EPILEPSY

Protease inhibitor implicated

James O. McNamara and Ram S. Puranam

THE culprit responsible for a rare form of inherited epilepsy, known as progressive myoclonic epilepsy of the Unverricht–Lundborg type (EPM1), has been collared. Writing in *Science*[1], Pennacchio *et al.* describe how they have exposed the identity of the mutant gene that is carried unbeknown by both parents and can manifest as EPM1 epilepsy in their offspring. The culprit, surprisingly, had not even been a suspect — of all the aberrant proteins that might have been expected to be encoded by this gene, which include ones affecting neuronal excitability or synaptic transmission, this turns out to be a cysteine protease inhibitor, whose normal function is to prevent the destruction of key proteins.

The epilepsies comprise a common collection of disorders that affect at least one per cent of the population worldwide and which consist of more than 40 distinct types, all sharing the common feature of an enduring increase of neuronal excitability that causes a periodic and unpredictable occurrence of seizures. EPM1 is an exceedingly rare form. Children afflicted with it start to suffer seizures between the ages of 8 and 13 (ref. 2). There follows a gradual decline in neurological function and the intellect deteriorates slowly, with a loss of roughly ten IQ points per decade. Survival into adulthood is common and some individuals reach the sixth decade. Autopsy discloses widespread neuronal degeneration.

The past decade has witnessed enormous progress in our understanding of the mechanisms that underlie a number of these disorders. But the restraint imposed by the daunting complexity of the mammalian nervous system makes the power of genetics especially appealing. Many of the epilepsies recognized to have genetic determinants (about one-third of cases) appear to be complex genetic disorders where several genes are probably at fault,

MEDICAL HISTORY

Country doctor and speckled monster

As the world knows, Edward Jenner (1749–1823) was the man who, through his observation of the immunity of milkmaids in his native Gloucestershire to the 'speckled monster', as smallpox was then known, established the means in 1796 by which the disease was eventually to be eradicated. A statue to commemorate him has long stood in Kensington Gardens, London; but it had become forgotten, and last week a ceremony was held there as a feature of the bicentennial celebrations of the advent of vaccination.

Jenner has sat there reflectively, chin in hand, for nearly 150 years. The sculptor, William Calder Marshall, gave a clue by inscribing a head of a cow on the chair on which his model sat. But only the name — Jenner — was inscribed on the pedestal and so, over time, the memorial became virtually forgotten. The Friends of Hyde Park and Kensington Gardens 'rediscovered' the memorial in their effort to foster recognition of the unique Victorian statues in Kensington Gardens. They were then joined by the Jenner Educational Trust and St George's Hospital Medical School, and together they have now placed a commemorative plaque at the memorial — at last giving full recognition to Jenner.

It is difficult to imagine the horror that smallpox brought in its train. No one was safe. In Jenner's time, it caused ten per cent of the total deaths and a third of those of children in London. It was the cruellest of the emissaries of the Grim Reaper.

On 14 May 1796, lymph from a pustule of dairymaid Sarah Nelmes was vaccinated by Jenner into James Phipps. Later, when the boy was inoculated with smallpox, the feared symptoms — high fever, followed by spots and, often, death — failed to appear. Vaccination was shown to give protection. It then took nearly 200 years before the World Health Organization, on completing an international eradication programme, declared in 1980 that the world was rid of the disease.

A number of statues of Jenner have been erected. The first was placed in 1825 in Gloucester Cathedral. Another, erected in 1865, stands in Bologne-sur-Mer, where the first vaccinations in France were carried out. Later, a memorial was placed in 1904 in Tokyo in the gardens of the National Museum. Perhaps the best known, with a cast in the Wellcome Building in London, is in the Museum of Fine Art in Genoa. It was by a leading Italian sculptor, Giulio Monteverde, and was commissioned in 1873 by Maria Brignole Sale, Duchess of Galliera, in recognition of the protection that vaccination gave her family.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Dan Atkin

Jenner reflects in Kensington Gardens.

But Jenner's statue in Kensington Gardens is his main memorial. Calder Marshall was a competent artist of the Victorian era and it was he, in fact, who first conceived the idea of the memorial. The necessary funds were slow in coming until an appeal was launched internationally. The United States headed the donations; Russia, despite the intervention of the Crimean War, came second; and Britain only third.

With the permission of Queen Victoria, a site in Trafalgar Square was secured and Prince Albert, the Prince Consort, presided over an inaugural occasion in 1858. Everybody who was anybody attended. But, soon afterwards, Jenner was banished. A non-military character was thought inappropriate in an area devoted to British success at arms. *The Times* spoke up for his removal, and it was demanded in Parliament. *Punch* took an ironic view:

England's ingratitude still blots
The escutcheon of the brave and free;
I saved you many million spots,
And now you grudge one spot for me.

And so, in 1862, Jenner was moved to become the first statue in Kensington Gardens. St George's Hospital, originally at Hyde Park Corner, put in a bid for the statue on the centennial anniversary of his discoveries. It had good grounds: the illustrious John Hunter, a surgeon at the hospital, was Jenner's mentor. But the attempt failed.

And so, today, his memorial still stands in Kensington Gardens. But now with acknowledgement of his place in medical history. John Empson

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■See also page 18.

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although a tiny fraction (less than one per cent of cases) do seem to be accounted for by a single mutant gene.

