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Regional localization of rat peripheral myelin protein 22 (*Pmp22*) gene to Chromosome 10q22 by FISH

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Species: *Rattus norvegicus*
Locus name: Peripheral myelin protein 22
Locus symbol: *Pmp22*
Map position: Chromosome (Chr) 10q22
Method of mapping: Fluorescence in situ hybridization (FISH): R-banding of chromosomes by DAPI-staining (after pretreatment with RNase and pepsin, followed by FISH procedure). Chromosomes were derived from the male fibroblast rat cell line RAT 1 [1], which has a karyotype containing only one marker chromosome (fusion between Chrs 1 and 3), which, however, had no disturbing influence on our study (Fig. 1A).
Hybridization, washing, detection, and evaluation were done as described elsewhere [2].

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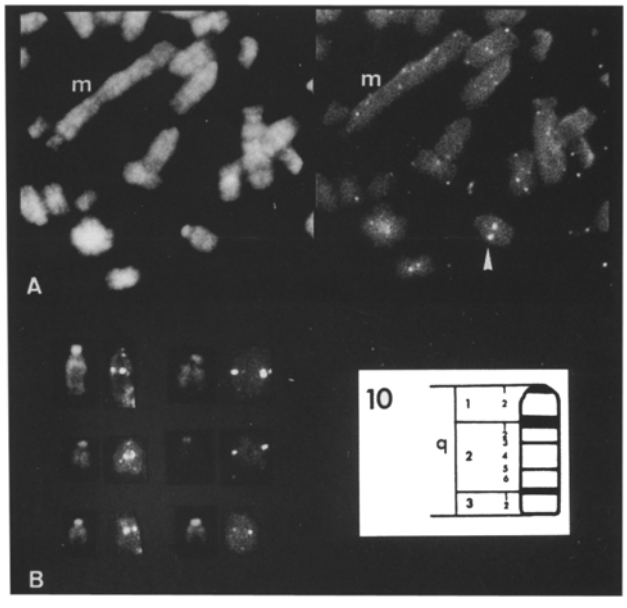

Fig. 1. FISH results after hybridization with the pCD25 probe specific for *Pmp22* DNA. **(A)** The partial metaphase shows a specific signal (arrowhead) on Chr 10, identified by DAPI banding pattern (left part of Fig. 1A). The marker chromosome (m) (fusion between Chr 1 and 3) of the used cell line RAT 1 is visible as well. **(B)** Six Chr 10 of five metaphases in two columns are shown. Chromosomes on the left are DAPI stained, and those on the right reveal the specific hybridization signals on Chromosome band 10q22 (idiogramm of Chr 10 on the right part of Fig. 1B).

Molecular reagents: pCD25, a cDNA clone containing an almost full-length insert (1.8 kB) of *Pmp22* gene [3].
Discussion: Gene dosage effects of human *PMP22* result in Charcot-Marie-Tooth disease (=CMT: increased dosage) or hereditary neuropathy with liability to pressure palsies (=HNPP: decreased dosage) [4]. The human *PMP22* gene is located on Chr 17p11.2 [5], and point mutations in this region are described to be pathogenetic, too [4]. One of these point mutations is found in the murine *Pmp22* gene as well, causing the Trembler^J mouse phenotype there [6]. Murine *Pmp22* gene is located on Chr 11 [7]. Rat *Pmp22* has been assigned to Chr 10 by Yeung and associates [8]. Our result confirms this finding and refines the chromosomal localization to 10q22.

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Multipoint genetic linkage analysis of the m2 human muscarinic receptor gene

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Species: Human
Locus name: Cholinergic receptor, muscarinic 2
Locus symbol: CHRM2
Map position: 7q31–35, D7S530–D7S512–D7S500–D7S509–CHRM2–D7S495, lod score 18.50
Method of mapping: Multipoint linkage analysis with LINKAGE [1], assuming codominant inheritance and no sex difference in recombination.
Database deposit information: Genbank accession number U19800
Molecular reagents: The downstream and upstream primers, m2p12A and m2p12B, had the following sequences: 5′-TTC AGC GTC TCT AAT ACC AAA TCT-3′ and 5′-AGC CAC CAT AAA CCA TAA ACA CTT-3′, respectively. These primers cover a predicted product of 157 bp.

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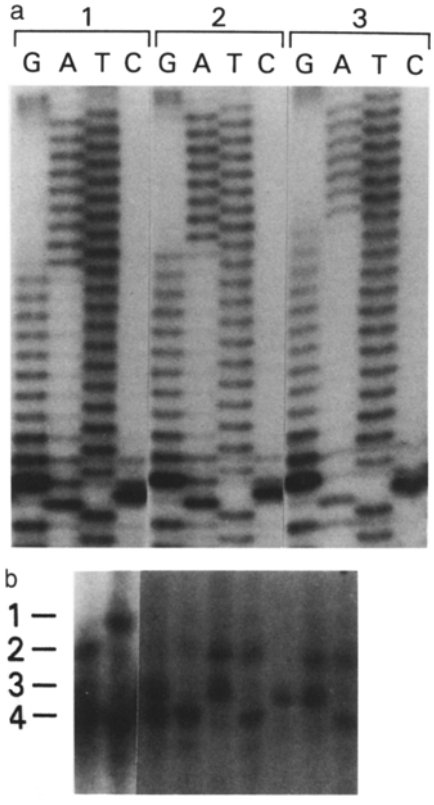

Fig. 1. SSCP polymorphic variants in the m2 muscarinic receptor gene. **A.** The four different alleles in nine individuals detected by SSCP are shown as indicated by the numbers. **B.** Sequence of the SSCP homozygotes for alleles 4 (panel 1), 3 (panel 2), and 2 (panel 3).

Allele detection: PCR products on nondenaturing SSCP gels detected four alleles. Sequencing of homozygotes revealed that alleles 2, 3, and 4 correspond to fragments containing $(GT)_{13}(AT)_7$, $(GT)_{12}(AT)_8$, and $(GT)_{11}(AT)_9$ respectively. Frequencies of alleles 1, 2, 3, and 4 were 0.01, 0.13, 0.45, and 0.41 respectively. No polymorphisms were detected on sequencing gels.

Previously identified homologs: Bovine (BOVMRM2SUB), pig (PIGACHRA1, PIGCHRA2, PIGCHRA3), chicken (CHKMRMR)

Discussion: Muscarinic receptors mediate many of the actions of the neurotransmitter acetylcholine in the central and peripheral nervous system. These receptors act through GTP binding proteins. The m2 receptor is one of five different muscarinic receptors that have been cloned [2]. The m2 receptor had been previously mapped by in situ hybridization to the 7q35-36 region [3]. Previous studies on CEPH data show the Chromosome 7q35-qter gene order to be D7S530–D7S512–D7S500–D7S509–D7S495 [4].

The finding of polymorphisms on SSCP but not on denaturing gels suggests that for compound dinucleotide repeats, conformation-based rather than size-based polymorphism might be easier to detect. It is, therefore, important to try both sequencing and SSCP gels when searching for individual variations in repeats.

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