

The Power of Two: Epigenetics and Twins

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There is little doubt that twins are at the forefront of complex disease research in the molecular era (van Dongen et al., 2012). This is highlighted by the increasing number of such studies aimed at unraveling the role of epigenetics in development and disease.

At a fundamental level, epigenetic processes, which regulate genomic activity in the absence of changes to DNA sequence, are critical for development in multicellular eukaryotes. They confer cell identity and function via gene regulation; and they control cell division, DNA replication, and telomere structure. The capacity for early life epigenetic change to respond to environmental influence is best illustrated by data showing clear differences in epigenetic profile in identical twins even at birth (Ollikainen et al., 2010). This is important because mounting evidence implicates epigenetic variation as a mediator of the effect of the early environment on risk for chronic disease in later life (Hanson et al., 2011). Studies characterizing epigenetic profile at birth are critical in building evidence of causality in this pathway and for understanding the biological effects of specific intrauterine exposures. Further, such studies have potential to reveal epigenetic biomarkers for modeling disease risk, for disease monitoring, and for informing novel interventions.

The studies in the special section of this issue of *Twin Research and Human Genetics* cover a broad spectrum of twin-based epigenetic research; from the importance of longitudinal birth cohorts in twins (reviewed by Chiarella et al.) to the potential for Epigenome Wide Association Studies (EWAS) to reveal insights into complex disorders in adults. Such studies use a platform that measures methylation throughout the genome to regress DNA methylation on a specific environment or on current or past disease stage (Michels et al., 2013). Each invariably uses regression analyses of hundreds of thousands, or even millions of data points, requiring stringent adjustment for multiple testing to identify genomic regions of differential methylation associated with each environment or disease state. Such studies often reveal differential methylation at many genes,

often showing evidence of common cellular pathways or functions.

The most common platform for EWAS is the Illumina Infinium HumanMethylation BeadChip array (HM450 or 450k array; (Dedeurwaerder et al., 2011)), measuring DNA methylation at over 480,000 CpG sites within the genome (soon to be 850,000; http://www.molmed.medsci.uu.se/digitalAssets/491/491080_1epic-data-sheet-2015.pdf). All but one of the EWAS in this special issue use this array platform.

The first four EWAS articles (Tsai et al., Bahl et al., Wong et al., Du et al.) focus on the most powerful twin model: the discordant MZ co-twin model, which controls for genetics, age, sex, and family environment, but not for twin-specific intrauterine or postnatal environment. Typically, within such studies, within-pair differences in DNA methylation are regressed against within-pair differences in environment or disease state, either as categorical or continuous measures. Tsai et al. focus on twins discordant for birth weight, reporting an association with a gene implicated in growth regulation. Bahl et al. focus on female MZ discordant for hormone replacement therapy (HRT) in the aim of identifying genes sensitive to methylation variation in association with this treatment during menopause. Wong et al. focus on adult twins discordant for diurnal preference ('day' and 'night' people), identifying a number of genes with differential methylation, some of which have previously been implicated in regulation of circadian rhythm.

In a novel twist on the co-twin study, Du and colleagues generate epigenome-wide methylation data using methylation-dependent immunoprecipitation from four twin pairs of different ages. Using the regions that are consistently differentially methylated across all pairs, they suggest

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that these regions can be used in forensic tests to distinguish genetically identical monozygotic twin pairs. Although of limited utility in forensics generally, the importance of this approach could be realized immediately in response to several international ongoing criminal investigations. Bui and colleagues tease out the effects of placentation on genome-wide DNA methylation. They conclude that subtle differences in the intrauterine experiences of these twin types may be reflected in epigenetic differences.

The next three studies come from the Netherlands Twin Registry, highlighting the power of collecting biological samples, from many hundreds, even thousands, of twin pairs. The three EWAS focus on aggressive behavior, tic disorders, and wellbeing. Consistently, these studies identify differentially methylated regions in genes expressed in the brain and central nervous system. Although this demonstrates the potential utility of peripheral samples for generating biomarkers for neurodevelopmental/psychiatric phenotypes, further work will need to be done to determine the relevance of such findings to disease aetiology.

Finally, Li and colleagues measure the differences between chronological age and methylation age as calculated from methylation data. Comparing data from MZ and DZ pairs they find little evidence for a genetic influences on epigenetics, and instead present evidence that shared environment contributes to familial correlations in DNA methylation.

In summary, this special issue provides a broad snapshot of twin-based epigenomics research at the present time, also highlighting the variety of analytical methods currently in use for the analysis of methylation data in twins. This reflects the broader field where consensus analytical approaches are few and far between.

Given the growth in epigenetics research internationally, the future will likely see many more epigenome-wide studies that explore both DNA methylation and of other epigenetic marks in twins, such as covalent histone modifications, chromatin accessibility, 3D nuclear architecture, and non-coding RNAs. Twin research over many decades has proven invaluable in many areas and has also provided many surprises. It is likely that this will also be the case when applied to our understanding of the role of epigenetic processes in human development and disease.

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