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Identification and Cloning of a Novel cDNA Belonging to Tetratricopeptide Repeat Gene Family from Down Syndrome-Critical Region 21q22.2[1]

Fujiko Tsukahara,*,[2] Masahira Hattori,† Takamura Muraki,* and Yoshiyuki Sakaki†

*Department of Pharmacology, Tokyo Women's Medical College, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162; and †Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108

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We identified and cloned a novel 9,078-bp cDNA, designated *TPRDI*, from the Down syndrome-critical region by exon trapping. The cDNA encodes a putative protein (TPRDI) of 2,025 amino acid residues. Two isoforms, *TPRDII* (8,992 bp) and *TPRDIII* (7,416 bp), were also isolated. *TPRDII*, which is probably an alternative splicing product from the *TPRD* gene transcript, encodes two large open reading frames (ORFs) of 200 amino acid residues and 1,792 amino acid residues, respectively. *TPRDIII*, which is probably generated by transcription from an alternative start site of the *TPRD* gene, encodes a putative protein of 1,715 amino acid residues (TPRDIII). Northern blot analysis revealed that *TPRDI* and its isoforms are present in 7-17 day mouse embryo and in all the human adult and fetal tissues examined. TPRDI has three units of a 34-amino-acid repeat similar to the tetratricopeptide repeat (TPR) motif, which may mediate interaction with various proteins. A larger ORF encoded by *TPRDII* also has three units of TPR motif, but TPRDIII has only two-thirds of this motif unit. Thus, the *TPRD* gene may belong to the TPR gene family. Near-central and C terminal regions of TPRDs showed some homology to several matrix proteins such as trichohyalin and bullous pemphigoid antigen. It is possible that the *TPRD* gene is one of the genes whose overexpression causes several morphological anomalies observed in Down syndrome.

Key words: cDNA cloning, Down syndrome, exon trapping, tetratricopeptide repeat.

Down syndrome (DS), the most common birth defect, is caused by trisomy of chromosome 21. Cytogenetic and clinical correlations of patients with partial trisomy 21 indicate that a region of 2-4 Mb in 21q22.2 is critical in the pathogenesis of DS (*1-3*), and this has been designated the Down syndrome-critical region (DCR). Thus, the cloning and characterization of genes in the DCR are important to elucidate the molecular basis of DS. Recently, a transgene study suggested that *Ets2* is involved in some skeletal abnormalities of DS (*4*). DS manifests complex phenotypes and may be caused by multiple genes. Several genes other than *Ets2*, including *hSIM* (*5, 6*), *ATP50* (*7*), *SLC5A3* (*8*), *KATP-2* (*9*), *erg* (*10*), *DSCR1* (*11*), potassium channel *Isk* (*12*), and *GABPA* (*13*), have been identified, but a number of unknown genes remain to be identified and characterized. For this reason, we have analyzed P1 clones mapped in this region by exon trapping. In this paper, we report a novel gene (cDNA) mapped in the DCR which encodes a protein(s) (230 kDa) possessing tetratricopeptide repeat (TPR) motif.

MATERIALS AND METHODS

Exon Trapping—Several P1 clones possessing a sequence-tagged site in the DCR (*14*) were isolated from a P1 phage library specific for chromosome 21 (*15*) by PCR-based screening, and subjected to exon-trapping analysis. The P1 DNA was isolated by the alkaline-SDS method (*16*), partially digested with *Sau*3AI, and the DNA fragments of 2 to 4 kb were ligated to the *Bam*HI-digested exon trapping vector pEXT3 (*17*). COS 7 cells were grown to 40-60% confluency and then transfected with the recombinant plasmids by Lipofectin (GIBCO BRL). After incubation at 37°C for 3 days, total RNA was isolated by using an ISOGEN (Nippongene) and subjected to RT-PCR by using primers of the splicing cassette of pEXT3. The PCR products were analyzed by 2% agarose gel electrophoresis and the bands containing the trapped fragments were cloned into pT7 Blue T-vector (Novagen) for sequencing.

Screening of cDNA Libraries—Among many trapped putative exons showing no homology to known genes,

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[2] To whom correspondence should be addressed. Phone: +81-3-3353-8111 (Ext. 22513), Fax: +81-3-5269-7417, E-mail: fuji@research.twmc.ac.jp

Abbreviations: DCR, Down syndrome-critical region; DS, Down syndrome; ORF, open reading frame; TPR, tetratricopeptide repeat.

(A)

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CTGAACTAGTTGCCAGTGATCTTGAAACGTGACAGTAACCAAGAGATAAATAGGTGACAATGACAGGAAAATTAGATGTAGTAAAAGAGAGTGTTTGAGA   100
GCAGAAGCTATGGCAACTAAAGACTGGATTTGAATCCTTCCTAGCTTGGTGACATGAGCAAATTACTTGATTTAAGTGAGCATTTTCCCATCTGTACAGT   200
GGAGATAACGATAATTGTGCCTGCTAAGAAGAATTGCTGTGAAGATTAGTGAAATAATGCATGTAAAACATTTGGTACAGTATGTGACACATAGTACAAA   300
TAGTTTGCTAGGAAGATTGTTATTATTCTTCACTTGTGATATTGTGAAGTTTTCATACAGCAAATTGGACATCATGAGATGGATTGATTAAATAAATAGA   400
TTTGAACTTCAAGGACTGGTAGTGTTCTTGCTTTGGAAAGAAGAAACTTGGTTTATCCTAATAATAGTAGGATAATAATGGTGAAGTGATAGGTACAAGT   500
AATAGTGTTTATGATGCGCTGGTGATGATAGGAAAAGAAAGCCATTATATGGGCAAGAGCTAGAAGTAATAAAATGGTGCATTTTTCAGTGATGTTTGGC   600
CTATGTAGCTATTCTCTGATAACTATAAAAATCCTTATTATTGAAGATTCTTCAGGAAAAAAAAACCCTTAGTCTGAAACTTTAGCACCAATCCCCCTTG   700
CCCCCCATTGAAATACGTATTTTTAAAACATGGCTTTTGATAATGTGAGGGTTTTTTCCTTTTTGCGATTTAGCAGTGCTGATTGTGTATTGCAGTAGTT   800
GTGAGAGCATTAGAAGCAGCAGTCGATAGGAGGATGGAAGGTCTGGATGCCGCCTTGGGGAGTTAGGAGATTGGCAGACTTACCCTGTACCACTCTAGCC   900
CTACTCCTTTGCCCAAGACAGAAACACACTGAGATGGATAGGAGAATATGAGCAGTTGATAGGAAAGTTCTCAGTGGAGTCAGGATTTAGGTTAGGCCAG  1000
GAGATTGAGAATATAACAGTTTGTGTATGATGAAATGGCATATTTCACAGAATGCAGTAAAAGCAGGTAGGGTACAAGTGCAGCAACAGGAAGATGTCTT  1100
TTCTTCATTCAGCAAACACTTATTTGAGAGCTTACCATGTGCTAGGCACATACAAAGATAAATAAGATGCCCTTGATGATCCTCTATTTAAAGGAGACAT  1200
GTAAACAGGTTAACTTAGAGTAGAGATGGTGAATATGTGAACCTGAGGAAAGGAAGAAATAGATTAAATTATCTGGAGAGAGAGGAAAAGTCAGCAGAAT  1300
GGGGACGAGAATCTTTCGGAGCTCAGTGTTCTGATAGGAGTTATTTCCTTGGGCATAGGTTCCAAGTATTTTTCTAATATACCATAGAAGCCAGGAAAAC  1400

