Polymorphism of apolipoprotein A-II (apoA-II) among inbred strains of mice

Relationship between the molecular type of apoA-II and mouse senile amyloidosis

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Three types of apolipoprotein A-II (apoA-II) proteins (A, B and C) were predicted from the nucleotide sequence of apoA-II cDNA. Substitution of amino acid residues was noted at four positions (type A: Pro-5, Asp-20, Met-26, Ala-38; B: Pro-5, Glu-20, Val-26, Val-38; C: Gln-5, Glu-20, Val-26, Ala-38). Each type was identifiable by digestion of amplified apoA-II DNA by PCR, using restriction-fragment-length polymorphism of the apoA-II gene for restriction enzymes *Cfr13I* and *MspI*. The molecular type of apoA-II was determined among 23 strains of mice including nine of the senescence accelerated mouse series developed in our laboratory. Examination of types of apoA-II and amyloid deposition in the F2 and F3 hybrid mice showed that apoA-II amyloid deposition was present only in the mice homozygous for type C apoA-II and which were 12–17 months of age. The molecular type of apoA-II may be a factor involved in the development of senile amyloidosis in mice.

INTRODUCTION

A unique senile amyloid protein 'AS_{SAM}' was isolated from the SAM-P/1 strain of mice (Matsumura *et al.*, 1982). Senescence-accelerated mice (SAM) are a group of mouse strains developed by Takeda and co-workers as a murine model of accelerated senescence (Takeda *et al.*, 1981). Accelerated senescence-prone mice (SAM-P) are a group of inbred strains of mice consisting of SAM-P/1, SAM-P/2, SAM-P/3, SAM-P/6, SAM-P/7, SAM-P/8 and SAM-P/9. All SAM-P strains show earlier onset and irreversible advancement of senescence (Hosokawa *et al.*, 1984; Takeda *et al.*, 1991). Accelerated-senescence-resistant mice (SAM-R) are a group of inbred strains which includes SAM-R/1 and SAM-R/2, both of which show normal aging. Systemic senile amyloidosis is one of the most characteristic findings in the SAM-P/1 and SAM-P/2 strains (Takeshita *et al.*, 1982; Higuchi *et al.*, 1983). Biochemical and immunochemical studies indicated that apolipoprotein A-II (apoA-II) in mouse serum high-density lipoprotein is deposited in the tissues of these two strains in the form of amyloid fibril 'AS_{SAM}' (Higuchi *et al.*, 1986a; Yonezu *et al.*, 1986). Sequence analysis of apoA-II in the SAM-P/1 strain and the SAM-R/1 strain (in which senile amyloidosis occurred with a low incidence) revealed an amino acid substitution (glutamine in SAM-P/1 and proline in SAM-R/1) at position 5 (Higuchi *et al.*, 1986b). These studies suggested that the molecular type of apoA-II may be linked to the development of murine senile amyloidosis. Spontaneous age-associated amyloidosis has been noted in various strains of mice (Glenner & Page, 1976; Scheinberg *et al.*, 1976; Koeger *et al.*, 1984). Thus elucidation of the structural polymorphism in apoA-II among inbred strains of mice is indispensable for a genetic analysis of the contribution of apoA-II to mouse senile amyloidosis.

Here, we observed three molecular types of mouse apoA-II and developed a procedure that makes use of PCR. Using this procedure, we identified apoA-II types in 23 inbred strains of

mice, including SAM-P and SAM-R strains, and a genetic analysis of murine senile amyloidosis was carried out.

EXPERIMENTAL

Mice

Strains SAM-P/1, SAM-P/2, SAM-P/3, SAM-P/6, SAM-P/7, SAM-P/8, SAM-P/9, SAM-R/1, SAM-R/2, (Takeda *et al.*, 1981; Matsushita *et al.*, 1986; Chen *et al.*, 1989; Yagi *et al.*, 1989; Takeda *et al.*, 1991) and DDD mice were maintained in our laboratory by sister–brother breeding. Strains A/J, AKR/N, BALB/c, B10.BR/Sg, B10.A/Sg, CBA/N, C3H/He, C57BL/6J, C57BL/10, DBA/2, NZB/N and Slc:ICR mice were purchased from The Shizuoka Laboratory Animal Center, Shizuoka, Japan. SJL/J mice were purchased from The Gokita Breeding Service, Tokyo, Japan.

