Am. J. Hum. Genet. 49:320–329, 1991

Genomic Structure of the Human Prion Protein Gene

Carmie Puckett,* Patrick Concannon,† Chris Casey,* and Leroy Hood*

*Division of Biology, California Institute of Technology, Pasadena; and †Department of Immunology, Virginia Mason Research Center, University of Washington, Seattle

Summary

Creutzfeld-Jacob disease and Gerstmann-Sträussler syndrome are rare degenerative disorders of the nervous system which have been genetically linked to the prion protein (PrP) gene. The PrP gene encodes a host glycoprotein of unknown function and is located on the short arm of chromosome 20, a region with few known genes or anonymous markers. The complete structure of the PrP gene in man has not been determined despite considerable interest in its relationship to these unusual disorders. We have determined that the human PrP gene has the same simple genomic structure seen in the hamster gene and consists of two exons and a single intron. In contrast to the hamster PrP gene the human gene appears to have a single major transcriptional start site. The region immediately 5′ of the transcriptional start site of the human PrP gene demonstrates the GC-rich features commonly seen in housekeeping genes. Curiously, the genomic clone we have isolated contains a 24-bp deletion that removes one of five octameric peptide repeats predicted to form a B-pleated sheet in this region of the PrP. We have also identified 5′ of the PrP gene an RFLP which has a high degree of heterozygosity and which should serve as a useful marker for the pter-12 region of human chromosome 20.

Introduction

Creutzfeld-Jacob disease (CJD) and Gerstmann-Sträussler syndrome (GSS) are rare degenerative disorders of man that demonstrate similar pathological changes in the nervous system. CJD seems to occur sporadically in all populations at a frequency of 1/1 million, although in 5%–15% of the cases it demonstrates a familial occurrence (Brown et al. 1979; Masters et al. 1981). The closely related disorder GSS is characterized by familial occurrence, progressive ataxia, and dementia. The pattern of transmission of both familial CJD and GSS suggests an autosomal dominant pattern of inheritance, yet paradoxically these disorders can be transmitted to primates and rodents by intracerebral inoculation of brain homogenates from individuals with CJD and GSS.

The brains of individuals affected with CJD and GSS accumulate amyloid-like deposits which contain a modified form of a host-encoded glycoprotein termed "prion protein" (PrP) (Bolton et al. 1982; Diringer et al. 1983; Oesch et al. 1985). A protease-resistant form of PrP has been shown, by a number of biochemical and biophysical methods, to be closely associated with the infectivity of brain homogenates (McKinley et al. 1983; Prusiner et al. 1983). After inoculation with the closely related disorder scrapie, various mouse strains demonstrate significant differences in the time to develop pathological changes similar to those seen in CJD. These differences can be correlated with two codon changes in the sequence of the murine PrP gene (Westaway et al. 1987).

In man PrP cDNAs have been used to localize the gene encoding this protein to the short arm of chromosome 20 (20pter-12), an area with few identified genes or anonymous markers (Liao et al. 1986; Robakis et al. 1986; Sparkes et al. 1986). The results cited above in mice have elicited a search for informative RFLPs in humans in order to examine the small number of familial cases of CJD and GSS for linkage. Utilizing the human PrP cDNAs, only a few RFLPs with low allele frequencies have been detected (Wu et al. 1987; Harris et al. 1990). Evidence for genetic linkage be-

Received January 3, 1991; revision received March 22, 1991.

Address for correspondence and reprints: Leroy Hood, Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125.

0002-9297/91/4902-0008$02.00

tween CJD and GSS has been obtained by direct nucleotide sequence analysis of the PrP genes in affected individuals. Hsaio et al. (1989) have demonstrated that in two unrelated pedigrees of GSS a single nucleotide change, which results in a proline-to-leucine change at codon 102 (L102), is genetically linked to GSS. This same change at codon 102 in the PrP gene has also been demonstrated to be associated with affecteds in a form of familial CJD (Doh-ura et al. 1989). Several other groups have also demonstrated that other changes in the PrP gene appear to be associated with familial CJD and GSS (Goldfarb et al. 1989, 1990; Goldgaber et al. 1989; Owen et al. 1990a). Thus nonsynonymous substitutions in the coding region of the human PrP gene have been demonstrated to be strongly associated with affecteds in a small number of CJD and GSS pedigrees. Because of the intriguing relationship between the PrP gene and these disorders, we have determined the complete genomic structure of the human PrP gene, including the entire first exon not previously isolated in cDNA clones. A comparison between the sequences of the genomic clone and those of the cDNA we have isolated reveals a unique eight-amino-acid deletion in the PrP gene. We have also identified a frequently heterozygous RFLP, which should be a useful polymorphic marker for this region of chromosome 20.

Material and Methods

Isolation, Restriction Mapping, and Sequence Analysis of the Genomic PrP Gene

The 2.4-kb human cDNA (pMPrP3) was isolated from a human brain lambda gt11 library by hybridization at reduced stringency (40% formamide, 1 M NaCl, 10% dextran sulfate, 1% SDS, and 100 μg salmon sperm DNA/ml at 42°C), using the hamster cDNA isolated by Oesch et al. (1985). A human cosmid library in the cosmid vector pTL5 (Lund et al. 1982) was screened by colony hybridization. This library was originally constructed from genomic DNA obtained from the human Hela cell line S3 (Lai et al. 1988). The hybridization conditions utilized to screen the library were 50% formamide, 5 × SSC, 10% dextran sulfate, 1% SDS, and 100 μg salmon sperm DNA/ml at 37°C. A single cosmid (designated pGPrP1) was isolated and analyzed by standard double-digest restriction mapping and by partial digest mapping (Rackwitz et al. 1985) with four different restriction enzymes—*Bam*HI, *Eco*RI, *Hin*dIII, and *Xba*I. Subclones of this cosmid that were identified as containing exons were cloned into M13, and the sequence was determined by the dideoxy chain-termination method.