TTTCTTCTGTTATCTCAAATGATTTAATTACTGACTTGAGTTTGTGTTGTCTCCTTAGACTTGTGCACCATGGACAATTTTGCTGAGGGAGATTTCACTG  1500
                                                                M  D  N  F  A  E  G  D  F  T  V                11

TGGCGGATTATGCCTTGTTAGAAGATTGCCCTCACGTGGATGATTGTGTCTTTGCTGCTGAATTTATGAGCAATGATTATGTTCGTGTGACTCAGCTTTA  1600
  A  D  Y  A  L  L  E  D  C  P  H  V  D  D  C  V  F  A  A  E  F  M  S  N  D  Y  V  R  V  T  Q  L  Y     44

CTGTGATGGGGTGGGTGTGCAATATAAAGATTATATCCAAAGTGAGAGGAATTTGGAATTTGACATCTGCAGTATATGGTGTAGTAAACCAATTTCTGTC  1700
  C  D  G  V  G  V  Q  Y  K  D  Y  I  Q  S  E  R  N  L  E  F  D  I  C  S  I  W  C  S  K  P  I  S  V     77

CTGCAAGATTATTGCGATGCCATTAAAATAAACATCTTCTGGCCACTTCTGTTTCAACATCAAAACAGTTCCGTAATATCACGATTGCATCCCTGTGTGG  1800
L  Q  D  Y  C  D  A  I  K  I  N  I  F  W  P  L  L  F  Q  H  Q  N  S  S  V  I  S  R  L  H  P  C  V  D    111

ACGCCAACAATTCACGTGCTTCTGAGATAAATTTGAAGAAACTACAACATCTTGAGTTGATGGAAGATATTGTGGATTTGGCAAAGAAAGTTGCTAATGA  1900
  A  N  N  S  R  A  S  E  I  N  L  K  K  L  Q  H  L  E  L  M  E  D  I  V  D  L  A  K  K  V  A  N  D    144

TTCATTCCTTATTGGAGGCTTATTGAGAATTGGTTGTAAAATAGAAAATAAAATCTTGGCAATGGAAGAAGCTCTGAATTGGATAAAATATGCAGGCGAT  2000
  S  F  L  I  G  G  L  L  R  I  G  C  K  I  E  N  K  I  L  A  M  E  E  A  L  N  W  I  K  Y  A  G  D    177

GTAACAATTCTAACTAAATTAGGATCAATTGACAATTGTTGGCCTATGTTAAGTATTTTCTTTACTGAATACAAGTACCACATAACTAAAATTGTAATGG  2100
V  T  I  L  T  K  L  G  S  I  D  N  C  W  P  M  L  S  I  F  F  T  E  Y  K  Y  H  I  T  K  I  V  M  E    211

AAGACTGCAATTTGCTTGAAGAACTTAAAACTCAAAGTTGTATGGATTGTATAGAGGAAGGAGAACTAATGAAAATGAAAGGAAATGAAGAGTTTTCCAA  2200
  D  C  N  L  L  E  E  L  K  T  Q  S  C  M  D  C  I  E  E  G  E  L  M  K  M  K  G  N  E  E  F  S  K    244
                                                                    (TPR)
AGAAAGATTTGATATAGCTATTATCTATTACACCAGAGCCATTGAATATAGACCTGAAAACTACCTTCTTTATGGTAACCGAGCTCTTTGTTTTCTTCGT  2300
  E  R  F  D  I  A  I  I  Y  Y  T  R  A  I  E  Y  R  P  E  N  Y  L  L  Y  G  N  R  A  L  C  F  L  R    277

ACTGGACAGTTTAGAAATGCACTCGGTGATGGAAAGAGAGCCACTATTCTGAAGAACACTTGGCCAAAGGGTCATTATCGTTATTGTGATGCTCTTTCTA  2400
  T  G  Q  F  R  N  A  L  G  D  G  K  R  A  T  I  L  K  N  T  W  P  K  G  H  Y  R  Y  C  D  A  L  S  M    311

TGCTGGGGGAATATGACTGGGCCCTGCAAGCAAACATAAAAGCTCAAAAACTCTGTAAAAATGACCCTGAGGGAATCAAGGATCTAATTCAGCAGCATGT  2500
    L  G  E  Y  D  W  A  L  Q  A  N  I  K  A  Q  K  L  C  K  N  D  P  E  G  I  K  D  L  I  Q  Q  H  V    344

AAAGTTACAAAAACAAATAGAAGACCTACAAGGTCGAACAGCAAATAAGGATCCAATTAAAGCCTTTTATGAAAACAGGGCCTACACACCTAGGAGTTTA  2600
K  L  Q  K  Q  I  E  D  L  Q  G  R  T  A  N  K  D  P  I  K  A  F  Y  E  N  R  A  Y  T  P  R  S  L    377

TCAGCACCTATATTTACTACTTCACTTAACTTTGTGGAGAAGGAAAGAGATTTCAGAAAAATTAATCACGAAATGGCCAACGGTGGTAATCAGAATCTAA  2700
S  A  P  I  F  T  T  S  L  N  F  V  E  K  E  R  D  F  R  K  I  N  H  E  M  A  N  G  G  N  Q  N  L  K    411

