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Partial Structure and Mapping of the Human Myelin P_2 Protein Gene

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Abstract: The myelin P_2 protein, a 14,800-Da cytosolic protein found primarily in peripheral nerves, belongs to a family of fatty acid binding proteins. Although it is similar in amino acid sequence and tertiary structure to fatty acid binding proteins found in the liver, adipocytes, and intestine, its expression is limited to the nervous system. It is detected only in myelin-producing cells of the central and peripheral nervous systems, i.e., the oligodendrocytes and Schwann cells, respectively. As part of a program to understand the regulation of expression of this gene, to determine its function in myelin-producing cells, and to study its role in peripheral nerve disease, we have isolated and characterized overlapping human genomic clones encoding the P_2 protein. We report here on the partial structure of this gene, and on its localization within the genome. By using a panel of human–hamster somatic cell hybrids and by in situ hybridization, we have mapped the human P_2 gene to segment q21 on the long arm of chromosome 8. This result identifies the myelin P_2 gene as a candidate gene for autosomal recessive Charcot–Marie–Tooth disease type 4A. **Key Words:** Myelin—P_2 protein—Human P_2 gene—Chromosomal mapping.
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Myelin, a structure unique to the nervous system, is a specialization of the plasma membrane of Schwann cells and oligodendrocytes. During development, the interaction between axon and myelinating cell triggers a cascade of events that results in the expansion of membrane surface area and expression of specific proteins that play a role in establishing and maintaining the lamellar structure of myelin. The major myelin proteins include myelin basic protein, proteolipid protein, P_0, and myelin-associated glycoprotein. These appear to serve a structural role during myelination (Martenson, 1992).

The P_2 protein is a minor component of the myelin proteins, making up less than 5% of the proteins of myelin (for review, see Martenson, 1992, Chapter 15). Although it is more easily detected in peripheral myelin, immunohistochemical studies have demonstrated its presence in the central nervous system as well (Trapp et al., 1983). P_2 has been purified from bovine, rabbit, and human nerves, and the amino acid sequences of these proteins determined (Kitamura et al., 1980; Ishaque et al., 1982; Suzuki et al., 1982). Comparison with sequences of other proteins revealed a striking similarity to members of the fatty acid binding protein (FABP) family, which includes rat liver, heart, and intestinal FABPs, cellular retinoic acid binding protein, cellular retinol binding protein, and an adipocyte lipid binding protein ($aP_2/422$) (Narayanan et al., 1988). The members of this family of proteins also have very similar tertiary structures, consisting of two sheets made up of antiparallel β-strands surrounding a hydrophobic core in which the ligand is carried (Jones et al., 1988; Sacchettini et al., 1988).

We have previously isolated and characterized a cDNA clone encoding the rabbit myelin P_2 protein (Narayanan et al., 1988) and have used this to study the structure of the mouse P_2 gene (Narayanan et al., 1991) and its regulation during myelination. Here we report the isolation and analysis of the structure of the human myelin P_2 gene and its mapping to the long arm of chromosome 8.

MATERIALS AND METHODS

A human (lung fibroblast) genomic library, constructed and amplified once in the phage λFIX, was purchased from

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Abbreviation used: FABP, fatty acid binding protein.

Stratagene, Inc. (La Jolla, CA, U.S.A.). Screening of this library with ^{32}P-labelled DNA probes and characterization of individual clones follow standard procedures (Sambrook et al., 1989).

DNA from human–CHO (Chinese hamster ovary) cell somatic cell hybrid lines was purchased from the BIOS Corp. (New Haven, CT, U.S.A.). Southern blots were prepared with these DNA samples digested with either *Eco*RI or *Hin*dIII, hybridized with a segment of the human P_2 gene, and washed at high stringency (65°C in 0.1 × SSC/0.1% sodium dodecyl sulfate, where 1 × SSC is 0.15 *M* NaCl/0.015 *M* sodium citrate, pH 7).

