

## Review Article

## Environmental Epigenomics in Human Health and Disease

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The epigenome consists of the DNA methylation marks and histone modifications involved in controlling gene expression. It is accurately reproduced during mitosis and can be inherited transgenerationally. The innate plasticity of the epigenome also enables it to be reprogrammed by nutritional, chemical, and physical factors. Imprinted genes and metastable epialleles represent two classes of genes that are particularly susceptible to environmental factors because their regulation is tightly linked to epigenetic mechanisms. To fully understand the etiology of the most devastating diseases that plague humans, the full complexity of the human epige-

nome will ultimately need to be characterized. Moreover, the elucidation of the interaction of the environment with the epigenome should allow for the development of novel epigenetic-based diagnostic, prevention, and therapeutic strategies for human diseases. Herein, we introduce the emerging field of environmental epigenomics, discuss the importance of imprinted genes and metastable epialleles as epigenetically labile genomic targets, and endorse the genome-wide identification of the full suite of epigenetically labile targets in both the mouse and human genomes. *Environ. Mol. Mutagen.* 49:4–8, 2008. © 2008 Wiley-Liss, Inc.

**Key words:** environmental epigenomics; genomic imprinting; metastable epiallele

## INTRODUCTION

Emerging research concerning environmental influences on health and disease has begun to determine the mechanisms responsible for phenotypic differences in genetically identical individuals. Specifically, the “fetal basis of adult disease” or “early origins hypothesis” postulates that nutrition and other environmental factors during prenatal and early postnatal development influence cellular plasticity, thereby altering susceptibility to adult cardiovascular disease, type 2 diabetes, obesity, and other chronic diseases [Barker, 1997; Barker et al., 2005]. Developmental plasticity occurs when environmental influences affect cellular pathways during gestation, enabling a single genotype to produce a broad range of adult phenotypes [Bateson et al., 2004].

Environmental exposure to nutritional, chemical, and physical factors can alter gene expression, and affect adult phenotype by not only mutating promoter and coding regions of genes but also by modifying CpG methylation and other epigenetic modifications at critical epigenetically labile genomic regions [Waterland and Jirtle, 2004]. Literally meaning “above the genome,” the epigenome comprises the heritable changes in gene expression that occur in the absence of changes to the DNA sequence itself. Epigenetic mechanisms include chromatin folding and attachment to the nuclear matrix, packaging of DNA around nucleosomes, covalent modifications of histone

tails (e.g. acetylation, methylation, phosphorylation), and DNA methylation. The influence of regulatory small RNAs and micro RNAs on gene transcription is also increasingly recognized as a key mechanism of epigenetic gene regulation [Matzke and Birchler, 2005].

Three potential epigenetic susceptibility targets for environmentally induced effects are transposable elements, the promoter regions of housekeeping genes, and cis-acting regulatory elements of imprinted genes. These genomic targets contain CpG islands that are normally methylated, unmethylated or differentially methylated, respectively. Of these epigenetically labile targets, transposable elements

Abbreviations: AS, Angelman syndrome;  $A^{vy}$ , viable yellow agouti; BPA, bisphenol A; BWS, Beckwith–Wiedemann syndrome; CpG, cytosine–guanine dinucleotide; ERV, endogenous retrovirus; IAP, intracisternal A particle; IVF, in vitro fertilization; LOH, loss of heterozygosity; LOI, loss of imprinting; LTR, long terminal repeat; PWS, Prader Willi syndrome.

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and imprinted genes are particularly important (see below). Furthermore, the epigenome is particularly susceptible to deregulation during gestation, neonatal development, puberty, and old age. Nevertheless, it is most vulnerable to environmental factors during embryogenesis because the DNA synthetic rate is high, and the elaborate DNA methylation patterning required for normal tissue development is established during early development.