AGGTGGCGGATGAGGCGTTGAAGGTAGATGATTGTGACTGTCATCCTGAATTTTCACCACCATCAAGTCAGCCTCCAAAACATAAAGGAAAACAAAAATC  2800
  V  A  D  E  A  L  K  V  D  D  C  D  C  H  P  E  F  S  P  P  S  S  Q  P  P  K  H  K  G  K  Q  K  S    444

TCGAAACAATGAATCAGAAAAGTTCAGTTCTAGTTCACCATTGACTTTACCAGCAGATTTGAAGAACATCTTGGAGAAACAGTTTTCTAAATCTTCCAGA  2900
R  N  N  E  S  E  K  F  S  S  S  S  P  L  T  L  P  A  D  L  K  N  I  L  E  K  Q  F  S  K  S  S  R    477

GCTGCACACCAGGATTTTGCTAATATAATGAAAATGCTGAGAAGCTTAATTCAAGATGGCTATATGGCCTTATTGGAGCAGCGTTGCCGCAGCGCTGCAC  3000
A  A  H  Q  D  F  A  N  I  M  K  M  L  R  S  L  I  Q  D  G  Y  M  A  L  L  E  Q  R  C  R  S  A  A  Q    511

AGGCCTTTACAGAGTTGCTGAACGGTTTAGATCCTCAAAAAATAAAGCAATTGAACCTGGCCATGATTAACTATGTTTTGGTCGTCTATGGACTTGCCAT  3100
  A  F  T  E  L  L  N  G  L  D  P  Q  K  I  K  Q  L  N  L  A  M  I  N  Y  V  L  V  V  Y  G  L  A  I    544

TTCTCTCCTTGGAATAGGACAGCCTGAGGAATTATCTGAAGCCGAAAACCAGTTTAAGAGGATTATTGAACACTACCCCAGTGAGGGCCTTGATTGCTTG  3200
  S  L  L  G  I  G  Q  P  E  E  L  S  E  A  E  N  Q  F  K  R  I  I  E  H  Y  P  S  E  G  L  D  C  L    577

GCCTACTGTGGAATTGGAAAAGTGTATTTGAAAAAAAACAGATTTCTAGAAGCTCTCAATCACTTTGAGAAAGCAAGAACCTTGATTTATCGTCTTCCTG  3300
A  Y  C  G  I  G  K  V  Y  L  K  K  N  R  F  L  E  A  L  N  H  F  E  K  A  R  T  L  I  Y  R  L  P  G    611

GAGTGTTAACTTGGCCCACGAGTAATGTGATTATTGAAGAGTCTCAGCCACAAAAAATAAAGATGCTGTTAGAGAAATTTGTTGAAGAATGCAAGTTCCC  3400
  V  L  T  W  P  T  S  N  V  I  I  E  E  S  Q  P  Q  K  I  K  M  L  L  E  K  F  V  E  E  C  K  F  P    644

TCCAGTGCCAGATGCCATTTGTTGCTATCAGAAGTGCCATGGATATTCTAAGATCCAGATATACATAACTGATCCAGACTTTAAGGGTTTTATACGCATC  3500
P  V  P  D  A  I  C  C  Y  Q  K  C  H  G  Y  S  K  I  Q  I  Y  I  T  D  P  D  F  K  G  F  I  R  I    677

AGCTGTTGCCAGTACTGTAAAATAGAATTTCACATGAATTGCTGGAAGAAGTTAAAAACTACAACCTTTAATGATAAAATTGACAAGGATTTTCTACAAG  3600
S  C  C  Q  Y  C  K  I  E  F  H  M  N  C  W  K  K  L  K  T  T  T  F  N  D  K  I  D  K  D  F  L  Q  G    711

GAATATGTCTTACCCCTGACTGTGAAGGTGTCATTTCTAAGATTATCATCTTCAGCAGTGGTGGTGAAGTTAAATGTGAATTTGAACACAAGGTCATAAA  3700
  I  C  L  T  P  D  C  E  G  V  I  S  K  I  I  I  F  S  S  G  G  E  V  K  C  E  F  E  H  K  V  I  K    744
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Fig. 1 (continued on next page)

