Genomic imprinting effects on complex traits in domestic animal species

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Abstract
Mammals are diploid organisms whose cells possess two matched sets of chromosomes, one inherited from the mother and one from the father. Genomic imprinting is an epigenetic mechanism that changes this potential because it restricts the expression of a gene to one of the two parental chromosomes. Monoallelically expressed genes that exert their phenotypic effect in a parent-of-origin specific manner are considered to be subject to genomic imprinting, the most well understood form of epigenetic regulation of gene expression in mammals. The observed differences in allele specific gene expression for imprinted genes are not attributable to differences in DNA sequence information, but to specific chemical modifications of DNA and chromatin proteins. Since the discovery of genomic imprinting some three decades ago, over 100 imprinted mammalian genes have been identified and considerable advances have been made in uncovering the molecular mechanisms regulating imprinted gene expression. While most genomic imprinting studies have focused on mouse models and human biomedical disorders, recent work has highlighted the contributions of imprinted genes to complex trait variation in domestic livestock species. Consequently, greater understanding of genomic imprinting and its effect on agriculturally important traits is predicted to have major implications for the future of animal breeding and husbandry. Imprinted gene expression can have a major effect on phenotypic traits in domestic livestock populations. Furthermore, imprinting is an important factor to consider in the models used for future the genetic improvement of domestic livestock for those genomic regions where imprinted gene expression is known to occur and to affect economically important traits included in the selection index.

Keywords: Genomic imprinting, Imprinted genes, long non coding RNA, Genetic disorders

Introduction
Mammalians are diploid (2n) organisms with two sets of chromosomes inherited from mother and father. Thus mammals have every gene in two copies. Genes from both the parental lines has the same potential to be expressed in any cell [1] (Barlow and Bartolomei, 2014) [3]. However, a few of mammalian autosomal genes has been identified where the gene expression is restricted to one of the two parentally inherited chromosomes in a parent-of-origin specific manner, those genes are called imprinted. An epigenetic mechanism that restricts the gene expression to one of the two parental chromosomes is genomic imprinting. This phenomenon has been displayed by only few hundred genes of our genome. Genomic imprinting is a consequence of parental inheritance, not of sex as it effects both male and female offsprings. That means, an imprinted gene that is active on the maternally inherited chromosome will be active on the maternal chromosome and silent on the paternal chromosome in both males and females offsprings [1]. Rather, ‘classically defined’ autosomal imprinting is a consequence of the parental origin of each allele such that, in general, paternally expressed/maternally imprinted genes are transcriptionally silenced on the maternally inherited chromosome only, while maternally expressed/paternally imprinted genes are silenced solely on the paternally inherited chromosome [1].

The concept of genomic imprinting introduced by Metz and Crouse, who coined the term in the context of the unique inheritance of sex chromosomes in the dipteran insect, Sciara coprophila. Zygote consisting of two maternal genomes is called gynogenones or parthenogenones and zygote, which contained two paternal genomes is called androgenones [2]. Neither of these two types of reconstituted zygote could develop to term but the former had better embryos, and the later, better development of placental tissues, which suggested that the parental genomes are functionally non-equivalent despite the fact that they have equivalent genetic information. This observation led to discovery of genomic imprinting, which indicate functional difference that is dictated by the parental origin of the genome [4, 5, 6].
Imprinting plays an important role in growth and development. There were reports showing that the imprinted genes have been contributed to economically important production traits [7].

Developments in the different theories of genomic imprinting discovery

The term “chromosome imprinting” was first coined to describe paternal-specific chromosome elimination that plays a role in sex determination in some Arthropod species [3]. Chromosomal imprinting of the mammalian X chromosome was also noted, which leads to paternal-specific inactivation of one of the two X chromosomes in all cells of female marsupials and the extraembryonic tissues of the mouse [9]. Classical geneticists were generating mouse mutants carrying chromosomal translocations that laid the foundation for the observation of imprinted gene expression. It showed a parental-specific phenotype when certain chromosomal regions were inherited as duplications of one parental chromosome in the absence of the other parental chromosome (known as uniparental disomy or UPD). These results indicated the possibility “that haploid expression of particular maternal or paternal genes is important for normal mouse development” [9]. At the same time, other geneticists used an unusual mouse mutant known as the “hairpin-tail” mouse that carried a large deletion of chromosome 17. It showed that, offspring who received the Hairpin-tail deletion from a maternal parent were of larger size and died midway through embryonic development, whereas paternal transmission of the genetically identical chromosome produced viable and fertile mice. So, a suggestion made that “the maternal genome might be normally active at the Hairpin-tail chromosomal region while its paternal counterpart is preferentially inactivated” [10].

A major step forward in establishing the existence of genomic imprinting in mammals came several years later with the development of an improved nuclear transfer technology being used to test the possibility of generating diploid uniparental embryos solely from mouse egg nuclei. It also confirmed the suggestion that genes on the maternal and paternal copy of chromosome 17 functioned differently during embryonic development [5]. Subsequently, nuclear transfer was used to show that embryos, reconstructed from two maternal pronuclei (known as gynogenetic embryos) or two paternal pronuclei (androgenetic embryos), failed to survive; whereas only embryos reconstructed from one maternal and one paternal pronucleus produced viable and fertile offspring [4, 6]. These experiments indicated the two parental genomes express different sets of genes needed for complete embryonic development.

