Molecular Medicine 4: 3-11, 1998

Molecular Medicine

Review

Charcot-Marie-Tooth Disease: Lessons in Genetic Mechanisms

James R. Lupski
Department of Molecular and Human Genetics, Department of Pediatrics, and Texas Children's Hospital, Baylor College of Medicine, Houston, Texas, U.S.A.

Introduction

In recent years, the application of molecular techniques to the study of human subjects has resulted in a virtual explosion of medical genetic information. This information has greatly expanded our understanding of disease and the mechanisms that cause them. One example is the molecular dissection of the Charcot-Marie-Tooth (CMT) peripheral neuropathy phenotype. The study of CMT has (*i*) revealed large DNA rearrangements as a frequent mutation mechanism, (*ii*) illuminated the importance of gene dosage as a mechanism, (*iii*) conceptually fused the seemingly disparate categories of Mendelian disorders and chromosomal syndromes, and (*iv*) illustrated that both allelic variations and locus heterogeneity could be responsible for a spectrum of clinical phenotypes which may include entities thought to be environmental or acquired. This review will briefly summarize what was known prior to molecular studies, what we have learned through molecular genetic analysis, and finally, what these lessons mean with respect to other human genetic disorders.

What Was Known?

In 1886, Charcot and Marie (1) in Paris, France and Tooth (2) in Cambridge, England independently described the disorder of the peripheral nerves that leads to distal muscle atrophy and weakness that now bears their names. Each recognized the hereditary nature of the disease by pointing to the more frequent occurrence in siblings and observing the disorder in multiple generations in one family. Their observations were reported decades before Mendel's laws were rediscovered. In 1895 Dejerine and Sottas described a more severe neuropathy (3), the Dejerine-Sottas syndrome (DSS), which was thought then to be clinically distinct from CMT.

In 1939 Allan (3) used Charcot-Marie-Tooth disease, also known as peroneal muscular atrophy, to derive two important principles for clinical genetic phenotypes. The first was that different patterns of inheritance could be observed in disorders thought to be caused by a single defective gene if enough families were examined. This Allan hypothesis formulated the concept of genetic heterogeneity (or locus heterogeneity)—that mutations at different loci could be responsible for the same disease phenotype in different families. The second general principle was that the age of onset and clinical severity of the disease were somewhat dependent upon the pattern of inheritance. Recessive disorders are caused by two mutant genes and have an earlier onset and increased severity when compared with dominant conditions in which only one gene in a pair is abnormal.

Throughout the 1960s and 70s, the clinical details of CMT subtypes and other related peripheral neuropathies were elucidated (reviewed in ref. 4). An important clinical observation was the recognition that two major CMT types could be distinguished on the basis of electrophysiologic and pathologic studies. CMT type 1

Address correspondence and reprint requests to: Dr. James R. Lupski, Baylor College of Medicine, One Baylor Plaza, Room 609E, Houston, TX 77030, U.S.A. Phone: 713-798-6530; Fax: 713-798-5073; E-mail: jlupski@bmc.tmc.edu

(CMT1), which is the demyelinating form primarily affecting the glial cells supporting the neuron, is characterized by reduced or slowed motor nerve conduction velocities (NCV) and "onion bulbs" consisting of defective Schwann cell processes on nerve biopsy. In contrast, CMT type 2 (CMT2) is characterized by normal or nearly normal NCV with decreased amplitudes reflecting the axonal involvement in this subtype.

Although the subtypes of CMT could be distinguished clinically and pathologically, it wasn't until the 1980s that the application of genetic linkage analysis enabled the identification of specific genetic loci responsible for CMT1 (5–7).

What Have We Learned?

Chromosomal Duplication as a Mutational Mechanism

The molecular mechanism responsible for the majority of patients with the CMT phenotype linked to the proximal short arm of chromosome 17 (17p11.2p12) is a submicroscopic DNA duplication. This CMT1A duplication is 3 million base pairs (3 megabases or 3 Mb) in length! It consists of a duplicated 1.5 Mb monomeric unit, arranged in tandem, and flanked by a 24,000 base pair (24 kilobases or 24 Kb) direct repeat, named CMT1A-REP (8–10). The molecular mechanism responsible for the CMT1A duplication is an unequal crossing-over event mediated by the homologous CMT1A-REP repeats. The proposed mechanism predicted a reciprocal recombination product resulting in a 1.5 Mb deletion (10). This was subsequently shown to be associated with the clinically distinct demyelinating peripheral neuropathy known as hereditary neuropathy with liability to pressure palsies (HNPP) (11,12).

Gene Dosage as a Mechanism for Disease

Several mechanisms were proposed to explain how the CMT1A duplication might affect a "CMT1 gene." These included (*i*) gene interruption at the duplication junction (most favored by this author), (*ii*) a position effect, and (*iii*) a gene dosage effect due to a dosage-sensitive gene located within the duplicated region. The identification of large, cytogenetically visible chromosomal duplications of chromosome 17p that contained the CMT1A locus in patients whose phenotype included slowed motor NCV supported the gene dosage model (13,14) (Fig. 1).

