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Mouse Models to Study Systemic Amyloidoses: Is Prion-Like Transmission a Common Pathogenic Mechanism?

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1. Introduction

The amyloidoses are a group of protein-misfolding disorders characterized by the accumulation of amyloid fibrils formed from a variety of proteins. Currently, twenty- eight different kinds of human and animal proteins, in intact or fragmented forms, have been found to be associated with pathological disorders such as Alzheimer's disease, type II diabetes, prion diseases, dialysis-related amyloidosis, and various familial, senile and sporadic amyloidosis (Sipe et al., 2010; Benson et al 2008). Amyloidoses have been divided into two major classes: 1) systemic and 2) localized amyloidoses. In systemic amyloidoses, precursor proteins circulating in the blood associate to form amyloid fibrils that are then deposited throughout the body. For example, immunoglobulin light chains form deposits in patients with myeloma in AL amyloidosis. In reactive AA amyloidosis, serum amyloid A (SAA) protein forms deposits in patients with chronic inflammation, and transthyretin (TTR) forms deposits in patients with familial amyloid polyneuropathy (FAP) and senile systemic amyloidosis (SSA). Patients on long-term hemodialysis develop dialysis-related amyloidosis (DRA) due to the deposition of amyloid fibrils (Aß$_2$M) of ß$_2$-microglobulin (ß$_2$m). In contrast to systemic amyloidosis, precursor proteins produced in local organs deposit in one particular area of the body in various localized amyloidoses.

In mice, apolipoprotein A-II (apoA-II) in serum high density lipoproteins (HDL) forms amyloid fibrils (AApoAII) in age-associated systemic amyloidosis (senile AApoAII amyloidosis). AA amyloidosis, known as reactive or secondary amyloidosis associated with inflammation, is generally recognized as the predominant form of systemic amyloidosis that occurs in humans, mice, domestic animals and many species in the animal kingdom. These amyloidoses are characterized by the systemic deposition of extracellular fibrils composed of apoA-II in AApoAII amyloidosis or SAA (serum AA) in AA amyloidosis, primarily in the spleen, liver, heart, kidney, vessels walls, and to a lesser extent in other organs. In most

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species, AApoAII and AA amyloidosis occurs sporadically and is associated with aging (AApoAII amyloidosis) and chronic inflammation (AA amyloidosis). However, intriguing recent data suggest that both AApoAII and AA amyloidoses could be transmitted by a prion-like infectious process through a seeding-nucleation mechanism (Qian et al., 2010; B. Zhang et al., 2008). In these amyloidoses, AApoAII and AA amyloid fibrils, abnormal forms of the host serum proteins (apoA-II and SAA), induce conformational changes in apoA-II and SAA to form AApoAII and AA fibrils, and this causes detectable phenotypes or diseases in affected individuals. Recently, Aß amyloid fibrils in Alzheimer's disease and intracellular amyloid fibril-like aggregated proteins were postulated to work as seeds for propagation of mis-folded and pathologic protein structures in various neurodegenerative disorders, including Huntington's disease, Parkinson's disease and tauopathies (Aguzzi et al., 2009; Brundin et al., 2010).

The prion-like transmission of amyloid fibrils or fibril-like materials, which could play an important role in the propagation of pathological events in systemic amyloidosis, will be discussed here.

2. Transmission of mouse senile AApoAII amyloidosis

Several senescence-prone inbred strains of mice (SAMP strains) have been developed with accelerated senescence, a shorter life span and various age-associated disorders and pathologic changes. These SAMP strains include SAMP1, SAMP6, SAMP8 and SAMP10. An accelerated senescence-resistant strain (SAMR1) has also been generated to serve as a control for the SAMP strains (Takeda et al., 1981; Higuchi et al., 1999). Severe senile amyloidosis is a characteristic age-associated disorder in the SAMP1 and SAMP10 strains, making them a valuable model to investigate amyloidosis pathogenesis and to assist in the development of effective therapeutic modalities. We identified apoA-II, a normal constituent of serum HDL, as the amyloid protein responsible for mouse senile amyloidosis in the SAMP1 strain, and the amyloid fibril was named AApoAII (Higuchi et al., 1983; Yonezu et al 1986; Higuchi et al. 1986). Recently we found that there was prion-like transmission in mouse AApoAII amyloidosis (Higuchi et al., 1998; Xing et al., 2001). Here, a brief history and the pathobiology of mouse AApoAII amyloidosis in SAMP1 and its related strains is described, followed by a discussion of AApoAII amyloidosis transmission.

2.1 Pathology of mouse AApoAII amyloidosis

Amyloid fibril deposition in aged SAMP1 mice is systemic and all organs except brain parenchyma are involved (Takeshita et al., 1982). The earliest AApoAII deposits are seen in the primary and secondary papillae of the tongue, the lamina propria and submucosa of the small intestine, the alveolar septa of the lungs and the squamous-glandular junction of the stomach. With advancing age, AApoAII deposits extend into the collecting tubules in the papillae of the kidneys, the perimedullary zone of the adrenal cortex, heart and skeletal muscle interstitium, thyroid gland interstitium, the papillary layer of the dermis, the testis interstitium, the corpora lutea, the atretic follicle and the ovarian interstitium, around the portal veins and in the spaces of Disse in the periportal sinusoid of the liver, the marginal zone around the lymphoid follicles of the spleen, and blood vessels throughout the body. In the final stage, the liver and spleen are enlarged and the kidneys are contracted with severe amyloid deposition (Higuchi et al. 1983) (Fig. 1).