It also proved that gynogenetic embryos at the time of death were defective in extraembryonic tissues that contribute to the placenta, whereas androgenetic embryos were defective in embryonic tissue. These outcomes led to the hypothesis that embryonic development required imprinted genes expressed from the maternal genome, whereas the paternal genome expressed imprinted genes required for extraembryonic development [11].

Despite the wealth of supportive data, final proof of the existence of genomic imprinting in mammals depended on the identification of genes showing imprinted parental specific expression. In 1991 when three imprinted mouse genes were described. The first of these, Igf2r (insulin-like growth factor type 2 receptor) was identified as a maternally expressed imprinted gene. This gene was later shown to explain the overgrowth phenotype of the Hairpin-tail mutant mouse [12]. For Igf2r, positional cloning was used to identify genes that mapped to the Hairpin-tail deletion on chromosome 17. A few months later, the Igf2 gene was identified as a paternally expressed imprinted gene [13, 14]. For Igf2, the physiological role of this growth factor in embryonic development was being tested by gene knockout technology. Finally, the H19 gene, an unusual long noncoding RNA (lncRNA) was subsequently shown to be a maternally expressed imprinted gene [15]. The H19 lncRNA was identified as an imprinted gene after this gene was mapped close to the Igf2 locus on chromosome 17, proving the hypothesis that imprinted genes could be clustered together.

Genomic imprinting-an epigenetic gene regulatory system

Genomic imprinting must therefore depend on an epigenetic system that modifies or “imprints” one of the two parental chromosomes. The most likely scenario is that gametic imprints are placed on paternally imprinted genes during sperm production and on maternally imprinted genes during egg formation. A key feature of the “imprinted” DNA sequence is that it would only be modified in one of the two parental gametes; thus, two types of recognition system are required, one sperm-specific and one oocyte-specific, each directed toward a different DNA sequence. First, once established, it must remain on the same parental chromosome after fertilization when the embryo is diploid. Second, the imprint must best ably inherited through mitosis of the embryo and adult animal. Last, it must be erasable.

Imprints are acquired by the gametes; hence, oocytes and sperm already carry imprinted chromosomes (first-generation imprints). After fertilization when the embryo is diploid, the imprint is maintained on the same parental chromosome after each cell division in cells of the embryo, yolk sac, placenta and also in the adult. The germ cells are formed in the embryonic gonad and the imprints are erased only in these cells before sex determination. As the embryo develops into a male, the gonads differentiate to testes that produce haploid sperm that acquire a paternal imprint on their chromosomes. Similarly, in developing females, chromosomes in the ovaries acquire maternal imprints (second-generation imprints).

How are gametic imprints identified?

An imprint can be defined as the epigenetic modification that distinguishes the two parental copies of a given gene. Once formed, the imprint must also allow the transcription machinery to treat the maternal and paternal gene copy differently within the same nucleus. A gametic imprint is predicted to be continuously present at all developmental stages, thus imprints can be found by comparing epigenetic modifications on maternal and paternal chromosomes in embryonic or adult tissues and tracing them back in development to one of the two gametes. Candidates for gametic imprints could be modifications of DNA or histone proteins that package DNA into chromosomes [18]. There are now two types of epigenetic DNA modification known in mammals; 5-methylcytosine and 5-hydroxymethylcytosine [19].

How does a gametic imprint control imprinted expression?

Three types of information needed:
1. Which parental chromosome carries the imprint,
2. Which parental chromosome carries the expressed allele,
of the imprinted gene
3. The position of the imprinted sequence relative to the expressed or silenced allele of the imprinted gene.

Gametic imprints can act on whole clusters of genes at once. These imprinted clusters contain 3-12 imprinted genes and span from 100–3700 kb of genomic DNA. The majority of genes in any one cluster are imprinted protein-coding mRNA genes; however, at least one is always an imprinted LNC RNA. It is possible to study the effect of the imprint on single genes in the cluster, but it may prove more informative to study the effects of the imprint on the entire cluster.

What is the function of genomic imprinting in mammals?
Determining function of imprinted genes can be performed by mutating the gene sequence to impair its function using the “homologous recombination” technique. The most significantly represented function among imprinted genes includes genes that affect growth of the embryo, placenta, and neonate. In this category are paternally expressed imprinted genes that function as growth promoters (Igf2, Peg1, Peg3, Rasgrf1 and Dlk1) and show growth retardation in embryos deficient for the gene. There are also maternally expressed imprinted genes that function as growth repressors (Igf2r, Gnas, Cdkn1c, H19 and Grb10), as shown by a growth enhancement in embryos deficient for the gene. The ability to regulate growth appears to be neatly divided with maternally expressed growth regulating genes acting to repress growth of the offspring, whereas paternally expressed genes in this category act to increase growth.

