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Molecular Characterization of the Mouse *mtprd* Gene, a Homologue of Human *TPRD*: Unique Gene Expression Suggesting Its Critical Role in the Pathophysiology of Down Syndrome[1]

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We and others recently isolated a human *TPRD* gene, possessing a motif of the tetratricopeptide repeat (TPR), from the Down syndrome-critical region (DCR) of chromosome 21q22.2. In this study, we isolated a mouse homologue of *TPRD* cDNA, *mtprd*, and examined its expression profile in mouse embryos. The gene was mapped to mouse chromosome 16C3.3-4, consistent with the location of DCR, and encodes 1,979 amino acid residues with 76% identity to TPRD. The mtprd protein has three units of the TPR motif with 91% homology to TPRD. The protein also has two regions homologous to several matrix proteins with 86 and 70% identities to those of TPRD. Several splicing variants of the 5′ portion of the open reading frame of *mtprd* were identified by RT-PCR and sequencing of mRNAs. *In situ* hybridization showed that *mtprd* is ubiquitously expressed in mouse embryos but predominantly in the central nervous system, including the telencephalon, mesencephalon, and metencephalon. These results suggest that the *TPRD* gene is one of the genes responsible for not only the morphological anomalies but also the neurological abnormalities observed in Down syndrome. The presence of splicing variants indicates that the protein may also have several isoforms in mice.

Key words: Down syndrome, *mtprd* gene, tetratricopeptide repeat, *TPRD* gene.

Down syndrome (DS), the most common chromosome aberration, is caused by trisomy of chromosome 21. DS manifests complex phenotypes, including a characteristic facial appearance, mental retardation, neonatal hypotonia, congenital heart defects, acute leukemia, and abnormalities of the gut and endocrine system (*1, 2*). In addition, all individuals with DS exhibit Alzheimer disease (AD)-like neuropathology by age 30-40 years. The cytogenetic and clinical correlations of patients with partial trisomy 21 indicate that one or multiple gene(s) in a region of 2-4 Mb in 21q22.2, designated as the Down syndrome-critical region (DCR), may contribute to the pathogenesis of DS (*3-5*). Thus, cloning and characterization of genes in the DCR are important for elucidating the molecular basis of DS.

We (*6*) and others (*7*) recently isolated a novel human cDNA, *TPRD*, which belongs to the tetratricopeptide repeat (TPR) gene family from the DCR. Northern blot analysis revealed that *TPRD* is expressed in all the human fetal and adult tissues examined. *TPRD* encodes a putative protein (TPRD) of 2,025 amino acid residues. Although the physiological function of TPRD has not been determined, protein database searches revealed that the N-terminal region of the predicted TPRD protein has three units of a 34-amino-acid repeat similar to the TPR motif, which may mediate protein-protein interactions. The TPR motif has been identified in proteins involved in the regulation of RNA synthesis or mitosis (*8-13*). The central and C-terminal regions of TPRD exhibit homology to several matrix proteins such as trichohyalin, an intermediate filament-associated protein (*14*), and bullous pemphigoid antigen, an adhesion junction plaque protein (*15*). In addition, TPRD has four hydrophobic regions, which are likely to be transmembrane domains, and three highly basic regions which resemble the consensus sequence of the bipartite nuclear location signal. From these data, it is possible that the *TPRD* gene encodes a matrix protein having some function in the nucleus and that the overexpression of the *mtprd* gene may be involved in several morphological anomalies observed in DS (*6, 7*).

To explore the pathophysiological significance of the gene

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Abbreviations: AD, Alzheimer disease; DCR, Down syndrome-critical region; DS, Down syndrome; ORF, open reading frame; TPR, tetratricopeptide repeat; 3′UTR, 3′-untranslated region.

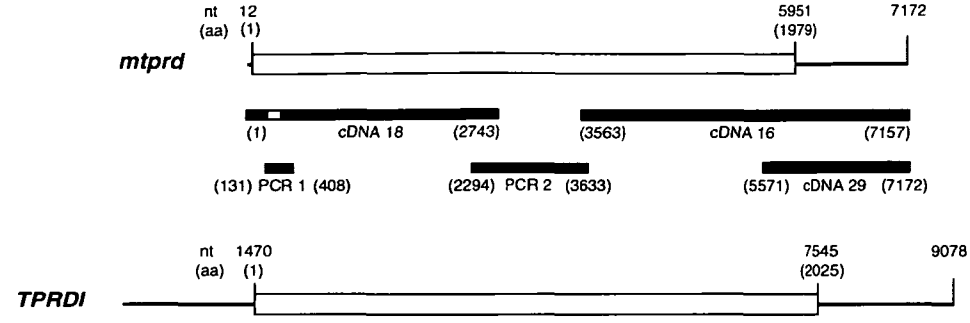

Fig. 1. **Schematic map showing the three overlapping mouse cDNA clones and two RT-PCR products encompassing 7,172 bases of the mouse *mtprd*.** ORFs are indicated by boxes and the non-coding regions by bars in both *mtprd* and *TPRDI* (*6*). cDNAs from library and RT-PCR products are indicated by black and gray bars, respectively. The lacking portion in cDNA clone 18 is shown by an open bar.

in DS, it is important to clarify its expression profile during the embryonic period. Thus, in the present study, we isolated a mouse homologue of *TPRD*, defined functionally relevant sequence regions *via* their evolutionary conservation, and then examined its expression profile in day 12 and 15 mouse embryos.

