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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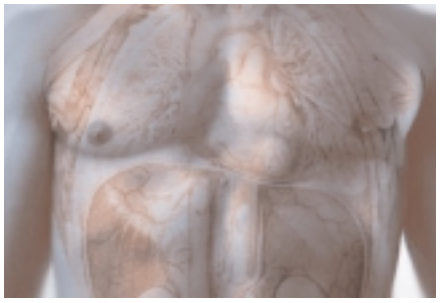

protein delivery or through gene transfer technology,' remarks Annex, 'have been disappointing,'

'The idea is that perhaps one isoform is not enough,' points out Matthew Springer, a Senior Research Scientist at Stanford University (http://www.stanford.edu). To get truly physiologically relevant angiogenesis, he explains, you might need all of the major isoforms in the ratios that the tissues themselves decide to produce.

And this is where Sangamo's approach gains one of its main advantages. Their synthetic three finger ZFP – linked to an activation domain – recognizes a nine-basepair DNase I-accessible region upstream from the coding region of gene [5]. The ZFP acts to turn on transcription of the endogenous VEGF-A gene, producing a full-length mRNA: the splicing machinery of the cell then takes over, 'allowing the body to produce whatever isoforms, in any combination, that the body would view as what it should produce,' says Annex.

Different means to an end

Ronald Crystal's group at Cornell University (http://www.med.cornell.edu) takes a different tack to achieve a similar result. They have created an adenoviral vector containing a cDNA–genomic DNA hybrid of the VEGF-A gene. When animals were treated with the AdVEGF-All vector the message is spliced to produce the three principle isoforms of VEGF-A, and blood flow was seen to increase dramatically in the ischaemic hind limb model. For comparison, they simultaneously treated animals with vectors for all three isoforms, obtaining similar results.

However, Crystal's work could encounter some intellectual property issues, Sangamo's Case speculates. 'The cDNA patents for the VEGF genes are very convoluted and very complex… The different isoforms are owned by different companies.'

This is where Sangamo's approach gains a second advantage. 'What we do is engineer transcription factors that work in trans, of course, and activate the endogenous gene,' explains Case. 'I've never seen a patent for an endogenous gene that is found in nature – there is no inventor involved in an endogenous gene.'

Hope for the future

Although hope for VEGF-A (in whatever form) as an angiogenic therapy might be high, it is not the only growth factor being investigated to induce the (re)generation of vasculature. There are currently 20 gene therapy trials for PAD, at various levels of review, listed in the National Institute of Health's Human Gene Transfer Protocols online database (http://www4.od.nih.gov/oba/rac/PROTOCOL.pdf); there are 19 for CAD. Molecules include fibroblast growth factor, hepatocyte growth factor, hypoxia inducible factor-1α, and developmental endothelial locus-1. In addition, research is also being done on direct protein delivery of a variety of angiogenic factors.

'The major difference between gene therapy and protein therapy is pharmacokinetics,' remarks Mark Post from the University of Maastricht (http://www.unimaas.nl). Protein therapies have thus far failed because patients have too short an exposure to the growth factor, but controlled- and sustained-release matrixes or gels are possible. Gene therapy, on the other hand, has the potential to produce a constant source of protein over a prolonged period of time, but is more difficult to gauge and predict,' he notes.

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