Why should genomic imprinting have evolved only in some mammals, but not in vertebrates in general?
Placental mammals such as mice and humans, and marsupials such as opossum and wallaby, have genomic imprinting. Egg-laying mammals, such as platypus and echidna, appear to lack imprinted genes. Placental mammals and marsupials are distinguished from egg-laying mammals by a reproductive strategy that allows the embryo to directly influence the amount of maternal resources used for its own growth. In contrast, embryos that develop within eggs are unable to directly influence maternal resources. The necessity of the paternal genome for fetal development, provide evidence that can fit two equally attractive hypotheses:

1. Parental Conflict Theory
Embryonic growth is dependent on one parent, but influenced by an embryo whose genome comes from two parents. Paternally expressed imprinted genes are proposed to increase embryonic growth, thereby maximizing the fitness of an individual offspring bearing a particular paternal genome. Maternally expressed imprinted genes are proposed to suppress fetal growth. This would allow a more equal distribution of maternal resources to all offspring and increase transmission of the maternal genome to multiple offspring, which may have different paternal genomes.

Trophoblast Defense Theory
This proposes that the maternal genome is at risk from the consequences of being anatomically equipped for internal reproduction should spontaneous oocyte activation lead to full embryonic development. Because males lack the necessary anatomical equipment for internal reproduction, they do not share the same risks should spontaneous activation of spermatozoa occur. Imprinting is thus proposed to either silence genes on the maternal chromosome that promote placental development or to activate genes that limit this process. The genes necessary for placental invasion of the maternal uterine vasculature would consequently only be expressed from a paternal genome after fertilization has occurred. Unfortunately, neither the parental conflict nor the trophoblast defense models can provide a full explanation for all the data.

Alternative explanations of the function of genomic imprinting in mammals could come from two sources
The first would be to examine the function of “imprinting” across a complete gene cluster in contrast to examining the phenotype of mice lacking a single imprinted gene product. This would require an ability to reverse an imprint and generate bi parental gene expression across the whole imprinted cluster. The second approach is to learn exactly how genes are imprinted. It is possible that not all genes in a cluster are deliberate targets of the imprinting mechanism and that some may just be “innocent bystanders” of the process, and their function would not be informative about the role of genomic imprinting.

Imprint Control Element’s control on imprinted genes in cluster
To date, about 150 imprinted genes have been mapped to 17 mouse chromosomes including the X chromosome. More than 80% of the identified imprinted genes are clustered into 16 genomic regions that contain two or more genes.
Name of the principle imprinted mRNA gene in the cluster or after a disease association

A common feature of these seven clusters is the presence of a DNA sequence carrying a gametic methylation imprint that is known as a gametic DMR (Differentially DNA-methylated region). A gametic DNA methylation imprint is defined as a methylation imprint established in one gamete and maintained only on one parental chromosome in diploid cells of the embryo. In five clusters (Igf2r, Kcnq1, Gnas, Grb10, and Pws), the gametic DMR has a maternal methylation imprint acquired in oogenesis, whereas in two clusters (Igf2 and Dlk1), it has a paternal methylation imprint acquired during spermatogenesis. In these examples, the gametic DMR controls imprinted expression of the whole or part of the cluster and is therefore designated as the imprint control element, or ICE, for the cluster [22].

The imprinted protein-coding genes in each cluster are expressed, for the most part, from the same parental chromosome, whereas the IncRNA is expressed from the opposite parental chromosome. Second, the ICE deletion causes loss of imprinted expression only when deleted from the parental allele expressing the IncRNA.

Presence of at Least One IncRNA Imprinted Gene Clusters

The majority of imprinted clusters contain an IncRNA, which is currently defined as a noncoding transcript more than 200 nucleotides [23]. Two features of imprinted IncRNAs indicate they may play a role in the silencing of the imprinted mRNA (i.e., protein-coding) genes in the cluster. The first is that the IncRNA generally shows reciprocal parental-specific expression compared to the imprinted mRNA genes (fig 1). Second, the DMR that carries the gametic methylation imprint, which controls imprinted expression of the whole cluster, overlaps with the IncRNA promoter in multiple instances (Airn region 2, KvDMR1, Snrpn-CGI, and Nespas-DMR). This finding could indicate that imprints evolved to regulate the IncRNA in each imprinted cluster. 3 maternally imprinted clusters (Igf2r, Kcnq1, and Gnas) share a common IncRNA-dependent silencing mechanism.