For chromosomal in situ hybridization studies, the DNA probe was nick-translated with biotin-14-dATP (BRL, Inc., Gaithersburg, MD, U.S.A.), with 40% incorporation as determined by tritium tracer incorporation. Slides with chromosome spreads were made from normal male lymphocytes cultured with bromodeoxyuridine (Bhatt et al., 1988). Fluorescence in situ hybridization was performed as described (Lichter et al., 1990) with modifications. Probe mix [2 × SSC, 50% formamide, 10% dextran sulfate, 10 ng/μl biotinylated probe, 2 μg/μl Cot-1 DNA (BRL), and 6 μg/μl salmon sperm DNA] was denatured at 70°C for 5 min, preannealed at 37°C for 30 min, placed on the slides, and hybridized at 37°C overnight. Slides were washed in 65% formamide/2 × SSC at 43°C for 20 min and two changes of 2 × SSC at 37°C for 5 min each. Biotinylated probe was detected with fluorescein isothiocyanate-avidin and amplified with biotinylated anti-avidin by using reagents from an in situ hybridization kit (Oncor Inc., Gaithersburg, MD, U.S.A.), following the manufacturer's instructions. These cells were photographed on color slide film (Kodak Ektachrome EES1600). To determine the specific chromosome and band location of the signals, the hybridized slide was G-banded by fluorescence plus Giemsa (Bhatt et al., 1988), photographed, and aligned with the color slides.

RESULTS AND DISCUSSION

The human genomic library was screened with the rabbit cDNA probe, and a single positive clone, HLF 14.2-1, containing a 13-kbp insert was identified (Narayanan and Tennekoon, 1989). The restriction sites on this clone were mapped (see Fig. 1A) and several overlapping *Xba*I and *Eco*RI segments subcloned into Bluescript (Stratagene) plasmid. By Southern blotting with the cDNA and by DNA sequencing, it was determined that this phage clone contained a single exon (exon 1). The 3′ end of this phage clone was then used to rescreen the genomic library, resulting in the isolation of an overlapping clone, HLF 32.1-1, as diagrammed in Fig. 1A. A 3-kbp *Sst*I fragment that hybridized with the cDNA probe, and hence was thought to contain exons, was sequenced. Comparison of this sequence with that of the rabbit cDNA allowed us to determine that this segment contained exons 1, 2, and 3 of the human P_2 gene. In order to be sure that the gene segment we had isolated was indeed the human P_2 gene and not another closely related gene, we isolated several cDNA clones from a human spinal cord cDNA library (ATCC no. 37434; R. Lazzarini), using the rabbit P_2 cDNA as probe. One of these strongly hybridizing clones, HC9.1, contained a 1,200-bp insert. The sequence of this human P_2 cDNA clone, HC9.1, is identical to the sequence reported by Hayasaka et al. (1991). Exon 4 of the human P_2 gene is located within a 3-kbp *Eco*RI fragment (Fig. 1A), as determined by Southern blot hybridization studies of phage DNA with segments of the human cDNA, and by partial sequencing of phage DNA directly with exon 4-specific primers (data not shown). Shown in Fig. 1B is partial sequence information from the genomic clone, HLF 32.1-1, including exons 1, 2, and 3, as determined by comparison with the human P_2 cDNA.