MATERIALS AND METHODS

Identification of the mtprd cDNA Sequence—A mouse embryo λgt11 cDNA library derived from a cross between ICR outbred females and outbred Swiss Webster males (Clontech) was screened by plaque hybridization. Partial human cDNAs covering the entire human *TPRDI* cDNA (*6*) were labeled by random priming (Megeprime; Amersham) with [α-^{32}P]dCTP and used as initial probes for hybridization. Hybridization was performed under appropriate conditions and the filters were finally washed with 0.1× SSC, 0.1% SDS at 60°C. Autoradiography was performed using X-ray films for 24-48 h. Both ends of the inserts of 21 positive clones were sequenced and assembled. Additional cDNA clones were identified by repeated screening with mouse-derived cDNAs and were then assembled. cDNA clones 16, 18, and 29 were purified, and then subcloned into the *Eco*RI site of pUC19 for sequencing. Two gaps in the cDNA sequence were amplified by PCR using two sets of primers flanking these regions [5′-AACCCAGCTTTACTATGAAGG-3′ (1F: nt 131-151) and 5′-CTTCCATCAACTCAAGATGTTG-3′ (4R: nt 387-408); and 5′-GAAGTTAAGACTGAAAGAAGAC-3′ (25F: nt 2294-2315) and 5′-GATTTAGTGGTAGTTTAACTCG-3′ (32R: nt 3612-3633)] with the mouse embryo cDNA library as a template. The PCR products were subcloned into the T-vector (Novagen) for sequencing.

Sequence Analysis—DNA sequencing was performed by the dideoxy chain terminator method (*16*) and analyzed by a HITACHI SQ-5500 sequencer. Both strands of the isolated subclones were sequenced. The subclones derived from the PCR product were sequenced several times to rule out the possibility of Taq polymerase errors. The sequence alignments were performed using a computer software program, GENETYX-MAC/ATSQ (Japan Software Development), and the DNA sequence and deduced amino acid residues were analyzed with GENETYX-MAC (Japan Software Development).

Chromosome Localization—The chromosomal localization of the *mtprd* gene was determined by fluorescence *in situ* hybridization (FISH) as described previously (*17*). The biotin-14-dATP-labeled probe derived from nt 1844-3376 of *mtprd* was hybridized to R-banded chromosomes from cultured splenocytes of male mice (BALB/c) prepared as described (*18*). After overnight hybridization, the slides were washed in 50% formamide/2×SSC at 42°C for 15 min, followed by a wash in 1×SSC at room temperature for 15 min. The slides were then blocked with 4% bovine serum albumin/4×SSC at 37°C for 30 min. Signal amplification was achieved using rabbit anti-biotin (ENZO), fluorescein-labeled goat anti-rabbit IgG (ENZO), and Cy2-labeled donkey anti-goat IgG (Amersham). Chromosomes were counterstained with propidium iodide. Only doublet hybridization signals were scored.

Northern Blot Analysis—The ^{32}P-labeled probe comprising nt 1844-2476 of *mtprd* was hybridized to 2 μg of poly(A)$^+$ RNA isolated from several tissues of adult mouse or from 7-17 day mouse embryos (Clontech). Hybridization was performed 1-2 h at 68°C with a ExpressHyb™ Hybridization solution (Clontech) under the conditions recommended by the supplier. The filters were finally washed with 0.1×SSC and 0.1% SDS at 50°C, and then exposed to X-ray film for 24-48 h.

RNA In Situ Hybridization—*In situ* hybridization of mouse embryos was performed as described previously (*19*) with several modifications. Antisense and sense riboprobes (nt 1844-2476 of *mtprd*) were generated with an RNA *in vitro* transcription system (Promega) and [α-^{35}S]-UTP. Sagittal sections of 12- and 15-day mouse embryos (Novagen) mounted on glass slides were hybridized with the antisense and sense riboprobes. The slides were hybridized at 55°C in 200 μl of a solution comprising 50% formamide, 10% dextran sulfate, 0.3 M NaCl, 20 mM Tris, pH 8.0, 10 mM sodium phosphate, pH 8.0, 1×Denhardt's reagent, 5 mM EDTA, 0.2% sarcosyl, 500 μg/ml yeast tRNA, 200 μg/ml salmon sperm DNA, and 5×10^5 cpm of an ^{35}S-labeled antisense or sense riboprobe in a humid chamber. The slides were washed for 20 min at 55°C with

Fig. 2. **Alignment of the mouse mtprd and human TPRD amino acid sequences.** Identical amino acids are boxed. The TPR motif (aa 234-335) and two regions showing homology to matrix proteins (aa 750-850 and 1464-1624) are overlined, and denoted by TPR, M1, and M2, respectively. Three highly basic regions (aa 1018-1029, 1171-1184, and 1547-1563) which resemble the consensus sequence of the bipartite nuclear location signal are also overlined, and denoted by n1, n2, and n3, respectively. The three hydrophobic regions of mtprd, which are likely to be transmembrane domains, are interrupted-overlined, and denoted by t1, t2, and t3, respectively. Amino acid residues are numbered on the left, one-letter amino acid designations being used.