Twenty-two F2 hybrid mice obtained by breeding SAM-P/9 mice with C57BL/6J mice and 15 F3 hybrid mice were obtained by breeding SAM-P/1 mice with B10.BR mice killed at 12–17 months of age. The liver, kidney, spleen, heart, lung, abdominal skin, stomach and small intestine were fixed in 10% neutral buffered formalin, embedded in paraffin and amyloid deposits were examined. DNA was isolated from the liver, and the molecular type of apoA-II in the hybrid mice was determined.

Amyloid deposits in liver, spleen, heart, skin and stomach in the SAM-P/3, SAM-P/6, SAM-P/9 and SAM-P/7 strains were examined. These mice had all been raised under conventional conditions at 24±2 °C with a light-controlled regimen (12 h light/dark cycle), and a commercial chow (CE-2; Nihon CLEA, Tokyo, Japan) was provided from weaning until death. Tap water was freely available for drinking.

Isolation of DNA and PCR amplification of the apoA-II gene

DNA was isolated from the livers by standard proteinase K digestion, phenol extraction and ethanol precipitation. Since

Abbreviations used: ABC, avidin–biotinylated horseradish peroxidase complex; AI, amyloid index; apoA-II, apolipoprotein A-II; RFLP, restriction-fragment-length polymorphism.

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the amino acid substitution in the apoA-II of the SAM-P/1 and SAM-R/1 strains was confirmed to be the result of substitution of two nucleotides in the third exon of the apoA-II gene (Yonezu *et al.*, 1989), a part of the apoA-II gene including the second and third exons was amplified by PCR, using two apoA-II-specific primers (SO-AII1 and SO-AII2) from 1 µg of genomic DNA (Fig. 1a). SO-AII1 (5'-TGAAGCTTCTCGCAATGGTCGCACTGCTGGT-3') is the coding strand and the 5' area has the *Hind*III-compatible end, and SO-AII2 (5'-AGTCATGCTCTGAAAGTACTGTGTG-3') is complementary to apoA-II mRNA and has the *Sca*I-recognition sequence. The mixture [final volume 100 µl, with conditions as specified in the GeneAmp Kit (Perkin-Elmer/Cetus, Norwalk, CT, U.S.A.)] was incubated at 94 °C for 6 min, followed by 40 cycles of successive denaturation (94 °C for 1 min), annealing (55 °C, 2 min) and extension (72 °C, 2.5 min), followed by a final 10 min extension at 72 °C. The PCR products were digested with restriction enzymes and loaded on 2.5% NuSieve (FMC Bioproducts, Rockland, ME, U.S.A.)/1% agarose gel.

Sequence analysis

PCR products of the apoA-II gene were digested with *Hind*III and *Sca*I and subcloned into the pUC19 vector. A cDNA library from livers of C57BL/6J mice (Clontech Laboratories, Palo Alto, CA, U.S.A.) in phage λgt11 was screened with a ³²P-labelled 320 bp *Bst*EII-*Bal*I fragment of mouse apoA-II cDNA (Kunisada *et al.*, 1986). Inserts of positive phages were subcloned into pUC19 for sequencing. Nucleotide sequencing was performed on double-stranded DNA by the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977).

Southern blotting

DNA (10 µg) from different inbred strains of mice was digested overnight with restriction enzymes and subjected to 1% (w/v) agarose-gel electrophoresis, transferred to a nitrocellulose membrane and hybridized with a ³²P-labelled apoA-II cDNA probe.

Detection of amyloid deposition

Identification of amyloid was made according to evidence of green birefringence in the Congo Red-stained section, under conditions of polarizing microscopy. Immunohistochemical identification of the deposited amyloid protein was performed using the avidin-biotinylated horseradish peroxidase complex (ABC) method with specific antisera against apoA-II and AA protein (Higuchi *et al.*, 1983).

