

Altered states

Changes to the genome that don't affect DNA sequence may help to explain differences between genetically identical twins. Might these 'epigenetic' phenomena also underlie common diseases? Carina Dennis investigates.

Marie and Jo — not their real names — are twins, genetically identical and raised in a happy home. Growing up, both enjoyed sport and art, were equally good at school, and appeared similar in almost every respect. But as adults, their lives and personalities diverged: in her early twenties, following episodes of hallucinations and delusions, Marie was diagnosed with schizophrenia.

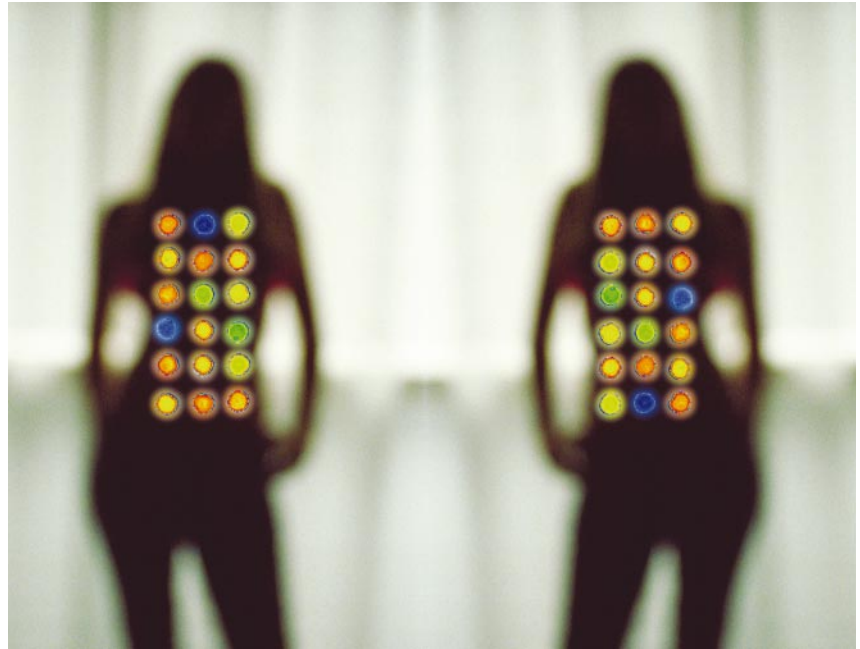
Such examples have long baffled geneticists. Despite sharing the same DNA and often the same environment, 'identical' twins can sometimes show striking differences. Now some researchers are beginning to investigate whether subtle modifications to the genome that don't alter its DNA sequence, known as epigenetic changes, may provide the answer. In doing so, they hope to shed light on the mysterious roots of common diseases — such as schizophrenia and diabetes — that burden society as a whole.

The idea that epigenetics underpins many of the world's health scourges is still highly speculative. "Most geneticists believe that the essence of all human disease is related to DNA-sequence variation," says Arturas Petronis, a psychiatrist at the University of Toronto in Canada. But with the genomics revolution having yet to yield the hoped-for avalanche of genes that confer susceptibility to common diseases, Petronis is not alone in believing that it's time to revisit the problem under the spotlight of epigenetics.

Each of our cells carries the genes for making all the building blocks of the body, but only some of them are active. Epigenetic modifications act like switches, helping to control gene activity so that only those that are required in a particular cell are actually turned on. They constitute a 'memory' of gene activity that can be passed on each time a cell divides, ensuring that liver cells beget more liver cells, and so on.

The best-known epigenetic signal is DNA methylation, which tags cytosine, one of the four chemical bases that make up the genetic code, with a methyl group. Although not a hard-and-fast rule, DNA methylation is generally associated with silencing of gene expression, whereas active genes are usually unmethylated.

Chromatin remodelling is another



Spot the difference: colour-coded epigenetic microarrays could show why 'identical' twins aren't the same.

