Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3077–3081, April 1990
Medical Sciences

Cerebellar degeneration-related antigen: A highly conserved neuroectodermal marker mapped to chromosomes X in human and mouse

(paraneoplastic syndrome/autoimmunity/chromosome mapping)

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Contributed by Lloyd J. Old, January 2, 1990

ABSTRACT **Cerebellar degeneration-related antigen (designated CDR34) was previously cloned by antibody screening of a cDNA library and was shown to be one of the target molecules recognized by autoantibodies in patients with paraneoplastic cerebellar degeneration. This molecule is distinctive in that it contains a tandem hexapeptide repetitive structure, presumably the basis for its high immunogenicity. In this study, we cloned the human *CDR34* gene and proved that the entire repetitive sequence is encoded by a single exon without introns. We also showed that the nucleotide repeats are preserved only in the protein-coding sequences, suggesting evolutionary constraint in this region of the gene. Corresponding mouse cDNA clones were also isolated, which encoded a larger molecule with very similar hexapeptide repeating units. Comparison of the human and mouse repeats revealed a highly conserved Glu-Asp core in each unit, implicating the functional significance of this motif. Chromosomal mapping by somatic cell hybrid analysis mapped *CDR34* to both human and mouse chromosomes X, and *in situ* hybridization further assigned *CDR34* to human Xq24–q27.**

Paraneoplastic cerebellar degeneration (PCD) is a disorder characterized by degeneration of Purkinje cells occurring in patients with occult or identifiable neoplasms (1, 2). In a subset of patients with PCD, who usually harbor gynecologic cancers, an autoantibody designated anti-Yo (2) identifies antigens expressed in cerebellar Purkinje cells and the underlying cancer (2). The anti-Yo antibody identifies two antigens, with molecular masses of 62 kDa and 34 kDa. Antibody screening of a λgt11 cerebellar cDNA library with IgG from a PCD patient led to the isolation of human CDR cDNA clones encoding the cerebellar degeneration-related 34-kDa antigen (designated CDR34) (3, 4). Sequencing of the 1.16-kilobase (kb) cDNA clone pCDR13 revealed a distinctive pattern consisting of units of 18 nucleotides (encoding 6 amino acids) repeated in tandem along the entire clone. RNA blotting analysis with normal tissues showed high level expression of the *CDR34* gene in the brain, particularly in the cerebellum; other normal tissue showed little or no CDR34 mRNA. Examination of human tumor cell lines demonstrated CDR34 mRNA in cancers of neuroectodermal origin—such as astrocytoma, melanoma, and neuroblastoma—but it was also detected in some epithelial cancer cell lines—e.g., kidney and lung cancers. Rabbit anti-peptide IgG raised against a synthetic peptide corresponding to amino acid residues 149–165 of the putative open reading frame recognizes Purkinje cells and tumor tissue from patients with PCD by immunohistochemistry and the 34-kDa band by Western blotting (4). Thus, CDR34 protein is a normal neuroectodermal marker with a potentially immunogenic, tandemly repeated, hexapeptide structure that can be anomalously expressed in certain nonneuroectodermal cancers. In the present study, we define human *CDR34* as a single-exon gene mapped to the X chromosome. In addition, the mouse *CDR34* gene was found to share important structural features with human *CDR34* and was similarly mapped to the X chromosome.‖

MATERIALS AND METHODS

Construction and Screening of Human Genomic Sublibrary. λgt.WES.λB *Eco*RI arms (Bethesda Research Laboratories) were annealed and ligated to *Eco*RI-digested 8- to 10-kb human genomic DNA in an equimolar ratio. The ligated DNA was packaged *in vitro* using Gigapack packaging extracts (Strategene), and 1.0×10^6 recombinant phages were obtained per μg of arms. Plaque hybridization with ^{32}P-labeled nick-translated probe was carried out as described (5).

DNA Sequencing and Analysis. DNA fragments were subcloned into M13mp18 and M13mp19, sequenced by the dideoxynucleotide chain-termination method (6), and analyzed with computer programs of BIONET.