Single mutant genes have been identified with three of the monogenic human epilepsies. A point mutation (caused by a base substitution in the DNA) in a gene encoding the α4 subunit of the neuronal nicotinic acetylcholine receptor underlies one form of frontal lobe epilepsy[3]. The other two monogenic epilepsies are like EPM1 in that they are myoclonic, with characteristic violent muscle contractions: a point mutation in a mitochondrial gene encoding a lysyl transfer RNA is responsible for myoclonic epilepsy and ragged red fibre disease[4], and deletions and a point mutation that alters a splice site in a novel gene have been linked to Batten disease[5].

Pennacchio *et al.*[1] also used a genetic approach to determine the nature of the defective protein in EPM1 epilepsy. Linkage analysis had already localized the gene underlying EPM1 to a region of 2 million base pairs on the long arm of chromosome 21, which was eventually narrowed down to 175 kilobases. Using direct complementary DNA selection, the authors looked for expressed DNA within this region of the genome: one cDNA was found to encode cystatin B, a protein inhibitor of cysteine proteases. This gene was expressed in all tissues examined.

Lymphoblastoid cell lines from affected and unaffected individuals were used to compare the expression of cystatin B messenger RNA. Affected individuals from four families had undetectable levels of cystatin B mRNA, unlike asymptomatic carriers and unaffected individuals. This suggested that a mutation was present in the cystatin B gene that resulted in decreased amounts of the mRNA.

Sequencing the gene from affected individuals disclosed different mutations in different families. A point mutation found in affected members of one family lay in a 3′ splice site, a G-to-C transversion at the last nucleotide of intron I, a position highly conserved in all introns. A second mutation, at amino-acid position 68, that generated a stop codon, was found in two additional families. Oddly, no mutation could be identified in the fourth family, despite sequencing the entire coding region and large tracts of non-coding regions. The authors conclude that there is a strong association between the symptoms suffered by patients with EPM1 (the phenotype) and the abnormal reduction of cystatin B mRNA, although a causal link still needs to be proven.

An odd feature peculiar to EPM1 that may provide a clue to understanding some of the phenotypic consequences of this mutation is the deleterious effect of the antiseizure drug phenytoin on these patients[6]. Phenytoin is widely used and is safe and effective in many patients with epilepsy, but not for those with EPM1, in whom it speeds neurological deterioration and may even shorten lifespan.

How might a mutation leading to reduced cystatin B mRNA (and presumably cystatin B protein) produce an epileptic phenotype? Cysteine proteases are small proteins that catalyse the destruction of diverse polypeptide substrates and are most active under mild reducing or acidic conditions, properties that render them well adapted to the environment of lysosomes[7], which are organelles inside the cell where unwanted macromolecules are broken down. These enzymes can work on a broad range of substrates and are important in regulating intracellular protein turnover.

The cystatins are naturally occurring protein inhibitors of cysteine proteases and include more than 20 related members of a superfamily; cystatin B is itself a member of a subgroup of small intracellular proteins. It is widely distributed in cells and tissues, including the brain. This ubiquitous distribution, together with its apparent locale in the cytosol, has led to the suggestion[8] that cystatin B protects the cell from cysteine proteases leaking out of the lysosomes.

The discovery by Pennacchio and colleagues[1] of the association of EPM1 with inadequate amounts of cystatin B mRNA raises many questions. Assuming that the phenotype is due to defective cystatin B activity intrinsic to the brain, in which brain regions is cystatin B expressed? Is it expressed in neurons and/or glia? Is it restricted to the cytosolic compartment of these cells? How is the expression of cystatin B and its target proteins regulated during development? What is the key enzyme(s) inhibited by cystatin B specifically in the brain? Alternatively, does cystatin B have functions apart from being a protease inhibitor?

The answers to these questions, together with the creation of mice carrying null mutations of cystatin B, should help us decipher how this mutation produces the EPM1 phenotype. Insight derived from the study of EPM1 may provide a clue to the cellular and molecular mechanisms underlying some of the more common forms of human epilepsy with similar features, and lead to more effective therapy. □

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OPTICS

Light bent by magnets

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On page 54 of this issue[1], Rikken and van Tiggelen report a new optical effect. In their experiment, light is injected into an isotropic scattering medium in a direction perpendicular to an applied magnetic field. They see a light flux induced in the third direction, perpendicular to the field and to the injected light, which is directly proportional to the magnetic field. Is this a trivial effect or is it surprisingly new?

An external magnetic field influences the refractive index of an isotropic dielectric medium. When the light travels parallel to the magnetic field, the refractive index for right circularly polarized light differs from the one for left circularly polarized light. This is known as the Faraday effect, and it is proportional to the magnetic field.

For linearly polarized light, the magnetic field causes the polarization plane to rotate in its passage through the medium. Part *a* of the figure shows this effect, where the electric field is represented by arrows and the wave vector and the external magnetic field are perpendicular to the plane of drawing. The charges in the dielectric are driven by the electric field of the light. Owing to the presence of an external magnetic field, the moving charges will experience a sideways Lorentz force. The net result is that the electric field vector is dragged along with the moving charges, thereby rotating. The Faraday effect cannot give rise to beam walk-off; in other words the Poynting vector, $\mathbf{S} = \mathbf{E} \times \mathbf{H}$, which describes the energy flow, will be parallel to the wave vector.

When we change the geometry so that the external magnetic field is perpendicular to the wave vector, the refractive index for light polarized parallel to the external field will differ from the one with perpendicular polarization. Only the latter case is of interest here and is shown in part *b* of the figure. By the reasoning above, the electric field vector will experience a wig-

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