The *PMP22* gene encoding peripheral myelin protein 22, which is mutated in the mouse models for human demyelinating neuropathies, *Trembler* and *Trembler*^{J} (15,16), was shown to map within the 1.5 Mb CMT1A duplication/HNPP deletion region (17–20). The absence of *PMP22* point mutation in CMT1A duplication patients further supported a gene dosage model (21). Rare demyelinating neuropathy patients without the CMT1A duplication were found to have *PMP22* point mutations, which are usually associated with a more severe CMT1 phenotype than observed with duplication (22,23). *PMP22* point mutations were also identified in patients with DSS (24). To underscore a *PMP22*-specific dosage effect, increased levels of *PMP22* mRNA were found in biopsied peripheral nerves of patients with the CMT1A duplication (25). Furthermore, multiple transgenic animals that overexpress wild-type *PMP22* (26–28), and a *PMP22* knockout mouse heterozygous for a *PMP22* null allele (29,30), recapitulated the phenotypic properties of the human demyelinating peripheral neuropathies. Thus, substantial evidence supports the notion that *PMP22* is the dosage-sensitive gene responsible for the demyelinating phenotype in patients with CMT1A duplication as well as HNPP deletion. This dosage effect is manifested by either trisomic overexpression in CMT1A or monosomic underexpression in HNPP (31,32).

Bridging the Gap between Chromosomal Syndromes and Mendelian Disorders

Traditionally, disorders that segregate as Mendelian traits have been believed to result from mutation in single genes. In contrast, chromosomal syndromes have been thought to result from effects of many genes within or flanking the region of chromosomal abnormality. Down syndrome associated with trisomy 21 is the most common genetic condition, and yet it has no mutant genes; however, a distinct clinical phenotype is observed.

Although the potential effects of gene dosage imbalance in chromosomal syndromes had already been appreciated (33), the concept of "gene dosage" or gene copy number effects was crystallized by findings at the CMT1A locus. A submicroscopic DNA duplication was passed through generations as a dominant trait and was responsible for the segregation of the CMT1A neuropathy phenotype observed by electrophysiologic studies revealing reduced motor NCV