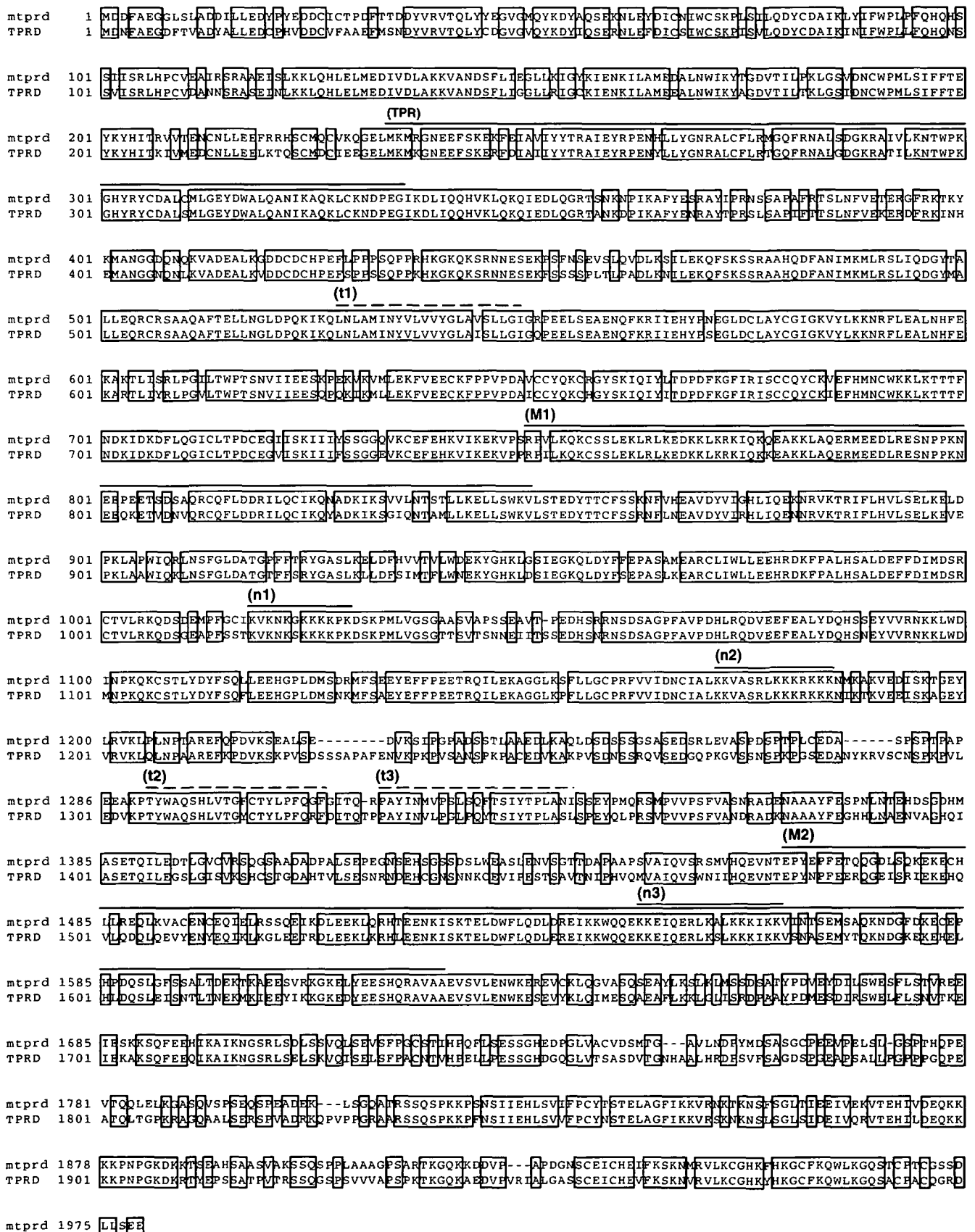

Fig. 2

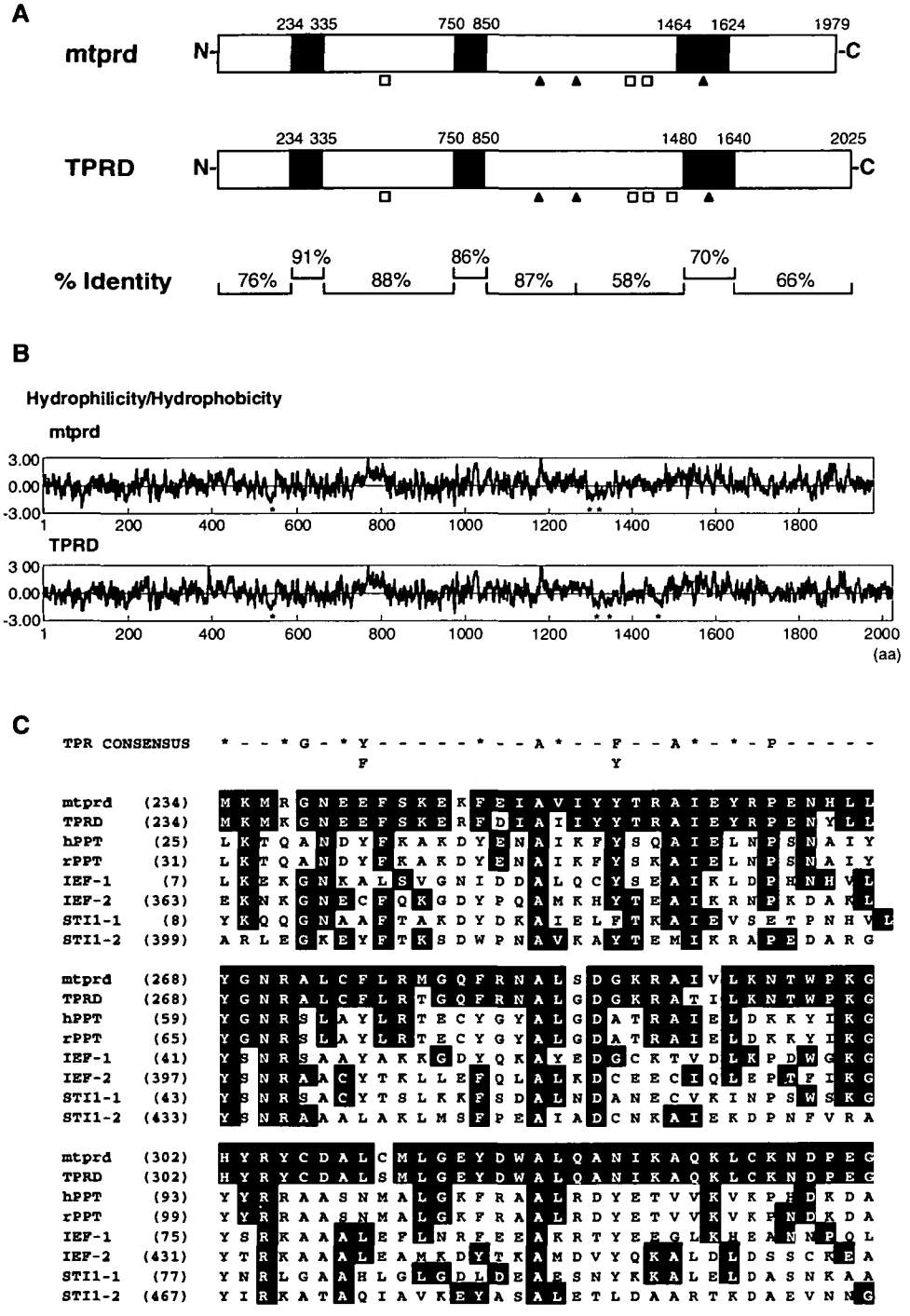

Fig. 3. **(A) Schematic comparison of predicted mtprd and TPRD proteins.** The three units of TPR motifs (TPR) are half-toned and the regions showing homology to matrix proteins (M1 and M2) are shown by filled boxes. Highly basic regions which resemble the consensus sequence of the bipartite nuclear location signal and hydrophobic regions which are presumably transmembrane domains are shown by filled triangles and open squares, respectively. The homologies of the regions of TPR motif, matrix proteins, and other regions between mtprd and TPRD are indicated as percentages. The region between M1 and M2 is divided into two regions (*aa* 851-1184 and *aa* 1185-1463) by a second nuclear location signal, and the homology of each region was calculated separately. **(B) Hydropathy analysis of mtprd and TPRD.** A hydropathy plot was obtained according to Hopp and Woods (*22*) with a window of 5 residues. The three and four hydrophobic regions which are likely to be transmembrane regions in mtprd and TPRD, respectively, are indicated by asterisks. **(C) Characterization of the TPR motif in mtprd.** The TPR motifs in mtprd, TPRD (*6, 7*), serine/threonine phosphatase of human (hPPT) (*10*), and rat (rPPT) (*11*), human IEF SSP 3521 (IEF) (*12*), and *S. cerevisiae* STI1 (*13*) are aligned in terms of the consensus TPR sequence (*9*). Residues identical to mtprd are shown as white letters. The asterisks in the consensus TPR sequence indicate hydrophobic residues. The position of the first amino acid residue of each TPR is given in parentheses.