PCR Amplification of the 5′ Region of the Human PrP Gene

In order to generate a probe for the 5′ region of the human PrP message, two PCR primers were synthesized that corresponded both to a sequence within the known coding region of the PrP gene (5′ GTCACTCCATGTGGCCACAAAGA 3′) and also to the sequence just 5′ of the *Eco*RI site in lambda gt11 (5′ GGTGGCGACGACTCCTGGAGCCCG 3′). Approximately 200 ng of DNA isolated from a phage stock of the lambda gt11 library utilized to isolate the human PrP cDNA was amplified for 30 cycles on a Perkin-Elmer thermo-cycler utilizing these primers and *Taq* polymerase (Saiki et al. 1988). The cycles utilized denaturation for 40 s at 94°C, annealing at 65°C for 50 s, and extension at 72°C for 60 s.

Primer-Extension Analysis of the Transcriptional Initiation Site

Total RNA was isolated (Chirgwin et al. 1979) from adult human cortex, and Poly A^+ RNA was isolated on an oligo dT cellulose column. Primer-extension analysis was performed utilizing a 30 mer (5′ AGGCTTCGGGCGCTTCTTGCAGAGGCCCAG 3′) end labeled with ^{32}P by T4 polynucleotide kinase. The hybridizations were performed with 4 μg of A^+ RNA and 1 fmol of primer at both 50°C and 55°C for 4 h. The hybridization mixture was extended with AMV reverse transcriptase (Calzone et al. 1988), and the products of the extension were analyzed on a 6% denaturing polyacrylamide gel by autoradiography. The size of extension products was determined by running a corresponding sequence ladder of M13 mp18.

PCR Amplification of the PrP Gene

Two oligonucleotide primers (5′ ATGGCGAACCTTGGCTGCTGGATG 3′ and 5′ GTAACGGTCCTCATAGTCACTGCC 3′) were synthesized on the basis of the sequence of the coding region of the second exon. The genomic DNAs used in PCR amplification were from unrelated individuals in the CEPH (Centre d'Etude du Polymorphisme Humain) collection (Dausset 1990), six additional unrelated individuals, and two separately maintained Hela cell lines. We also

examined DNA isolated from a phage stock of the human brain lambda gt11 library used to isolate the human PrP cDNA. The amplification utilized 30 cycles with 30 s denaturation at 94°C, 45 s annealing at 62°C, and 90 s extension at 72°C with a final 7-min extension at 72°C. To radioactively label the PCR products, 3 μCi ^{32}P dCTP (3,000 Ci/nmol) were added to the reaction, and the products were analyzed on a 6% polyacrylamide denaturing gel.

RFLP Identification

High-molecular-weight human genomic DNA was supplied by CEPH, and probes were prepared by the random priming method (Feinberg and Vogelstein 1983). These probes were used in hybridizations with genomic blots containing DNA from six unrelated Caucasian individuals that was cleaved with each of the following restriction enzymes: *Eco*RI, *Bam*HI, *Hin*dIII, *Pst*I, *Bgl*I, *Bgl*II, *Sac*I, *Msp*I, *Taq*I, *Rsa*I, PvuII, *Kpn*I, *Xba*I, *Bst*EII, *Eco*RV, *Sca*I, *Ava*I, and *Stu*I. Radiolabeled probes were hybridized to Southern blots transferred to nylon membranes (Reed and Mann 1985) as described by Gatti et al. (1984). Hybridizations were carried out in the presence of 100 μg sheared and denatured human placental DNA/ml to block hybridization of highly receptive sequences. Blots were washed to a final stringency of 0.1 × SSC and 1% SDS at 65°C. Genetic linkage analysis was carried out using the program MENDEL (Lange et al. 1988).

Results and Discussion

Genomic Structure of the PrP Gene in Man

To characterize the human PrP gene we first isolated a human cDNA (pMPrP3) by utilizing the hamster PrP cDNA as probe in a reduced-stringency screen of a human brain cDNA library. We determined that the sequence of the 2.4-kb human PrP cDNA contains the known open reading frame (ORF) of human PrP (Kretzschmar et al. 1986; Liao et al. 1986). This cDNA was then used to probe a human cosmid library. The single cosmid pGPrP1 isolated from this screen contained a 36-kb insert, the restriction-endonuclease sites of which are indicated in figure 1. The entire cosmid was then subcloned as seven *Bam*HI fragments. The subclone pRB9.4 was demonstrated to contain the coding and 3′ untranslated region of the PrP gene. Detailed restriction maps of the 9.4-kb *Bam*HI genomic fragment containing this exon of the PrP gene were identical with the restriction map of the human cDNA for the region containing this exon. This region of the genomic fragment was sequenced on one strand by chain-termination methods. This sequence was then compared with that of the human cDNA which was sequenced on both strands, and areas of ambiguity were resolved by sequencing the opposite strand on the genomic clones. The exon contained on the 9.4-kb genomic *Bam*HI fragment encodes a 245-amino-acid protein which is identical both to the human cDNA isolated by us and to the sequence previously published by Kretzschmar et al. (1986), with the exception of a 24-bp deletion. The general features of the predicted amino acid sequence of this exon correspond to those previously described in the cDNAs isolated by Kretzschmar et al. (1986) and Liao et al. (1986). However, the cDNA we isolated contained 5′ sequence which diverged from the corresponding region of the genomic clone. This implied that there was at least one or more additional exons present in the human PrP gene.

