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Cerebrovascular amyloidosis in squirrel monkeys and rhesus monkeys: apolipoprotein E genotype

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Abstract Some neuropathological changes characteristic of aging and Alzheimer's disease (AD) in humans are present also in senescent non-human primates. The human apoE4 allele is associated with an increased risk of developing late-onset familial and sporadic AD. We found that rhesus monkeys and three subspecies of squirrel monkeys are homozygous for apoE phenotype with arginine at positions 112 and 158 as in human apoE4. However, in both species threonine replaces arginine at position 61 of human apoE. It was previously shown that arginine 61 was critical in determining apoE4 lipoprotein distribution in humans.

Key words: Aging; Alzheimer's disease; Amyloid; Apolipoprotein E

1. Introduction

Sporadic and inherited cerebral amyloidoses are characterized by the deposition of β-amyloid (Aβ) fibrils, which are formed by aggregation of a fragment of a larger β-amyloid precursor protein (βPP) [1 7], predominantly in the extracellular space of the brain parenchyma. Patients with sporadic cerebral amyloid angiopathy (SCAA), hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D) and some patients with familial Alzheimer's disease, display vascular syndromes ranging from multiinfarct dementia to single massive intracerebral hemorrhages, as a result of extensive amyloid deposition within cerebral vessel walls [8–16].

Similar to humans, aged non-human primates manifest Aβ deposits in the cerebrum and the amount and localization of amyloid varies among individuals of similar age [17–19]. In aged rhesus monkeys (*Macaca mulatta*), Old World primates with a life span of 35–40 years [20], cerebral amyloid deposition occurs most commonly in association with senile plaques and less frequently in blood vessels. These aged monkeys exhibit behavioral abnormalities that are similar to the minor cognitive deficits manifested by aged humans [21]. Conversely, in aged squirrel monkeys (*Saimiri sciureus*), New World primates with a life span of 25–30 years [18], cerebrovascular deposits usually are more conspicuous than are senile plaques [18,22]. In the brains of all aged squirrel monkeys tested amyloid was associated primarily with intracerebral and meningeal capillaries and arterioles and occurs to a lesser degree as senile plaques [18]. Vascular amyloid deposition is an age-associated process, with marked differences in the degree of vascular amyloidosis and the incidence of non-vascular lesions between monkeys of the same chronological age [18,22]. The brains of aged rhesus monkeys as well as squirrel monkeys lack neurofibrillary tangles, another neuropathological hallmark of AD in humans.

Apolipoprotein E (apoE) is a plasma protein that, in the human population, presents three major alleles (e2, e3 and e4) which encode three apoE isoforms (E2, E3 and E4) [23]. The e4 allele is associated with an increased risk of developing AD in late-onset familial as well as sporadic AD [24–28]. Using two-dimensional polyacrylamide-gel-electrophoresis it was shown that ninety non-human primates of nine different species, including squirrel monkeys (12 monkeys), have a homozygous apoE phenotype, which in accordance with the human nomenclature was designated E3/E3 [29]. However, PCR analysis revealed the apoE allele in rhesus monkey to resemble the human apoE4 isotype [30]. To clarify this issue, we compared the apoE isotype in rhesus monkeys and three subspecies of squirrel monkeys to test the hypothesis that species differences and inter-individual variability in the histological locus of amyloid deposition are related to the apoE allelotype in non-human primates.

2. Materials and methods

2.1. Tissues

DNA was isolated from frozen tissues of nine squirrel monkeys of unknown subspecies and from blood and frozen liver tissues of 30 monkeys from The Primate Research Center at the University of South Alabama (10 squirrel monkeys of each of 3 distinct phenotypes: *Saimiri boliviensis, Saimiri sciureus and Saimiri boliviensis peruviensis*). DNA was also isolated from blood of four rhesus monkeys, and from a human with known apoE phenotype.

2.2. APOE genotyping

APOE genotyping was performed as previously described [31,32]. Genomic DNA was isolated by the phenol/chloroform extraction method [33]. DNA fragments of 227 bp, which span both apoE human polymorphic sites, were PCR-amplified using the primers underlined with a double line (Fig. 1), and were digested with *Hha*I. Digestion products were analyzed on 10% PAGE, stained with ethidium bromide and viewed under UV light. These fragments, as well as fragments that were PCR amplified using the primers underlined with a single line (Fig. 1), were sequenced. For sequence analysis, 1 μl of amplified fragments

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Abbreviations: AD, Alzheimer's disease; apoE, apolipoprotein E; CAA, cerebral amyloid angiopathy; SCAA, sporadic cerebral amyloid angiopathy; Aβ, β-amyloid; βPP, β-amyloid precursor protein; HCHWA-D, hereditary cerebral hemorrhage with amyloidosis, Dutch type.

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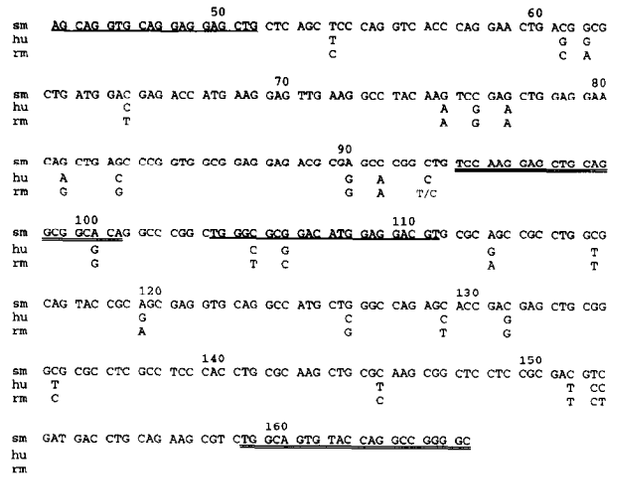

Fig. 1. Nucleotide sequence of squirrel monkey (sm) APOE cDNA, shown in the first line. Nucleotide substitutions found in human (hu) and in rhesus monkey (rm) are indicated in the second and third lines, respectively. Numbering according to the human amino acid sequence.

