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Regulating PMP22 Expression as a Dosage Sensitive Neuropathy Gene

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Abstract

Structural variation in the human genome has emerged as a major cause of disease as genomic data have accumulated. One of the most common structural variants associated with human disease causes the heritable neuropathy known as Charcot-Marie-Tooth (CMT) disease type 1A. This 1.4 Mb duplication causes nearly half of the CMT cases that are genetically diagnosed. The PMP22 gene is highly induced in Schwann cells during development, although its precise role in myelin formation and homeostasis is still under active investigation. The PMP22 gene can be considered as a nucleoprotein complex with enzymatic activity to produce the PMP22 transcript, and the complex is allosterically regulated by transcription factors that respond to intracellular signals and epigenomic modifications. The control of PMP22 transcript levels has been one of the major therapeutic targets of therapy development, and this review summarizes those approaches as well as efforts to characterize the regulation of the PMP22 gene.

Copy Number Variants in the PMP22 Locus and Charcot-Marie-Tooth Disease

There are >100 genes that are mutated in CMT or syndromic conditions involving peripheral neuropathy (Rossor et al., 2017). However, the most common subtype of CMT, called CMT Type 1A (CMT1A), accounting for 40-50% of genetically diagnosed CMT cases results from a 1.4 Mb duplication on chromosome 17, which includes the myelin gene Peripheral Myelin Protein 22 (*PMP22*) (Lupski et al., 1991; Patel et al., 1992; Raeymaekers et al., 1991; Snipes et al., 1992). The reciprocal 1.4 Mb deletion is also linked to a second distinct disease (see Figure 1), Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) (Chance et al., 1993). Transgenic studies linked aberrant *Pmp22* expression to peripheral

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neuropathy in rodent models of CMT1A (Huxley et al., 1996; Magyar et al., 1996; Sereda et al., 1996) and studies of knockout alleles revealed symptoms in the *Pmp22* heterozygotes that resemble aspects of HNPP (Adlkofer et al., 1995; Adlkofer et al., 1997; Guo et al., 2014). Furthermore, reduction of the *Pmp22* transcript has been shown to ameliorate the symptoms of neuropathy in rodent models of *Pmp22* overexpression (Passage et al., 2004; Perea et al., 2001; Sereda et al., 2003; Zhao et al., 2018), indicating that transcriptional regulation is a valid target of therapies for CMT1A.

Pathomechanisms Caused by the CMT1A Duplication

The development of rodent models of CMT1A have enabled studies of how Pmp22 overexpression causes neuropathy. Some of the mechanisms are thought to be downstream of the PMP22 duplication, such as formation of PMP22 aggregates (Fortun et al., 2003), imbalanced ERK/AKT kinase regulation (Fledrich et al., 2014), altered lipid metabolism (Fledrich et al., 2018), and elevated calcium through P2X7 channels (Nobbio et al., 2009). Despite a number of significant advances, several issues have been raised regarding the validity of rodent models (Jouaud et al., 2019; Li, 2017). One important point is that transgenic insertion of even the large genomic clone in the C3, C61, and C22 models (Huxley et al., 1998) likely does not recapitulate all of the genomic/epigenomic effects of the tandem duplication of the 1.4 Mb segment on chromosome 17. For example, there are other transcripts (including microRNAs or long noncoding RNAs) within the interval, which could play a role. In this respect, it is thought that studies of CMT1A-derived induced pluripotent stem cells could provide a superior model (Juneja et al., 2019; Mukherjee-Clavin et al., 2019), although challenges remain in obtaining fully differentiated Schwann cells.

Recently, an alternate hypothesis for CMT1A has been presented, in which the chromosome 17 duplication causes destabilized PMP22 transcription due to disruption of long range intra- and inter-chromosomal interactions (Li, 2017). It is possible to address the hypothesis that the duplication per se causes CMT1A rather than gene dosage, since patients have been analyzed that have a co-occurring HNPP-associated deletion on one copy of chromosome 17 and the CMT1A-associated duplication on the other copy of chromosome 17 (Hirt et al., 2015). These patients therefore have the correct gene dosage but still have the duplication, and the absence of the typical CMT1A neuropathy symptoms strongly suggests that gene dosage is the critical factor.

Analysis of rodent models do not clearly show overexpression of PMP22 protein, and in fact some report decreased levels of PMP22 protein, although this could be due to the lack of PMP22 stabilization that occurs when it is not efficiently incorporated into healthy myelin (Pareek et al., 1997). Transcript measurements do show elevated *Pmp22* mRNA in animal models, but this is also dependent upon the stage at which it is measured (Fledrich et al., 2014). In human samples, immuno-EM studies have shown elevated levels of PMP22 protein in the myelin of dermal nerves in CMT1A skin biopsies, although these levels are quite variable and do not correlate with the severity of symptoms (Katona et al., 2009). Other quantitative RT-PCR studies in human skin samples have not shown a clear elevation of *PMP22* transcript in CMT1A compared to controls (Nobbio et al., 2014). As detailed below, reduction of *PMP22* transcript levels in CMT1A animal models has been successful

in resolving the neuropathy, but the therapeutic validity of lowering PMP22 in human CMT1A has not yet been demonstrated.

PMP22 Gene Regulation

PMP22 is most highly expressed in peripheral nerve, and its transcripts are among the most abundantly expressed genes in mature Schwann cells after a dramatic induction during myelination. Initial studies of *Pmp22* regulation had focused on *Pmp22* promoters (Suter et al., 1994). The two major promoters P1 and P2 drive expression of two alternate noncoding exons (1A or 1B respectively) and these transcripts are approximately in 3:1 ratio in rodents and 1:1 ratio in human Schwann cells (as recently confirmed in gtexportal.org). The P1 promoter is expressed exclusively in myelinating Schwann cells, although both P1 and P2 transcripts are induced during myelination. In other tissues where Pmp22 is expressed at a lower level, the P2 promoter is the major transcription start site. Subsequent transgenic analysis identified an enhancer region upstream of the P1 promoter (Maier et al., 2002; Maier et al., 2003), known as the late myelinating Schwann cell enhancer (LMSE). However, neither the promoters nor the LMSE could recapitulate the large developmental induction of *Pmp22* in transgenic assays.

To perform a more comprehensive analysis of *Pmp22* regulation, chromatin immunoprecipitation (ChIP) techniques were used to map binding sites of EGR2 and SOX10 (Jones et al., 2011; Srinivasan et al., 2012), which are required for myelination and high level expression of PMP22. Peripheral nerve is an excellent substrate for ChIP analysis: the sciatic nerve contains no neuronal nuclei, and is highly enriched in Schwann cells, which selectively express *Pmp22*. Most Schwann cells, moreover, are myelinating, whereas the remaining nonmyelinating Remak Schwann cells do not express high levels of myelin genes. Our analysis identified binding sites for EGR2 and SOX10 within an intronic regulatory element in *Pmp22.* The intronic site responds to EGR2 and SOX10 activity in transient transfection assays, and also drives tissue-specific expression to peripheral nerve in mouse transgenic assays (Jones et al., 2011).

Extension of this approach with ChIP-seq identified a super-enhancer domain upstream of *Pmp22* (Jones et al., 2012; Lopez-Anido et al., 2016; Pantera et al., 2018). Super-enhancers are large genomic regions containing multiple enhancer elements (Whyte et al., 2013). The enhancers within these domains display hallmarks of enhancer status and activity (e.g. the presence of the enhancer-associated histone mark H3K27 acetylation, H3K27ac) at levels greater than those observed at typical enhancers. The major elements of this super-enhancer (labeled A, B, C in Figure 2) have several characteristics of Schwann cell-specific enhancer elements (Jones et al., 2012). First, all three show binding of EGR2 and/or SOX10 and have conserved consensus binding sites for these factors. Second, the three genomic segments show EGR2- and/or SOX10-dependent activity in reporter assays. Third, chromatin structure analysis of the *Pmp22* locus revealed that regions A, B, and C bear chromatin modifications typically found in regulatory regions (Hung et al., 2015; Lopez-Anido et al., 2016), such as H3K27 acetylation. This H3K27ac signature is lost at these elements following nerve injury, an event that coincides with loss of *Pmp22* transcription (Figure 2) (Hung et al., 2015; Welcher et al., 1991). Interestingly, Pmp22 is much more highly expressed in Schwann cells

compared to oligodendrocytes, and Sox10 binding and H3K27ac modifications in the super enhancer are present in peripheral nerve but not in ChIP assays of spinal cord oligodendrocytes (Lopez-Anido et al., 2015). Finally, deletion of this super-enhancer in the S16 Schwann cell line significantly reduced *Pmp22* transcription in these cells, with a larger impact on transcription from the Schwann cell-specific P1 promoter (Pantera et al., 2018).