Southern Blot Analysis. High molecular weight genomic DNA (15 μg) was digested with restriction endonucleases, electrophoresed in 0.7% agarose gels, and transferred to nitrocellulose (7). Filters were prehybridized and hybridized to nick-translated probes at 65°C for 15 hr. Hybridization was performed in 5× SSC/4× Denhardt's solution/50 mM phosphate buffer/50 μg of salmon sperm DNA per ml/10% dextran sulfate (1× SSC = 0.15 M NaCl/0.015 M sodium citrate; 1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone). Filters were then washed and autoradiographed.

Hybrid Cell Lines. Human cell lines and rodent–human hybrid clones used for human chromosome mapping, as well as mouse–Chinese hamster hybrid clones for mouse chromosome mapping, have been described (8–11).

***In Situ* Hybridization.** *In situ* hybridization of molecular probes to somatic and meiotic chromosomes was performed as described (12).

Neuroblastoma Cell Variants. The derivation and maintenance of human neuroblastoma cell lines have been described (13). The cloned sublines of neuroblastoma cell variants

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Abbreviation: PCD, paraneoplastic cerebellar degeneration.
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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M31423).

representing neuroblastic (N-type) and substrate-adherent (S-type) subpopulations were isolated as described (13).

RESULTS

Genomic Organization of the Human *CDR34* Gene. DNA blot analysis of human DNA showed hybridization of the 1.2-kb pCDR13 probe to an 8.5-kb *Eco*RI fragment and a 2.4-kb *Pst* I fragment (3). A subgenomic human library enriched for 8.5-kb *Eco*RI species was constructed in λgt.WES.λB and screened with pCDR13. Fig. 1 shows the restriction map of a representative positive clone, λCDR34. It contains a 2.4-kb pCDR13-positive *Pst* I fragment, consistent with the genomic Southern blot analysis. This fragment is entirely sequenced (Fig. 2).

The 1.2-kb pCDR13 cDNA sequence, which includes the entire coding region and part of the 5′ and 3′ untranslated regions of the *CDR34* gene, is identified as a single exon. The tandem-repeated sequences characteristic of the *CDR34* gene are found to extend over a 1.4-kb DNA stretch in the genomic clone. They are absent outside this 2.4-kb *Pst* I fragment as indicated by negative hybridization to pCDR13. There are 77 inexact repeating units in total, with 14 units 5′ and 26 units 3′ to the mRNA coding sequences. The coding sequences contain 37 nucleotide repeats but 34 hexapeptide repeats; a single nucleotide insertion disrupts the repeating reading frame 17 amino acids proximal to the termination codon (amino acid position 207) (3). The repeating units in both flanking regions, although maintaining the fundamental repeating structure, show great variation in nucleotide number and composition among units, indicating an absence of evolutionary constraints in these regions.

Comparison of Human and Mouse *CDR34* Genes. DNA blot analysis of mouse DNA showed strong cross-hybridization with pCDR13, and RNA blot analysis of the mouse brain RNA indicated that the mouse CDR34 transcript is 3.5 kb in size, in contrast to the 1.3- to 1.5-kb transcripts in human (3). A mouse brain cDNA library constructed in λgt10 was screened with pCDR13, and five overlapping clones were obtained. These five clones, designated mCDR19, -20, -35, -65, and -68, span a total length of 2.4 kb (Fig. 3). Sequence analysis revealed similar tandem repeats throughout the entire sequence, significantly longer than the 1.4-kb repeats in the human genomic clone λCDR34.

Detailed examination of the cDNA sequence showed a 1.2-kb 5′ untranslated region, evidenced by the lack of long open reading frames. This DNA segment contains >60 nucleotide repeats, with the nucleotide number in each repeating unit varying between 17 and 21 nucleotides. This lack of precision, similarly observed in the 5′ and 3′ untranslated regions of the human *CDR34* gene (see above), further suggests assignment to 5′ untranslated sequences.

