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The case for transgenerational epigenetic inheritance in humans

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Abstract Work in the laboratory mouse has identified a group of genes, called metastable epialleles, that are informing us about the mechanisms by which the epigenetic state is established in the embryo. At these alleles, transcriptional activity is dependent on the epigenetic state and this can vary from cell to cell in the one tissue type. The decision to be active or inactive is probabilistic and sensitive to environmental influences. Moreover, in some cases the epigenetic state at these alleles can survive across generations, termed transgenerational epigenetic inheritance. Together these findings raise the spectre of Lamarckism and epigenetics is now being touted as an explanation for some intergenerational effects in human populations. In this review we will discuss the evidence so far.

Introduction

The term "epigenetics" was coined in the 1950's to describe the mechanism by which multicellular organisms develop different tissue types from a single genotype and we now recognise that this process is associated with detectable molecular marks. These epigenetic marks take various forms, including both DNA methylation and modifications to the proteins that package DNA. These modifications affect the transcriptional activity of the underlying genes and, once established, are relatively stable through rounds of cell division.

Some genes, termed metastable epialleles, have been identified in the mouse at which the establishment of the epigenetic state is probabilistic and as a result they exhibit variegation, i.e., cells of the same type do not all express the gene. They also exhibit variable expressivity in the context of isogenicity, i.e., mice of the same genotype (inbred) do not all express the gene to the same extent. The *agouti viable yellow* (A^{vy}) allele and the *axin-fused* ($Axin^{Fu}$) allele are two examples (Rakyan et al. 2002). These alleles have taught us much about the process by which the decision to be active or inactive is made.

The A^{vy} allele is a dominant mutation of the *agouti* (*A*) locus, caused by the insertion of an intracisternal A-particle (IAP) retrotransposon upstream of the agouti coding exons (Duhl et al. 1994). Contained within this IAP is a promoter which can drive constitutive expression of the *agouti* gene, resulting in a yellow coat colour. When the cryptic promoter is silenced, the coat colour reverts to agouti. Many mice within a litter have both yellow and agouti patches, called mottled, indicating a clonal pattern of epigenetic silencing, i.e., variegation (Fig. 1). DNA methylation at this promoter correlates with silencing (Morgan et al. 1999).

So what determines the probability of methylation at such a locus? We have known for some time that the genetic background is critical (Wolff 1978; Blewitt et al. 2005; Wolff 1978) and now we know that it is also sensitive to environment. Changes to the dam's diet during pregnancy, can alter the proportion of yellow mice within a litter. For example, when the dam's diet is supplemented with methyl donors, including betaine, methionine, and

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Fig. 1 Isogenic mice carrying the A^{vy} allele display a range of phenotypes, from completely yellow, through degrees of yellow/agouti mottling, to completely agouti (termed pseudoagouti). Yellow coat colour correlates closely with adult body weight. (Morgan et al. 1999)

folic acid, there is a shift in the colour of their offspring away from yellow and towards agouti (Wolff et al. 1998; Waterland and Jirtle 2003). Similar effects have been observed following the feeding of the dams with genistein, which is found in soy milk (Dolinoy et al. 2006).

Transgenerational epigenetic inheritance in mice

Although epigenetic states, once established, are maintained for the life of the organism, it is rare for these states to be passed to the next generation. Between generations, the epigenetic state of the genome undergoes two dynamic reprogramming events, first in the gametes of the parent and later in the zygote (Dean et al. 2003). This enables the zygote to acquire the totipotent state needed for the differentiation of all cell types. In spite of this, there is strong evidence for transmission of the epigenetic state through the gametes to the next generation at a small number of loci in the mouse. This is known as transgenerational epigenetic inheritance and was first demonstrated convincingly at the A^{vy} locus (Morgan et al. 1999), following evidence for such a phenomenon at other loci (Allen et al. 1990; Hadchouel et al. 1987; Roemer et al. 1997). Morgan and colleagues (1999) showed that in an inbred colony the distribution of phenotypes among offspring is related to the phenotype of the dam; agouti dams are more likely to produce agouti offspring and yellow dams are more likely to produce yellow offspring. They ruled out the possibility that this was the result of the uterine environment by embryo transfer experiments. This inheritance is the result of incomplete erasure of epigenetic marks as they pass through the female germline. Transgenerational epigenetic inheritance has since been reported at another metastable epiallele, *Axin-fused* (Rakyan et al. 2003), at a genetically modified locus (Herman et al. 2003), and at *c-kit* (Rassoulzadegan et al. 2006).

Sensitivity to environment, combined with transgenerational epigenetic inheritance, suggests that the diet of a pregnant female could affect not only her offspring's coat colour, but also that of subsequent generations. This idea was supported by the findings of Cropley, et al. (2006). They showed that methyl donor dietary supplementation can change the epigenetic state of the A^{vy} allele in the germline and that these modifications can be retained through the epigenetic reprogramming that occurs during early embryogenesis. However, this report has been tempered by the failure of another study to replicate the transgenerational effect (Waterland et al. 2007).