Studies of both rodent and human cDNAs have demonstrated numerous cloning artifacts that have precluded the identification of the complete 5′ region of the human PrP message (Kretzschmar et al. 1986; Liao et al. 1986). To generate a probe for the isolation of the genomic region encoding this 5′ region, we utilized PCR amplification and oligonucleotide primers corresponding to sequences within lambda gt11 and the ORF of the human PrP cDNA. By PCR we amplified DNA isolated from a phage stock of the human brain gt11 library previously used to isolate the human cDNA. A 234-bp fragment was seen as one of several products of this amplification. The sequence of this 234-bp fragment was determined to begin at the PCR primer corresponding to the sequence within the second exon and to extend 45 bp beyond the first ATG of this exon. This PCR product was utilized as a probe to identify the corresponding restriction-endonuclease fragment of the cosmid pGPrP1 encoding this 5′ region of the PrP message. This 234-bp PCR product identified a 5.4-kb *Eco*RI fragment of the cosmid pGPrP1. This *Eco*RI fragment was 9 kb 5′ to the exon encoding the PrP protein (fig. 1). A 300-bp *Eag*I fragment of this 5.4-kb *Eco*RI piece was subcloned into M13, and the sequence was determined to have a 45-bp overlap with the PCR product.

The restriction map and localization of exon 1 and exon 2 (fig. 1) were confirmed by utilizing this 234-bp PCR product and the human PrP cDNA as probes on genomic blots of human DNA cleaved with the same

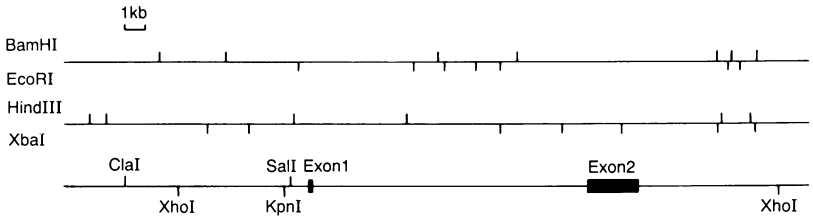

Figure 1 Restriction map of human PrP gene. This restriction map was derived from the cosmid pGPrP1 containing the human PrP gene. Restriction sites are indicated by vertical lines, and the respective restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, and *Xba*I are indicated on the left. The two exons of the human gene are indicated as black boxes on the bottom line, and other, less common restriction sites in this region are also indicated. The exact location of exon 1 could be as much as 1 kb 5′ or 3′ of the depicted location.

restriction endonucleases utilized to generate the cosmid map. Fragments of the size predicted by this restriction map were identified, confirming the genomic structure of the human PrP gene. Therefore, the genomic organization of the human PrP gene consists of two exons separated by a single 13-kb intron and is virtually identical to the exon-intron structure described, by Basler et al. (1986), in hamster.

The 5′ Region of the Human PrP Gene

The 300-bp *Eag*I genomic fragment containing exon 1 has 71 bp of the 5′ region of the human gene and possesses the same general features of the 5′ region of the hamster gene (Basler et al. 1986). The region contains no identifiable TATA box, is very GC rich (83% GC residues), and contains numerous GC repeats. The 9-bp repeat GCCCCGCCC present in the upstream region of the hamster gene is not repeated verbatim in the upstream region of the human gene. The six-base sequence CCGCCC, which is similar to its seven-base complement GGGCGGG found in the human adenosine deaminase gene (Valerio et al. 1985), is repeated four times between bases 1 and 105 (fig. 2*A*). This same CCGCCC repeat or its inverted complement GGGCGG is also seen in the human hypoxanthine phosphoribosyltransferase gene (Patel et al. 1986) and in the 21-bp repeat of SV40 (Hansen and Sharp 1983). In the human PrP gene three of these repeats occur after the major start site of transcription, and there are numerous GC-rich repeats (allowing for two or fewer gaps or mismatches) in the sequences from base 1 to base 210 (fig. 2*A*). The sequence $\text{GCCGGC}\underset{\text{C}}{\overset{\text{G}}{-}}\text{GCC}\underset{\text{C}}{\overset{\text{G}}{-}}\text{GC}$ is repeated at nucleotides 2–14 and 35–48. Thus, the human PrP gene displays the same GC-rich features seen in a number of both genes which are expressed in a wide variety of tissues (Dynan et al. 1986). In the absence of a deletional analysis of the PrP promoter, it is not clear which, if any, of these repeats is critical to the transcription of the PrP gene. When the 300-bp *Eag*I fragment is utilized as a probe on human genomic blots, it identifies a 5.4-kb *Eco*RI fragment or a 5.2-kb *Hin*dIII fragment. These fragments are the same size as seen when the cosmid pGPrP1 is cleaved with identical restriction endonucleases and is hybridized with the same genomic fragment. In addition to these bands the *Eag*I fragment generates some background smearing in each lane, suggesting that under high-stringency conditions it hybridizes to other GC-rich regions.

