

Rapid Quantitative PCR Diagnostic Strategy for Charcot-Marie-Tooth 1A and Hereditary Neuropathy with Liability to Pressure Palsies

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Charcot-Marie-Tooth (CMT) disorders are an extremely heterogeneous group of diseases of the peripheral nervous system in humans, with a prevalence of around 1 in 2,500.¹ To date, mutations in 30 genes have been reported in various CMT forms. In numerous CMT types, only the locus is known, but some CMT forms were shown not to be linked with any known locus. Genetic studies in CMT disorders cover a wide spectrum of problems ranging from identification of novel mutations through studies of the pathogenic nature of mutations to genotype-phenotype correlations.

CMT type 1 (CMT1) is a genetically heterogeneous group of chronic demyelinating polyneuropathies with loci mapping to chromosome 17 (CMT1A), chromosome 1 (CMT1B), chromosome 16 (CMT1C), and chromosome 10 (CMT1D). CMT1A is most often associated with a tandem 1.5-megabase (Mb) duplication in chromosome 17p11.2-p12. Rarely, it may result from a point mutation in the peripheral myelin protein-22 (PMP22) gene. CMT1B is associated with point mutations in the myelin protein zero (Po or MPZ) gene. Mutations in the SIMPLE gene cause CMT1C, and CMT1D is the result of mutations in the early response 2 (ERG2 or Krox-20) gene. An X-linked form of CMT1 (CMT1X) maps to Xq13 and is associated with mutations in the connexin32 (Cx32) gene. CMT neuropathy type 2 (CMT2) is an axonal neuropathy that maps to chromosome 1p35-p36 (CMT2A), chromosome 3q13-q22 (CMT2B), chromosome 7p14 (CMT2D), chromosome 8p21 (CMT2E), chromosome 1q22-q23 (CMT2F), or chromosome 3q13 (CMT2G).

Dejerine-Sottas disease (DSD), also called hereditary motor and sensory neuropathy type III (HMSNIII), is a severe, infantile-onset demyelinating polyneuropathy syndrome that may be associated with point mutations in either the PMP22 gene, PO gene, EGR2 gene or the PRX gene (for the recessive form). It shares considerable clinical and pathologic features with CMT1. Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant disorder that results in a recurrent, episodic demyelinating neuropathy. HNPP is associated with a 1.5-Mb deletion in chromosome 17p11.2-p12 that results in reduced expression of the PMP22 gene. CMT1A and HNPP are reciprocal duplication/deletion syndromes that originate from unequal crossover during germ cell meiosis.²

Southern blots, pulsed-field gel electrophoresis (PFGE), fluorescence *in situ* hybridization (FISH), rapid real-time PCR gene dosage, and polymorphic marker analysis are the currently used diagnostic methods for CMT1A and HNPP detection.³ In a recent study comparing real-time PCR with the other methods currently used for the diagnosis of CMT, by using a combination of junction fragment PCR, analysis of microsatellite markers, and PFGE, 76 unrelated patients with 17p12 duplication were identified. In these patients, junction fragment PCR detected 63% of cases of duplication, the microsatellite markers method revealed 74%, while the combined use of microsatellite markers and junction fragment PCR revealed 91% of cases of CMT1A. PFGE detected 100% of the cases with duplication, even in the presence of atypical 17p12 duplication. Real-time PCR detected

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100% of the cases with CMT1A and was comparable to PFGE. However, in contrast to PFGE, real-time PCR does not need fresh blood, and minimizes diagnosis time and cost.⁴ A conclusion might be made that the previous methods are time-consuming, labor-intensive, and have some significant limitations, especially difficulties in interpretation of dosage for different alleles.⁵

As reported in this issue of the *Journal of the Chinese Medical Association*, Lin et al⁶ developed allele-specific primers to amplify genomic DNA of patients with CMT1A and HNPP, which provides a rapid quantitative PCR method for determining gene copy number for the identification of DNA duplication or deletion occurring in CMT1A or HNPP. They could identify the mutations by means of agarose gel electrophoresis after PCR amplification without restriction enzyme digestion from 33 (82.5%) of the 40 CMT1A and 19 (76.0%) of the 25 HNPP samples. All CMT1A and HNPP patients had previously been identified with microsatellite mapping. Stringently specific primers were used to overcome the problem of nonspecific amplification and provide a rapid, all-or-none PCR product and efficient screening test for CMT1A and HNPP. This new method is also faster and easier than currently available methods. The question has been raised whether the absence of PCR products in some patients was related to technical reasons in the CMT1 or HNPP assays or not, and the design of primers for multiplex PCR has been suggested for improving the detection rate.

Currently, mutations in multiple different genes expressed in Schwann cells and neurons cause a variety of overlapping clinical phenotypes of CMT and HNPP.

Recent studies have clarified the molecular pathways involved in the pathogenesis of these disorders, and for the first time provide rational treatment strategies for the most common forms of CMT, especially CMT1 and HNPP.⁷ Taken together, results from these studies support the concept that genetic causes of CMT serve as a living microarray system to identify molecules necessary for normal peripheral nervous system function. When we can make sense of these microarrays, we are likely to understand the pathogenesis and develop rational therapies for CMT1A and HNPP.⁷

References

1. Kochanski A. Molecular genetics studies in Polish Charcot-Marie-Tooth families. *Folia Neuropathol* 2005;43:65-73.
2. Chance PF. Genetic evaluation of inherited motor/sensory neuropathy. *Suppl Clin Neurophysiol* 2004;57:228-42.
3. Choi JR, Lee WH, Sunwoo IN, Lee EK, Lee CH, Lim JB. Effectiveness of real-time quantitative PCR compared to repeat PCR for the diagnosis of Charcot-Marie-Tooth Type 1A and hereditary neuropathy with liability to pressure palsies. *Yonsei Med J* 2005;46:347-52.
4. Patitucci A, Muglia M, Magariello A, Gabriele AL, Peluso G, Sprovieri T, Conforti FL, et al. Comparison of different techniques for detecting 17p12 duplication in CMT1A. *Neuromuscul Disord* 2005;15:488-92.
5. Naimi M, Tardieu S, Depienne C, Ruberg M, Brice A, Dubourg O, Leguern E. Detection of genomic rearrangements by DHPLC: a prospective study of 90 patients with inherited peripheral neuropathies associated with 17p11.2. *Am J Med Genet* 2005;136:136-9.
6. Lin KP, Chou CH, Lee HY, Soong BW. Allele-specific all-or-none PCR product diagnostic strategy for Charcot-Marie-Tooth 1A and hereditary neuropathy with liability to pressure palsies. *J Chin Med Assoc* 2006;69:68-73.
7. Shy ME. Charcot-Marie-Tooth disease: an update. *Curr Opin Neurol* 2004;17:579-85.