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A Novel Homozygous Mutation of the Myelin Po Gene Producing Dejerine–Sottas Disease (Hereditary Motor and Sensory Neuropathy Type III)

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We have previously reported that heterozygosity for myelin Po gene mutations were associated with Charcot–Marie–Tooth disease type 1B (CMT1B) or Dejerine–Sottas disease. We investigated the Po gene in a family with clinical Dejerine–Sottas disease and found two children were homozygous for a deletion of Phe 64. The parents were heterozygous first cousins with subclinical CMT1B and slow nerve conduction velocities. These results suggest that the effect of homozygous Phe 64 deletion on impairment of myelination is dosage-dependent. Clinical phenotype and/or myelin impairment may be determined both by the type of mutation and by the dosage of mutated gene.

Charcot-Marie-Tooth neuropathy type 1 (CMT1; Hereditary Motor and Sensory Neuropathy type 1, HMSNI) is a genetically heterogenous inherited peripheral neuropathy and is characterized clinically by distal muscle weakness and atrophy, absence of deep tendon reflexes, and pes cavus deformity of the feet (1). The major electrophysiological and neuropathological features are reduced nerve conduction velocities (NCV), and segmental de- and remyelination with onion-bulb formation on peripheral nerve biopsy. CMT1 loci map to chromosome 1q21.3-q23 (CMT1B) (2), chromosome 17p11.2 (CMT1A) (3), and chromosome Xq13.1 (CMTX) (4). CMT1A is the most common subtype of CMT1, and the majority of patients have a large DNA duplication in 17p11.2 including peripheral myelin protein-22 gene (*PMP22*) (5). Several point mutations of *PMP22* have been identified in non-duplication families (6). These findings suggest that alterations in the structure or expression of PMP-22, are responsible for CMT1A. CMT1B is associated with mutations in the major peripheral myelin protein (Po) gene (*Po*) (7–9) and CMTX is related to mutations in the connexin 32 gene (*Cx32*) (10). Dejerine-Sottas disease (DS; HMSN III) has represented a severe and early onset demyelinating disorder pathologically more severe than CMT1. Recently, de novo mutations of *Po* or *PMP22* have been identified in several patients (11–14). In addition, individuals homozygous for a 17p11.2 DNA duplication also present severe clinical manifestations with severe slowing of nerve conduction velocities (5). In the present study, we analyzed *Po* in a family with clinical DS and found two patients homozygous for a deletion of Phe 64.

METHODS AND MATERIALS

Clinical materials. The proband of the family first walked at 18 months. She was referred for medical attention at the age 3 because of an unusual gait with frequent falls and difficulty in standing still without support. On examination, cranial nerves were found to be normal except for fine nystagmus on lateral gaze. She showed swaying gait, hyperextending knees, and steppage gait on a wide base. She could not hop or elevate her forefoot when walking on her heels. She had distal

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weakness of lower limbs and a mild pes cavus deformity of the feet. Romberg's test was positive and deep tendon reflexes were absent except in the triceps. She could not detect vibration, had poor joint position sense, and showed reduced two-point recognition in her hands. At age 11, she was slow with writing and had normal upper limit strength with some distal weakness. She could walk all day without any problems. All, except for one male sibling, were asymptomatic and not available for examination. The affected male sibling failed to walk until 1 year and 6 months old. Cranial nerves were normal except for fine nystagmus on lateral gaze. At age 5, he was found to be hypotonic with no deep tendon reflexes. He had mild pes cavus and palpable enlargement of peripheral nerves. He walked by holding onto furniture on a wide base with hyperextension of the knees. The parents were first cousins and had no symptoms. They were average runners at school with no history of ankle sprains. The father had occasional severe muscle cramps. On examination, they had no muscle wastage, but had moderate pes cavus with no tendon reflexes. The father could not walk on his heels. The father's median and peroneal motor conduction velocities were 31 and 31 m/sec, respectively and the mothers' were 36 and 29 m/sec, respectively. Both parents lacked median and sural sensory action potentials. Both affected children had no elicitable motor or sensory potentials in the sural, median, and peroneal nerves. Sural nerve biopsy of the proband revealed severe loss of fibers of all diameters and incorporation of surviving axons into onion bulb formations. All axons were either demyelinated or surrounded with a thin myelin sheath, and no myelinated fibers were found in teased fibers.

