

The Charcot-Marie-Tooth diseases: how can we identify and develop novel therapeutic targets?

Genetic studies have provided significant insights towards understanding inherited neurological diseases. As the most common inherited neuromuscular disorder, Charcot-Marie-Tooth disease (CMT) is a prime example of how the identification during the past 20 years of underlying genetic mutations in patients and their families has led to the realization that we can no longer regard CMT as a single disease but rather a collection of hereditary peripheral neuropathies resulting from pathogenic mutations in more than 40 distinct genes (<http://www.molgen.ua.ac.be/CMTMutations/Mutations/MutByGene.cfm>). It is no surprise therefore that diagnostic whole-genome sequencing methods were first applied to patients with CMT (Lupski *et al.*, 2010; Montenegro *et al.*, 2011).

With this wealth of genetic information, a key question is whether all the pathogenic mutations associated with CMT lead to disease by mechanisms converging on a limited number of dysfunctional pathways, or, alternatively, does each genetic mutation lead to peripheral nerve degeneration by a distinct mechanism? The answer will have obvious implications as we attempt to develop effective treatments. Moreover, these questions highlight the fact that the identification of each genetic mutation is only the first step in deciphering molecular mechanisms that fail at the protein and cellular level in patients with CMT.

The classification of CMT into 'demyelinating' and 'axonal' forms, reflecting the presumed main site of pathology, i.e. the Schwann cell or axon, respectively, has proved useful in terms of targeted genetic testing (Reilly *et al.*, 2011). Although most genes associated with axonal CMT are yet to be identified, many of those mutated in the demyelinating forms are known. Because of the role of the Schwann cells in axon myelination, it is no surprise that among the genes identified as being associated with demyelinating CMT are components of the myelin sheath [e.g. peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), connexin-32 and periaxin], transcriptional regulators (e.g. EGR2 and SOX10) and proteins known to be involved in intracellular membrane trafficking (e.g. SH3TC2, MTMR2/MTMR13, FIG4, LITAF and NDRG1). The identification of a large number of genes encoding proteins that influence intracellular membrane trafficking underlies the critical importance of ensuring that cellular components are targeted to the correct intracellular destination, particularly in a structure such as the Schwann cell that needs to mobilize and regulate vast amounts of membrane and cargo during formation and maintenance of the insulating myelin sheath (Niemann *et al.*, 2006; Pereira *et al.*, 2012). Dysfunction can therefore result from the absence of a specific protein or lipid at the intended site, or the accumulation of mistargeted components within intracellular compartments. Furthermore, the canonical protective responses that cells have evolved to overcome cellular stresses caused by

mistargeting can, in certain circumstances, become paradoxically deleterious by activating pathways leading to programmed cell death.

One such protective but potentially deleterious cellular mechanism is the unfolded protein response (Wang and Kaufman, 2012). The unfolded protein response is activated when misfolded proteins are detected in the endoplasmic reticulum, the first organelle traversed by synthesized proteins *en route* to specific destinations. A number of pathogenic mutations in the myelin proteins PMP22 and MPZ can lead to their retention in the endoplasmic reticulum with resultant activation of the unfolded protein response. Saporta *et al.* (2012) recently reported in *Brain* the development of an *Mpz* R98C 'knock-in' mouse which mimics a form of CMT type 1B (CMT1B). The R98C mutation in *MPZ* has been associated with a severe early onset dysmyelinating CMT1B, and belongs to a group of severe congenital neuropathies previously referred to as Dejerine-Sottas disease. By being retained in the endoplasmic reticulum, expression of *Mpz* harbouring the R98C mutation in mouse Schwann cells leads to unfolded protein response activation.