The First Exon of the Human PrP Gene

Primer-extension results indicate that exon 1 is approximately 136 bp in length (fig. 3). (The exact nucleotide of initiation is unclear, since we did not run a corresponding sequence ladder of the genomic clone containing the first exon.) In contrast to the results of primer-extension experiments in mouse and hamster, the human gene appears to have a single major transcriptional start site, whereas the rodent genes have multiple transcriptional start sites (Basler et al. 1986; Westaway et al. 1987). There are three other faint extension products visible above and below the major transcriptional start site, suggesting that there are additional start sites for the human PrP gene. However, these appear to be infrequently utilized, since we visually estimate that these extension products constitute less than 5% of the total product seen with primer extension. The sequence of the 300-bp *Eag*I genomic fragment containing this PCR product is identical from nucleotide 168 to nucleotide 206 (fig. 2*A*), with the consensus sequence being derived from two different human PrP cDNAs (Kretzschmar et al. 1986). Both of these cDNAs contained probable cloning artifacts in this region. The human PrP cDNA published by Liao et al. (1986) contains both an inver-

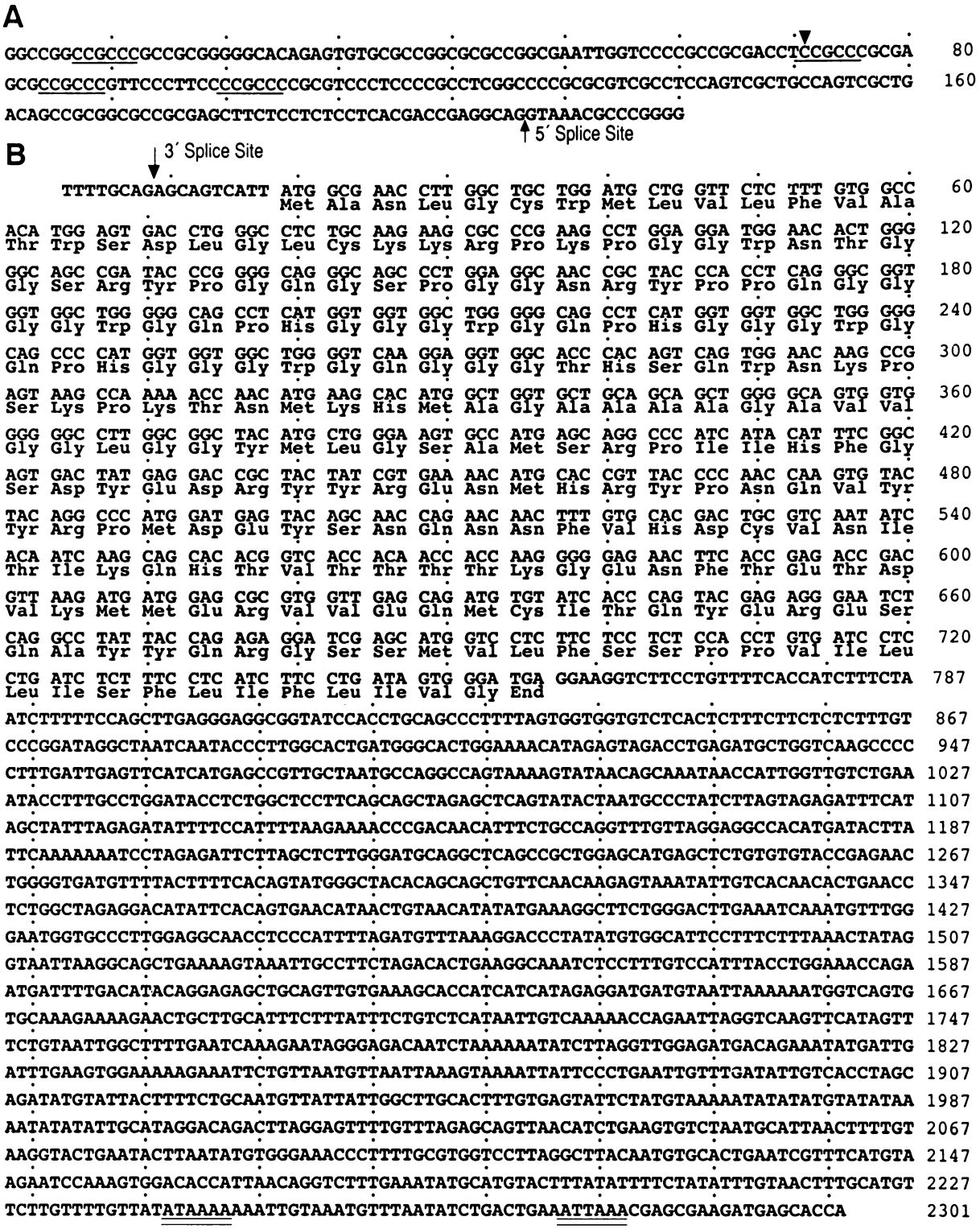

Figure 2 Nucleotide sequence of genomic fragments containing exon 1 and exon 2 of human PrP gene. The upper sequence (*A*) contains exon 1, with the approximate transcriptional initiation indicated by the arrowhead (▼) and with the 5′ splice donor site indicated by the upward-pointing arrow (↑). The underlined sequences in the upper sequence (*A*) indicate the location of the CCGCCC repeats. The lower sequence contains exon 2, with the 3′ splice acceptor site indicated by the downward-pointing arrow (↓), the conceptual translation of the ORF contained within this exon, and the two possible polyadenylation sites, which are underlined.