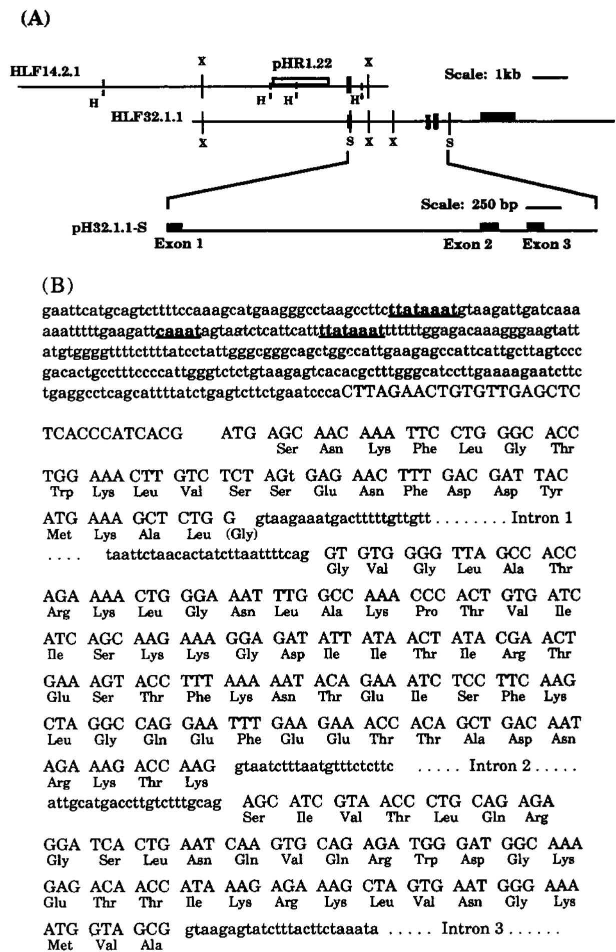

FIG. 1. A: Restriction map of overlapping segments of the human P_2 gene. The segment of clone HLF 14.2-1 that was used as a probe for Southern analysis of somatic cell hybrid DNAs, pHR1.22, is indicated as a box and is a 2-kbp *Eco*RI fragment. Exons are shown as black boxes. H, *Hin*dIII; X, *Xba*I; S, *Sac*I. **B:** Partial DNA sequence of the human P_2 gene. The segments that are identical to the human P_2 cDNA (exons 1, 2, and 3) are uppercase. A single nucleotide difference in the third position for serine (amino acid no. 13) between this sequence and that of Hayasaka et al. (1991) is shown in lowercase. Indicated below the nucleotide sequence is the corresponding human P_2 amino acid sequence. Exon–intron splice junctions conform to the GT-AG rule. Potential TATA and CAAT boxes within about 250 bp upstream of the initiator ATG are underlined.

% Discordant

Human Chromosomes

Bands	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Hind III 2.1, 0.7 kb	32	24	36	12	76	28	20	0	32	32	24	36	36	40	28	24	20	28	40	32	40	28	16	36
EcoRI 2.0 kb	28	22	33	11	83	28	17	0	28	33	28	39	28	44	28	22	22	28	39	28	39	28	11	39

% discordant = (presence of chromosome / absence of band) + (absence of chromosome / presence of band)
total of 25 hybrid cell lines screened with Hind III digests or a total of 18 hybrid cell lines screened with EcoRI digests

FIG. 2. DNA from somatic cell hybrid lines were digested with either *Hin*dIII (25 lines tested) or *Eco*RI (18 lines tested), and Southern blots hybridized with a 2-kbp human P_2 genomic segment. Cell lines were scored according to the presence of 2.1-kbp and 0.7-kbp bands in the *Hin*dIII-digested DNA and a 2-kbp band in the *Eco*RI-digested DNA. Summarized in this diagram is the analysis of discordance between presence of the hybridizing *Hin*dIII and *Eco*RI bands and each of the human chromosomes. There is 100% concordance between the presence of chromosome 8 and these hybridizing bands, and greater than 11% discordance between the presence of these bands and each of the chromosomes other than 8.

Potential TATA and CAAT boxes lying within about 250 bp upstream of the initiator methionine are underlined. The locations of the exon–intron boundaries in this gene segment conform to the GT-AG rule, and are very similar to that of the mouse P_2 gene and the other FABP genes (Narayanan et al., 1991), reinforcing the idea that these all belong to a family of homologous genes.

Localization of the P_2 gene within the human genome was done by Southern blot analysis of somatic cell hybrids, and by in situ hybridization on metaphase spreads of human chromosomes. A 2-kbp fragment of the cloned human P_2 gene containing sequences upstream of exon 1 (pHR1.22; see Fig. 1A) was radiolabelled with [^{32}P]dCTP and used to probe Southern blots prepared from a set of somatic cell hybrid DNAs (BIOS Corp.) digested with *Hin*dIII or *Eco*RI. Under wash conditions of high stringency (65°C in 0.1 × SSC), the probe detected 2.1-kbp and 0.7-kbp bands in the *Hin*dIII-digested DNA and a 2-kbp band in the *Eco*RI-digested DNA (data not shown). The result of our analysis of 25 somatic cell hybrid lines is summa-