The Role of DNA Methylation in Genomic Imprinting

DNA methylation, a modification in mammals that covalently adds a methyl group to the cytosine residue in CpG dinucleotides. DNA methylation is widely considered as a repressive gene expression mechanism that regulates imprinted gene expression by promoting chromatin condensation, rendering the DNA less accessible to the cell’s transcriptional machinery. Thus, silenced or repressed gene expression is generally observed from the hyper methylated DMR [24]. DNA methylation is acquired through the action of de novo methyltransferases and maintained in situ each time the cell divides by the action of maintenance methyltransferases [19]. Hence, this modification fulfills the criteria for a parental identity mark or “imprint” because

1. It can be established in either the sperm or oocyte by de novo methyltransferases that act only in one gamete
2. It can best ably propagated at each embryonic cell division by a maintenance methyltransferase
3. It can be erased in the germline to reset the imprint in the next generation, either by passive demethylation (DNA replication followed by the failure to undergo maintenance methylation) or through the action of a demethylation activity possibly through conversion of 5-methylcytosine to 5-hydroxymethylcytosine by the ten-eleven translocation family of enzymes or through excision of 5-methylcytosine by the DNA repair machinery [25].

It could act as the imprinting mark by being acquired de novo only by the chromosomes in one gamete. It could also serve to silence one of the parental alleles because DNA methylation is associated with gene repression [19]. If it forms during
Imprinted Disorders in Domestic Livestock Species

In domesticated species, the importance of establishing appropriate epigenetic marks at imprinted loci has been highlighted largely through assisted reproductive technologies (ART) including somatic cell nuclear transfer (SCNT) cloning studies. It has been proposed that ART exposes the epigenome to external factors that may interfere with the correct establishment and maintenance of genome imprints. For example, superovulation, embryo culturing and cryopreservation can affect methylation profiles and gene expression at imprinted loci [31, 32]. Epigenetic perturbations, associated with ART and SCNT, may contribute to developmental issues such as increased abortion rate, perinatal death, enlarged placenta, enlarged umbilical cords, high-birth weight and large offspring syndrome [33, 34, 35].

LOS is an overgrowth disorder in domesticated ruminants bearing phenotypic similarities to Beckwith-Wiedemann syndrome (BWS, an overgrowth disorder in humans), and is characterized by excessive birth weight, enlarged tongue, umbilical hernia, enlarged internal organs and hypoglycemia [36]. Both BWS and LOS can occur naturally; however, there is evidence that these disorders have an increased incidence in individuals generated from ART [32]. Ablation methylation at the H19-IGF2 and the KCNQ1OT1-CDKN1C loci and ART-generated fetuses, especially in offspring displaying LOS or which had died shortly after birth [37, 38].

Young also demonstrated that sheep fetuses displaying LOS has reduced maternal IGF2R mRNA and protein levels relative to control fetuses, which was correlated with a loss of methylation at the IGF2R ICR on the maternally active allele [39].

In humans, gain of methylation epimutations at the maternal IGF2/H19 ICR, resulting in increased expression of IGF2, can account for 2-7% of all BWS cases, while 50% of cases are due to loss-of-methylation epimutations at the maternal ICR. Examples of genomic imprinting disorders has been reviewed by Butler [40]. These include Prader-Willi and Angelman syndromes (the first examples of genomic imprinting in humans), Silver-Russell syndrome, Beckwith-Wiedemann syndrome, Albright hereditary osteodystrophy and uniparental disomy 14.

Prader-Willi syndrome

Chromosome 15 deletion was de novo or due to a new event and found that the chromosome 15 leading to the deletion was donated only from the father. In about 70% of subjects with PWS, the 15q11-q13 deletion was present while about 25% of individuals with PWS had either maternal disomy 15 (both 15s from the mother) or defects in the imprinting center controlling the activity of genes in the chromosome 15 region (about 5% of cases). Characterized by infantile hypotonia, early childhood obesity, short stature, small hands and feet, growth hormone deficiency, hypogonadism/hyponadism, mental deficiency and behavioral problems including temper tantrums and skin picking and a characteristic facial appearance with a narrow bifrontal diameter, short upturned nose, triangular mouth, almond-shaped eyes, and oral findings (sticky saliva, enameled hypoplasia). It affects an estimated 350,000–400,000 people worldwide.

Angelman syndrome (AS)

AS is characterized by seizures, severe mental retardation, ataxia and jerky arm movements, hypopigmentation, inappropriate laughter, lack of speech, micro Brachycephaly, maxillary hypoplasia, a large mouth with protruding tongue, prominent nose, wide spaced teeth, and usually a maternal 15q11-q13 deletion.

Silver-Russell syndrome

SRS affects approximately 1 in 75,000 births. SRS is clinically heterogeneous with prenatal and postnatal growth retardation, a characteristic facial appearance including a small, triangular face with frontal prominence and a normal head circumference, growth asymmetry particularly of the limbs, and small incurved fifth fingers (clinodactyly). Several abnormalities have been reported involving chromosomes 7, 8, 15, 17, and 18, in the form of rings, deletions, and translocations. However, the majority of Silver-Russell syndrome patients have a normal karyotype.

Beckwith-Wiedemann syndrome

Characterized by macrosomia with large muscle mass at birth, Craniofacial features (macroglossia, prominent eyes, periorbital fullness, ear creases and/or pits), Omphalocele, hypoglycemia, Organomegaly (kidneys, liver, spleen), abdominal tumors, Hemihypertrophy. Paternal uniparental disomy 11 (in 15% of cases); loss of imprinting of IGF2 (hypermethylation of telomeric imprinting center region) (in 5%); mutations in KCN1 in centromeric imprinting center region (in 10%); hypomethylation of centromeric imprinting center region (about 50%); unknown (15%).