Curcumin, the active component of the curry spice, turmeric, has been proposed to attenuate endoplasmic reticulum stress by a number of possible mechanisms. Previous examples of curcumin's potential therapeutic benefit include correcting the endoplasmic reticulum retention of the cystic fibrosis-related CFTR $\Delta F508$ ion channel (Egan *et al.*, 2004), truncated constructs of MPZ (Khajavi *et al.*, 2005) and PMP22-containing CMT1E-associated point mutations (Khajavi *et al.*, 2007). Furthermore, motor deficits in Trembler-J mice (harbouring CMT1E-associated point mutations in PMP22) improved when these mice were fed curcumin. Against this background, and based on their previous work in developing the *Mpz* R98C mouse models, Patzkó and colleagues describe in this issue the effects of giving oral curcumin to their mice and show promising results in terms of motor function, neurophysiological parameters and histological analysis when compared with untreated littermates. The beneficial effects appear to be associated with increased levels of promyelinating and decreased levels of demyelinating factors in treated mice, presumably maintaining Schwann cells in a myelinating state. Moreover, curcumin attenuates unfolded protein response activation, albeit without altering the endoplasmic reticulum retention of *Mpz* R98C.

In their manuscript, however, Patzkó *et al.* (2012) are careful to point out and discuss at length a number of important mechanistic issues that arise. First, the carrier in which curcumin was dissolved appeared to be critical for efficacy. The authors found that only curcumin or phosphatidylcurcumin dissolved in sesame oil led to beneficial effects when given to the mice, and,

surprisingly, sesame oil alone appeared also to improve motor and neurophysiological parameters, albeit without reaching statistical significance. These data were in contrast to previous studies showing beneficial effects of curcumin in the Trembler-J mice using other carriers such as Alimentum® (hypoallergenic milk powder) (Khajavi *et al.*, 2007), which had no effect in the *Mpz* R98C mice. Second, it is worth noting that curcumin treatment of *Mpz* R98C mice did not result in any histological or neurophysiological changes to suggest improved myelination of large axons, implying that other mechanisms convey the observed phenotypic benefit. Third, although this study provides evidence that the unfolded protein response activation seen in *Mpz* R98C mice can be attenuated by curcumin, the precise downstream effects are yet to be fully understood and appear to differ from those seen in the *Mpz* S63del mouse, which is also characterized by retention of mutant protein in the endoplasmic reticulum and subsequent unfolded protein response activation (Pennuto *et al.*, 2008).

There is no doubt that we are entering an era where therapeutic strategies for genetic diseases are being pursued. With respect to CMT, a number of promising targets are continually being identified and treatment strategies formulated based on data generated through basic scientific investigation (Garofalo *et al.*, 2011). However, translating these exciting findings into clinically proven treatments in patients remains a challenge (Pareyson *et al.*, 2011). It is almost certain that, if we are to develop effective treatment strategies, CMT will need to be considered as a collection of distinct genetic diseases manifesting as similar clinical syndromes, predominantly affecting peripheral nerves, rather than one clinical disease due to a number of underlying genetic mutations. With this in mind, it is sobering to consider that the R98C mutation in *MPZ* studied by Patzkó *et al.* (2012) is only one of over 120 different CMT-associated mutations in the *MPZ* gene, perhaps reflecting a wide variety of distinct underlying pathogenic cellular mechanisms, with retention of misfolded proteins in the endoplasmic reticulum and activation of the unfolded protein response relevant only in a small proportion of cases. As with the less prevalent G551D mutation in *CFTR* shown to be amenable to treatment with ivacaftor in patients with cystic fibrosis (Ramsey *et al.*, 2011), treatments for CMT are likely to have to be specific for each underlying mutation, even within the same gene.

Patzkó *et al.* (2012) show that the development of specific animal models of disease can prove informative in terms both of understanding pathology and testing treatment strategies. However, building on the advances made in identifying mutations in genes associated with CMT, it is imperative that we continue to invest resources in understanding the basic dysfunctional pathways implicated in each of these inherited peripheral neuropathies at the molecular and cellular level if novel and effective treatments are to become available. Alongside clinical observation, further genetic studies and the development of relevant animal models will eventually lead to the therapeutic breakthroughs in treating hitherto incurable heterogeneous genetic diseases such as CMT that patients and their families now expect in the 21st century.

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Rhys C. Roberts

Department of Clinical Neurosciences, Cambridge Institute for Medical Research, Cambridge, UK

Correspondence to: Rhys C. Roberts,
Department of Clinical Neurosciences,
Cambridge Institute for Medical Research,
Wellcome Trust/MRC Building,
Hills Road,
Cambridge CB2 0XY, UK
E-mail: rcr20@cam.ac.uk

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