The regulatory elements identified thus far are highly conserved between rodents and humans. Interestingly, the orthologous human super-enhancer resides within two independent duplications (of <200 kb) identified by copy number variation (CNV) analysis of patients with a mild form of CMT (Weterman et al., 2010; Zhang et al., 2010). In these patients, only the super-enhancer is duplicated, not the *PMP22* gene itself. The two duplicated regions overlap by 168kb and encompass not only the super-enhancer, but also the intervening *TEKT3* gene, which is primarily expressed in testis. The *Tekt3* gene is inactive in Schwann cells (Jones et al., 2012), and therefore is not likely the target of these enhancers. Our data are consistent with the possibility that the upstream duplications alter *PMP22* regulation by inserting an extra set of enhancers upstream of the gene, stimulating increased expression of the gene. The upstream super-enhancer is not absolutely required for *Pmp22* expression, since genomic clones of *Pmp22* lacking this domain have been used to make transgenic models of CMT1A (Sereda et al., 1996; Sereda and Nave, 2006). However, the lack of the upstream cluster in these genomic clones may explain why several copies (>3) of the locus must be inserted in order to significantly increase *PMP22* mRNA levels.

Since *Pmp22* regulation appears to involve several enhancers, several of which are remote, we used genome editing to embed reporters in the endogenous *Pmp22* locus, so that reporters would reflect the long range enhancers and also epigenetic regulation (e.g. chromatin structure, microRNA regulation) of the *Pmp22* gene. We used TALEN-mediated recombination to insert reporters in the S16 rat Schwann cell line as a fusion within the endogenous *Pmp22* gene with an intervening 2a self-cleaving sequence so that reporter protein(s) are not fused to PMP22 (Dranchak et al., 2018; Inglese et al., 2014; Jang et al., 2012). The S16 Schwann cell line is unique among Schwann cell lines in having a high level of PMP22 expression, which is comparable to that found *in vivo* (Hai et al., 2002), and our ChIP studies have consistently found transcription factor binding patterns are similar in S16 cells and rat sciatic nerve (Jones et al., 2011; Jones et al., 2012; Lopez-Anido et al., 2016; Srinivasan et al., 2012).

As one of the first examples of a drug screen employing a genome edited reporter, this approach was able to identify candidate compounds that were not identified using single *Pmp22* enhancer constructs (Dranchak et al., 2018; Inglese et al., 2014; Jang et al., 2012). These studies identified PKC activators and HDAC inhibitors as regulators of *Pmp22* expression; HDAC 1/2 inhibitor romidepsin was a particularly potent inhibitor of expression. Other studies showed that while HDAC1/2 inhibitors could reduce *Pmp22* expression, HDAC8 inhibition had no effect and HDAC3 inhibition increases expression of several myelin genes including *Pmp22,* demonstrating that specific HDAC subtypes are involved (He et al., 2018). Histone deacetylases often work within large complexes, and we had previously found a reduction of *Pmp22* mRNA and protein levels in mice with a Schwann

cell-specific deletion of the CHD4 subunit of the NuRD (nucleosome remodeling and deacetylase) complex that also contains HDAC1/2 (Hung et al., 2012).

Other lines of evidence have highlighted the growth-regulating Hippo signaling pathway as an important regulator of Schwann cell differentiation and myelin gene expression (Deng et al., 2017; Lopez-Anido et al., 2016; Poitelon et al., 2016). This pathway features a kinase cascade that, when active, inhibits nuclear localization and transcriptional activity of its terminal effectors, the co-activators YAP and TAZ. The YAP/TAZ complex, along with other coactivators including the TEAD family of transcription factors, drive expression of genes involved in cell growth and proliferation (Chen et al., 2010; Deng et al., 2017; Zhao et al., 2010). Following the discovery that TEAD binding motifs were enriched in peripheral nerve SOX10-bound enhancers (Lopez-Anido et al., 2015), including enhancers associated with *Pmp22,* we examined whether TEAD1 might be an important regulator of *Pmp22* transcription (Lopez-Anido et al., 2016). Our analysis in primary rat Schwann cells and the S16 cell line showed that siRNA-mediated knockdown of *Tead1* reduces *Pmp22* transcription, as does loss of the YAP/TAZ co-activators *in vivo.* Furthermore, ChIP assays detected TEAD1 binding at multiple *Pmp22* enhancers, including the intronic element and the B and C elements within the distal super-enhancer, both in the S16 cell line and in peripheral nerve. Mutational analyses of luciferase constructs bearing the sequences of the B, C, or intronic elements suggested that many of the TEAD binding sites within these elements are important for activation of those enhancers. Similarly, examination of TAZ ChIP-seq data (Figure 2) generated from purified newborn rat Schwann cells (Deng et al., 2017) indicated TAZ binding at the enhancers previously shown to bind TEAD1 (Lopez-Anido et al., 2016). Together, these data point to YAP/TAZ and TEAD1 being important direct regulators of *Pmp22* expression. Other potential direct regulators of PMP22 expression are the LXR transcription factors, which are proposed to mediate the effects of oxysterols on myelin genes (Makoukji et al., 2011).

In addition to transcriptional regulation, transcript levels are also subject to post-transcriptional regulation through microRNAs (Gokey et al., 2012; Svaren, 2014), and several studies have identified microRNAs that appear to modulate *Pmp22* expression. One of the earliest analyses of microRNAs identified miR-29a as a modulator of *Pmp22* levels (Verrier et al., 2009). PMP22 levels decrease after nerve injury, which correlates with increased levels of miR29a (Arthur-Farraj et al., 2017; Verrier et al., 2009; Viader et al., 2011). Another microRNA, miR-381, was identified as being down in the C22 mouse model of CMT1A, and was subsequently found to reduce *PMP22* RNA and protein levels in transfection experiments. Intraneural injection of lentivirus expressing miR381 resulted in downregulation of PMP22 protein and improved myelination in the C22 model (Lee et al., 2019).

Modifiers of CMT1A

Recent studies have begun to identify modifier loci that modulate the severity of CMT1A. For example, polymorphisms in regulatory elements of the *SH3TC2* gene—which is itself mutated in CMT4C--were shown to correlate with various parameters in a carefully phenotyped cohort of ~400 CMT1A patients (Brewer et al., 2014). Some modifier genes

could directly modulate the levels of *PMP22* transcripts. One potential microRNA modulator of *PMP22* was identified through genetic analysis of a polymorphism in miR149 that correlated with age of onset and severity of CMT1A patients. Analysis of the polymorphism in miR149 suggested that it may affect its ability to repress *PMP22* and possibly other myelin genes (Nam et al., 2018). In addition, a study of genetic modifiers in 907 CMT1A patients identified several polymorphisms in the *SIPA1L2* gene that correlate with foot dorsiflexion weakness (Tao et al., 2019). The function of this gene is unknown, although the encoded protein has predicted GTPase domains and was shown to interact with actin and MYH9. Interestingly, *SIPA1l2* is itself regulated by SOX10 and EGR2, and siRNA-mediated depletion of the *SIPA1L2* gene in the S16 Schwann cell line resulted in loss of PMP22 expression along with other myelin genes.

Therapeutic Agents that Reduce *PMP22* Expression.

Ascorbic acid.

Based on its important role in promoting *in vitro* myelination (Eldridge et al., 1987), ascorbic acid (AA) was evaluated as a potential therapy to restore myelination in the C22 mouse model. In these mice, high-dose AA administration at 57 mg/kg suppressed the human *PMP22* transcript, improved locomotor function, and partially rescued defects in myelination (Passage et al., 2004). A follow-up study suggested that this AA-induced suppression of the *PMP22* gene was mediated by inhibition of adenylate cyclase and reduction of cyclic AMP levels in Schwann cells (Kaya et al., 2007). However, several clinical trials in which patients were given AA over one or two years did not find evidence that AA is effective at ameliorating neuropathy in CMT1A patients (Gess et al., 2015; Lewis et al., 2013; Pareyson et al., 2011).

