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Infectious amyloid precursor gene sequences in primates used for experimental transmission of human spongiform encephalopathy

[Creutzfeldt–Jakob disease/transmissible spongiform encephalopathy/*PRNP* (chromosome 20 amyloid precursor) gene/primates]

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ABSTRACT **Based on the analysis of genomic DNA from single healthy animals of each of five primate species, nucleotide and predicted amino acid sequences of the infectious amyloid precursor gene of higher apes (*Gorilla* and *Pan*) and Old World (*Macaca*) and New World (*Ateles, Saimiri*) monkeys showed 95–99% homology to the human sequences, corresponding to their phylogenetic distance from humans. Two of 18 amino acids that differed from humans resulted from nucleotide changes at sites of mutations in humans with familial forms of spongiform encephalopathy (a deleted codon within the codon 51–91 region of 24 bp repeats and a substitution at codon 198). In each of the five animals, codon 129 specified methionine, the more common of the two polymorphic genotypes in humans. Because genotypic homology did not correlate with experimental transmission rates of human spongiform encephalopathy, primary structural similarity of the infectious amyloid precursor protein in humans and experimental primates may not be an important factor in disease transmissibility.**

The human spongiform encephalopathies have been experimentally transmitted with varying success to numerous primate and nonprimate species, including higher apes, monkeys, prosimians, ruminants, felines, and rodents. Genetic differences might influence the ease with which human spongiform ecephalopathy can be experimentally transmitted, and the discovery and sequencing of the human gene on chromosome 20 that encodes the pathogenetic amyloid protein has made it possible to test the thesis. We report here the gene sequences of five different nonhuman primate species and compare the degree of homology with humans to experimental transmission rates for each inoculated species.§

MATERIALS AND METHODS

Genomic DNA was extracted from frozen brain tissue taken from a single healthy animal of each of the following primate species: gorilla (*Gorilla gorilla*), chimpanzee (*Pantroglodytes*), Old World rhesus monkey (*Macaca mulatta*), New World spider monkey (*Ateles paniscus/fusciceps*), and New World squirrel monkey (*Saimiri sciureus*). Two overlapping fragments of the open reading frame of the infectious amyloid precursor gene were amplified by PCR using *Taq* polymerase and two sets of oligonucleotide primers: 5′-TAC TGA GAA TTC ATG GCG AAC CTT GGC TAC TGG-3′ and 5′-TAC TGA TCT AGA TGC TCA TGG CAC TTC CCA GCA T-3′ (for the 5′ fragment), and 5′-TAC TGA GCG GCC GCC AAC ATG AAG CAC ATG GCT GGT-3′ and 5′-TAC TGA GTC GAC CCT TCC TCA TCC CAC TAT CAG G-3′ (for the 3′ fragment). Generated fragments were ligated into plasmid vectors and transfected into *Escherichia coli* (1). DNA inserts from five to nine colonies were sequenced in both directions using Prism cycle sequencing kits (Applied Biosystems) on a Catalyst LabStation or a Perkin–Elmer 9600 thermal cycler following the protocol outlined by the manufacturer and electrophoresed on an Applied Biosystems model 373 DNA sequencer.

A single nucleotide in the sequences of each monkey species gave an ambiguous signal in the sequencing procedure, which was resolved by single nucleotide primer-extension analysis (2) using the following primer pairs: 5′-GCC GCC ACC ATG AGG CTG TCC CCA-3′ and 5′-TGG GGA CAG CCC CAT GGT GGC GG-3′ (for nt 240 in the squirrel monkey), 5′-CAT CAT CTT AAC GTC GGT CTC AGT GA-3′ and 5′-ACC ACC ACC ACC AAA GGG GAG AAC-3′ (for nt 594 in the spider monkey), and 5′-GAA GAT GAG GAA AGA AAT CAG GAG G-3′ and 5′-GTC CTC TTC TCC TCC CCG CCT GTG A-3′ (for nt 723 in the rhesus monkey).