5×SSC and 10 mM dithiothreitol, and then for 30 min at 65°C with 50% formamide, 2×SSC, and 0.1 mM dithiothreitol. After RNase A digestion, the slides were finally washed for 10 min at 65°C with 0.1×SSC. After drying, they were exposed to X-ray films for 2-3 days.

Identification of Alternative Splicing Sites of mtprd by RT-PCR—Several primers were designed according to the exon/intron organization of the *TPRD* gene, which were recently defined by our group (unpublished data). Among these primers, three sets [5′-AACCCAGCTTTACTATGAAGG-3′ (1F: nt 131-151) and 5′-CTTCCATCAACTCAAGATGTTG-3′ (4R: nt 387-408); 5′-CATTAAGCTGTACATCTTCTGG-3′ (3F: nt 263-284) and 5′-TGTCAACTGATCCTAATTTAGG-3′ (6R: 555-576); and 5′-GATGGAAAGAGGGCCATTGT-3′ (10F: 870-889) and 5′-TTGTTTCGTGATTTTTGTTTTCC-3′ (15R: 1329-1351)] were used for the detection of splice variants corresponding to exons 2, 5, and 11-14 of the *TPRD* gene. Total RNA was isolated from several tissues of ICR adult mice using ISOGEN (Nippongene) and then reverse transcribed with a Superscript Preamplification System (BRL) with oligo(dT) primers. PCR amplifications were performed for 35 cycles of 1 min at 94°C, 1 min at 54°C and 1 min at 72°C. The PCR products were analyzed by agarose or acrylamide gel

electrophoresis, and the bands were cloned into the T-vector for sequencing.

