Epigenetic epidemiology of obesity: application of epigenomic technology

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Compelling human epidemiologic and animal model data indicate that during critical periods of prenatal and postnatal mammalian development, nutrition and other environmental stimuli influence developmental pathways and thereby induce permanent changes in metabolism and chronic disease susceptibility. The biologic mechanisms underlying such ‘metabolic imprinting’ are poorly understood, but epigenetic mechanisms are likely involved. Epigenetics is the study of mitotically heritable alterations in gene expression potential that are not caused by changes in DNA sequence. Transient environmental influences during development can cause permanent changes in epigenetic gene regulation, and accumulating evidence links epigenetic dysregulation to human disease.

The worldwide increase in the prevalence of obesity in recent decades has occurred too rapidly to be explained completely by genetic variation, suggesting the involvement of epigenetic mechanisms. Indeed, data from animal models and humans demonstrate that epigenetic dysregulation can cause obesity. Several years ago Levin proposed that maternal obesity during pregnancy and lactation might cause metabolic imprinting of neural networks in the offspring, perpetuating, or even amplifying, obesity susceptibility across generations. This postulate is supported by recent studies in the mouse, which demonstrate that the formation of hypothalamic projections during early postnatal development is dependent upon cues from outside the brain. Leptin-deficient (ob/ob) mice fail to form hypothalamic connections necessary for normal energy homeostasis. Remarkably, transient administration of exogenous leptin during postnatal development rescues hypothalamic innervation and normalizes adult body weight. Analogously, maternal obesity during pregnancy and/or lactation could alter hormonal or other signaling mechanisms that affect morphological development of the fetal or postnatal hypothalamus, with permanent consequences for offspring body weight.

In addition to such morphological effects, maternal obesity could affect hypothalamic development at the epigenetic level. All monogenic forms of human obesity are associated with mutations affecting leptin signaling in the hypothalamus. The most common forms of monogenic obesity in humans are caused by mutations in the gene encoding melanocortin 4 receptor (MC4R), which integrates opposing peptide signals from the arcuate nucleus. The latest edition of the human obesity gene map includes data on associations between candidate genes and human obesity. Only recently have we begun to learn about the epigenetic regulation of genes that play a central role in food intake regulation. During differentiation from preadipocytes to adipocytes in vitro, hypomethylation of specific CpG sites in the promoter of the human gene encoding leptin (LEP) correlates with upregulation of LEP expression. Also, the POMC promoter is hypomethylated in human tissues that express POMC, relative to nonexpressing tissues. We need to learn a great deal more about the developmental epigenetics of genes important to body-weight regulation and the potential for early environment to influence these processes.

"Epigenetic epidemiology" describes the study of the associations between epigenetic variation and risk of disease. The overall hypothesis underlying the epigenetic epidemiology of obesity is that maternal obesity and/or

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nutrition before and during pregnancy induces epigenetic alterations that perpetuate obesity in her offspring. The greatest single obstacle to testing this hypothesis in humans is the intrinsic tissue-specificity of epigenetic regulation. It is likely that important interindividual epigenetic variation occurs, specifically in the hypothalamus. Also, whereas initial studies will focus on DNA methylation due to the relative simplicity and stability of this epigenetic modification, epigenetic dysregulation relevant to obesity will almost certainly involve additional interacting epigenetic mechanisms, including myriad histone modifications and autoregulatory DNA-binding proteins. Lastly, we now have a relatively poor ability to predict the genomic regions in which epigenetic modifications contribute to the transcriptional regulation of specific genes. Over the last decade, most studies have focused on epigenetic modifications at gene promoters. It is now clear that epigenetic regulation is integrative and can function over vast genomic distances.

For all of these reasons, extensive research in animal models will be necessary to facilitate future human studies of the epigenetic epidemiology of obesity. Animal models will enable invasive studies of tissues in which interindividual epigenetic variation is most likely to cause individual differences in food intake regulation, energy expenditure, and adiposity. Rather than studying candidate genes in these animal models, epigenomic technologies now enable genome-wide screens for epigenetic alterations associated with obesity. For example, methylated CpG island amplification coupled with microarray hybridization (MCAM) has recently been validated as an effective tool to screen the genome to identify regions of

Figure 1: Illustration of the methylated CpG island amplification coupled with microarray hybridization (MCAM) method. At the top are two alleles drawn from hypothetical genomic DNA samples. The ‘lollipops’ represent CpG sites with Smal sites (filled = methylated, empty = unmethylated). Genomic DNA is digested with Smal, which cuts at CCCGGG sequences only when the CG site is unmethylated and leaves blunt ends. Next, the same DNA samples are digested with Xmal. Xmal also cuts at CCCGGG, but regardless of methylation at the CpG site and, importantly, leaves a 4 nucleotide overhang. Hence, following ligation of PCR adaptors to these Xmal ‘sticky ends’, ligation-mediated PCR results in the amplification of Smal fragments in which the Smal sites at both ends are methylated (methylation-specific amplification). Subsequent differential labeling of the two MCA products with Cy3 and Cy5, followed by hybridization to oligonucleotide arrays, enables the identification of differentially methylated genomic loci. Reprinted from Shen et al. (2007) with permission.
hypermethylation.\textsuperscript{11} (CpG islands are discrete genomic regions with a high density of CpG sites, and are often located at gene promoters.)

MCAM involves two steps (Figure 1). The first step is methylated CpG island amplification (MCA), which enriches for methylated fragments by serial digestion of genomic DNA with \textit{SmaI} (methylation sensitive) and \textit{XmaI} (methylation insensitive) endonucleases, followed by ligation-mediated PCR. The second step involves cohybridization of MCA products to oligonucleotide arrays to identify sites of differential methylation between two samples.\textsuperscript{11} We recently used MCAM to identify a class of human promoter-region CpG islands that are silenced by CpG methylation in a tissue-specific manner in humans.\textsuperscript{11} We performed cohybridizations comparing genomic DNA from peripheral blood leukocytes with in vitro-methylated genomic DNA, and identified 258 dense, autosomal promoter-region CpG islands that are hypermethylated in peripheral blood leukocytes. We randomly selected 28 of the array hits for validation by bisulfite-pyrosequencing,\textsuperscript{12} confirming the MCAM results for 26 of the 28 genes (90\% specificity). Hence, MCAM provides a new capability to perform genome-wide screens for locus-specific DNA methylation alterations in normal tissues. MCAM is currently being used in animal models to test the hypothesis that interindividual variation in locus-specific CpG methylation contributes to individual susceptibility to obesity.

Understanding the specific biologic mechanisms linking early nutrition to later disease will ultimately enable focused early-life nutritional interventions to achieve long-term improvements in human health. Given the current dearth of knowledge regarding the potential role of epigenetic mechanisms in body weight regulation, extensive research in appropriate animal models is now required to develop specific hypotheses that can be tested in epigenetic epidemiologic studies of human obesity.

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\textbf{REFERENCES}

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