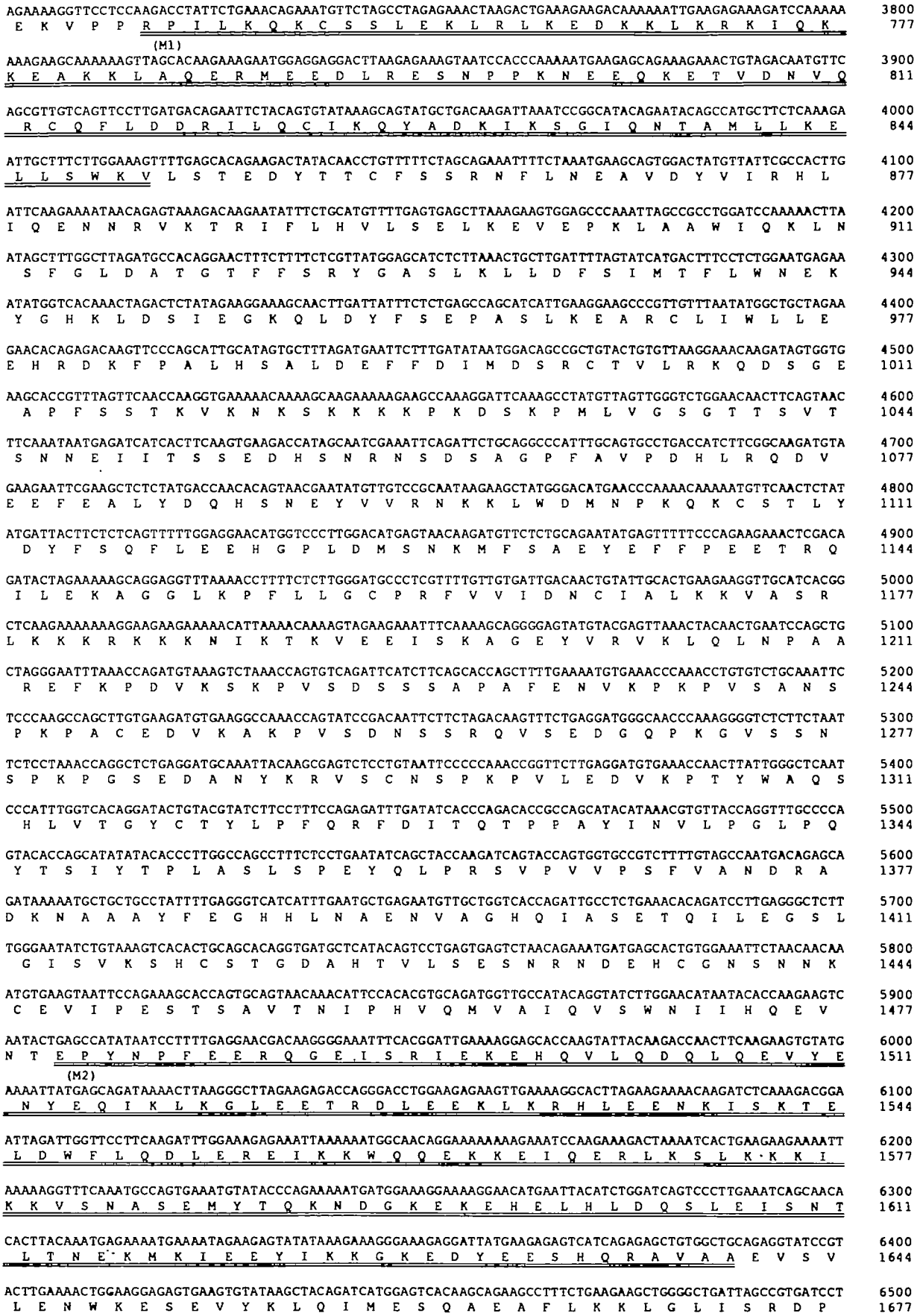

Fig. 1 (continued on next page)

several exons isolated from a P1 clone (T1212) hit by a STS marker 238wc3 (*14*) were found to hybridize with large transcripts of about 7.4 and 9 kb. These exons were then used as probes to screen the human fetal brain and heart cDNA libraries (Clontech). Hybridization was performed with ^{32}P-labeled probes under appropriate conditions. The filters were washed with 0.1×SSC, 0.1% SDS at 65°C and analyzed by using the Fuji Bio Imaging Analyzer BAS2000 system. The positive cDNA clones were sequenced and overlapped. The terminal regions of the overlapped sequences were furthermore used as probes to obtain the extended clones. The 5′ and 3′ ends of the cDNA were also isolated by the 5′ and 3′ rapid amplification of cDNA ends system (5′ RACE and 3′ RACE, GIBCO BRL) under the conditions recommended by the supplier.

Northern Blot Analysis—The ^{32}P-labeled probe from a region common to *TPRDI* and its isoforms (nt 2711-3222 of *TPRDI*) was hybridized to 2 μg of poly(A)$^+$ RNA isolated from several tissues of human fetus or adult or from 7-17 day mouse embryo (Clontech). After hybridization under appropriate conditions, the filters were washed with 0.1× SSC, 0.1% SDS at 65°C and analyzed by using the Fuji Bio Imaging Analyzer BAS2000 system.

RT-PCR—To prove the existence of *TPRDII*, RT-PCR was performed with primers flanking the presumed deletion by alternative splicing (5′-ATGGAAGATATTGTGG-

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GCAGCATATCCTGACATGGAGTCTGATATACGTTCATGGGAATTGTTTCTTTCTAATGTTACAAAAGAAATTGAGAAAGCAAAGTCTCAGTTTGAAGAAC   6600
A  A  Y  P  D  M  E  S  D  I  R  S  W  E  L  F  L  S  N  V  T  K  E  I  E  K  A  K  S  Q  F  E  E  Q   1711

AAATTAAGGCAATTAAAAATGGTTCTCGGCTCAGTGAACTTTCTAAAGTGCAGATTTCTGAGCTTTCATTTCCTGCCTGTAACACGGTTCATCCCGAGTT   6700
   I  K  A  I  K  N  G  S  R  L  S  E  L  S  K  V  Q  I  S  E  L  S  F  P  A  C  N  T  V  H  P  E  L   1744

ACTCCCTGAGTCTTCAGGCCACGATGGCCAAGGGCTTGTGACTTCTGCAAGCGACGTGACTGGAAACCACGCAGCACTTCACAGGGATCCTAGTGTGTTC   6800
 L  P  E  S  S  G  H  D  G  Q  G  L  V  T  S  A  S  D  V  T  G  N  H  A  A  L  H  R  D  P  S  V  F    1777

TCTGCTGGTGATTCCCCAGGGGAGGCTCCTTCTGCGCTGTTGCCAGGGCCACCCCCTGGTCAGCCTGAAGCCACTCAGCTGACAGGGCCAAAACGGGCTG   6900
S  A  G  D  S  P  G  E  A  P  S  A  L  L  P  G  P  P  P  G  Q  P  E  A  T  Q  L  T  G  P  K  R  A  G    1811

GCCAGGCAGCTCTGTCAGAACGAAGCCCTGTGGCTGATCGGAAGCAGCCTGTTCCTCCAGGACGTGCTGCGCGTTCAAGCCAGTCTCCAAAAAAGCCGTT   7000
  Q  A  A  L  S  E  R  S  P  V  A  D  R  K  Q  P  V  P  P  G  R  A  A  R  S  S  Q  S  P  K  K  P  F    1844

CAATAGTATTATTGAGCACCTGTCAGTGGTATTCCCATGTTACAACAGCACTGAGCTTGCTGGTTTTATTAAAAAAGTGCGAAGCAAAAACAAGAACTCA   7100
 N  S  I  I  E  H  L  S  V  V  F  P  C  Y  N  S  T  E  L  A  G  F  I  K  K  V  R  S  K  N  K  N  S    1877

CTCTCAGGATTGAGTATTGATGAAATTGTCCAAAGAGTGACAGAACACATTCTAGATGAACAGAAAAAGAAAAAGCCAAACCCAGGAAAGGACAAGAGGA   7200
L  S  G  L  S  I  D  E  I  V  Q  R  V  T  E  H  I  L  D  E  Q  K  K  K  K  P  N  P  G  K  D  K  R  T    1911

