



# Epigenetics – an introductory overview

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Epigenetics or imprinting refers to the process of ‘chemically’ marking the central A, C, G, T double-stranded DNA code. In the past several decades a deeper understanding has been gained of a wide range of mechanisms that have been invoked in biology for the purpose of regulating gene expression and dosage of protein products that are effectively translated and functional in the cells. We mostly carry two copies of a gene (one per non-sex chromosome inherited from each of our parents) in each of our somatic cells; however, some genes are required to have two functional copies in each cell, while others require no less than one copy, but are tolerant of more than one copy. There are very specific genes, often dealing with growth and development, which are, however, only ever needed in a single copy; any more or any less is problematic and even lethal. Epigenetics is the process of reversibly ‘marking’ genes, either driven by endogenous processes or through exposure to environmental factors, such that they may be effectively silenced. Human development requires an orderly and systematic means of switching on and off of genes; understandably, if there is a mutation affecting this process, it leads to disease, while exposure to chemicals or other experiential stimuli may have a similar effect. This review provides a broad outline of epigenetics and genetic imprinting on human and mammalian development and disease.

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## What is epigenetics?

Epigenetics refers to the phenomenon of differential expression of genes as a result of non-genetic changes to the underlying DNA. Simply put – it is the reversible ‘marking’ or ‘tagging’ (i.e. *epi* = ‘on top of’, or ‘outside off’ the structural genomic code) of regions of the genome, which leads to the adjacent gene not being expressed, at least until the ‘tag’ remains.<sup>[1]</sup> This brief review attempts to cover the subject from describing the role of endogenous programmed epigenetics influencing the transition of different cell types during development and growth, to discussing exogenous or environmental influences on gene expression and health and behavioural outcomes.

## Understanding gene expression during development and differentiation

Gene expression is regulated in four dimensions, i.e. the three dimensions of space, within which the cell types divide, and the fourth dimension of time. It should be obvious that not all of the ~20 000 genes (in humans) are expressed in every cell, from conception onwards. This would simply result in a multiplication of cells (1, 2, 4, 8, 16, 32, 64 ...  $2^n$ ) with something like a bulbous mass of trillions of cells needing to exit the womb at gestational maturity, hinting at a complex post-conceptual process of different genes switching on and off, temporally and spatially, guiding a conceptus through a process of differentiation and development.

We generally carry two copies (‘2n’) of every gene (except for those on the Y chromosome and mitochondria), one inherited paternally and the other maternally. However, some gene products are required in different functional doses. As a gross generalisation, and on the one hand, genes for **structural proteins** need a full complement of two functioning genes to produce a ‘2n’ product, e.g. to make collagen for bones, skin and teeth. Any compromise or mutation of these genes, to produce less than ‘2n’ of protein, results in a disorder, e.g. osteogenesis imperfecta or brittle bone disease (OMIM 166200). However, for most process-mediating functions, e.g. **enzymes**, one requires at least ‘1n’ amount of product, although ‘2n’ would generally

be produced. This provides tolerance for mutations, as is evident in recessively inherited disorders. It is why **parents** of children with recessive conditions, e.g. mucopolysaccharidoses (MPS) and Sanfilippo syndrome C (OMIM 252930), with just ‘1n’ copy of the gene, fare as well as the background population (who would have ‘2n’ copies of functional gene product). However, children with both copies of the gene rendered non-functional (= ‘0n’ dose of the product), suffer with a severe and generally life-threatening condition.

For a range of other genes, however, there is an obligatory need for only ‘1n’ copy of the gene ever needing to be expressed, and where either less than or more than ‘1n’ would be undesirable, or indeed pathogenic. In this regard, **DNA methylation**, i.e. the covalent addition of a methyl or  $-CH_3$  group on to a cytosine residue in the genome, was the first mechanism recognised that regulates gene expression ephemerally, i.e. in a generation of cells or in one generation of an organism. It is only when a cytosine occurs in sequence next to a guanine (so-called *CpG* island – where the *-p* represents the phosphodiester link between the C and G bases), that such a methylation is possible. This process of methylating genes to control expression is known as imprinting. Imprinted genes on the genome are generally involved in the control of embryonic growth and development, including that of the placenta.<sup>[2]</sup>

For this review, the author considers this a simple intergenerational switching on or off of these genes. Generally, these non-permanent ‘tags’ are erased completely during gametogenesis (sort of putting the genome through a ‘car-wash’), while at the same time undergoing parent-gender specific reprogramming of genes. Therefore, all sperm produced during spermatogenesis would have a certain complement of genes that are imprinted, while all eggs will have a different set of genes imprinted – and any resultant conceptus would only ever be producing ‘1n’ of each of those gene products. It is important to note that the biological mechanisms that underpin the parental gamete-specific epigenetics are programmed into the genetic machinery and are essential for normal development and differentiation. The reader

is referred to the 'parental conflict hypothesis' for more information on the evolution of genomic imprinting, and thoughts about why specific genes may have been selected for differential imprinting by the male and female lineages, respectively.<sup>[3]</sup>