Amplification and sequencing of the coding region of the Po Gene. Genomic DNA was extracted from the leukocytes from the members of the family and healthy control (15). Coding regions of the Po gene were obtained as three DNA fragments from genomic DNA by use of the polymerase chain reaction (PCR) and sequenced as previously described (9).

Allele-specific PCR (*ASPCR*). To confirm the allelism of the candidate mutations, ASPCR analysis was performed essentially by the method of Newton et al. (16) using the following primers; g: 5′-TCTCAGATGACATCTCCTT-3′ and d: 5′-TCCCAGAGCCTGAATAAAGG-3′ resulting in a 397 bp fragment from the normal allele, and h: 5′-CTCAGATGACATCTCCAC-3′ and d resulting in a 393 bp fragment from the mutant allele. The templates used for ASPCR were first PCR products using primers c: 5′-CCATAGGTGCATCTGATTCC-3′ and d. ASPCR products were electrophoresed on a 3% agarose gel and stained with ethidium bromide.

RESULTS

All coding regions of *Po* were sequenced after PCR amplification of genomic DNA. Direct sequencing of DNA from the proband of the family revealed homozygosity for a three base pair deletion in exon 2, which resulted in the deletion of Phe 64 (Fig. 1). Allele-specific PCR (ASPCR) analyses were performed to confirm the allelism of the candidate mutation and confirmed that the proband was homozygous for the mutation (Fig. 2). Her parents were heterozygotes and her brother was also homozygous for the mutation.

DISCUSSION

Po is the most abundant protein of peripheral nervous system myelin and is a homophilic adhesion molecule with a single variable-like immunoglobulin (Ig) domain (17). *Po* spans about 7 kilobases and consists of six exons. Each exon of *Po* corresponds to a proposed functional domain; exon 1 corresponds to signal sequence, exons 2 and 3 to the extracellular domain, exon 4 to the transmembrane domain, and exons 5 and 6 to the cytoplasmic domain. The extracellular domain contains a single N-linked glycosylation site and two cysteines, considered to be responsible for the

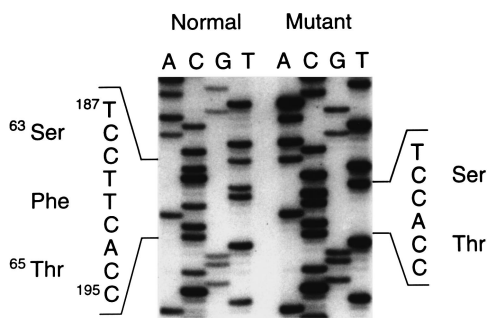

FIG. 1. Deletion of Phe 64 in the Po gene. Genomic DNA samples from the proband of the family and a healthy control were amplified and sequenced. The sequence shown was obtained with primer P02R: 5′-AGCACTTTCTGTTATCC-3′ (7). Nucleotide numbering of the Po cDNA starts at the initiation site of translation.

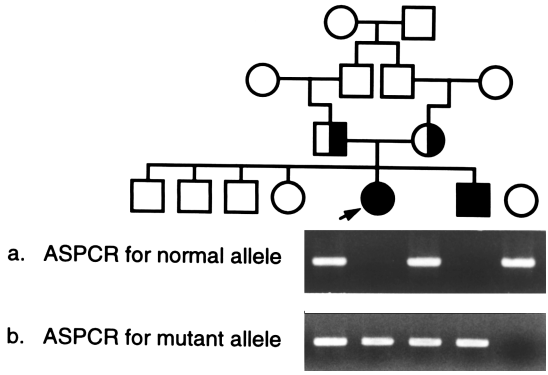

FIG. 2. Analysis of the family using allele-specific PCR. a. ASPCR analysis for the normal allele produced a 397 bp fragment from the normal allele using normal allele-specific primers g and d. The parents and healthy control were positive. b. ASPCR analysis for the mutant allele produced a 393 bp fragment from the mutant allele using mutant allele-specific primers h and d. The parents, the proband, and her brother were positive. The arrow indicates the proband.

