is a strong correlation between the concentration of certain antibodies and the extent of atherosclerosis,' said Nilsson. 'The vaccine would be unlikely to work like familiar vaccines, such as those for measles. It is more probable that the vaccine would be used in a combined therapy with statins to combat atherosclerosis.'

Future developments

Nilsson expects the vaccine to enter Phase I clinical trials in the next two years. 'It's always difficult to predict at this stage whether there will be any side effects when the vaccine is used in humans,' said Nilsson, although he remains optimistic about side effects from the results with the mouse model. 'The vaccine could be administered by the traditional subcutaneous injection, although with the rate that delivery technology is progressing, an alternative method, a nasal spray, for example, could be used.'

Wulf Palinski of the University of San Diego California (USDC; CA, USA), one of the first investigators to study this area, believes that such a vaccine is still a long way off. 'The efficacy of immunizations in various animal models of atherosclerosis has now been confirmed by at least six published papers, but we are a long way from proposing this as a preventive approach in humans,' he commented. 'From a scientific point, the main unresolved question is that the mechanism responsible is not known. Both humoral and cellular immune responses have been implicated. The nature of the immunogen and the frequency of immunization will also need much more work.'

The teams from Lund and Cedars-Sinai will now focus on increasing the efficacy of the vaccine and investigating the regulatory factors involved in the immune response to LDL.

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2 Palinski, W. *et al.* (1995) Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 92, 821–825

3 Palinski, J. and Witztum, J.L. (2000) Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. *J. Intern. Med.* 247, 371–380

News in brief

Targets and mechanisms

Could exercise be a thing of the past?

Researchers have discovered a biochemical pathway in muscle cells that can generate the beneficial effects of exercise, without the hard work [1]. The research was conducted at Duke University Medical Center (Durham, NC, USA) and the University of Texas Southwestern Medical Center (Dallas, TX, USA). R. Sanders Williams, the Dean of Duke, said: 'We think this discovery could lead to the synthesis of new drugs that will allow individuals to acquire the health benefits of regular exercise, even if they cannot exercise. It has the potential to improve the lives of patients with heart failure, pulmonary disease, renal failure, diabetes and other chronic diseases,' says Williams.

In this study, the scientists discovered a cellular signalling pathway involving calmodulin-dependent protein kinases (CaMKs); these control genes that are responsible for the physiological and metabolic properties of muscle cells. The researchers generated transgenic mice that express a constitutively active form of CaMK IV in skeletal muscle, which subsequently showed increased levels of mitochondrial biogenesis, as well as the upregulation of enzymes involved in fatty acid metabolism and electron transport, and reduced susceptibility of the muscle cells to fatigue.

Skeletal muscle is made up of two types: muscle that handles long-term low-level loads and muscle that responds to sudden heavy loads. Exercise such as weightlifting makes muscles larger, while sustained exercise, such as long-distance running, increases resistance to fatigue and thus reduces the risk of disorders such as cardiovascular disease and diabetes.

Rhonda Bassel-Duby, Associate Professor of Internal Medicine and co-author of the study, said: 'The muscles of individuals who are on bed-rest resemble type II muscle fibres; they fatigue quickly and the muscles are tired.'

CaMK also induced expression of peroxisome proliferator-activated receptor γ co-activator (PGC1), a regulator of *in vivo* mitochondrial biogenesis. 'Activation of CaMK recapitulated the effects of exercise indicating that this is a central pathway by which exercise modifies the metabolic properties of skeletal muscles,' said Williams. 'Until now, scientists did not suspect that this particular enzyme was involved in that control.'

Hai Wu, postdoctoral research fellow and lead author of the study commented that, because CaMK is also responsible for neuron plasticity and is involved in learning and memory: 'Both neurons and muscle cells are excitable, and they share a lot of

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common signalling pathways in response to either brain activity or exercise.'

The team is continuing to study the pathway to identify the best targets for drug discovery, and will also research whether this pathway is involved in other tissues, such as fat, or even in the biology of cancer cells. Williams added that mitochondria are fundamental to the function of all cells and the pathway that controls mitochondria could possibly be manipulated in other kinds of medical conditions.