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ATGTTGGTTCATAAGATCTGGTCTATAAGGAGGAATGTCCCATTAAATGTTTTTGAAGCTAATTCAACTAGAAG   74
CAGAAATAGTTGAGTTGGAAGATTTTCTGTAGAGTGATTTTAACATGGGAAGGCTCAGACAGGGGAAGCCTAGA  148
TTTGAAAAGGCCTGGACCTGGGGAAAGGCTGGCAAGATCTGGACTATAGAACATGTTAGAATACTGATATTCGC  222
AGACACCTGGAAGACTGAATGTCAGAAGATCAGCACACTGGAGACGTTGGAAGACATGGATATTGAGCCAGTT  295
GATGGAAGACTGGGTAGTTGTTGGAAGACATCAAGGTGCTGGAAGACACAGCAGCATGCTGGAAGACCTGGAGA  369
TGTTGGAAGACGAGCAGACTCCTGGAAGCCCTGGAGATGCTGCAAGACCTGGAGATATAGGAAGACACTGGACT  443
TTGTTGCGAGCTTAGTTGGAAGACATATATTTTTGGAAGACGTGGATTTTCTGGAAGAC ATG GCT TGG TTG  514
GAA GAC GTG GAT TTT CTG GAA GAC GTA CCT TTG TTG GAA GAC ATA CCT TTG TTG GAA GAC GTA  577
CCT TTG TTG GAA GAC GTA CCT TTG TTG GAA GAC ACA AGT AGG CTG GAA GAC ATT AAT TTG ATG  640
GAA GAC ATG GCT TTG TTG GAA GAC GTG GAT TTG CTG GAA GAC ACG GAT TTC CTG GAA GAC CTG  703
GAT TTT TCG GAA GCT ATG GAT TTG AGG GAA GAC AAG GAT TTT CTG GAA GAC ATG GAT AGT CTG  766
GAA GAC ATG GCT TTG TTG GAA GAC GTG GAC TTG CTG GAA GAC ACG GAT TTC CTG GAA GAC CCG  829
GAT TTT TTG GAA GCT ATA GAT TTA AGG GAA GAC AAG GAT TTT CTG GAA GAC ATG GAT AGT CTG  892
GAA GAC CTG AGG CCA TTG GAA GAT GTG GAT TTT CTG GAA GAC ATG GCT TTT TTG GAA GAC GTA  955
GAT TTT CAG GAA GAC CCA AAT TAT CCG GAA GAC TTG GAT TGT TGG GAA GAC GTG GAT TTT CTG  1018
GAA GAC TGG AGG TTA CTG GAA GAC ATG GAT TTT CTG GAA GAC ATG GAT TTT CTG GAA GAC GTG  1081
GAT CTT CAG GAA GAC ATA TAT TGG CTG GAA GAC CTG GAT TTT TTC CGG AAG ATG TGG ATT GAC  1144
TGG AAG ACC TGG ATT TGG TGG AAG ACG TAG ATTTTTCTGGAAGACACTGACTGACTGGAAGACACTGA  1212
TTGACTGGAAGACCTGGATTTCTTTCTGGAAGACACTGATTGACTGGAAGATCTAGATTTTTCTGGAAGAACTA  1286
GATTTACTGGAAGACTTGGATTTGGTGGAAGACACAGATTTTTCTGGAAGACATGGATTAGCTGGAAGATCTGT  1360
ATTTGATGGAAGACCTTGAAATTATTGGAAGACATGGATTTCCTGGAAGACGTGGATTTTCCTGGAAGATCTGG  1434
ATTTGGTGGAAGACCAGTAATTGCTGGAAGACTGGATTTGCTGGAAGACTTGATTTACTGGAAGACTTGGAGCT  1508
TCTTGGAAGACATGGATTGTCCGGAAGACATGGATTGTCTGGAAGATGTGGATTTTCTGGAAGCTCAGGATTAT  1582
CTGGAAGACCTTGAGATTATTGGAACACTTGAAGTCGCTGGAAGACCCGAGTTGTTGGAAGACCTTGTACACAG  1656
GTGCCATCGGAACTCCTGACATTGAAACATTGTAAGCACAGGATATTGAGACATTGCAAGCCTTGATTTTAAGA  1730
CATGGTACTCTGGACATTGATATTTCTGAGGCCCTGAACATTGGGATATTAATATTGGAAGTCATAGACACTGA  1804
AATCTCTGGAAATTAGAGATATTGTAAGTCCTGTACCTTGGAACTCCTAAATACTGGCAGATATAAACAACAG  1877
CAGATGTAGACATTTATAAATCCTAAAATGAGAAGCCCTGGATATTGGGAGACATTGGTAAGCATGGATACTT  1950
GACATATTTATGTCAAAAAGACAGTTTGGAAGAATTAAATTTTAAAGATGCTCCATGTCAAGAATACTGGCAG  2023
CCTGGACAATATGAGACCAGGATATTAAGAGGTCTATTCATTCAGACATTGAGGATATTGATGTACCTGAAAG  2096
TTCTTGCAGGTATTTAAAGACTTGAGCATTGGAGGAATTGGCGATAAAAATACACTGTAAAACTAGAAAGTAG  2169
GAGACATTTAAAAATGTAAAAACTGAATGATGTAAGTGCTGGAAGACATTGAAGAATCTAGAAGACCTGTAT  2241
ATAGGAGACATTGGAGGATTAGGACCATGGCCGACTTGTAATTTAGAACTCTGGATTCTGAAAGACAAGACCTG  2315
GACTTTGAAGAAGGGTTGTTGGAGATATTAGAAGACCTAAATTTTTAATGACTTGAATACTGGGAGTTTAGAA  2388
AACAAGGGCATTTGAGATGCTGCAG                                                    2403
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FIG. 2. Nucleotide sequence of the 2.4-kb *Pst* I fragment containing the genomic *CDR* gene. The protein coding region is shown in italics, and the initiation and termination codons are boldface. The sequence GAAGAC (and its analogs) represents the core Glu-Asp in the repeating unit and is underlined throughout the flanking sequence to illustrate the extent of the repeating units.

