptor-bound protein 10 (*GRB10*), paternally expressed 3 (*PEG3*), pleckstrin homology-like domain, family A, member 2 (*PHLDA2*), RAS protein-specific guanine nucleotide-releasing factor 1 (*RASGRF1*), tetraspanin 32 (*TSPAN32*), zinc-finger, imprinted 2 (*ZIM2*) and zinc-finger protein 215 (*ZNF215*)) and found that six had significant associations with various traits. However, it should be noted that only *PEG3* has been shown to be imprinted in cattle, and the associations were not examined with regard to allelic parent of origin.

Other studies have focused on the contribution of the imprinted gene insulin-like growth factor 2 (*IGF2*) to traits such as variation in meat-quality characteristics in pigs after a QTL mapping study found that a locus containing this gene was strongly associated with variation in muscle mass[35–38]. This paternally expressed locus was found to have major effects on lean meat content in ham, accounting for 30% of the phenotypic variance in this trait[37]. The QTL was fine-mapped[35], and an intronic SNP affecting *IGF2* expression in postnatal muscle was identified[38]. This SNP was found to abate binding of the repressor zinc-finger BED domain-containing protein 6 (ZBED6) to the *IGF2* locus, leading to a rise in *IGF2* expression and increased muscle mass[39]. Other SNPs in *IGF2* have been associated with milk-quality traits in dairy cows[40]. It is hypothesized that breeding schemes focusing intensive selection on males could favour such variation at paternally expressed loci[37].

Human complex traits. In human studies, parent-of-origin effects have been implicated in many complex disorders, including Alzheimer disease[41], autism[42], bipolar disorder and schizophrenia[43,44], cancer[29], adiposity and type 2 diabetes[29,45,46], and type 1 diabetes[26]. Although most of these results have not been validated, they do suggest that imprinting effects underlie some of the variation observed in these traits. Unfortunately, human samples often do not have data available to determine the parent of origin of alleles and/or are unlikely to incorporate this information into their analyses owing to small sample sizes. However, recent large-scale studies have found interesting parent-of-origin effects associated with variation in multiple complex disorders[26,29,46], which implies that incorporating the parent of origin of alleles into mapping models will increase the power of a study to account for the heritable variation of a trait. Large-scale studies with pedigree information will be important for developing models and tools that can accommodate the extra degrees of freedom resulting from distinguishing among heterozygote classes.

Complex imprinting patterns. In addition to suggesting that there are more imprinted genes than are currently characterized and that allelic parent of origin contributes to complex trait variation, studies of imprinted genetic effects have revealed that imprinting patterns can be complex. Complex imprinting patterns (BOX 1) can arise when a locus contains multiple genes that can differ in their imprinting status. Imprinted genes tend to have a clustered distribution[11], and within imprinted gene clusters there can be both maternally and paternally expressed genes regulated by the same imprint control region. For example, at the *H19–IGF2* locus, which is associated with Beckwith–Weidemann syndrome, *H19* is expressed from the maternal allele and *IGF2* is expressed from the paternal allele[47].

The first example of a locus with a complex imprinting pattern is the callipyge (*CLPG*) locus in sheep[48,49], which shows polar overdominance (BOX 1). Polar overdominance has also been described at the delta-like 1 homologue (*DLK1*) gene in humans, which is associated with juvenile obesity[50]. Early work hypothesized that the complex pattern results from a mutation that switches parent-of-origin-specific expression from the paternal chromosome to the maternal chromosome (or vice versa)[48,51]. The *CLPG* mutation is an A-to-G transition in the intergenic region flanked by the paternally expressed protein-coding gene *DLK1* and the maternally expressed non-coding RNA gene *GTL2* (also known as *MEG3*). Focused studies of the *CLPG* mutation show that it affects molecular marks, including local DNA hypomethylation and DNase 1 hypersensitivity, and long-range bidirectional transcription throughout the intergenic region[52]. In addition, microRNAs processed from a maternally expressed transcript that runs antisense to the *PEG11* transcript repress PEG11 expression. Both *PEG11* and *antiPEG11* expression are affected by the *CLPG* mutation[53]. Precise details of how the polar overdominance phenotype is achieved remain unclear, but the predominant model is that it is the result of upregulation of a paternally expressed 'effector' and a maternally expressed 'repressor'[49,54], which may be linked to the molecular mechanisms described at the locus (FIG. 2A).

