Dermal Nerve Assessment: A Window to the Mechanism of Neuropathies in Outcome Measures

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Hairy Skin v.s. Glabrous Skin

Epidermis

Dermis

Hypodermis

2mm

4mm

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• Safe, validated and reliable technique
• High specificity, but variable sensitivities
• Has been successfully applied in a few clinical trials as one of End-point measurements

Lauria et al. Euro J Neurol 2005
CLASSIFICATION

• CMT1 Gene
  CMT1A 17p11.2
  CMT1B P0
  CMT1C SIMPLE
  CMT1D EGR2

• CMT2A-F
  CMT2A Mitofusin

• CMTX
  CMTX1 Connexin32

• CMT4A-J
  CMT4J Fig4

www.molgen.ua.ac.be/CMTmutations/default.cfm
Advantages with immunoEM?
Stoichiometric Alteration of PMP22 Protein Determines the Phenotype of Hereditary Neuropathy With Liability to Pressure Palsies

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**Background:** Hereditary neuropathy with liability to pressure palsies (HNPP) is caused by a 1.4-megabase deletion at chromosome 17p11.2, which bears the PMP22 gene and other genes. However, whether other genes besides PMP22 contribute to the phenotype is unknown. Whether any mutation within the coding region of the PMP22 gene ultimately causes HNPP by reducing the amount of peripheral myelin protein 22 (PMP22) expressed in myelin is also unknown.

**Objectives:** To determine whether affected patients develop a phenotype identical to that found in HNPP and whether the leucine 7 frameshift (Leu7fs) mutation reduces PMP22 levels in myelin.

**Design:** We evaluated affected family members by neurological examination, electrophysiology, and skin biopsies. We identified a large family with a Leu7fs mutation of PMP22 (11 affected members across 3 generations) that predicts truncation of the protein prematurely and eliminates PMP22 expression from the mutant allele.

**Results:** We found that PMP22 levels were reduced in peripheral nerve myelin in dermal skin biopsies in patients with an Leu7fs mutation. Through clinical and electrophysiological evaluation, we also found that patients with the Leu7fs mutation were indistinguishable from patients with HNPP caused by deletion. We also found that a length-dependent axonal loss became pronounced in elderly patients with Leu7fs mutations, similar to what has been described in heterozygous knockout mice (pmp22+-).

**Conclusions:** Taken together, these results confirm that the phenotypic expression is identical in patients with Leu7fs mutation and patients with HNPP caused by chromosome 17p11.2 deletion. They also demonstrate that reduction of PMP22 is sufficient to cause the full HNPP phenotype.

*Arch Neurol.* 2007;64(7):974-978
Variation of PMP22 levels, not the absolute value of PMP22 expression, matters.

Katona et al. PMP22 Expression in Dermal Myelin Nerve from Patients with CMT1A. Brain 2009.
Skin biopsies demonstrate MPZ splicing abnormalities in Charcot-Marie-Tooth neuropathy 1B

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Abstract—Objective: To demonstrate that intronic mutations in the myelin protein zero (MPZ) cause Charcot-Marie-Tooth neuropathy 1B (CMT1B) by disrupting MPZ splicing. Methods: We report a family with a T>G transversion at the invariant +2 position in intron 4 of MPZ (c.614 +2T>G) that abolishes 5' donor site recognition and is predicted to alter MPZ splicing. We obtained detailed clinical and neurophysiologic analysis of the family. We performed skin biopsies to investigate splicing abnormalities, MPZ protein levels, and localization in myelinated nerves. Results: Patients developed a late onset neuropathy with minimally slow nerve conduction velocities. Skin biopsies confirmed the predicted skipping of exon 4 and downstream frameshift of the mutant MPZ. Quantitative immuno-EM demonstrated normal nerve MPZ levels, suggesting that the mutant MPZ was transported to compact myelin. Conclusions: Intronic mutations cause CMT1B by disrupting splicing and certain MPZ mutations may cause neuropathy by interacting with the wild type MPZ in the extracellular space of compact myelin.

NEUROLOGY 2006;67:1141-1146
Molecular Architecture on Myelinated Nerve Fibers
Saporta et al. Brain 2009; Accepted
Sodium channels are correctly localized at nodes of Ranvier in CMT1A

Saporta et al. Brain 2009; Accepted
Axonal Loss in Patients with CMT1A

Saporta et al. Brain 2009; Accepted
Intra-axonal Mitochondrial Density is Increased in CMT 1A Patients

Saporta et al. Brain 2009; Accepted
LETTERS

Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J

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1. Plt mice have homozygous mutation in Fig4 gene. Loss of function of Fig4 reduces PI3,5P₂.
2. Neuronal loss and vacuoles in plt mice; death in 6wks
3. Fig4 with 907 AA; chromosome 6q21; ubiquitous expression of mRNA
4. Screen human subjects - CMT4J
Mutation of Fig4 causes a rapidly progressive, asymmetric neuronal degeneration

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Recessive Charcot-Marie-Tooth disease type-4J (CMT4J) and its animal model, the pale tremor mouse (plt), are caused by mutations of the FIG4 gene encoding a PI(3,5)P2 5-phosphatase. We describe the 9-year clinical course of CMT4J, including asymmetric, rapidly progressive paralysis, in two siblings. Sensory symptoms were absent despite reduced numbers of sensory axons. Thus, the phenotypic presentation of CMT4J clinically resembles motor neuron disease. Time-lapse imaging of fibroblasts from CMT4J patients demonstrates impaired trafficking of intracellular organelles because of obstruction by vacuoles. Further characterization of plt mice identified axonal degeneration in motor and sensory neurons, limited segmental demyelination, lack of TUNEL staining and lack of accumulation of ubiquitinated protein in vacuoles of motor and sensory neurons. This study represents the first documentation of the natural history of CMT4J. Physical obstruction of organelle trafficking by vacuoles is a potential novel cellular mechanism of neurodegeneration.
Zhang et al. Brain 2008

Images A and B show fibroblasts under different conditions: control and CMT4J, respectively. Images C and D illustrate the percentage of fibroblasts with vacuoles and the percentage of organelles with vectorial movement, respectively.

Zhang et al. Brain 2008
Time-Lapse Imaging
Zhang et al. Brain 2008
Summary:

• While studying small fibre sensory neuropathies is an important advance, skin biopsy technique can be utilized to probe many other aspects of neurological diseases.
• Pathological alterations in myelinated nerve fibres
• Regulation of gene expression at mRNA or protein level
• Molecular architecture in myelinated nerve fibres
• Mechanism of cell biology, such as trafficking of intracellular organelles