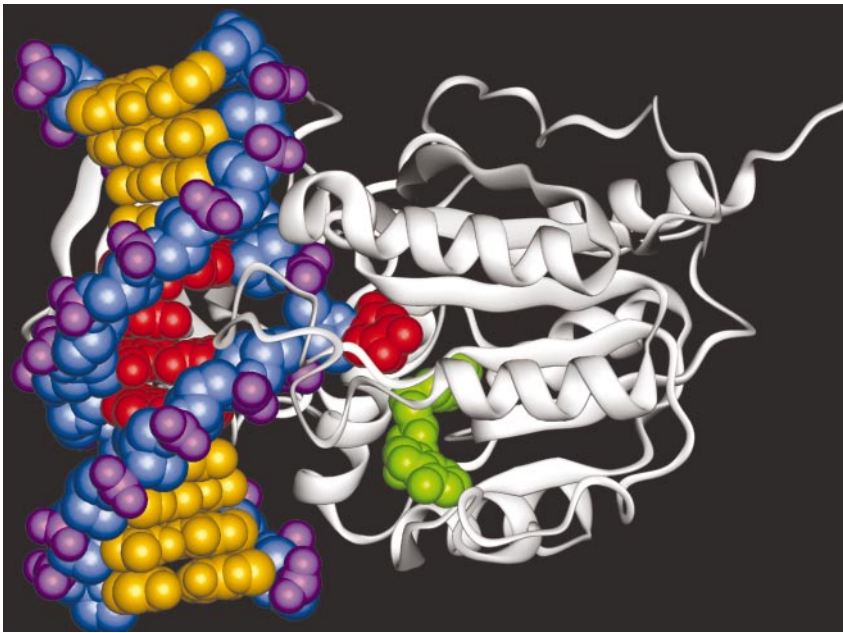
important epigenetic mechanism. Inside the nucleus, strands of DNA wrap around proteins called histones, and then coil up again into a densely packed structure called chromatin. Chemical modification of the protruding histone tails — by the addition of acetyl, methyl or phosphate groups — can alter chromatin structure, which in turn influences the activity of adjacent genes. There are some predictable patterns — for example, genes associated with acetylated histones are usually switched on. But the consequences of the many different combinations of histone modifications — known as the histone code — have yet to be fully deciphered.

Together, these mechanisms modulate gene expression throughout the genome, and underpin several unusual phenomena, including the shutting down of one copy of the X chromosome that occurs in female mammals, and parental 'imprinting' — in which a gene's activity depends on whether it is inherited from the mother or the father.

Over the past few years, some researchers studying rare diseases have found themselves delving into epigenetics. In some cases, they

stumbled into the subject from conventional genetics, discovering that the mutated gene for a rare condition exerts its effect by influencing epigenetic modifications elsewhere in the genome, for instance. Children born with mutations in a gene called *MECP2*, which influences chromatin remodelling, gradually lose their ability to speak and walk¹. People with a mutant form of a gene for an important DNA-methylating enzyme suffer from immune-system deficiencies and facial deformities². And mutations in a gene called *ATRX* cause mental retardation, urogenital abnormalities and a form of anaemia — apparently the result of changes in both chromatin structure and DNA methylation³.

Among oncologists, the idea that epigenetic changes might be linked to disease is already well established. "Epigenetic changes are terribly important in cancer," says Andrew Feinberg, a geneticist at Johns Hopkins University in Baltimore, Maryland. Two decades ago, Feinberg and his colleague Bert Vogelstein were among the first to show that cancer cells may exhibit unusual patterns of DNA methylation⁴. Since then, similar reports of epigenetic



A methylating enzyme (white) binds to its target site (red) on DNA; the methyl donor is shown in green.

abnormalities in cancer have proliferated⁵.

Yet researchers who are interested in other common diseases — such as diabetes, obesity, heart disease and a host of psychiatric disorders — have shown relatively little interest in epigenetics. Whereas tumours arise from aberrant cells, making it easy to imagine how an epigenetic mix-up might be involved, the origins of other conditions are more complex, which made the idea seem less plausible for these diseases. “It’s not been on people’s radar screens,” says Feinberg.

Disease by descent

Besides, many common diseases seem to be inherited within families, which is incompatible with the long-standing idea that the epigenetic slate is wiped clean in the embryo shortly after fertilization, except for parentally imprinted genes. Only now is evidence emerging that other epigenetic changes can be inherited (see ‘Keep it in the family’, right).

The lure of genomics has also been instrumental in keeping medical geneticists focused on DNA sequence rather than what goes on around it. One consequence of the Human Genome Project has been the mapping of more than a million single-nucleotide polymorphisms (SNPs) — positions in the genome at which the sequence varies between individuals by a single letter in the genetic code. By seeing which SNPs are inherited alongside common, complex diseases, it should be possible to pin down the locations of genes that make some people particularly susceptible to certain conditions. But SNP-based approaches have so far yielded a disappointing harvest, which has led some researchers to wonder whether sequence variation is just part of the story. “The role of epigenetics in common diseases has been underestimated —

it’s the new frontier,” claims Feinberg.

That frontier is still a fairly lonely place, but pioneers are starting to explore. “Eight years ago, I didn’t even know what the word ‘epigenetics’ meant,” says Petronis. Today, he feels that some features of complex diseases are easier to explain in terms of epigenetic changes than through conventional genetics. Some conditions, such as multiple sclerosis, fluctuate in severity as they progress. Although the case is far from cut-and-dried, Petronis believes this to be more consistent with shifting epigenetic profiles than with the constant nagging presence of a susceptibility gene⁶.