Line-cross design
An approach to quantitative trait locus mapping in which two non-inbred lines are crossed to produce a mapping population. The approach assumes that the two lines are fixed for different quantitative trait locus alleles, but there is variation at marker loci segregating within the lines.

Quantitative trait locus
(QTL). A region of the genome in which genetic variation at a marker locus is significantly correlated with phenotypic variation for a complex trait.

Parental genetic effects
Effects that occur when genes expressed in the mother or father have a causal influence on the phenotype of the offspring.

Parental imprinting
A phenomenon that occurs when either only the maternally or only the paternally inherited allele affects a phenotype. In a two-allele system, genotypes will group into two phenotypic classes based on the maternally or paternally expressed allele.

Advanced intercross
The result of continued random mating of a population derived from a cross between inbred lines. Advanced intercrosses provide higher resolution for quantitative trait loci than traditional (for example, F_2) mapping approaches because of the accumulation of recombination through each generation of random mating.

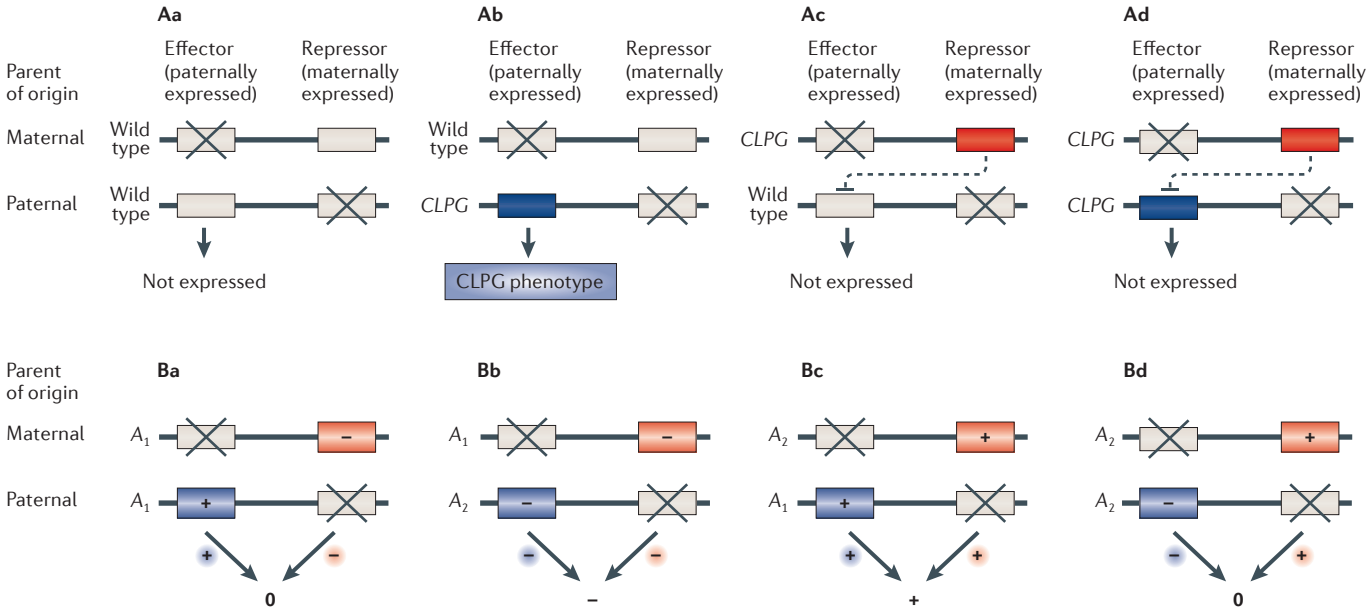


Figure 2 | **Molecular mechanisms that generate complex phenotypic patterns associated with genomic imprinting.** In each panel, the locus is composed of two imprinted genes, with one showing maternal expression and the other showing paternal expression. The imprinted copies are shown with a cross through them. Genes in grey are not expressed, either because they are imprinted or because they are inactive alleles. Genes in blue are paternally expressed, whereas those in red are maternally expressed. **A** | A working model for polar overdominance following Georges *et al.*[49] (for simplicity, the long-range control element is not included). **Aa** | Individuals homozygous for the wild-type allele do not express the effector or repressor and show the wild-type phenotype. **Ab** | Heterozygotes that inherit the active effector (callipyge (*CLPG*) allele) from their fathers and the inactive (wild-type) repressor from their mothers manifest the CLPG phenotype. **Ac** | Heterozygotes that inherit the active repressor (*CLPG* allele) from their mothers but the inactive effector from their fathers have a wild-type phenotype (the repressor is expressed but has no effect, as there is no effector to block in *trans*). **Ad** | The CLPG homozygote expresses the effector from the paternally inherited copy and the repressor from the maternally inherited copy. The repressor blocks the effector in *trans* and results in a wild-type phenotype. **B** | A hypothetical model for the origin of bipolar dominance imprinting[8]. The A_1 allele has a positive effect on the phenotype when paternally inherited (because of a paternally expressed gene) but a negative effect when maternally inherited (because of a maternally expressed gene), whereas the A_2 allele has the opposite pattern of effect. The effects of the two genes are summed together to determine the influence of the *A* locus on the phenotype. Maternally inherited alleles are given first in the genotypes listed. **Ba** | In the A_1A_1 homozygote, the paternally inherited positive effect (+) cancels out the maternally inherited negative effect (–) to result in a net effect of zero. **Bb** | In the A_1A_2 heterozygote, both the paternally inherited (A_2) and maternally inherited (A_1) alleles have a negative effect, resulting in a net negative phenotypic effect. **Bc** | In the A_2A_1 heterozygote, both the paternally inherited (A_1) and maternally inherited (A_2) alleles have a positive effect, resulting in a net positive phenotypic effect. **Bd** | In the A_2A_2 homozygote, the paternally inherited negative effect cancels out the maternally inherited positive effect to result in a net effect of zero.

