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Population Epigenetics

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Abstract

The field of epigenetics is maturing, with increased interest in understanding the normal regulation of the genome and the possibility that it becomes reprogrammed aberrantly as part of the cause of disease phenotypes. Applying the current technologies and insights to the study of human populations is potentially a way of understanding mechanisms and consequences of these diseases. When extended to encompass health care disparities, understanding why certain populations are unusually prone to specific conditions, there is certainly some potential for gaining new and valuable insights, but these studies are likely to be unusually prone to the effects of confounding influences and need to be designed, executed and interpreted with extra care.

INTRODUCTION

The first uses of the term “population epigenetics” were by Keller [1], who created a conceptual framework for understanding the inheritance of traits mediated by autoregulatory transcription factors, and by Richards [2], whose focus was on the natural variation in DNA methylation that occurs in plants. The intriguing possibility being raised was that this variability could in some way influence and modulate the effects of genetic variability. The implicit assumption at that time was that epigenetic variability was a completely distinct layer of information from that encoded in the genome, an assumption based on the then-current definition of “epigenetics”, which back-translates epi- and –genetics as an influence residing above or upon that of the DNA sequence itself [3], usually taken to mean something mediated by a molecular regulator of genomic function.

An implicit hope was probably that we could use the lessons of the field of population genetics to apply to population epigenetics. This has unfortunately proven to be very difficult. As we have recently reviewed [4], the patterns of variability of molecular regulators of genomic function are themselves phenotypes, and subject to multiple influences, unlike the genotype, which is fixed within the individual. Apart from genotype, using any other kind of –omics data in a phenotypic association study is basically correlating a phenotype with a phenotype. This leads to problems when performing the typical cross-sectional study design approach employed when associating molecular genomic regulators with a phenotype

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– how do you know that the molecular changes observed lead to the phenotype, when the phenotype could instead be leading to the molecular changes? Such reverse causation is now demonstrated to occur for DNA methylation in peripheral blood leukocytes in a study of individuals with increased body mass index [5] and another looking at people with altered blood lipid profiles [6].

Furthermore, in recent years there have been numerous studies that have revealed an interplay between DNA sequence variability and the functional properties of the genome. One source of insight has been through observational studies, such as the identification of loci where the different parental alleles have markedly distinct DNA methylation patterns at a site of DNA sequence polymorphism [7], or larger-scale studies in which DNA sequence variants such as single nucleotide polymorphisms (SNPs) have been significantly correlated with different levels of DNA methylation at local sites, or more distantly or on other chromosomes [8–17]. These have been referred to as methylation quantitative trait loci (meQTLs or mQTLs), and have counterparts described for gene expression (eQTLs) [18] and chromatin states (chrQTLs) [19]. Through twin studies and other approaches, the proportion of inter-individual variability of DNA methylation that can be accounted for by DNA sequence variation has been calculated, and estimated to be between 22% to 80% in humans [7,8,20].

The initial hope that insights into epigenetic variability in a population could complement information about DNA sequence variability has therefore been complicated by this strong association between the two types of information. However, it should be noted that DNA sequence variability does not account for 100% of variation of DNA methylation, indicating that if we can dissect out the interactions of these molecular events, we should be able to find two interesting types of information – the potential genomic regulatory mechanisms through which DNA sequence variants work, and the independent variation in genomic regulatory mechanisms that may be a source of modulation of sequence-based phenotypes, as originally hoped [2].

MAIN TEXT

Keys to interpreting DNA methylation variability

The key to a population epigenetics study is to understand the influences affecting inter-individual variability in the genomic regulator being studied, which is usually DNA methylation. This turns out to be a surprisingly complicated area of research, as DNA methylation is influenced by a large number of factors. For example, as DNA methylation differs in each cell type in the body, and DNA methylation assays are performed on pools of cells, any systematic difference in a cell subtype proportion within the pool of cells tested will be reflected by changes in DNA methylation at loci where the pattern is distinctive in that cell type [21]. This is how the testing of pools of cells can generate differences in DNA methylation, without any cells present necessarily having changed their innate patterns of DNA methylation. The influence of cell subtype composition is now a well-recognized problem in studies of DNA methylation and has been the focus of a number of thoughtful analytical approaches [21–24]. Another unexpected problem is a molecular example of reverse causation. While DNA methylation is usually thought to be a regulator of gene

expression, the act of transcribing a locus can alter its DNA methylation [25,26], requiring that we concurrently test the samples for their transcriptional profiles when testing DNA methylation.