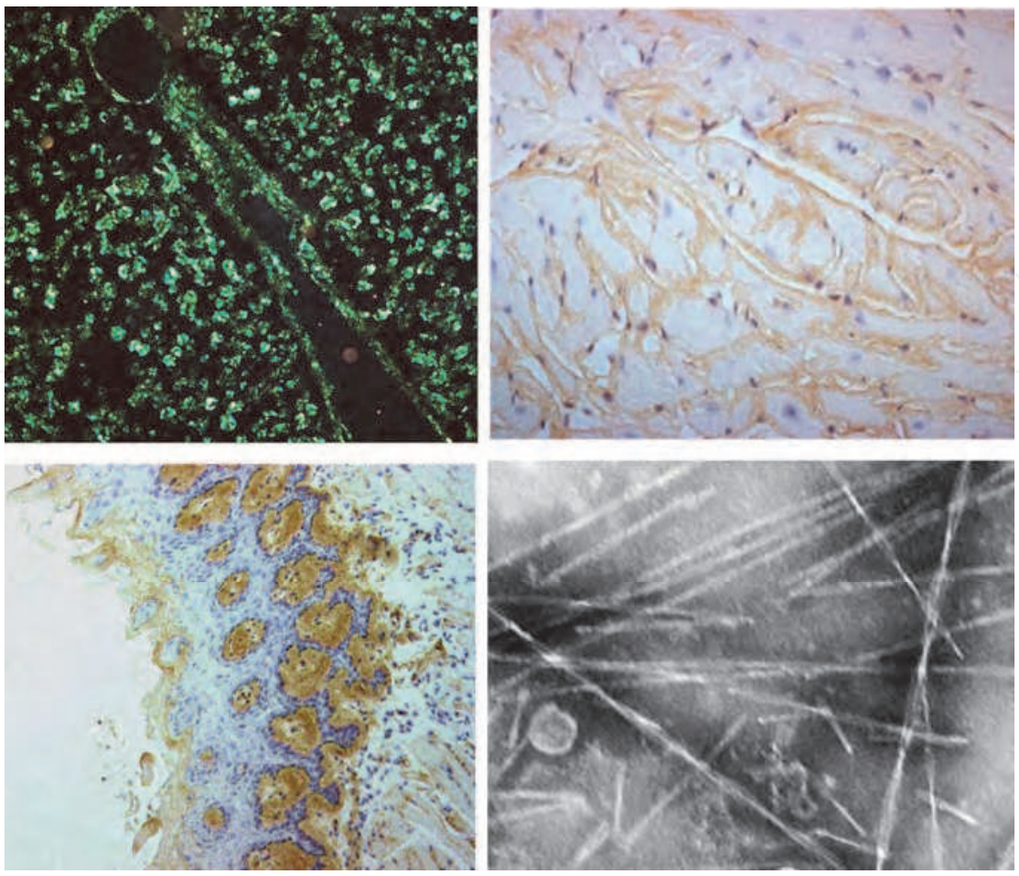

Fig. 1. **Mouse systemic senile amyloidosis in SAMP1 mice.** AApoAII amyloid fibrils were deposited in the whole body except for the brain parenchyma. **Upper-left**: heavy amyloid deposition in a liver section strained with Congo red was detected as green birefringence under the polarizing microscope (X100). **Upper-righ**t and **Lower-left**: AApoAII amyloid depositions were determined immunohistochemically in the heart and tongue, respectively (X400). **Lower-right**; Electron microscopic observation of AApoAII amyloid fibrils isolated from the liver (X50,000).

Although senile AApoAII amyloidosis is common in most mouse strains, severe senile amyloidosis has been reported in only a few strains, i.e. SAMP1, SAMP10, SJL/J, LLC, and PS strains. The complete nucleotide sequence of the apoA-II (gene is *Apoa2*) cDNA was determined in 41 inbred strains of mice (Kunisada et al., 1986; Kitagawa et al., 2003). Among these strains, six alleles containing amino acid substitutions (*Apoa2*^{a}, *Apoa2*^{b}, *Apoa2*^{c}, *Apoa2*^{d}, *Apoa2*^{e} and *Apoa2*^{f}) were identified. Severe amyloid deposition was observed in the strains with the *Apoa2*^{c} allele (Higuchi et al., 1991). Several genetic analyses indicated that the presence of the *Apoa2*^{c} allele markedly accelerates age-associated deposition of AApoAII and reduces the lifespan (Naiki et al., 1993; Higuchi et al., 1995; 1996). These results show that type C apoA-II protein in mice is highly amyloidogenic, while SAMR1 and strains with type B apoA-II protein are resistant to the development of amyloidosis. The mice expressing type A apoA-II protein, such as C57BL/6, are moderately amyloidogenic. *In vitro* amyloid fibril formation (fibril extension) has been reported with the type C apoA-II protein, but not with the type B apoA-II (Naiki et al., 1992; Fu et al., 2001). Recently we demonstrated that

the combination of N- and C-terminal sequences of mouse apoA-II and a conformational change in their secondary structure are essential for polymerization into AApoAII amyloid fibrils (Sawashita et al., 2009). However, the mechanism behind the high amyloidogenicity of type C apoA-II protein *in vivo* is not yet known.

Congenic R1.P1-*Apoa2*^{c} mice have the amyloidogenic *Apoa2*^{c} SAMP1 gene on the genetic background of SAMR1 (Higuchi et al., 1993). Comparable to the donor SAMP1 strain, severe amyloid deposition is present in the R1.P1-*Apoa2*^{c} strain (Higuchi et al., 1995), and the R1.P1-*Apoa2*^{c} mice are more convenient for use in amyloidosis experiments. A transgenic mouse strain that over-expresses *Apoa2*^{c} mRNA has been established on the genetic background of R1.P1-*Apoa2*^{c}. This strain shows higher concentration of apoA-II and greater susceptibility to amyloidosis than the control R1.P1-*Apoa2*^{c} strain and should prove valuable in future studies of amyloidosis (Ge et al., 2007).