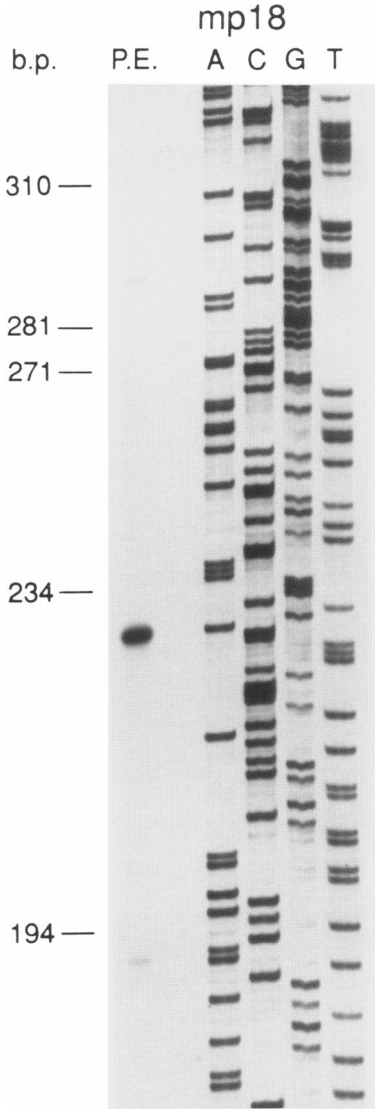

Figure 3 Transcriptional start of human PrP gene as determined by primer extension. The primer-extension products seen by autoradiography on a 6.0% polyacrylamide denaturing gel are indicated (lane P.E.) and are sized by comparison to radiolabeled PhiX-174 (lane b.p.) and by comparison to a sequence ladder of M13 mp18.

sion of the 23 bp 5′ of the first ATG of the second exon and another inversion and rearrangement of sequences present in the first exon. Thus all three of the published cDNAs for the human PrP gene are incomplete and contain probable cloning artifacts. The sequences at the donor and acceptor sites of the single splice junction conform to those usually utilized in eukaryotes (Mount 1982). The first exon, when spliced to the second exon, generates an ORF which extends 38 amino acids 5′ of the first ATG in exon 2. The differences between the N-terminal sequences of the 33–35-kD form of PrP protein and the predicted amino acid sequence of the cDNA are believed to be secondary to posttranslational cleavage of the signal sequence. Since there is no methionine present in the predicted amino acid sequence of the first exon, we presume that this region is not translated into a precursor protein. However, the presence of this ORF does raise the possibility that the mature protein has a precursor that, because of posttranslational cleavage of this region, has not been identified.

The Second Exon of the Genomic PrP Clone Contains an Eight-Amino-Acid Deletion

The second exon contained on the 9.4-kb genomic *Bam*HI fragment differs, from both our cDNA and published cDNAs (Liao et al. 1986; Kretzschmar et al. 1986), in the absence of an eight-amino-acid segment of the protein from either residues 81–88 or residues 82–89 (fig. 4). We cannot discriminate between these two possibilities, because this deletion results in the loss of one of five octameric peptide repeats of the following sequence: Pro His Gly Gly Gly Trp Gly Gln. Determination of the nucleic acid sequence fails to resolve this ambiguity, because the region deleted results in the reconstitution of the ORF in such a manner that it is unclear whether the deletion begins after the last base in codon 80 or after the third base in codon 82 (fig. 4*A* and *B*) (codon numbering corresponds to that of the cDNA previously published by Kretzschmar et al. [1986]). The restriction map of the cosmid corresponds to the fragment sizes seen on genomic blots in which genomic DNA from unrelated individuals was restricted with the same enzymes and in which the entire cosmid was used as probe (data not shown). This result strongly suggests that there are no gross rearrangements in this cosmid but does not exclude the possibility that this deletion represents a cloning artifact or somatic mutation in this cell line. To exclude a deletion that occurred during the growth of M13 phage, we also directly sequenced the original genomic subclone, pGPrP1, and observed the same deletion. To see whether we could detect this deletion in other human DNA samples, we synthesized a pair of 25-base oligonucleotides to serve as PCR primers. If the 24-bp deletion was present in the PrP gene, these primers would generate predicted PCR products of 563 bp or 539 bp. We were unable to demonstrate this deletion in 30 unrelated individuals (data not shown). However, we were able to demonstrate a 24-bp dele-