RESULTS

Identification of three types of apoA-II

The apoA-II DNA fragment of 424 bp was amplified by PCR from DNA of SAM-P/1, SAM-P/2, SAM-R/1, SAM-R/2, A/J, AKR/N, C57BL/6J, DDD and SJL/J mice and subcloned into pUC19. Five randomly selected clones of each strain were sequenced to avoid error caused by PCR (Fig. 1a). The results of the sequence analysis are shown in Fig. 1(b). The PCR products of SAM-P/1, SAM-P/2, A/J and SJL/J mice had the same sequence as the sequence of the apoA-II gene in the SAM-P/1 strain (Yonezu *et al.*, 1989). PCR products of the SAM-R/1 and DDD strains had a two-nucleotide substitution in the third exon from the PCR product of the SAM-P/1 strain and this substitution provided a recognition site for restriction enzyme *Cfr*13I. In the PCR products of the SAM-R/2, AKR/N and C57BL/6J strains, there was a substitution of seven nucleotides from the PCR product of the SAM-P/1 strain. The one substitution at

nucleotide 322 from guanosine (G) to adenosine (A) provided a recognition site for the restriction enzyme *Rsa*I. The substitution at nucleotide 712 from A to cytidine (C) in the third exon led to the appearance of the *Cfr*13I and *Msp*I site.

To determine the structure of apoA-II molecules observed in the SAM-R/2, AKR/N and C57BL/6J strains, we isolated eight phages including apoA-II cDNA inserts from the liver cDNA library of the C57BL/6J strain. The apoA-II cDNA sequence of the C57BL/6J strain had seven nucleotide substitutions, as compared with the apoA-II cDNA in the SAM-P/1 strain (Fig. 2). Three of these nucleotide substitutions caused replacements in the deduced amino acid sequence from glutamine to proline at position 5, from glutamic acid to aspartic acid at position 20 and from valine to methionine at position 26. No amino acid replacement was observed in the pre- and pro-peptides. The substitution at nucleotide 14 from A to C produced a new *Msp*I and *Cfr*13I site. The substitutions at nucleotide 39 from G to A removed the recognition site for *Rsa*I and *Sca*I, and the substitution at nucleotide 210 from C to thymidine (T) removed the recognition site for *Xho*I. All the eight clones sequenced had 3' untranslated regions that were seven nucleotides longer than previously determined in SAM-P/1 and SAM-R/1 apoA-II cDNA.

Determination of types of apoA-II among inbred mouse strains

For determination of the types of apoA-II among various inbred strains of mice, we used the PCR products of the apoA-II gene demonstrated in Fig. 1(a). PCR products have one *Cfr*13I site (nucleotide 503) and one *Msp*I site (nucleotide 528) in common and were polymorphic for *Cfr*13I and *Msp*I sites located at nucleotides 608–614 (Fig. 1b). PCR products were digested with *Cfr*13I or *Msp*I and separated by agarose-gel electrophoresis (Fig. 3). After digestion with *Cfr*13I, the PCR product of the SAM-P/1 strain showed two bands. PCR products of the SAM-R/1 and C57BL/6J strains showed three bands, and the PCR product of the F1 hybrid mouse obtained by crossing SAM-P/1 mice with SAM-R/1 mice showed all four bands. After digestion with *Msp*I, the PCR products of SAM-P/1 and SAM-R/1 mice showed two bands, and the PCR product of the C57BL/6J strain showed three bands. PCR products of the F1 hybrid mouse between the SAM-P/1 and C57BL/6J strains had all four bands.

The result of determinations of the genetic types of apoA-II among 23 strains, including nine of the SAM series, is summarized in Table 1. We termed the genetic type of apoA-II found in AKR/J, B10.A, B10.BR, C57BL/6J, C57BL/10, DBA/2, SAM-P/3, SAM-P/8 and SAM-R/2 strains as 'type A', since the phenotype of apoA-II (Apoa2) in strains AKR/J, C57BL/6J, C57BL/10 and DBA/2 had been determined as 'A' by isoelectric focusing (Lusis *et al.*, 1983). We termed the genetic type of apoA-II found in strains BALB/c, CBA/N, C3H/He, DDD, NZB/N, ICR:slc, SAM-P/6 and SAM-R/1 as 'type B', since the phenotype of apoA-II in BALB/c, CBA/Caj, C3H/HeJ and NZB/BINJ had been determined as 'B' by isoelectric focusing. Finally we termed the genetic type of apoA-II found in strains A/J, SJL/J, SAM-P/1, SAM-P/2, SAM-P/7 and SAM-P/9 as 'type C'. The phenotype of A/J and SJL/J had been determined as 'B' by isoelectric focusing.

