Jpn J Human Genet 37, 303–306, 1992

Short Communication

LOCALIZATION OF PMP-22 GENE (CANDIDATE GENE FOR THE CHARCOT-MARIE-TOOTH DISEASE 1A) TO BAND 17p11.2 BY DIRECT R-BANDING FLUORESCENCE *IN SITU* HYBRIDIZATION

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Summary We mapped PMP-22 gene, candidate gene for the Charcot-Marie-Tooth disease (CMT) 1A, by direct R-banding fluorescence *in situ* hybridization. The signals of PMP-22 probe were localized to chromosome band 17p11.2. The present result was within the map position of the CMT 1A gene by genetic linkage analysis, and strongly indicated that PMP-22 gene is a candidate gene for the CMT 1A.

Key Words mapping of PMP-22 gene, direct R-banding FISH, 17p-11.2

INTRODUCTION

Myelin is a multilamellar compacted membrane structure that surrounds and insulates the axon, facilitating the conduction of nerve impulses. Schwann cells are responsible for myelin formation in the peripheral nervous system. P0, P2, myelin basic protein (MBP) and myelin associated glycoprotein (MAG) are the major myelin proteins of peripheral nervous system (see for review, Morell *et al.*, 1989). In addition, PMP-22 is integral membrane protein of the myelin in the peripheral nervous system (Suter *et al.*, 1992). The mutation of PMP-22 gene was identified as a primary defect of autosomal dominant trembler mouse (Suter *et al.*, 1992), which maps to mouse chromosome 11 (Davisson and Roderick, 1978). In the peripheral myelin disorders, Charcot-Marie-Tooth disease (CMT) 1A is autosomal dominant and linked to chromosome 17p11.2-p12 (MacAlpine *et al.*, 1990).

Received July 6, 1992; Accepted August 10, 1992.
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Genes in that region are conserved on mouse chromosome 11 (Münke and Francke, 1987). By analogy, PMP-22 could be a candidate gene for CMT 1A.

Recently, direct R-banding fluorescence *in situ* hybridization (FISH), which is based on FISH combined with replicated prometaphase R-bands, has proved powerful for the precise localization of genes and DNA markers on the bands (Takahashi *et al.*, 1990, 1991; Yamakawa *et al.*, 1991). For the detection of single copy DNA markers with inserts of less than 5 kb, an amplification procedure with goat antibiotin antibody and fluorescent antigoat IgG has been developed (Viegas-Péquignot *et al.*, 1989). Till now, this amplification procedure have been applied using a genomic clone containing a 2.1 kb insert and cDNA clone with a 0.5 kb insert (Takahashi *et al.*, 1991; Lemieux *et al.*, 1992).

We have cloned a full length of a cDNA of human PMP-22 (Hayasaka *et al.*, 1992). In the present communication, we report a localization of PMP-22 gene by the combined FISH system.

MATERIALS AND METHODS

DNA probe. Isolation of the cDNA clone encoding PMP-22 has been previously reported (Hayasaka *et al.*, 1992). A plasmid clone (J1h), which is a subclone of a 1.8 kb *Eco*RI fragment in pUC 19 derived from the lambda gt11 J1h clone, was used as a DNA probe in the present study.

Direct R-banding FISH. The procedures of direct R-banding FISH have been described elsewhere (Takahashi *et al.*, 1990). For the detection of the signals of PMP-22 probe, we applied the amplification procedures reported by Viegas-Péquignot *et al.* (1989) with slight modification (Takahashi *et al.*, 1991).

RESULTS AND DISCUSSION

We have examined 100 (pro)metaphase plates showing typical R-bands. Of them, 1% of such R-banded chromosomes exhibited complete double spots on both chromatids of both homologs, 20% were incomplete single and/or double spots on either or both homologs, and in the others (79%) no spots were detectable. The fluorescent signals were localized to the R-positive p11.2 band very close to R-negative p12 band of chromosome 17 (Fig. 1, a–d). No other double spots were observed. Thus, PMP-22 gene could be assigned to band 17p11.2. The CMT 1A has been localized to 17p11.2-p12 by genetic linkage and cytogenetic analyses (McAlpine *et al.*, 1990; Patel *et al.*, 1990; Vance *et al.*, 1991; Lupski *et al.*, 1991; Raeymaekers *et al.*, 1991). The present result was within the map position of the CMT 1A gene by genetic linkage analysis, and strongly indicated that PMP-22 gene is a candidate gene for the CMT 1A.

As for the assignments of relevant genes, CMT 1B (1q21.1-q23.3), CMT X1 (Xq11-q13), CMT X2 (Xp22.2), and CMT X3 (Xq26) have been localized by genetic

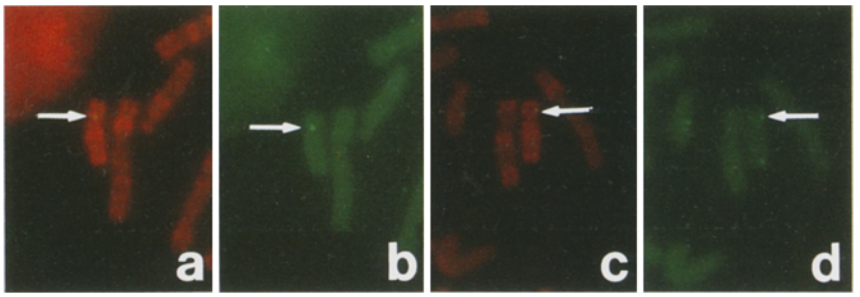

Fig. 1. Partial R-banded (pro)metaphases after FISH with biotinylated PMP-22 gene. Arrows indicate signals on 17p11.2. a and c, Nikon B-2A filter; b and d, Nikon B-2E filter.

linkage analyses (see for review, Frézal and Schinzel, 1991).

It is worth noting that PMP-22 gene was located to R-positive 17p11.2 band. In mammals, early replicating R-positive bands have been known to contain housekeeping genes, which are GC-rich in their DNA sequences (Holmquist *et al.*, 1982; Goldman *et al.*, 1984). Further work is being directed at examining the structure of genomic PMP-22 DNA sequences for this notion.

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