1 Wu, H. *et al.* (2002) Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* 296, 349–352

Muscular dystrophy: an end to muscle waste?

Scientists have found a way to halt the muscle destruction that is associated with Duchenne muscular dystrophy (DMD) [2]. The research, performed at the University of California, San Diego (UCSD) School of Medicine (La Jolla, CA, USA) showed that the addition of a naturally occurring enzyme, cytotoxic T cell GalNAc transferase, halted muscle wasting in mice.

DMD is a congenital X-linked myopathy caused by the lack of expression of the protein dystrophin. Many dystrophin-associated proteins (DAPs) are also underexpressed at the sarcolemmal membrane; however, they remain concentrated at the neuromuscular junction where a dystrophin homologue, utrophin, is expressed. This led to the theory that expression of these DAPs and utrophin along myofibres could counteract the effects of DMD.

This study showed that overexpression of the synaptic cytotoxic T (CT) cell GalNAc transferase increased the expression of utrophin and other DAPs.

Paul Martin, UCSD Associate Professor of Neurosciences and senior author of the study, said: 'We hope this enzyme can eventually be used as a therapy for DMD. It has the potential for managing the disease, much like we manage diabetes with insulin medication or injections.' In research reported earlier this year [3], Martin and co-workers described the role of CT GalNAc transferase in the development of mice that had the enzyme in skeletal muscles, as well as at the neuromuscular junction: these mice did not develop the muscle wasting that is associated with DMD.

As for the potential of a therapy for DMD, Martin says: 'There are two schools of thought about how to develop a therapy. One option is gene therapy. Although a small amount of enzyme would have a big effect, the mechanics of administering the gene to all muscles in the body would not be an easy task. We're more excited about the possibility of creating an easy-to-administer oral medication that could globally stimulate this enzyme in skeletal muscles.'

2 Nguyen, H.H. *et al.* (2002) Overexpression of the cytotoxic T cell GalNAc transferase in skeletal muscle inhibits muscular dystrophy in mdx mice. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5616–5621

3 Xia, B. *et al.* (2002) Overexpression of the CT GalNAc transferase in skeletal muscle alters myofiber growth, neuromuscular structure and laminin expression. *Dev. Biol.* 242, 58–73

Mitochondrial DNA data made freely available

A study of the variations that occur in the mitochondrial (mt) DNA of geographically distinct subgroups has been published by MitoKor (San Diego, CA, USA) in collaboration with the University of Newcastle-upon-Tyne (Newcastle, UK), the VA Medical Center (San Francisco, CA, USA), the University of California, San Diego (CA, USA) and Harvard Medical School, Massachusetts General Hospital (Harvard, MA, USA) [4].

The study, of more than 500 individuals, analyzed 560 complete European, Asian and African mtDNA coding-region sequences from unrelated individuals and identified a total of 497 haplogroup-associated polymorphisms. The data produced, available at no cost from http://www.mitokor.com/science/560mtdnas.php, will help researchers understand how changes in mtDNA sequence can affect susceptibility to disease. 'Mitochondrial genetics is improving our understanding of human evolution and prehistoric migratory patterns. In addition, mitochondrial sequence variation has been implicated as a causative or contributing factor in a number of human diseases,' said Neil Howell, Vice-President of Research at MitoKor. 'We are now combining these two aspects to understand the genetics of these complex disorders.' The study will form the basis of ongoing investigations into the association of variations in mitochondrial DNA with diseases of ageing such as Alzheimer's and type 2 diabetes.

4 Herrnstadt, C. *et al.* (2002) Reduced-median-network analysis of complete mitochondrial DNA coding region sequences for the major African, Asian and European haplogroups. *Am. J. Hum. Genet.* 70, 1152–1171

Unravelling the genetics of Hirschsprung disease

A multi-national team of researchers has unravelled the genetics of Hirschsprung disease (HSCR) in one go. This is possibly the first time that the complicated genetics of a disease has been ascertained and leads the way for the unravelling of the genetics of other complex diseases [5].