Although the most common mechanism involved in epigenetics (or perhaps the one most often heard about) is that of methylation, other pretranscriptional and post-translational mechanisms include histone modification (through de/acetylation), ubiquitination, sumoylation, and RNA- and polycomb-based functions, although these are not fully understood.<sup>[4,5]</sup>

Many of the problems in generating cloned animals (starting with somatic cells derived from a mature individual), or understanding the limitations of parthenogenesis, or why two sperm cells or two eggs cannot produce a normal fetus/individual, stem from understanding the preferential imprinting of genes (both copies would either be switched off, i.e. '0n' or '2n' of the gene product would be produced when just '1n' is required). It has been reported that even *in vitro* fertilisation, including intracytoplasmic sperm injection, is associated with an increased risk of imprinting disorders.<sup>[6]</sup>

### Some human diseases associated with genomic imprinting

Prader-Willi syndrome (OMIM 176270) and Angelman syndrome (OMIM 105830) are associated with loss of the chromosomal region, 15q11-13. This region of the genome contains the *SNRPN* and *NDN* genes, which are paternally expressed (i.e. the maternal allele is imprinted), and *UBE3A*, which is maternally expressed. Inheritance of a paternal deletion of this region results in Prader-Willi syndrome, which is characterised by obesity, hypotonia and hypogonadism. However, if one inherited a maternal deletion, this is associated with Angelman syndrome, characterised by epilepsy, tremors and a permanently smiling facial expression.

Beckwith-Wiedemann syndrome (OMIM 130650) is another genetic disorder that is often associated with imprinting. It maps to chromosomal region 11p15.5, which contains two domains of imprinted genes: domain one contains genes such as *Igf2*, which is paternally expressed, and *H19*, which is maternally expressed;<sup>[7]</sup> the second or the *KIP/LIT1* domain has at least six imprinted genes.<sup>[8,9]</sup> Deletions or mutations of the respective genes may remain masked if they are meant to be imprinted – or they may be exposed if they are meant to be expressed – depending on gender of their parent of origin.

Another range of disorders associated with epigenetic changes are often due to expanded non-coding triplet repeats that have an impact on chromatin packaging of proximal genes and their interaction with histones. Examples include Friedreich ataxia (OMIM 229300), myotonic dystrophy (OMIM 160900) and fragile X syndrome (OMIM 158900). The reader is referred to other sources for a detailed explanation of these disorders.<sup>[10]</sup>

### Diet and environmental exposure

Although it has long been understood that epigenetic processes may be constant for certain genes, i.e. to be developmentally expressed only from the maternal or paternal allele, similar but more ephemeral processes influence tissue differentiation and development during embryogenesis. In this regard, different cell lines and tissue types reflect epigenotypes, which may be in flux throughout life, and it has been observed that the regulatory influence is greatest during intrauterine development. During this period, the crucial windows of influence/effect are narrow, pointing to the nuanced interface of embryonic development and a high level of plasticity.

Although it has long been recognised that normal development (i.e. defining cellular, tissue and organogenic differentiation) requires

endogenous mechanisms of switching on and off of genes, more recent research has focused on epigenetic changes that occur as a result of environmental factors interacting with the genome. Exposures that may result in a gene-specific or genome-wide methylation include cigarette smoke<sup>[11]</sup> and toxic trace metals, such as arsenic, manganese and mercury.<sup>[12]</sup> Air pollution has been reported to induce methylation and switching off of important genes, resulting in neurodegenerative diseases.<sup>[13,14]</sup>

Although it seems common sense that a pregnant woman's dietary habits have an impact on her developing fetus, the extent to which this happens is less well understood. A human tragedy during World War II, known as the Dutch *hongerwinter* (hunger winter), which began in November 1944 and lasted to late spring in 1945, provided an opportunity to observe the transgenerational impact that the environment has on human development.<sup>[15]</sup> During this period, the blockade of supplies to the northern and western Nazi-occupied regions of the Netherlands resulted in a catastrophic decrease in caloric consumption by residents to a quarter of the daily recommended intake. Before the liberation of the Netherlands at the end of the war, close on 20 000 people died as a result of starvation.

The healthcare infrastructure and records maintained in the Netherlands have facilitated a long-term epidemiological study of the effects of the *hongerwinter* famine.<sup>[16,17]</sup> In the first instance, it was observed that many children born to parents who endured the *hongerwinter* were small and underdeveloped. In some instances, health problems persisted throughout the lives of these children. However, there were noticeable differences in birthweight whether the mother was malnourished very early or later during her pregnancy. Where the mother was relatively well fed during and post conception, but malnourished towards the latter part of her pregnancy, the baby was likely to be born small. If the mother was undernourished during the first 3 months of her pregnancy, but was then well fed, the baby would likely be born a normal size. This seemed normal, as fetuses do most of their growing at the latter end of gestation.<sup>[17-19]</sup>

However, in longitudinal studies it has been observed that children whose mothers were undernourished early in pregnancy, but relatively well fed towards the end of their pregnancy, exhibited higher rates of obesity than normal, as well as a greater incidence of other health problems, including a range of other developmental and mental disorders. Seemingly, early events during pregnancy, when the fetus is rapidly developing crucial cell lineages, have an influence on individuals for the rest of their lives. Relatively recent genetic and genomic studies of *hongerwinter* babies have shown differential levels of methylation of the genes for insulin-like growth factor II (IGF2), interleukin 10 (IL-10), and others involved with cholesterol transport and ageing.<sup>[20]</sup> IGF2 is an important growth hormone, especially during gestation, and IL-10 has been associated with schizophrenia.