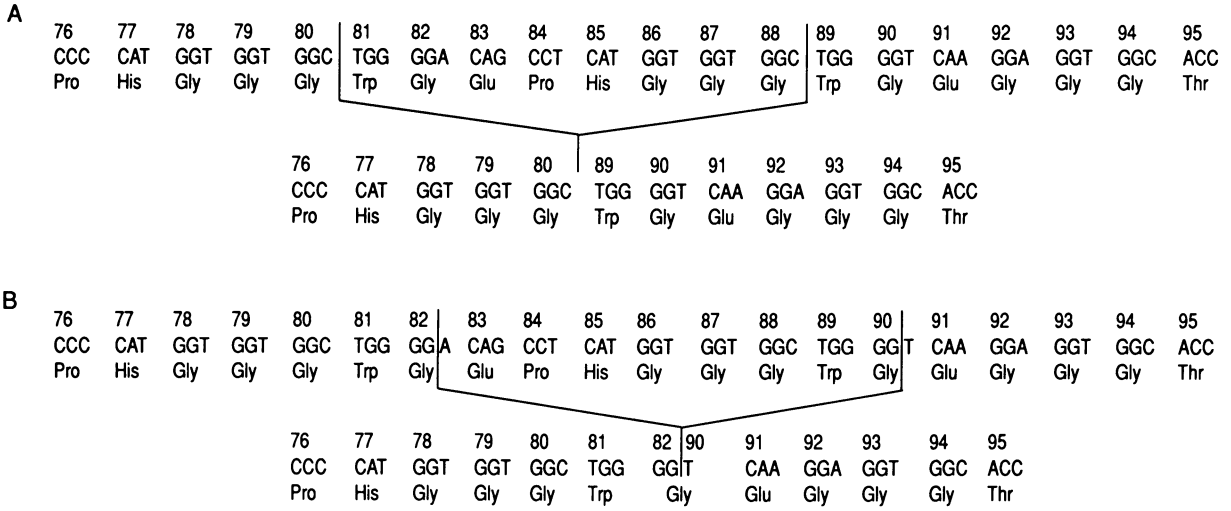

Figure 4 Two potential sites of 24-bp nucleotide deletion of human PrP gene. Brackets indicate the potential sites of the 24-bp nucleotide deletion. The upper sequences in each panel represent the codons and corresponding amino acids as numbered in the published cDNA (Kretzschmar et al. 1986). The lower sequences represent the predicted amino acid sequence of the isolated cosmid (pGPrP1) containing the human PrP gene. Either 24-bp deletion would generate the same predicted amino acid sequence with the loss of one eight-amino-acid repeat.

tion both in genomic DNA derived from two separately maintained Hela cell lines and in the human brain cDNA library used to isolate the PrP cDNA (fig. 5). In the absence of genomic DNA from the same individual whose DNA was used to construct this cDNA library, we cannot prove that this transcript is derived from an allele containing this deletion. Although this deletion could represent a deletion specific to the Hela cell line, the fact that it is also present in our cDNA library suggests that it is an uncommon polymorphic variant in the coding region of the PrP gene.

The deletion we describe results in the deletion of one of five octameric peptide repeats (Pro His Gly Gly Gly Trp Gly Gln) encoded by codons 51–91 in the human PrP gene. In one English GSS pedigree studied by Owen et al. (1990*b*), the presence of an increased number of these repeats was associated with the individuals affected with GSS. There has been one prior report of an allele in a Moroccan family (Laplanche et al. 1990) containing a deletion in the human PrP gene. The location of the deletion was shown by restriction enzymes to be between basepairs 277 and 397 of the second exon and occurred in a family without any history of GSS or CJD. Since the nucleotide sequence of this allele was not determined, it is not clear that this deletion represents a loss of one of these octameric repeats. It will prove very interesting if the insertion or deletion of these eight-amino-acid repeats cosegregates with affected individuals in GSS and CJD pedigrees. These octameric repeats are conserved in mouse (Westaway et al. 1987), hamster (Oesch et al. 1985), and human proteins and are predicted to contribute to a B-pleated sheet conformation in this region of the PrP protein. In the hamster PrP gene the overlapping region from codon 74 to codon 114 has also been shown to contain sequences which determine whether the PrP protein is secreted or an integral membrane protein (Lopez et al. 1990). The insertion or deletion of these octameric peptide repeats could conceivably alter either the posttranslational processing or the conformation of the PrP protein. Such a result could then alter the cellular localization or solubility of the PrP protein and could therefore have some interplay in the genesis of the PrP deposits seen in CJD and GSS. If insertions and deletions within this region do segregate with affecteds in GSS families (Owen et al. 1989), it will pose another interesting paradox, because this region is not seen in the protease-resistant PrP protein seen in CJD.

A PvuII *Polymorphism*

Only a few RFLPs have been identified by utilizing the human PrP cDNAs, and these have a low frequency of occurrence in European Caucasians (Wu et al. 1987; Harris et al. 1990). One of these RFLPs is a

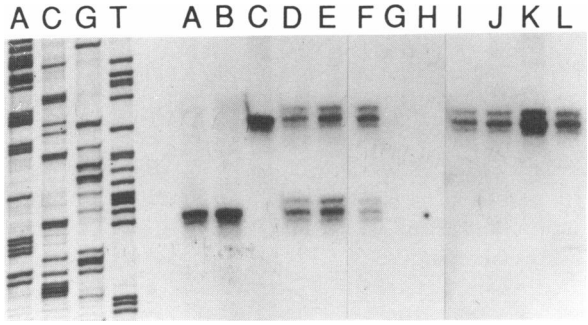

Figure 5 Autoradiograph of results of PCR amplification of DNAs from clones, cell lines, and unrelated individuals, run on a 6% polyacrylamide denaturing gel next to sequence ladder of M13 mp18, with respective sequencing lanes indicated as A, C, G, and T (the four leftmost columns). The 12 lettered lanes to the right are PCR-amplification products derived from the following DNA samples: cosmid clone pGPrP1 (lane A); subclone pRB9.4 containing the exon 2 genomic fragment (lane B); plasmid pmPrP3, the human PrP cDNA (lane C); Hela cell genomic DNA (UCLA) (lane D); Hela cell genomic DNA (Caltech) (lane E); lambda phage DNA from the human brain cDNA library (lane F); PCR-negative controls (lanes G and H); genomic DNA from unrelated individuals (lanes I, J, K, and L). The multiple bands seen within a few bases above and below the PCR products are attributed to heterogeneity in the major PCR products seen on this 6% denaturing polyacrylamide gel. These small differences we attribute to incomplete extensions of the final products by *Taq* polymerase.