Albright Hereditary Osteodystrophy (AHO)

Characterised by small stature (final height, 54 to 60 inches) and short metacarpals, rounded face with short neck. Delayed dental eruption or enamel hypoplasia, areas of mineralization in subcutaneous tissues with variable hypocalcemia and
hyperphosphatemia, defects of the GNAS gene associated with different forms of PHP and PPHP depending on the parent of origin. For example, maternal inheritance leads to PHP-1a, i.e., AHO plus hormone resistance while paternal inheritance leads to PHPP or AHO without evidence of resistance to parathyroid hormone.

**Uniparental disomy 14**

Clinical findings in maternal disomy 14 include growth retardation, congenital hypotonia, joint laxity, psychomotor retardation, truncal obesity and minor dysmorphic facial features. Clinical features are more severe in paternal disomy 14 including polyhydramnios, thoracic and abdominal wall defects, growth retardation and severe developmental delay. Imprinting errors with imprinted locus at 14q32 including the paternally expressed DLK1 gene and maternally expressed GTL2 gene. Uniparental disomy, copy number changes and disruption of regulatory sequences or mutations of a single active allele leads to the disorder.

Mainly, mammalian genes displaying genomic imprinting are distinguishable from genes that display apparent parental specific expression due to unequal or unique genetic contributions from male and female parents such as the expression of Y-linked genes in XY males, the expression of maternally derived mitochondrial genes, and the expression of X-linked genes that evade the process of X-chromosome inactivation in XX females. X-chromosome inactivation, in particular, has been extensively studied in mammals since it was first described by Lyon (1961) 67. During early female embryonic development, one of the two X chromosomes is randomly inactivated to equalize the X-linked gene dosage difference between XX females and XY males. This process, called ‘random X-inactivation’, involves the decoration of one X-chromosome with a non-protein coding RNA. Not all imprinted genes adhere to this classic definition; for some genes transcriptional repression of the ‘imprinted’ parental allele is partial where both alleles are expressed but one is expressed more strongly than the other in a parent-of-origin specific way. Preferential expression relates to imprinted genes where both alleles are expressed but one is expressed more strongly than the other in a parent-of-origin specific way.

Most of the paternally expressed genes showed exclusive monoallelic expression, while most of the maternally expressed genes showed preferential expression. Using the Fisher’s Exact Test, a significant difference was found (P < 0.0001) between the proportions of genes displaying strict monoallelic expression and preferential expression for maternally versus paternally expressed genes. These preferential expression differences could be attributed to sequence and methylation characteristics that distinguish paternally expressed from maternally expressed genes. Comparison between the two sub groups in mouse and human revealed that maternally expressed genes have a higher density of short interspersed transposable elements (SINEs) than paternally expressed genes. Paternally expressed genes were also found to be associated with a lower density of GC content. Also, it has been found that paternally methylated differentially methylated regions (DMRs) have a lower CpG content than maternally methylated DMRs, and that the average GC content of the paternally methylated DMRs was significantly lower than that of the maternally methylated DMRs. Furthermore, it was reported that, after establishment of methylation imprints, male germ cells have more cell divisions than female germ cells. Therefore, the paternally methylated DMRs might have more C/T mutations than the maternally methylated DMRs, leading to partial silencing of the paternal alleles.

**Three possible mechanisms of preferential expression**

1. The overlapping of different transcripts from different genes.
2. The existence of two promoters that can give rise to overlapping transcripts.
3. The transition of the imprinting status of some genes from imprinted to a non-imprinted status.

**The Discovery of Callipyge Phenotype in Sheep and Epigenetic Mechanisms in Regulating Gene Expression**

The complex interplay between different epigenetic and genetic mechanisms in regulating mammalian imprinted gene expression is aptly illustrated by the callipyge phenotype in sheep, which is responsible for a ~30% increase in skeletal muscle (Hindquarters), a corresponding ~8% reduction in fat content and improved feed efficiency 41. This phenotype is observed only in heterozygous individuals that carry the causative mutation on the paternal chromosome (i.e., mat+/patC, where ‘mat’ and ‘pat’ denote maternal and paternal chromosomes, respectively and superscript ‘+’ and ‘C’ represent wild-type and callipyge alleles, respectively) - a mode of non-Mendelian inheritance termed ‘Polar Overdominance’ 41. The callipyge phenotype is caused by an A-to-G single nucleotide polymorphism (SNP; i.e., the callipyge mutation) located between the paternally expressed/maternally imprinted DLK1 protein-coding gene and the maternally expressed/paternally imprinted MEG3 long non-coding RNA (ncRNA) gene within the imprinted DLK1-DIO3 gene cluster on ovine chromosome 18 42. Callipyge individuals (i.e., mat+/patC) display overexpression of the paternally expressed DLK1 and PEG11 protein-coding transcripts in skeletal muscle tissue relative to non-callipyge animals (i.e., mat+/patpat; matC/pat+; mat C/patC). In contrast, individuals that inherit the callipyge mutation on the maternal chromosome (i.e., matC/patC or matC/patpat) display upregulation of maternal long ncRNAs and miRNAs in cis relative to wild-type (i.e., mat+/patpat) and callipyge animals 43.

