Imprint regulatory elements as epigenetic biosensors of exposure in epidemiological studies

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Mapping the human genome has provided the foundation for studies investigating the role of genetic and environmental factors in the aetiology of complex diseases and neurological disorders. Assessment of genetic risk factors is becoming increasingly comprehensive with the advent of high-throughput, genome-wide approaches. The constancy of genotypes has enabled their use as risk factors in case–control and related hybrid study designs. These genetic risk factors not only carry a low misclassification potential, but they also promise to result in epidemiological studies focused on individuals at high risk of developing chronic complex disease.

Exposure ascertainment for complex diseases largely relies on participants to recall past lifestyle behaviours and diet. Poor recall, especially differential recall between cases and controls, remains a fundamental concern threatening the validity of epidemiological studies. As we embark on studies evaluating exposure throughout human life, such as testing for the developmental origins of adult disease,[1–3] we can anticipate misclassification resulting from poor recall to worsen. A growing body of evidence now suggests that epigenetic features of the genome that regulate phenotype without changing the nucleotide sequence may provide a means of ''recording'' past exposures,[4] and could thus be exploited to improve exposure assessment.

DNA methylation is the most studied of the epigenetic modifications, owing to its heritable nature, stability and ease of measurement. To determine the role of epigenetic changes in the aetiology of diseases, gene promoters are most commonly screened for aberrant CpG methylation, and then evaluated with respect to outcome. This experimental approach is analogous to that used in genetic epidemiology studies. Unfortunately, promoter methylation can vary between tissues and as a function of life stage,[5–8] making it difficult to use promoter methylation as an epigenetic biosensor for environmental exposures.

In contrast, the imprint regulatory elements that result in parent-of-origin-dependent monoallelic expression of imprinted genes are normally differentially methylated (ie, one parental allele methylated and the other unmethylated),[9] providing a 50% methylation baseline in diploid cells. This provides an advantage over the study of non-imprinted gene promoter or transposable element methylation profiles, where methylation alterations are unidirectional. The establishment of the parental allele-specific methylation patterns of imprint regulatory elements occurs very early in development, prior to germ layer specification.[10] As DNA methylation patterns are faithfully transmitted during somatic cell division, the unique differential methylation pattern of the imprint regulatory elements is perpetuated throughout life in all tissues. Hence, methylation shifts at these differentially methylated regions (DMRs) have the potential to function as genome-wide, epigenetic biosensors for environmental exposures that either increase or decrease methylation, particularly during early development when they are most vulnerable to deregulation.

Individuals exposed to severe famine prenatally have a higher incidence of chronic diseases, including a doubling in the incidence of schizophrenia, type 2 diabetes, coronary heart disease, hypercholesterolaemia and some cancers.[11–16] A recent study of the Dutch famine victims illustrates the utility of using DMRs in investigating the role of environmental exposures during gestation in the aetiology of complex diseases later in life.[17] Exposure to severe caloric restriction periconceptionally was associated with hypomethylation of a DMR that controls *IGF2*[17] expression. Interestingly, this aberrant methylation was detected in peripheral blood specimens six decades after nutrient privation. Whether a concomitant change in *IGF2* expression resulted in the enhanced incidence of chronic diseases observed in these individuals is presently unknown.

The evaluation of overnutrition in the aetiology of complex diseases could also benefit from the use of epigenetic biosensors. In the last decade, there has been an increase in the prevalence of folic acid intake worldwide.[18] Folate is a vitamin necessary as a source of carbon moieties used for nucleotide synthesis and DNA methylation.[19 20] Periconceptional folate deficiency is associated with risk of neural tube defects in the offspring, prompting several countries, including the US, to fortify milled grain with 140 μg of folic acid per 100 g,[21] in addition to advising women of child-bearing age to supplement diets with folic acid. As a consequence, circulating folate levels among American women have doubled in the last decade.[18]

Animal experiments have shown that manipulating maternal folate levels permanently alters DNA methylation of transposable elements, leading to increases in obesity.[22 23] It is unclear whether similar modulations of the epigenetic profile occur in response to increased maternal folic acid intake in humans. What is known, however, is that case–control studies conducted in the 1980s suggested a lower risk of several cancers in folic acid users. More recent studies show either no association between folic acid exposure and cancer incidence[24] or enhanced risk of cancer formation since folic acid fortification began.[25 26] While these findings may result from the dysregulation of gene expression, testing this possibility will be difficult unless an epigenetic measure of past exposure to overnutrition is developed.

We briefly discussed the possibility of using imprint regulatory elements throughout the genome as epigenetic biosensors for improving exposure assessment in epidemiological studies. This will undoubtedly require a more detailed understanding of environmental exposures that induce epigenetic alterations at these DMRs. It will also require fully

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defining the entire repertoire of human imprinted genes and their imprint regulatory elements—*the imprintome*. Without this critical information, our ability to diagnose, prevent and treat chronic diseases and neurological disorders that plague us will remain compromised.

Acknowledgements: The authors acknowledge Joellen Schildkraut, Dora Ilyasova, Truls Ostbye and Robert Millikan for their comments on the text.

Competing interests: None.

Accepted 20 April 2009

J Epidemiol Community Health 2009;**63**:683–684. doi:10.1136/jech.2009.090803

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