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REVIEW ARTICLE

# Animal Models of Charcot-Marie-Tooth Disease Type 1A

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## Abstract

The most frequent genetic subtype of Charcot-Marie-Tooth disease is CMT1A, linked to chromosome 17p11.2. In the majority of cases, CMT1A is a gene dosage disease associated with a 1.5 Mb large genomic duplication. Transgenic models with extra copies of the *Pmp22* gene have provided formal proof that overexpression of only this candidate gene is sufficient to cause peripheral demyelination, onion bulb formation, secondary axonal loss, and progressive muscle atrophy, the pathological hallmarks of CMT1A. The transgenic CMT rat with about 1.6-fold PMP22 overexpression exhibits clinical abnormalities, such as reduced nerve conduction velocity and lower grip strength that mimick findings in CMT1A patients. Also transgenic mice, carrying yeast artifical chromosomes as *Pmp22* transgenes, demonstrate the variability of disease expression as a function of increased gene dosage. Recently, the first rational experimental therapies of CMT1A were tested, using transgenic animal models. In one proof-of-principle study with the CMT rat, a synthetic antagonist of the nuclear progesterone receptor was shown to reduce PMP22 overexpression and to ameliorate the clinical severity. In another study, administration of ascorbic acid, an essential factor of in vitro myelination, prolonged the survival and restored myelination of a dysmyelinated mouse model. Application of gene expression analysis to nerve biopsies that are readily available from such CMT1A animal models might identify additional pharmacological targets.

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**Index Entries:** Antiprogesterone; ascorbate; axon loss; CMT rat; demyelination; epigenetic factors; neuroprotection; onapristone; onion bulbs; PMP22; progesterone; transgenic CMT model; trembler; YAC.

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## Introduction

The normal function of peripheral nerves requires myelination of axons by Schwann cells. These highly specialized glial cells form myelin sheaths that facilitate the rapid impulse propagation along the axon. In recent years, numerous membrane proteins that are localized to myelin and axon–glia junctions have been molecularly cloned and functionally characterized (Nave and Suter, 2004; Sherman and Brophy, 2005). Many of these proteins could also be associated with inherited myelin diseases and neuropathies in humans, as discussed elsewhere in this issue.

Peripheral neuropathies are common neurological diseases in which peripheral nervous system function is impaired, owing to very different underlying causes. Diabetes and alcoholism cause the more frequent acquired neuropathies. Hereditary neuropathies (defined by single gene mutations) are less common. Hereditary motor and sensory neuropathies constitute a heterogeneous group of diseases, referred to as Charcot-Marie-Tooth (CMT) disease by geneticists. With the identification of numerous responsible disease genes significant progress has been made in understanding CMT disease mechanisms (Suter and Scherer, 2003). An updated list of CMT mutations can be found at www.molgen.ua.ac.be/CMTMutations/.

The main clinical feature of all forms of CMT is the symmetric and distally pronounced muscle weakness of the lower limb, and to a lesser extent of the upper limb, which slowly progresses proximally. Initially, the muscle atrophy affects the distal leg and intrinsic foot muscles, causing steppage gait and foot deformities. Sensory deficits are a common feature but less prominent (Shy et al., 2005).

In CMT type 1 (CMT1), a genetically heterogeneous subgroup, the primary genetic defect affects the myelin forming Schwann cell and causes abnormalities of the myelin sheaths. The loss of myelin (demyelination) leads to reduced nerve conduction velocity (NCV). The measurement of NCVs is indeed the most important diagnostic tool for the classification of CMT. It allows to differentiate between CMT1 patients, having a significantly reduced NCV (< 38 m/s), and CMT2 patients with a normal NCV (> 38 m/s). In the less frequent CMT2, the underlying mutations are thought to primarily affect neuronal/axonal functions (Harding and Thomas, 1980). We note, however, that some mutations of oligodendrocyte-specific genes cause axonal loss without a primary myelin pathology (Lappe-Siefke et al., 2003). Also, the degree of NCV reduction in CMT1 does not correlate with the clinical picture (Killian et al., 1996). Reduced NCV can be demonstrated before clinical handicaps become apparent, and can be used for the early diagnosis of CMT1 (Birouk et al., 1997). Electromyography (EMG) shows spontaneous activity as signs of chronic muscle denervation and axonal loss. In contrast to NCV, the severity of EMG alterations correlates with the clinical phenotype (Berciano et al., 2000; Krajewski et al., 2000). Thus, axonal loss and consecutive muscle atrophy and sensory loss are the clinically relevant changes in CMT1 (Lewis et al., 2003), whereas histologically, the prominent features are segmental demyelination and the formation of "onion bulbs." This pictorial term describes demyelinated axons that are surrounded by concentric layers of dedifferentiated Schwann-cells and their processes (Guenard et al., 1996).