Following this 1.2-kb segment is the translation initiation codon ATG, and this is followed by ≈50 tandem repeats, estimated from restriction mapping. The sequencing of this segment is hampered by the highly repetitive nature and lack of any restriction site in this region, but exact sequences were obtained for 38 of the 50 repeats. All 38 repeats contain 18

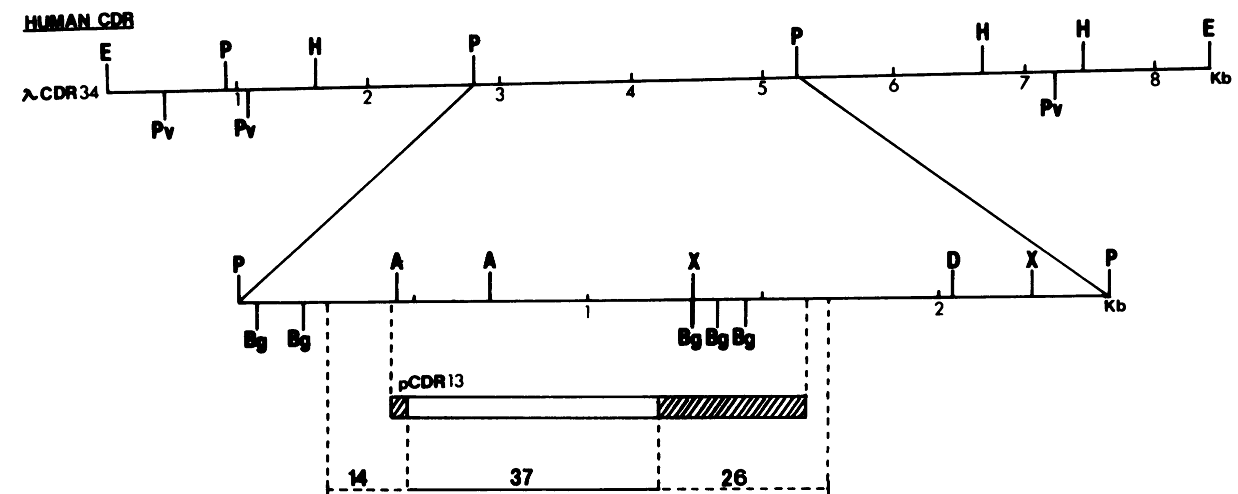

FIG. 1. Restriction map of the human genomic *CDR34* clone λCDR34. The 2.5-kb *Pst* I fragment contains the cDNA clone pCDR13 previously described (3). Hatched areas indicate 5′ and 3′ untranslated regions. Number of repeating units in 5′ flanking, protein-coding, and 3′ flanking regions are shown in the bottom line. A, *Ala* I; Bg, *Bgl* II, D, *Dra* I; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; Pv, *Pvu* II; X, *Xba* I.

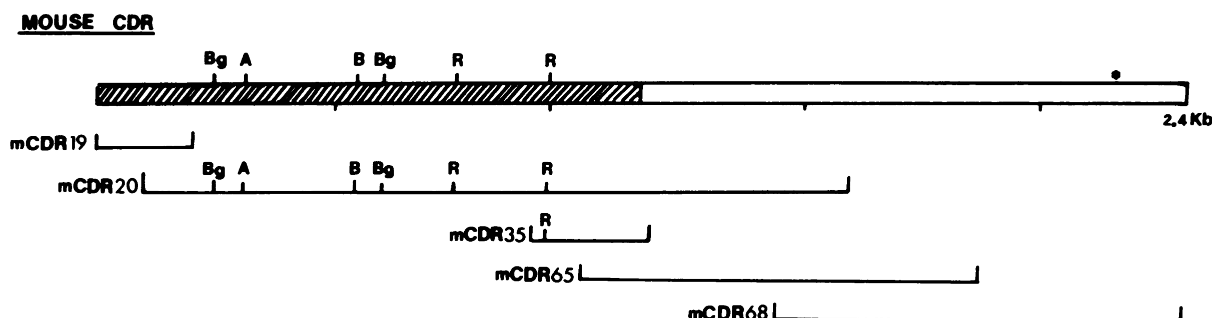

FIG. 3. Restriction map of the mouse CDR34 cDNA clones. Five overlapping clones were isolated, spanning 2.4 kb. Hatched area indicates the 5′ untranslated region, and asterisk indicates the end of the coding sequences for the hexapeptide repeats. The repeats are disrupted due to a 2-nucleotide insertion (see text). A, *Alu* I; B, *Bam*HI; Bg, *Bgl* II; R, *Rsa* I.