Progesterone antagonists.

Following the discovery that progesterone and progesterone derivatives could activate the Schwann cell-specific P1 promoter and drive transcription of *Pmp22* in rats (Desarnaud et al., 1998; Melcangi et al., 1999), the progesterone/glucocorticoid receptor antagonist onapristone was evaluated for its potential as a therapeutic in CMT1A rats (Sereda et al., 2003). Administration of 20 mg/kg onapristone from P5 to seven weeks of age led to specific down-regulation of the *Pmp22* gene, while CMT1A rats administered the same dose of progesterone displayed elevated transcription of both *Pmp22* and *Mpz.* Onapristone-treated rats displayed improved motor performance, an increase in the number of axons, and proportion of myelinated axons compared to rats given progesterone. A follow-up study evaluating the effects of longer-term onapristone treatment in older CMT1A rats found similar beneficial outcomes (Meyer zu Horste et al., 2007). Rats treated with onapristone at 20 mg/kg/day from 5 weeks to 26 weeks of age showed improved CMAP amplitudes and reduction in loss of large-caliber axons, and skin biopsies from rats at 26 weeks showed reduction in *Pmp22* mRNA compared to rats on placebo (Meyer zu Horste et al., 2007). Despite these outcomes, concerns over toxicity of current progesterone antagonists have precluded trials in CMT1A patients, and therefore the efficacy of progesterone receptor inhibition as a CMT1A therapy is still unknown. Interestingly, no differences in overall severity have been found between men and women with CMT1A (Fridman et al., 2014;

Swan et al., 2007). Mechanistically, it is not known if progesterone (and/or glucocorticoids) would regulate Pmp22 through direct binding of its receptor to the PMP22 gene, or rather through regulation of EGR2/SOX10 (Magnaghi et al., 2007).

PXT3003.

A small molecule therapy in clinical trials for CMT1A is a cocktail of three approved drugs: the GABAB agonist baclofen, the opioid receptor antagonist naltrexone, and D-sorbitol. These drugs were identified in a systems biology screen intended to single out compounds capable of acting on different G protein-coupled receptors to influence a wide swath of signaling pathways upstream of myelin gene regulation (Chumakov et al., 2014). While all three individual drugs proved capable of improving myelination in DRG co-cultures from CMT1A model rats, the PXT3003 cocktail displayed synergistic improvement both in myelination and in selective down-regulation of *Pmp22* transcription *in vitro* and *in vivo,* with rats displaying improved motor function at nine weeks of treatment and improved myelination and nerve conduction velocity at four months of treatment. Concurrent trials in patients with mild-to-moderate CMT1A showed similarly promising results (Attarian et al., 2014). Patients given PXT3003 daily for one year, particularly at the trial's high dose, demonstrated improvements to both motor function and electrophysiological parameters associated with neuropathy. Inspired by these results, more recent work sought to evaluate whether PXT3003 treatment has a lasting protective effect on promoting healthy Schwann cell differentiation when administered early in life, from postnatal day six to postnatal day 18 in CMT1A rats (Prukop et al., 2019). In this study, rats were segregated into groups treated with three different doses of PXT3003 and examined for longterm changes to the CMT1A phenotype at the age of three, nine, or 12 weeks. While there were persisting improvements to some functional measurements (e.g. hindlimb strength) and reduction in *Pmp22* transcript particularly in the high-dose group, other measures including nerve conduction velocity and CMAP were not improved by treatment. Similarly, while there was a shift toward maintenance of larger-caliber axons, the total number of myelinated axons was not significantly improved compared to CMT1A controls.

Antisense oligonucleotides.

One of the emerging platforms for therapeutic reduction of disease-associated transcripts is the use of antisense oligonucleotides (ASOs) that hybridize with and promote RNAse H-dependent degradation of their target mRNAs, thus suppressing effective expression of the target. Recent work in CMT1A rodent models evaluated the efficacy of ASO therapy targeting *Pmp22* transcription (Zhao et al., 2018). In this study, five-week-old C22 mice received weekly injections of a PMP22 ASO at 25, 50, or 100 mg/kg for nine weeks and demonstrated dose-dependent reduction of both the human transgene and endogenous *Pmp22* mRNA. This reduction in mRNA was accompanied by improved motor function and partial rescue of both motor nerve conduction velocity and CMAP amplitude, as well as improvement in the proportion of myelinated axons and reduction of onion bulb formation. The ASO approach was similarly effective in CMT1A rats, showing significant reduction of *Pmp22* mRNA, improvements in CMAP amplitude and proportion of myelinated axons, and rescue of nerve conduction velocity changes in this model. While small molecule therapies operate via broader mechanisms more susceptible to off-target effects, successful application

of ASO therapy to CMT1A patients could be a safer, more focused method of treating the disease.

Measuring PMP22 levels as a Marker of Target Engagement

If reducing PMP22 levels is a valid therapeutic target, the assessment of PMP22 levels as a target engagement assay is an important gap in developing effective clinical trials. As noted above, treatment-induced decreases in Pmp22 transcripts can be detected in rodent skin (Meyer zu Horste et al., 2007; Zhao et al., 2018). In humans, dermal skin biopsies allow for the relatively non-invasive evaluation of myelinated nerve fibers innervating structures such as Meissner's corpuscles (Li et al., 2005), compared to the more invasive sural nerve biopsies. While quantitative PCR measures of *PMP22* measurements have been employed in clinical trials for CMT1A, they unfortunately did not show changes in the ascorbic trials, and in fact do not discriminate CMT1A patients from controls (Katona et al., 2009; Lewis et al., 2013; Nobbio et al., 2014; Pareyson et al., 2011). Lack of consistency may be attributed to loss of dermal nerve fibers that has been documented in CMT1A patients (Manganelli et al., 2015; Nolano et al., 2015), which may require a robust normalization strategy to account for variation in Schwann cell content in skin biopsies. Levels of PMP22 in immuno-EM of CMT1A patient skin samples were elevated, although the levels were more variable than in non-CMT1A samples (Katona et al., 2009; Li et al., 2005).

In our recent study, a pilot RNA-seq analysis from skin biopsies from nine CMT1A patients and seven controls showed that *PMP22* levels are ~1.55 fold higher in CMT1A samples than in control samples ($p=0.016$). Because a more digital method of RNA detection—compared to quantitative PCR used in previous studies (Nobbio et al., 2014)—revealed an elevated level of *PMP22* (Svaren et al., 2019), we therefore sought to develop a method to normalize to variations in Schwann cell content. With the ultimate goal of developing a clinically applicable platform for analysis of skin biopsies, we employed Nanostring detection, which uses a unique barcode-based probe design to digitally count numbers of transcripts in biological samples (Geiss et al., 2008). Since no amplification or cDNA synthesis is involved, the precision of the assay is substantially better than qPCR techniques.

For Nanostring analysis, we assembled a pilot 50+ gene panel of Schwann cell genes that are a) dysregulated in CMT1A, or b) Schwann cell-specific genes that are relatively stable and therefore will serve as normalization controls. Several resources are available to identify Schwann cell-specific genes, including expression profiles of peripheral nerve development and response to injury (Araki et al., 2001; Arthur-Farraj et al., 2012; Barrette et al., 2010; Bosse et al., 2006; Kim et al., 2012; Nagarajan et al., 2002; Schneider et al., 2001), as well as profiling of sorted cell types in mouse embryonic skin (Sennett et al., 2015) and the recent RNA-seq profiling of >400 human tibial nerve samples along with many other tissues (Broad Institute, gtexportal.org). Using these resources, we identified a series of Schwann cell-specific genes that are relatively unchanged in nerve injury and CMT1A models. Using a set of CMT1A and control skin biopsies and normalizing to two Schwann cell-specific genes, the results are shown as a volcano plot in Figure 3, in which gene expression changes in the upper right quadrant show increased levels (y-axis) in CMT1A samples compared to controls plotted against the significance of the changes (negative log p value). Using

Nanostring probes to detect total *PMP22* mRNA and also the two major transcripts (exon1a and exon1b, driven by P1 and P2 promoters, respectively), *PMP22* levels were elevated in CMT1A vs. control (i.e. log2=~1). The levels of PMP22 were more variable in CMT1A samples compared to controls, which was consistent with the earlier immuno-EM analysis (Katona et al., 2009), but levels of *PMP22* did not correlate with age nor with severity of the neuropathy (Svaren et al., 2019). To address the variability of *PMP22* levels in CMT1A, we also employed two independent punches from each patient, and found that PMP22 levels were fairly consistent within a patient if they were normalized to Schwann cell-specific genes. In addition, we also saw changes in *L1CAM* and *NGFR/p75*, which have been shown to be elevated in rodent models of CMT1A (Hanemann et al., 1996; Klein et al., 2014; Magyar et al., 1996; Niemann et al., 2000). The increase in *PMP22* levels is also apparent in unnormalized data, or after normalization to other Schwann cell-specific genes (Svaren et al., 2019). Overall, our data suggest that employing more digital methods of analysis coupled with normalization to appropriate genes can create a more reliable and precise method for measuring *PMP22* levels in skin biopsies for clinical trials.