HSCR is an inherited intestinal condition that occurs in ~1 in 5000 births and is characterized by a loss of the nerves that usually control the bowel. There are two forms of the disease – the most common and more genetically complex short-segment form (S-HSCR, 80% of cases) and the long-segment form (L-HSCR, 20% of cases).

The team, led by the Case Western Reserve University-based laboratory of Aravinda Chakravarti, who is now at Johns Hopkins University, had previously shown that the *RET* gene was the main gene involved in HSCR. By mapping the disease to regions of chromosomes, and then identifying the changes within those regions, the researchers found that there are three regions from chromosomes 3, 10 and 19 that are integral to HSCR and which, taken together, explain its complicated inheritance pattern. They also showed that *RET* is the region identified on chromosome 10.

Their results show how important it is to look at all parts of a gene, and not just the areas that encode proteins. The researchers found that the protein-encoding regions are often unchanged: 'just because coding sequences aren't changed, doesn't mean that a gene isn't involved', said Chakravarti. Changes in non-coding regions can also affect sites for gene regulation and how the gene is read, or can alter gene function and expression, explained Chakravati. The group hopes to find a single gene in the regions from chromosomes 3 and 19, although they do not rule out the possibility of further genes being involved.

Thus, with carefully designed studies that incorporate both mapping and searching for specific genetic changes, it might be possible to ascertain the genetics of other complex diseases.

5 Gabriel, S.B. *et al.* (2002) Segregation at three loci explains familial and population risk in Hirschsprung disease. *Nat. Genet.* 10.1038/ng868 (http://www.nature.com/ng/)

CNS-related disorders

Statins could prevent damage by AD protein

Commonly used cholesterol-lowering drugs known as statins can prevent the damage caused to blood vessels by a protein associated with Alzheimer's disease (AD), researchers from the University of South Florida's Roskamp Institute (Tampa, FL, USA) have found [6].

It was already known that statins could reduce the risk of developing AD, but the reasons were unclear. In addition, high cholesterol levels have been linked to a greater risk of developing the disease. The Roskamp researchers were struck by the similar effects in blood vessels of cholesterol and the Aβ protein, a crucial protein in AD pathology. Both compounds promote the constriction of blood vessels through a similar mechanism. The researchers therefore investigated whether anti-cholesterol drugs would have an effect on the Aβ protein.

They studied the effects of the two most commonly used statins, mevinolin (lovastatin) and mevastatin (compactin). Mevastatin prevented the toxicity associated with Aβ in human neuronal cells that had been cultured in the laboratory. Furthermore, both statins reduced the propensity of Aβ protein to cause constriction of laboratory-prepared blood vessels from rats.

'Statins block the vasoconstrictive effects of Aβ protein,' said Daniel Paris, Assistant Professor at the USF Roskamp Institute and first author of the study. 'These drugs appear to have anti-inflammatory properties, independent of their benefit in lowering cholesterol, that may help protect against dementia.' The Roskamp researchers are currently investigating the effectiveness of statins to prevent or slow the progression of AD in a mouse model.

6 Paris, D. *et al.* (2002) Statins inhibit Aβ-neurotoxicity *in vitro* and Aβ-induced vasoconstriction and inflammation in rat aortae. *Atherosclerosis* 161, 293–299

Probing the genetics of autism

New insights into the genetics of autism, a disorder beginning in early childhood that impairs thought, feelings, language and social skills, have been reported in several recent papers. Although its causes and effective treatments have eluded science, evidence suggests that the disorder is highly heritable and is probably the result of interactions among multiple unknown genes. Recent studies have investigated several genes that might predispose individuals to autism.

Edwin Cook and co-workers from the University of Chicago (Chicago, IL, USA) have investigated the serotonin transporter gene *SLC6A4* as a candidate gene in autism [7]. The gene and its flanking regions were sequenced, followed by typing of single nucleotide polymorphisms and simple sequence repeats. Four markers showed evidence of transmission disequilibrium, indicating that these variants might be linked with the disorder.

Elena Maestrini of the University of Bologna (Italy) and co-workers have studied chromosome 7q, which had previously been identified as a likely host for an autism susceptibility locus [8]. They examined four adjacent genes using a positional candidate gene approach. Analysis of these genes strongly suggested that they do not have a major role in autism, and further candidate genes at this locus should now be screened.