The results to date of DNA methylation studies associated with phenotypes are not frequently replicated, with the exception of the association between cigarette smoking and DNA methylation in peripheral blood leukocytes, which has revealed the same loci to undergo changes in multiple studies [27–29]. However, even this paradigm of epigenetic association may be undermined by what appear to be substantial effects of DNA sequence variation and cell subtype effects for the informative loci [30–32], raising the possibility that these DNA methylation changes are substantially due to allelic variants for these meQTLs segregating non-randomly into the smoker and non-smoker groups, and blood cell subtypes being altered as a response to cigarette smoking.

When these cell subtype and transcriptional influences are combined with the strong effect of DNA sequence variation on DNA methylation, it becomes clear that any study of a molecular regulator like DNA methylation is by itself uninterpretable [4], and has to be studied with parallel genotyping and transcriptomic studies, and detailed insights into the cell subcomposition present. This makes epigenetic studies complex and demanding of resources, but allows the generation of rich data sets that allow interpretation of the results generated.

Transcription factors

Epigenetics, defined as an influence above or upon that of the DNA sequence itself, also has generally been taken to indicate an influence that can override transcriptional regulatory mechanisms. Observations that DNA methylation could inhibit the binding of DNA-binding proteins like transcription factors (TFs) [33–35] lent support to the implicit model that there exists a generic transcriptional regulatory program that could be overridden by “epigenetic” mechanisms.

The problem is that the evidence for epigenetic reprogramming, usually tested by studying patterns of DNA methylation, involves the same loci changing their patterns in multiple individuals. For the same sequences in the genome to be selected in this way, the mediators have to have the ability to recognize complex DNA sequences, which is not a property of DNA methyltransferases, histone modifying or nucleosomal remodeling enzymes. The potential mediators with the required sequence specificity are transcription factors or possibly some examples of small non-coding RNAs. We have recently noted that TFs not only have a primary role in transcriptional regulation, they drive cell fate choices and maintain cellular identities through autoregulatory mechanisms [36]. Their activities are also influenced by environmental factors, making them very attractive candidates for mediating the cellular reprogramming sought in studies associating molecular reprogramming of cells with phenotypes. As we also note [36], molecular processes like DNA methylation and chromatin modifications are influenced by the local binding of TFs, so that when these kinds of “epigenetic” regulators are noted to be altered in association with a phenotype, they may merely be footprinting where TFs have altered their activities, rather than representing the primary mediators of the regulatory process.

It is therefore essential to consider the possibility that TFs represent the primary mediators of cellular changes associated with phenotypes. Studying DNA methylation would remain of value even in this revised perspective, with its potential to define the sites at which these TF-mediated events are occurring. However, it is probably an over-interpretation to assume that sequence-specific DNA methylation changes occur autonomously.

Performing a population epigenetics study

Having defined the challenges above, a study could be designed that took into account all of these issues. The question is how such a study could address a population epigenetics issue. Given the strong link between genetic and DNA methylation variability, we can no longer consider population epigenetics as a separate influence on a phenotype, as once proposed [2]. Epigenetic association studies currently aim to understand the relationship between molecular processes like DNA methylation and phenotypes. As discussed above, any such associations need to be interpreted carefully – they could represent genuine cellular reprogramming, although more likely to be mediated primarily by TFs. They could also reflect consistent bias of cell subpopulation proportions, DNA sequence differences or transcriptional variability between control and test individuals, each of which would also be interesting in terms of insights into the development of phenotypes.

The issues above apply to any epigenetic association study. A population epigenetics study would be likely to have a more specific focus on a racial or ethnic minority, socioeconomically disadvantaged, and rural groups, who are disproportionately affected by many diseases, and can have distinct environmental exposures. Clearly there are many contributors to health care disparities, including issues of access to health care, cultural influences, racial discrimination, and differences in health literacy [37]. A study that includes racial and ethnic minorities as a comparison group is likely to be influenced by meQTLs, the DNA sequence diversity that leads to DNA methylation variability. The interpretation of such studies therefore has to be performed with great care. Genotyping is therefore especially important in these studies, as is having a cohort size large enough to identify meQTLs in each of the populations being studied, which is driven by the need to include enough individuals with alternative alleles to recognize their effects.