RESULTS

Identification of the mtprd cDNA and Nucleotide Sequence Analysis—mtprd cDNA clones were initially isolated by screening a mouse embryo cDNA library with partial human cDNAs covering the entire *TPRDI* cDNA (*6*), which encodes the same amino acid residues as *TPRD* cDNA (*7*). Additional cDNA clones were obtained by repeated screening with the derived mouse cDNAs and were then assembled. The sequences of the two gaps in mouse cDNA were amplified by PCR using primers flanking these regions with the mouse embryo cDNA library as a template. Finally, three cDNA clones and two RT-PCR products encompassed 7,172 bases of the mouse *mtprd* cDNA (DDBJ, EMBL, and GenBank accession number AB008516), covering a large open reading frame (ORF) corresponding to *TPRD* (*TPRDI*) (Fig. 1).

mtprd contains a 5′-untranslated region of 11 nt followed by a single large ORF of 5,937 nt and a 3′-untranslated region (3′UTR) of 1,224 nt with a polyadenylation signal (AATAAA) 17 nt before the 3′-end. The coding region and 3′UTR of *mtprd* exhibit identities of 82 and 66% to *TPRD*, respectively. The sequence around the initiation codon (AGCATGG) of *mtprd* has a favorable translation initiation sequence (*20*) similar to that (ACCATGG) of *TPRD*.

Analysis of the Predicted Amino Acid Residues (Figs. 2 and 3)—mtprd encodes 1,979 amino acid residues with a calculated molecular mass of 224 kDa. The alignment of the mtprd and TPRD proteins revealed that mtprd is 46 amino acid residues shorter than TPRD and exhibits 76% identity to TPRD. The N-terminal region of mtprd is more conserved than the C-terminal region. The C-terminal region has eight deletions of one to 8 amino acid residues (aa 1051-1052, aa 1224-1225, aa 1277-1278, aa 1315-1316, aa 1749-1750, aa 1772-1773, aa 1805-1806, and aa 1924-1925) and lacks the C-terminal 20 amino acid residues corresponding to TPRD.

Like TPRD, mtprd has three units of a 34-amino-acid repeat, the TPR motif, in its N-terminal region (aa 234-335). Comparison of the TPR motif sequence between human and mouse revealed high homology (91% identity) (Figs. 2 and 3). Protein database searches using the BLASTP revealed sequence similarity to several proteins containing TPR motifs, such as human and rat serine/threonine phosphatase (*10, 11*), human transformation-sensitive protein IEF SSP 3512 (*12*), and yeast heat shock protein STI1 (*13*). mtprd also has regions homologous to several matrix proteins, such as trichohyalin (*14*) and bullous pemphigoid antigen (*15*), in the central and C-terminal regions (aa 750-850 and aa 1464-1624). The sequences of these regions in human and mouse showed lower homology (M1: 86%, and M2: 70% identity). Chou-Fasman analysis (*21*) indicated the presence of many α-helical regions in mtprd, almost the same as in TPRD. mtprd is quite hydrophilic, as estimated by Hopp and Woods hydropathy plots (*22*), and three of the four hydrophobic regions in TPRD, which are likely to be transmembrane domains, are conserved in mtprd. In addition, mtprd also has three highly basic regions (aa 1018-1029, aa 1171-1184, and aa 1547-1563), which resemble the consensus sequence of the bipartite nuclear location signal (*23*), like in TPRD.

Chromosome Localization—Comparative mapping between mouse and human has revealed that the distal region of mouse chromosome 16 is homologous to the DCR of human chromosome 21 (*24*). We therefore performed *in situ* hybridization to determine if the *mtprd* gene maps to chromosome 16 (Fig. 4). Fifty metaphase cells were