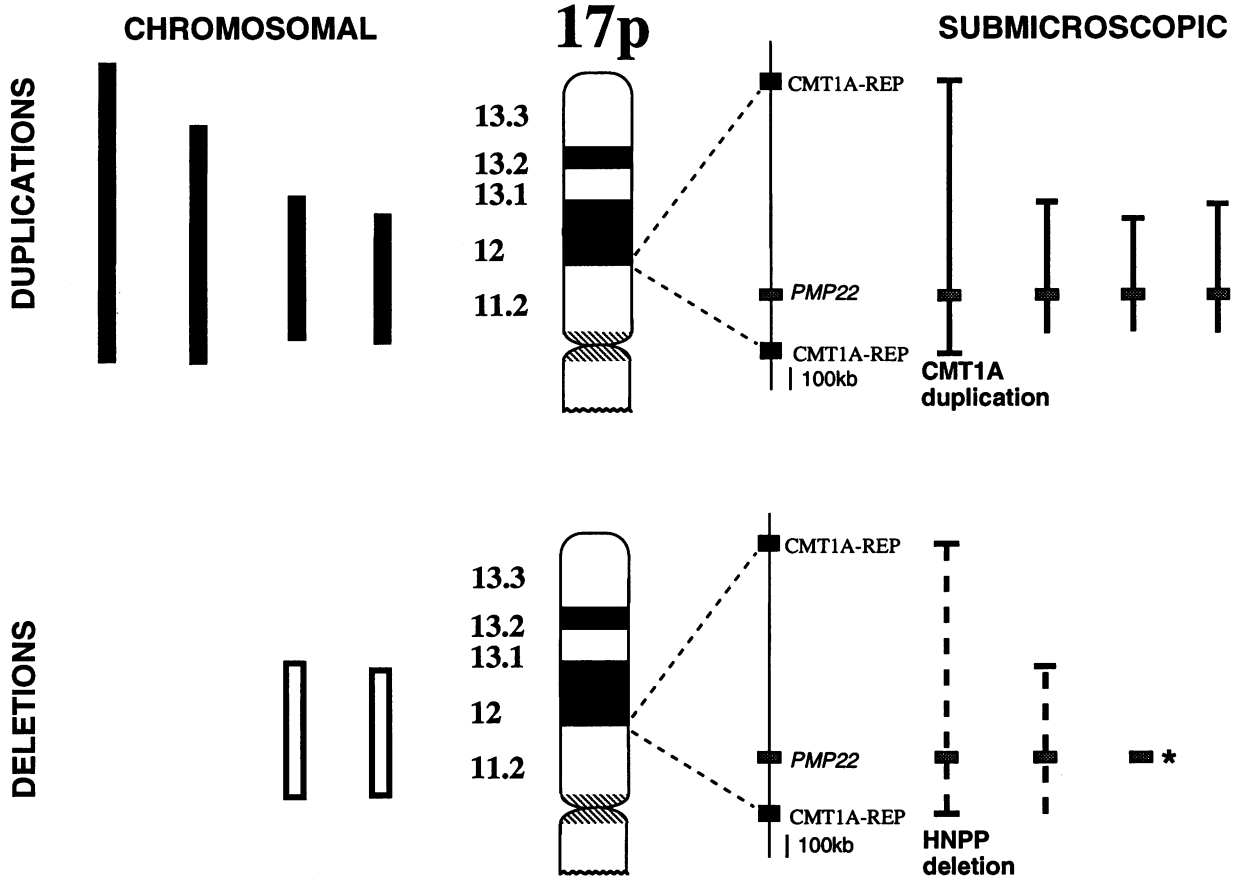

Fig. 1. Chromosomal syndrome versus Mendelian disorders. In the middle of the figure the G-banded ideogram for the short arm of chromosome 17-17p is shown; to the left are cytogenetically visible chromosomal DNA rearrangements, while to the right are submicroscopic rearrangements. Bold vertical rectangles represent the 17p region duplicated (top left), open vertical rectangles show the region deleted (bottom left). Shown to the right of the karyogram is an expansion of the submicroscopic 17p12 region with the *PMP22* gene (hatched box) flanked by CMT1A-REP repeats (closed box). The region duplicated is shown by a bold vertical line (top right) whereas that deleted is shown by a dashed vertical line (bottom right). The asterisk represents the point mutation in *PMP22* that can be associated with HNPP.

(34). Likewise, cytogenetically visible chromosomal duplications involving the same region presented with the motor NCV abnormality as a distinct part of their more complex phenotype (35).

The HNPP deletion is a 1.5 Mb submicroscopic DNA rearrangement. Although as many as 30 to 50 genes are likely deleted, only one gene, *PMP22*, appears to be dosage-sensitive, resulting in a haploinsufficiency phenotype. The proof for this concept is provided by the identification of frameshift mutations in *PMP22*, presumably resulting in null alleles, in some nondeletion HNPP patients (36,37).

Cytogenetically visible deletion of 17p11.2 results in the Smith-Magenis syndrome (SMS) (38). Most SMS patients have a common deletion that appears mechanistically to occur by homologous recombination of a flanking repeat gene cluster (39). However, some rare patients have larger deletions which can include the CMT1A/HNPP genomic region in 17p12 (40,41). When SMS patients have a larger deletion that includes *PMP22*, they exhibit eletrophysiologic features consistent with HNPP (40,41). Thus, manifesting a single Mendelian disorder versus a chromosomal syndrome may be reflective of the size of the DNA rearrangements and the number of dosage-sensitive genes involved.

Chromosomal Duplication Can Be a High-Frequency Mutation

Chromosomal duplications have been known for decades in fruit flies and have been extensively

analyzed both genetically and physically in bacteria during the last 20 years (42). They have been demonstrated to occur at a surprisingly high frequency. As there is no net loss of genetic information, duplications are essentially unrestricted in size and location on the chromosome.

To date, at least seven loci have been associated with a CMT1 phenotype, yet 70–90% of all patients have the CMT1A duplication (43). These observations suggest that duplication may be ten times or more likely to cause CMT1 than other mutational mechanisms. Furthermore, CMT1 is one of the most common inherited disorders with an estimated prevalence of 1/2500 individuals. One study estimates that 10% of the CMT1A duplication cases result from de novo events (44), suggesting a mutation frequency on the order of 10^{-4}. This is 2 to 4 orders of magnitude greater than the spontaneous mutation frequency usually associated with single-gene disorders.

A Recombination Hotspot Associated with Reciprocal Homologous Recombination

The CMT1A-REP repeats flanking the CMT1A duplication/HNPP deletion (45) are ~99% identical across a 24,011 bp region. This provides a substantial region of homology for crossover events to occur, yet the majority of crossover events resulting in these DNA rearrangements occur within an approximately 1.7 Kb region (46,47). Analysis of the DNA sequence surrounding the crossover hotspot region reveals no significant increase in sequence identity between the proximal and distal CMT1A-REPs when compared with surrounding sequence (46). These data suggest that some other signal must be present at or near the site of strand exchange to generate the hotspot. Intriguingly, a *mariner* transposon-like element, termed MITE, maps near the hotspot (46). We have proposed that MITE may stimulate homologous recombination between CMT1A-REPs by providing a target for double-strand breaks (46) (Fig. 2). The analysis of the DNA sequence of the recombinant CMT1A-REP in HNPP patients reveals homologous recombination products consistent with a double-strand break model (48). Furthermore, these DNA sequencing studies of recombinant CMT1A-REP elements have revealed what may be minimal efficient processing segments (MEPS) required for human homologous meiotic recombination.

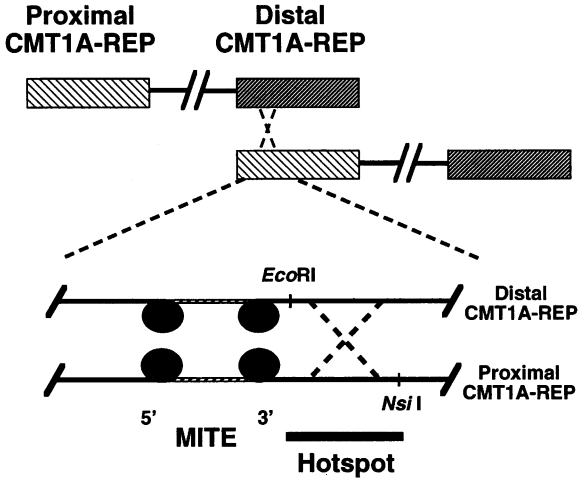

Fig. 2. *Mariner* transposon mediated recombination hot spot. The unequal crossing-over event with the distal CMT1A-REP aligning with homologous proximal CMT1A-REP is shown at the top of the figure; the bottom section shows an enlargement of the recombination hotspot region. The *mariner* insect transposon-like element (MITE) is depicted with four hypothetical transposase molecules (filled circles) at the ends. The model consist of three parts: (*1*) *trans*-acting transposase initiating a double-strand break at MITE, (*2*) CMT1A-REP providing 24 Kb of ~99% homology for recombination mediated repair, and (*3*) the hotspot reflecting resolution of the Holliday junction.