## GENOMIC IMPRINTING

The vast majority of autosomal genes are expressed from both parentally contributed alleles; however, the expression of an increasing number of growth regulatory genes is controlled by an unusual epigenetic phenomenon referred to as genomic imprinting [Reik and Walter, 2001; Murphy and Jirtle, 2003]. Genomic imprinting is a form of gene regulation in which epigenetic chromosomal modifications drive differential gene expression in a parent-of-origin manner. Imprinted genes were first hypothesized following nuclear transplantation studies conducted by Surani and colleagues in the 1980s in which diploid androgenotes derived from two male pronuclei and diploid gynogenotes derived from two female pronuclei developed improperly [Barton et al., 1984; Surani et al., 1984]. It was not until 1991, however, that the first imprinted genes were identified. Since the demonstration that *insulin-like growth factor 2 (Igf2)*, a potent growth factor [DeChiara et al., 1991], and *insulin-like growth factor 2 receptor (Igf2r)* [Barlow et al., 1991] are imprinted, ~80 imprinted genes have been identified in mice and humans, with 29, or about one-third being imprinted in both species [Morison et al., 2005].

Because imprinted genes are functionally haploid, the health consequences of genomic imprinting are potentially disastrous. Monoallelic expression eliminates the protection that diploidy normally affords against deleterious effects of recessive mutations. Imprinted gene dysregulation can occur in somatic cells, either by epigenetic or genetic mutations, causing cancer [Feinberg, 2004; Feinberg and Tycko, 2004]. Imprinted genes are therefore at a much greater risk of somatic cell inactivation by mutation, loss of heterozygosity (LOH), and epigenetic alterations in gene expression because one allele is already inactive because of imprinting. The imprinted, silenced allele has been equated to the “first hit,” as proposed by Knudson in his two-step model for carcinogenesis. Furthermore, abnormal expression of imprinted genes during development results in a number of severe pediatric developmental disorders such as Prader–Willi syndrome (PWS), Angelman syndrome (AS), and Beckwith–Wiedemann syndrome (BWS) [reviewed in Murphy and Jirtle, 2003]. In all three of these imprinting disorders, epigenetic alterations have an important contributory or causative role. Additionally, loss of imprinting (LOI) during in vitro fertilization (IVF) is associated with a significant

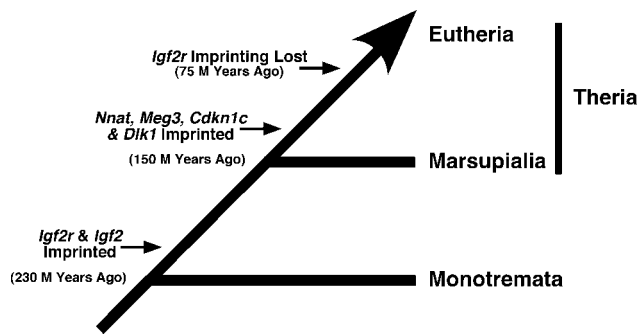
increase in the incidence of these developmental disorders [Niemitz and Feinberg, 2004].

Mutations in non-imprinted regions can also influence the regulation of imprinted genes and result in phenotypic consequences. The *Callipyge (CLPG1)* gene is named after the Greek goddess of love, Aphrodite Kallipygos whose name means “beautiful buttocks.” Mutation of this apparent single locus gene in the telomeric region of ovine chromosome 18 results in fast twitch muscle hypertrophy in sheep [Cockett et al., 1994]. The only genotype that expresses this muscle hypertrophy is one in which the mutant callipyge allele is inherited from the sire and a normal allele is inherited from the dam. The homologous regions on human chromosome 14 and mouse chromosome 12 have been intensively studied because the genes *DLK1* and *MEG3*, which are present in this region, are reciprocally imprinted and expressed from the paternal and maternal alleles, respectively [Schmidt et al., 2000; Takada et al., 2000; Wylie et al., 2000]. Mutation detection in a ram of callipyge phenotype revealed a single A/G polymorphism in the *CLPG1* gene that causes muscle hypertrophy in sheep [Murphy et al., 2005].

