nature genetics
volume 1 no.3 june 1992

Dose response in Charcot-Marie-Tooth disease

It is more than 100 years since the first descriptions of a novel form of progressive muscular atrophy were reported by J. M. Charcot and P. Marie in France, and independently by H. H. Tooth in Britain, in 1886. And yet it has been only within the past 12 months or so that researchers have begun to recognize the genetic events underlying at least one form of Charcot-Marie-Tooth (CMT) disease. Although the four papers published in this issue of *Nature Genetics* are not the last word in the story, they offer a satisfying and plausible explanation for a partial understanding of the molecular pathology of CMT.

CMT is one of the most common hereditary motor and sensory neuropathies, affecting roughly 1 in 2,500 people. The disease affects the peripheral nerves — causing atrophy of the distal muscles — and patients often have clawed hands and deformed feet that may require surgery. CMT is clinically and genetically heterogeneous: clinically the disease can be diagnosed by decreases in nerve conduction velocity (NCV), but these are highly variable and may not differ significantly from the normal range. There are several autosomal and X-linked loci for CMT, including a locus on chromosome 17 responsible for the most common sub-type, CMT type 1A (CMT1A).

The linkage of CMT1A to markers on the proximal short arm of chromosome 17 (17p11.2), first reported in 1989 by Vance *et al.* (*Exp. Neurol.* **104**, 186–189), was soon confirmed by others. But there was a puzzling aspect to the linkage data as more probes in the estimated vicinity of the CMT1A locus were used: markers that were predicted to be so close to the gene as to be most unlikely to yield recombinants did so, confounding attempts to resolve the location of the locus. Finally, two groups unravelled the confusion. J. Lupski *et al.* (*Cell* **66**, 219–132; 1991) and P. Raeymaekers *et al.* (*Neuromusc. Disorders* 1, 93–97; 1991) found that certain markers were duplicated in CMT1A patients (as revealed by the presence of multiple alleles, dosage differences and *in situ* hybridization). Both groups found that the duplication, which can arise *de novo*, was linked to the CMT1A phenotype. In addition, Lupski and colleagues detected a novel 500 kb *Sac*II fragment on pulsed field gels of patients' DNA, suggesting that the genomic rearrangement was of significant size.

The phenomenon of a large-scale DNA duplication in a dominant hereditary disorder was without precedent, but naturally raised more questions than it answered. One that has yet to be solved is the precise molecular basis for the duplication, now known to embrace more than one megabase. A more immediate question was how the duplication led to the manifestation of the disease. Although a simple dosage effect (caused by having three copies of a gene or genes, rather than two) was the most appealing explanation, there were at least three others: gene expression could be altered by a position effect (a different physical location), or abrogated by interruption of the coding sequence at one of the boundaries of the chromosomal rearrangement, or perhaps a new, deleterious mutation was introduced into the duplicated gene. Evidence in

favour of a gene dosage mechanism recently came to light when Lupski *et al.* (*Nature Genet.* 1, 29–33; 1992) reported decreased NCVs (reminiscent of CMT patients) in an individual with a partial trisomy of chromosome 17p, which included all of the CMT1A candidate region.

However, the impetus for the current flood of papers came less from studies in humans than from a mouse model for CMT called *Trembler* (*Tr*). *Tr* mice move awkwardly and suffer from seizures and tremors; at the cellular level they suffer from hypomyelination in the peripheral nervous system and continuing Schwann cell proliferation. In a recent paper in *Nature*, U. Suter *et al.* (**356**, 241–244; 1992) described a point mutation in *Tr* mice in the gene for peripheral myelin protein-22 (*pmp-22*) and have subsequently found a second allelic mutation (both occur in membrane-spanning regions of pmp-22) in *Trembler-J* (*Tr*^{J}) mice (*Proc. natn. Acad. Sci. U.S.A.* **89**, 4382–4386; 1992). Not only is *Trembler* a legitimate model for CMT on phenotypic grounds, but *Tr* maps to murine chromosome 11, in a region syntenic with human chromosome 17p (and the CMT1A locus). Taken together, the evidence was sufficiently persuasive that Suter *et al.* predicted that 'the human PMP-22 gene will be found on the proximal short arm of chromosome 17, identifying *PMP-22* as a candidate gene for the Charcot-Marie-Tooth disorder'.

Evidently the same thought occurred to several groups who have now confirmed that prediction and their findings are published in this issue of *Nature Genetics* (P.I. Patel *et al.* page 159; L.J. Valentijn *et al.* page 166; V. Timmerman *et al.* page 171; N. Matsunami *et al.* page 176). In each case, the groups have produced direct evidence that *PMP-22* maps to the duplicated region in CMT1A, using methods such as pulsed field gel electrophoresis, fluorescent *in situ* hybridization and dosage analysis. In particular, Patel *et al.* have cloned the human PMP-22 gene, noting strong homology with *pmp-22* and finding expression in the spinal cord and femoral nerve. *PMP-22* maps firmly in the middle of the duplication, again suggesting that overexpression of the gene is the cause, at least in part, of CMT1A.

Settling the issue may not be straightforward, however. The onset of disease symptoms differs markedly in *Tr* mice (caused by a point mutation) compared to humans (predominantly a duplication). It is possible that transgenic mice, the obvious means with which to test the dosage hypothesis, will not live long enough to manifest the disease, which in humans does not usually appear until the second decade. And what about other genes in the region, of which there could be be 30 or more? Some of these genes may contribute to the CMT phenotype as well as other disorders that map in close proximity to CMT1A (for example an REM sleep deficit associated with Smith-Magenis syndrome).

There is, however, a reasonable chance that formal proof of the *PMP-22* candidacy for CMT1A is at hand. There are a few CMT1A patients who do not appear to possess the DNA duplication, and like the *Tr* (*Tr*^{J}) mice, they may harbour a defect within the PMP-22 gene. *PMP-22* defects may also give rise to similar neurological disorders such as Roussy-Levy syndrome, which compounds CMT symptoms with tremor of the hands. Such studies are undoubtedly in progress. □

Pioneer proteins

Norrie disease is a rare but extremely severe neurodevelopmental disorder characterized by congenital blindness, mental retardation and progressive hearing loss. The locus maps to the short arm of the X chromosome which has revealed a multitude of genes that can give rise to hereditary blindness. One of these, choroideremia, was isolated by positional cloning almost two years ago, and now in this issue, two European groups report the isolation of a candidate gene for Norrie disease using a similar approach (W. Berger *et al.* page 199; Z-Y. Chen *et al.* page 204). Several Norrie patients are found to have deletions including or contained within the putative Norrie gene.

Unfortunately, for the moment, the predicted sequence has not proved particularly helpful in elucidating its likely function. The Norrie gene appears to encode a novel polypeptide—otherwise referred to as a 'pioneer protein' by some investigators—with no relatives in the databases. Not that discerning homology is necessarily that informative, but in this case there are virtually no clues from the predicted protein structure as to its normal role. The protein is small (133 amino acids), relatively polar and conserved in evolution. Indeed, study of a related gene in *Drosophila* may prove to be more fruitful. □