**Association of Imprinted genes with complex phenotype with mammals**

In mice, for example, studies have demonstrated the contribution of imprinted genes to variation in adiposity and body weight, muscle traits, metabolism, and disease susceptibility and resistance to infectious disease 44, 45, 46. Similarly, while investigations of the callipyge phenotype have demonstrated a role for imprinting in sheep muscle traits, studies in pigs have identified a single SNP (G-to-A mutation) in the paternally expressed/maternally imprinted porcine IGF2 gene that is responsible for ~30% of the variance for lean meat, 15–30% of the variance for muscle mass and 10-20% of the variance for back fat content.
(Jeon et al., 1999). Animals inheriting a sire-derived ‘A’ nucleotide display a three-fold increase in IGF2 expression in post-natal muscle relative to those animals inheriting a sire-derived ‘G’ nucleotide, which results in increased muscle mass and a corresponding reduction in body fat [47].

<table>
<thead>
<tr>
<th>Gene symbol/Alias</th>
<th>Gene name</th>
<th>Expressed allele</th>
<th>Species in which gene is imprinted</th>
<th>Phenotypic trait associations</th>
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</thead>
<tbody>
<tr>
<td>DIC3</td>
<td>Deiodinase, iodothyronine, type III</td>
<td>Paternal</td>
<td>Pigs, sheep</td>
<td>Fertility traits</td>
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<td>Pigs</td>
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<td>Growth traits; fertility traits; milk traits</td>
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<td>Milk traits; body conformation traits</td>
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<td>PEG3</td>
<td>Paternally expressed gene 3</td>
<td>Paternal</td>
<td>Cattle, pigs, sheep</td>
<td>Fertility traits</td>
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</tbody>
</table>

**Fig 2:** Phenotypic traits associated with the imprinted genes in various species

**Imprinted Genes as Candidates for Genotype-Phenotype Association Studies in Domestic Livestock**

Animals carrying a marker allele(s) or genotype(s) known to associate with a desired complex phenotype (often referred to as ‘quantitative trait loci’) maybe selected as parental candidates for subsequent generations; this approach underpinned marker assisted selection (MAS) strategies that were proposed for the genetic improvement of domestic livestock populations [48]. Early studies based on STR genotypes uncovered parent-of-origin QTL for a series of phenotypic traits in pigs, sheep and cattle. For example, parent-of-origin QTL influencing body composition, carcass and meat quality traits, growth traits and reproductive traits in the F2 progeny of experimental crossbred pig populations [49, 50, 51]. Interestingly, a theoretical approach to identifying parent-of-origin effects on body composition data (eye muscle area, rib fat, rump fat and intramuscular fat percent) collected from ultrasonic measurements revealed that a mean of 28% of the total genetic variance for these traits was due to parent-of-origin effects [52]. A recent comprehensive genome-wide scan in cattle that specifically included a parent-of-origin inheritance model identified 24 parent-of-origin QTL (six were significant at the 5% genome-wide level and 18 were significant at the 5% chromosome-wide level) distributed across 15 bovine autosomes influencing growth and carcass traits; two of these QTL encompassed the bovine imprinted GNAS and PEG3 genes [53]. Subsequent studies have revealed associations between SNPs in the bovine PEG3 and GNAS genes and growth related traits, calving and fertility traits and animal health traits (e.g., somatic cell count, a marker of
mastitis infection and susceptibility). Collectively, these results suggest that the GNAS and PEG3 loci play an important role in bovine growth and development, fertility and health \[7\]. Additional studies revealing associations between imprinted loci and livestock production traits include the imprinted bovine IGF2 and IGF2R genes and meat quality, milk production and growth traits in beef and dairy cattle populations \[54, 55, 56, 57, 58\]. Associations between SNPs at the mammalian DLK1-DIO3 imprinted gene cluster and production traits such as growth, fatness and body composition have also been reported in pigs and cattle \[59, 7\]. Recent survey of SNPs in the imprinted paternally expressed/ maternally imprinted DIO3 gene—which is involved in thyroid metabolism and has been shown to be highly expressed in uterine tissues in humans and rodents was associated with fertility traits in pigs. It has been proposed that DIO3 influences porcine fertility through the regulation of placental and/ fetal growth \[60\].