Clinical symptoms in CMT are characterized by a high degree of interindividual variability. The age at which patients notice first symptoms ranges between 10 and 40 yr and also disease severity varies greatly, from asymptomatic patients to others who become wheel chair dependent (Shy et al., 2005). Magnetic resonance imaging of muscles (Gallardo et al., 2005) or nerves (Ellegalo et al., 2006) may be useful diagnostic tools in presymptmatic CMT1A patients. This clinical variability has even been described in identical twins (Garcia et al., 1995), and some of this variability is also seen in animal models.

## CMT1A: A Gene Dosage Disease

The most common inherited demyelinating neuropathy is CMT1A, accounting for about 60–70% of all CMT forms (Ionasescu, 1995). It is defined by linkage to chromosome 17p11.2-p12, and is caused in the majority of cases by a genomic duplication spanning of about $1.5 \times 10^6$ bp (Lupski et al., 1991; Raeymaekers et al., 1991). The duplication results from an unequal meiotic crossover mediated by highly homologous repeat sequences flanking the duplicated region. Contained in the duplicated region is the disease gene *Pmp22*, encoding a small myelin protein of 22 kDa in size (PMP22) (Matsunami et al., 1992; Patel et al., 1992; Timmerman et al., 1992; Valentijn et al., 1992 a,b). *Pmp22* was a particular attractive

candidate, as natural point mutations of this gene in Trembler mice underlie a severe peripheral neuropathy similar in severity to Dejerine-Sottas syndrome in humans (Suter et al., 1992 a,b; Tobler et al., 1999). PMP22 itself is a hydrophobic protein with four transmembrane domains located in compact myelin. In the adult nervous system, PMP22 is expressed by myelin-forming Schwann cells. There is also transient expression in embryonic motorneurons and outside the nervous system (Baechner et al., 1995). Null mutations of this gene cause peripheral myelin abnormalities, such as widespread tomacula, but do not interfere with myelination per se (Adlkofer et al., 1995). The biological function of this protein in myelin assembly or myelin preservation remains poorly understood (Suter and Scherer, 2003).

Patients with a homozygous genomic duplication (2.0-fold *Pmp22* gene dosage) have been described who suffer from an even aggravated disease (Lupski et al., 1991). That a predicted 1.5-fold increase in *Pmp22* copy number causes CMT1A, and that also *Pmp22* haplo-insufficiency (in patients with "hereditary neuropathy with liability to pressure pulses," HNPP) is not tolerated, poses a biological challenge to explain. The physical interaction between PMP22 and myelin protein P0 (MPZ) could play an important role if the altered ratio between these proteins destabilizes compact myelin (D'Urso et al., 1999; Hasse et al., 2004). It was also proposed that PMP22 overexpression is associated with abnormal intracellular sorting and toxicity of this protein. In transfected Schwann cells, PMP22 reaches late endosomes and might also form intracellular protein aggregates (aggresomes), that contain PMP22 associated with ubiquitin immunoreactivity (Notterpek et al., 1999; Fortun et al., 2005). These aggresomes were initially described as a general cellular response to an overload of misfolded proteins (Johnston et al., 1998). Thus, PMP22 overexpression may disturb Schwann cell function by unspecifically interfering with the intracellular sorting of other proteins, and possibly by overloading the protein degradation machinery.

