that, although the correlative data are compelling, they do not prove directly that the heterochromatin is the key determinant. Although it was proposed that the Rb suppressor acts directly by recruiting heterochromatin proteins to certain genomic regions, it is formally possible that Rb acts in the process indirectly.

'If cellular senescence happens not only *in vitro* but also *in vivo*, this work may have significant relevance,' says Manel Esteller, Director of the Cancer Epigenetics Laboratory at the Spanish National Cancer Center (CNIO; http://www.cnio.es). 'We know that the Rb and $p16^{INK4a}$ tumour suppressors are inactivated in many human tumours, mainly due to methylation-associated silencing. Thus, if both proteins are involved in cellular senescence, their inactivation may predict putative responses to certain chemotherapy agents,' Esteller continues. However, this is just a hypothesis that needs to be tested.

What next?

Scientists anticipate that the results from this study could have significant future implications for cancer diagnosis and treatment. Lerner notes that it will now be of great interest to examine, in residual primary tumour cells from patients who have undergone chemotherapy, whether SAHF and the chromatin changes described can be used as markers for cells that are alive but incapable of replication.

In contemplating ways of harnessing these new results, Lowe remarks: 'More information concerning two related issues needs to be obtained to know for sure: (1) to what extent does the senescence program we study in cell culture contribute to 'tumour suppression' or certain diseases in humans? (2) What are the more detailed molecular mechanisms of the process?' Lowe thinks that cancer genetics argues strongly for the importance of overriding senescence during tumour evolution, but there are no good 'markers' of senescence *in vivo*. He also thinks that the current information does not suggest obvious therapeutic targets, but with further study these might be identified.

However, Esteller argues that although the paper shows how the transition of certain human genome regions from active euchromatic to inactive heterochromatic state can be mediated by the Rb tumour suppressor gene, the Rb protein is known to trigger epigenetic changes, which cause gene silencing too. Also important is that these experiments were done with human fibroblast cell cultures and that it is not straightforward to translate such results to organs and tissues of the human body. 'We do not yet know how generalizable the results are, but this is certainly an important issue to resolve,' Lowe notes.

'There still exists a lot of concern that the senescence phenotype is a pure cell culture event and it does not occur in 'real life' or in primary human tumours,' adds Esteller. To this end, the Lowe's group hopes to resolve major uncertainties in future studies but for now most of these questions remain open.

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Bipolar disorder gene identified

Jane Bradbury, freelance writer

A single nucleotide polymorphism in the promoter of the G-protein receptor kinase 3 (GRK3) gene is associated with bipolar disorder (BPD) [1], reported US and Canadian researchers recently. 'Our paper provides the strongest, most definitive evidence for this gene's involvement in BPD to date,' explains John Kelsoe, Professor of Psychiatry at the University of California, San Diego (http://psychgenes.ucsd.edu/). Discoveries like this, he says, should lead to the development of rational drug therapies for BPD.

A complex genetic disease

BPD, which affects 1% of people during their lifetime, is characterized by extreme mood swings between mania and depression [2]. Family and twin studies indicate a genetic basis for BPD, but its mode of inheritance is complex, indicating the involvement of many genes in its aetiology, 'maybe as many as 10 or 20,' says Professor Peter McGuffin, Director of the Social, Genetic and Developmental Psychiatry

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Centre (http://www.iop.kcl.ac.uk/iop/Departments/SGDPsy/index.shtml).

Researchers have been using linkage analysis for many years to home in on BPD susceptibility loci. Recently, for example, the US National Institutes of Mental Health Genetics Initiative (http://www.nimh.nih.gov) reported linkages on 17q, 6q, 2q, 3q and 8q in a study of 1152 individuals [3]. By contrast, a new meta-analysis of 18 genome data sets reported linkage on chromosomes 9p, 10q, 14q, 8q and 18 [4]. However, says Kelsoe, 'a previous meta-analysis highlighted chromosomes 13 and 22. All these studies really show is that many genes are involved in BPD. Every time you reach into the bag and grab another handful of families, you come out with another collection of linkages,' he observes.

Table 1. Treatment of bipolar disorder (BPD)

Long-term prophylactic treatment	Lithium (mood stabilizer)
	Carbamazepine (mood stabilizer)
	Valproate (mood stabilizer)
	Atypical neuroleptics (e.g. clozapine, olanzapine)
	Anticonvulsants (e.g. lamotrigine)
	Thyroid hormones
Treatment of acute mania (mild, moderate and euphoric)	Lithium or carbamazepine with benzodiazepines for first 7 days
	Valproate
	Olanzapine
Treatment of acute mania (severe, uncooperative, psychotic and euphoric)	Lithium, carbamazepine or valproate plus antipsychotics
	Olanzapine plus one mood stabilizer
Treatment of depression (mild, moderate)	Lithium
	Lamotragine
	Brief treatment with an antidepressant
	Psychotherapy, cognitive behavioural therapy
Treatment of severe depression	Lithium plus antidepressant

For all patients with BPD, combinations of medications are often required, and types of medication must be titrated against response and the clinical picture of each individual patient. For prophylaxis, the established options are shown first; for medications lower down the list there is less evidence of efficacy at present. The treatments for depression shown are in the absence of prophylactic treatment. If depression occurs in a patient being treated with mood stabilizers, the treatment must be optimized and/or additional mood stabilizers or selective serotonin reuptake inhibitors added in.