The ultimate use of this technique would presumably not be restricted to target engagement, but perhaps to also inform the appropriate dosing. One might expect that a therapeutic agent's effect on *PMP22* transcript levels in a clinical trial could be measured in skin within a few weeks of administration, although that remains an important question. With regard to dosing, one potential risk is that over-suppression of PMP22 could cause HNPP or even more significant symptoms associated with biallelic loss of *PMP22*. On this point, it is not yet clear that the myelin abnormalities or conduction block in HNPP patients and rodent models (Guo et al., 2014) arises from PMP22 deficiency during myelin development or is dependent upon ongoing depletion of PMP22 in mature Schwann cells. Therefore, it is possible that PMP22-lowering therapies may be more tolerated in mature myelin, but this has not been tested in a quantitative fashion in rodent models.

Conclusion.

As one of the more abundant myelin genes in Schwann cells, the *PMP22* gene is important to understanding the integration of all the pro-myelinating signaling pathways that culminate in the large induction of this gene and the myelin gene network during myelination. In addition, the modulation of *PMP22* transcript levels remains a target of therapeutic development for CMT1A, and new tools are available to assess target engagement in future clinical trials.

References

Adlkofer K, Martini R, Aguzzi A, Zielasek J, Toyka KV, Suter U, 1995 Hypermyelination and demyelinating peripheral neuropathy in Pmp22-deficient mice.Nat Genet. 11, 274–80. [PubMed: 7581450]

Adlkofer K, Frei R, Neuberg DH, Zielasek J, Toyka KV, Suter U, 1997 Heterozygous peripheral myelin protein 22-deficient mice are affected by a progressive demyelinating tomaculous neuropathy. J Neurosci. 17, 4662–71. [PubMed: 9169527]

Araki T, Nagarajan R, Milbrandt J, 2001 Identification of genes induced in peripheral nerve after injury. Expression profiling and novel gene discovery. J Biol Chem. 276, 34131–41. [PubMed: 11427537]

Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, Woodhoo A, Jenkins B, Rahman M, Turmaine M, Wicher GK, Mitter R, Greensmith L, Behrens A, Raivich G, Mirsky R, Jessen KR, 2012 c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. Neuron. 75, 633–47. [PubMed: 22920255]

Arthur-Farraj PJ, Morgan CC, Adamowicz M, Gomez-Sanchez JA, Fazal SV, Beucher A, Razzaghi B, Mirsky R, Jessen KR, Aitman TJ, 2017 Changes in the Coding and Non-coding Transcriptome and DNA Methylome that Define the Schwann Cell Repair Phenotype after Nerve Injury. Cell Rep. 20, 2719–2734. [PubMed: 28903050]

Attarian S, Vallat JM, Magy L, Funalot B, Gonnaud PM, Lacour A, Péréon Y, Dubourg O, Pouget J, Micallef J, Franques J, Lefebvre MN, Ghorab K, Al-Moussawi M, Tiffreau V, Preudhomme M, Magot A, Leclair-Visonneau L, Stojkovic T, Bossi L, Lehert P, Gilbert W, Bertrand V, Mandel J, Milet A, Hajj R, Boudiaf L, Scart-Grès C, Nabirotchkin S, Guedj M, Chumakov I, Cohen D, 2014 An exploratory randomised double-blind and placebo-controlled phase 2 study of a combination of baclofen, naltrexone and sorbitol (PXT3003) in patients with Charcot-Marie-Tooth disease type 1A. Orphanet J Rare Dis. 9, 199. [PubMed: 25519680]

Barrette B, Calvo E, Vallières N, Lacroix S, 2010 Transcriptional profiling of the injured sciatic nerve of mice carrying the Wld(S) mutant gene: identification of genes involved in neuroprotection, neuroinflammation, and nerve regeneration. Brain Behav Immun. 24, 1254–67. [PubMed: 20688153]

Bosse F, Hasenpusch-Theil K, Küry P, Müller HW, 2006 Gene expression profiling reveals that peripheral nerve regeneration is a consequence of both novel injury-dependent and reactivated developmental processes. J Neurochem. 96, 1441–57. [PubMed: 16478531]

Brewer MH, Ma KH, Beecham GW, Gopinath C, Baas F, Choi B-O, Reilly MM, Shy ME, Zuechner S, Svaren J, Antonellis A, INC, 2014 Haplotype-specific modulation of a SOX10/CREB response element at the Charcot Marie Tooth disease type 4C locus SH3TC2. Human Molecular Genetics. 23, 5171–5187. [PubMed: 24833716]

Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Disteche CM, Bird TD, 1993 DNA deletion associated with hereditary neuropathy with liability to pressure palsies. Cell. 72, 143–51. [PubMed: 8422677]

Chen L, Loh PG, Song H, 2010 Structural and functional insights into the TEAD-YAP complex in the Hippo signaling pathway. Protein Cell. 1, 1073–83. [PubMed: 21213102]

Chumakov I, Milet A, Cholet N, Primas G, Boucard A, Pereira Y, Graudens E, Mandel J, Laffaire J, Foucquier J, Glibert F, Bertrand V, Nave KA, Sereda MW, Vial E, Guedj M, Hajj R, Nabirotchkin S, Cohen D, 2014 Polytherapy with a combination of three repurposed drugs (PXT3003) down-regulates Pmp22 over-expression and improves myelination, axonal and functional parameters in models of CMT1A neuropathy. Orphanet J Rare Dis. 9, 201. [PubMed: 25491744]

Deng Y, Wu LMN, Bai S, Zhao C, Wang H, Wang J, Xu L, Sakabe M, Zhou W, Xin M, Lu QR, 2017 A reciprocal regulatory loop between TAZ/YAP and G-protein Gαs regulates Schwann cell proliferation and myelination. Nat Commun. 8, 15161. [PubMed: 28443644]

Desarnaud F, Do Thi AN, Brown AM, Lemke G, Suter U, Baulieu EE, Schumacher M, 1998 Progesterone stimulates the activity of the promoters of peripheral myelin protein-22 and protein zero genes in Schwann cells. J Neurochem. 71, 1765–8. [PubMed: 9751213]

Dranchak P, Moran JJ, MacArthur R, Lopez-Anido C, Inglese J, Svaren J, 2018 Genome-Edited Cell Lines for High-Throughput Screening. Methods Mol Biol. 1755, 1–17. [PubMed: 29671259]

Eldridge CF, Bunge MB, Bunge RP, Wood PM, 1987 Differentiation of axon-related Schwann cells in vitro. I. Ascorbic acid regulates basal lamina assembly and myelin formation. J Cell Biol. 105, 1023–34. [PubMed: 3624305]

Fledrich R, Stassart RM, Klink A, Rasch LM, Prukop T, Haag L, Czesnik D, Kungl T, Abdelaal TA, Keric N, Stadelmann C, Brück W, Nave KA, Sereda MW, 2014 Soluble neuregulin-1 modulates disease pathogenesis in rodent models of Charcot-Marie-Tooth disease 1A. Nat Med. 20, 1055–61. [PubMed: 25150498]

Fledrich R, Abdelaal T, Rasch L, Bansal V, Schutza V, Brugger B, Luchtenborg C, Prukop T, Stenzel J, Rahman RU, Hermes D, Ewers D, Mobius W, Ruhwedel T, Katona I, Weis J, Klein D, Martini R, Bruck W, Muller WC, Bonn S, Bechmann I, Nave KA, Stassart RM, Sereda MW, 2018 Targeting

myelin lipid metabolism as a potential therapeutic strategy in a model of CMT1A neuropathy. Nat Commun. 9, 3025. [PubMed: 30072689]

Fortun J, Dunn WJ, Joy S, Li J, Notterpek L, 2003 Emerging role for autophagy in the removal of aggresomes in Schwann cells. J Neurosci. 23, 10672–80. [PubMed: 14627652]

Fridman V, Bundy B, Reilly MM, Pareyson D, Bacon C, Burns J, Day J, Feely S, Finkel RS, Grider T, Kirk CA, Herrmann DN, Laura M, Li J, Lloyd T, Sumner CJ, Muntoni F, Piscosquito G, Ramchandren S, Shy R, Siskind CE, Yum SW, Moroni T, Pagliano E, Zuchner S, Scherer SS, Shy ME, 2014 CMT subtypes and disease burden in patients enrolled in the Inherited Neuropathies Consortium natural history study: a cross-sectional analysis. J Neurol Neurosurg Psychiatry.

Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, Fell HP, Ferree S, George RD, Grogan T, James JJ, Maysuria M, Mitton JD, Oliveri P, Osborn JL, Peng T, Ratcliffe AL, Webster PJ, Davidson EH, Hood L, Dimitrov K, 2008 Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol. 26, 317–25. [PubMed: 18278033]

Gess B, Baets J, De Jonghe P, Reilly MM, Pareyson D, Young P, 2015 Ascorbic acid for the treatment of Charcot-Marie-Tooth disease. Cochrane Database Syst Rev. CD011952. [PubMed: 26662471]

Gokey NG, Srinivasan R, Lopez-Anido C, Krueger C, Svaren J, 2012 Developmental Regulation of MicroRNA Expression in Schwann Cells. Molecular and Cellular Biology. 32, 558–568. [PubMed: 22064487]

Guo J, Wang L, Zhang Y, Wu J, Arpag S, Hu B, Imhof BA, Tian X, Carter BD, Suter U, Li J, 2014 Abnormal junctions and permeability of myelin in PMP22-deficient nerves. Ann Neurol. 75, 255–65. [PubMed: 24339129]

Hai M, Muja N, DeVries GH, Quarles RH, Patel PI, 2002 Comparative analysis of Schwann cell lines as model systems for myelin gene transcription studies. J Neurosci Res. 69, 497–508. [PubMed: 12210843]

Hanemann CO, Gabreëls-Fasten AA, Müller HW, Stoll G, 1996 Low affinity NGF receptor expression in CMT1A nerve biopsies of different disease stages. Brain. 119 (Pt 5), 1461–9. [PubMed: 8931571]

He X, Zhang L, Queme LF, Liu X, Lu A, Waclaw RR, Dong X, Zhou W, Kidd G, Yoon SO, Buonanno A, Rubin JB, Xin M, Nave KA, Trapp BD, Jankowski MP, Lu QR, 2018 A histone deacetylase 3-dependent pathway delimits peripheral myelin growth and functional regeneration. Nat Med. 24, 338–351. [PubMed: 29431744]

Hirt N, Eggermann K, Hyrenbach S, Lambeck J, Busche A, Fischer J, Rudnik-Schöneborn S, Gaspar H, 2015 Genetic dosage compensation via co-occurrence of PMP22 duplication and PMP22 deletion. Neurology. 84, 1605–6. [PubMed: 25795643]

Hung H, Kohnken R, Svaren J, 2012 The Nucleosome Remodeling and Deacetylase Chromatin Remodeling (NuRD) Complex Is Required for Peripheral Nerve Myelination. Journal of Neuroscience. 32, 1517–1527. [PubMed: 22302795]

Hung HA, Sun G, Keles S, Svaren J, 2015 Dynamic Regulation of Schwann Cell Enhancers after Peripheral Nerve Injury. J Biol Chem. 290, 6937–50. [PubMed: 25614629]

Huxley C, Passage E, Manson A, Putzu G, Figarella-Branger D, Pellissier J, Fontés M, 1996 Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. Hum Mol Genet. 5, 563–9. [PubMed: 8733121]

Huxley C, Passage E, Robertson A, Youl B, Huston S, Manson A, Sabéran-Djoniedi D, Figarella-Branger D, Pellissier J, Thomas P, Fontés M, 1998 Correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in nerve conduction velocity in transgenic mice. Hum Mol Genet. 7, 449–58. [PubMed: 9467003]

Inglese J, Dranchak P, Moran JJ, Jang S-W, Srinivasan R, Santiago Y, Zhang L, Guha R, Martinez N, MacArthur R, Cost GJ, Svaren J, 2014 Genome Editing-Enabled HIS Assays Expand Drug Target Pathways for Charcot-Marie-Tooth Disease. Acs Chemical Biology. 9, 2594–2602. [PubMed: 25188731]

Jang SW, Lopez-Anido C, MacArthur R, Svaren J, Inglese J, 2012 Identification of drug modulators targeting gene-dosage disease CMT1A. ACS Chem Biol. 7, 1205–13. [PubMed: 22530759]

Jones EA, Lopez-Anido C, Srinivasan R, Krueger C, Chang L-W, Nagarajan R, Svaren J, 2011 Regulation of the PMP22 Gene through an Intronic Enhancer. Journal of Neuroscience. 31, 4242–4250. [PubMed: 21411665]

Jones EA, Brewer MH, Srinivasan R, Krueger C, Sun G, Charney KN, Keles S, Antonellis A, Svaren J, 2012 Distal enhancers upstream of the Charcot-Marie-Tooth type 1A disease gene PMP22. Human Molecular Genetics. 21, 1581–1591. [PubMed: 22180461]

Jouaud M, Mathis S, Richard L, Lia AS, Magy L, Vallat JM, 2019 Rodent models with expression of PMP22: Relevance to dysmyelinating CMT and HNPP. J Neurol Sci. 398, 79–90. [PubMed: 30685714]

Juneja M, Burns J, Saporta MA, Timmerman V, 2019 Challenges in modelling the Charcot-Marie-Tooth neuropathies for therapy development. J Neurol Neurosurg Psychiatry. 90, 58–67. [PubMed: 30018047]

Katona I, Wu X, Feely SM, Sottile S, Siskind CE, Miller LJ, Shy ME, Li J, 2009 PMP22 expression in dermal nerve myelin from patients with CMT1A. Brain. 132, 1734–40. [PubMed: 19447823]

Kaya F, Belin S, Bourgeois P, Micaleff J, Blin O, Fontés M, 2007 Ascorbic acid inhibits PMP22 expression by reducing cAMP levels. Neuromuscul Disord. 17, 248–53. [PubMed: 17303424]

Kim Y, Remacle AG, Chernov AV, Liu H, Shubayev I, Lai C, Dolkas J, Shiryaev SA, Golubkov VS, Mizisin AP, Strongin AY, Shubayev VI, 2012 The MMP-9/TIMP-1 axis controls the status of differentiation and function of myelin-forming Schwann cells in nerve regeneration. PLoS One. 7, e33664. [PubMed: 22438979]

Klein D, Groh J, Wettmarshausen J, Martini R, 2014 Nonuniform molecular features of myelinating Schwann cells in models for CMT1: distinct disease patterns are associated with NCAM and c-Jun upregulation. Glia. 62, 736–50. [PubMed: 24526449]

Lee JS, Kwak G, Kim HJ, Park HT, Choi BO, Hong YB, 2019 miR-381 Attenuates Peripheral Neuropathic Phenotype Caused by Overexpression of PMP22. Exp Neurobiol. 28, 279–288. [PubMed: 31138995]

Lewis RA, McDermott MP, Herrmann DN, Hoke A, Clawson LL, Siskind C, Feely SM, Miller LJ, Barohn RJ, Smith P, Luebbe E, Wu X, Shy ME, 2013 High-Dosage Ascorbic Acid Treatment in Charcot-Marie-Tooth Disease Type 1A: Results of a Randomized, Double-Masked, Controlled Trial. JAMA Neurol. 70, 981–7. [PubMed: 23797954]