2.2 Transmission of mouse AApoAII amyloidosis

Nucleation-dependent polymerization is a postulated model consistent with the kinetics of *in vitro* amyloid protein fibrillization in amyloidoses such as prion diseases, Alzheimer disease and mouse AApoAII amyloidosis (Jarrett et al., 1993; Harper et al., 1997; Naki et al., 1991). This model is comprised of two phases: 1) nucleation and 2) extension. 1) Nucleus formation requires a series of thermodynamically unfavorable monomer association steps. Thus, the nucleation phase is the rate-limiting step in the development of amyloidosis. 2) Once the nucleus has formed, further addition of monomers becomes thermodynamically favorable, resulting in rapid extension of amyloid fibrils. The dramatically hastened *in vitro* fibril formation fueled by the addition of amyloid fibrils (nucleus) to a solution of amyloid protein monomers is an example of nucleation-dependent polymerization.

Prion diseases are associated with the accumulation of a pathologic conformational isomer (PrP^{Sc}) by a host-derived prion protein (PrP^{c}). Prion transmission or propagation involves the conversion of cellular PrP^{c} into PrP^{Sc} via an increase in its ß-sheet secondary structure content. According to the protein-only hypothesis, introduction of the abnormal conformer PrP^{Sc} into an organism would accelerate the conversion of PrP^{c} into its pathological conformation (Prusiner et al., 1998; 2006). Thus, the nucleation-dependent polymerization model provides a feasible mechanism for the *in vivo* conformational conversion of PrP^{c} to PrP^{Sc} via transmission of prions (Weissmann et al, 1999).

As predicted by the nucleation-dependent polymerization model, *in vitro* fibril formation experiments in which apoA-II monomers are converted to AApoAII fibrils indicate that the addition of AApoAII amyloid fibrils can facilitate the formation of amyloid fibrils from apoA-II monomers (seeding reaction)(Fig. 2A). Amyloid deposition was notably accelerated after a very small quantity of AApoAII fibrils was administered to R1.P1-*Apoa2*^{c} mice by peripheral injection (intravenous, intraperitoneal or intragastric) or in the diet (Fig. 2B) (Higuchi et al., 1998; Xing et al., 2001). Thus, fibril formation is greatly accelerated in mice through seeding with pre-formed fibrils *in vivo* in mice (transmission of amyloidosis). The acceleration of amyloidosis fibrils (transmissibility) disappeared completely after treatment with protein denaturing reagents including 6 M guanidine hydrochloride, strong alkaline solution or formic acid. Acceleration was slowed by 6 M urea or by autoclaving, and it was not changed by formalin, DNAse or RNAse treatments. This finding revealed that transmissibility of AApoAII depends on fibril conformation (H. Zhang et al., 2006).

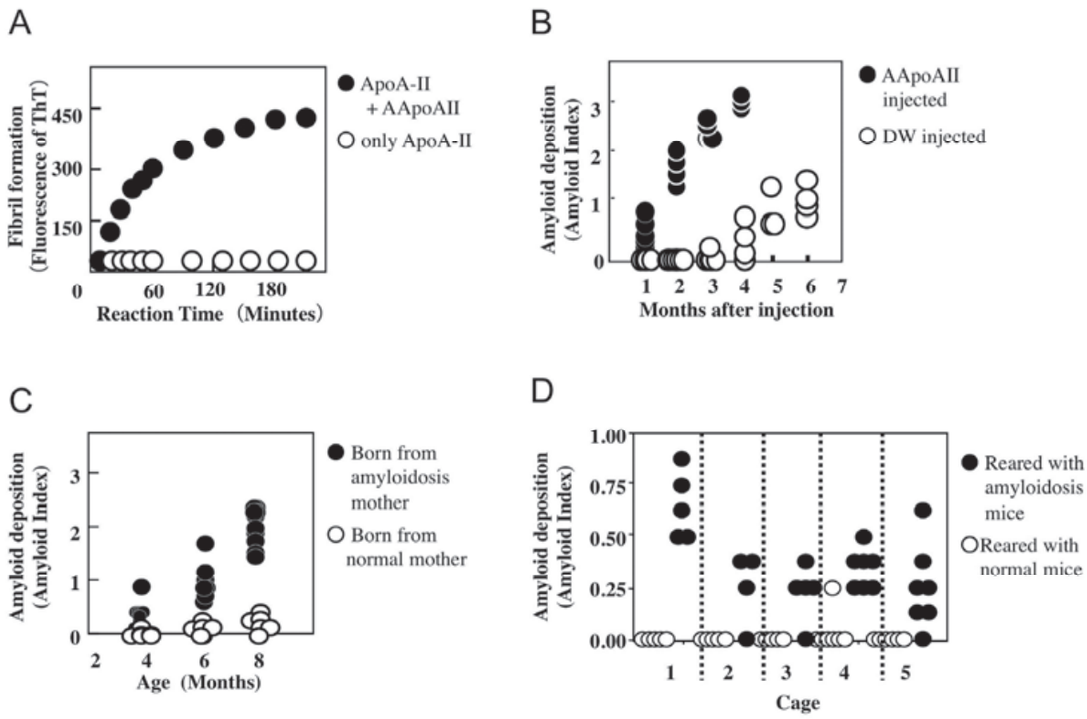

Fig. 2. **Transmission of mouse AApoAII amyloidosis in vitro and in vivo. A:** Addition of AApoAII amyloid fibrils to a solution of apoA-II monomers accelerates fibril formation in test tubes. **B:** Injection of AApoAII amyloid fibrils into R1.P1- *Apoa2*^{c} mice accelerates amyloid deposition. **C:** Amyloid deposition is accelerated in offspring born to and nursed by mothers with amyloidosis. **D:** Young R1.P1- *Apoa2*^{c} mice that were kept in the same cage with mice having amyloidosis for 3 months developed amyloidosis. The intensity of amyloid deposition in mice was determined semi-quantitatively using the amyloid index (AI) as a parameter. The AI parameter represents the average degree of deposition, graded 0 to 4, in Congo red - stained sections from the seven organs examined (liver, heart, spleen, tongue, stomach, intestine and skin).