Genome Evolution and Consequences of DNA Rearrangements in the CMT1A/HNPP Region

The nucleotide sequence of CMT1A-REP has revealed one other coding exon in addition to that for the putative transposase of MITE. This exon is part of the *COX10* gene encoding heme A: farnesyltransferase which farnesylates the heme A moiety incorporated into cytochrome oxidase (45). The human *COX10* gene was cloned by human cDNA complementation of a cytochrome oxidase-deficient yeast but was never mapped in the human genome. Subsequent analysis revealed that the entire *COX10* gene spans the distal CMT1A-REP (45,49). Exon VI of *COX10* and 24 Kb of surrounding sequence appear to have been duplicated during genome evolution, at a time of divergence between gorilla and chimpanzees (45,50), as there are two copies present in chimpanzees and only one in gorilla, and copied 1.5 Mb proximally on chromosome 17p to yield the proximal CMT1A-REP (Fig. 3). Thus, the HNPP deletion results in one null allele of *COX10* (45). These findings suggest a further complexity to the consequences of DNA rearrangements in

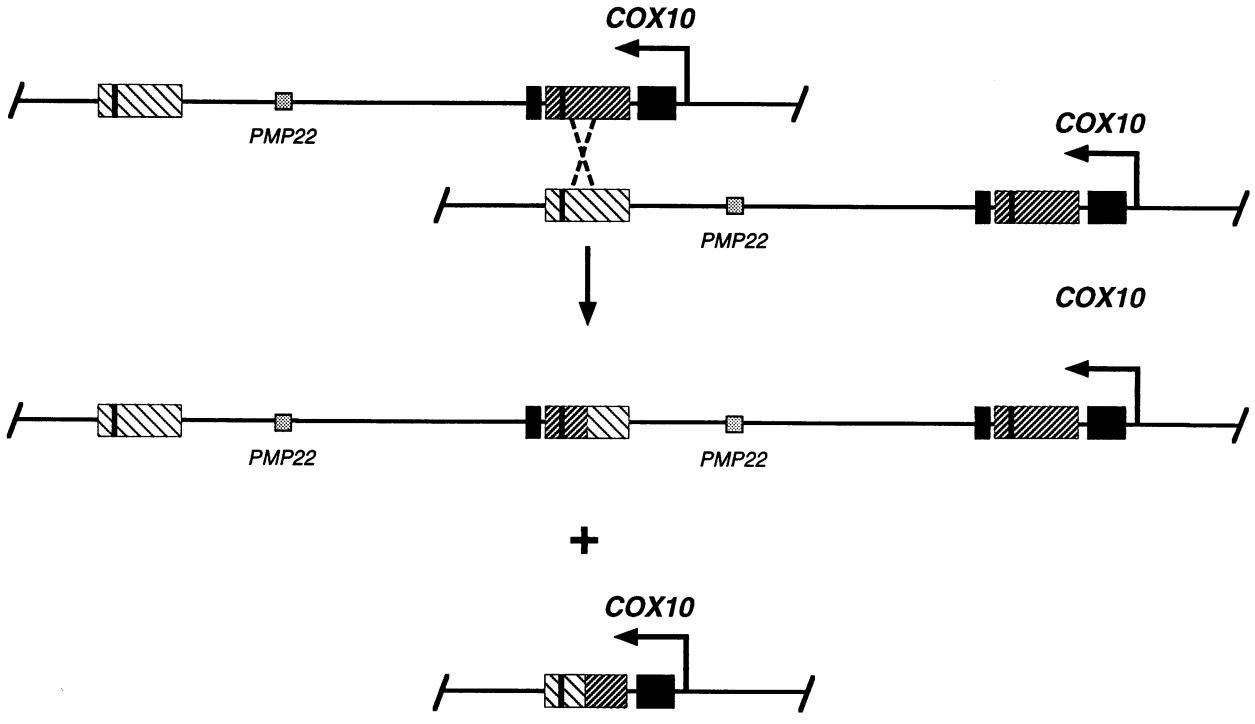

Fig. 3. HNPP deletion interrupts the *COX10* gene. The unequal crossing-over between distal CMT1A-REP and proximal CMT1A-REP is shown at the top of the figure. The *COX10* gene (bold) spans distal CMT1A-REP with one coding exon (exon VI) embedded within distal CMT1A-REP. The *PMP22* gene (stippled box) is located between the flanking CMT1A-REPs. The direction of transcription for *COX10* is depicted by the horizontal arrow. The 5′ end of *COX10* is located outside the genomic region duplicated in CMT1A and deleted in HNPP while the 3′ end is within this genomic region. Note the unequal crossover results in recombination products consisting of the CMT1A duplication, with the recombinant CMT1A-REP missing the 5′ end of *COX10* but having one normal copy present, and the HNPP deletion with the recombinant CMT1A-REP missing the 3′ end of *COX10*.

the CMT1A/HNPP region in addition to altering the copy number of the dosage-sensitive *PMP22* gene. The disruption of *COX10* may have ramifications for phenotype expression in individuals with the HNPP deletion (45).