Factors such as lifestyle and diet undoubtedly influence our susceptibility to disease, and there is mounting speculation that they leave a trail of epigenetic footprints across our genome. Alexander Olek, chief executive officer of Epigenomics, a Berlin-based company that aims to hunt down the epigenetic markers of disease, is hot on the trail of changes associated with diet. Olek hopes that this will lead him to an epigenetic fingerprint for diabetes⁷. Epigenetics may also help to explain why many diseases only appear late in life. “Age is accompanied by changes in DNA methylation,” Olek points out.

While Olek’s firm is launching a wide-ranging effort to study disease epigenetics, Petronis was drawn to the subject by his long-standing quest to understand the most debilitating conditions that haunt the human mind, including bipolar disorder — popularly known as manic depression — and schizophrenia. Many features of these conditions, such as the differences between twins like Marie and Jo, cannot be explained readily by DNA-sequence variation. And although some psychiatric disorders seem to run in families, your chance of succumbing depends

Keep it in the family

You are what you eat, or so we are told. But how could what your grandmother ate make any difference? That’s the mystery posed by the grandchildren of women who were pregnant during the famine that hit the Netherlands at the end of the Second World War. Not only were the babies born to malnourished women smaller than normal, but the next generation also had unusually low birth weights¹³.

A similarly perplexing finding emerged last November, in a study of the grandchildren of Swedish men born in 1890–1920. From crop records, Gunnar Kaati and his colleagues at the University of Umeå calculated how much the grandparents had eaten just before puberty. They found that the grandchildren of well-fed adolescents had a greater risk of dying from diabetes, whereas those descended from famine survivors were less likely to die of heart disease¹⁴.

How can this be? It smacks of Lamarckism, the discredited theory that evolution occurs by the inheritance of acquired characteristics. Now some researchers argue that the answer lies in epigenetics. “Changes in diet could activate certain pathways that then leave an imprint that is passed to the next generation,” says Alexander Olek of the Berlin-based company Epigenomics.

It’s a contentious idea — biologists have long thought that the genome is reprogrammed around the time of fertilization. But recently, evidence has emerged that some epigenetic changes survive this process.

At the University of Sydney in Australia, researchers led by Emma Whitelaw have studied a strain of genetically identical mice whose fur ranges in colour from yellow to black, depending on the methylation of a single gene. They found that coat colour tends to pass from mothers to their pups¹⁵. And now a study of fruitflies is generating a similar stir. Douglas Ruden, of the University of Alabama at Birmingham, and his colleagues found evidence that reductions in the activity of a protein called Hsp90 cause heritable changes in eye structure that are the result of epigenetic changes in chromatin¹⁶.

If similar phenomena occur in humans, it is possible that epigenetic modifications, and not just mutations in DNA sequence, could lie at the root of inherited diseases.



Hungry hopefuls: a Dutch food queue in 1945.



DNA detective: Stephan Beck is aiming to uncover modifications that underlie immune diseases.

in some cases on whether this history is on your mother's or father's side — suggesting, perhaps, that parental imprinting is involved.

Previous genetic studies of schizophrenia and bipolar disorder had seemed to implicate regions of chromosome 22 (refs 8, 9), but the search for a susceptibility gene has drawn a blank. So Petronis is now analysing methylation patterns across the chromosome in DNA from post-mortem samples of brain tissue from affected and unaffected individuals. He then plans to home in on those regions that show clear differences, and look for altered patterns of gene expression.

Attracting more researchers to the epigenetic frontier may require the counterpart of today's high-density SNP maps: a systematic effort to map epigenetic variation across the genome. That is the mission of the Human Epigenome Consortium¹⁰, which is cataloguing the genomic positions of distinct methylation variants. The consortium, established in December 1999, includes Epigenomics, as well as Britain's Wellcome Trust Sanger Institute near Cambridge, the French National Genotyping Centre near Paris, the German Cancer Research Centre in Heidelberg, the Technical University of Berlin and the Max Planck Institute for Molecular Genetics, also in Berlin.

The consortium is now documenting the position of methylation variants across the major histocompatibility complex (MHC), a region on chromosome 6 that contains roughly 150 active genes, many of which are involved in immune recognition. "We chose this region because it is associated with more human diseases than any other," says Stephan Beck, the Sanger Institute's head of sequencing.

A pilot study involving eight initial tissue types has so far identified 4,500 sites within the MHC at which DNA methylation can occur. Information about these sites, which

will shortly be made freely available to other researchers, should prove especially useful for researchers looking for clues to why some people develop autoimmune diseases.