Li J, Bai Y, Ghandour K, Qin P, Grandis M, Trostinskaia A, Ianakova E, Wu X, Schenone A, Vallat JM, Kupsky WJ, Hatfield J, Shy ME, 2005 Skin biopsies in myelin-related neuropathies: bringing molecular pathology to the bedside. Brain. 128, 1168–77. Epub 2005 Mar 17. [PubMed: 15774502]

Li J, 2017 Caveats in the Established Understanding of CMT1A. Ann Clin Transl Neurol. 4, 601–607. [PubMed: 28812050]

Lopez-Anido C, Sun G, Koenning M, Srinivasan R, Hung HA, Emery B, Keles S, Svaren J, 2015 Differential Sox10 genomic occupancy in myelinating glia. Glia. 63, 1897–1914. [PubMed: 25974668]

Lopez-Anido C, Poitelon Y, Gopinath C, Moran JJ, Ma KH, Law WD, Antonellis A, Feltri ML, Svaren J, 2016 Tead1 regulates the expression of Peripheral Myelin Protein 22 during Schwann cell development. Hum Mol Genet. 25, 3055–3069. [PubMed: 27288457]

Lupski JR, de Oca-Luna RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo-Cardenas O, Barker DF, Killian JM, Garcia CA, Chakravarti A, Patel PI, 1991 DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell. 66, 219–32. [PubMed: 1677316]

Magnaghi V, Ballabio M, Roglio I, Melcangi RC, 2007 Progesterone derivatives increase expression of Krox-20 and Sox-10 in rat Schwann cells. J Mol Neurosci. 31, 149–57. [PubMed: 17478888]

Magyar J, Martini R, Ruelicke T, Aguzzi A, Adlkofer K, Dembic Z, Zielasek J, Toyka K, Suter U, 1996 Impaired differentiation of Schwann cells in transgenic mice with increased PMP22 gene dosage. J Neurosci. 16, 5351–60. [PubMed: 8757248]

Maier M, Berger P, Nave KA, Suter U, 2002 Identification of the regulatory region of the peripheral myelin protein 22 (PMP22) gene that directs temporal and spatial expression in development and regeneration of peripheral nerves. Mol Cell Neurosci. 20, 93–109. [PubMed: 12056842]

Maier M, Castagner F, Berger P, Suter U, 2003 Distinct elements of the peripheral myelin protein 22 (PMP22) promoter regulate expression in Schwann cells and sensory neurons. Mol Cell Neurosci. 24, 803–17. [PubMed: 14664827]

Makoukji J, Shackleford G, Meffre D, Grenier J, Liere P, Lobaccaro JM, Schumacher M, Massaad C, 2011 Interplay between LXR and Wnt/β-catenin signaling in the negative regulation of peripheral myelin genes by oxysterols. J Neurosci. 31, 9620–9. [PubMed: 21715627]

Manganelli F, Nolano M, Pisciotta C, Provitera V, Fabrizi GM, Cavallaro T, Stancanelli A, Caporaso G, Shy ME, Santoro L, 2015 Charcot-Marie-Tooth disease: New insights from skin biopsy. Neurology. 85, 1202–8. [PubMed: 26362287]

Melcangi RC, Magnaghi V, Cavarretta I, Zucchi I, Bovolin P, D'Urso D, Martini L, 1999 Progesterone derivatives are able to influence peripheral myelin protein 22 and P0 gene expression: possible mechanisms of action. J Neurosci Res. 56, 349–57. [PubMed: 10340743]

Meyer zu Horste G, Prukop T, Liebetanz D, Mobius W, Nave KA, Sereda MW, 2007 Antiprogesterone therapy uncouples axonal loss from demyelination in a transgenic rat model of CMT1A neuropathy. Ann Neurol. 61, 61–72. [PubMed: 17262851]

Mukheijee-Clavin B, Mi R, Kern B, Choi IY, Lim H, Oh Y, Lannon B, Kim KJ, Bell S, Hur JK, Hwang W, Che YH, Habib O, Baloh RH, Eggan K, Brandacher G, Hoke A, Studer L, Kim YJ, Lee G, 2019 Comparison of three congruent patient-specific cell types for the modelling of a human genetic Schwann-cell disorder. Nat Biomed Eng. 3, 571–582. [PubMed: 30962586]

Nagarajan R, Le N, Mahoney H, Araki T, Milbrandt J, 2002 Deciphering peripheral nerve myelination by using Schwann cell expression profiling. Proc Natl Acad Sci U S A. 99, 8998–9003. [PubMed: 12084938]

Nam SH, Kanwal S, Nam DE, Lee MH, Kang TH, Jung SC, Choi BO, Chung KW, 2018 Association of miR-149 polymorphism with onset age and severity in Charcot-Marie-Tooth disease type 1A. Neuromuscul Disord. 28, 502–507. [PubMed: 29729827]

Niemann S, Sereda MW, Suter U, Griffiths IR, Nave KA, 2000 Uncoupling of myelin assembly and schwann cell differentiation by transgenic overexpression of peripheral myelin protein 22. J Neurosci. 20, 4120–8. [PubMed: 10818147]

Nobbio L, Sturla L, Fiorese F, Usai C, Basile G, Moreschi I, Benvenuto F, Zocchi E, De Flora A, Schenone A, Bruzzone S, 2009 P2X7-mediated increased intracellular calcium causes functional derangement in Schwann cells from rats with CMT1A neuropathy. J Biol Chem. 284, 23146–58. [PubMed: 19546221]

Nobbio L, Visigalli D, Radice D, Fiorina E, Solari A, Lauria G, Reilly MM, Santoro L, Schenone A, Pareyson D, Group, C.-T., 2014 PMP22 messenger RNA levels in skin biopsies: testing the effectiveness of a Charcot-Marie-Tooth 1A biomarker. Brain. 137, 1614–20. [PubMed: 24812204]

Nolano M, Manganelli F, Provitera V, Pisciotta C, Stancanelli A, Caporaso G, Iodice R, Shy ME, Santoro L, 2015 Small nerve fiber involvement in CMT1A. Neurology. 84, 407–14. [PubMed: 25540311]

Pantera H, Moran JJ, Hung HA, Pak E, Dutra A, Svaren J, 2018 Regulation of the neuropathy-associated Pmp22 gene by a distal super-enhancer. Hum Mol Genet. 27, 2830–2839. [PubMed: 29771329]

Pareek S, Notterpek L, Snipes G, Naef R, Sossin W, Laliberté J, Iacampo S, Suter U, Shooter E, Murphy R, 1997 Neurons promote the translocation of peripheral myelin protein 22 into myelin. J Neurosci. 17, 7754–62. [PubMed: 9315897]

Pareyson D, Reilly MM, Schenone A, Fabrizi GM, Cavallaro T, Santoro L, Vita G, Quattrone A, Padua L, Gemignani F, Visioli F, Laurà M, Radice D, Calabrese D, Hughes RA, Solari A, CMT-TRIAAL, groups, C.-T., 2011 Ascorbic acid in Charcot-Marie-Tooth disease type 1A (CMT-TRIAAL and CMT-TRAUK): a double-blind randomised trial. Lancet Neurol. 10, 320–8. [PubMed: 21393063]

Passage E, Norreel J, Noack-Fraissignes P, Sanguedolce V, Pizant J, Thirion X, Robaglia-Schlupp A, Pellissier J, Fontés M, 2004 Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. Nat Med. 10, 396–401. [PubMed: 15034573]

Patel PI, Roa BB, Welcher AA, Schoener-Scott R, Trask BJ, Pentao L, Snipes GJ, Garcia CA, Francke U, Shooter EM, Lupski JR, Suter U, 1992 The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. Nat Genet. 1, 159–65. [PubMed: 1303228]

Perea J, Robertson A, Tolmachova T, Muddle J, King R, Ponsford S, Thomas P, Huxley C, 2001 Induced myelination and demyelination in a conditional mouse model of Charcot-Marie-Tooth disease type 1A. Hum Mol Genet. 10, 1007–18. [PubMed: 11331611]

Poitelon Y, Lopez-Anido C, Catignas K, Berti C, Palmisano M, Williamson C, Ameroso D, Abiko K, Hwang Y, Gregorieff A, Wrana JL, Asmani M, Zhao R, Sim FJ, Wrabetz L, Svaren J, Feltri ML, 2016 YAP and TAZ control peripheral myelination and the expression of laminin receptors in Schwann cells. Nat Neurosci. 19, 879–87. [PubMed: 27273766]

Prukop T, Stenzel J, Wernick S, Kungl T, Mroczek M, Adam J, Ewers D, Nabirotchkin S, Nave KA, Hajj R, Cohen D, Sereda MW, 2019 Early short-term PXT3003 combinational therapy delays disease onset in a transgenic rat model of Charcot-Marie-Tooth disease 1A (CMT1A). PLoS One. 14, e0209752. [PubMed: 30650121]

Raeymaekers P, Timmerman V, Nelis E, De Jonghe P, Hoogendijk JE, Baas F, Barker DF, Martin JJ, De Visser M, Bolhuis PA, et al., 1991 Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a). The HMSN Collaborative Research Group. Neuromuscul Disord. 1, 93–7. [PubMed: 1822787]

Rossor AM, Carr AS, Devine H, Chandrashekar H, Pelayo-Negro AL, Pareyson D, Shy ME, Scherer SS, Reilly MM, 2017 Peripheral neuropathy in complex inherited diseases: an approach to diagnosis. J Neurol Neurosurg Psychiatry.