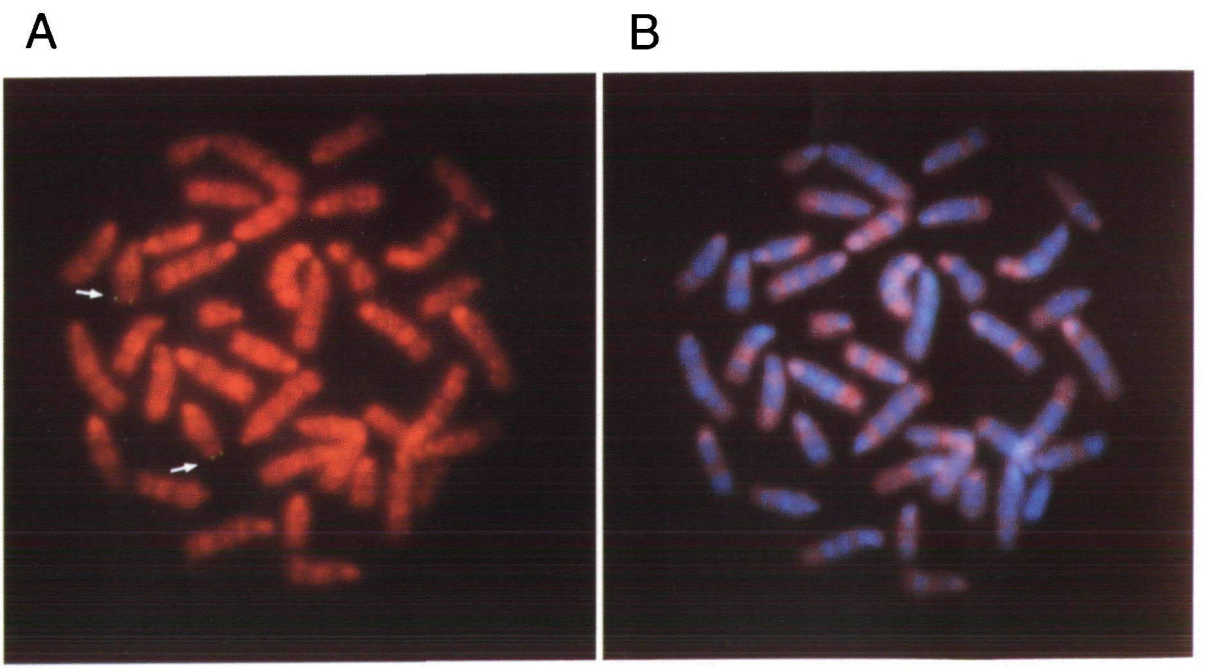

Fig. 4. **Chromosomal localization of the *mtprd* gene by FISH.** The radiolabeled probe comprising nt 1844-3376 in *mtprd* was hybridized to chromosomes from cultured splenocytes of male mice. (A) Metaphase plate showing doublet signals on chromosome 16 in region C3.3-C4 (arrows). (B) The same metaphase plate as in (A) viewed with a UV filter for G-band analysis.

evaluated with a probe comprising nt 1844-3376 in *mtprd*. Specific hybridization signals were observed at region C of one (16 cells) or both (3 cells) chromosome 16. All these doublet signals were located on band C4 or on the distal portion of band C3 (C3.3). No other hybridization sites were detected. From these results, the *mtprd* gene was assigned to the most distal region of mouse chromosome 16C3.3-C4.

Expression of mtprd (Fig. 5)—Northern blot analysis showed that a probe comprising nt 1844-2476 in *mtprd* gave two bands, corresponding to approximately 8 and 7 kb, which are shorter than the 9 and 7.4 kb bands for the *TPRD* gene (*6*). Although the transcripts of the *mtprd* gene were expressed in 7-17 day mouse embryos and all of the adult mouse tissues examined, the highest expression was found in the brain and heart among the adult tissues examined.

We further examined the expression profile of the *mtprd* gene during the embryonic period by RNA *in situ* hybridization. Sagittal sections of 12- and 15-day mouse embryos were hybridized with antisense and sense riboprobes comprising nt 1844-2476 in *mtprd*. *mtprd* was ubiquitously expressed in the mouse embryos. The most predominant expression was seen in the central nervous system, including the telencephalon, mesencephalon, metencephalon, retina, and spinal cord. Definite expression was also observed in other tissues including the heart and gut. No label was observed with a control ^{35}S-labeled sense *mtprd* riboprobe, as shown in adjacent sagittal sections.

Identification of Alternative Splicing Sites of mtprd by RT-PCR (Fig. 6)—Several skipped exons in the mouse cDNA sequence, which may be generated by alternative splicing, were suggested from the alignment of mouse and human cDNAs. To identify other splicing variants, we designed several primers according to the exon/intron organization of the *TPRD* gene, which was recently defined by our group (unpublished data). Bands of 278 and 235 bp, and 314 and 260 bp were detected with the primer sets of 1F and 4R, and 3F and 6R, respectively, by the RT-PCR of tissues from adult mice. These bands were due to a lack of nt 156-198 (aa 49-63) and nt 438-491 (aa 143-160) in *mtprd*, corresponding to exons 2 and 5 of *TPRD*, respectively. The relative intensity of each set of bands was almost the same for all tissues examined in mice. We also identified four bands of 482, 319, 273, and 85 bp using the primer set of 10F and 15R. These bands were due to a lack