We have described an analogous 'effector–repressor' model to explain the appearance of bipolar dominance imprinting, in which homozygotes are phenotypically identical but heterozygotes have different phenotypes (BOX 1; FIG. 2B). An implication of a bipolar dominance effect in a disease context is that the same allele can be both protective and a potential risk factor in the heterozygote, depending on the parent of origin. Kong *et al.*[29] recently identified a pattern consistent with bipolar dominance in a large genotyped human population. In this case, a particular SNP variant was associated with type 2 diabetes risk when paternally inherited but was found to be protective when maternally inherited.

Context-dependent imprinting effects. Studies identifying imprinted genetic effects on complex traits strongly suggest that these effects can be context dependent, and imprinting patterns are not consistent among traits and environments or between sexes. Mapping results for multiple metabolic parameters in mice (adiposity, serum lipids, glucose and insulin levels) found context dependency to be prevalent at candidate imprinted QTLs[55]. For example, a locus associated with the levels of free fatty acids (known as *DMetS1b*) showed apparent imprinted effects in females, but high-fat-fed females showed maternal expression, whereas low-fat-fed females showed paternal expression[55]. This result indicates that imprinted genetic effects may occur at many levels. Owing to the complexity of the genotype–phenotype relationship, it can be difficult to systematically study these effects in human samples. This constraint may be especially true for more 'plastic' complex traits such as obesity, in which the genetic architecture results from

Dominance imprinting
A complex imprinting pattern in which the parent of origin of alleles affects dominance at a locus. For example, bipolar dominance imprinting occurs when one heterozygote shows overdominance and the reciprocal heterozygote shows underdominance.

Box 3 | **Epistasis and genomic imprinting**

Analyses of interactions among imprinted genes suggest that these genes are particularly 'interactive', as they are enriched in complex networks that include both imprinted and non-imprinted genes[76,77]. Effects of epistatic interactions involving imprinting effects on complex traits occur when the effect of one locus depends on the parent of origin of alleles at another locus and/or the imprinting effect of a locus changes as a function of the alleles present at another locus (or loci); this latter scenario can potentially include cases in which one locus modifies the imprinting status of another locus. Here, we briefly discuss the contribution of these types of epistatic interactions involving imprinting effects to variation in complex traits and to the evolution of imprinting patterns. We provide a formal framework for dissecting epistatic interactions involving imprinting effects elsewhere[77]. Readers are referred to Li *et al.*[78] for methods to identify epistatic interactions involving imprinted genes in a Bayesian framework.

