



Development of congenic strains of mice carrying amyloidogenic apolipoprotein A-II (Apoa2^c)

Apoa2^c reduces the plasma level and the size of high density lipoprotein

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A congenic strain of mice with amyloidogenic apolipoprotein A-II (Apoa2^c) on the genetic background of the amyloidosis-resistant SAM-R/1 strain was produced by 12 generations of backcrossing. Genome mapping using endogenous murine leukemia proviral markers was done in the congenic strain, termed R1·P1-Apoa2^c. We confirmed that only a small region surrounding the apoA-II gene on chromosome 1 was transferred from the genome of the donor SAM-P/1 strain. The level and particle size of plasma high density lipoprotein were decreased in R1·P1-Apoa2^c mice compared to those in the progenitor SAM-R/1 mice. The function of apoA-II can be studied using this strain of mice.

Amyloidogenic apolipoprotein A-II; Congenic Mouse; High density lipoprotein; Senescence accelerated mouse (SAM)

1. INTRODUCTION

Amyloidosis is characterized by extracellular deposition of fibrillar amyloid protein. Apolipoprotein A-II (apoA-II) in mouse serum high-density lipoprotein (HDL) is a serum precursor of murine senile amyloid protein (AApoA-II) [1,2]. Three variants of apoA-II protein (A, B and C) with different amino acid substitutions at four positions had been found among inbred strains of mice [3]. The SAM-P/1 and SJL/J strains with a high incidence of senile amyloidosis have type C apoA-II with glutamine at position 5, whereas the SAM/R-1, C57BL/6J and DDD strains with a low incidence of amyloidosis have type A or B apoA-II with proline at position 5 [4]. Examination of the type of apoA-II and amyloid deposition in the F1, F2 and backcrossed mice between strains with Apoa2^c and Apoa2^a or Apoa2^b showed that acceleration of murine senile amyloidosis is linked to Apoa2^c and is transmitted in an autosomal recessive manner [3,5].

The SAM-P/1 strain with Apoa2^c is a murine model of accelerated senescence [6] and has the following characteristics of lipid metabolism: (1) low levels of serum

apoA-II and HDL, (2) age-associated decreases in serum levels of apoA-I and apoA-II, and (3) age-associated increases in clearance rate of serum apoA-II [7,8]. To examine if the molecular type of apoA-II is a factor involved in the regulation of lipid metabolism and development of senile amyloidosis, we developed a congenic strain, R1·P1-Apoa2^c containing amyloidogenic type C apoA-II gene of the SAM-P/1 strain on the genetic background of the SAM-R/1 strain, by 12 generations of backcrossing. Lipoproteins in the congenic strain were compared to those in the progenitor strains and our findings are described herein.

2. MATERIALS AND METHODS

2.1. Production of congenic strains of mice

The original parents, the SAM-R/1 strain with type B apoA-II (Apoa2^b) and the SAM-P/1 strain with type C apoA-II (Apoa2^c) were crossed and the F1 progeny were backcrossed to SAM-R/1. In each subsequent generation, progeny were selected that possessed the donor strain allele (Apoa2^{bc}) at the differential locus, Apoa2. After 6 and 12 backcrosses, heterozygous siblings were interbred and progeny with the Apoa2^{cc} genotype were selected. The genotype of ApoA-II was determined by polymorphism of the polymerase chain reaction (PCR)-amplified apoA-II gene for digestion by restriction enzyme Cfr13I [3].

2.2. Genetic analysis of the congenic mice

To analyze the genetic identity of congenic strains with the progenitor strain, SAM-R/1, we used endogenous murine leukemia virus (MuLV) proviral markers which are dispersed over mouse chromosomes [9]. High molecular weight DNA prepared from the livers was digested with *PvuII* or *EcoRI*, separated by electrophoresis on 0.8%

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Abbreviations: apoA-II, apolipoprotein A-II, HDL, high-density lipoprotein, PCR, polymerase chain reaction, MuLV, murine leukemia virus.