Southern hybridization analysis

Restriction-fragment-length polymorphism (RFLP) analysis was made on the DNA of 23 strains with restriction enzymes *Rsa*I, *Msp*I, *Sca*I and *Xho*I, the polymorphisms of which were predicted from the sequence analysis of apoA-II cDNA. After digestion with *Rsa*I, the SAM-P/1, SAM-R/1 and C57BL/6J DNA showed three, two and one hybridization bands respectively

Twenty-two F2 hybrid mice obtained by crossing F1 hybrid mice (parental strains were C57BL/6J and SAM-P/9) were killed at age 12–17 months (Fig. 5a). The C57BL/6J strain has the type A apoA-II (Apoa2^{2a/a}) and the SAM-P/9 strain has the type C apoA-II (Apoa2^{2c/c}). The genetic type of apoA-II in the individual hybrid mice was determined by RFLP analysis with *AceI* and was confirmed by PCR amplification. Three homozygous mice for type C apoA-II had apoA-II deposits as senile amyloid fibrils, in the stomach, skin, liver, spleen, kidney, small intestine, lung and heart. Two male F2 homozygous mice for type C apoA-II (Apoa2^{2c/c}), obtained by crossing progenitor strains SAM-P/1 (Apoa2^{2c/c}) and B10.BR (Apoa2^{2a/a}), were mated with two female F2 heterozygous mice for apoA-II (Apoa2^{2a/c}) obtained from the same litter (Fig. 5b). Amyloid deposits were present in all five homozygous mice (Apoa2^{2c/c}), but there was no amyloid deposition in the ten heterozygous mice (Apoa2^{2a/c}). A wide variation in the intensity of the amyloid deposition was evident among F2 and F3 mice homozygous for type C apoA-II.

SAM-P/1	CTGCAGCATAGAATCGCAGCACTGTTCTTAGGCCATAG [†] CTGCCATC	ATG AAG CTG	-61
SAM-R/1		<u>Met Lys Leu</u>	(-21)
C57BL/6J	C		
SAM-P/1	CTC GCA ATG GTC GCA CTG CTG GTC ACC ATC TGT AGC CTG GAA GGA GCT TTG GTT AAG AGA	-1	
SAM-R/1	<u>Leu Ala Met Val Ala Leu Leu Val Thr Ile Cys Ser Leu Glu Gly</u>	<u>Ala Leu Val Lys Arg</u>	(-1)
C57BL/6J			
	Cfr13I MspI RsaI ScaI		
SAM-P/1	CAG GCG GAC GGA CAG GAT ATG CAG AGC CTG TTC ACT CAG TAC TTT CAG AGC ATG ACT GAA	60	
SAM-R/1	Gln Ala Asp <u>Gly</u> Gln Asp Met Gln Ser Leu Phe Thr Gln Tyr Phe Gln Ser Met Thr Glu	(20)	
C57BL/6J	A C Pro A T		
SAM-P/1	TAT GGC AAA GAT TTC GTG GAG AAG GCC AAG ACC TCA GAG ATT CAG AGC CAG GCC AAG GCA	120	
SAM-R/1	Tyr Gly Lys Asp Leu Val Glu Lys Ala Lys Thr Ser Glu Ile Gln Ser Gln Ala Lys Ala	(40)	
C57BL/6J	A Met		
SAM-P/1	TAC TTT GAG AAG ACA CAC GAG CAG CTG ACA CCC CTT GTC AGG TCA GCA GGA ACT AGT CTG	180	
SAM-R/1	Tyr Phe Glu Lys Thr His Glu Gln Leu Thr Pro Leu Val Arg Ser Ala Gly Thr Ser Leu	(60)	
C57BL/6J			
	XhoI		
SAM-P/1	GTG AAC TTC TTC AGC AGT TTA ATG AAC CTC GAG GAG AAA CCG GCT CCT GCG GCT AAG TGA	240	
SAM-R/1	Val Asn Phe Phe Ser Ser Leu Met Asn Leu Glu Glu Lys Pro Ala Pro Ala Ala Lys ***	(79)	
C57BL/6J	T		
SAM-P/1	GATGTGCCAGG CCAGTCTTCCCACCCAGCTGCTCCACTGGCCACCGCTAGAGCCCCTCTCCCTACCTTCTGCCTGTT	319	
SAM-R/1			
C57BL/6J	C		
SAM-P/1	TTCTCCAATAAATGCGGAAGGAGTT-AAAAAAAA(A)n	343	
SAM-R/1			
C57BL/6J	AAACTTC-AAAAAAAA(A)n	351	

Fig. 2. Comparison of the nucleotide sequences of the three types of mouse apoA-II cDNA

The sequence of SAM-P/1 apoA-II cDNA determined by Kunisada *et al.* (1986) is presented on the top line and the amino acid encoded is shown below the DNA sequence. Prepeptide and propeptide are shown by solid and broken underlines respectively. Amino acid residues from 1 to 78 correspond to mature apoA-II. Substitution of nucleotides and amino acid residues observed in SAM-R/1 and C57BL/6J apoA-II compared with SAM-P/1 apoA-II are represented on the second and the third lines respectively. Polymorphisms of the restriction-enzyme sites are presented by short solid lines above the DNA sequence of each type of apoA-II cDNA.