*Pvu*II polymorphism which occurs within the ORF of exon 2 (Wu et al. 1987) and can also be identified by the human PrP cosmid (pGPrP1). By utilizing the entire cosmid as a probe, we were able to identify a new biallelic polymorphism represented by bands at 5.1 kb and 4.2 kb on genomic blots of human DNA digested with *Pvu*II. Segregation of these fragments as alleles was observed in a large multigeneration Amish pedigree comprising more than 60 members (AT012) (Gatti et al. 1988). The average heterozygosity of this marker was 52% in 46 unrelated CEPH individuals that were typed. The polymorphic restriction site was mapped to a single *Bam*HI fragment located 5′ of exon 1 of the PrP gene in the cosmid pGPrP1. A subclone containing the *Bam*HI fragment pRB6.2 was used to detect the RFLP in subsequent analyses. Genotypes for the PrP/*Pvu*II RFLP were collected from 11 informative CEPH pedigrees. Two-point linkage calculations utilizing genotypes for the PrP/*Pvu*II RFLP revealed significant linkage (Lod > 3) to several markers on chromosome 20, notably an RFLP detected with the prodynorphin gene (peak Lod 4.8, recombination fraction .0). However, there was no evidence for linkage disequilibrium between the PrP/*Pvu*II RFLP and the prodynorphin gene. This is consistent with previous analyses utilizing in situ hybridization which localizes the PrP gene to the 20pter-12 region (Sparkes et al. 1986), and it confirms that the cosmid pGPrP1 is derived from this chromosome. The RFLP identified with probe pRB6.2 has a high degree of heterozygosity and should therefore be a useful marker for the short arm of chromosome 20.

Acknowledgments

The authors wish to thank Dr. Brian Popko for providing the cosmid library, Dr. Stanley Prusiner for providing the hamster PrP cDNA, and Drs. Steven Dlouhy and Karen Hsiao for discussing, prior to its publication, their work on the PrP gene. We also wish to thank Dr. Robert Lazzarini, in whose laboratory the human PrP cDNA was characterized, and the following individuals for critical reading of the manuscript: Drs. Sally Orr, Patrick Charmley, and Bill Funkhouser. The National Neurological Research Bank, VAMC, Wadsworth Division, Los Angeles, provided adult human cortex. This investigation received support from NINCDS Clinical Investigator Development Award NS 01163-04 and NIH grant NS 22786.

References

Basler K, Oesch B, Scott M, Westaway D, Walchli M, Groth DF, McKinley MP, et al (1986) Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell 46:417–428

Bolton DC, McKinley MP, Prusiner SB (1982) Identification of a protein that purifies with the scrapie prion. Science 218:1301–1311

Brown P, Cathala F, Gajdusek DC (1979) Creutzfeld-Jacob disease in France. III. Epidemiological study of 170 patients dying during the decade 1968–1977. Ann Neurol 6:438–446

Calzone FJ, Britten RJ, Davidson E (1988) Mapping of gene transcripts by nuclease protection assays and cDNA primer extension. Methods Enzymol 142:611–632

Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294–5299

Dausset J, Cann H, Cohen D, Lathrop M, Lalouel J-M, White R (1990) Program description: Centre d'Etude du Polymorphisme Humain (CEPH) collaborative genetic mapping of the human genome. Genomics 6:575–577

Diringer H, Hilmert H, Simon D, Werner E, Ehlers B (1983) Towards purification of the scrapie agent. Eur J Biochem 134:555–560

Doh-ura K, Tateishi J, Sasaki H, Kitamoto T, Sakaki Y (1989) Pro-Leu change at position 102 of prion protein is

the most common but not the sole mutation related to Gerstmann-Sträussler syndrome. Biochem Biophys Res Commun 163:974–979

Dynan WS, Sazer S, Tijan R, Schimke RT (1986) Transcription factor Sp1 recognizes a DNA sequence in the mouse dihydrofolate reductase promoter. Nature 319:240–248

Feinberg AP and Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gatti RA, Berkel I, Boder E, Braedt G, Charmley P, Concannon P, Ersoy F, et al (1988) Localization of an ataxia-telangiectasia gene to chromosome 11 q 22–23. Nature 336:577–580

Gatti RA, Concannon P, and Salser W (1984) Multiple use of Southern blots. Biotechniques 2:148–154

Goldfarb LG, Brown P, Goldgaber D, Asher DM, Strass N, Granpera G, Piccardo P, et al (1989) Patients with Creutzfeldt-Jacob disease and kuru lack the mutation in the PRIP gene found in Gerstmann-Sträussler syndrome, but they show a different double-allele mutation in the same gene. Am J Hum Genet 45 [Suppl]: A189