nucleotides in each unit and constitute an open reading frame of hexapeptide repeats. Similar to the human CDR34, this repeat is subsequently disrupted by a 2-nucleotide insertion, leading to a frameshift event and, thus, termination of the amino acid repeats. The available mouse CDR34 cDNA clones, however, indicate at least 37 nonrepetitive amino acid residues in the carboxyl end of the peptide, longer than the 17-residue tail in the human CDR34 peptide.

Fig. 4 shows the sequence comparison between human and mouse CDR34 repeats. The most distinct feature shared by the two species is the invariant Glu-Asp (codons GAA-GAC) core, a finding previously noted for the human CDR34 repeats (3). The other aspartic acid residue in the repeat, frequently observed (21/34 repeats) in the human *CDR34* gene, is markedly conserved in the mouse (34/38 repeats). Not unexpectedly, the positions that are more variable in human *CDR34* are also less conserved in the mouse.

Chromosome Mapping of the Human *CDR34* Gene. The human *CDR34* gene was mapped with a panel of 27 rodent–human somatic cell hybrids using pCDR12 as the probe. pCDR13 detects a 2.4-kb fragment in *Pst* I-digested human genomic DNA and cross-hybridizes to a 9.0-kb mouse CDR34 fragment, allowing a distinction between the mouse and human CDR34 in the hybrid DNA. Discordancy analysis (Table 1) shows that the human *CDR34* gene segregates with the human X chromosome and excludes all other chromosomes as potential sites. Furthermore, a hybrid containing the long arm of human chromosome X, GM7298, is positive for the CDR34 sequence. Human Y chromosome, present in hybrid LM29, does not contain a CDR34 homolog.