The Effects of Imprinted Gene Expression on Phenotype

Genomic imprinting raises several interesting theoretical considerations for genotype–phenotype association studies. For example, classic imprinted gene expression (i.e., complete parent-of-origin monoallelic expression) is expected to generate patterns of phenotypic expression whereby phenotype is solely determined by the expressed allele. Consequently, classically defined imprinted loci with two alleles can be regarded as being functionally hemizygous \[15\]. This reduces the number of phenotypic classes at such loci from three (as expected under an additive genetic model) to two such that the heterozygote class is functionally equivalent to one of the two homozygote classes. It is important to note that for loci exhibiting complete imprinting, heterozygous individuals expressing the allele with the greatest phenotypic effect may display similar phenotypic scores to those traits controlled by loci with dominance effects. However, for many imprinted loci, transcriptional silencing is only partial \[61\], which can generate functional differences between reciprocal heterozygotes (i.e., heterozygous individuals that have inherited the same allele from different parents) and can lead to four potential phenotypic classes.

Polar overdominance, as exemplified by the callipyge phenotype, can result in phenotypic differences between reciprocal heterozygotes; in addition, under a model of polar overdominance, one of the heterozygous states will display a phenotypic value greater than all three other genotypes, which themselves show no differences in phenotypic values. Conversely, a model of polar under dominance, whereby one of the two reciprocal heterozygotes has a phenotypic value less than all three other phenotypically equivalent genotypes, has been reported in mice (Wolf et al., 2008). Finally, bipolar dominance can exist at imprinted loci such that one heterozygote displays larger phenotypic values and the other heterozygote exhibits lower phenotypic values than both homozygotes, which have the same phenotypic value \[62\].

Methods to investigate the Imprinting effect

To investigate the impact of imprinted genes on important traits in domestic animals, diverse methods were used. These can broadly be categorized as expression studies, candidate gene approach and variance component analyses.

Variance component analysis: (Neugebauer model)

According to Tier and Meyer \[52\], the neglecting of genomic imprinting in animal breeding programs could bias, e.g., breeding values and estimated genetic parameters. The first results on how much imprinted genes contribute to genetic variation in livestock were presented by De Vries \[63\] with estimates of genetic variance components of carcass and growth traits in pigs. They found that about 5% of the phenotypic variance in the back fat thickness and up to 4% of growth rate variance. De Vries \[63\] used an animal model augmented by either an additional paternal or maternal gametic effect, an approach, which was adopted by nearly all researchers in this field. Engellant and Tier found a significant paternal gametic variance for two fatness traits

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*Fig 3: Phenotypic score plotted against different dominance and imprinting conditions*
and, economically most important, for carcass meat content of German Gelbvieh finishing bulls \(^{[52]}\). However, these analysis were limited to consider imprinting effect from one parents only. Another approach was used by Essl and Voith suggesting to employ separate animal and dam model analysis to assess the difference between paternal and maternal imprinting \(^{[64]}\).

Statistical methods are available to incorporate genomic imprinting in breeding value prediction programs \(^{[65, 66]}\). In order to assess the relative importance of genomic imprinting for the genetic variation of traits economically relevant for beef and pork production. A model with two gametic effects for each animal was applied: the first corresponds to a paternal and the second to a maternal expression pattern of imprinted genes. The imprinting variance was estimated as the sum of both corresponding genetic variances per animal minus twice the covariance. Genetic variance components are expressed in terms of gametic variance as sire \(\sigma^2_s\), maternal variance as dam \(\sigma^2_d\) and their gametic covariance \(\sigma_{sd}\). The additive genetic variance is the sum of Mendelian and imprinting variance. For each observation paternal inheritance in the pedigree was traced back until the first male founder was identified. The number of this male founder was assigned to this observation as the corresponding \(y\)-chromosomal effect. We used the same system for the mitochondrial inheritance and traced back the maternal inheritance.

The imprinting effect were estimated by taking the difference between estimated genetic effects as sire and as dam correspond to the imprinting effect; for animal in the pedigree. The estimated imprinting variances are a result of the incomplete genetic correlation between both genetic effects and the differences between their variances. Several traits had a genetic correlation close to one, but showed a significant imprinting variance, which is mainly caused by a difference between both genetic variance components. Genomic imprinting significantly contributed to the genetic variance of 19 and 10 traits in pigs and beef cattle. The proportion of the total additive genetic variance that could be attributed to genomic imprinting was of the order between 5% and 19% and 8% to 25% in pigs and beef cattle respectively.

Expression studies

Such a studies are increasingly used due to the recent progress in next generation sequencing technologies. Using phasing information the parental allele expression levels are investigated and tested for a preferential expression. According to the tug-of-war theory of genomic imprinting \(^{27}\), imprinting should not be present in birds. An in-depth review on parent-of-origin QTL effects in chickens and their overlap with imprinted regions in mammals was provided by \(^{68}\). They argued that while chickens may not show genomic imprinting in the same way as mammals, several characteristics of imprinted genes are also found in the chicken genome \(^{67}\).