## CMT1A Animal Models

The spontaneous mouse mutants Trembler and Trembler$^J$ both carry point mutations in the Pmp22 gene (Suter et al., 1992 a,b) and share pathological features with CMT1A (Henry et al., 1983). Indeed, the same mutation has been identified in mouse and in one human CMT1A family (Valentijn et al., 1992a). However, the subcellular consequences of PMP22 misfolding (in trembler mice) and PMP22 overexpression (in the majority of CMT1A cases) are different (Giambonini-Brugnoli et al., 2005), and in the context of this review we do not consider trembler mice as models for the human gene dosage disorder CMT1A. Overexpression of wild-type PMP22 can only be modeled by transgenic extra copies of the wildtype gene.

Transgenic animals are generated by injection of purified DNA restriction fragments or larger genomic fragments (such as yeast artificial chromosomes/YACs or bacterial artificial chromosomes/BACs) into fertilized oozytes, a standard procedure in mice, technically more demanding for rats and other species. Recombination of the cloned DNA into the host genom is random. Typically, the integration process creates multiple copies of smaller transgene fragments that are then found in a tandem head-to-tail orientation. For smaller transgenes, there is also a poor correlation between copy number and gene expression level, the latter strongly depending on positional effects. PMP22 transgenic animal models have been generated in both rats (Sereda et al., 1996, 2003) and mice (Huxley et al., 1996; Magyar et al., 1996; Huxley et al., 1998; Robertson et al., 2002). The CMT-like phenotype of these rodents was the first direct proof that in humans *Pmp22* is the responsible disease gene within the duplicated 1.5 Mb region. Collectively, these transgenic animals demonstrate that the clinical phenotype correlates well with the transcription of the *Pmp22* transgenes (rather than nominal transgene copy number). Rodents with moderately increased PMP22 mRNA provide models for the human disease CMT1A, whereas higher expression levels lead to severe manifestation of peripheral neuropathy, similar to Dejerine-Sottas syndrome or even congenital hypomyelination. An interesting "inducable" model of CMT1A has also been generated in which PMP22 overexpression was achieved under cell-type-specific control of a tetracyclin-activatable transactivator (Mansuy and Bujard, 2000). Mice in which PMP22 overexpression was first turned "on" and then "off" could demonstrate that the PMP22-dependent demyelination is reversible (Perea et al., 2001), a finding of principle interest for translational research aimed at reducing PMP22 overexpression

Table 1
CMT1A Animal Models

| Transgenic line | Pmp22 transgene | Copy number | PMP22 mRNA (fold wild-type) | Clinical phenotype | Nerve pathology | Survival time | Motor NCV (m/s) | Reference |
|---|---|---|---|---|---|---|---|---|
| **(1) CMT1A rat model** | | | | | | | | |
| CMT rat[a] | Mouse cosmid | 3 | 1.6 (1–4) | Gait abnormalities, slowly progressive muscle weakness | Demyelination, many onion bulbs | Normal | 14.7 (wt:36.4) | Sereda et al. (1996) also (13), (14) |
| (homo-zygous) | Mouse cosmid | 6 | (4–6) | Gait abnormalities, Rapid progression to complete paralysis | Amyelination | 1–8 mo | n.a. | (6), (12) |
| **(2) CMT1A mouse models** | | | | | | | | |
| C61[a] | Human YAC | 4 | 2 | No obvious phenotype, weak motor impairment at 2 mo (on testing) | Mild demyelination, basal laminal onion bulbs | Normal | 25 (wt: 38) | Huxley et al. (1998); also (3), (6), (10), (11) |
| C22 | Human YAC | 8 | 2.6 | Gait abnormalities, muscle weakness, paralysis at 6 mo | Demyelination, basal laminal onion bulbs | 2–10 mo (average 6 mo) | 4 (wt: 38) | (1), (2), (6), (7), (8), (10), 11) |
| TgN248 | Mouse cosmid | 16 | 2 | Shivering at 2 wk , paralysis of hindlimbs | Dysmyelination | Exceeding 8 mo | 2 (wt: 41) | (4) |
| My41 | Mouse YAC | n.a. | n.a. | Gait abnormalities, progression to severe weakness | Dysmyelination | 4–5 mo | n.a. | (11) |
| JP18 (JY13[a]) | Inducable Mouse cDNA | n.a. | n.a. | Slightly abnormal gait | Demyelination | n.a. | 34.5 (wt: 44.2) | (9) |

[a]Denotes a clinically relevant disease model of human CMT1A.

n.a., not analyzed; NCV, nerve conduction velocity; wt, age-matched wildtype controls; mo, months; wk, weeks.