Certain environmental exposures occur non-randomly with respect to race, ethnicity and socioeconomic status. For example, ethnic minorities often reside in poor urban neighborhoods that are located in close proximity to major highways [38], pollution from which is associated with asthma [39]. Traffic related air pollution (TRAP) is an excellent example of an environmental exposure leading to a disease phenotype, children residing within 500 feet of a highway documented to have a substantially higher incidence of asthma than those residing further away [40]. TRAP-attributable emergency room visits for asthma are 4.5 times higher in high-poverty as compared to low-poverty neighborhoods [41]. Moreover, early life exposure to TRAP is associated with incident wheeze [42] and increased airway sensitization and responsiveness to classical asthma triggers later in life [43], suggesting a long-term effect of early life exposures, a key feature supporting an epigenetic reprogramming by TRAP of cells mediating the inflammatory phenotype. Such examples prompt interest in whether the effects of the environment and the memory of these

exposures are mediated by epigenetic processes. A study design that compares those living in proximity to the highway with individuals living further away is likely to be confounded by socio-economic factors, as those families with more resources will avail of options to live more distantly from an obvious source of pollution. An alternative study design should be considered, comparing those who are susceptible with those resilient to the same stress living in the same environment [44,45] with very similar environmental exposures, socio-economic status, diet and indoor allergen exposure. This disease resiliency comparison could be a very effective study design in population epigenomics studies focused on effects of pollution.

The choice of cells to sample in an epigenetic association study involves a number of considerations. Of these, the most important is that the cells either mediate or are affected by the phenotype of interest. If a change in the phenotype of the cells themselves can be demonstrated, this is clearly a valuable indication that they have underlying molecular changes, and may manifest the transcriptional regulatory perturbations sought in an epigenetic association study. Getting a relatively homogeneous sample of cells is desirable, if only to enhance the “signal” of alteration of transcriptional regulation. It cannot be assumed, however, that purification of cells using surface markers is going to isolate cells that are homogeneous in terms of their molecular properties. We have previously made this point when studying CD34+ hematopoietic stem/progenitor cells, which, while representing a purified population of cells, have an underlying molecular heterogeneity, so that any epigenome-wide assay is, in effect, testing a collection of epigenomes, what we refer to as a meta-epigenome [46].

A study that includes population groups with unusual environmental exposures also needs to pay attention to the fact that certain environmental exposures are associated with cell fate alterations [47–49]. While this is a confounding variable when seeking evidence for cellular reprogramming associated with exposures, it could also be an immensely valuable observation in understanding why the exposure resulted in a specific phenotype. It is essential therefore that what would otherwise be considered confounding variables be assessed specifically and included as potentially informative results of a population epigenetics study.

A TF-centric perspective allows a focus on some potential outcomes. Micronutrients like vitamin D are much more likely to be deficient in African-Americans [50]. The vitamin D receptor (VDR) acts as a TF to mediate the effects of dietary vitamin D. If vitamin D deficiency is the cause for a difference in cellular states, studies of molecular genomic regulators like DNA methylation or chromatin states might be expected to reveal enrichment at these loci for the DNA sequence characterizing the binding site of the VDR. As vitamin D deficiency is associated with a wide range of diseases [51], it would not be unexpected for it to emerge as a candidate when interpreting a population epigenetics study using a focus on the effects of TFs.

CONCLUSIONS

The original hope for population epigenetics was that variability of DNA methylation could influence the development of phenotypic traits, interacting with the influence of DNA sequence variability. Now that we recognize that DNA methylation is strongly influenced by DNA sequence, we will find a substantial proportion of the variability of each to be correlated, but as DNA sequence does not explain all DNA methylation variability there remains potential for studies of their interactions. A population epigenetics study involving racial and ethnic diversity is going to be unusually prone to the effects of meQTLs, while environmental exposures have the potential to induce cell subpopulation changes, so these kinds of studies need to be interpreted with great caution. However, with care in design, execution and interpretation, they can reveal a number of different influences (cellular, sequence and genomic regulatory) that could potentially be contributing to causing the phenotype being studied.

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HIGHLIGHTS

- Population epigenetics studies have potential to reveal insights into why certain conditions occur at different frequencies in distinct population groups.
- Because the multiple influences upon epigenetic regulators are likely to be unusually marked in diverse populations, these studies require unusually rigorous design, execution and interpretation.
- However, the ability to identify specific effects of even these confounding influences will allow insights into many types of potential mechanisms of these human phenotypes.

Population diversity

Figure.

A summary of the issues to be overcome when performing a population epigenetics study. If the focus is on health disparities, the study will be including individuals of diverse ancestry, wealth and geography. For the reasons stated in this review, this will increase the DNA sequence diversity of the population studied, while the altered profile of environmental exposures also has the potential to influence cell repertoires present in the samples obtained. Each of these has potential to influence the interpretation of epigenomic association studies, but if the study is carefully designed and performed, the interpretation may allow insights into a number of possible mechanisms mediating the phenotype being studied.