integrity of the single Ig-domain. Giese et al. (18) reported that mice homozygous for a null mutation (Po^{-}/Po^{-}) had severely hypomyelinated nerves ten weeks after birth similar to observations in DS, indicating a significant role of Po in compaction of peripheral myelin. In 1993, *Po* point mutations were confirmed in four pedigrees with CMT1B (7–9) and *de novo Po* point mutations were found in two patients with DS (11). At least fourteen variants of *Po* mutations have been reported to date. Recently, mice heterozygous for a null mutation (Po^{+}/Po^{-}) were reported to develop progressive demyelination after long-term observation (19). These findings indicated that reduction in the gene dosage of *Po* similar to point mutations in *Po* would also impair normal development and maintenance of peripheral myelin. Gene dosage effects were demonstrated in *PMP22* duplication and deletion mutations in mice and human (5, 20). All reported patients with *Po* mutations have been heterozygotes and may thus produce only half a dose of normal protein, as in Po^{+}/Po^{-} mice. However, the heterozygotes presumably produce the mutant Po protein, resulting in the formation of homodimers of wild-type Po, heterodimers of wild and mutant-type Po, and homodimers of mutant-type Po (Fig. 3). If only homodimers of wild-type Po are functional, functional dimers would be present in quantities equivalent to 1/4 of the wild-type level in the heterozygotes and 1/2 of the wild-type level in Po^{+}/Po^{-} mice. This dosage effect could explain the discrepancy in phenotype, i.e. symptoms seem to be more severe in the heterozygous patients compared to those in Po^{+}/Po^{-} mice. The parents of this family were heterozygotes with subclinical

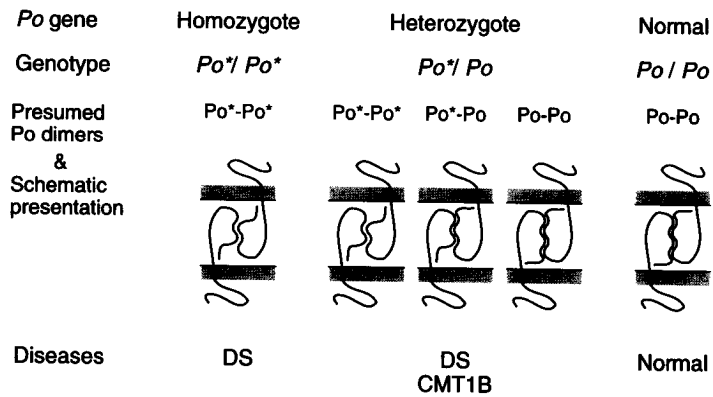

FIG. 3. *Po* gene mutations and clinical phenotypes. *Po** represents mutant *Po* gene.

CMT1B and moderately slow nerve conduction velocities. The gene dosage effects would explain the severe phenotype in the proband and her brother, who were homozygous for a deletion mutation and thus had no normally functional Po homodimers.

On the other hand, some heterozygous Po mutations can demonstrate very severe clinical manifestations (11). Mutated Po protein may play a significant role in the impairment of myelination, which may be toxic for Schwann cells or disturb the function of the normal counterpart via a dominant-negative mechanism (7). In addition, clinical phenotypes are variable within families with CMT1B as in families with CMT1A, and therefore additional factors including unknown compensation mechanisms for Po or disproportional expression of allelic genes in each Schwann cell should also be considered. Further analysis of patients with various phenotypes and generation of targeted mutations will help in understanding the relationship between the genotype and phenotype in *Po* mutations.