References: 1) Huxley et al., 1996; 2) Huxley et al., 1998; 3) Kobsar et al., 2005; 4) Magyar et al., 1996; 5) Niemann et al., 2000; 6) Norreel et al., 2001; 7) Norreel et al., 2003; 8) Passage et al., 2004; 9) Perea et al., 2001; 10) Robertson et al., 1999; 11) Robertson et al., 2002; 12) Sereda et al., 1996; 13) Sereda et al., 2003; 14) Grandis et al., 2004.

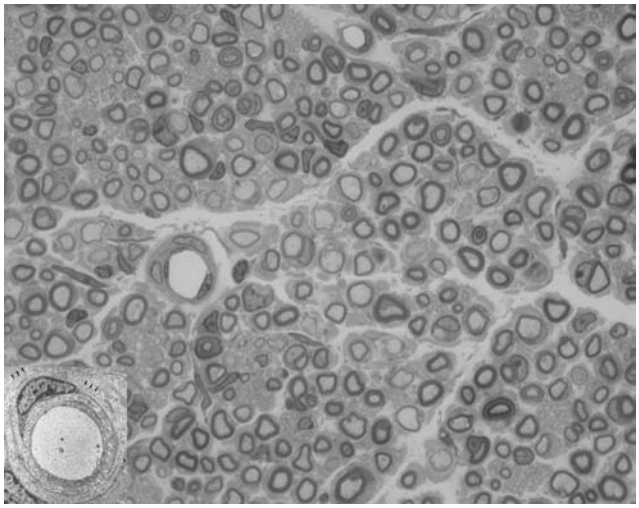

Fig. 1. Sciatic nerve neuropathology of an adult CMT rat. Histological section of a 7-wk-old PMP22 transgenic rat, stained with methylen-blue. Numerous hypomyelinated and demyelinated axons are revealed next to apparently regular myelin profiles. Onion bulbs that consist of demyelinated axons, surrounded by concentric layers of Schwann cell membrane, and redundant basal lamina are best depicted by electron microscopy (small insert with onion bulb from a 6-mo-old CMT rat taken from Sereda et al., [1996] with permission from Elsevier).

pharmacologically (*see* "Testing Experimental Therapies in CMT1A Disease Models"). An overview of published CMT1A models is given in Table 1.

## PMP22-Transgenic "CMT Rats"

The CMT rat and first animal model of CMT1A was generated by pronuclear injection of a cosmid-derived 43 kb DNA fragment, harboring the entire murine *Pmp22* gene (Sereda et al., 1996). The same construct was used also for the generation of a transgenic mouse model (Magyar et al., 1996). In the transgenic rat, approx 3 additional copies of the genomic fragment are autosomally transmitted. However, the degree of transcriptional *Pmp22* overexpression is only 1.6-fold, when quantified by real-time RT-PCR for a large number of independent sciatic nerve preparations (Sereda et al., 2003).

All PMP22 transgenic rats show signs of dysmyelination and demyelination in peripheral (Fig. 1) and cranial nerves, with histological and physiological changes preceding the overt clinical symptoms (Sereda et al., 1996; Grandis et al., 2004). "Onion-bulbs" can also be detected at the age of 2.5 mo on histological sections that are more extensive in older animals. In agreement with the clinical phenotype (an unsteady gait of the rats that move clumsily, with feet spread outward), motor fibers are more severely affected than sensory fibers. Also, large caliber axons are more severely hypomyelinated than small caliber fibers (Sereda et al., 1996). It is possible to clinically score the animals with respect to motor impairment, similar to the MRC clinical score of muscle strength. Using a simple "bar test" (measuring holding time on a 2.5 cm thick horizontal bar) to quantify motor performance in wild-type and PMP22-transgenic rats, a clinical phenotype was obvious at about 3 wk of age. In a more detailed behavioral profiling at later timepoints ("rotarod," grip strength analysis) also subtle differences in motor performance could be recorded and yielded clinical scores for animals that were more or less severely affected (Fig. 2). This clinical variability is not fully understood but is also well documented for CMT1A patients. There is no correlation to the development of anti-PMP22 antibodies in the sera of some rats (Sereda and Kan, 2006). We note that the original rat strain (Sprague-Dawley), used for transgenesis, was genetically outbred, leaving room for the activity of modifier genes and unknown epigenetic factors.