The localization of the *CDR34* gene on human chromosome X was confirmed by *in situ* hybridization of pCDR13 to normal human metaphase cells, which mapped CDR34 to Xq24–q27 (Fig. 5).

Chromosome Mapping of the Mouse *CDR34* Gene. The mouse *CDR34* gene was similarly mapped with a panel of 25 Chinese hamster–mouse somatic cell hybrids (12) containing reduced numbers of mouse chromosome. In *Bam*HI digests, pCDR13 hybridizes to an 11-kb fragment in mouse genome, and a 23-kb fragment in Chinese hamster DNA. Discordancy analysis (Table 2) concludes that the mouse *CDR34* gene is also located on the mouse X chromosome.

Human *CDR34* Expression in Human Neuroblastoma Cell Variants. All human neuroblastoma cell lines that have been examined express the *CDR34* gene (3). Two morphologically distinct cell types have been described in cultured neuroblastoma cell lines—namely, neuroblastic (N-type) cells and substrate-adherent (S-type) cells (13). The S-type variant cells were shown to have a molecular phenotype similar to ectomesenchymal cells, in contrast to the neuronal molecular phenotypes of N-type variants. Two pairs of N-type and S-type variants [LA-N-1 and LA1-5s, BE(2)-88n and BE(2)-99s], each derived from the same primary culture, were analyzed for *CDR34* expression by RNA dot blot analysis. The results indicate down-regulation of *CDR34* expression in the S-type variants [LA1-5s and BE(2)-99s] to 5–10% of the level of the N-type (Fig. 6). This is supportive of our previous concept that *CDR34* is predominantly a neuronal marker and has little or no expression in mesenchymal cells.

HUMAN	Nucleotide	TT G/T	C/T TG	GAA	GAC	A/G TG	GAT
	Amino Acid	Leu/Phe	Leu	Glu	Asp	Met/Val	Asp
	No. appeared	26	26	34	32	18	21
MOUSE	Nucleotide	TT C/T	CA/TG G	GAA	GA C/T	TT G/C	GAT
	Amino Acid	Phe	Trp/Gln	Glu	Asp	Leu/Phe	Asp
	No. appeared	37	26	38	37	27	34

FIG. 4. Comparison of the tandem repeats in the human and mouse CDR34 sequences. The 34 human repeats and 38 mouse repeats sequenced are summarized to illustrate the consensus 18-nucleotide/hexapeptide unit.

Table 1. Human chromosome content and presence of human CDR34 sequences in human–mouse somatic cell hybrids

Human chromosome	CDR34 DNA hybridization/ chromosome retention,* no. of hybrid clones				% discordant
	+/+	−/−	+/−	−/+	
1	5	2	5	0	42
2	1	8	16	0	64
3	1	5	16	1	74
4	4	6	11	1	55
5	6	7	13	1	52
6	6	5	12	3	58
7	4	4	15	4	70
8	2	6	17	2	70
9	2	7	17	1	67
10	7	4	10	3	54
11	5	5	4	3	41
12	5	3	13	4	68
13	1	8	18	0	67
14	12	3	6	4	40
15	13	4	6	2	32
16	4	6	13	0	57
17	2	3	17	2	79
18	0	8	19	0	70
19	4	6	9	2	52
20	12	3	5	3	35
21	10	2	5	4	43
22	2	8	14	0	58
X	19	8	0	0	0
Y	1	7	19	0	70

*By karyotyping and/or testing for human isozymes and cell surface antigens.