DISCUSSION

Mature apoA-II protein is a peptide with 78 amino acids and is produced by removing the propeptide from the *N*-terminus and the lysine residue from the C-terminus in mice (Kunisada *et al.*, 1986). A definite physiological role for apoA-II, other than being the major constituent of high-density lipoprotein, has not been defined, although it appears to activate hepatic lipase and may also affect phosphatidylcholine:cholesterol acetyltransferase activity (Jahn *et al.*, 1983). One of the most interesting biological events in which apoA-II plays an important role is senile amyloidosis in mice. ApoA-II is deposited extracellularly in the form of twisted-wire-like amyloid fibrils in the SAM-P/1 and SAM-P/2 strains (Higuchi *et al.*, 1986a). Sequence analysis of apoA-II protein in the SAM-P/1 and SAM-R/1 strains revealed the existence of two molecular types of apoA-II. A heterogeneity in isoelectric point of apoA-II among inbred strains of mice has been reported (Lusis *et al.*, 1983; Green, 1989). Here we have attempted to discern the molecular basis for the structural difference in apoA-II detected by our group and that of Lusis *et al.* (1983).

First, we sequenced PCR products of the apoA-II gene obtained from nine strains of mice. We found that the PCR products of strains SAM-R/2, AKR/N and C57BL/6J had a sequence that differed in seven nucleotides from the PCR products of the SAM-P/1 and SAM-R/1 strains. Since the infidelity of *Taq* polymerase under certain conditions is known (Saiki *et al.*, 1988; Innis *et al.*, 1988), we screened apoA-II cDNA

clones from a cDNA library of C57BL/6J mice, and the sequence analysis confirmed the presence of the third type of apoA-II.

Next, we developed an easy and suitable method for identifying the three types of apoA-II, using the RFLP patterns of the apoA-II gene amplified by PCR. Determination of the types of apoA-II among 23 mouse strains revealed that ApoA2;B, a basic phenotype in Lusis's classification could be divided into two types, B and C (Table 1). Polymorphisms at the restriction enzyme site and the amino acid sequences observed for the three types of mouse apoA-II are summarized in Fig. 6. Since these amino acid substitutions would not explain the greater acidity of the type A apoA-II, some modification of the protein, such as oxidation of methionine (Anantharamaiah *et al.*, 1988) or deamination of glutamine or asparagine residues, would need to be considered. It has been reported that apoA-II cDNAs in strains C57BL/6J, DBA/2, BALB/c and C3H/HeJ have the same sequences as type A, A, B and B apoA-II respectively in our classification (Doolittle *et al.*, 1990).

The A/J, SJL/J, SAM-P/1, SAM-P/2, SAM-P/7 and SAM-P/9 strains were revealed to have type C apoA-II. In the SJL/J strain, a high incidence of severe age-associated senile amyloidosis has been reported (Scheinberg *et al.*, 1976). Several investigators have reported age-associated systemic amyloidosis in the A strain of mice (Thung, 1957; Zschiesche, 1972). A high incidence of amyloid deposition was observed in the SAM-P/7 and SAM-P/9 strains. On the other hand a low incidence of amyloid deposition was observed in SAM-P/3, SAM-P/6 (Table 2), SAM-P/8 (Miyamoto *et al.*, 1986), SAM-R/1 and SAM-R/2

Fig. 3. Determination of the molecular types of apoA-II using PCR

(a) 3.5 % agarose-gel electrophoresis of PCR products. One-tenth of the total PCR products without (left half of the gel) and with (right half) digestion with *Cfr131* were loaded and stained with ethidium bromide. F1 hybrid mouse (P/1 × R/1) was obtained by crossing SAM-P/1 and SAM-R/1 strains. (b) PCR products were digested with *MspI* and loaded on a 3.5 % agarose gel. The F1 hybrid strain (P/1 × B6) was obtained by crossing SAM-P/1 and C57BL/6J strains. Sizes of the bands of the molecular-size markers (M) are 2027, 1353, 1078, 872, 603, 310, 271, 234 and 194 (in bp) from top to bottom. Molecular mass of the digested DNA fragments is shown on the right of each gel (in bp).