Importantly, these ongoing observational and molecular investigations indicate that the epigenetic effects exist trans-generationally. Considerable effort is currently being put into determining the exact nature of the persistence. In the most recent publication on a genetic study of this cohort, the authors observed that their data were consistent with the hypothesis that adverse environmental effects in early life influence long-term metabolic health, and that the specific mechanisms whereby this occurs await elucidation.<sup>[20]</sup>

### Social environment and behaviour-impacting epigenetics

Behavioural epigenetics examines the epigenetic mechanisms that shape development in response to social experiences, while also being responsible for variations in social behaviour. The variation

of epigenetic mechanisms (such as methylation and histone modifications) in response to social interaction and experience implies that epigenetics is an early evolutionary process facilitating the adaptation of organisms to different social and environmental conditions.<sup>[21]</sup>

Studies have recently noted a correlation between poor care during infancy and epigenetic changes; this in turn was shown to correlate with long-term behavioural impairments linked to neglect. In mice, maternal care, as reflected by parental licking of offspring, has been shown to be correlated with epigenetic changes.<sup>[22]</sup> A heightened level of care, as exhibited by greater frequency of licking, resulted in long-term reduction in stress response in the pups. Specifically, the high level of licking was shown to be correlated with decreased methylation of the glucocorticoid receptor gene, the product of which plays a pivotal role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. It is noted that epigenetic variations as a result of parental care are passed from mother to female offspring. Female offspring who received increased parental care tended to exhibit increased parental care themselves, and those who received less parental care became neglectful mothers.

In an attempt to understand the role of exposure to prenatal mood in humans, Moore<sup>[23]</sup> compared the status of the glucocorticoid receptor gene expression in infants born to mothers who were: (i) depressed and on serotonin reuptake inhibitors; (ii) depressed and not on medication; and (iii) non-depressed. Prenatal exposure to depressed mood, regardless of treatment status, correlated with decreased expression of the glucocorticoid receptor as a result of increased methylation of this gene, and the consequent increased HPA reactivity.

A number of studies have implicated differentially methylated status of a range of genes (e.g. brain-derived neurotrophic factor, glucocorticoid receptor, membrane-bound catechol-O-methyl transferase and the glutamatergic genes), and anatomical sites (the hypothalamus, nucleus accumbens and prefrontal cortex) in individuals affected with a range of behavioural disorders, such as drug addiction/abuse,<sup>[23]</sup> eating disorders and obesity,<sup>[24]</sup> major depressive disorder, bipolar disorder, schizophrenia, psychopathy and suicide.<sup>[25]</sup> Importantly, these epigenetic changes are influenced by exposure to environmental agents, such as alcohol, amphetamine, cocaine, methamphetamine, nicotine and opiates.

## Therapeutics/reversal of methylation

Importantly, the discovery that environmental exposures lead to epigenetic changes has led to the investigation of exposure to other environmental/dietary and pharmacological agents that may render these changes reversible and potentially make health outcomes amenable to human intervention.

Drugs targeting imprinted sites of the genome are currently being used for certain cancers, which are the result of a dysregulated imprinting programme.<sup>[26]</sup> The dysfunction of these mechanisms results in abnormal gene expression and progression of pathologies. It has been noted that epigenetic changes act as valuable biomarkers for the detection and diagnosis of disease, and in some instances may also be used in predicting response to treatment.<sup>[27]</sup>

Interestingly, B vitamins may protect against harmful epigenetic effects of pollution.<sup>[28,29]</sup> Other studies that have shown evidence of environmental exposures increasing the epigenetic imprint on the genome include a ketogenic diet, which essentially is the consumption of a diet high in fats, adequate protein and low carbohydrates. The recent study by Moreno and Mobbs<sup>[30]</sup> was based on the protective effect of dietary restriction against a broad range of age-related diseases. In their investigation of how dietary restriction

and a ketogenic diet might exert life-extending effects, they attempted to identify potential targets for pharmacological intervention.<sup>[30]</sup> They also concluded that it was possible to target the epigenome of especially the hypothalamus towards reducing the negative cognitive effects of ageing.

## Conclusions

Imprinting through its multiple endogenous and exogenous elicitors and mechanisms adds an inordinate complexity to our understanding of the potential functioning and pliability of the genomic message. Equally, a clearer understanding of these mechanisms also provides great promise for using epigenetic signatures as markers for disease and potential targets for therapeutics.

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