Table 2. Mouse chromosome content and presence of mouse CDR34 sequences in mouse–Chinese hamster somatic cell hybrids

Mouse chromosome	Mouse CDR/chromosome retention,* no. of hybrid clones				% discordant
	+/+	−/−	+/−	−/+	
1	7	6	3	4	35
2	8	4	3	7	45
3	7	6	2	4	32
4	6	8	5	2	33
5	2	8	7	2	47
6	8	8	3	3	27
7	8	3	3	8	50
8	6	8	5	3	36
9	5	8	5	3	38
10	2	11	9	0	41
11	0	9	11	0	55
12	7	4	3	6	45
13	2	4	3	4	54
14	3	8	7	3	48
15	9	2	0	7	39
16	3	2	4	6	67
17	11	4	0	7	32
18	3	5	3	3	43
19	5	7	5	3	37
X	10	11	0	0	0

*Fourteen hybrids were karyotyped, and the rest were typed for markers on specific chromosomes.

DISCUSSION

Sequence analysis of the human *CDR34* gene revealed tandem hexapeptide repeats unlike any previously described in eukaryotic genes. DNA blot analysis yielded single genomic fragments using a number of restriction enzymes, suggesting that the entire repetitive structure is derived from one exon or from exons lacking restriction sites (3). In this study, we isolated and sequenced the human *CDR34* genomic clone and proved that the entire repetitive structure, indeed, is encoded by a single exon. This finding is not surprising for two reasons: (*i*) the disruption of such a repetitive structure into more than one exon would create the probability of splicing errors, leading to a nonfunctional protein product; (*ii*) the entire repeating structure is best considered as a single domain and, based on the notion of one exon/one domain, would reasonably be encoded by a single exon. Literature review of other proteins with similar tandem repeats, such as

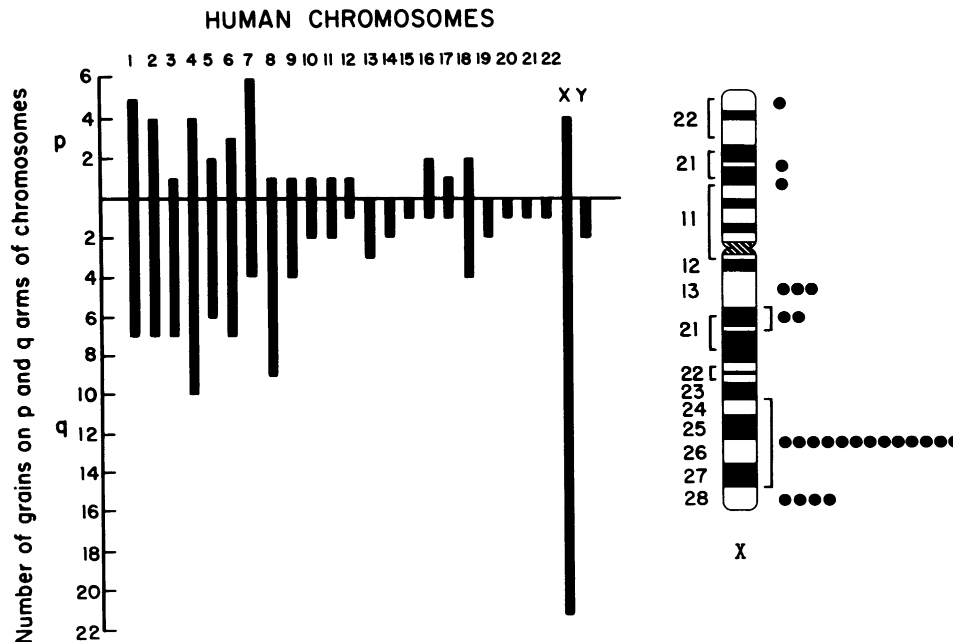

FIG. 5. *In situ* hybridization of pCDR13 to chromosomal preparation from cultured lymphocytes of a normal male. A total of 100 metaphases with 145 silver grains were evaluated and 25 grains were found on the X chromosome, corresponding to 34% of grains per haploid genome. Twenty-two grains were on Xq and 13 grains were on Xq24–q27.