Peripheral Neuropathies Represent a Spectrum of Genotypically Related Entities

While the majority of patients with CMT1 or HNPP have DNA rearrangements that are responsible for their disease, the discovery of point mutations in genes encoding major myelin proteins have yielded new insights into the pathogenesis of these disorders. The three genes identified to date are *PMP22, MPZ* encoding myelin protein P_0, and *Cx32* encoding the gap junction protein connexin 32 (51). The analysis of disease-associated sequence alterations in patients reveals that point mutations via methylation-mediated deamination is an important mutational mechanism in demyelinating peripheral neuropathy. However, this mechanism does not constitute a mutation hotspot in these three genes that is significantly different from other disease-associated genes (51).

Mutations in *MPZ* and *PMP22* have been identified in patients with the related peripheral neuropathy Dejerine-Sottas syndrome, while *MPZ* mutations have also been observed in patients with congenital hypomyelination (Fig. 4). The crystal structure of the extracellular domain of P_0 has recently been determined (52). This information, in conjunction with natural mutations observed in demyelinating neuropathy patients, has enabled attempts at genotype/phenotype correlations (53). Such analyses suggest that these neuropathies represent a spectrum of clinical severity and that the phenotype is dependent upon the nature of the mutation and its effect on the gene and protein product. Furthermore, more severe phenotype associated with carboxy

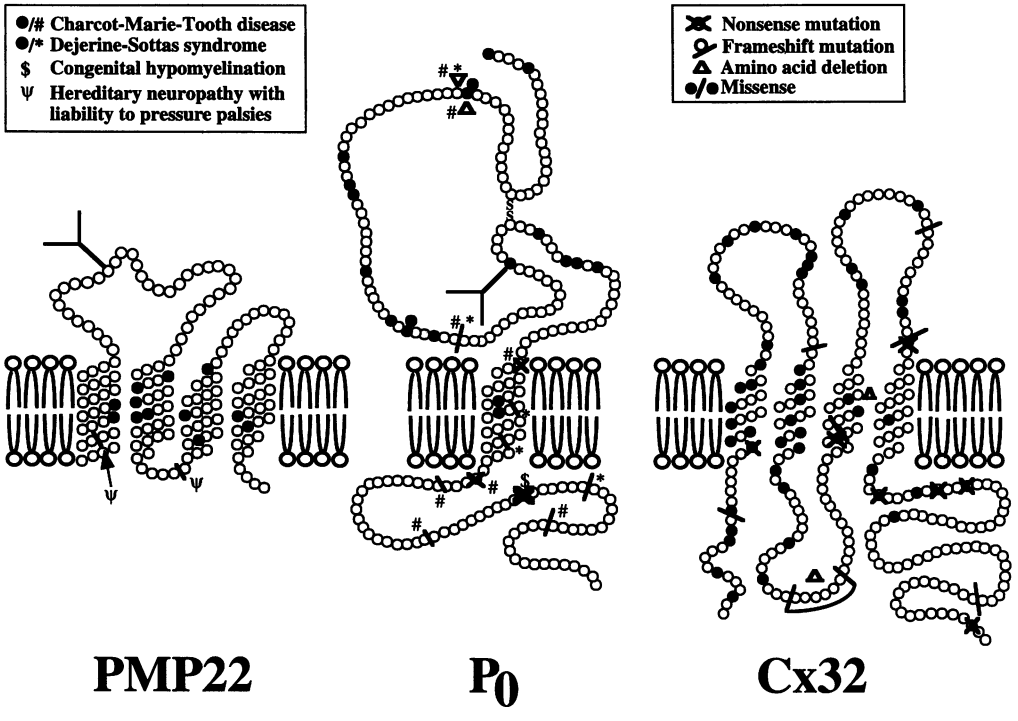

Fig. 4. Myelin gene mutations and myelinopathies. Protein structure models for myelin gene products associated with myelinopathies. The upper left portion of the figure shows the symbols for the specified myelinopathy phenotypes while the upper right gives the key to the mutation types. Note that the *PMP22* mutations are located in the predicted transmembrane domain whereas P_0 mutations are predominantly in the extracellular domain. The *Cx32* mutations associated with X-linked CMT are located throughout the molecule.

terminal mutations suggests an important function for the P_0 intracellular domain (53).

What Does It Mean?

The identification of the CMT1A duplication suggests that other clinical phenotypes may result from submicroscopic DNA rearrangements as opposed to mutations within a gene classically thought of for Mendelian diseases. These rearrangements may reflect structural features of the human genome. The mutation frequencies for DNA rearrangements, and thus de novo mutation in sporadic cases, may be quite high when compared with point mutations. Since these rearrangements may reflect intrinsic structural properties of the genome, the mutation and thus the disease frequency may be similar in different populations.