Schneider S, Bosse F, D'Urso D, Muller H, Sereda MW, Nave K, Niehaus A, Kempf T, Schnolzer M, Trotter J, 2001 The AN2 protein is a novel marker for the Schwann cell lineage expressed by immature and nonmyelinating Schwann cells. J Neurosci. 21, 920–33. [PubMed: 11157078]

Sennett R, Wang Z, Rezza A, Grisanti L, Roitershtein N, Sicchio C, Mok KW, Heitman NJ, Clavel C, Ma'ayan A, Rendl M, 2015 An Integrated Transcriptome Atlas of Embryonic Hair Follicle Progenitors, Their Niche, and the Developing Skin. Dev Cell. 34, 577–91. [PubMed: 26256211]

Sereda M, Griffiths I, Pühlhofer A, Stewart H, Rossner MJ, Zimmerman F, Magyar JP, Schneider A, Hund E, Meinck HM, Suter U, Nave KA, 1996 A transgenic rat model of Charcot-Marie-Tooth disease. Neuron. 16, 1049–60. [PubMed: 8630243]

Sereda MW, Meyer zu Horste G, Suter U, Uzma N, Nave KA, 2003 Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A). Nat Med. 9, 1533–7. [PubMed: 14608378]

Sereda MW, Nave KA, 2006 Animal models of Charcot-Marie-Tooth disease type 1A. Neuromolecular Med. 8, 205–16. [PubMed: 16775377]

Snipes GJ, Suter U, Welcher AA, Shooter EM, 1992 Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). J Cell Biol. 117, 225–38. [PubMed: 1556154]

Srinivasan R, Sun G, Keles S, Jones EA, Jang SW, Krueger C, Moran JJ, Svaren J, 2012 Genome-wide analysis of EGR2/SOX10 binding in myelinating peripheral nerve. Nucleic Acids Res. 40, 6449–6460. [PubMed: 22492709]

Suter U, Snipes GJ, Schoener-Scott R, Welcher AA, Pareek S, Lupski JR, Murphy RA, Shooter EM, Patel PI, 1994 Regulation of tissue-specific expression of alternative peripheral myelin protein-22 (PMP22) gene transcripts by two promoters. J Biol Chem. 269, 25795–808. [PubMed: 7929285]

Svaren J, 2014 MicroRNA and transcriptional crosstalk in myelinating glia. Neurochemistry International. 77, 50–57. [PubMed: 24979526]

Svaren J, Moran JJ, Wu X, Zuccarino R, Bacon C, Bai Y, Ramesh R, Gutmann L, Anderson DM, Pavelec D, Shy ME, 2019 Schwann cell transcript biomarkers for hereditary neuropathy skin biopsies. Ann Neurol. 85, 887–898. [PubMed: 30945774]

Swan ER, Fuerst DR, Shy ME, 2007 Women and men are equally disabled by Charcot-Marie-Tooth disease type 1A. Neurology. 68, 873. [PubMed: 17353481]

Tao F, Beecham GW, Rebelo AP, Svaren J, Blanton SH, Moran JJ, Lopez-Anido C, Morrow JM, Abreu L, Rizzo D, Kirk CA, Wu X, Feely S, Verhamme C, Saporta MA, Herrmann DN, Day JW, Sumner CJ, Lloyd TE, Li J, Yum SW, Taroni F, Baas F, Choi BO, Pareyson D, Scherer SS, Reilly MM, Shy ME, Züchner S, Consortium, I.N., 2019 Variation in SIPA1L2 is correlated with phenotype

modification in Charcot- Marie- Tooth disease type 1A. Ann Neurol. 85, 316–330. [PubMed: 30706531]

Verrier JD, Lau P, Hudson L, Murashov AK, Renne R, Notterpek L, 2009 Peripheral myelin protein 22 is regulated post-transcriptionally by miRNA-29a. Glia. 57, 1265–79. [PubMed: 19170179]

Viader A, Chang LW, Fahrner T, Nagarajan R, Milbrandt J, 2011 MicroRNAs Modulate Schwann Cell Response to Nerve Injury by Reinforcing Transcriptional Silencing of Dedifferentiation-Related Genes. J Neurosci. 31, 17358–69. [PubMed: 22131398]

Welcher AA, Suter U, De Leon M, Snipes GJ, Shooter EM, 1991 A myelin protein is encoded by the homologue of a growth arrest-specific gene. Proc Natl Acad Sci U S A. 88, 7195–9. [PubMed: 1714591]

Weterman MA, van Ruissen F, de Wissel M, Bordewijk L, Samijn JP, van der Pol WL, Meggouh F, Baas F, 2010 Copy number variation upstream of PMP22 in Charcot-Marie-Tooth disease. Eur J Hum Genet. 18, 421–8. [PubMed: 19888301]

Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA, 2013 Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell. 153, 307–19. [PubMed: 23582322]

Zhang F, Seeman P, Liu P, Weterman MA, Gonzaga-Jauregui C, Towne CF, Batish SD, De Vriendt E, De Jonghe P, Rautenstrauss B, Krause KH, Khajavi M, Posadka J, Vandenberghe A, Palau F, Van Maldergem L, Baas F, Timmerman V, Lupski JR, 2010 Mechanisms for nonrecurrent genomic rearrangements associated with CMT1A or HNPP: rare CNVs as a cause for missing heritability. Am J Hum Genet. 86, 892–903. [PubMed: 20493460]

Zhao B, Li L, Lei Q, Guan KL, 2010 The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version. Genes Dev. 24, 862–74. [PubMed: 20439427]

Zhao HT, Damle S, Ikeda-Lee K, Kuntz S, Li J, Mohan A, Kim A, Hung G, Scheideler MA, Scherer SS, Svaren J, Swayze EE, Kordasiewicz HB, 2018 PMP22 antisense oligonucleotides reverse Charcot-Marie-Tooth disease type 1A features in rodent models. J Clin Invest. 128, 359–368. [PubMed: 29202483]

Highlights

The most common cause of the hereditary neuropathy known as Charcot-Marie-Tooth disease is a structural variant in chromosome 17 that consists of a duplicated 1.4 Mb segment encompassing the PMP22 gene, which is classified as CMT1A.

Animal models overexpressing PMP22 recapitulate several aspects of neuropathy, and experimental interventions that reduce PMP22 show benefit in preclinical studies.

Therapeutic agents that reduce PMP22 are in development for clinical trials for CMT1A.

Several aspects of PMP22 gene regulation have been identified, including transcription factors SOX10 and EGR2 among others that activate PMP22 transcription through an upstream superenhancer.

Recent biomarker studies have identified novel means to measure PMP22 in dermal nerves of skin biopsies, which will allow assessment of target engagement and dosing in clinical trials.