RESULTS

Fig. 1 compares the sequence of the infectious amyloid precursor gene coding region in each tested primate species to the previously published human sequence (3), and Fig. 2 shows the position of amino acids predicted to be different from those of the human protein. It is apparent that the two higher apes (gorilla and chimpanzee) had considerably fewer nucleotide and amino acid coding differences than did either the phylogenetically more distant Old World rhesus monkey or New World spider and squirrel monkeys, ranging from only 3-nt (2 amino acid) differences in the gorilla to 43-nt (12 amino acid) differences in the squirrel monkey. Of a combined total of 60 nt that differed from humans, 18 resulted in predicted amino acid substitutions or deletions. Nine amino acids differed from humans in a single primate species (rhesus, spider, or squirrel monkeys); in the other nine positions, differences occurred in two or more species, but at any given position the change was always identical; for example, at codon 168 the same G → C nucleotide substitution resulted in a change from glutamic acid (human) to glutamine (all animals).

Table 1 shows the degree of homology between humans and animals for nucleotide and predicted amino acid sequences and compares them to our previously published experimental transmission rates for spongiform ecephalopathy (4). The chimpanzee showed a somewhat greater degree of homology and an equal or slightly higher transmission rate than the New World spider or squirrel monkeys, but the Old World rhesus monkey, with a similar degree of homology, had a much lower transmission rate.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U15039 and U15163–U15166).

DISCUSSION

In agreement with phylogenetic relationships among primate species deduced from classical anthropological studies, apes had a higher degree of amyloid precursor gene homology with humans (99%) than did monkeys (94–96%), and the Old World rhesus monkey had a slightly higher degree of homology than either of the New World spider or squirrel monkeys. Nucleotide differences in each primate species were randomly scattered throughout the gene, but most of the predicted amino acid substitutions occurred within the middle portion of the encoded protein (positions 92–171), especially