The high frequency of new mutations suggests that the disease prevalence could be increasing unless reversions are in equilibrium. After the CMT1A duplication occurs, the 1.5 Mb duplicated segment of the genome provides an even larger region for homologous recombination and reversion of the duplication mutation. At least one such case has been suggested by the identification of a patient mosaic for the CMT1A duplication who displayed a milder phenotype than that in the affected parent with the duplication (54,55). However, if this were occurring to a significant extent, one might expect to observe segregation distortion, or less than the expected 50% affected individuals, in large autosomal dominant pedigrees.

The CMT1A duplication frequency also likely reflects the recombination hotspot in CMT1A-REP. Whether DNA transposons such as MITE or other *mariner*-like elements (MLEs) are involved in initiating double strand breaks that stimulate homologous recombination events elsewhere in the human genome remains to be determined.

DNA duplications can be quite large since there is no net loss of genetic information. Given the potential for large size, a duplication may encompass several genes, but it appears from

studies of CMT1A and HNPP that only a subset of genes are dosage-sensitive. The *PMP22* dosage phenomenon also suggests that the stoichiometry of this protein is important in maintaining normal myelin structure and function. The finding of only one dosage-sensitive gene in a relatively large 1.5 Mb genomic region suggests that the phenotypic manifestations of chromosomal aneuploidy syndromes may represent the effects of a small subset of dosage-sensitive genes. Furthermore, the identification of specific clinical features that can sometimes be found as dominant traits (e.g., syndactyly, brachydactyly, microcornea, etc.) in patients with cytogenetically visible duplications may suggest a potential localization for a dosage-sensitive gene associated with the trait present within the region of segmental aneuploidy.

In summary, there recently has been remarkable progress in elucidating the molecular genetic bases of inherited peripheral neuropathies. These investigations confirm the long-held suspicion that inherited peripheral neuropathies are the clinical manifestations of peripheral nerve dysfunction resulting from abnormalities in Schwann cells and their myelin sheath. These studies have also revealed much about the biology and structure of the peripheral nerve and uncovered novel genetic mechanisms. In addition, the new molecular knowledge has ramifications for patient care. The findings provide the clinician with diagnostic tools to enable a precise and secure diagnosis (56–58), enable accurate recurrence risk estimates, and provide prognostic information (59) and the potential to design rational therapeutic approaches. However, perhaps most importantly, genetic findings in this group of disorders has widespread implications for human genetics and the molecular medicine of other disease phenotypes.

Acknowledgments

I thank the members of my laboratory for their critical reviews, for providing a stimulating environment, and for continuing to make doing science fun. The work described here has been generously supported by the National Institute of Neurological Disorders and Stroke (NIH), the Muscular Dystrophy Association, and the Pew Scholars Program in Biomedical Sciences.

References

1. Charcot J-M, Marie P. (1886) Sur une forme particulaiere d'atrophie musculaire progressive souvent familiale debutant par les pied et les jambes et atteignant plus tard les mains. *Rev. Med.* **6:** 97–138.
2. Tooth H. (1886) *The Peroneal Type of Progressive Muscular Atrophy*. H. K. Lewis, London.
3. Allan W. (1939) Relation of hereditary pattern to clinical severity as illustrated by peroneal atrophy. *Arch. Intern. Med.* **63:** 1123–1131.
4. Dyck PJ, Chance P, Lebo R, Carney JA. (1993) Hereditary motor and sensory neuropathies. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds). *Peripheral Neuropathy*. W. B. Saunders, Philadelphia, pp. 1094–1136.
5. Bird TD, Ott J, Giblett ER. (1982) Evidence for linkage of Charcot-Marie-Tooth neuropathy to the Duffy locus on chromosome 1. *Am. J. Hum. Genet.* **34:** 388–394.
6. Gal A, Mucke J, Theile H, Wieacker PF, Ropers H-H, Wienker TF. (1985) X-linked dominant Charcot-Marie-Tooth disease: Suggestions of linkage with a cloned DNA sequence from the proximal Xq. *Hum. Genet.* **70:** 38–42.
7. Vance JM, Nicholson GA, Yamaoka LH, et al. (1989) Linkage of Charcot-Marie-Tooth neuropathy type 1a to chromosome 17. *Exp. Neurol.* **104:** 186–189.
8. Lupski JR, Montes de Oca-Luna R, Slaugenhaupt S, et al. (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* **66:** 219–232.
9. Raeymaekers P, Timmerman V, Nelis E, et al. (1991) Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT1a). *Neuromuscul. Disord.* **1:** 93–97.
10. Pentao L, Wise CA, Chinault AC, Patel PI, Lupski JR. (1992) Charcot-Marie-Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. *Nat. Genet.* **2:** 292–300.
11. Chance PF, Alderson MK, Leppig KA, et al. (1993) DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell* **72:** 143–151.
12. Chance PF, Abbas N, Lensch MW, et al. (1994) Two autosomal dominant neuropathies result from reciprocal DNA duplication/deletion of a region on chromosome 17. *Hum. Mol. Genet.* **3:** 223–228.
13. Lupski JR, Wise CA, Kuwano A, et al. (1992) Gene dosage is a mechanism for Charcot-Marie-Tooth disease type 1A. *Nat. Genet.* **1:** 29–33.
14. Chance PF, Bird TD, Matsunami N, Lensch MW, Brothman AR, Feldman GM. (1992) Trisomy 17p associated with Charcot-Marie-Tooth neuropathy type 1A phenotype: Evidence for gene dosage as a mechanism in CMT1A. *Neurology* **42:** 2295–2299.