CTTATGAGCCCAGCTCTGCCACCCCCGTGACCAGGTCCTCCCAGGGCTCACCCTCGGTGGTTGTTGCACCATCACCCAAAACCAAGGGGCAGAAAGCAGA   7300
  Y  E  P  S  S  A  T  P  V  T  R  S  S  Q  G  S  P  S  V  V  V  A  P  S  P  K  T  K  G  Q  K  A  E    1944

AGATGTCCCTGTGAGGATTGCACTGGGTGCAAGTTCCTGTGAAATATGCCACGAGGTGTTCAAATCAAAAAACGTGCGTGTGCTCAAATGTGGGCACAAG   7400
 D  V  P  V  R  I  A  L  G  A  S  S  C  E  I  C  H  E  V  F  K  S  K  N  V  R  V  L  K  C  G  H  K    1977

TATCACAAAGGGTGCTTTAAGCAGTGGCTTAAAGGGCAGAGCGCTTGCCCGGCCTGCCAGGGTCGTGATCTCCTGACAGAAGAGTCACCTTCTGGAAGAG   7500
Y  H  K  G  C  F  K  Q  W  L  K  G  Q  S  A  C  P  A  C  Q  G  R  D  L  L  T  E  E  S  P  S  G  R  G    2011

GCTGGCCCAGTCAGAATCAGGAGCTGCCTTCCTGCTCTTCTAGGTAGTCACACTTCACTAAAGTGTCATCCACCAGTGTGTTGAATCCGAAGAATGACAA   7600
  W  P  S  Q  N  Q  E  L  P  S  C  S  S  R  *                                                          2025

TTTTCTACCACTGGTGTAAAAAACAAACATTTGAAGACCCTTGTGCATTGTGTGTCACAAAGCTAAATACATGGAAATCGTTAATATCGCTGATATTAAG   7700
TAATTTCCCCACTCTGAGTGAATACTTTGATGATTGCCAACAGTGGCTAATAAAATGACGGCTACCACACTCATGGGTCACTGGGGCTGCGCAGGGCTCT   7800
TTGAGGTGGGTGGCTTCTTTTGGAAAGTACTATGAACGTCTCGAAGCAGTATTCTAGTGATAAGAATTCTTAACATAGCCAAGCGCCCCACGTTTGTTCC   7900
CCACGTTTGTTCCCCTTTTCTGTTTGAAAAACCTGTTCTGGTAGCTCCACAAGAGAGATGATACTGACTTTTTAAATTTTTTACAAGAGTCTGTATTCCT   8000
GATATGCCTATATTTTTCCTCAAAGATTCTGCATTTTAAGGATGGGCATAAGCAAACTATATTTTAATAATTTATAGTTAATGTTAAAATATTGGCTGAT   8100
TTAGACCAAAAGATTCAAATCTCCTCTTTGTGAAATCCCATCTGCATTTGATTTTTATTATTTTTATGTTCCCCCGTTAGATTGTTTTAAGTGTTTGCTT   8200
TTCATCTTTTATAGATGTAATCTGATTTTCAAAAATCATTAACACTTTTTAATTAGTATCGACTAAGACTTTTTCCCCCTGGAATCGAGGCTGTGTGTCC   8300
GTCATCCCAGCCCCCGGTTGGAGCCTGCTCTTTGAACTCCGCTGCCTTCCTTAGCAGCTTCTGTCCTCTTCTGTGAGTCAGTCAGCGAGTGCTTGGGATC   8400
CGCATCCAGCCGTGCTGAGCACACAACAGGCTGTGTGTGGAAATGGCCACCACCATTCTCCTTCCCCACCCCACCACAAAAAGAGAAGCTGTGTCTTTAG   8500
ACAACCCTGAGGTATCTGTGTTACAATCGTTCTGTGTTTGATATTTGTGTAAAGTATGCATGCAGTCTTGTACTGTGACCTAAGAACAAAACTGTAACTG   8600
CATTAGAAACCATGAAAAAATTAGATATTGTTTTGTGACTTTTAGACAGTGGTAAATATAGAACCATGAATTCTGGTCACATTCCATTTCTCTCCAACAT   8700
GAAGGATCAAAAAATGTTTTTCAATGTGTTCTTTGTTCCACTGGAAACTTAGAGTCATGAGTTTATGAGCTGATTTGGTCACCTTCCTCTGCCTTTGTTC   8800
ACTGTGAGTTCTGATGTCTTAGTGACTTAGTTCTTAGAAGCTCACGCCTTAGTTTGAAACAGATTCTCCACGGTGGTCCCCAAAACACTGTCTGCATATC   8900
CATAAGAATTGAGCGCTATGGGTGTTAACGTGCATGAGGATCAGTTTGCAGCAGCAAGTACAAAAGGAGAAGAGGAACATCCGTTGAATGAGTGTGTTTT   9000
GTACATAACTTCAGATACTTGTGAACATGCCTTATATTTGTCCAACAACTGTCAGAATAAAGAACATTCTAAAATGAG                          9078
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(B)

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                                                                                    (Alu)
TACATTTGGAAAGTCTTACTGACATGCAGAAATAGTACAGAAAAACATACAAATAGGAATGTTATTGGCTGGGCATGGTGGCTCACACCTGTAATCCCAG   100
CACTTTGGGAGGCCAAGGCGGGTGGATCACAAGGTCAGGAGATCGAGACCATCGTGGCTAACATGGTGAAACCCTGTGCCTACTAAAAATTCAAAAAATT   200
AGCCAGGTGTACTGGCATGTGCTGTAATCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCACTTGAACCCGGGAGCAAAGGTTGCAGTGAGCCAAGATC   300
ACGCCACTGCACTCCAGCCTGGGCGACAGAGCGAGACTCTGTCTCAAAAAAAACAAAAGAATGCTATGCATAGGTACAATGTCGAAATGTGCAAGAAATA   400
CTTCAGAAATATTAAAAGTAGTTATTCCTGGGTAGTTGTGATTGTGACTCTGGGTGACTTTTTCCCTTTGTTTTATTTTTCTGTATTTTCCAAATTTCCT   500
ATAATGGACATATATTATATGGATTTTTTTTAATTAAAATTATCTTTTGACTAGATAATATACATGGAAAATTCACAAAGTACACATATGCAAGATATAA   600
TACTTTTACTATAAAAGAGTGAAAATTTTTAAATACTTGTTTTTCCTTTTAG                                                      652
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Fig. 1. **(A) Nucleotide and deduced amino acid sequences of *TPRDI*.** The ATG initiation and the TAG stop codons and a poly A signal are underlined. TPR motif (aa 234-335) and two regions showing some homology to matrix proteins (aa 750-850 and aa 1480-1640) are double-underlined and indicated by TPR, M1, and M2, respectively. **(B) A unique 5′-end sequence (652 nt) of *TPRDIII*.** An Alu-like sequence (nt 80-345) is underlined and indicated by Alu. Nucleotide and amino acid residues are numbered on the right, and one-letter amino acid designations are used.