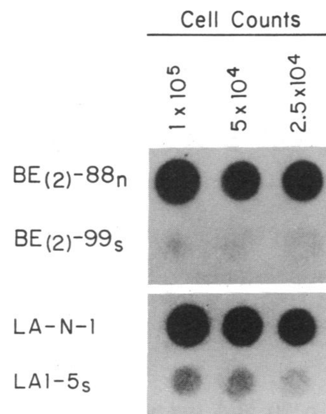

FIG. 6. CDR34 mRNA expression in N- and S-type neuroblastoma wall lines. Total cellular RNA was prepared from two neuroblastic (N-type) neuroblastoma cell lines [LA-N-1 and BE(2)-88n] and their substrate-adherent (S-type) variants [LA1-5s and BE(2)-99s], transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled nick-translated pCDR13.

mouse RNA polymerase II (14) and involucrin (15), confirms that the repetitive parts of the molecule, indeed, are always encoded by one exon. However, in both RNA polymerase II and involucrin, there are separate exons 5′ to the repeats that code for either the nonrepetitive part of the protein (14) or the 5′ untranslated region (15). This has not been found in the CDR34 clones analyzed. However, the 5′ boundary of the CDR34 mRNA has not been completely defined. Primer-extension studies using the preexisting clones would help elucidate the transcription unit of the *CDR34* gene and pave the way for the study of how tissue-specific expression of *CDR34* is regulated.

The mouse CDR34 transcript is significantly larger than the human transcript, and this could be explained by longer untranslated regions, extra tandemly repeated units, or the presence of nonrepetitive coding sequence. This last possibility could lead to significant changes in protein configuration and modification in biological activity. Our results to date indicate that the size difference between mouse and human CDR34 transcripts is due to longer untranslated regions and extra tandemly repeated units, neither of which would change the basic structure of the protein product. Comparison of human and mouse CDR34 sequences showed two interesting features regarding the repeated hexapeptide units. First, the invariant Glu-Asp core in the human hexapeptide repeats is also strictly preserved in the mouse CDR34; second, the more variable residues in human—e.g., the residues immediately 5′ to the Glu-Asp core—are also the residues that display marked interspecies differences. This strongly implies the importance of the Glu-Asp core in the biological function of this molecule.

CDR34 expression in normal tissues appears to be restricted to neuronal tissue, with particularly high expression in Purkinje cells (3, 4). In screening cancer cell lines, all neuroblastoma cell lines tested showed *CDR34* expression. An interesting property of the neuroblastoma cell lines is the spontaneous conversion between two morphologically distinct cell types: neuroblastic (N-type) cells that are only loosely substrate adherent, and the substrate-adherent (S-type) cells. Previous studies have shown that N-type cells have neuronal characteristics, whereas S-type cells most closely resemble the fibroblast-like meningeal cells. In this study, we demonstrated that the loss of neuronal features in S-type variants is closely associated with a parallel decrease in *CDR34* expression. Although the function of the protein remains unclear, the availability of the mouse *CDR34* gene now provides us a tool to examine developmental regulation of *CDR34*, as well as its expression in normal mouse and neurological mouse mutants—e.g., Purkinje cell-loss mutants.

By *in situ* hybridization and somatic cell hybrid analysis, we have mapped *CDR34* to human and mouse X chromosomes. There are nearly 20 human X chromosome-linked disorders involving the nervous system (16). One of them, the fragile X disease, is mapped closely to the *CDR34* locus. Additional fine mapping further narrowed the *CDR34* gene to human Xq27.3, at the upper boundary of *FRAXA*, the locus of fragile X syndrome (17). Molecular analysis of the *CDR34* gene from patients with X chromosome-linked neurological disorders, therefore, may reveal changes in *CDR34* gene organization and/or expression and may shed light on the normal function of the CDR34 product.

We are grateful to Dr. J. Bielder for providing the N- and S-type variants of neuroblastoma cell lines. This research was supported by the Samuel Freeman Charitable Trust, and BIONET National Computer Resource for Molecular Biology (Grant 1 U41 RR-01685-03) sponsored by the National Institutes of Health.

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