Amyloidosis in the offspring of R1.P1- *Apoa2*^{c} mice was examined to further characterize this transmissibility. Acceleration of amyloidosis was observed in offspring born to, and nursed by, mothers with amyloidosis that was induced by injection of amyloid fibrils 3 months before pregnancy (Fig. 2C) compared with the offspring of control mothers that did not receive injections of amyloid fibrils (Korenaga et al., 2006). Acceleration of amyloidosis was also observed in offspring born to mothers that were not injected, but that were nursed by mothers injected with amyloid fibrils. However, this phenomenon was not observed in offspring born to amyloidosis mothers and nursed by control mothers. Injection of milk obtained from amyloid fibril-injected mothers induced AApoAII amyloidosis in young mice, and amyloid fibrils were detected in the milk of amyloid fibril-injected mothers by electron microscopy. Young mice were reared for 3 months in cages with old R1.P1- *Apoa2*^{c} mice that had severe amyloid-depositions. All of the young mice developed amyloid deposits (Fig. 1D)(Xing et al., 2001). Amyloid fibrils were detected in feces of mice with amyloidosis. Injection of amyloid fibrils found in feces induced amyloidosis in mice, raising

the possibility that the oral transmission of amyloid fibrils through feces leads to acceleration of amyloidosis in AApoAII amyloidosis. AApoAII amyloid fibril deposits were found in the skeletal muscles of amyloid-affected mice, primarily in the blood vessels and in the interstitial tissues (endomysium) surrounding muscle fibers (Qian et al., 2010). Amyloid fibril fractions isolated from the muscles could also induce amyloidosis in young mice.

2.3 Cross-seeding of amyloidosis

The induction of AApoAII amyloidosis was studied *in vivo* using various kinds of amyloid fibrils that were isolated from human and mouse tissues or were formed *in vitro* from synthetic peptides and recombinant proteins. The fibrils were injected intravenously into young R1.P1-*Apoa2*^{c} mice. At 3 and 6 months after injection, the most severe amyloid depositions were detected in mice injected with mouse AApoAII(C) amyloid fibrils composed of amyloidogenic C type apoA-II, (Fu et al 2004, Yan et al., 2007). Mild amyloid depositions were also detected in tissues of mice that had been injected with other types of fibrils, including synthetic peptides and recombinant proteins. However, no amyloid depositions were found in mice injected with non-amyloid fibril proteins such as serum albumin, transthyretin and mouse apoA-II. This cross-seeding model postulates that there is a direct interaction between newly forming and preexisting heterologous amyloid fibrils *in vivo*. Thus, induction by various amyloid fibrils supports the prospect of amyloidosis acceleration in animals and humans by heterogeneous amyloid and amyloid-like fibrils in foods or the environment.

AApoAII(C) fibrils were injected intravenously into 2-month-old SAMR1 mice, which carry the less amyloidogenic apoA-II allele (*Apoa2*^{b}) and develop few, if any, spontaneous amyloid deposits. Ten months after the amyloid injection, deposits were detected on the tongue, and the intensity of deposition increased thereafter, whereas no amyloid was detected in SAMR1 mice injected with distilled water, even after 20 months (Xing et al., 2002). The deposited amyloid fibrils were composed of endogenous type B apoA-II protein, with a different amyloid fibril conformation of the proto-fibril-like figure. Subsequent injection of these AApoAII(B) fibrils induced earlier and more severe amyloidosis in SAMR1 mice than did the first injection of AApoAII(C) fibrils. Thus, adaptation of amyloid fibril structure might happen during deposition in SAMR1 mice. AApoAII(A) fibrils composed of mild amyloidogenic apoA-II allele (*Apoa2*^{a}) were isolated from the intestine and liver of C57BL/6 mice. Atomic force microscopy and transmission electron microscopy revealed that the majority of isolated AApoAII(A) fibrils have fine, proto-fibril-like shapes. The AApoAII(A) fibril has a much weaker affinity for thioflavin T than does the AApoAII(C) fibril. The injection of AApoAII(A) fibrils induced amyloid deposition in C57BL/6 mice (*Apoa2*^{a}) as well as in R1.P1-*Apoa2*^{c} mice (*Apoa2*^{c}). However, the AApoAII(A) fibrils induced more severe amyloidosis in *Apoa2*^{a} strains than in the *Apoa2*^{c} strain (Korenaga et al., 2003).

These findings indicate that AApoAII(A) fibrils isolated from mice having the mild-amyloidogenic type A apoA-II, and AApoAII(B) fibrils isolated from mice having the less-amyloidogenic type B apoA-II, have distinct morphological, pathological and structural characteristics that differ from those of the AApoAII(C) fibrils of amyloidogenic type C apoA-II. Consequently, cross-seeding with the amyloid fibrils induced amyloid deposition in mice that had amyloid protein monomers with different primary structures.