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REFERENCES

1. Chance, P. F., and Reilly, M. (1994) *Current Opinion Neurol.* **7,** 367–371.
2. Bird, T. D., Ott, J., and Giblett, E. R. (1982) *Am. J. hum. Genet.* **34,** 388–394.
3. Vance, J. M., Nicholson, G. A., Yamaoka, L. H., Stajich, J., Stewart, C. S., Speer, M. C., Hung, W.-Y., Roses, A. D., Barker, D. F., and Pericak-Vance, M. A. (1989) *Exp. Neurol.* **104,** 186–189.
4. Gal, A., Mucke, J., Theile, H., Wieacker, P. F., Ropers, H.-H., and Wienker, T. F. (1985) *Hum. Genet.* **70,** 38–42.
5. Patel, P. I., and Lupski, J. R. (1994) *Trends Genet.* **10,** 128–133.
6. Valentijn, L. J., Baas, F., Wolterman, R. A., Hoogendijk, J. E., van den Bosch, N. H. A., Zorn, I., Gabreëls-Festen, A. A. W. M., de Visser, M., and Bolhuis, P. A. (1992) *Nature Genet.* **2,** 288–291.
7. Hayasaka, K., Himoro, M., Sato, W., Takada, G., Uyemura, K., Shimizu, N., Bird, T. D., Conneally, P. M., and Chance, P. F. (1993) *Nature Genet.* **5,** 31–34.
8. Kulkens, T., Bolhuis, P. A., Wolterman, R. A., Kemp, S., teNijenhuis, S., Valentijn, L. J., Hensels, G. W., Jennekens, F. G., de Visser, M., Hoogendijk, J. E., and Baas, F. (1993) *Nature Genet.* **5,** 35–39.
9. Hayasaka, K., Takada, G., and Ionasescu, V. V. (1993) *Hum. Molec. Genet.* **2,** 1369–1372.
10. Bergoffen, J., Scherer, S. S., Wang, S., Oronzi Scott, M., Bone, L. J., Paul, D. L., Chen, K., Lensch, M. W., Chance, P. F., and Fischbeck, K. H. (1993) *Science* **262,** 2039–2042.
11. Hayasaka, K., Himoro, M., Sawaishi, Y., Nanao, K., Takahashi, T., Takada, G., Nicholson, G. A., Ouvrier, R. A., and Tachi, N. (1993) *Nature Genet.* **5,** 266–268.
12. Rautenstrauß, B., Nelis, E., Grehl, H., Pfeiffer, R. A., and Van Broeckhoven, C. (1994) *Hum. Molec. Genet.* **3,** 1701–1702.
13. Valentijn, L. J., Ouvrier, R. A., van den Bosch, N. H., Bolhuis, P. A., Baas, F., and Nicholson, G. A. (1995) *Hum. Mut.* **5,** 76–80.
14. Ionasescu, V. V., Ionasescu, R., Searby, Ch., and Neahring, R. (1995) *Neurol.* **45,** 1766–1767.
15. Kunkel, L. M., Smith, K. D., Boyer, S. H., Borgaonkar, D. S., Wachtel, S. S., Miller, O. J., Breg, W. R., Jones Jr., H. W., and Bary, J. M. (1977) *Proc. natn. Acad. Sci. USA* **74,** 1245–1249.
16. Newton, C. R., Graham, A., Heptinstall, L. E., Powell, S. J., Summers, C., Kalsheker, N., Smith, J. C., and Markham, A. F. (1989) *Nucl. Acids Res.* **17,** 2503–2516.
17. Lemke, G., Lamar, E., and Patterson, J. (1988) *Neuron* **1,** 73–83.
18. Giese, K. P., Martini, R., Lemke, G., Soriano, P., and Schachner, M. (1992) *Cell* **71,** 565–576.
19. Martini, R., Zielasek, J., Toyka, K. V., Giesel, K. P., and Schachner, M. (1995) *Nature Genet.* **11,** 281–286.
20. Adlkofer, K., Martini, R., Aguzzi, A., Zielasek, J., Toyka, K. V., and Suter, U. (1995) *Nature Genet.* **11,** 274–280.