Jonathan Mill

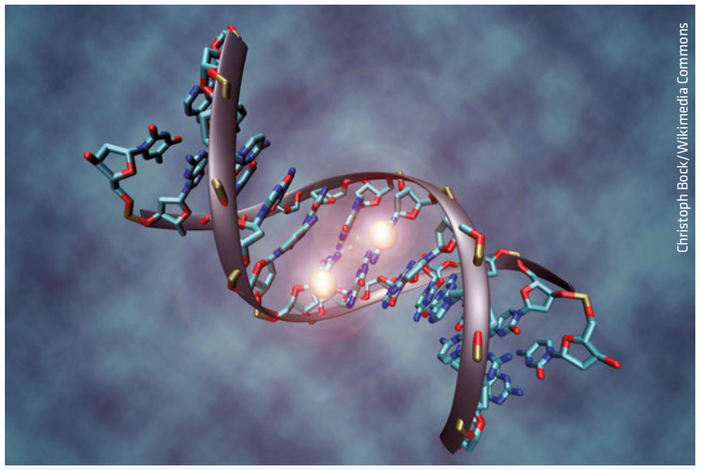
Methylome sweet methylome

A better understanding of epigenetic modifications in the brain is providing new insight into both neurodevelopmental and neurodegenerative disorders.

After the Human Genome Project revealed that humans had roughly the same number of genes as a fruit fly, attention rapidly refocused on gene regulation, with the realisation that control of gene expression was a key factor in human biology (and disease). In particular, work on epigenetic modifications of DNA and histones has exploded in popularity. Based at Exeter and King's College London, **Jonathan Mill** is examining modifications to DNA – particularly cytosine methylation – in the human brain, and the potential consequences it could have for both neurodevelopmental and neurodegenerative disorders.

Methylation of cytosine residues in DNA affects the binding of transcription factors and other proteins that regulate access to DNA. DNA methylation is generally associated with gene silencing. "That's the classical view," says Professor Mill. "But actually it's much more complex than that." For example, methylation can also affect sites within the coding regions of genes, sometimes increasing gene expression or altering splicing patterns so different protein variants are generated.

One reason for the growing popularity of epigenetics is its potential to explain how environmental factors exert a lasting influence on gene expression and human biology. "There's some truth in that," notes Professor Mill. "There are a number of well-characterised examples where this does seem to be the case. Smoking is a big one, it's now very robustly associated with changes in DNA methylation at multiple sites in the human genome. In fact, if you gave me a DNA sample I'd be able to tell if you were a smoker and how much you'd smoked."



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Methylation of cytosine residues in DNA.

However, he urges caution, arguing that much work is preliminary. Furthermore, he adds, "We know that one of the biggest determinants of variation in DNA methylation is genotype. Epigenetics is often seen in opposition to genetics, but we see it as a way of understanding the mechanisms by which either genetic variation or factors in the environment might developmentally lead to changes in gene regulation that lead to disease."

Epigenetic dynamics

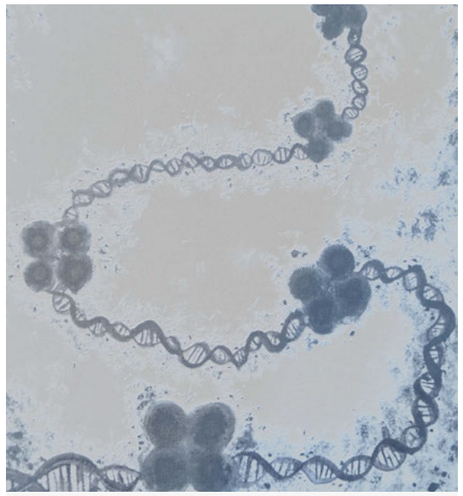
Unlike the genome, which is essentially fixed from conception in all cells, DNA methylation varies between cell types and over time. (Remarkably, epigenetic markers can reveal someone's age to an accuracy of two to three years.) For diseases that become manifest at particular stages of life, such as neurodevelopmental and neurodegenerative disorders, it is therefore plausible that dynamic changes in methylation could be significant.

"The reason we were interested in understanding what was taking place in brain development was that some of the diseases we focus on – for example, schizophrenia and autism – are thought to have a neurodevelopmental origin. The general consensus is these conditions have their origins very early on in brain development. Subtle changes in how the brain is wired or develops can put an individual on a trajectory to be at increased risk of neurodevelopmental disease."

Working with material from the Human Developmental Biology Resource, Professor Mill was able to track how patterns of DNA methylation across the genome changed during fetal development. By comparing these results with DNA methylation patterns seen in the brains of adults with schizophrenia, he was able to pinpoint sites that showed methylation changes during early brain development and were also abnormally methylated in a disease state.