ATTTGG-3′ and 5′-CTAAACTGTCCAGTACGAAGA-3′ corresponding to nt 1860-1881 and nt 2294-2314 of *TPRDI*, respectively). cDNA libraries of human fetal brain, heart, and kidney (Clontech) were used as templates. PCR involved was denaturation at 94°C for 5 min, followed by 30 cycles of amplification at 94°C for 1 min, 54°C for 1 min and 72°C for 1 min, with a final elongation at 72°C for 10 min. The RT-PCR products were analyzed by 2% agarose gel electrophoresis and subcloned into pT7 Blue T-vector (Novagen) for sequencing.

Sequence Analysis—DNA sequencing was performed by the dideoxy chain terminator method (*18*) and analyzed by HITACHI SQ-5500 and ABI 373A sequencers. Both strands of isolated cDNA clones were sequenced. Three or more separate subclones of the PCR product were sequenced to rule out the possibility of any Taq polymerase errors. The sequence alignments were done by using a computer software program, GENETYX-MAC/ATSQ, and the deduced amino acid residues were analyzed by GENETYX-MAC (Software Development, Tokyo). Sequence identity search was carried out with the BLAST program in databases: GenBank (release 92.0), EMBL (release 45), SwissProt (release 32.0), PIR (release 47.0), and PRF (release 96-01).

RESULTS AND DISCUSSION

We identified several possible exons from a P1 clone (T1212) hit by a STS marker 238wc3 in DCR (*14*) by exon trapping. They showed no homology to human genes. Northern analysis using these exons revealed two large transcripts of about 7.4 and 9 kb. To isolate a full-length clone, we then screened human fetal brain and heart cDNA libraries by plaque hybridization using these trapped exons as probes. In combination with 5′ and 3′ RACE, we finally obtained a novel cDNA of 9,078 bp, designated *TPRDI*.

TPRDI possesses a 5′-untranslated sequence of 1,469 nt followed by a single large open reading frame (ORF) of 6,075 nt and a 3′-untranslated sequence of 1,534 nt with a polyadenylation signal (AATAAA) 17 nt before the 3′-end (Figs. 1A and 2). The sequence around the initiation codon has the most favorable translation initiation sequence ACCATGG (*19*). Thus, we concluded that *TPRDI* encodes a putative protein of 2,025 amino acid residues (TPRDI) with a calculated molecular mass of 230 kDa and pI of 7.3. The expression sequence tags (ESTs) in 21q22.2, 21ES-0203, and 21ES0084 (*20*), are present at nt 5933-6075 and

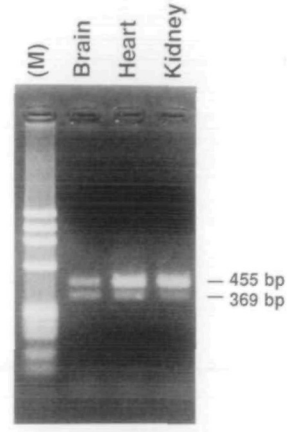

Fig. 3. **RT-PCR analysis of *TPRDI* and *TPRDII*.** RT-PCR was performed with primers flanking the presumed deletion by alternative splicing. PCR products were electrophoresed in a 2% agarose gel and stained with ethidium bromide. Two RT-PCR products of 455 and 369 bp corresponding to sequences from *TPRDI* and *TPRDII*, respectively, were detected in human fetal brain, heart, and kidney. The size markers (M) are ϕX174-*Hae*III digest.

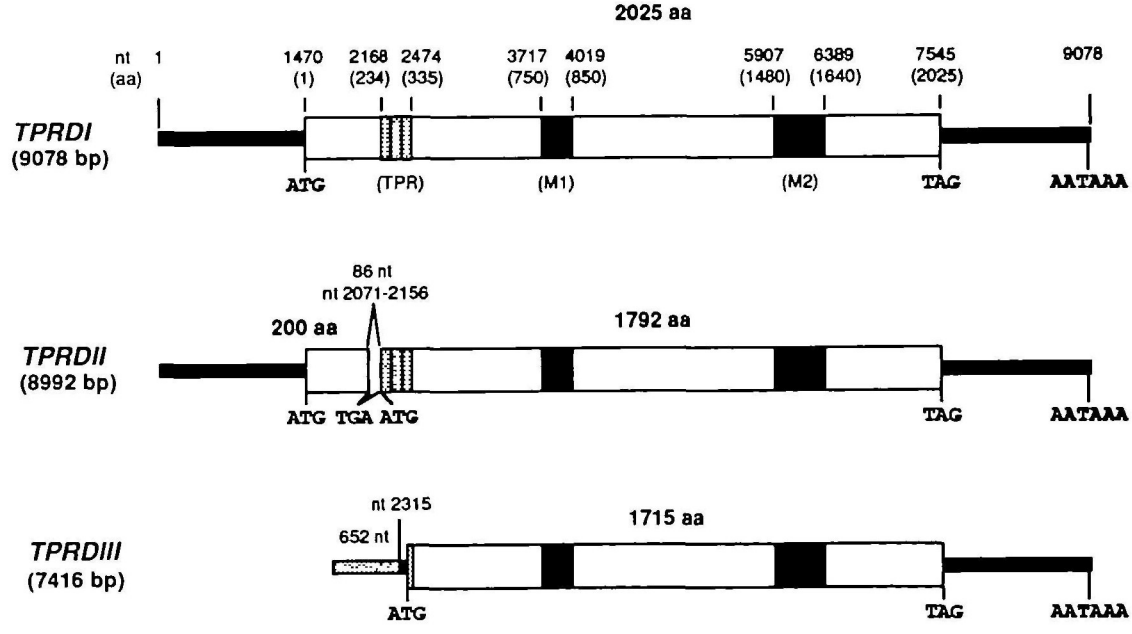

Fig. 2. **Schematic comparison of the *TPRD* gene transcripts.** ORFs of *TPRDI*, *TPRDII*, and *TPRDIII* are indicated by box columns and the non-coding regions are indicated by bars. Three units of TPR motifs (TPR) are half-toned and the regions showing some homology to matrix proteins (M1 and M2) are shown by filled boxes. *TPRDII* has a small deletion sequence of 86 nt and *TPRDIII* has a unique 5′-end sequence of 652 nt which is indicated by half-tone. See text for further detail.

nt 8546-8915, respectively. Thus, the *TPRD* gene is mapped to DCR.