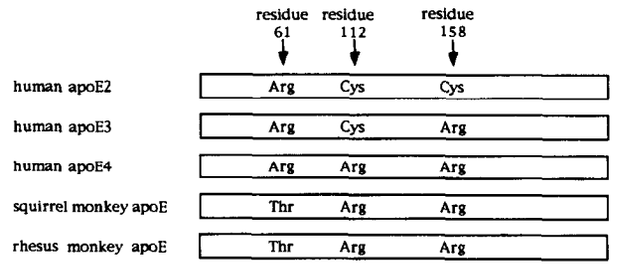

Fig. 2. Diagram of allelic variants of apoE. The amino acid sequences at residues 61, 112 and 158, according to the human numbering and nomenclature, are indicated.

was cloned into pCRII vector (Invitrogen, San Diego, California). Several colonies, derived from at least two unrelated PCR reactions, were sequenced by the dideoxy chain termination method with sequenase (USB) and the sequence was determined in both strands.

3. Results and discussion

Allelic polymorphism in the human APOE gene results in three major isoforms. The E2 isoform differs from the E3 isoform by an Arg to Cys substitution at position 158. The E4 isoform differs from the E3 isoform by a Cys to Arg substitution at position 112 (Fig. 2). The nucleotide substitutions that account for the allelic variation result in polymorphic restriction sites for *Hha*I. All squirrel monkeys tested showed the same arrangement of bands, different from that of rhesus monkeys and humans (Fig. 3). Sequence analysis of the PCR-amplified APOE DNA fragments revealed the sequence differences between the squirrel monkey, the rhesus monkey and the human (Fig. 1) that are responsible for the difference in the restriction patterns. Within the DNA fragment sequenced, the squirrel monkey's APOE sequence differs from the human sequence by 18 nucleotides. The rhesus monkey's sequence differs from that of the human by 15 nucleotides, 8 of which are identical to the squirrel monkey's sequence. The first nucleotide of codon 93 of the rhesus monkey's sequence is polymorphic, but does not result in an amino acid change.

Both rhesus and squirrel monkeys are homozygous for apoE4 with Arg at positions 112 and 158, according to the human numbering and nomenclature. Eight deduced amino acid differences between the squirrel monkey and human sequences were found, five of which are shared with the rhesus monkey: Arg^{61}Thr, Thr^{83}Ser, Gly^{113}Ser, Gly^{120}Ser, Val^{135}Ala. Three differences are unique to the squirrel monkey: Val^{116}Ala, Glu^{131}Asp and Ala^{152}Val, and two differences are unique to the rhesus monkey: Ser^{54}Pro and Ala^{62}Thr. We have analyzed a large sample of squirrel monkeys, searching for possible subspecies or individual differences in APOE genotype that could be responsible for the variability in amyloid deposition found in these monkeys. However, the APOE genotype in all monkeys tested was identical.

In humans the E3 allele, with Cys at position 112, is the most common. The apoE proteins of the squirrel monkey, rhesus monkey [30], cynomolgus monkey [34], baboon [35], cow [36], pig [37], rat [38] and mouse [39] all contain an Arg at this residue. The rabbit apoE contains a Cys at residue 112 [40]. The Cys–Arg interchange at position 112, which distinguishes apoE3 from apoE4, influences the class of lipoprotein to which the carboxyl-terminal domain will bind; apoE3 displays a preference for high density lipoproteins and apoE4 for very low density lipoproteins. The involvement of Arg^{61} in modulating domain interaction appears to be a key factor in determining the isoform-specific lipoprotein preferences of apoE [41]. Of the ten animal species for which the sequence of apoE is known, only human apoE has an arginine at position 61; all of the other species, including rhesus and squirrel monkeys, contain threonine at this position (or its equivalent) (Fig. 1). When the Arg61 in the human apoE4 was mutated to threonine, the binding preference of apoE shifted from very low density lipoproteins

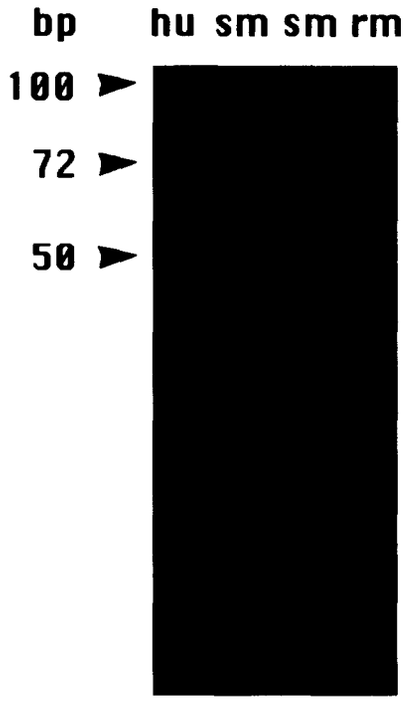

Fig. 3. APOE genotyping of human and monkeys DNA by *Hha*I restriction fragment analysis of PCR products. The patterns of human APOE3/E2 (hu), two squirrel monkeys APOE (sm) and a rhesus monkeys APOE (rm) are presented. The 100, 72 and 50 bp markers are marked by arrowheads.

to high density lipoproteins [41]. It is therefore likely that the apoE in the monkeys behaves like human apoE3, at least with regard to its interaction with lipoproteins.

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