Chromosome 6q21 has been implicated as a candidate region for a gene contributing to autism [9]. The area contains the glutamate receptor 6 gene, which is known as a functional candidate for the syndrome. The work of Thomas Bourgeron of the Pasteur Institute (Paris, France) and co-workers suggests that a mutant version of the glutamate receptor gene is in linkage disequilibrium with autism.

In a fourth study, Joseph Buxbaum and co-workers of the Mount Sinai School of Medicine (New York, NY, USA) applied a transmission disequilibrium test to the *155CA2* [10] gene. They demonstrated an association between autism and the *155CA2* gene, which is part of the GABA receptor gene complex.

Further research into the genetic basis of autism has been boosted by a five year, US$6 million grant from the National Institute of Mental Health that has been awarded to Daniel Geschwind, Professor of Neurology at the University of California, Los Angeles (CA, USA).

7 Kim, S-J. *et al.* (2002) Transmission disequilibrium mapping at the serotonin transporter gene (SLC6A4) region in autistic disorder. *Mol. Psychiatry* 7, 278–288

8 Bonora, E. *et al.* (2002) Mutation screening and imprinting analysis of four candidate genes for autism in the 7q32 region. *Mol. Psychiatry* 7, 289–301

9 Jamain, S. *et al.* (2002) Linkage and association of the glutamate receptor 6 gene with autism. *Mol. Psychiatry* 7, 302–310

10 Buxbaum, J.D. *et al.* (2002) Association between a GABRB3 polymorphism and autism. *Mol. Psychiatry* 7, 311–316

Function of reelin protein revealed

The role of the brain protein reelin, whose function in the adult brain has long been a mystery, has recently been identified [11]. The new findings also implicate the protein in a possible molecular mechanism for schizophrenia.

Scientists at the University of Illinois (UIC; Chicago, IL, USA) discovered that reelin is responsible for the migration of neural stem cells to their appropriate location in the brain as it adapts to new information. 'Triggered by unknown environmental cues or factors, they migrate to specific areas to become a glial cell or a neuron, forming a link in the adult brain's complex neural network,' said Kiminobu Sugaya, Assistant Professor of Psychiatry and the principal investigator of the study.

In the study, human neural stem cells were injected into the lateral ventricle of the brains of normal and 'reeler' mice, which cannot produce reelin. In the normal mice, symmetrical migration and differentiation of the cells could be detected. However, according to Sugaya, the cells in the reeler mice 'got lost'. 'They failed to migrate,' he said.

If neural stem cells do not migrate to the correct place in the brain and differentiate into the right type of cell, cognitive and psychological functions fail. This suggests that a lack of reelin is involved in psychiatric disorders. In an earlier postmortem study, Erminio Costa, Scientific Director of UIC's Psychiatric Institute and a co-author on the neural stem cell study, found that the level of reelin in the brains of schizophrenics was half that in normal brains. 'Perhaps in schizophrenics, who

lack reelin, the brain's stem cells can't find their way to make the appropriate neural connections,' explained Sugaya. 'As a result, perception and thinking may break down.'

11 Kim, H.M. *et al.* (2002) Reelin function in neural stem cell biology. *Proc. Natl. Acad. Sci. U. S. A.* 99, 4020–4025

Miscellaneous

Pharma snubbed as biotech opts to keep business in the family

Biotechnology companies are increasingly choosing to sign licensing agreements with other wealthier biotechnology companies rather than their traditional partners, the pharmaceutical companies, because of their more similar culture, says a new report from Datamonitor (London, UK) *Biotech Company Growth Strategies: Intra-biotech Collaborations Bring Independence from Pharma One Step Closer*.

Larger biotechnology companies, such as Amgen (Boulder, CO, USA) and Genzyme (Cambridge, MA, USA), have been using mergers and acquisitions as their strategy to boost pipelines. However, as mergers and acquisitions are typically paid for with stock, this is highly reliant on stock prices. Hence, in-licensing presents a better option for these companies, claims the report. Furthermore, because a fully integrated biotech company has been through the same growth stages as the smaller biotech companies that it is partnering, the relationship is more likely to be successful and to avoid the pitfalls of bad communications and conflicting goals that often lead to the failure of pharma–biotech collaborations.