3. Transmission of mouse reactive AA amyloidosis

AA amyloidosis, also known as reactive or secondary amyloidosis, is generally recognized as the predominant form of systemic amyloidosis that occurs in the human and animal kingdoms (Sipe & Cohen, 2000). The disease is characterized by systemic deposition, primarily in the spleen, liver and, to a lesser extent, in other organs, of extracellular fibrils composed of amyloid A protein. In most species, AA amyloidosis typically occurs secondary to chronic inflammation, infection or neoplasia. SAA protein is an acute phase apolipoprotein reactant primarily produced by hepatocytes under the control of interleukin-1, interleukin-6, and tumor necrosis factor-α (Betts et al., 1993; Hagihara et al., 2005). The plasma concentration of SAA is normally very low, but it can increase to > 1,000 mg/liter following an inflammatory stimulus. This protein can be proteolytically processed to produce an N-terminal cleavage product of approximately 44 to 100 residues that is deposited as amyloid in vital organs including the spleen, liver, and kidneys (Kisilevsky et al., 1994). AA amyloidosis occurs in patients with rheumatoid arthritis and other chronic inflammatory diseases. AA can also be induced experimentally in mice by injecting them with silver nitrate, casein, or lipopolysaccharide (LPS), which greatly increases the concentration of circulating SAA (Hoffman & Benditt, 1982). Intriguing recent data have led to the suggestion that AA amyloidosis might also be transmitted by a prion-like infectious process that involves a seeding-nucleation mechanism, a model that is widely accepted (Lundmark, et al., 2002, 2005; Cui et al., 2002). The lag phase of AA amyloidogenesis can be dramatically shortened by co-injection of "amyloid enhancing factor (AEF)" with an acute inflammatory stimulus. There is evidence that AEF is actually AA fibrils, and that AA amyloidosis might be transmitted by a prion-like mechanism.

3.1 Transmission of AA amyloidosis in cheetah and cattle

The cheetah species (*Acinonyx jubatus*) is in danger of extinction and is included on The World Conservation Union list of vulnerable species. Although efforts have been made in wildlife sanctuary parks and zoos worldwide to prevent extinction, a steady increase in the size of the cheetah population is hampered by the high prevalence of systemic AA amyloidosis, which is regarded as an increasingly important cause of morbidity and mortality in captive cheetahs (Papendick et al 1997). Inflammatory diseases, especially chronic lymphoplasmacytic gastritis, were found in 100% of cheetahs with AA amyloidosis, and environmental epidemiological studies indicate that breeding conditions have a prominent effect on the incidence of AA amyloidosis. A high rearing density is always associated with early age of onset and with a high incidence and severity of AA amyloidosis. We hypothesize that the propagation of AA amyloidosis among captive cheetah populations may depend on a horizontal transmission pathway (B. Zhang et al., 2008).

Captive cheetahs with severe AA amyloidosis were studied. AA amyloid fibrils were isolated from several tissues and the biochemical and pathological future outcomes of the animals were recorded. In particular, we hypothesized that amyloid fibrils in feces, urine and saliva would be important for the conveyance of amyloid fibrils from the body into the environment and hence for horizontal transmission. We found that cheetah feces contained AA amyloid fibrils that were different from those in the liver, having a smaller molecular weight and a shorter and finer shape. However, we could not find amyloid fibrils in the urine, and unfortunately we could not collect saliva from cheetahs. We

compared the transmissibility of fecal and liver AA amyloid fibrils using the mouse experimental AA amyloidosis system. Fecal amyloid fibrils had significantly greater transmissibility (Fig. 3A). The infectious activity of fecal AA amyloid fibrils was reduced or abolished by the protein denaturants 6 M guanidine HCl and formic acid or by AA immunodepletion. With regard to the liver fraction, formic acid treatment caused a nearly complete loss of amyloid-inducing activity, whereas the guanidine-HCl-treated fraction retained high amyloid-inducing activity (Fig. 3B). Thus, we unexpectedly found that the amyloid fibril fraction from feces had smaller amyloid fibrils and higher sensitivity to denaturation treatment than the liver amyloid fibril fraction. In yeast prions, it also has been indicated that [PSI$^+$] with stronger infectivity typically have less stable fibrils *in vivo* than strains with weaker infectivity (Krishnan & Lindquist, 2005), and the prion strain with relatively smaller prion particles is always associated with greater fragility and increased sensitivity to denaturants (Tanaka et al., 2006). Thus, we propose that feces are a potential transmission vehicle that may accelerate AA amyloidosis in captive cheetah populations. These results provide a pathogenic mechanism for AA amyloidosis and suggest possible measures for rescuing cheetahs from extinction.

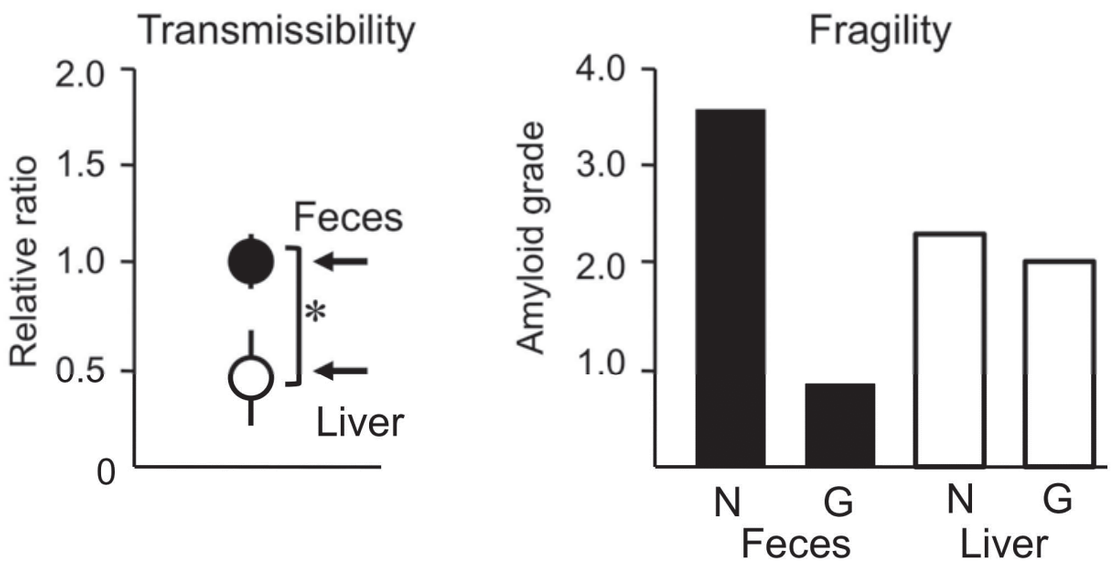

Fig. 3. **Transmissibility and fragility of fecal AA amyloid fibrils. A**: Quantification of transmissibility of AA amyloid fibrils from feces and liver. The degree of AA deposition in AA-induced mice was determined by isolation of AA amyloid fibril fractions from the spleens of mice in each group (filled circle is fecal; open circle is liver) followed by Western blot analysis and quantification using an image analyzer. The means and SE were determined by the relative ratios of AA amyloid protein levels versus the group receiving 10 μg of amyloid fibrils fraction from the feces (*, $P < 0.05$). **B**: Fecal and liver AA amyloid fibril fractions were untreated (N), or treated with guanidine-hydrochloride (G) or formic acid (F) and injected into mice to induce AA amyloidosis. Equal quantities of amyloid fractions (100 μg) were used in each experiment. The degree of amyloidosis was determined by the amyloid deposition observed in Congo red-stained sections of the spleen.

4. Analysis of transmission in mouse models of another systemic amyloidosis

Patients on long-term hemodialysis can develop dialysis-related amyloidosis (DRA) due to deposition of ß$_2$-microglobulin (ß$_2$m) into amyloid fibrils (Aß$_2$M) (Gejyo et al., 1985). Despite intensive biochemical studies, the pathogenesis of amyloid deposition in DRA patients remains poorly understood. Intact wild type ß$_2$m is unable to form amyloid fibrils under physiological conditions in test tubes. However, the addition of Aß$_2$M amyloid fibrils induces fibril formation following a nucleation dependent polymerisation model (Yamaguchi et al., 2001; Xue et al., 2008). To elucidate the mechanisms that underlie Aß$_2$M fibril formation in DRA, transgenic mice were generated that overexpress human ß$_2$m protein on a mouse ß2m gene knockout background (hB2MTg$^{+/+}$mB2m $^{-/-}$), and the possibility of transmission was examined using these model mice (P. Zhang et al., 2009).

Families with a variant transthyretins (TTR V30M)-associated familial amyloidotic polyneuropathy (FAP) exhibit genetic anticipation, with TTR V30M-amyloid depositing noted at an earlier age in successive generations (Yamamoto et al., 1998). The molecular basis of anticipation in FAP remains to be determined. The possibility that ATTR amyloid fibrils might be excreted in the milk of the FAP patients was suggested (Tokuda et al., 2007). We asked if administration of TTR-amyloid fibrils (ATTR) extracted from the heart of an FAP TTR V30M patient would accelerate ATTR deposition in transgenic mice expressing the human mutant TTR gene responsible for FAP TTR V30M (Wei et al., 2004).

4.1 Analysis of the transmission of Aß$_2$M amyloidosis in human ß$_2$m transgenic mice

Transgenic mice that overexpress human ß2m protein were generated on a mouse B2m gene knockout background (hB2MTg$^{+/+}$, mB2m$^{-/-}$). First, the pCAGGS-hB2M vector was created, and a human ß2m gene (hB2M) cDNA fragment was isolated by reverse transcription-PCR (RT-PCR) of messenger RNA extracted from human liver and cloned into pCAGGS. The hB2M cDNA was expressed under the control of the cytomegalovirus immediate early gene enhancer (CMV-IE)/chicken ß-actin promoter and rabbit ß-globin poly(A) signal (Fig. 4). The hB2M transgene copy number was determined by real-time PCR and calculated as 10 for homozygous hB2MTg$^{+/+}$. To exclude possible effects of endogenous mouse ß2m, transgenic mice were crossed with mouse ß2m (mB2m) knockout mice. The hB2MTg$^{+/+}$, mB2m$^{-/-}$ mice express a high level of human ß2m protein in many tissues and also have a high plasma ß2m concentration (192.8 mg/L). This concentration is >100 times higher than that observed in healthy humans and >4 times higher than that detected in patients on dialysis. These mice were checked for spontaneous amyloidosis, but amyloid deposition of ß2m protein was not observed in aged (~2 years) mice. Next we attempted to accelerate amyloidosis by injecting human Aß2M amyloid fibrils isolated from the amyloid-laden ligaments of the patient, and artificial amyloid fibrils were produced from recombinant human ß2m protein. Amyloid deposition of ß2m protein was not observed in animals injected with amyloid fibrils. However, mouse senile AApoAII amyloidosis was detected, particularly in the joints of mice that were injected with amyloid fibrils.

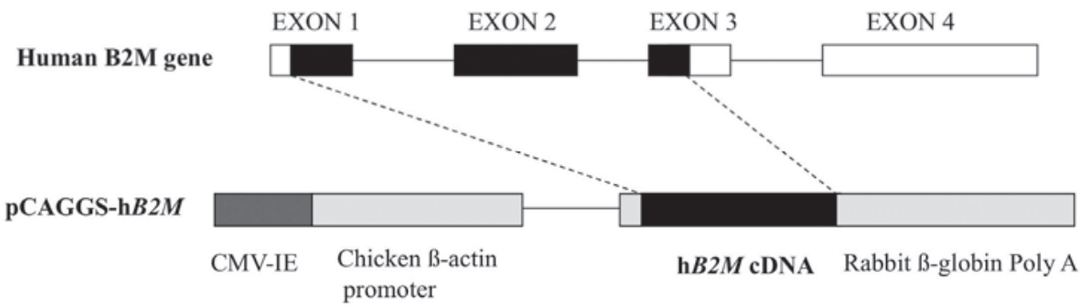

Fig. 4. **Establishment of hB2M transgenic mice.** The pCAGGS-hB2M targeting vector containing the human ß$_2$m cDNA inserted between the CMV-IE enhancer/chicken ß-actin promoter and the rabbit ß-globin poly(A) site.

This study demonstrates that this mouse model could be valuable in studying the components and conditions that promote DRA, and the results indicate that neither high plasma concentrations of hß$_2$m nor seeding with pre-existing amyloid fibrils may be sufficient to induce Aß$_2$M. We attempted to enhance amyloidosis by inducing arthritis through injection of the following substances: a monoclonal antibody against type II collagens, LPS (which induces inflammatory reactions), type I collagen and heparin solution, all of which are known to induce amyloid fibril formation in the test tube (Relini et al., 2006; Bellotti & Chiti, 2008). However, we have not been able to induce Aß$_2$M amyloid deposition in mice.

4.2 Analysis of the transmission of TTR amyloidosis in human variant TTR transgenic mice

Transgenic mice producing human variant TTR due to a mutant TTR (V30M) gene with its endogenous 6.0 kb upstream region were generated by Maeda et al (Kohno et al., 1997). The variant TTR transgene copy number was determined to be ~ 60 for homozygous h*TTR-V30M*Tg$^{+/+}$. To exclude possible effects of endogenous mouse *Ttr*, transgenic mice were crossed with *Ttr* knockout mice. The *Ttr*$^{-/-}$, h*TTR-V30M*Tg$^{+/?}$ mice expressing a high level of human variant TTR protein, which was produced mainly in the liver, were used (Fig. 5).

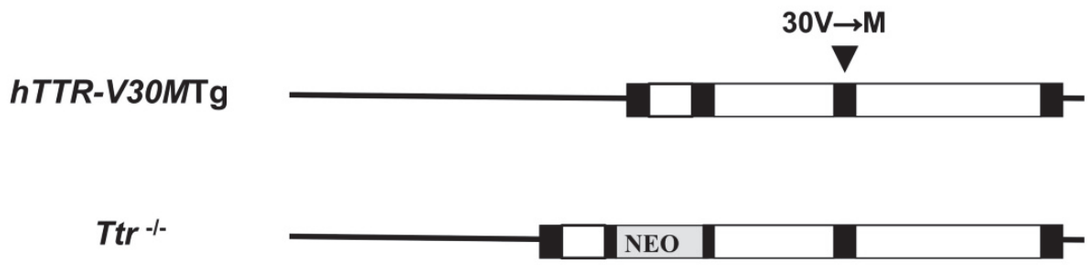

Fig. 5. **Establishment of Ttr$^{-/-}$, hTTR-V30MTg$^{+/?}$ mice.** Variant human *Ttr* transgenic mice were crossed with mouse *Ttr* knockout mice.

We asked if administration of TTR-amyloid fibrils (ATTR) extracted from the heart of an FAP TTR V30M patient would accelerate ATTR deposition in these transgenic mice. Although the administration of amyloid fibrils did accelerate deposition of AApoAII fibrils

in several organs including intestine, esophagus, heart kidney, liver and so on by a cross-seeding effect, deposition of ATTR was not observed. Thus, these experiments present, for the first time, evidence that the degree of ATTR inducibility is low relative to that of AApoAII. This leads us to suggest that administration of ATTR may not explain the genetic anticipation that occurs in FAP.

5. Discussion and conclusion

The amyloidogenic SAMP1, congenic R1.P1-*ApoA2*^{c} and transgenic m*ApoA2*^{c}Tg strains did not experience spontaneous amyloid deposition when they were reared in specific pathogen free (SPF) and amyloidosis-free conditions. This finding suggested that environmental conditions affect amyloidosis and that pre-existing amyloid fibrils make a significant contribution to the transmission or induction of AApoAII amyloidosis *in vivo*. (Higuchi et al., unpublished data). Moreover, cross-seeding between heterogeneous amyloid fibrils or fibril-like structures and amyloidogenic proteins has been reported *in vitro* and *in vivo* in both mouse AApoAII and AA amyloidosis (Fu et al., 2004; P. Westermark et al., 2009). Thus, the possibility of acceleration and induction of amyloidosis in animals and humans by heterogeneous amyloid fibrils in foods or the environment should be considered (Fig.6).

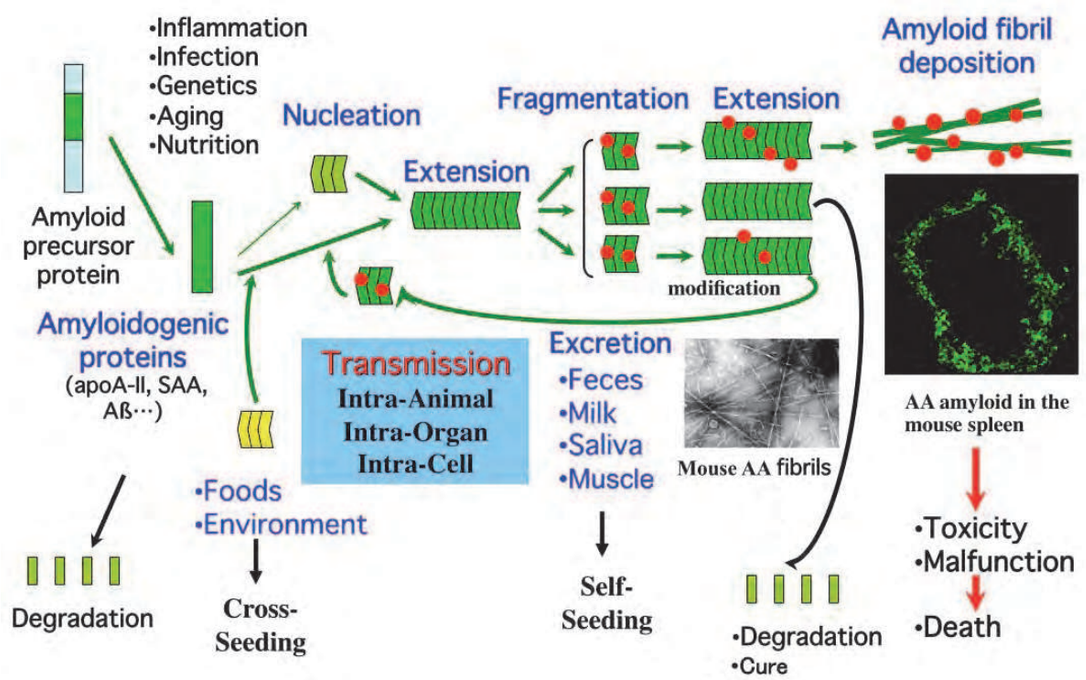

Fig. 6. **Transmission mechanism in systemic AApoAII and AA amyloidosis**. HDL-associated ApoA-II and SAA circulate in the blood. Spontaneous formation of the nucleus is slow and rare. Homologous or heterologous amyloid fibrils act as seeds and facilitate the formation of amyloid fibrils (self and cross-seeding). Extended amyloid fibrils were fragmented to make new seeds. ●: Fibril associated molecules that stabilize or modify the amyloid fibril structure. Amyloid fibrils are excreted from the body into feces, milk, saliva and muscle, and other animals may then take these into their bodies. Transmission should be observed at 3 levels; intra-animal, intra-organ and intra cells.

Recently, researchers have recognized that misfolded proteins play important roles in many neuro- degenerative diseases, including Alzheimer's disease (Eisele et al., 2010), Parkinson's disease (Hansen et al., 2011), Huntington's disease (Ren et al., 2009), tauopathies (Clavaguera et al 2009) and so on. These disorders resemble classic prion diseases, as the disease can be spread by imparting the pathological structure of the proteins to new and normal cellular counterparts, and these seeds can recruit endogenous proteins (Angot et al., 2010). Although none of these mis-folded proteins behave like typical infectious agents, the possible exception is systemic AApoAII and AA amyloidosis (Walker et al., 2006; G.T. Westermark & P. Westermark, 2010). Inter-individual infectivity of these two amyloidoses has been revealed experimentally and clinical outcomes are similar to those of the prion diseases. However, a paucity of epidemiological evidence argues against the role that transmission of amyloidosis may play in the human amyloidosis. How do the pathologic agents (amyloid fibrils) penetrate the recipient body? How do they spread throughout the body? Our studies have revealed that feces, milk, saliva and muscle are possible transporters through nasal and gastric pathways for inter-animal transmission. In particular, the amyloid fibrils in feces are noteworthy, since these fibrils showed higher fragility and transmissibility. Unstable species of infections prion (PrP^{Sc}) and yeast prion (Sup35) fibrils, which readily break and generate further free ends that can then act as seeds, have been reported to be important for transmission (Tanaka et al., 2006; Wille et al., 2009).

Analysis of amyloid fibril formation of $ß_2$m protein in test tubes revealed clearly the acceleration of fibril extension in the presence of pre-existing amyloid fibrils. However, injection of A$ß_2$M fibrils did not induce amyloidosis in transgenic mice having a high blood concentration of human $ß_2$m. Thus, transmission seems to not contribute to the development of systemic A$ß_2$M amyloidosis associated with long-term dialysis. However, the connections between *in vitro* studies performed in extreme and simplified conditions and *in vivo* observations in more complex organisms are important, and we need to improve and extend future experiments using model systems in order to understand the pathogenesis of human diseases.

The concept of transmission via self-propagating structures of proteins or by seeding is unique, and it is important to understand the pathogenesis of protein mis-folding diseases. The animal models of systemic amyloidosis described here should prove valuable in further studies of the pathogenesis, genetics, therapeutics and transmission of amyloidosis.

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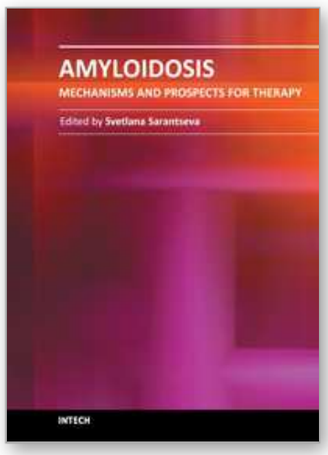

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Amyloidoses are a heterogeneous group of diverse etiology diseases. They are characterized by an endogenous production of abnormal proteins called amyloid proteins, which are not hydrosoluble, form depots in various organs and tissue of animals and humans and cause dysfunctions. Despite many decades of research, the origin of the pathogenesis and the molecular determinants involved in amyloid diseases has remained elusive. At present, there is not an effective treatment to prevent protein misfolding in these amyloid diseases. The aim of this book is to present an overview of different aspects of amyloidoses from basic mechanisms and diagnosis to latest advancements in treatment.

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