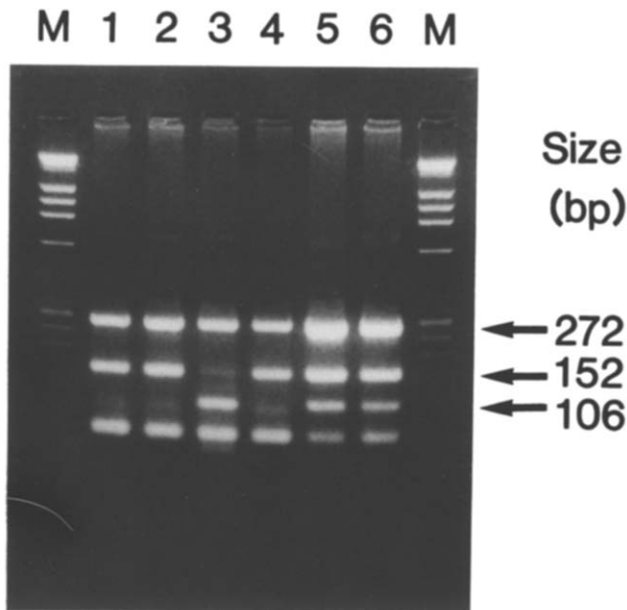


Fig. 1. Determination of the genetic type of apoA-II gene in the congenic strains. The DNA was isolated from tails of SAM-P/1 (lane 1), R1-P1-Apoa2^c (lane 2), SAM-R/1 (lane 3), R1-P1-Apoa2^c#6 (lane 4), a male (lane 5) and a female (lane 6) progeny used to produce R1-P1-Apoa2^c. ApoA-II PCR products digested with *Cfr*13I were analyzed on a 3.5% agarose gel. The type C and type B apoA-II gene showed characteristic 152 and 106 bp bands, respectively. Molecular mass of the digested DNA is shown on the right (in bp).

agarose gels, transferred to nylon membranes (GeneScreen Plus, NEN Products, Boston, MA, USA) and hybridized to ³²P-labeled oligonucleotide probes identifying xenotropic, polytropic and modified polytropic proviruses [9–11]. Membranes were exposed to -80°C for one week.

2.3. Lipoprotein quantitations

All mice had been raised on a 12 h light/dark cycle and were fasted for 12–16 h prior to collection of blood samples obtained by cardiac puncture following ether anesthesia. The mice were fed a commercial chow (CE-2, Nihon CLEA, Tokyo, Japan) containing 4% fat. Plasma total cholesterol and triglyceride levels were determined using enzymatic procedures (Cholesterol C-Test Wako and Triglyceride G-Test Wako, Wako Pure Chemical Industries Osaka, Japan). HDL cholesterol was estimated according to a modified heparin-manganese precipitation procedure (HDL cholesterol C-Test Wako).

Non-denaturing gradient polyacrylamide gel electrophoresis was performed on minigels 84 mm wide × 90 mm high. Gels containing a 4–15% linear polyacrylamide gradient were electrophoresed in 25 mM Tris, 192 mM glycine. Prior to electrophoresis, plasma samples (3 μl) were stained for lipid by incubation for 3 h at room temperature with 24 μl freshly prepared Sudan black B dye solution (5 parts 1.0% Sudan Black B in ethylene glycol; 3 parts 40% sucrose). Electrophoresis was carried out at 30 mA for 2 h.

3. RESULTS AND DISCUSSION

After 6 and 12 generations of backcrossing, heterozygous male and female progeny (Apoa2^{bc}) were mated to produce congenic mice carrying type C apoA-II, homozygously (Fig. 1). We termed the congenic strains produced by 6 and 12 backcrosses as R1-P1-Apoa2^c#6 and R1-P1-Apoa2^c, respectively.

After 6 and 12 backcrosses, we calculated that 3.13% and 0.05% of the genome of the donor strain (SAM-P/1) might be transferred to the genome of the inbred partner (SAM-R/1). We analyzed the genetic identity of the congenic strains with the progenitor SAM-R/1 strain by genome mapping, using Southern blot hybridization with synthetic oligo-nucleotide probes specific to three classes of endogenous MuLV proviruses (Fig. 2). Since the classes of MuLV proviruses are reasonably stable and many copies are usually found in inbred mouse strains, they can serve as genetic markers when attempting to map genetic determinants for pathologic phenotypes [9–12]. A pair of Southern blots of DNA isolated from SAM-P/1 and SAM-R/1 mice (digested with either *Pvu*II or *Eco*RI) were carefully compared with the data of Frankel et al. [9–11] for fragment size for each class of MuLV proviruses. The 34 identifiable fragments were assigned locus numbers according to Frankel et al. and the 4 fragments not found in their table were given arbitrary alphabetical letters. The 15 fragments which were specific to either strain, Xenotropic (Xmv)-21 on chromosome 1, Xmv-h on unassigned locus, Xmv-27 on chromosome 13, Xmv-15 on chromosome 9, Xmv-26 on chromosome 8, Xmv-24 on chromosome 6, modified