The CMT rat appears well suited to be a CMT1A model, also from a clinical point of view. Electrophysiologically, adult CMT rats exhibit a slowing of motor nerve conduction velocity (14.7 m/s compared to 36.4 m/s in the wild-type controls) with values similar to those of CMT1A patients, i.e., less than 50%. After sciatic nerve stimulation, compound muscle action potentials show reduced amplitudes and desynchronization. Although degenerating fibers are difficult to visualize microscopically, axonal loss could be confirmed by direct histological counts (Sereda et al., 2003). By electromyographic (EMG) recordings, spontaneous muscle fiber activity has been taken as an indirect sign of denervation (Sereda et al., 1996). Axonal loss and distally pronounced muscle atrophy thus match the human CMT1A symptoms.

At the mRNA level, an average 1.6-fold overexpression of PMP22 in heterozygous transgenic rats correlates with the clinical phenotype. However, it appears that minor differences of transcriptional PMP22 overexpression can have significant

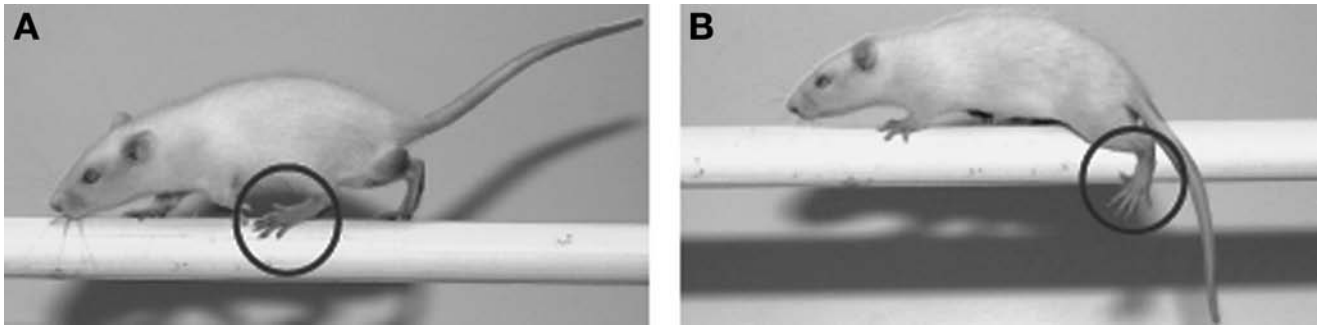


Fig. 2. Interindividual differences in the phenotype of CMT rats. In PMP22-transgenic rats (age 2 mo), there is significant interindividual variability of clinical symptoms, ranging from mild weekness to severe motor impairment. When placed on a horizontal bar, some animals hold on and move across the bar **(A)**, whereas other rats defined by the same transgene lack the necessary grip strength **(B)**. All animals show some motor impairment, however, which is due to axonal loss. Understanding the underlying epigenetic factors may help to develop neuroprotective treatment strategies.

impact on the development and course of disease. A putative threshold level of PMP22 overexpression (at which the wildtype gene turns into a disease gene) is an obvious "target" to reach by therapeutic approaches aimed at reducing PMP22 gene expression (*see* "Antiprogesterone").

A biochemical analysis of CMT rats led to the "paradoxical" finding of elevated PMP22 expression at the transcriptional level in contrast to an apparent decrease of PMP22 at the protein level, when monitored by Western blotting of sciatic nerve extracts (Sereda et al., 1996). This is most likely explained by the overall reduction of myelin in the adult nerve. In fact, when sciatic nerve myelin is purified by sucrose gradient centrifugation, CMT rats exhibit a higher content of PMP22 than age-matched controls (Sereda and Nave, 2006).