From a statistical perspective, epistatic interactions involving imprinting effects appear logically as interaction terms in the multi-locus extension to the mapping model framework[77] (BOX 2). For example, there may be a statistical interaction between the additive effect of one locus and the imprinting effect of another locus (known as 'additive-by-imprinting' epistasis), meaning that the additive effect of one locus depends on the type of heterozygote present at another locus, whereas the imprinting effect of that other locus depends on the type of homozygote present at the first locus. More generally, epistasis involving imprinting occurs whenever one must consider the parent of origin of alleles to understand how effects at one locus change as a function of the genotype present at another locus (and vice versa). In some scenarios, the change in the effect of a locus as a function of the genetic background provided by another locus can correspond to a change in the pattern of imprinting. For example, a locus may show a pattern of maternal expression in one genetic background and a pattern of paternal expression in a different genetic background. Consequently, the status of imprinting at the locus could evolve as the genetic background changes.

Few studies have considered epistatic interactions between imprinted genes in the context of quantitative genetic variation, but some studies have shown that epistatic interactions in the broad sense can involve imprinted genes. For example, Reilly *et al.*[79] found that the development of neural tumours in a mouse model is influenced by epistatic interactions involving an imprinted locus and tumour suppressor genes. The effects of various combinations of uniparental disomies containing imprinted regions have been studied in mice, and these studies have shown that the combinations often have non-additive effects on developmental traits[80]. Studies have also shown that *trans*-acting factors can change the imprinting status of a locus. For example, imprinting of the gene *MEDEA* in *Arabidopsis thaliana* endosperm is controlled by antagonism between at least two other genes, and hence changes at those other genes can disrupt the presence of imprinting at *MEDEA*.

multidimensional interactions among genes and environment as well as from inter-organ crosstalk (see BOX 3 for a discussion of epistasis and genomic imprinting).

Results can be further confounded by the fact that the trait being studied might depend on a combination of tissues or developmental stages that include both imprinted and non-imprinted expression. For example, many genes are imprinted only in the placenta[56]; ubiquitin protein ligase E3A (*UBE3A*), which is involved in Angelman syndrome, is biallelically expressed in most tissues but is maternally expressed in most neurons[57]; and *IGF2* is biallelically expressed in some tissues[11]. This can result in a phenotypic difference between reciprocal heterozygotes, but the difference is not as pronounced as the difference between the homozygotes. This scenario is called partial imprinting and has been observed both in the phenotypic manifestation at imprinted QTLs[8,16,23] and in gene expression differences[58].

Thus, animal models can be important complements to human studies because developmental stage, genetics and environment can be controlled and monitored. Imprinted patterns and genes identified in animal studies can inform human studies by revealing an imprinted 'locus' or the pathway containing the imprinted gene (or genes). However, it is unclear how often genes that are imprinted in one species are also imprinted in another. Studies comparing sets of predicted imprinted genes in humans and mice have suggested that 32%[59] to 87%[60] of imprinted genes are imprinted in both species. Much work is required to validate such estimates, let alone to determine whether conserved imprinted genes are also conserved in their phenotypic effects and/or underlying molecular mechanisms (not to mention tissue, developmental or environmental contexts)[61,62].

Identifying molecular signatures of imprinted loci

The ultimate support for an imprinted effect comes from molecular characterization of a locus. Such support is especially important when putative imprinted genetic effects are mapped to genomic regions that do not contain confirmed imprinted genes. Advances in whole-genome sequencing technologies can facilitate molecular characterization of candidate loci using DNA sequence features that, in some contexts, distinguish imprinted from non-imprinted genes. Features such as the concentration and orientation of short interspersed repetitive elements (SINEs) and local GC content in conjunction with epigenetic features such as histone modification sites have been used to train algorithms that bioinformatically predict imprinted loci from whole-genome sequences[63]. Some of these predictions have been integrated with data from genes that have known parent-of-origin allele-specific biases and have been used to further classify a predicted imprinted gene as maternally or paternally expressed, as well as to identify potential patterns (and hence molecular mechanisms) that might distinguish the parent of origin of alleles[30,59]. For example, a study[59] using such computational predictions identified two novel imprinted genes in humans: potassium channel, subfamily K, member 9 (*KCNK9*), which is maternally expressed in fetal brain, and discs large homologue-associated protein 2 (*DLGAP2*), which is paternally expressed in testes. It has been hypothesized that different mechanisms control maternal versus paternal expression biases[11,64], and the identification of patterns associated with allele-specific regulation can provide a framework for clarifying the relationships between DNA sequence and gene expression that underlie the phenotypic signatures of imprinting.