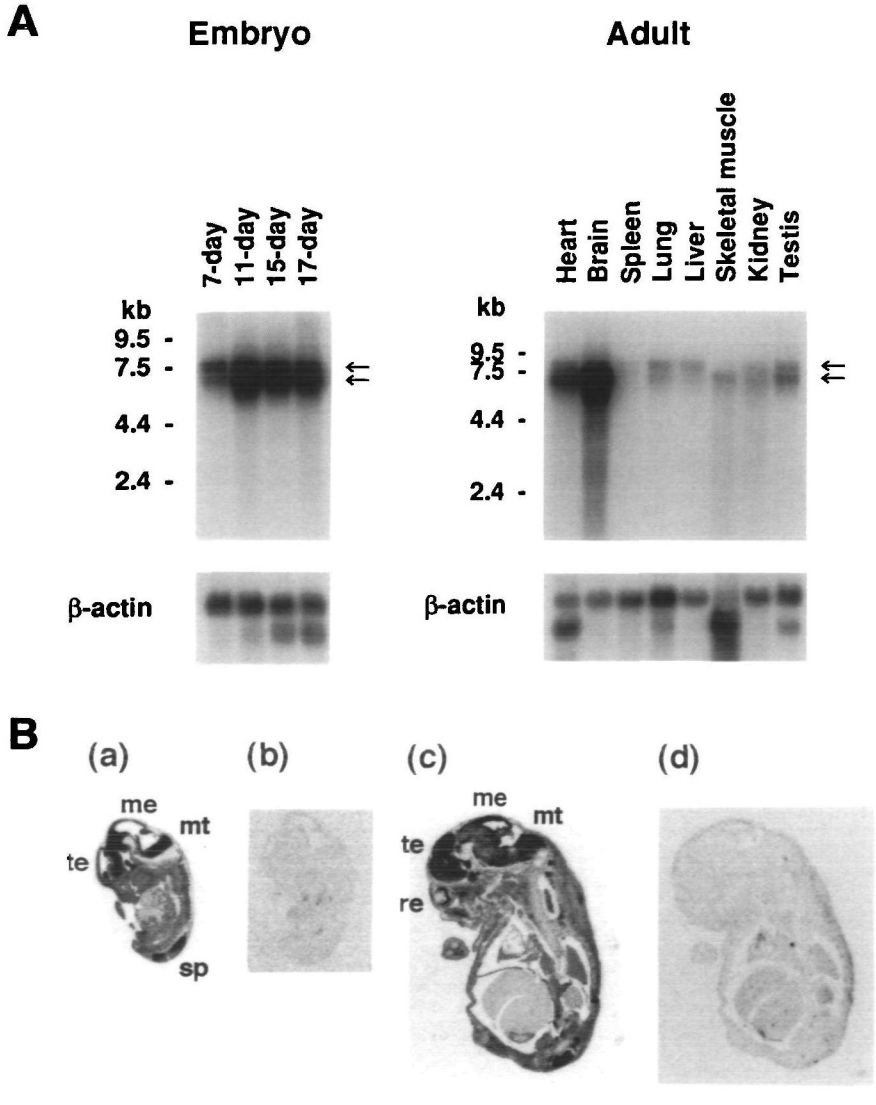

Fig. 5. **(A) Northern blot analysis of *mtprd*.** The radiolabeled probe comprising nt 1844-2476 in *mtprd* was hybridized to poly(A)$^+$ RNAs (2 μg) isolated from several tissues of adult mice and from 7-17 day mouse embryos. Two bands corresponding to approximately 7 and 8 kb (indicated by arrows) were observed in all tissues examined. A β-actin blot is shown at the bottom. The molecular sizes are shown in the left column. **(B) *In situ* hybridization of 12 (a and b) and 15 (c and d) day mouse embryos.** A radiolabeled antisense (a and c) or sense (b and d) riboprobe comprising nt 1844-2476 in *mtprd* was hybridized to a sagittal section of a mouse embryo. *mtprd* is ubiquitously expressed in all tissues of mouse embryos, predominantly in the central nervous system. No signal was observed with the sense riboprobe in adjacent sagittal sections. te, telencephalon; me, mesencephalon; mt, metencephalon; re, retina; sp, spinal cord.

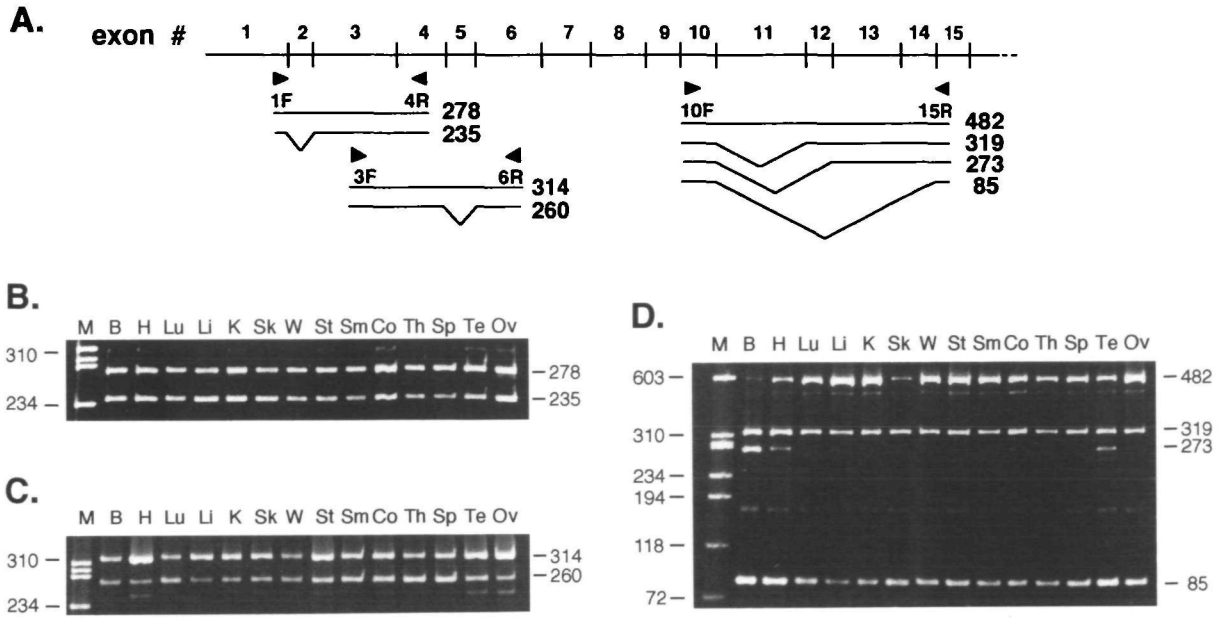

Fig. 6. **RT-PCR analysis of *mtprd*.** (A) A map of exons 1 to 15 of *TPRD*. The locations of introns are indicated by vertical bars. Arrows indicate the locations of the oligonucleotides used for RT-PCR assays. The predicted full-length and spliced sizes of the RT-PCR products in base pairs (bp) are shown by lines underneath the arrows. (B-D) RT-PCR of mRNA from several tissues using primer sets 1F and 4R (B), 3F and 6R (C), and 10F and 15R (D) to detect the deletion of exons 2, 5, and 11-14, respectively. The PCR products were analyzed by acrylamide gel electrophoresis. M, ϕX174 *Hae*III size marker; B, brain; H, heart; Lu, lung; Li, liver; K, kidney; Sk, skeletal muscle; W, white adipose tissue; St, stomach; Sm, small intestine; Co, colon; Th, thymus; Sp, spleen; Te, testis; Ov, ovary.