Epidemiological evidence for transgenerational epigenetic inheritance in humans

Although information in addition to DNA sequence can be inherited from parent to offspring in mice, there is little direct evidence for transgenerational epigenetic inheritance in humans despite a number of reports describing effects that appear to be similar. One frequently cited example, the Dutch Famine Birth Cohort Study (Lumey 1992), reported that offspring born during times of famine in World War II were smaller than average and that the effects could last two generations. However, a subsequent report by the same authors failed to reproduce some of the findings (Stein and Lumley 2002).

More recently, Pembrey, et al. (2006) reported transgenerational effects in humans. Using the Avon Longitudinal Study of Parents and Children (ALSPAC) and Överkalix cohorts they found that pre-adolescent paternal smoking was associated with greater body mass index (BMI) in their sons, but not daughters. They also found that the paternal grandfather's food supply in pre-adolescence was linked to the mortality risk ratio of grandsons, while the paternal grandmother's food supply was linked to the mortality risk ratio of the granddaughters. Although these studies appear to demonstrate transgenerational effects induced by environmental factors, the suggestion that this is the result of the direct transfer of epigenetic information via the gametes, is not yet supported by any molecular evidence (Pembrey et al. 2006). Intergenerational effects of this type could be explained by societal factors.

Identifying metastable epialleles in humans

So how might we set about identifying transgenerational epigenetic inheritance in humans at the molecular level? As mentioned above, most of the alleles that display

transgenerational epigenetic inheritance in the mouse are metastable epialleles. It follows that metastable epialleles are good candidates for transgenerational epigenetic inheritance in humans. But how do we find them? Human populations are outbred, making it difficult to identify phenotypic variation due solely to epigenetic causes. One way to circumvent this "genetic noise" is to study monozygotic (MZ) twin pairs and look for epigenetic variation between individuals within a twin pair. Genes resembling metastable epialleles have been found in humans (Fraga et al. 2005; Oates et al. 2006; Petronis 2006). However, there is currently no evidence of transgenerational inheritance of their epigenetic state and we still have no method to test this. Moreover, the assumption that MZ twins are genetically identical has recently been challenged by the finding of a high degree of copy-number variation within twin pairs (Bruder et al. 2008).

Human disease associated with transgenerational inheritance of an aberrant epigenetic state

A number of recent studies in humans suggest that diseases that result from disruption to the epigenetic state, "epimutations", can be inherited across generations. Prader–Willi syndrome is a rare disease characterised by decreased mental capacity and obesity. The syndrome is generally associated with mutation in a set of genes on chromosome 15, but some cases have been reported where there is no apparent mutation, but, instead, aberrant methylation, i.e., an epimutation (Buiting et al. 2003). This appears to be the result of an allele that has passed through the male germline without clearing of the silent epigenetic state previously established in the grandmother, indicative of transgenerational epigenetic inheritance.

Similarly, there is some evidence that epimutations can be inherited transgenerationally in a couple of families with an increased risk of colorectal cancer resulting from heterozygosity for epimutations at tumour suppressor genes. The best evidence comes from an individual with hereditary nonpolyposis colorectal cancer (HNPCC) (Hitchins et al. 2007). The subject had abnormal DNA methylation and silencing of one allele of the DNA mismatch repair gene, *MLH1*, in all three germ layers, suggesting that it arose in the parental germline. No novel DNA mutations were identified in the region and some siblings inherited the same allele (as determined by SNP analysis) in an unmethylated state. This is in contrast to another case of HNPCC associated with an epimutation of a different tumour suppressor gene (*MSH2*) (Chan et al. 2006). In this case the haplotype associated with the epimutation segregated in a Mendelian manner, suggesting that the epimutation was caused by a linked DNA mutation. Unfortunately, in humans it is almost impossible to prove that an epimutation was inherited because of a failure to reprogram in the germline because we cannot rule out unlinked genetic causes. Even if the DNA in the region of the epimutation has no mutation, copy-number- variation must now be considered. There are several reports of HNPCC with *MLH1* epimutations (Hitchins and Ward 2008), suggesting that it may be a hotspot for abnormal DNA methylation or genetic instability. It is worth noting that one of the earliest reports suggesting transgenerational epigenetic inheritance at *MLH1* (Suter et al. 2004) has been partially retracted (Hitchins and Ward 2007).The authors failed to replicate methylation of the allele in the sperm of the affected individual. A more detailed discussion of some of these findings can be found in a series of Commentaries published in *Nature Genetics* (Chong et al. 2007; Leung et al. 2007; Suter and Martin 2007).

Summary

While it is clear that some sporadic diseases in humans are associated with epimutations, the notion that these epimutations (in the absence of underlying genetic changes) are passed down from one generation to the next, is still unsubstantiated. The strongest argument for transgenerational epigenetic inheritance in humans remains the evidence from mice.

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