Another strategy has been to look at cohorts of twins. "Identical twins are a great resource for epigenetic epidemiology," says Professor Mill. "We can look for DNA methylation differences between them that might tell us something about why they differ." His group has examined potential epigenetic contributions to psychotic symptoms and neurobehavioural conditions such as autism in pairs of identical twins where only one twin is affected.

Research



Digitally edited pen and pencil illustration of DNA and histones by Helen Spiers (www.helenspiers.com).

"...IF YOU GAVE ME A DNA SAMPLE I'D BE ABLE TO TELL IF YOU WERE A SMOKER AND HOW MUCH YOU'D SMOKED."

Stress testing

Professor Mill is also interested in how epigenetic mechanisms might mediate the impact of stress. "It's a very robust association between stress and certain mental health outcomes," he points out. "We know that stress is leading to some long-term biological changes, some kind of programming."

Human studies are challenging because of the sheer number of potential confounders that could influence methylation patterns – for example, people subject to chronic stress might smoke more. However, in animal models, work with Hans Reul in Bristol has shown that methylation plays a critical role in stress-induced changes in gene expression and behaviour.

Furthermore, the appalling neglect suffered by Romanian orphans during the Ceausescu regime has provided a rare opportunity to examine the impact of early-life stress in people. In collaboration with Edmund Sonuga-Barke in Southampton, Professor Mill has examined what impact the harrowing conditions experienced in early childhood has had on the orphans, in comparison to matched UK adoptees. Professor Mill acknowledges the study's drawbacks, including its small numbers of participants and use of peripheral tissue samples. "But we were able to identify some regions that were potentially interesting for follow-up studies."

Later-life diseases

For neurodegenerative conditions, Professor Mill is more confident that findings hold up. " I think that's some of the most robust findings we have now."

One noticeable feature of conditions such as Alzheimer's disease is their tissue specificity – some parts of the brain are badly affected while others are untouched. "We were interested in comparing different areas of the cortex, which are affected early in disease, to the cerebellum, which is largely protected from neuropathology in these individuals," says Professor Mill.

These studies revealed methylation changes in multiple genomic regions, most notably around the *ANK1* gene. "We saw this very consistently across different regions of the cortex. It was particularly marked in areas of the cortex that are affected very early on in disease." Conversely, DNA methylation differences were not apparent in cerebellum and blood. "So it tells us there is something very specific about the regions of the brain affected in Alzheimer's disease."

Of course, DNA methylation changes could be a consequence of disease rather than a cause. So how important does he think epigenetic changes are to disease? "The real answer is I don't know," he acknowledges. But he points out a notable feature of neurodevelopmental diseases: "If you take a disease like autism or schizophrenia, aetiologically they're incredible heterogeneous – there's probably no two individuals with these diseases who have the same underlying genetic mutations or underlying environmental exposures. But when you do genomic profiling in the brain, you see this very convergent molecular pathology. So it tells us despite this underlying heterogeneity, at a molecular level these different routes to disease perhaps operate through a common functional pathway."

Looking ahead, Professor Mill is examining other forms of DNA modification, including DNA hydroxymethylation which, interestingly, seems to be particularly important in controlling gene activity in the brain. And with Matthew Grubb at King's he is examining how epigenetic patterns are altered by activation of individual neurons. Through single-cell profiling, the pair are hoping to examine how neural activity changes DNA modifications, gene activity and the electrophysiological properties of cells.

Spiers H *et al.* Methylomic trajectories across human fetal brain development. *Genome Res.* 2015;25(3):338–52.
Pidsley R *et al.* Methylomic profiling of human brain tissue supports a neurodevelopmental origin for schizophrenia. *Genome Biol.* 2014;15(10):483.
Wong CC *et al.* Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Mol Psychiatry.* 2014;19(4):495–503.
Saunderson EA *et al.* Stress-induced gene expression and behavior are controlled by DNA methylation and methyl donor availability in the dentate gyrus. *Proc Natl Acad Sci* USA. 2016;113(17):4830–5.
Kumsta R *et al.* Severe psychosocial deprivation in early childhood is associated with increased DNA methylation across a region spanning the transcription start site of CYP2E1. *Transl Psychiatry.* 2016;6(6):e830.
Lunnon K *et al.* Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nat Neurosci.* 2014;17(9):1164–70.

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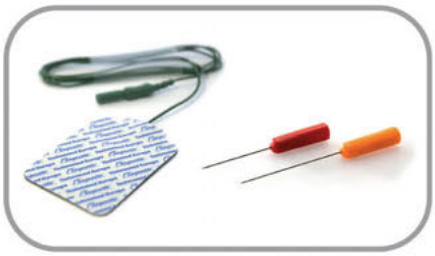

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