Such studies will involve screening tissue samples from large numbers of people with the condition, and from unaffected controls, to see if a particular methylation fingerprint is strongly correlated with the disorder. Here, the bottleneck is the current lack of high-throughput technology to allow hundreds or thousands of samples to be screened rapidly.

On the map

One way of mapping methylation sites is to treat DNA with a chemical called sodium bisulphite, which converts unmethylated cytosine to a base called uracil, but leaves methylated cytosine intact. The altered bases can then be detected using standard molecular-genetic probes. Several groups, including researchers at Epigenomics, are now working to incorporate these methods into DNA microarrays that could allow samples to be screened for methylation at thousands of genomic sites in parallel.

High-throughput methods are also being developed to screen for histone modifications. The idea is to treat tissue samples with chemicals that 'freeze' modified histone proteins and their associated DNA in their living state. Antibodies targeted at a particular histone modification can then be used to fish out fragments of chromatin that bear these changes. Finally, their genomic location can be established by analysing their associated DNA, again using microarrays. Currently, however, attempts to develop such methods are dogged by problems, including antibodies' tendency to cross-react with different histone modifications.

While the technology for high-throughput studies of large samples of the general popula-

tion is being perfected, some researchers are turning to pairs of identical twins in which only one suffers from a disease. With the variable of DNA-sequence variation removed, it should be simpler to spot distinctive epigenetic modifications that correlate with the condition. Emma Whitelaw, an epigeneticist at the University of Sydney in Australia, for instance, has teamed up with Nick Martin of the Queensland Institute of Medical Research in Brisbane, who has co-founded a large twin registry. "We are keen to start with diseases with an unambiguous clinical diagnosis," says Whitelaw.

Petronis, meanwhile, is already studying pairs of twins in which one suffers from schizophrenia, and has found substantial differences in DNA methylation. "Not all of these changes will be related to the disease," he warns. "The quest we are now facing is distinguishing epigenetic background 'noise' from functionally relevant signals."

Establishing cause and effect in disease epigenetics is a major problem, agrees Olek. This quest is further complicated by the fact that different tissues — and even different cells within tissues — may show epigenetic differences. Epigenetic modifications can even vary with the conditions under which cells are grown in the lab. "It's overwhelmingly complex," concludes Whitelaw.

To establish links between an epigenetic modification and a disease, researchers might look to see whether the change precedes the occurrence of symptoms in affected tissues. A stronger case could be made if the epigenetic variant is shown to alter gene expression and so affect cells' biochemical activities in a way that can explain the condition's pathology — which may require new animal models of important human diseases.

But in the long run, will such studies offer hope to patients like Marie? One drug that inhibits DNA methylation, called azacitidine, is already being tested for the treatment of some cancers^{11,12}. But it acts across the entire genome. If epigenetic changes are shown to underpin psychoses, diabetes and other diseases, we may need more specific treatments. That will pose a challenge, but not an insurmountable one, argues Feinberg. "I think it will be much easier to treat an epigenetic aberration than a sequence mutation," he says. ■

Carina Dennis is Nature's Australasian correspondent.

1. Amir, R. E. *et al. Nature Genet.* **23**, 185–188 (1999).
2. Xu, G.-L. *et al. Nature* **402**, 187–191 (1999).
3. Gibbons, R. J. *et al. Nature Genet.* **24**, 368–371 (2000).
4. Feinberg, A. P. & Vogelstein, B. *Nature* **301**, 89–92 (1983).
5. Feinberg, A. P. *Proc. Natl. Acad. Sci. USA* **98**, 392–394 (2001).
6. Petronis, A. *Trends Genet.* **17**, 142–146 (2001).
7. Maier, S. & Olek, A. *J. Nutr. Sci.* **132**, 2440–2443 (2002).
8. DeLisi, L. E. *et al. Am. J. Psychiatry* **159**, 803–812 (2002).
9. Kelsøe, J. R. *et al. Proc. Natl. Acad. Sci. USA* **98**, 585–590 (2001).
10. Beck, S., Olek, A. & Walter, J. *Nature Biotechnol.* **17**, 1144 (1999).
11. Silverman, L. R. *et al. J. Clin. Oncol.* **20**, 2429–2440 (2002).
12. Kornblith, A. B. *et al. J. Clin. Oncol.* **20**, 2441–2452 (2002).
13. Lumey, L. H. *Paediatr. Perinat. Epidemiol.* **6**, 240–253 (1992).
14. Kaati, G., Bygren, L. O. & Edvinsson, S. *Eur. J. Hum. Genet.* **10**, 682–688 (2002).
15. Morgan, H. D., Sutherland, H. G. E., Martin, D. I. K. & Whitelaw, E. *Nature Genet.* **23**, 314–318 (1999).
16. Sollars, V. *et al. Nature Genet.* **33**, 70–74 (2003).