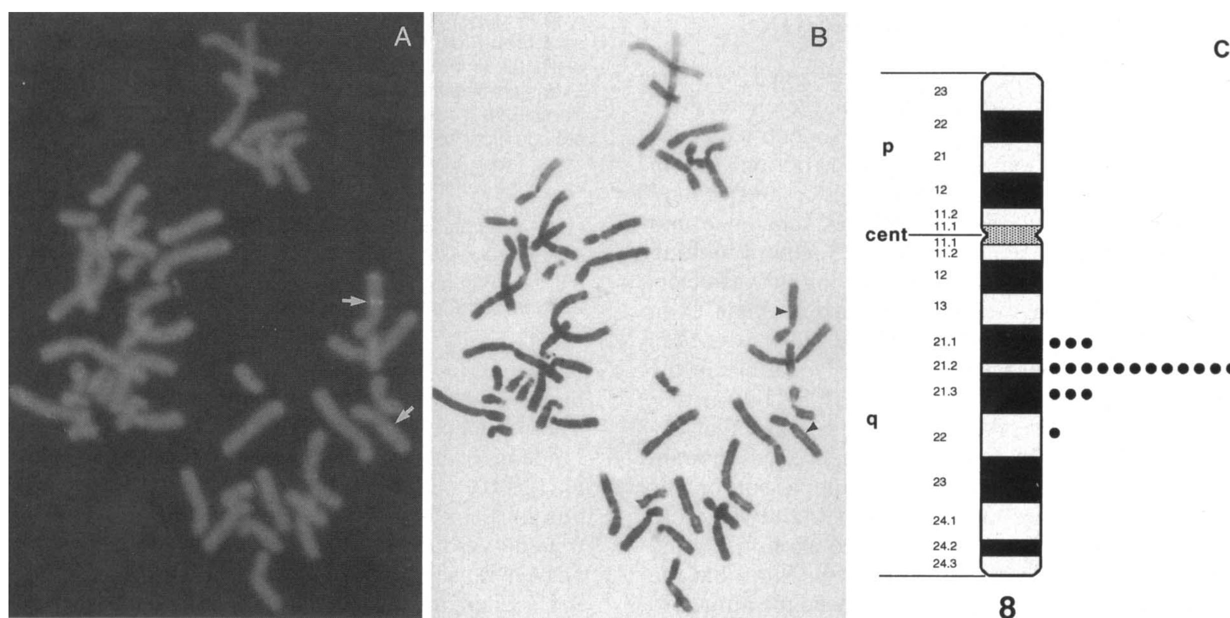

FIG. 3. A: Metaphase chromosomes hybridized with genomic clone HLF 32.1-1. Arrows indicate paired signals. **B:** Same metaphase as A, after G-banding identifies chromosomes with signal (arrowheads) as chromosome 8. **C:** Ideogram of human chromosome 8, showing localization of the myelin P_2 gene to 8q21.2. Each dot represents a paired signal seen on metaphase chromosomes, diagrammed to the right of the band on which it was localized.

rized in Fig. 2. There is 100% concordance between the presence of the 2.1- and 0.7-kbp *Hin*dIII bands and chromosome 8 in hybrids (nos. 803, 811, 909, 967, 1,006) of the panel. The bands are absent in hybrids without chromosome 8, and there is 12% or greater discordance between the presence of this band and all chromosomes other than 8. There is 100% concordance between the presence of the 2-kbp *Eco*RI band and chromosome 8 in hybrids (nos. 909, 967, 1,006) of a subset of 18 hybrid lines digested with *Eco*RI. There was greater than 11% discordance between the presence of this band and all chromosomes other than chromosome 8. No homologous CHO *Hin*dIII or *Eco*RI DNA fragments were detected at this stringency. This allows us to conclude that the human myelin P_2 protein gene maps to chromosome 8.

The entire phage clone, HLF 32.1-1 (see Fig. 1A), was used as probe for localization on cytogenetic preparations by in situ hybridization. Purified phage DNA was nick-translated with biotin-14-dATP and hybridized to metaphase chromosomal spreads from normal male lymphocytes. Analysis of 28 metaphase cells showed that 18 cells (64%) had at least one pair of signals (involving both chromatids of a single chromosome). A total of 25 paired signals were seen, all located on the long arm of a small C-group chromosome. The hybridized slides were then G-banded, photographed, and aligned with the color slides. Twenty signals were analyzable after banding; all were on chromosome 8, on band q21, with most on q21.2 (Fig. 3). This allowed us to localize the P_2 gene to chromosomal band 8q21 on the long arm of chromosome 8, confirming the result obtained independently by somatic cell hybrid analysis.