We also isolated cDNA isoforms of *TPRDI*, designated *TPRDII* (8,992 bp) and *TPRDIII* (7,416 bp) (Fig. 1, A and B, and Fig. 2). *TPRDII* had a small deletion from nt 2071 to 2156 in comparison with *TPRDI*. As shown in Fig. 3, two RT-PCR products of 455 and 369 bp, corresponding to sequences from *TPRDI* and *TPRDII*, respectively, were detected in human fetal brain, heart, and kidney by using primers flanking this deletion. Thus, *TPRDII* is probably an alternative splicing product from the *TPRD* gene transcript. This small deletion generated a stop codon TGA so that *TPRDII* has two putative ORFs: one ORF of 600 nt begins at the same initiator codon in *TPRDI* and ends at the newly generated stop codon, and the other ORF of 5,376 nt begins at a possible initiator codon (ATG) at nt 2169-2171. It remains to be elucidated which ORF(s) is really translated into protein(s). In contrast, *TPRDIII* has a different 5′-end sequence of 652 nt which is connected to adenosine residue at nt 2315 of *TPRDI*, suggesting that it is generated by transcription from an alternative start site of the *TPRD* gene. Since the different 5′-end sequence of *TPRDIII* was mapped back to the original P1 clone (T1212), it may not be a cloning artifact. Thus, *TPRDIII* possesses a 5′-untranslated sequence of 737 nt, followed by a single ORF of 5,145 nt which begins at a possible initiator codon (ATG) at nt 2400-2402 in *TPRDI*. Therefore, *TPRDIII* encodes a putative protein of 1,715 amino acid residues (TPRDIII) with a calculated molecular mass of 194 kDa and pI of 8.0. It is of interest to note that the 5′-untranslated sequence of *TPRDIII* has an Alu-like sequence.

As shown in Fig. 4, Northern blot analysis showed that a probe from an identical region among these isoforms (nt 2711-3222) revealed two bands, one at the position of 9 kb

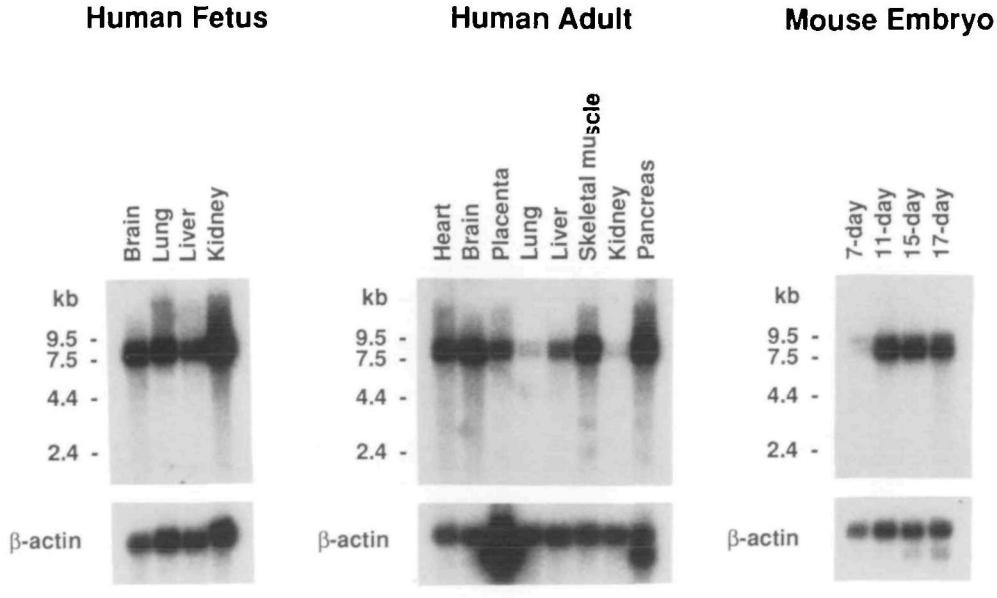

Fig. 4. **Northern blot analysis of the *TPRD* gene.** The radiolabeled probe from a region common to *TPRDI* and its isoforms (nt 2711-3222 in *TPRDI*) was hybridized to poly(A)$^+$ RNAs (2 μg) isolated from several tissues of human fetus and adult and from 7-17 day mouse embryo (Clontech). Two bands, one at the position of approximately 9 kb corresponding to *TPRDI* and *TPRDII* and the other at approximately 7.4 kb for *TPRDIII*, were observed in all the tissues examined. A β-actin control for RNA integrity is shown at the bottom. The size marker is indicated to the left of the figures.

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TPR CONSENSUS  * - - * G - * Y - - - - - * - - A * - - F - - A * - * - P - - - - -
                                F                       Y

TPRDI    (234)  M K M K G N E E F S K E R F D I A I I Y Y T R A I E Y R P E N Y L L
hPPT      (25)  L K T Q A N D Y F K A K D Y E N A I K F Y S Q A I E L N P S N A I Y
rPPT      (31)  L K T Q A N D Y F K A K D Y E N A I K F Y S K A I E L N P S N A I Y
IEF(1)     (7)  L K E K G N K A L S V G N I D D A L Q C Y S E A I K L D P H N H V L
IEF(2)   (363)  E K N K G N E C F Q K G D Y P Q A M K H Y T E A I K R N P K D A K L
STI1(1)    (8)  Y K Q Q G N A A F T A K D Y D K A I E L F T K A I E V S E T P N H V L
STI1(2)  (399)  A R L E G K E Y F T K S D W P N A V K A Y T E M I K R A P E D A R G