15. Suter U, Welcher AA, Özcelik T, et al. (1992) *Trembler* mouse carries a point mutation in a myelin gene. *Nature* **356:** 241–244.
16. Suter U, Moskow JJ, Welcher AA, et al. (1992) A leucine-to-proline mutation in the putative first transmembrane domain of the 22-kDa peripheral myelin protein in the *trembler-J* mouse. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 4382–4386.
17. Patel PI, Roa BB, Welcher AA, et al. (1992) The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. *Nat. Genet.* **1:** 159–165.
18. Valentijn LJ, Bolhuis PA, Zorn I, et al. (1992) The peripheral myelin gene *PMP22/GAS-3* is duplicated in Charcot-Marie-Tooth disease type 1A. *Nat. Genet.* **1:** 166–170.
19. Timmerman V, Nelis E, Van Hul W, et al. (1992) The peripheral myelin protein gene *PMP22* is contained within the Charcot-Marie-Tooth disease type 1A duplication. *Nat. Genet.* **1:** 171–175.
20. Matsunami N, Smith B, Ballard L, et al. (1992) Peripheral myelin protein-22 gene maps in the duplication in chromosome 17p11.2 associated with Charcot-Marie-Tooth 1A. *Nat. Genet.* **1:** 176–179.
21. Warner LE, Roa BB, Lupski JR. (1996) Absence of PMP22 coding region mutations in CMT1A duplication patients: Further evidence supporting gene dosage as a mechanism for Charcot-Marie-Tooth disease type 1A. *Hum. Mutat.* **8:** 362.
22. Roa BB, Garcia CA, Suter U, et al. (1993) Charcot-Marie-Tooth disease type 1A: Association with a spontaneous point mutation in the *PMP22* gene. *N. Engl. J. Med.* **329:** 96–101.
23. Valentijn LJ, Baas F, Wolterman RA, et al. (1992) Identical point mutations of *PMP22* in *Trembler-J* mouse and Charcot-Marie-Tooth disease type 1A. *Nat. Genet.* **2:** 288–291.
24. Roa BB, Dyck PJ, Marks HG, Chance PF, Lupski JR. (1993) Dejerine-Sottas syndrome associated with point mutation in the peripheral myelin protein 22 (*PMP22*) gene. *Nat. Genet.* **5:** 269–273.
25. Yoshikawa H, Nishimura T, Nakatsuji Y, et al. (1994) Elevated expression of messenger RNA for peripheral myelin protein 22 in biopsied peripheral nerves of patients with Charcot-Marie-Tooth disease type 1A. *Ann. Neurol.* **35:** 445–450.
26. Sereda M, Griffiths I, Pühlhofer A, et al. (1996) A transgenic rat model of Charcot-Marie-Tooth disease. *Neuron* **16:** 1049–1060.
27. Huxley C, Passage E, Manson A, et al. (1996) Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. *Hum. Mol. Genet.* **5:** 563–569.
28. Magyar JP, Martini R, Ruelicke T, et al. (1996) Impaired differentiation of Schwann cells in transgenic mice with increased *PMP22* gene dosage. *J. Neurosci.* **16:** 5351–5360.
29. 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:** 274–280.
30. Adlkofer K, Frei R, Neuberg DH-H, Zielasek J, Toyka KV, Suter U. (1997) Heterozygous *PMP22*-deficient mice are affected by a progressive demyelinating tomaculous neuropathy. *J. Neurosci.* **17:** 4662–4671.
31. Lupski JR. (1997) Charcot-Marie-Tooth Disease: A gene-dosage effect. *Hosp. Prac.* **32:** 83–122.
32. Lupski JR. (1998) Molecular genetics of peripheral neuropathies. *Scientific American Neurology*, Scientific American Press, New York (in press).
33. Epstein CJ, (ed). (1986) *The Consequences of Chromosome Imbalance: Principals, Mechanisms, and Models.* Cambridge University Press, Cambridge, U.K.
34. Kaku DA, Parry GJ, Malamut R, Lupski JR, Garcia CA. (1993) Nerve conduction studies in Charcot-Marie-Tooth polyneuropathy associated with a segmental duplication of chromosome 17. *Neurology* **43:** 1806–1808.
35. Roa BB, Greenberg F, Gunaratne P, et al. (1996) Duplication of the *PMP22* gene in 17p partial trisomy patients with Charcot-Marie-Tooth type 1A neuropathy. *Hum. Genet.* **97:** 642–649.
36. Nicholson GA, Valentijn LJ, Cherryson AK, et al. (1994) A frameshift mutation in the PMP22 gene in hereditary neuropathy with liability to pressure palsies. *Nat. Genet.* **6:** 263–266.
37. Young P, Wiebusch H, Stogbauer F, Ringelstein B, Assman G, Funke H. (1997) A novel frameshift mutation in *PMP22* accounts for hereditary neuropathy with liability to pressure palsies. *Neurology* **48:** 450–452.
38. Smith ACM, McGavran L, Robinson J, et al. (1986) Interstitial deletion of (17)(p11.2 p11.2) in nine patients. *Am. J. Med. Genet.* **24:** 393–414.
39. Chen K-S, Manian P, Koeuth T, et al. (1997) Homologous recombination of a flanking repeat gene cluster is a mechanism for a common contiguous gene deletion syndrome. *Nat. Genet.* **17:** 154–163.
40. Zori RT, Lupski JR, Heju Z, et al. (1993) Clinical, cytogenetic, and molecular evidence for an infant with Smith-Magenis syndrome born from a mother having mosaic 17p11.2p12 deletion. *Am. J. Med. Genet.* **47:** 504–511.
41. Trask BJ, Mefford H, van den Engh G, et al. (1996) Quantification by flow cytometry of chromosome-17 deletions in Smith-Magenis syndrome patients. *Hum. Genet.* **98:** 710–718.
42. Lupski JR, Roth JR, Weinstock GM. (1996) Chromosomal duplications in bacteria, fruit flies, and humans. *Am. J. Hum. Genet.* **58:** 21–27.
43. Nelis E, Van Broeckhoven C. (1996) Estimation of the mutation frequencies in Charcot-Marie-Tooth disease type 1 and hereditary neuropathy with liability to pressure palsies: A European collaborative study. *Eur. J. Hum. Genet.* **4:** 25–33.
44. Blair IP, Nash J, Gordon MJ, Nicholson GA. (1996) Prevalence and origin of de novo duplica-