When *Pmp22* transgenic rats were bred to homozygosity, PMP22 mRNA expression increased accordingly, causing a dramatic increase of clinical severity. Animals never developed a normal gait and were largely paralyzed at 3–4 wk of age (Niemann et al., 2000). Homozygous animals died prematurely, most probably as a result of respiratory insufficiency, as the peripheral nervous system was virtually amyelinated. At the ultra-structural level, Schwann cells were able to single-out individual axons, but appeared "arrested" at this promyelin stage. The failure to assemble myelin contrasted with an ongoing differentiation at the molecular level, which included the transcription of myelin genes (Niemann et al., 2000). Similar clinical phenotypes have been reported for transgenic mice overexpressing PMP22 at higher levels.

The CMT rat has been used as a readily available tissue source for experiments hardly possible with human biopsy samples. These include primary cocultures of CMT rat Schwann cells and in vitro myelination studies by Schenone and colleagues (Nobbio et al., 2001), transcriptome analysis of CMT nerves (Vigo et al., 2005), as well as in vivo proliferation analysis of CMT Schwann cells (Atanasoski et al., 2002). A proteome analysis of purified myelin from CMT rats is currently underway (Stassart, Meyer zu Hörste, Sereda, and Nave, 2006). The CMT rat was also taken as a model for an experimental CMT1A therapy with antiprogesterone (*see* below).

## PMP22-Transgenic Mice

For the generation of transgenic mice, Magyar et al. used a 43 kb cosmid-derived DNA fragment with the murine *Pmp22* gene, and obtained lines with 16 (TgN248) and 30 transgene copies (TgN247 and TgN249), respectively (Magyar et al., 1996). These mice exhibited a very similar and severe dysmyelinated phenotype. At the mRNA level, PMP22 was twofold upregulated, but only when "normalized" to P0 mRNA, as both messages were reduced in sciatic nerve RNA. This is in contrast to the elevated expression of PMP22 mRNA in the CMT rat (Sereda et al., 1996) and suggests that at higher *Pmp22* gene dosage in the mouse, transgenic Schwann cells are

developmentally dysregulated. Moreover, myelin was absent from the majority of axons in histological sections, yet most Schwann cells were associated 1:1 with these axons at the promyelin stage. This degree of dysmyelination is very similar to that of homozygous CMT rats (Niemann et al., 2000), confirming that a threshold level of PMP22 overexpression suffices to completely block myelination. Instead, these Schwann cells remain immature, with reduced overall myelin gene expression and elevated proliferation. Classical "onion bulbs" were not a feature. Muscle histology showed abnormal fiber-type grouping and signs of myotubular degeneration at older age. Thus, TgN248 mice confirm the gene dosage hypothesis for CMT1A but are models for the more severe Dejerine-Sottas syndrome.

Independently, Huxley and colleagues generated several lines of *Pmp22* transgenic mice by pronuclear injection of a YAC containing the 40 kb human *Pmp22* gene, including 100 kb of 5′ flanking and 300 kb of 3′ flanking sequence (Huxley et al., 1996, 1998). One line (C22), with about eight YAC copies, had a strong behavioural phenotype at 3 wk of age, followed by progressive hind-limb weakness. Histologically, there was pronounced absence of myelin and occasional "basal laminal onion bulbs" (King, 1999) both of which were thought to result from demyelination and remyelination. *Pmp22* overexpression was estimated to be 2.7-fold at the RNA level. C22 mice were used later for an experimental therapy with ascorbate (*see* "Ascorbate").

Another line from the same series of YAC-transgenic mice (C61) harbors four additional copies of the human *Pmp22* gene, and exhibits a lower degree of PMP22 overexpression. These mice show no major signs of motor impairment. However, when analyzed in more detail, younger C61 mice (age 2 mo) revealed reduced muscle strength and deficits in motor coordination and balancing (Norreel et al., 2001). Histologically, the development of peripheral myelin was initially normal, but followed by demyelination, mainly of larger caliber axons, at approximately 4 wk of age (Robertson et al., 1999, 2002). Electron microscopy of sciatic nerves taken from 14 wk old mice revealed demyelinated and thinly remyelinated axons, supernumerary Schwann cells, as well as hypermyelinated small caliber axons, a pattern very similar to the morphological description of CMT rats. Electrophysiologically, NCV were reduced (Huxley et al., 1998) and F-wave latencies measured in the sciatic nerve were prolonged compared with wild-type controls, confirming another clinical feature of CMT1A (Kobsar et al., 2005). The latter report also demonstrated a peculiar involvement of invading B and T cells, as seen before in other mice with demyelinating neuropathies. Taken together, the C61 line represents a useful mouse model for human CMT1A.