TPRDI    (268)  Y G N R A L C F L R T G Q F R N A L G D G K R A T I L K N T W P K G
hPPT      (59)  Y G N R S L A Y L R T E C Y G Y A L G D A T R A I E L D K K Y I K G
rPPT      (65)  Y G N R S L A Y L R T E C Y G Y A L G D A T R A I E L D K K Y I K G
IEF(1)    (41)  Y S N R S A A Y A K K G D Y Q K A Y E D G C K T V D L K P D W G K G
IEF(2)   (397)  Y S N R A A C Y T K L L E F Q L A L K D C E E C I Q L E P T F I K G
STI1(1)   (43)  Y S N R S A C Y T S L K K F S D A L N D A N E C V K I N P S W S K G
STI1(2)  (433)  Y S N R A A A L A K L M S F P E A I A D C N K A I E K D P N F V R A

TPRDI    (302)  H Y R Y C D A L S M L G E Y D W A L Q A N I K A Q K L C K N D P E G
hPPT      (93)  Y Y R R A A S N M A L G K F R A A L R D Y E T V V K V K P H D K D A
rPPT      (99)  Y Y R R A A S N M A L G K F R A A L R D Y E T V V K V K P N D K D A
IEF(1)    (75)  Y S R K A A A L E F L N R F E E A K R T Y E E G L K H E A N N P Q L
IEF(2)   (431)  Y T R K A A A L E A M K D Y T K A M D V Y Q K A L D L D S S C K E A
STI1(1)   (77)  Y N R L G A A H L G L G D L D E A E S N Y K K A L E L D A S N K A A
STI1(2)  (467)  Y I R K A T A Q I A V K E Y A S A L E T L D A A R T K D A E V N N G
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Fig. 5. **Characterization of the TPR motif in TPRDI.** The TPR motifs in TPRDI, serine/threonine phosphatase of human (hPPT) (*21*) and rat (rPPT) (*22*), human IEF SSP 3521 (IEF) (*23*) and *S. cerevisiae* STI1 (*24*) are aligned in terms of the consensus TPR sequence (*31*). IEF SSP 3521 and STI1 have two TPR motifs. Residues identical to TPRDI are boxed. The asterisks in the consensus TPR sequence indicate any hydrophobic residues. The position of the first amino acid residue of each TPR is given in parentheses.

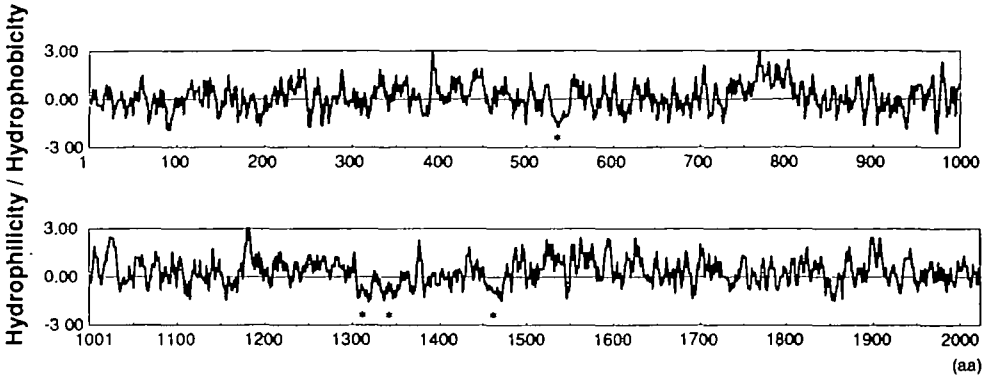

Fig. 6. **Hydropathy analysis of TPRDI.** Hydropathy plot was made according to Hopp and Woods (*28*) with a window of 5 residues. Four hydrophobic regions which might be transmembrane regions are indicated by asterisks.

corresponding to *TPRDI* and *TPRDII*, and the other at approximately 7.4 kb for *TPRDIII*. The transcripts of the *TPRD* gene were expressed in 7-17 day mouse embryo and in all the human fetal and adult tissues examined.

Protein database searches for the predicted protein, TPRDI, using the BLAST program revealed the tetratricopeptide repeat (TPR) motif homologous to three units of a 34-amino-acid repeat in several proteins, including serine/threonine phosphatase of human (*21*) and rat (*22*), human transformation-sensitive protein IEF SSP 3512 (*23*) and yeast heat shock protein STI1 (*24*), in an N-terminal region (aa 234-335) (Figs. 2 and 5). The TPR consensus sequence is well conserved in TPRDI. A larger ORF encoded by *TPRDII* also has three units of TPR motif, but TPRDIII has only two-thirds of this motif unit. In addition, the near-central and C terminal regions of TPRDI (aa 750-850 and aa 1480-1640), which are predicted to have α-helical structures by the Chou-Fasman analysis (*25*), also showed some homology to several peptide repeat regions of several matrix proteins, such as human trichohyalin, an intermediate filament-associated protein (*26*), human bullous pemphigoid antigen, an adhesion junction plaque protein (*27*), and myosin heavy chain of several species. A larger ORF encoded by *TPRDII* and TPRDIII also have these regions. Hydropathy analysis using Hopp and Woods hydropathy plot (*28*) revealed four hydrophobic regions, which might be transmembrane domains (Fig. 6). These data suggest that the *TPRD* gene belongs to the TPR gene family and encodes a matrix protein containing transmembrane structure.

The TPR motif was initially identified in several cell-division cycle gene products (cdc16, cdc23, nuc2$^+$, bimA) and proteins involved in the regulation of RNA synthesis (SSN6, SKI3) (*29-32*). Now it has been found in about 20 different proteins. It has been suggested that TPR-containing proteins physically interact with themselves, with each other and with the cytoskeleton *via* their TPR domains (*30, 32*). Some TPR-containing proteins may interact functionally with proteins containing a 43-amino-acid repeat called the beta-transducin repeat (*30*). It is possible, therefore, that the overexpression of the *TPRD* gene causes imbalance of the protein-protein interaction during cell growth and differentiation, and consequently leads to several morphological anomalies observed in Down syndrome. Recently, Ohira *et al.* also identified a novel cDNA of 9,045 bp, designated *TPRD*, from the same P1 clone (*33*). Their *TPRD* may be an another cDNA isoform, since it has a different 5′ end sequence of 1,385 nt which is connected to adenosine residue at nt 1459 of *TPRDI*. Further study will clarify the role of these isoforms in the pathogenesis of Down syndrome.

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