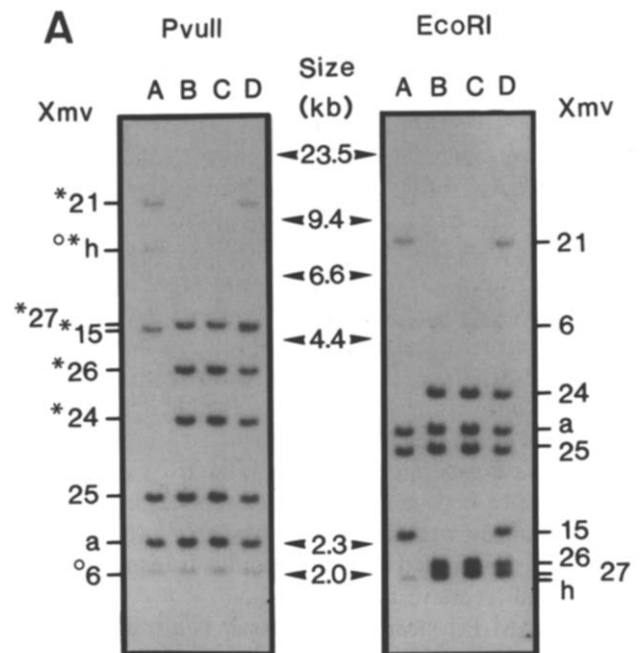


Fig. 2. A comparison of: A, xenotropic (Xmv); B, modified polytropic (Mpmv); and C, polytropic (Pmv) provirus contents of SAM-P/1 (lane A), R1-P1-Apoa2^c (lane B), SAM-R/1 (lane C) and R1-P1-Apoa2^c#6 (lane D). Also shown are location of fragments corresponding to Xmv, Mpmv and Pmv loci numbered as described by Frankel et al. [9]. Fragments represented by alphabetical letters could not be found in common mouse strains defined by Frankel et al. Fragments present only in either progenitor SAM-P/1 and SAM-R/1 strain are represented by asterisks. Xmv loci that hybridized poorly to the probe are represented by circles. *Hind*III-digested DNA molecular size standards are shown.

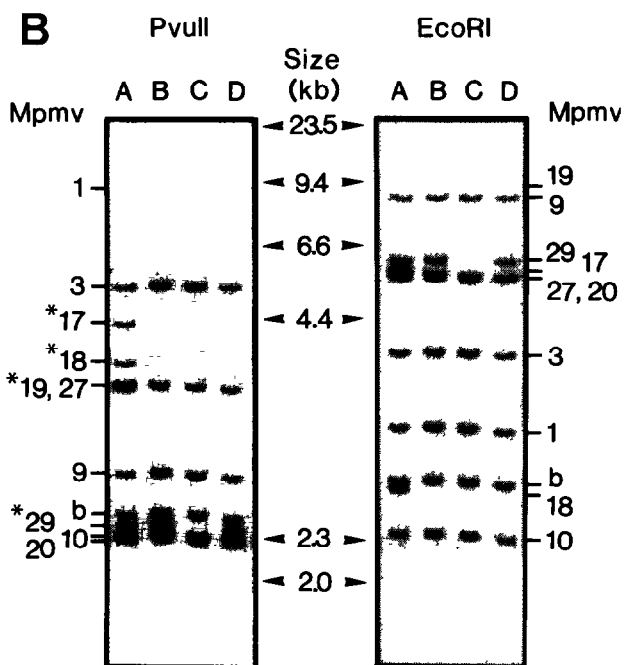


Fig. 2B.

polytropic (Mpmv)-17 on chromosome 16, Mpmv-18 on chromosome 11, Mpmv-19 on chromosome 4, Mpmv-29 on chromosome 1, polytropic (Pmv)-25 on chromosome 4, Pmv-35 on chromosome 16, Pmv-14 on chromosome 16, Pmv-32 on unassigned locus and Pmv-20 on chromosome 18 are represented by asterisks in Fig. 2. Comparison of profiles of provirus fragments of the congenic strains with those of their progenitor strains showed that almost all fragments were identical with those of the SAM-R/1 strain, except for one fragment (Mpmv-29) in R1·P1-Apoa2^c and three fragments (Mpmv-29, Xmv-15 and Xmv-21) in R1·P1-Apoa2^{#6}. Mpmv-29 are closely linked with the apoA-II gene on chromosome 1 and may be transferred from the SAM-P/1 genome to the SAM-R/1 genome together with the apoA-II gene. The presence of Xmv-15 on chromosome 9 and Xmv-21 located 15 cM proximal to the centromere from apoA-II gene in R1·P1-Apoa2^{#6} revealed that 6 backcrosses were not sufficient and parts of the SAM-P/1 genome remained in R1·P1-Apoa2^c#6.

Plasma levels of total and HDL cholesterol in R1·P1-Apoa2^c and R1·P1-Apoa2^{#6} were significantly lower than those of progenitor SAM-R/1 which has type B apoA-II (Student's *t*-test $P < 0.001$) (Table I). Values of the congenic strains were much the same as values in the donor strain (SAM-P/1). Heterozygous F1 hybrid mice produced by crossing between SAM-R/1 and R1·P1-Apoa2^c (R1·P1-Apoa2^{b/c}) revealed intermediate values of total and HDL cholesterol levels. There was no difference in plasma levels of triglycerides among the strains and no significant differences in the levels of hepatic apoA-II mRNA were observed among the strains (K. Kitagawa et al., unpublished data).

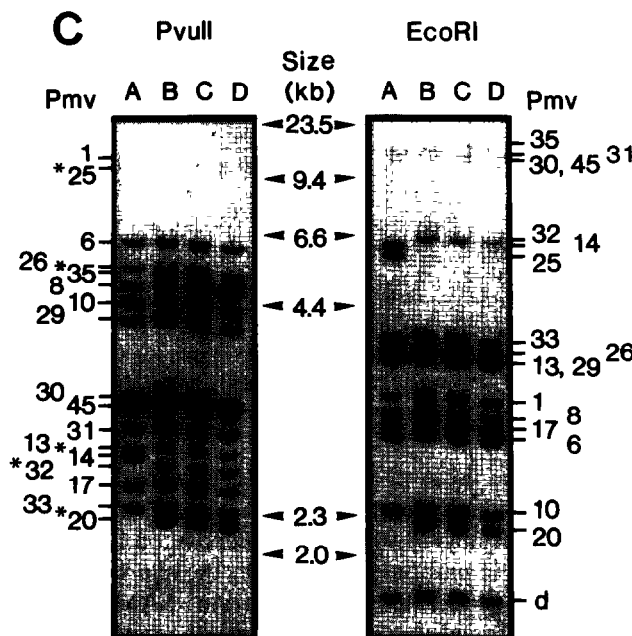


Fig. 2C.

Non-denaturing polyacrylamide gradient gel electrophoresis was used to determine size distribution on HDL stained with Sudan black B (Fig. 3). A clear shift in the distribution of HDL particle was observed with HDL from the SAM-R/1 strain (larger HDL), as compared to the SAM-P/1 strain (smaller HDL). The congenic strains resembled the donor SAM-P/1 strain in terms of HDL size (smaller). Heterozygous F1 hybrid mice and HDL of an intermediate size of the progenitor

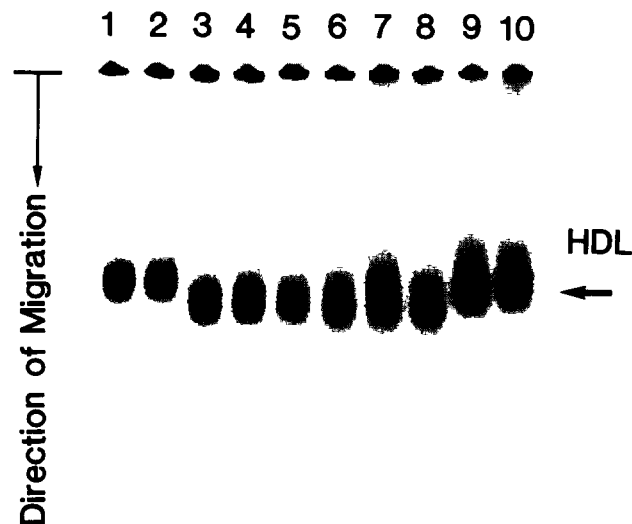


Fig. 3. Plasma HDL from strains SAM-R/1 (lanes 1,2), SAM-P/1 (lanes 3,4), R1·P1-Apoa2^c (lanes 5,6), R1·P1-Apoa2^{#6} (lanes 7,8), R1·P1-Apoa2^{b/c} (lane 9,10) was stained with Sudan black B and subjected to non-denaturing polyacrylamide gradient (4-15%) gel electrophoresis.

Table I
Plasma lipid levels in congenic and progenitor strains

Strains	Type of apoA-II	n	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	Triglycerides (mg/dl)
SAM-P/1	C	5	79.91 ± 11.09	51.86 ± 1.69	142.22 ± 47.80
SAM-R/1	B	5	135.34 ± 12.05	84.30 ± 6.29	134.78 ± 36.52
R1·P1-Apoa2 ^c	C	7	66.99 ± 7.06*	53.35 ± 5.47*	120.09 ± 23.62
R1·P1-Apoa2 ^c #6	C	6	67.71 ± 10.68*	57.61 ± 3.71*	119.11 ± 26.07
R1·P1-Apoa2 ^{b/c}	B/C	4	89.07 ± 13.40**	73.67 ± 3.73**	97.71 ± 40.43

Results represent mean ± S.D.

* Values in R1·P1-Apoa2^c and R1·P1-Apoa2^c#6 mice were significantly lower than those in SAM-R/1 mice ($P < 0.001$, Student's *t*-test).

**Values in R1·P1-Apoa2^{b/c} were significantly higher than those in R1·P1-Apoa2^c mice ($P < 0.01$, Student's *t*-test).

strains. Thus the level and size of HDL were determined by a molecular type of apoA-II.

Age-associated severe amyloid deposition is the most characteristic pathologic finding in the short-lived and senescence-accelerated mouse strain SAM-P/1. Although we found that the phenotype of accelerated amyloid deposition co-segregated with the type C apoA-II gene locus [3,5], we had no definite data concerning the interaction between accelerated senescence and accelerated amyloid deposition in the SAM-P/1 strain. Using the congenic strains carrying only a small portion of chromosome 1 surrounding the apoA-II gene, from the SAM-P/1 strain on the genetic background of the SAM-R/1 strain with normal aging process, will shed light on this interesting and important question. This approach will avoid inestimable effects by unknown genes dispersed over the mouse genome.

The SAM-P/1 strain has a characteristic lipoprotein metabolism. The presence of type C apoA-II reduced the plasma level and particle size of HDL, determined by using apoA-II congenic strains. Ongoing investigations on age-associated changes in lipoprotein metabolism in the congenic strain will shed light on the role of a characteristic lipoprotein metabolism in SAM-P/1 in the development of senile amyloidosis.

The functions and biogenesis of HDL have yet to be entirely elucidated. Epidemiological data suggest an inverse correlation between HDL cholesterol levels and risk of coronary artery disease [13]. In mice, genetic studies revealed that decreases in HDL cholesterol levels in response to a high fat diet co-segregated with susceptibility to diet-induced atherosclerosis [14]. Although apoA-II is the second most abundant protein constituent of human and mouse HDL, the physiological role of apoA-II in HDL metabolism has not been clearly defined. Recent studies revealed that structural differences between mouse type A and type B apoA-II affects the apoA-II translation efficiency and the size of the HDL is altered [15,16]. The R1·P1-Apoa2^c strain could be an excellent model to study the function of apoA-II.

Human apoA-II amyloid protein or apoA-II vari-

ants, except for *NspI* restriction enzyme polymorphism in the apoA-II gene reported by Scott et al. [17] have not been found. Our finding will aid in the search for apoA-II variants affecting amyloidosis and lipid metabolism in humans.

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