## Testing Experimental Therapies in CMT1A Disease Models

CMT1A is an incurable disease and rational treatment strategies are desperately needed. So far no treatment strategy has reached the stage of a clinical trial in humans (Shy, 2004; Grandis and Shy, 2005), one reason being that safety concerns are very high for treating a nonlethal disease, such as neuropathy. Thus, any proof-of-principle trial in CMT1A would benefit from the availability of a bona fide animal model. Recently, two such trials have been published that made use of the CMT rat and mouse model, respectively (Sereda et al., 2003; Passage et al., 2004).

## Antiprogesterone

The steroid hormone progesterone stimulates transcription of the endogenous *Pmp22* gene (Desarnaud et al., 1998; Melcangi et al., 1999), suggesting that the nuclear progesterone receptor is a possible target to modulate *Pmp22* expression and therefore the CMT1A phenotype. In a proof-of-principle study, aimed at reducing total Pmp22 expression in the CMT rat model, a selective progesterone receptor antagonist (Onapristone) was tested for its ability to reduce PMP22 overexpression (Sereda et al., 2003). Indeed, the non-agonistic (type II) progesterone antagonist reduced transcriptional overexpression by about 15%, thereby preventing a significant fraction (30%) of the axonal loss and improving the clinical neuropathy phenotype after 7 wk of treatment. In contrast, the agonist progesterone further increased PMP22 overexpression and accelerated CMT disease progression. Ongoing experiments demonstrate effectiveness for longer (5 mo) treatment duration (Meyer, zu Hörste, Prukop, Nave, and Sereda, 2006). These results confirm that

*Pmp22* transcription is coregulated by the nuclear progesterone receptor in vivo, and that antiprogesterones (which were primarily developed for breast cancer patients) are candidate drugs to be tested in duplication patients with CMT1A (Sereda et al., 2003; Shy, 2004; Grandis and Shy, 2005). Progesterone-containing drugs, however, might enhance CMT symptoms and should be avoided in this patient group.

## Ascorbate

Ascorbic acid (vitamin C) is required for collagen synthesis and basal lamina formation (Carey and Todd, 1987; Podratz et al., 2001), and is necessary to induce myelin formation in neuron–Schwann cell cocultures (Podratz et al., 2004). Passage et al. (2004) tested the hypothesis that a high dose of ascorbic acid treatment alters the course of disease in the more severely dysmyelinated CMT1A mouse model (strain C22 of Huxley et al., 1996; Norreel et al., 2003). Following a 3 mo treatment with weekly ascorbate force-feeding (57 mg/kg), *Pmp22* transgenic mice and controls were behaviorally tested and histologically analyzed. The authors report a nearly fourfold increase of myelinated axons (or 80% of normal), major improvements in motor performance, and the normalization of average life expectancy from 5 mo (untreated mutants) to 20 mo (ascorbate treated). Although the molecular mechanism underlying some of these findings may have to be clarified (such as the effect of ascorbic acid on peripheral myelin gene expression), the study is attractive because ascorbate is not expensive and clinically safe. Because vitamin C is an over-the-counter drug, a clinical trial in human CMT patients is considered for the near future.

## Neuroprotection

Axonal loss marks the final common pathway of pathology in CMT1A and most other forms of neuropathy, both genetic and acquired forms. Genetic models that mimic human patients well lend themselves to experimental treatment studies. They exhibit a more "reproducable" phenotype (in comparison with most acquired neuropathies) and fewer interactions with other aspects of the disease (such as in diabetic neuropathy). Recently, neuroprotection mediated by erythropoietin, a hematopoetic growth factor, has been identified as a promising new strategy to overcome neuronal and axonal loss in neurological disease, including stroke (Siren et al., 2001; Ehrenreich et al., 2004). Indeed, attenuated axonal loss has been described in cell culture models of HIV-induced neuropathy (Keswani et al., 2004). The application of erythropoietin as a neuroprotective drug to prevent axonal loss in the CMT rat is currently under investigation (Prukop, Ehrenreich, Sereda, and Nave, 2006).

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