(Yonezu *et al.*, 1987) strains. These results support the idea that age-related amyloid deposition might be accelerated in strains with one molecular type of apoA-II (type C).

Genetic analysis using F2 and F3 hybrid mice indicated that apoA-II deposition, as senile amyloid fibril, was observed only in mice homozygous for type C apoA-II (Apoa2^{cc}), at the age of 12–17 months. Thus it was shown that the structure of apoA-II is probably a major factor that determines the development of

Fig. 4. RFLPs of the mouse apoA-II gene

Genomic DNA was digested with restriction enzymes, subjected to 1 % agarose-gel electrophoresis, transferred to filters and hybridized with labelled apoA-II cDNA. (a) Typical results for *RsaI*-digested DNA. Three F1 hybrid mice were obtained by crossing SAM-P/1 mice with SAM-R/1 mice (P/1 × R/1), SAM-P/1 mice with C57BL/6J mice (P/1 × B6) and SAM-R/1 mice with C57BL/6J mice (R/1 × B6). (b) RFLP of the apoA-II gene obtained with *AccI* is shown. Molecular size markers (in kb) are indicated at the left of each gel.

senile amyloidosis in mice. A comparison of the amino acid sequences of the three types of apoA-II may support the idea that the glutamine residue at position 5 is the key residue for the susceptibility to amyloid fibril formation, because three other amino acid substitutions are not specific for type C apoA-II. Another notion is that the coexistence of type A and type C apoA-II in high-density lipoproteins may inhibit fibril formation. The mechanisms of acceleration of amyloid deposition associated with structural differences in apoA-II proteins have to be clarified. Age-associated phenomena such as senile amyloidosis might be a polygenetically controlled event. The finding of a large variation in the AI values among F2 and F3 hybrid mice which were

Table 1. Different genetic types of apoA-II among different mouse strains

Genetic types of apoA-II were determined by combining the results obtained by the 3.5 %-agarose-gel electrophoresis of PCR products of the apoA-II gene digested with restriction enzymes *Cfr131* and *MspI* as described in the text. The pattern of restriction fragment observed for PCR products of the apoA-II gene after digestion with *Cfr131* and *MspI* is designated with a '1' or '2'. For *Cfr131* the 1 pattern corresponds to 272 bp, 106 bp and 46 bp fragments while the 2 pattern corresponds to 272 bp and 152 bp fragments. For *MspI* the 1 pattern corresponds to the 296 bp, 84 bp and 44 bp fragments while the 2 pattern corresponds to 296 bp and 128 bp fragments. The pattern of restriction fragments observed for Southern blot hybridization of the apoA-II gene after digestion with *RsaI* is designated with '1', '2' and '3'. The 1 pattern corresponds to a 2.2 kb fragment. The 2 pattern corresponds to 1.8 kb and 1.2 kb fragments. The 3 pattern corresponds to 1.5 kb, 1.2 kb and 0.3 kb fragments. The phenotypes 'A' and 'B' were determined by isoelectric focusing of the apoA-II protein (Lusis *et al.*, 1983).

Genetic type of apoA-II	Strain	PCR		RFLP <i>RsaI</i>	Apoa2
		<i>Cfr131</i>	<i>MspI</i>		
A	AKR/N, B10.A, B10.BR, C57BL/6J, C57BL/10, DBA/2 SAM-P/3, SAM-P/8, SAM-R/2	1	1	1	A
B	BALB/c, CBA/N, C3H/He, DDD, NZB/N, ICR SAM-P/6, SAM-R/1	2	1	2	B
C	A/J, SJL/J SAM-P/1, SAM-P/2, SAM-P/7, SAM-P/9	2	2	3	B

Table 2. Senile amyloidosis in strains SAM-P/3, SAM-P/6, SAM-P/7 and SAM-P/9

Amyloid deposition was examined in the Congo Red-stained sections under the conditions of polarizing microscopy. The intensity of amyloid deposition in liver, spleen, heart, skin and stomach was graded at five levels: 4, most intense deposits; 3, intense deposits; 2, moderate deposits; 1, slight deposits; 0, no deposits. The score of the five organs was summed and divided by 5 to obtain the average intensity, amyloid index (AI).