Meanwhile, the influx of investor income in 1999/2000 into smaller biotech companies means that many can now afford later-stage clinical trials and their own sales and marketing without pharma investment. If pharmaceutical companies are to compete in this trading, they should offer technology, drug development expertise and long-term support rather than cash, says the report.

New contraceptive patch will be a serious challenger to the pill

The launch of a new hormonal contraceptive patch by Johnson & Johnson looks set to offer the first real challenge to 'the pill', says a recent Datamonitor (London, UK) report, *Strategic Perspectives 2001: Hormonal Contraceptives*. The patch – called Ortho Evra – lasts a week, unlike oral contraceptives, which need to be taken daily, and has none of the discomfort associated with implants and injections.

Public uptake is expected to be high in the USA, where direct-to-consumer advertising is permitted, although it will be dependent on physicians' willingness to prescribe a new, more expensive contraceptive. Global sales of the patch are expected to reach US$300 million by 2008, claims the report. However, the visibility of the patch could be a hindrance to sales for those that that do not wish their choice of contraceptive to be on public display.

Clinical Research Organizations band together

The Association of Clinical Research Organizations (ACRO) has been formed to represent the views of the drug development industry before legislative and regulatory groups in the USA and other countries. The founding member companies of ACRO, Covance (Princeton, NJ, USA), Kendle International (Cincinnati, OH, USA), Parexel International Corp. (Waltham, MA, USA), PPD Development (Wilmington, NC, USA) and Quintiles Transnational Corp. (Research Triangle Park, NC, USA), have elected Quintiles Transnational chairman Dennis Gillings as ACRO Chairman.

'We will work to provide a heightened awareness of the role that CROs play in expediting the introduction of important new medicines… [and] will be active in the development of regulations, legislation or initiatives that may affect our industry,' said Gillings.

Cancer targets and mechanisms

Contradictory action of breast cancer drug explained

Scientists have exposed the mechanism that causes the unwanted side effects of tamoxifen [12]. The findings could enable researchers to design new drugs that reduce the risk of certain types of breast cancer but do not have the same side effects as tamoxifen.

'Tamoxifen was the first drug shown to be capable of reducing the chances of breast cancer development in some women at risk of the disease,' says Myles Brown, researcher at the Dana-Farber Cancer Institute (Boston, MA, USA) and senior author of the study. 'But many women have been reluctant to take it because of the potential side effects.'

Although the drug is effective in preventing breast cancer, it is associated with an increased incidence of endometrial cancer. A related drug, raloxifene, also prevents breast cancer, but appears not to increase the risk of endometrial cancer.

Both drugs are selective oestrogen receptor modulators (SERMs), which bind to the oestrogen receptor (ER). In the breast, binding of oestrogen to the ER induces cell growth, allowing carcinomas to spread, whereas binding of tamoxifen or raloxifene restricts cell growth. In the uterus, however, binding of oestrogen or tamoxifen promotes growth.

Brown and co-author Yongfeng Shang, an instructor in medicine at the Dana-Farber Cancer Institute and Harvard Medical School (Cambridge, MA, USA), revealed that the action of SERMs in the breast and uterus depends on co-regulatory proteins recruited to the site. They showed that, in breast cancer cells, both tamoxifen and raloxifene induce recruitment of co-repressors. However, in uterine cells, tamoxifen, but not raloxifene, induced recruitment of co-activators, potentially stimulating cell proliferation.

'Our findings demonstrate that the make-up of the co-regulating proteins present in a cell determines how it will respond to treatment with different SERMs,' Brown says. 'This knowledge will help us better predict the range of effects of newly developed SERMs, and study those that are likely to have the best spectrum of benefits. Also, it will help researchers 'look for medications whose profile of effects is more advantageous to patients,' he says.

12 Shang, Y. and Brown, M. (2002) Molecular determinants for the tissue specificity of SERMs. *Science* 295, 2465–2468

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