Homing in on GRK3

In 1997, Kelsoe found a susceptibility locus for BPD on chromosome 22. Concurrently, data from the Human Genome Project began to emerge. Says Kelsoe, 'although if you throw a dart into the genome, there will be maybe a dozen compelling candidate genes in the region where it lands, we were intrigued to find that the GRK3 gene was right under this linkage peak'.

GRK3 helps to regulate neurotransmitter signal transduction. When G-protein-coupled neurotransmitter receptors, including dopaminergic receptors, are stimulated by their neurotransmitter, desensitization to further challenge is achieved by receptor removal from the cell surface. When there is a high level of signalling, explains Kelsoe, 'GRK3 migrates to the cell surface, phosphorylates the receptor and initiates a series of events that ultimately triggers receptor endocytosis'.

A good hunch

Kelsoe's hunch that GRK3 might be the chromosome 22 BPD susceptibility gene was strengthened by microarray experiments. 'If you give amphetamines, which are dopamine agonists, to rats or people,' he explains, 'you get a response that mimics mania. When we used the Affymetrix rat chip to look at gene expression in amphetamine-treated rats, GRK3 gene expression showed the greatest increase.' This, together with his linkage analysis, led Kelsoe to look for single nucleotide polymorphisms in the GRK3 gene. He now reports that two promoter variants are associated with BPD. The P-5 variant has an allele frequency of 3% in people with BPD and increases the risk of developing the disorder threefold [1].

'These data are interesting but need robust replication in large samples,' notes Nick Craddock, Professor of Psychiatry at the University of Wales College of Medicine (http://www.uwcm.ac.uk/study/medicine/psychological_medicine). McGuffin agrees, adding that there are many other potentially interesting genes in this region. Kelsoe, however, thinks it is 'pretty likely given the combined animal and human data' that GRK3 is involved in BPD aetiology. However, 'the P-5 variant may only be a marker for the true functional variant of GRK3,' a possibility that he is now investigating.

Certainly, a role for the GRK3 signalling pathway in BPD ties in well with what is known about dopamine and BPD, including the positive response of patients in the manic phase to dopamine blockers. But, says Craddock, it would be difficult to explain all the features of BPD on the basis of alterations in neurotransmission through dopamine receptors or any other single neurotransmitter receptor. Instead, says Craddock, 'it is likely that

a complete description of pathogenesis will include more subtle alterations in second messenger systems or other signalling pathways that are distant from neurotransmitters and their receptors'.

Treatment today and tomorrow

The search for gene variants associated with BPD will continue for some years to come but why is it so important to understand the genetic basis of BPD? One reason, says Kelsoe, is that although there are several mainstay treatments for BPD (Table 1), 'no-one really knows how these medications work.' And, adds McGuffin, 'although we are pretty good at treating manic depression, 30–40% of patients fail to respond to the drugs we have currently'.

For both these reasons, says Craddock, 'genetic studies are important because they may identify a pathway that has not previously attracted much attention from the pharmaceutical industry and open up a whole avenue of potential targets.' Furthermore, he says, 'at present we can not predict very well who will respond to which treatment and all of the treatments have their own spectrum of side-effects, which we are also poor at predicting. My hope is that as our understanding of BPD increases, we will eventually be in a position where laboratory tests will be able to help us decide the best solution for each patient.' This, says Kelsoe, 'is the promise of pharmacogenomics' which should, he says, see clinical application in the relatively near future for BPD.

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Zinc fingers on the pulse of cardiovascular disease

Josh P. Roberts, freelance writer

There could soon be a new way to treat peripheral arterial disease (PAD), a progressive affliction that affects about one in eight of all adults [1]. Results from a recent study were presented in June 2003, simultaneously to the annual meetings of the *American Society for Gene Therapy* (http://www.asgt.org) in Washington DC [2], and the *Society for Vascular Medicine and Biology* (http://www.svmb.org) in Toronto, Canada [3].

'There are no therapies that are really designed to improve blood flow, which is the major problem in PAD,' emphasizes Brian Annex, lead author of a preclinical study from Duke University (http://www.duke.edu) designed to help alleviate that problem.

Peripheral arterial disease

PAD, which is often accompanied by coronary artery disease (CAD), can result in severe reductions in mobility and can even lead to loss of life or limb. It is generally treated by managing symptoms and the underlying causative atherosclerosis [4]. Although surgical techniques such as bypass, balloon angioplasty and stents can sometimes be used to treat blocked heart vessels, these options are generally not feasible to treat obstructed arteries in the limbs.

Annex' team used a zinc finger protein (ZFP), engineered to bind to a regulatory region of the vascular endothelial growth factor-A (VEGF-A) gene. When the ZFP–VEGF-A plasmid was injected into the limb of rabbits whose femoral artery had been ligated, both overall blood flow and new vessel formation were significantly improved. The ischaemic hind limb model is the standard laboratory system for studying PAD.

'This was, from our perspective, the main efficacy experiment,' explains Casey Case, Vice President of research at Sangamo BioSciences (http://www.sangamo.com), the company that engineered the ZFP. Sangamo, in partnership with Edwards Lifesciences (http://www.edwards.com), hopes to file an Investigational New Drug application for the treatment of PAD during the first half of 2004.

VEGF-A isoforms: how many is enough?

This would not be the first clinical trial attempting to induce blood vessel formation in patients, nor would it even be the first to use VEGF-A to do so. But attempts with this angiogenic growth factor thus far have introduced only one of the (at least) four VEGF-A isoforms. And those, 'be it through

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