For instance, Lopes Pinto examined allele-specific RNA expression levels in three different tissues of F1 progeny of a reciprocal cross between The Virginia Tech high and low body weight line in chicken \(^{65}\). The aim of the experiment was to test for the preferential expression of alleles for parent-of-origin effects in six F1 individuals from reciprocal crosses of generation 54 parents. Pyro sequencing was used for whole transcriptome sequencing to generate generated circa 250 million RNA sequencing reads from RNA samples extracted from liver, hypothalamus and breast muscle. Only SNPs for which all four parents were fully informative (e.g. homozygous for line-specific alleles) were retained for further analysis. For the parent-of-origin effect we tested whether the maternally inherited alleles were preferentially expressed over the paternally inherited alleles or vice versa. The number of SNPs with parent-of-origin effects without allelic imbalance was between 500 and 650 for each tissue.

Candidate gene approach

Studies in mice and humans have shown that imprinted genes, whereby expression from one of the two parentally inherited alleles is attenuated or completely silenced, have a major effect on mammalian growth, metabolism and physiology. More recently, investigations in livestock species indicate that genes subject to this type of epigenetic regulation contribute to, or are associated with, several performance traits, most notably muscle mass and fat deposition. In the present study, a candidate gene approach was adopted to assess 17 validated single nucleotide polymorphisms (SNPs) and their association with a range of performance traits in 848 progeny tested Irish Holstein-Friesian artificial insemination sires. These SNPs are located proximal to, or within, the bovine orthologs of eight genes (CALCR, GRB10, PEG3, PHLD2A, RASGRF1, TSPAN32, ZIM2 and ZNF215) that have been shown to be imprinted in cattle or in at least one other mammalian species (i.e. human/ mouse/ pig/ sheep).

Of the eight candidate bovine imprinted genes assessed, DNA sequence polymorphisms in six of these genes (CALCR, GRB10, PEG3, RASGRF1, ZIM2 and ZNF215) displayed associations with several of the phenotypes included for analyses. The genotype-phenotype associations detected here are further supported by the biological function of these six genes, each of which plays important roles in mammalian growth, development and physiology. The associations between SNPs within the imprinted PEG3 gene cluster and traits related to calving, calf performance and gestation length suggest that this domain on chromosome 18 may play a role regulating pre-natal growth and development and fertility. SNPs within the bovine ZNF215 gene were associated with bovine growth and body conformation traits and studies in humans have revealed that the human ZNF215 ortholog belongs to the imprinted gene cluster associated with Beckwith-Wiedemann syndrome-a genetic disorder characterised by growth abnormalities. Similarly, the data presented here suggest that the ZNF215 gene may have an important role in regulating bovine growth. Collectively, our results support previous work showing that (candidate) imprinted genes/loci contribute to heritable variation in bovine performance traits and suggest that DNA sequence polymorphisms within these genes/loci represents an important reservoir of genomic markers for future genetic improvement of dairy and beef cattle populations.

1. bovine CALCR : angularity and body condition
2. GRB10 : angularity, body conditioning score and rump angle
3. PEG3 and ZIM2: animal stature, angularity
4. RASGRF1: milk protein percentage, somatic cell count
5. ZNF215: growth-related traits

Imprinting effects in GBLUP

Genomic best linear unbiased prediction (GBLUP) is a statistical method used to predict breeding values using single nucleotide polymorphisms for selection in animal and plant breeding. Genetic effects are often modeled as additively acting marker allele effects. However, the actual mode of
biological action can differ from this assumption. Many livestock traits exhibit genomic imprinting, which may substantially contribute to the total genetic variation of quantitative traits. Here, the stud presented with two statistical models of GBLUP including imprinting effects (GBLUP-I) on the basis of genotypic values (GBLUP-II) and genetic values (GBLUP-I2). The performance of these models for the estimation of variance components and prediction of genetic values across a range of genetic variations was evaluated in simulations.

Estimates of total genetic variances and residual variances with GBLUP-I and GBLUP-I2 were close to the true values and the regression coefficients of total genetic values on their estimates were close to 1. Accuracies of estimated total genetic values in both GBLUP-I methods increased with increasing degree of imprinting and broad-sense heritability. When the imprinting variances were equal to 1.4% to 6.0% of the phenotypic variances, the accuracies of estimated total genetic values with GBLUP-I exceeded those with GBLUP by 1.4% to 7.8%. In comparison with GBLUP-I, the superiority of GBLUP-I2 over GBLUP depended strongly on degree of imprinting and difference in genetic values between paternal and maternal alleles. When paternal and maternal alleles were predicted (phasing accuracy was equal to 0.979), accuracies of the estimated total genetic values in GBLUP-I and GBLUP-I2 were 1.7% and 1.2% lower than when paternal and maternal alleles were known. This simulation study shows that GBLUP-I and GBLUP-I2 can accurately estimate total genetic variance and perform well for the prediction of total genetic values. GBLUP-I is preferred for genomic evaluation, while GBLUP-I2 is preferred when the imprinting effects are large, and the genetic effects differ substantially between sexes.

Conclusion

Imprinting parent-of-origin effects may complicate quantitative genetic models used in phenotypic association studies. Important factor for models in future genetic improvement program would be handy to understand the imprinting effect on the phenotype. GBLUP models could help in assertive mating in field performance. Still lots of scope for improvement and revision needed in the models.

References

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