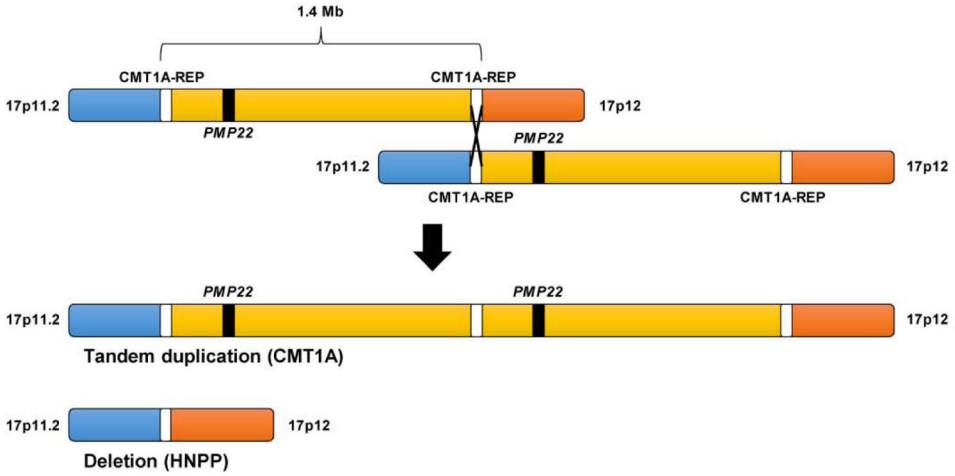

Figure 1.

Genomic rearrangement on 17p11.2 leads to distinct inherited peripheral neuropathies. Misalignment of sister chromatids at highly-homologous CMT1A-REP regions (white bars) during meiosis enables an unequal crossing over event. This results in a tandem duplication of a 1.4 megabase region including the *PMP22* gene (black bars) on one sister chromatid and the reciprocal deletion of the same region on the second sister chromatid. Inheritance of the chromatid possessing the tandem duplication leads to development of CMT1A, while inheritance of the chromatid possessing the deletion leads to HNPP. Colors in chromatid segments are added to ease visualization of the rearrangement.

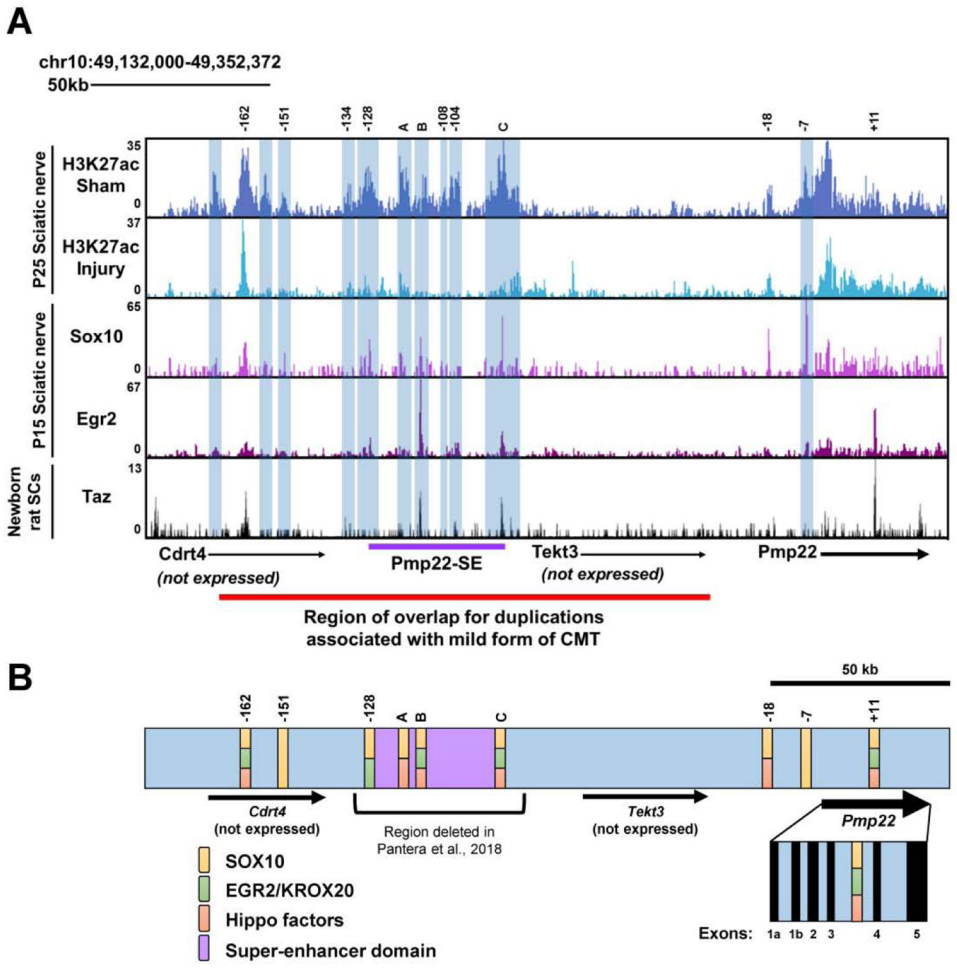

Figure 2.

Chromatin structure of a dosage-sensitive myelin gene.

The diagram shows ChIP-seq data for the Pmp22 gene, which is duplicated in the most common form of CMT, known as CMT1A. The rat Pmp22 gene is shown by the bold arrow and the tracks show ChIP-seq data for the Sox10, Egr2, and Taz transcription factors, along with a marker of actively engaged enhancers, histone H3K27 acetylation. Enhancers that lose H3K27 acetylation following peripheral nerve injury are shaded blue, and the super-enhancer domain labelled Pmp22-SE containing several of these enhancers is indicated by the purple bar. While the normal duplication in CMT1A is 1.4 Mb surrounding the entire PMP22 gene, there have been smaller duplications associated with mild forms of CMT, indicated by a red bar, which would duplicate the super-enhancer. Other genes partially included in this smaller duplication, Cdrt4 and Tekt3, are transcriptionally silent in Schwann cells.

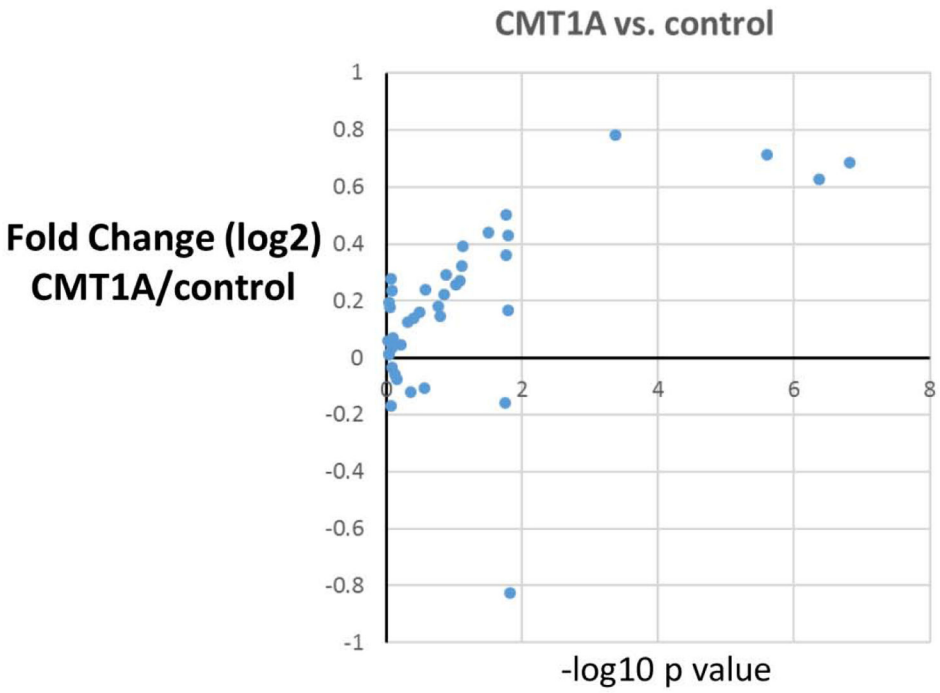

Figure 3.

Nanostring measurements of PMP22 in skin biopsies

These data were published as part of a recent study(Svaren et al., 2019). Punch biopsies were collected from distal forearm (9 cm from ulnar crease). Equal amounts of skin biopsy RNA's were applied to the custom Nanostring panel. After normalizing to two Schwann cell-specific genes, Nanostring results from skin biopsies are summarized in the volcano plot with levels (log 2, y-axis) plotted against the significance of the changes (x-axis, negative log10 p value). The most statistically significant changes between control and CMT1A skin biopsies are in the upper right quadrant.