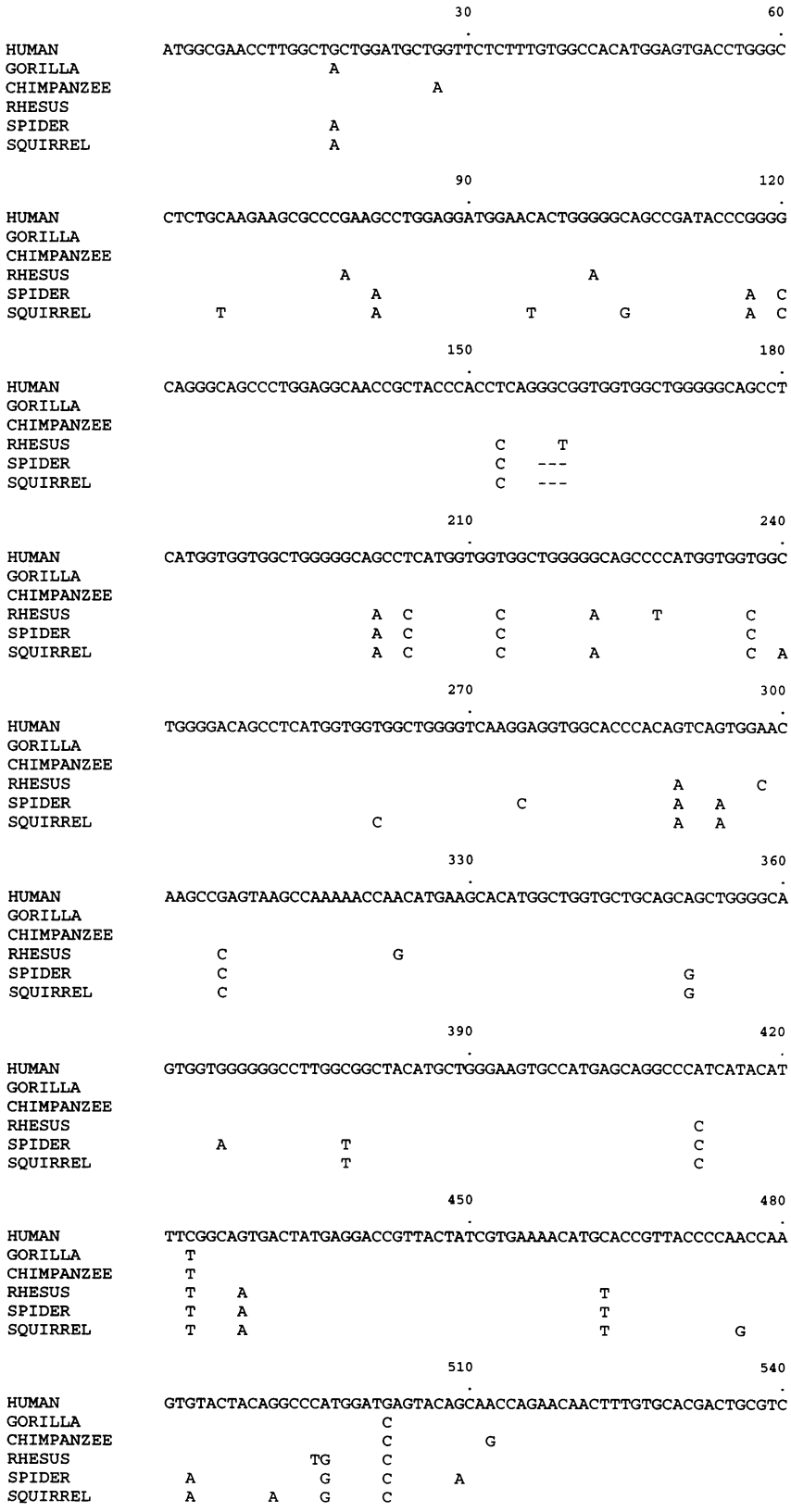

FIG. 1. (*Figure continues on the opposite page.*)

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                                                570                             600
                                                 .                               .
HUMAN           AATATCACAATCAAGCAGCACACGGTCACCACAACCACCAAGGGGGAGAACTTCACCGAG
GORILLA
CHIMPANZEE                G
RHESUS                    G  T             A         C         A
SPIDER                                     A         C         A          C    T
SQUIRREL          CG      G                A         C         A               T

                                                630                             660
                                                 .                               .
HUMAN           ACCGACGTTAAGATGATGGAGCGCGTGGTTGAGCAGATGTGTATCACCCAGTACGAGAGG
GORILLA
CHIMPANZEE
RHESUS                                                                          A
SPIDER                                     T                         T
SQUIRREL                                                             T          A

                                                690                             720
                                                 .                               .
HUMAN           GAATCTCAGGCCTATTACCAGAGAGGATCGAGCATGGTCCTCTTCTCCTCTCCACCTGTG
GORILLA
CHIMPANZEE
RHESUS                  A           T                                 C  G
SPIDER                  C                                             C
SQUIRREL                C                                             C

