A new era for epigenetic epidemiology

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Methylation of CpG dinucleotides is a mitotically heritable and highly stable epigenetic mechanism fundamental to cellular differentiation. Establishment of cell type-specific DNA methylation occurs throughout embryonic, fetal and early postnatal development, and stably regulates gene expression potential throughout life [1]. The ability of DNA methylation to stably regulate gene expression makes epigenetic and genetic etiology of disease equally plausible. Accordingly, since the first published use of the term in 2004 [2], there has been growing interest in the field of epigenetic epidemiology, defined as the study of the associations between interindividual epigenetic variation and risk of disease [3]. Because of its long-term stability and ability to be assayed in minute quantities of DNA, nearly all epigenetic epidemiologic studies have focused on CpG methylation. Compared with genetic epidemiology, epigenetic epidemiology presents major challenges [3]. The inherent cell-type specificity of DNA methylation means that we cannot generally 'epigenotype' an individual using peripheral blood DNA, complicating large-scale population studies of most human diseases. Also, even when the 'tissue of interest' can be obtained (in a case-control design, for example) [4–6], the disease process itself can induce epigenetic changes, compromising the ability to draw causal inferences.

The vast majority of so called 'epigenome-wide association studies' (EWAS) to date have employed Illumina methylation arrays. The earliest version, the Infinium HumanMethylation27 BeadChip (27 K) array, interrogated 27,578 CpG sites across 14,495 promoter regions of genes implicated in cancer. The subsequent Illumina HumanMethylation450 BeadChip (450 K) array was designed to measure methylation at approximately 450,000 CpG sites covering 99% of RefSeq genes [7], facilitating comprehensive assessment of associations between tissue-specific differential methylation and gene expression [8]. In 2016 Illumina introduced an expanded version of the 450 K array, the HumanMethylationEPIC BeadChip (EPIC) array, which measures methylation at approximately 850,000 CpG sites, including 90% of those on the 450 K platform. The additional probes improved coverage of CpG sites in regulatory elements, including 58% of FANTOM5 enhancers, 7% of distal and 27% of proximal ENCODE regulatory elements [9].

We recognize the myriad advantages of a standardized commercial platform, the data from which can easily be compared across different studies, populations, etc. For population-level epigenetic epidemiology, however, the designs of the 450 K and EPIC arrays are far from ideal. Many of the probes on the 450 K and EPIC arrays cover CpGs that are generally not methylated in normal somatic tissues [9,10]. On both platforms, most probes do not show appreciable interindividual variation [10–12]. Consistent with these observations (and particularly troubling for studies attempting to prospectively predict later outcomes) a recent study using the EPIC array [12] observed generally poor test–retest reproducibility in peripheral blood DNA of the same individuals over a 1-year period.

A logical approach to overcome these limitations is to focus on genomic regions that display systemic interindividual variation (SIV) in DNA methylation [13,14]. Gunasekara *et al.* recently reported the largest SIV screen to date [15]. Previous studies identifying SIV regions [13,14,16] pursued the specific goal of identifying metastable epialleles [17], that is, stochastic (nongenetically mediated) systemic epigenetic variants. Given the difficulty of ruling out genetic influences in outbred human populations, Gunasekara *et al.* focused on identifying regions of SIV regardless of whether they are genetically or stochastically mediated. A computational algorithm was developed to analyze deep whole-genome bisulfite-sequencing (WGBS) data on tissues representing all three germ layers (thy-

roid, heart and brain) from each of ten donors from the NIH Genotype-Tissue Expression (GTEx) project. The authors identified 9926 correlated regions of systemic interindividual variation (CoRSIVs) in the human genome. CoRSIVs, although involving just 0.1% of the genome, are intercorrelated over long genomic distances, associated with transposable elements and sub-telomeric regions, conserved across diverse human ethnic groups, sensitive to periconceptional environment and associated with genes implicated in a wide range of human disorders and phenotypes [15]. Remarkably, only 1% of 450 K or EPIC probes assay CpG sites within CoRSIV regions, rendering these platforms nearly blind to SIV in the human genome. This is extremely unfortunate given their preponderance among EWAS reported in the last decade. A database of published EWAS data (EWASdb) [18] currently hosts about 900 450 K and 65 EPIC datasets.

Analogous to genetic variants, CoRSIVs are stable, systemic interindividual variants – essentially epigenetic polymorphisms. Notably, in the last year or two, previously identified regions of SIV (candidate metastable epialleles) have gained the attention of epigenetic epidemiologists, who have uncovered links to disease based on approximately 150 previously identified candidate metastable epialleles [13,14,16]. For instance, DNA methylation at SIV regions has recently been associated with Alzheimer's disease [19], autism [20,21], cancer [22], cleft palate [23], obesity [24], Parkinson's disease [6], rheumatoid arthritis [25] and schizophrenia [26]. These associations were among top hits in many cases, despite the underrepresentation of SIV regions on the Illumina arrays. Although causality remains unclear in most cases, prospective studies have identified predictive associations providing evidence of causality. For example, at SIV regions, DNA methylation in peripheral blood of healthy adults is associated with cancer diagnosed years later [27], and methylation in newborn blood samples is associated with BMI at age 5 [28]. Individual DNA methylation at metastable epialleles is stable over time [12], over 10 years in one example [13], underscoring the potential for CoRSIV methylation to contribute to disease prediction, diagnosis and prognosis. Further, SIV regions are particularly sensitive to periconceptional environment [14–16,29,30], making them an obvious focus for studies of the developmental origins of health and disease [3,31].

In summary, unlike the vast majority of HM450 and EPIC probe CpGs, CoRSIVs are systemic, highly variable among individuals, and stable over time within individuals. Focusing on these regions, investigators can employ genomic DNA from easily obtainable tissues like peripheral blood to draw inferences about epigenetic regulation throughout the body – akin to genotyping sequence variants. Although CoRSIVs are grossly underrepresented on the 450 K and EPIC arrays, accumulating data from these established platforms indicate that individual epigenetic variation at CoRSIVs is associated with a wide range of human diseases. To launch a new era in understanding how interindividual epigenetic variation affects risk of disease, we encourage others in the worldwide epigenomics community to join us in advocating for development of a standardized commercial platform focused on human CoRSIVs.

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