of nt 912-1074 (aa 301-355), nt 912-1120 (aa 301-370) and nt 912-1308 (aa 301-433) in *mtprd*, corresponding to exons 11, 11-12, and 11-14 of *TPRD*, respectively. Although the intensities of the bands of 319 and 85 bp were almost the same in all tissues examined, the band of 482 bp was faint in the brain and skin, and that of 273 bp was predominantly detected in the brain, heart and testis.

DISCUSSION

We have cloned and characterized *mtprd* cDNA, a mouse homologue of *TPRD*, which was recently isolated from the DCR by our group and others (*6, 7*). The *mtprd* and *TPRD* open reading frames show marked conservation at both the nucleotide sequence (82% identity) and amino acid sequence (76% identity) levels. Like TPRD, the mtprd protein has three units of the TPR motif and two regions homologous to several matrix proteins. The *mtprd* gene was mapped to region C3.3-C4, which is located at the distal end of mouse chromosome 16. This region of mouse chromosome 16 is homologous to that of human chromosome 21, that contains much of the genetic material responsible for the DS phenotype (*24*). These results confirmed that *mtprd* is a homologue of human *TPRD*.

The gene responsible for the pathogenesis of DS must be expressed during the embryonic period. Thus, we examined the expression profile of the gene in mouse embryos. *In situ* hybridization revealed that *mtprd* is expressed ubiquitously in day 12 and 15 mouse embryos. Therefore, the gene may be involved in the pathogenesis of a variety of developmental morphological anomalies observed in DS (*1, 2*), which further supports our previous proposal regarding the function of the *TPRD* gene (*6*). In particular, *mtprd* was expressed in the central nervous system, such as the telencephalon, mesencephalon, metencephalon, and retina in mouse embryos. The neuropathological changes in DS are characterized by a decreased neuronal number and abnormal neuronal differentiation (*25-29*). In addition, all individuals with DS exhibit AD-like neuropathology by 30-40 years of age (*1, 2*). Our preliminary experiment showed that *mtprd* is expressed in the areas of the adult rat brain with the highest neural density, including the hippocampus, cerebral cortex and cerebellar granular layer. The high-level expression of *mtprd* in the central nervous system indicates that this gene may be of importance in DS, especially in the pathogenesis of mental retardation. In addition, *mtprd* is also expressed in the heart and gut, suggesting that this gene is associated with congenital heart defects and abnormalities of the gut.

Recently, a new genetic model for DS, Ts65Dn mice, which have an extra copy of the distal aspect of chromosome 16, was developed (*24, 30, 31*). Ts65Dn mice show a developmental delay during the postnatal period as well as abnormal behavior in both young and adult animals that may be analogous to mental retardation (*24, 31*). This mouse model also shows age-related degeneration of septo-hippocampal cholinergic neurons and astrocytic hypertrophy (*30*). The expression of *mtprd* in Ts65Dn mice may provide further evidence that *mtprd* is a critical gene for DS.

We identified several alternative splicing variants in the 5′ portion of ORF of *mtprd* by RT-PCR. These variants correspond to skipped exons 2, 5, and 11-14 of the *TPRD* gene. These missing exons, except for exon 5, may cause a shift of the reading frame of amino acid residues. The relative intensities of two of the four RT-PCR products derived from skipped exons 11-14 were different among

the tissues examined. All transcripts may be detected by Northern blot analysis and by RNA *in situ* hybridization because probes derived from a region common to these variants (nt 1844-2476) have been used. The two bands detected by Northern blot analysis may be related with alternatively spliced variants. The relative intensities of these bands were different among the tissues examined. These results suggest that mtprd has several isoforms which might have different functions and localization. Our previous study demonstrated human cDNA isoforms: *TPRDI* and *TPRDIII*, which are transcribed from the altered transcription start site of the *TPRD* gene; and *TPRDII*, which is an alternative splicing product (lacking exon 7) (*6*). Therefore, there might be several transcription start sites and/or alternative splicing sites in the *mtprd* and *TPRD* genes. The physiological role of each isoform in both human and mouse remains to be determined.

Several genes other than *TPRD* (*6, 7*), including *Ets2* (*32*), *MNB* (*33-35*), *SIM2* (*36, 37*), *GIRK2* (*38-41*), *HCS* (*42, 43*), and *IRKK* (*44*), have been mapped to the DCR. Recently, our group isolated another two genes, *DCRA* (*45*) and *DCRB* (*46*). A transgene study suggested that *Ets2* is involved in some skeletal abnormalities in DS (*32*). In addition, another transgene study using yeast artificial chromosome showed that *MNB* causes learning defects relevant to DS (*33*). The generation of transgenic mice that overexpress these genes, either singly or in combination, will reveal their involvement in the pathophysiology of DS. The present work lays the groundwork for further analysis of the *TPRD/mtprd* function, including the development of specific antibodies and cDNA probes to assess the cellular and subcellular distribution of the gene products, and also transgene studies. These experiments will provide essential information on the potential involvement of this gene in the phenotype of DS.

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