The most widely debated theory of why imprinting evolved, “the conflict hypothesis,” predicts that imprinting arose because of a genetic tug-of-war between the parents to control the amount of nutrients extracted from the mother by her offspring [Haig and Graham, 1991; Wilkins and Haig, 2003]. We demonstrated that imprinting evolved ~230–150 million years ago following the divergence of Prototherian (i.e. monotremes) from Therian (i.e. marsupials and eutherians) mammals (Fig. 1) [Killian et al., 2000; Murphy and Jirtle, 2003]. Thus, genomic imprinting arose in mammals with the evolution of the placenta and advent of viviparity. Although *Igf2* is imprinted in all Therian mammals investigated including humans, imprinting at the *Igf2r* locus was lost ~75 million years ago in an ancestral mammal that ultimately gave rise to primates (Fig. 1) [Killian et al., 2001; Murphy and Jirtle, 2003]. *NNAT* and *MEG3* are imprinted genes found only in eutherian mammals [Evans et al., 2005]. Although *DLK1* and *CDKN1C* are present in marsupials, they are only imprinted in eutherians (Fig. 1) [Suzuki et al., 2005; Weidman et al., 2006]. These findings demonstrate that the expression of imprinted genes is species, tissue, and developmental stage dependent, and indicate that imprinting may play an important role in mammalian speciation.

## METASTABLE EPIALLELES

Metastable epialleles are alleles that are variably expressed due to epigenetic modifications that are established in very early development [Rakyan et al., 2002]. They are most often associated with retroelements and transgenesis. Three of the identified murine metastable epialleles (*A<sup>vy</sup>*, *Axin<sup>Fu</sup>*, *Cabp<sup>IAP</sup>*) are associated with contraoriented IAP



**Fig. 1.** Evolution of imprinted genes. Phylogenetic analysis demonstrates that imprinting at the *IGF2R* and *IGF2* loci evolved ~230–150 million years ago following the divergence of Prototherian (i.e. monotremes) from Therian (i.e. marsupials and eutherians) mammals [Killian et al., 2000]. Studies of *NNAT* and *MEG3* reveal that they are eutherian-specific and imprinted. Although *DLK1* and *CDKN1C* are present in marsupials, they are only imprinted in eutherians [Suzuki et al., 2005; Weidman et al., 2006]. Finally, although *IGF2* is clearly imprinted in all Therian mammals including humans, imprinting at the *IGF2R* locus was lost ~75 million years ago in an ancestral mammal that ultimately gave rise to primates [Killian et al., 2001]. Thus, imprinted genes evolved at multiple times in the course of mammalian evolution, and gene imprinting has been both gained and lost.

insertions [Duhl et al., 1994; Vasicek et al., 1997; Druker et al., 2004]. The extent of DNA methylation at each allele is stochastic and dependent upon maternal nutrition and environmental exposures during early development [Waterland and Jirtle, 2003; Dolinoy et al., 2006; Waterland et al., 2006; Dolinoy et al., 2007]. Approximately 1,000 copies of IAP retrotransposons are present in the mouse genome [Kuff and Lueders, 1988], and about 40% of the human genome is comprised of transposable elements, of which ~9% are retrotransposons [International Human Genome Sequencing Consortium, 2001].

The viable yellow agouti ( $A^{vy}$ ) allele is the most extensively studied murine metastable epiallele. The  $A^{vy}$  mouse model, in which coat color variation is correlated to epigenetic marks established early in development, has been used to investigate the impacts of nutritional and environmental influences on the fetal epigenome. The wildtype murine *Agouti* gene encodes a paracrine signaling molecule that produces either black eumelanin (*a*) or yellow pheomelanin (*A*). Both *A* and *a* transcriptions are initiated from a developmentally regulated hair-cycle specific promoter in exon 2. Transient *A* expression in hair follicles during a specific stage of hair growth results in a sub-apical yellow band on each black hair shaft, causing the brown agouti coat color of wild-type mice [Duhl et al., 1994]. The  $A^{vy}$  metastable epiallele resulted from the insertion of an intracisternal A particle (IAP) murine retrotransposon upstream of the transcription start site of the *Agouti* gene [Duhl et al., 1994; Waterland and Jirtle, 2003]. A cryptic promoter in the proximal end of the  $A^{vy}$  IAP promotes constitutive ectopic *Agouti* transcription not only in hair follicles, but throughout all cells, leading to yellow fur, as well as adult onset obesity, diabetes, and tumorigen-

esis [Miltenberger et al., 1997; Morgan et al., 1999]. Interestingly, CpG methylation in the  $A^{vy}$  IAP correlates inversely with ectopic *Agouti* expression. The degree of methylation within the 5' IAP long terminal repeat (LTR) varies dramatically among individual isogenic  $A^{vy}/a$  mice, causing a wide variation in coat color ranging from yellow (unmethylated) to pseudoagouti (methylated).

We initially utilized the  $A^{vy}$  mouse model as an epigenetic biosensor to characterize nutritional factors affecting epigenetic gene regulation and subsequent adult phenotype. In 2003, Waterland and Jirtle fed agouti dams a diet high in methyl donors, such as folic acid and betaine. A marked shift in offspring coat color distribution toward brown was observed as well as increased DNA methylation near the  $A^{vy}$  metastable epiallele [Waterland and Jirtle, 2003]. The  $A^{vy}$  model was also employed to investigate the effects of plant phytoestrogens on the fetal epigenome [Dolinoy et al., 2006]. Maternal dietary supplementation with genistein (250 mg/kg diet), the major isoflavone in soy, also shifted the coat color distribution of  $A^{vy}/a$  offspring toward brown and increased DNA methylation of six CpG sites within the  $A^{vy}$  IAP.

The  $A^{vy}$  model was used recently to evaluate the effects on the fetal epigenome of maternal exposure to toxicological agents. Maternal dietary exposure to the endocrine active compound, bisphenol A (BPA), shifted the coat color of  $A^{vy}/a$  offspring toward yellow (Fig. 2A), and decreased the methylation of nine CpG sites within the  $A^{vy}$  IAP [Dolinoy et al., 2007]. CpG methylation was also decreased at the *Cabp<sup>IAP</sup>* metastable locus, indicating that BPA-induced hypomethylation is not gene locus specific, and may also impact yet unidentified epigenetically labile genes in the mouse, and potentially, human genome. Moreover, the BPA-induced hypomethylation of the fetal epigenome was abolished by maternal dietary nutritional supplementation with either methyl donors (folic acid, betaine, vitamin B<sub>12</sub>, and choline) (Fig. 2B) or the phytoestrogen genistein (Fig. 2C). These findings demonstrate that simple dietary changes can protect against the deleterious effects of environmental toxicants on the fetal epigenome.

In all of these studies, the extent of DNA methylation in tissues from the three germ layers (brain, kidney, and liver) was similar, indicating that nutritional and environmental influence on DNA methylation occurs during early embryonic development [Waterland and Jirtle, 2003; Dolinoy et al., 2006, 2007]. Clearly, embryogenesis is a critical window of vulnerability for environmentally induced epigenetic alterations. In fact, epigenetic marks, including CpG methylation are generally stable in somatic cells; however, during at least two developmental time periods, the epigenome undergoes extensive reprogramming. These critical windows of development include gametogenesis as well as early pre-implantation embryos [Reik et al., 2001]. Therefore, to fully characterize environmental epigenomics, an expanded analysis of timing of exposure will be essential.

## BIOINFORMATICS APPROACH TO IDENTIFY EPIGENETICALLY LABILE GENES

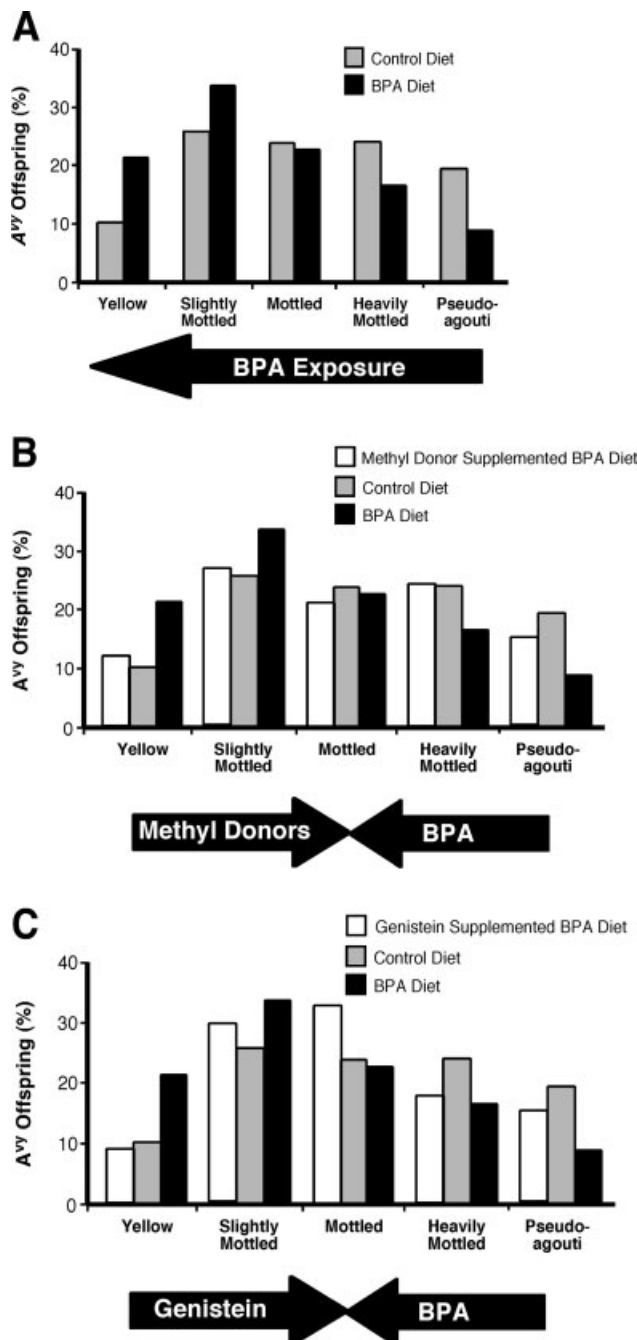
Until recently, most attempts to identify imprinted genes were experimental, focusing on small regions of a chromosome. In 2005, a robust method for genome-wide identification of imprinted genes was developed using machine-learning algorithms trained to identify genomic motifs predictive of imprinted genes [Luedi et al., 2005]. Luedi et al. [2005] developed a bioinformatics approach for interrogating the entire mouse genome to identify genes

with high probability of being imprinted. Data were collected on repeated elements, CpG islands, transcription factor binding sites, and other DNA characteristics within the upstream and downstream regions, introns, and exons of all annotated genes in the mouse genome. The most important genomic features for predicting the imprint status of a gene include the intronic presence of endogenous retrovirus (ERV) elements, and their insertion orientation relative to that of the gene. Of the 23,788 annotated autosomal mouse genes, the imprinted gene prediction algorithm identified 600 (2.5%) candidate imprinted genes, 64% of which are predicted to exhibit maternal expression.

The real power of a bioinformatics approach for predicting imprinted genes, however, lies in its ability to readily interrogate the genomes of any eutherian species for which complete genomic sequence is available. Therefore, Luedi et al. [2007] applied and extended the machine learning approach to identify candidate imprinted genes in the human genome. Of the 20,770 annotated autosomal genes in the human genome, 156 (0.75%) are predicted to be imprinted, and 56% of them are likely to be expressed only from the maternal allele. Moreover, only 32% of these genes are predicted to be imprinted in both mice and humans. Thus, the repertoire of imprinted genes appears to be highly species dependent, indicating that the mouse may not be an appropriate model for assessing human disease risk resulting from epigenetic deregulation of imprinted genes. The development of similar bioinformatics models to identify metastable epialleles is imperative to characterize the full suite of genes susceptible to environmentally induced changes of the epigenome.

## CONCLUSION

Once the identification of key epigenetically labile loci in humans is accomplished, epigenetic approaches for screening and diagnosis will become highly useful in enabling



**Fig. 2.** Maternal nutrient supplementation counteracts BPA-induced DNA hypomethylation in the offspring. (A) Coat color distribution of  $A^{vy}/a$  offspring born to control and BPA-exposed litters (50 mg BPA/kg diet). Maternal BPA exposure shifts offspring coat color distribution toward yellow (indicated by left facing "BPA Exposure" arrow) ( $P = 0.007$ ). (B) Coat color distribution of  $A^{vy}/a$  offspring born to BPA-exposed/methyl donor supplemented, control and BPA-exposed mothers. Maternal nutritional supplementation with methyl donors (4.3 mg folic acid/kg diet, 0.53 mg vitamin  $B_{12}$ /kg diet, 5 g betaine/kg diet, and 7.97 g choline chloride/kg diet) counteracts BPA-induced DNA hypomethylation reducing the shift in coat color distribution toward yellow (indicated by right facing "Methyl Donors" arrow). (C) Coat color distribution of  $A^{vy}/a$  offspring born to BPA-exposed/genistein supplemented, control and BPA-exposed mothers. Maternal nutritional supplementation of the BPA diet with the phytoestrogen genistein (250 mg genistein/kg diet) also counteracts BPA-induced DNA hypomethylation and the shift in coat color distribution toward yellow (indicated by right facing "Genistein" arrow) (Redrawn with permission from Dolinoy DC, Huang D, Jirtle RL, Proc Natl Acad Sci USA, 2007, 104, 13056–13061, © National Academy of Sciences).

clinicians to identify at-risk individuals prior to disease onset. For example, screening individuals at an early age for epigenetically susceptible disease profiles will allow for closer monitoring and more frequent follow-up. Additionally, unlike genetic mutations, epigenetic profiles are potentially reversible. Therefore, epigenetic approaches for prevention and treatment, such as nutritional supplementation and/or pharmaceutical therapies may be developed to counteract negative epigenomic profiles. The future of epigenomics therapy holds tremendous potential for not only individualized health care but also for population-wide disease diagnostic, screening, and prevention strategies.

## REFERENCES

- Barker D. 1997. Intrauterine programming of coronary heart disease and stroke. *Acta Paediatr* 423 (Suppl):178–182.
- Barker DJP, Osmond C, Forsen TJ, Kajantie E, Eriksson JG. 2005. Trajectories of growth among children who gave coronary events as adults. *N Engl J Med* 353:1802–1809.
- Barlow DP, Stoger R, Herrmann BG, Saito K, Schweifer N. 1991. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature* 349:84–87.
- Barton S, Surani M, Norris M. 1984. Role of paternal and maternal genomes in mouse development. *Nature* 311:374–376.
- Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, Gluckman P, Godfrey K, Kirkwood T, Lahr MM, McNamara J, Metcalfe NB, Monaghan P, Spencer HG, Sultan SE. 2004. Developmental plasticity and human health. *Nature* 430:419–421.
- Cockett NE, Jackson SP, Shay TL, Nielsen D, Moore SS, Steele MR, Barendse W, Green RD, Georges M. 1994. Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. *Proc Natl Acad Sci USA* 91:3019–3023.
- DeChiara TM, Robertson EJ, Efstratiadis A. 1991. Parental imprinting of the mouse insulin-like growth factor ii gene. *Cell* 64:849–859.
- Dolinoy DC, Wiedman J, Waterland R, Jirtle RL. 2006. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ Health Perspect* 114:567–572.
- Dolinoy DC, Huang D, Jirtle RL. 2007. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc Natl Acad Sci USA* 104:13056–13061.
- Druker R, Bruxner TJ, Lehrbach NJ, Whitelaw E. 2004. Complex patterns of transcription at the insertion site of a retrotransposon in the mouse. *Nucleic Acids Res* 32:5800–5808.
- Duhl D, Vrieling H, Miller K, Wolff G, Barsh G. 1994. Neomorphic agouti mutations in obese yellow mice. *Nat Genet* 8:59–65.
- Evans HK, Weidman JR, Cowley DO, Jirtle RL. 2005. Comparative phylogenetic analysis of *Bicap1/Nnat* reveals eutherian-specific imprinted gene. *Mol Biol Evol* 22:1740–1748.
- Feinberg AP. 2004. The epigenetics of cancer etiology. *Semin Cancer Biol* 14:427–432.
- Feinberg AP, Tycko B. 2004. The history of cancer epigenetics. *Nat Rev Cancer* 4:143–153.
- Haig D, Graham C. 1991. Genomic imprinting and the strange case of the insulin-like growth factor II receptor. *Cell* 64:1045–1046.
- International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. *Nature* 209:860–921.
- Killian J, Byrd J, Jirtle J, Munday B, Stoskopf M, MacDonald R, Jirtle R. 2000. M6P/IGF2R imprinting evolution in mammals. *Mol Cell* 5:707–716.
- Killian JK, Nolan CM, Wylie AA, Li T, Vu TH, Hoffman AR, Jirtle RL. 2001. Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. *Hum Mol Genet* 10:1721–1728.
- Kuff E, Lueders K. 1988. The intracisternal A-particle gene family: Structure and functional aspects. *Adv Cancer Res* 51:183–276.
- Luedi PP, Hartemink AJ, Jirtle RL. 2005. Genome-wide prediction of imprinted murine genes. *Genome Res* 15:875–884.
- Luedi PP, Dietrich FS, Weidman JR, Bosko JM, Jirtle RL, Hartemink AJ. 2007. Computational and experimental identification of novel human imprinted genes. *Genome Res* 17:1723–1730.
- Matzke M, Birchler J. 2005. RNAi-mediated pathways in the nucleus. *Nat Rev Genet* 6:24–35.
- Miltenberger R, Mynatt R, Wilkinson J, Woychik R. 1997. The role of the agouti gene in the Yellow Obese Syndrome. *J Nutr* 127:1902S–1907S.
- Morgan H, Sutherland H, Martin D, Whitelaw E. 1999. Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 23:314–318.
- Morison IM, Ramsay JP, Spencer HG. 2005. A census of mammalian imprinting. *Trends Genet* 21:457–465.
- Murphy SK, Jirtle RL. 2003. Imprinting evolution and the price of silence. *Bioessays* 25:577–588.
- Murphy SK, Freking BA, Smith TPL, Leymaster K, Nolan CM, Wylie AA, Evans HK, Jirtle RL. 2005. Abnormal postnatal maintenance of elevated *DLK1* transcript levels in callipyge sheep. *Mamm Genome* 16:171–183.
- Niemitz E, Feinberg A. 2004. Epigenetics and assisted reproductive technology: A call for investigation. *Am J Hum Genet* 74:599–609.
- Rakyan VK, Blewitt ME, Druker R, Preis JJ, Whitelaw E. 2002. Metastable epialleles in mammals. *Trends Genet* 18:348–351.
- Reik W, Walter J. 2001. Genomic imprinting: Parental influence on the genome. *Nat Rev Genet* 2:21–32.
- Reik W, Dean W, Walter J. 2001. Epigenetic reprogramming in mammalian development. *Science* 293:1089–1093.
- Schmidt JV, Matteson PG, Jones BK, Guan XJ, Tilghman SM. 2000. The *Dlk1* and *Gtl2* genes are linked and reciprocally imprinted. *Genes Dev* 14:1997–2002.
- Surani MA, Barton SC, Norris ML. 1984. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature* 308:548–550.
- Suzuki S, Renfree M, Pask A, Shaw G, Kobayashi S, Kohda T, Kaneko-Ishino T, Ishino F. 2005. Genomic imprinting of IGF2, p57(KIP2) and PEG1/MEST in a marsupial, the tammar wallaby. *Mech Dev* 122:213–222.
- Takada S, Tevendale M, Baker J, Georgiades P, Campbell E, Freeman T, Johnson MH, Paulsen M, Ferguson-Smith AC. 2000. Delta-like and gtl2 are reciprocally expressed, differentially methylated linked imprinted genes on mouse chromosome 12. *Curr Biol* 10:1135–1138.
- Vasicek T, Zeng L, Guan X, Zhang T, Costantini F, Tilghman S. 1997. Two dominant mutations in the mouse fused gene are the result of transposon insertions. *Genetics* 147:777–786.
- Waterland R, Jirtle R. 2003. Transposable elements: Targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23:5293–5300.
- Waterland R, Jirtle R. 2004. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition* 20:63–68.
- Waterland R, Dolinoy DC, Lin J-R, Smith CA, Shi X, Tahiliani K. 2006. Maternal methyl supplements increase offspring DNA methylation at Axin(fused). *Genesis* 44:401–406.
- Weidman J, Maloney K, Jirtle R. 2006. Comparative phylogenetic analysis reveals multiple non-imprinted isoforms of opossum *Dlk1*. *Mamm Genome* 17:157–167.
- Wilkins JF, Haig D. 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nat Rev Genet* 4:359–368.
- Wylie AA, Murphy SK, Orton TC, Jirtle RL. 2000. Novel imprinted *DLK1/GTL2* domain on human chromosome 14 contains motifs that mimic those implicated in *IGF2/H19* regulation. *Genome Res* 10:1711–1718.