Strain	Type of apoA-II	Mouse no.	Age (months)	Sex	Amyloid deposition					AI
					Spleen	Liver	Heart	Skin	Stomach	
SAM-P/3	A	1	13	M	0	0	0	0	0	0.0
		2	13	F	0	0	0	0	0	0.0
		3	14	F	0	0	0	0	1	0.2
		4	15	F	0	0	1	0	1	0.4
		5	17	M	0	1	1	0	2	0.8
		6	18	M	0	0	0	0	0	0.0
		7	18	M	0	0	0	0	0	0.0
		8	19	M	0	0	0	0	0	0.0
		9	19	M	0	1	1	1	1	0.8
SAM-P/6	B	1	11	M	3*	2	0	0	1	1.2
		2	11	F	0	0	0	0	0	0.0
		3	15	F	1	0	0	0	0	0.2
		4	18	M	3*	1	0	0	2	1.2
		5	20	F	1	0	0	0	0	0.2
		6	21	F	0	0	0	0	0	0.0
SAM-P/7	C	1	12	M	2	2	2	2	2	2.0
		2	13	M	1	1	1	2	1	1.2
		3	13	M	0	2	3	3	3	2.2
		4	13	M	2	2	2	3	4	2.6
		5	13	M	3	2	2	3	3	2.6
SAM-P/9	C	1	10	F	1	3	2	3	3	2.4
		2	11	M	3	1	2	2	3	2.2
		3	14	F	1	1	2	1	1	1.2

* Intense amyloid deposits in the spleen and liver of the SAM-P/6 strain were stained positively with anti-AA serum.

Fig. 5. Molecular types of apoA-II and senile amyloidosis in F2 and F3 hybrid mice

(a) Twenty-two F2 hybrid mice were obtained by crossing F1 hybrid mice, whose parental strains were SAM-P/9 and C57BL/6J. Squares and circles represent male and female mice respectively. Open symbols indicate the homozygous mice for type A apoA-II (*Apoa2^{8/a}*) and solid symbols indicate homozygous mice for type C apoA-II (*Apoa2^{c/c}*). Half-solid symbols represent heterozygous mice for apoA-II (*Apoa2^{8/c}*). Amyloid index (AI) was calculated using immunohistologically stained tissue sections. The age when the mice were killed is given on the bottom line. (b) Fifteen F3 hybrid mice were obtained by crossing two male F2 homozygous mice for type C apoA-II and two female heterozygous F2 mice, the progenitor strains being SAM-P/1 and B10.BR.

homozygous for type C apoA-II and the fact that calorie restriction reduced amyloid deposition in the SAM-P/1 strain (Kohno *et al.*, 1985) support the idea that other genetic factors may control senile amyloid deposition. The possibility that the actual 'amyloidogenic gene' located in close proximity to the apoA-II gene might co-segregate with the type of apoA-II in F2 and F3 mice has not been ruled out.

In humans, senile amyloidosis is also a common age-associated disease, and the putative precursor in serum may be prealbumin (Pitkanen *et al.*, 1983; Cornwell *et al.*, 1988; Gorevic *et al.*, 1989). Patients in several different families with familial amyloid

polyneuropathy have different prealbumin variants with a single amino acid substitution and have heavy amyloid deposition in peripheral nerves and other organs even from early or middle stages of life (Tawara *et al.*, 1983; Husby & Sletten 1985; Wallace *et al.*, 1988). Structural changes in the senile amyloid precursor proteins, apoA-II in mice and prealbumin in humans, may possibly accelerate the age-associated construction of the amyloid fibril. Although it is not yet clear if the same mechanism functions in human and murine senile amyloidosis, the murine system will contribute to a better understanding of these interesting but poorly elucidated age-associated phenomena.

Fig. 6. Schematic diagram of the polymorphisms of apoA-II gene and apoA-II protein

The four exons of the apoA-II gene are illustrated by solid boxes. The mRNA encoding the mature apoA-II is shown by a solid box, and the 5' and 3' untranslated region and mRNA encoding the prepeptide and propeptide are shown by open boxes. ○ and × above the restriction enzymes indicate presence and absence of the recognition sequence of the enzymes.

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