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Original Article

Spontaneous amyloidosis in senile NSY mice

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Senile Nagoya, Shibata, Yasuda (NSY) mice developed amyloidosis and died from renal failure as a result of amyloidosis. NSY mice were first reported as experimental congenital diabetic mice by Shibata *et al.* in 1980. This study questioned whether NSY mice died from diabetic nephropathy. The authors of the present study investigated the life span and cause of death in these mice. The life span of NSY mice was found to be 618.7 ± 72.5 days. NSY mice that lived for more than 400 days showed rising blood urea nitrogen and large amounts of amyloid deposits in the glomerulus of the kidneys. NSY mice died of renal amyloidosis. Immunological methods revealed that AApoAII was evident in the amyloid deposits of NSY mice. Apart from the kidneys, amyloid deposition was also found in the tongue, esophagus, stomach, small intestine, large intestine, rectum, lung, heart and adrenal glands. Amyloid deposits were found to a slight degree in the liver and the spleen. The most dominant amyloid deposition in NSY mice was seen in the glomerulus of the kidneys. From the point of view of amyloid depositional distribution, NSY mice were unique compared with other spontaneous amyloid mice.

Key words: AApoAII, amyloidosis, NSY mice, renal amyloidosis

Nagoya, Shibata, Yasuda (NSY) mice were reported as new experimental diabetic mice by Shibata *et al.* in 1980 (Fig. 1).[1–4] Streptozotocin (80 mg/kg body weight) was injected into the adult ICR mice and these mice were mated to obtain F_1 mice. The brother and sister of the same generation were then mated. Glucose tolerance after the F_6 generation was impaired. There is a relationship between abnormal glucose tolerance and the body weight of NSY mice. The NSY mice became widely used as models of type 2 diabetes mellitus. The F_{38} generation is now being used.

It was questioned whether NSY mice would die of diabetic nephropathy or not. The purpose of this study was to investigate the cause of death and the life span of NSY mice. The study showed spontaneous age-associated systemic amyloidosis in senile NSY mice and also that mice died of renal amyloidosis.

MATERIALS AND METHODS

Animals

Fifty-four males and 15 female NSY mice (F_{29}–F_{33}) were used. The mice had been raised under conventional conditions at 22 ± 2°C. The mice were maintained on a commercial diet (Funahashi Farm, Chiba, Japan) and tap water *ad libitum*. Nine mice were dissected under ether anesthesia before day 400. After 400 days, 60 mice were observed until their death. