



Epigenetic gene regulation: early environmental exposures



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Environmental epigenetics

Traditional research on the combined effects of genetics and the environment investigates how individuals differ in susceptibility to disease and how susceptibility changes over time. The majority of these gene–environment interaction studies focus on genetic sequence variants, including single nucleotide polymorphisms (SNPs), which influence toxicant uptake, metabolism and subsequent disease susceptibility. Others hold genetics constant to evaluate the effects of differential nutritional, environmental and occupational exposures on health and disease. However, it has become clear that while genetics and the environment play an important role in the manifestation of many disorders, they do not fully explain all variation in human disease susceptibility.

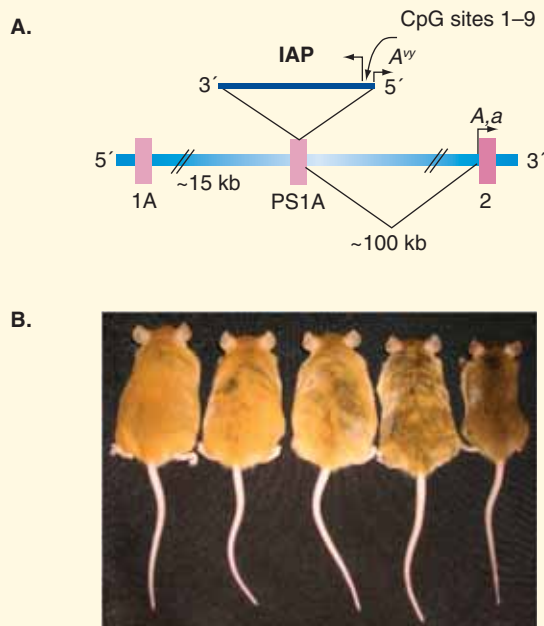
However, a growing body of evidence suggests that epigenetic gene regulation, including DNA methylation and histone modifications, are also influenced by the environment and may play a role in the fetal basis of adult disease [1]. Specifically, the ‘early origins hypothesis’ postulates that nutrition and other environmental factors during prenatal and early postnatal development program the risks for adverse health outcomes in adult life [2–4]. Developmental plasticity occurs when environmental influences affect cellular pathways during gestation, enabling a single genotype to produce a broad range of adult phenotypes [1]. Recently, human epidemiologic and animal model data have suggested that developmental plasticity is influenced by persistent epigenetic adaptations that occur early in development in response to environmental factors [5–8].

Epigenetics is defined as the study of heritable changes in gene expression that occur in the absence of a change in the DNA sequence itself. These include DNA methylation, covalent modifications to histone tails, packaging of DNA around nucleosomes, chromatin folding and chromatin attachment to the nuclear matrix. Additionally, the influence of regulatory small/micro RNAs on gene transcription is an emerging mechanism of epigenetic gene regulation. The epigenome is particularly susceptible to environmental influences during embryogenesis because the DNA synthetic rate is high and the elaborate DNA methylation patterning required for normal tissue development is established during this period. Furthermore, aberrant epigenetic gene regulation has been proposed as a mechanism of action for nongenotoxic carcinogenesis [9], imprinting disorders [10,11] and complex disorders including schizophrenia [12] and asthma [13]. Despite a growing consensus on the role of epigenetic gene regulation in susceptibility to chronic diseases, we have yet to identify the majority of epigenetically labile regions of the genome, fully characterize the important environmental exposures affecting the epigenome and determine the critical windows of vulnerability to environmentally-induced epigenetic alterations.

Proof of concept: viable yellow agouti mice

Within human, mouse, and other animal species, individuals considered genetically identical, such as monozygotic twins and isogenic mouse strains, often display phenotypic discordance in various traits and disease susceptibility, even after controlling for environment. A handful of murine metastable epialleles have been identified (*A^v*, *Axin^{Fu}*, *Cabp^{LA}*) in which the activity of a retrotransposon controls expression of an adjacent gene (Figure 1A) [14–16]. Metastable epialleles are alleles that are variably expressed in genetically identical individuals due to epigenetic modifications that were established during early development [16]. Variable expressivity of murine metastable epialleles results from stochastic DNA methylation of the retrotransposon, producing genetically identical individuals with

Figure 1. The viable yellow agouti (A^{vy}) mouse model.



(A) A contra-oriented IAP insertion within pseudoexon 1A of the murine agouti gene contains a cryptic promoter (short arrowhead labeled A^{vy}) that drives ectopic Agouti expression. Transcription of A and a alleles initiates from a hair-cycle specific promoter in exon 2 (short arrowhead labeled A, a). **(B)** Genetically identical 3-month old viable yellow agouti mice representing the five coat color phenotypes. Yellow mice are hypomethylated at the transposable element upstream of the agouti gene, allowing maximal ectopic expression, whereas hypermethylation of this site silences ectopic agouti expression in the pseudoagouti animals. Mice that are predominately yellow are also clearly more obese than brown mice. Maternal supplementation with methyl donating compounds, such as folic acid, or the phytoestrogen, genistein, is associated with a coat color distribution shift toward the brown pseudoagouti phenotype, resulting in population level protection from adult onset diseases. Reprinted with permission from [17].

A^{vy} : Viable yellow agouti; IAP: Intracisternal A particle; PS1A: Pseudoexon 1A.

varying phenotypes. Interestingly, the normal distribution of variable expressivity can be shifted at these metastable epialleles following early exposure to various environmental factors [8,17–19].

One model for such epigenetically-based phenotypic variability is the viable yellow agouti (A^{vy}) mouse (Figures 1A and B), in which coat color variation is influenced by epigenetic marks established early in development. The A^{vy} allele resulted from the insertion of an intracisternal A particle (IAP) murine retrotransposon upstream of the transcription start site of the agouti gene (Figure 1A) [19,20]. A cryptic promoter in the proximal end of the A^{vy} IAP promotes constitutive ectopic agouti transcription, leading to yellow fur, obesity and tumorigenesis [21,22]. CpG

methylation in the A^{vy} IAP correlates inversely with ectopic agouti expression. The degree of methylation varies dramatically among individual isogenic A^{vy}/a mice, causing a wide variation in coat color ranging from yellow (unmethylated) to pseudoagouti (methylated) [21].

Recently, the A^{vy} model has been used as an epigenetic biosensor for determining whether maternal nutritional supplementation affects the fetal epigenome. Cooney and colleagues [8] and Waterland and colleagues [19] demonstrated that maternal dietary methyl supplementation with extra folic acid, vitamin B12, choline and betaine shifts the coat color distribution of the offspring towards the pseudoagouti phenotype. Waterland and colleagues further demonstrated that the shift in coat color distribution was caused by increased methylation near the A^{vy} retrotransposon [19]. Methylation profiles were highly correlated in tissues from ectodermal (brain and tail), endodermal (liver) and mesodermal (kidney) lineages, indicating that methylation profiles at the A^{vy} locus are established prior to embryonic stem cell differentiation. In addition, methylation in day 21 of life (d21) tissues is correlated to methylation in d100 tissues, demonstrating that methylation of this metastable epiallele is efficiently maintained over time. Subsequently, Cropley and colleagues reported that nutritional influences on coat color distribution in A^{vy} mice are inherited in the F2 generation via germline epigenetic modifications [23]. Pseudoagouti female offspring of mothers supplemented with methyl donors (folic acid, betaine, choline, methionine and vitamin B12) produced more pseudoagouti offspring than pseudoagouti females who were fed a normal control diet. This striking shift in coat color indicates that increased methylation at the A^{vy} locus persists into the F2 generation, even though only the grandmother received nutrient supplementation. Thus, it is clear that environmental effects on the epigenome can be inherited in the mammalian germline.

More recently, Dolinoy and colleagues demonstrated that maternal dietary supplementation of mice with the phytoestrogen genistein, at levels comparable with humans consuming high soy diets, shifted the coat color of offspring toward pseudoagouti by increasing methylation of the A^{vy} retrotransposon. Methylation profiles were consistent across tissues from the three germ layer lineages, supporting the postulate that the methylation profiles genistein is influencing are also established very early in development. Moreover,

the genistein-induced hypermethylation persisted into adulthood and protected A^{Y} animals from adult-onset obesity [17].

Until now, the A^{Y} model has been employed chiefly as a biosensor for epigenetic alteration following maternal nutritional exposure; however, the opportunity exists to utilize this model as an epigenetic biosensor for xenobiotic, behavioral, pharmaceutical and physical environmental factors. Moreover, for environmental agents that shift the coat color distribution toward the yellow (unmethylated) phenotype, it will be important to determine whether coexposure with methyl donors and/or genistein negates the hypomethylation effect. The determination that nutritional supplementation counteracts epigenetic hypomethylating effects would hold tremendous potential for public health prevention and intervention.

Early environmental exposures, the fetal epigenome & transgenerational inheritance

Prenatal and early postnatal environmental factors, including nutritional supplements [5,8,17–19], xenobiotic chemicals [6,24,25], behavioral cues [26,27], reproductive factors [28,29], and even low-dose radiation [30], have been linked to altered epigenetic programming and subsequent changes in gene-expression patterns. Furthermore, epigenetic alterations have been observed in response to adolescent and adult exposure to environmental factors [31,32]. These important observations have recently been summarized [33]. Therefore, in this editorial I have focused on current literature demonstrating transgenerational inheritance of epigenetic marks influenced by environmental exposures.

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 preimplantation embryos [34]. Importantly, some genomic loci fully or partially escape epigenetic reprogramming during gametogenesis, leading to transgenerational inheritance of phenotype via epigenetic, not genetic mechanisms. In the late 1970s breeding studies involving both A^{Y} and axin-fused ($Axin^{Fu}$) mice revealed inheritance of coat color [35] or tail kink phenotype [36], respectively. For example, pseudoagouti A^{Y} mothers but not fathers produce more pseudoagouti offspring. In contrast, penetrant $Axin^{Fu}$ mothers and fathers produce more

offspring with tail kinks. Recently, DNA methylation in A^{Y} gametes, zygotes and blastocysts was assessed to determine if methylation was the transgenerationally inherited epigenetic mark [37]. Interestingly, DNA methylation was absent from the blastocyst, indicating that an epigenetic mechanism other than DNA methylation is the inherited mark.

“These findings indicate that you are not only what you eat, but what your mother and grandparents ate as well.”

Incomplete germline reprogramming of epigenetic marks implies that environmental-induced changes in the epigenome may be inherited transgenerationally, even in the absence of continued exposure. These findings indicate that you are not only what you eat, but what your mother and grandparents ate as well. A flurry of recent animal and human epidemiological studies supports this tenet. Drake and colleagues reported the transgenerational inheritance of low birth weight and adult diabetes in rats following maternal or paternal exposure to abnormal levels of glucocorticoids [38]. The authors hypothesized that germline epigenetic reprogramming rather than genetic mutations is responsible for the observed phenotypic changes. Subsequently, Newbold and colleagues reported transgenerational inheritance of tumor susceptibility in mice following perinatal maternal exposure to diethylstilbestrol, a synthetic estrogenic chemical [39]. Increased risk is thought to result from both genetic and epigenetic mechanisms, including decreased methylation in uterine genes such as lactoferrin and c-fos. Koturbash and colleagues also recently observed global decreases in methylation, reduced DNA methyltransferase concentration, and reduced methyl binding protein levels in mouse offspring of parents who were both exposed to radiation [40]. These epigenetic mechanisms are postulated to underlie observed transgenerational genome instability and carcinogenesis risk following parental radiation exposure. Finally, using epidemiological, crop yield and economic data from Sweden, Pembrey and colleagues reported sex-specific male germline transgenerational inheritance of health endpoints, including increased mortality [41]. Researchers hypothesize underlying epigenetic and sex-linked genetic mechanisms are associated with these inherited phenotypes.

In a series of recent reports, Anway and colleagues report transgenerational reproductive toxicity, decreased epididymal sperm count, and reduced sperm motility in male rats following maternal exposure to the endocrine active fungicide vinclozolin and the pesticide methoxychlor [42]. The reduced reproductive outcomes were observed not only in the first generation, but also in generations two to four. The high frequency of these pathologies, coupled with a sustained high risk of developing them in subsequent generations, led to the exclusion of genetic mutation in their etiology. In addition, as the animals aged, a number of adult onset disorders were observed transgenerationally, including breast cancer and prostate disease [43]. These transgenerational effects were associated with inherited epigenetic marks, including DNA methylation, in several genes in the male germ line [42,44].

Incorporation of epigenomics into personalized healthcare

The mounting evidence summarized in this editorial emphasizes the potential for environmental factors to influence fetal, adult and transgenerational epigenetic gene regulation, resulting in numerous phenotypic consequences. Nevertheless, despite the weight of this evidence, little is known about the genes in the human genome that are most susceptible to environmentally induced epigenetic changes. Analysis of epigenetic profiles of monozygotic twins offers confirmation that epigenetic dysregulation results in differential phenotype in genetically identical humans [45,46]. For example, epigenetic profiles of monozygotic twin sets, as measured by global CpG methylation and histone H3 and H4 acetylation, exhibited greater divergence in older twins as well as twins who had spent more than 50% of their lives apart [45]. These findings indicate that the adult environment influences epigenetic gene regulation as individuals age. Additionally, a twin discordant for a set of birth defects known as caudal duplication syndrome exhibited markedly decreased methylation of the *AXIN 1* gene when compared with her affected twin sister [46]. Whether this phenotypic difference, as well as others displayed in monozygotic twin sets, stems from intrauterine environment remains to be determined.

However, environmental epigenetic research has revealed that two distinct sets of epigenetically labile genes link early environmental exposures to adult disease. Genes with metastable epialleles have highly variable expression due to stochastic allelic changes in the epigenome. In contrast,

imprinted genes display monoallelic, parent-of-origin-dependent expression, which is regulated by a combination of DNA methylation, histone modification, and antisense transcript epigenetic mechanisms. The development of genome-wide approaches, including expression arrays and novel bioinformatic tools [47], to determine those epigenetically labile targets in the human genome that are involved in the etiology of chronic diseases is critical to the development of prevention, screening and therapeutic strategies. While the Human Epigenome Project [101] has achieved advances in the categorization of genome-wide DNA methylation patterns of human genes in major tissues [48], this approach will be incomplete without further identification of environmentally responsive epigenetically labile loci in the human genome.

“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.”

Once the identification of key genomic loci is accomplished, epigenetic approaches for screening and diagnosis will become highly useful in enabling 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 healthcare, but also population-wide diagnostic screening and prevention strategies, an exciting possibility indeed.

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Website

101. Human Epigenome Project homepage www.epigenome.org/index.php