tions in Charcot-Marie-Tooth disease type 1A: First report of a de novo duplication with a maternal origin. *Am. J. Hum. Genet.* **58:** 472–476.
45. Reiter LT, Murakami T, Koeuth T, Gibbs RA, Lupski JR. (1997) The human *COX10* gene is disrupted during homologous recombination between the 24-Kb proximal and distal CMT1A-REPs. *Hum. Mol. Genet.* **6:** 1595–1603.
46. Reiter LT, Murakami T, Koeuth T, et al. (1996) A recombination hotspot responsible for two inherited peripheral neuropathies is located near a *mariner* transponson-like element. *Nature Genet.* **12:** 288–297.
47. Timmerman V, Rautenstrauss B, Reiter LT, et al. (1997) Detection of the CMT1A/HNPP recombination hotspot in unrelated patients of European decent. *J. Med. Genet.* **34:** 43–49.
48. Reiter LT, Hastings PJ, Nelis E, DeJohnge P, Van Broeckhoven C, Lupski JR. (1998) Human meiotic recombination products revealed by sequencing a hotspot for homologous strand exchange in multiple HNPP patients. *Am. J. Hum. Genet.* (in press).
49. Murakami T, Reiter LT, Lupski JR. (1997) Genomic structure and expression of the human heme A: Farnesyltransferase (*COX10*) gene. *Genomics* **42:** 161–164.
50. Kiyosawa H, Chance P. (1996) Primate origin of the CMT1A-REP repeat and analysis of a putative transposon-associated recombinational hotspot. *Hum. Mol. Genet.* **5:** 745–753.
51. Warner LE, Reiter LT, Murakami T, Lupski JR. (1996) Molecular mechanisms for Charcot-Marie-Tooth disease and related demyelinating peripheral neuropathies. *Cold Spring Harbor Symposia on Quantitative Biology* **61:** 659–670.
52. Shapiro L, Doyle JP, Hensley P, Colman DR, Hendrickson W. (1996) Crystal structure of the extracellular domain from P_0, the major structural protein of peripheral nerve myelin. *Neuron* **17:** 435–449.
53. Warner LE, Hilz M, Appel S, et al. (1996) Clinical phenotype of different *MPZ* mutations may include Charcot-Marie-Tooth type 1B, Dejerine-Sottas, and congenital hypomyelination. *Neuron* **17:** 451–460.
54. Liehr T, Rautenstrauss B, Grehl H, et al. (1996) Mosaicism for the Charcot-Marie-Tooth disease type 1A duplication suggests somatic reversion. *Hum. Genet.* **98:** 22.
55. Grehl H, Rautenstrauss B, Liehr T, et al. (1997) Clinical and morphological phenotype of HMSN 1A mosaicism. *Neuromuscul. Disord.* **7:** 27–31.
56. Lupski JR. (1996) DNA diagnostics for Charcot-Marie-Tooth disease and related inherited neuropathies. *Clin. Chem.* **42:** 995.
57. Roa BB, Ananth U, Garcia CA, Lupski JR. (1995) Molecular diagnosis of CMT1A and HNPP. *Lab. Med. Int.* **12:** 22–24.
58. Shaffer LG, Kennedy GM, Spikes AS, Lupski JR. (1997) Diagnosis of CMT1A duplications and HNPP deletions by interphase FISH: Implications for testing in the cytogenetics laboratory. *Am. J. Med. Genet.* **69:** 325–331.
59. Killian JM, Tiwari PS, Jacobson S, Jackson RD, Lupski JR. (1996) Longitudinal studies of the duplication form of Charcot-Marie-Tooth polyneuropathy. *Muscle Nerve* **19:** 74–78.