                                                750
                                                 .
HUMAN           ATCCTCCTGATCTCTTTCCTCATCTTCCTGATAGTGGGATGA
GORILLA
CHIMPANZEE
RHESUS                        T
SPIDER
SQUIRREL
```

FIG. 1. Comparison of nucleotide sequences of the translated portion of the infectious amyloid precursor gene in humans and five primate species. Every 30th nucleotide is dotted and numbered. Only nucleotide differences from humans are shown for the experimental primates (the codon 53 nucleotide triplet GGC in the human sequence is missing in the spider and squirrel monkeys).

toward its C-terminal end, where five of eight successive amino acids differed from humans in one or another of the animal species.

Two amino acid-altering nucleotide differences occurred in codons known to be sites of pathogenetic mutations or polymorphisms in humans: (*i*) a deletion of codon 53, in the area of repeating 24-bp inserts in several families with atypical forms of Creutzfeldt–Jakob disease and (*ii*) a substitution at codon 198, the site of a point mutation in a family with atypical Gerstmann–Sträussler–Scheinker disease. Also of possible interest is the fact that all three monkey species had a "silent" nucleotide substitution at codon 102, and the two New World monkeys had a "silent" substitution at codon 117, both of which are sites of amino acid-altering mutations in Gerstmann–Sträussler–Scheinker disease. Codon 129, which in humans is polymorphic with allelic frequencies of 0.68 for methionine and 0.32 for valine (in Caucasians), encoded methionine in each of the five nonhuman primates, but we obviously cannot say from this study of individual animals whether it is polymorphic in any of the species.

Position	6	53	92	97	100	108	138	143
Human	Cys	Gly	Gly	Ser	Asn	Asn	Ile	Ser
Gorilla	Tyr							
Chimpanzee								
Rhesus				Asn	His	Ser	Leu	Asn
Spider	Tyr	-	Ala	Asn			Leu	Asn
Squirrel	Tyr	-		Asn			Leu	Asn

Position	155	159	164	166	168	170	171	182
Human	His	Asn	Arg	Met	Glu	Ser	Asn	Ile
Gorilla					Gln			
Chimpanzee					Gln		Ser	
Rhesus	Tyr			Val	Gln			
Spider	Tyr			Val	Gln	Asn		
Squirrel	Tyr	Ser	Lys	Val	Gln			Val

Position	198	220
Human	Phe	Arg
Gorilla		
Chimpanzee		
Rhesus		Lys
Spider	Leu	
Squirrel		Lys

FIG. 2. Comparison of predicted amino acid sequences encoded by the infectious amyloid precursor gene in humans and five primate species. Only positions at which amino acids in animals differ from humans are indicated (Gly-53 in the human sequence is missing in the spider and squirrel monkeys).

In our experience, primates have been considerably more susceptible to experimental infection with human transmissible spongiform encephalopathy than nonprimate species, and among the primates, chimpanzees and New World monkeys (especially the spider and squirrel monkeys) have, as a group, been more susceptible than Old World monkeys (4). Compared with transmission rates of 97% in the chimpanzee and 70–95% in most monkey species, the mouse and hamster have shown much lower transmission rates of 8–12% (4).

Both rodents show substantially less genetic homology to humans (85–87%) than do the experimental primates, and their predicted amino acid sequences each differ from humans at 27 positions. Many of these differences occur in the posttranslationally excised N- and C-terminal regions of the protein (and thus presumably do not influence its folding pattern), and the remainder are distributed in much the same pattern as that of the nonhuman primates—that is, in the middle third of the protein.

Overall gene homology and transmission rates thus appear to show a positive correlation when the experimental host species are separated into these three groups. However, this broad correlation does not withstand further subdivision, as the Old World rhesus monkey showed a slightly greater homology to humans than either of the New World spider and squirrel monkeys, despite their quite different experimental transmission rates of 73% and 93–95%, respectively.

This discrepancy could have several explanations. (*i*) The human brain tissue specimens inoculated into different primate groups were not identical, and the rhesus monkeys

Table 1. Comparison of infectious amyloid precursor nucleotide and predicted amino acid homology to transmission rates of human spongiform encephalopathy in experimental primates

Species	Nucleotide homology, %	Amino acid homology, %	Transmission rate (positive/total animals)
Gorilla (ape)	99.6	99.2	Not inoculated
Chimpanzee (ape)	99.3	99.2	97 (28/29)
Rhesus (Old World) monkey	95.8	96.8	73 (19/26)
Spider (New World) monkey	95.3	96.1	97 (30/31)
Squirrel (New World) monkey	94.3	96.3	93 (196/211)

could have received a less infectious group of human spongiform encephalopathy samples than did the other species. Against this explanation is the fact that all seven of the tissue specimens that failed to transmit disease to rhesus monkeys were also inoculated into other species (mostly squirrel monkeys) in which transmission was successful. (*ii*) The gene sequence in the single healthy rhesus monkey analyzed might not be identical to that of the inoculated monkeys. In particular, codon 129 might be polymorphic in nonhuman primates as well as in humans, or there could be polymorphisms at other sites, such as codons 100 and 108, where predicted amino acid differences from humans were unique to the rhesus monkeys. (*iii*) Genetic homology might not be a determining influence for disease transmissibility.

Extending these studies to include additional animals (for example, comparing the sequence of rhesus monkeys that did or did not develop disease after inoculation with spongiform encephalopathy agents) should provide a firmer basis on which to evaluate the role of host genotype in susceptibility to experimental infection, as should molecular genetic studies of breeds of sheep with different susceptibilities to scrapie infection. The question is not unimportant, as codon 129 homology has been shown to play a role in all forms of human spongiform encephalopathy, including iatrogenic disease resulting from accidental inoculation or grafting of contaminated tissues (5–8), and studies in transgenic mice with either human or hamster gene insertions have demonstrated the importance of overall homology for replication of the infectious agent in both intra- and interspecies models (9).

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