As part of a program aimed at obtaining a better understanding of the molecular biology of myelination, we have cloned and characterized rabbit, murine, and human genes encoding the myelin P_2 protein. Based on the structure of these genes, the P_2 protein has been assigned to a family of FABPs. Purified bovine P_2 protein specifically binds oleic acid, retinoic acid, and retinol (Uyemura et al., 1984). Together with data on the crystal structure of the bovine P_2 protein, this reinforces the idea that the P_2 protein may serve as a fatty acid carrier. Whether the ability of P_2 to bind specifically to fatty acids is related to its role in myelination is unknown. No human or animal disorders are known to be caused by a defect in the P_2 gene. A recessive neuropathy, Charcot–Marie–Tooth disease type 4A, has been linked recently to markers from chromosome 8q13-21.1 (Ben Othmane et al., 1993), raising the possibility that this is due to a defect in the P_2 gene. The mapping of the myelin P_2 gene to chromosome 8q21 reported in this article may aid in the study of this and other inherited peripheral nerve disorders.

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REFERENCES

Ben Othmane K., Hentati F., Lennon F., Ben Hamida C., Blel S., Roses A. D., Pericak-Vance M. A., Ben Hamida M., and Vance J. M. (1993) Linkage of a locus (CMT4A) for autosomal recessive Charcot–Marie–Tooth disease to chromosome 8q. *Hum. Mol. Genet.* **2,** 1625–1628.

Bhatt B., Burns J., Flannery D., and McGee J. (1988) Direct visualization of single copy genes on banded metaphase chromosomes by nonisotopic *in situ* hybridization. *Nucleic Acids Res.* **16,** 3951–3961.

Hayasaka K., Nanao K., Tahara N., Sato W., Takada G., Miura M., and Uyemura K. (1991) Isolation and sequence determination of cDNA encoding P_2 protein of human peripheral myelin. *Biochem. Biophys. Res. Commun.* **181,** 204–207.

Ishaque A., Hoffman T., and Eylar E. H. (1982) The complete amino acid sequence of the rabbit P_2 protein. *J. Biol. Chem.* **257,** 592–595.

Jones T. A., Bergfors T., Sedzik J., and Unge T. (1988) The three-dimensional structure of the P_2 myelin protein. *EMBO J.* **7,** 1597–1604.

Kitamura K., Suzuki M., Suzuki A., and Uyemura K. (1980) The complete amino acid sequence of the P_2 protein in bovine peripheral nerve myelin. *FEBS Lett.* **115,** 27–30.

Lichter P., Tang C., Call K., Hermanson G., Evans G., Housman D., and Ward D. (1990) High resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. *Science* **247,** 64–69.

Martenson R. E., ed (1992) *Myelin: Biology and Chemistry.* CRC Press, Boca Raton, Florida.

Narayanan V., and Tennekoon G. (1989) Cloning of the human myelin P_2 protein gene: analysis of its structure and regulation. *Soc. Neurosci. Abstr.* **15,** 1269.

Narayanan V., Barbosa E., Reed R. R., and Tennekoon G. I. (1988) Characterization of a cloned cDNA encoding rabbit myelin P_2 protein. *J. Biol. Chem.* **263,** 8332–8337.

Narayanan V., Kaestner K. H., and Tennekoon G. I. (1991) Structure of the mouse myelin P_2 protein gene. *J. Neurochem.* **57,** 75–80.

Sacchettini J. C., Gordon J. I., and Banaszak L. J. (1988) The structure of crystalline. *Escherichia coli*-derived rat intestinal fatty acid-binding protein at 2.5 A resolution. *J. Biol. Chem.* **263,** 5815–5819.

Sambrook J., Fritsch E. F., and Maniatis T. (1989) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Suzuki M., Kitamura K., Sakamoto Y., and Uyemura K. (1982) The complete amino acid sequence of human P_2 protein. *J. Neurochem.* **39,** 1759–1762.

Trapp B. D., Itoyama Y., MacIntosh T. D., and Quarles R. H. (1983) P_2 protein in oligodendrocytes and myelin of the rabbit central nervous system. *J. Neurochem.* **40,** 47–54.

Uyemura K., Yoshimura K., Suzuki M., and Kitamura K. (1984) Lipid binding activities of the P_2 protein in peripheral nerve myelin. *Neurochem. Res.* **9,** 1509–1514.