Phenotypic variation at imprinted loci can be directly linked to genomic variation through the analysis of parent-of-origin-dependent gene expression. RNA sequencing is the gold standard for quantifying whole-genome allele-specific biases in transcription (in which there is an unequal number of transcripts from the maternally and paternally derived alleles of a gene), and several studies have made use of this technology in an effort to identify novel imprinted genes in reciprocal crossings of inbred model organisms[65–67]. Inconsistent criteria for ascertaining parent-of-origin-dependent biases have led to substantial discrepancies among results and failure to validate most predictions[68]. Further confounding factors include

Allele-specific biases
Biases that occur when the two alleles in a heterozygote are not functionally equivalent. This situation can arise from an expression bias wherein one allele is expressed at a higher level than the other (the null expectation being that both alleles will be expressed at approximately the same level). There can also be methylation biases, wherein one allele is preferentially methylated (or unmethylated); this can underlie allele-specific expression biases.

Differentially methylated regions
(DMRs). Genomic regions in which the pattern of methylation (the ratio of methylated to unmethylated sequence) is different between two alleles at the same locus.

the context dependency of parent-of-origin-dependent effects, as discussed above.

A potential mechanism underlying allele-specific expression is DNA methylation. The addition of a methyl group to DNA nucleotides can occur in an allele-specific manner, and allele-specific methylation in imprint control regions (referred to as differentially methylated regions (DMRs)) is associated with parent-of-origin-dependent gene expression. For example, the DMR at the *H19–IGF2* locus is unmethylated on the maternal allele, allowing binding of the transcription factor CCCTC-binding factor (CTCF) and preventing *IGF2* promoter activation. The methylated paternal allele prevents CTCF binding, and the downstream enhancer is able to activate *IGF2* transcription[69,70]. Next-generation sequencing technologies that specifically target methylated DNA have been used to identify DMRs that might be associated with imprinted genes[71–73]. A promising avenue of research is to integrate RNA sequencing data with whole-genome methylation data to identify regions where both allele-specific expression and methylation correlate in a parent-of-origin-dependent manner that associates with phenotypic variation.

In addition to DMRs in imprint control regions, imprinted gene clusters often contain non-coding RNAs that have regulatory roles. Hence, the phenotypic manifestation of a particular 'locus' can be determined by the joint action of multiple imprinted coding genes and non-coding elements. Thus, characterizing the genomic context — such as identifying clusters of non-coding RNA elements that may affect local gene expression in potentially complex interacting combinations — can be a tool for identifying loci that show complex imprinting patterns such as polar overdominance or bipolar dominance.

Concluding remarks

Recent empirical research indicates that parent-of-origin effects which are putatively caused by genomic imprinting are an important component of the genetic architecture of complex traits. In animal research on parent-of-origin effects, there is a need to develop and incorporate models that consider developmental stage, tissue specificity and context dependency into models of discovery research. In human association studies, there is a need to develop and incorporate models that allow parental alleles in heterozygotes to be functionally non-equivalent. Most studies of complex traits in both model organisms and human samples are underpowered (or the data are just not available), and there is currently no way to predict these complicated epigenetic effects from DNA sequence alone. What is lacking is a framework of DNA sequence–imprinted function relationships. There is a clear need for further research integrating complex trait mapping results with next-generation sequencing data to understand how imprinted genes contribute to the patterns of phenotypic variation seen in both natural and experimental populations. Studies that consider how and when imprinted genetic effects are conserved among species and/or are modified by environmental factors or genetic background will be particularly relevant for advancing the field of complex trait research.

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