Goldfarb LG, Mitrová E, Brown P, Hock Toh B, Gajdusek CD (1990) Mutation in codon 200 of scrapie amyloid protein gene in two clusters of Creutzfeld-Jacob disease in Slovakia. Lancet 336:514–515

Goldgaber D, Goldfarb LG, Brown P, Asher DM, Brown W, Lin S, Teener JW, et al (1989) Mutations in familial Creutzfeldt-Jacob disease and Gertsmann-Sträussler Scheinker's syndrome. Exp Neurol 106:204–206

Hansen U, Sharp PA (1983) Sequences controlling *in vitro* transcription of SV40 promoters. EMBO J 2:2293–2303

Harris MS, Devine-Gage E, Robakis NK (1990) An Alu I RFLP detected in the human prion protein (PrP) gene. Nucleic Acids Res 18:385

Hsiao K, Baker HF, Crow TJ, Poulter M, Owen F, Terwilliger JD, Westaway D, et al (1989) Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome. Nature 338:342–345

Kretzschmar HA, Stowring LE, Westaway D, Stubblebine WH, Prusiner SB, Dearmond SJ (1986) Molecular cloning of a human prion protein cDNA. DNA 5:314–324

Lai E, Concannon P, Hood L (1988) Conserved organization of the human and murine T-cell receptor B gene families. Nature 331:543–546

Lange K, Weeks D, Boehnke M (1988) Programs for pedigree analysis: MENDEL, FISHER and dGENE. Genet Epidemiol 5:471–472

Laplanche J-L, Chatelain J, Launay J-M, Gazengel C, Vibaud M (1990) Deletion in prion protein gene in a Moroccan family. Nucleic Acids Res 18:6745

Liao YJ, Lebo RV, Clawson GA, Smuckler EA (1986) Human prion protein cDNA: molecular cloning, chromosomal mapping, and biological implications. Science 233: 364–367

Lopez CD, Yost CS, Prusiner SB, Myers RM, Lingappa VR (1990) Unusual topogenic sequence directs prion protein biogenesis. Science 248:226–229

Lund T, Grosveld FG, Flavell RA (1982) Isolation of transforming DNA by cosmid rescue. Proc Natl Acad Sci USA 79:520–524

McKinley MP, Bolton DC, Prusiner SB (1983) A protease resistant protein is a structural component of the scrapie prion. Cell 35:57–62

Masters CL, Gajdusek DC, Gibbs CJ (1981) The familial occurrence of Creutzfeld-Jacob disease and Alzheimers disease. Brain 104:535–538

Mount SM (1982) A catalogue of splice junction sequences. Nucleic Acids Res 10:459–472

Oesch B, Westaway D, Walchli M, McKinley M, Kent SBH, Aebersold R, Barry RA, et al (1985) A cellular gene encodes scrapie PrP 27–30 protein. Cell 40:735–746

Owen F, Poulter M, Collinge J, Crow TJ (1990*a*) Codon 129 changes in the prion protein gene in Caucasians. Am J Hum Genet 46:1215–1216

Owen F, Poulter M, Lofthouse R, Collinge J, Crow TJ, Risby D, Baker HF, et al (1989) Insertion in prion protein gene in familial Creutzfeldt-Jacob. Lancet 1:51–52

Owen F, Poulter M, Shah T, Collinge J, Lofthouse R, Baker H, Ridley R, et al (1990*b*) An in-frame insertion in the prion protein gene in familial Creutzfeld-Jacob disease. Mol Brain Res 7:273–276

Patel PI, Fromson PE, Caskey CT, Chinault AC (1986) Fine structure of the human hypoxanthine phosphoribosyl transferase gene. Mol Cell Biol 6:393–403

Prusiner SB, McKinley MP, Bowman KA, Bolton DC, Bendheim PE, Groth DF, Glenner GG (1983) Scrapie prions aggregate to form amyloid-like birefringent rods. Cell 35: 349–358

Rackwitz HR, Zehetner G, Murialdo H, Delius H, Chai JH, Poustka A, Frischauf A, et al (1985) Analysis of cosmids using linearization by phage lambda terminase. Gene 40: 259–266

Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res 13: 7207–7221

Robakis NK, Devine-Gage EA, Jenkins EC, Kascak RJ, Brown TW, Krawczun MS (1986) Localization of a human gene homologous to the PrP gene on the p arm of chromosome 20 and detection of Prp-related antigens in normal brain. Biochem Biophys Res Commun 140:758–765

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sparkes RS, Simon M, Cohn VH, Fournier REK, Lem J, Klisak I, Heinzman C, et al (1986) Assignment of the human and mouse prion protein genes to homologous chromosomes. Proc Natl Acad Sci USA 83:7358–7362

Valerio D, Duyvesteyn MGC, Dekker BMM, Weeda G,

Berkvens Th M, van der Voorn L, van Ormondt H, et al (1985) Adenosine deaminase: characterization and expression of a gene with a remarkable promoter. EMBO J 4:437–443

Westaway D, Goodman PA, Mirenda CA, McKinley MP, Carlson GA, Prusiner SB (1987) Distinct prion proteins in short and long scrapie incubation period mice. Cell 51: 651–652

Wu Y, Brown WT, Robakis NK, Dobkin C, Devine-Gage E, Merz P, Wisniewski HM (1987) A Pvu II RFLP detected in the human prion protein (PrP) gene. Nucleic Acid Res 15:3191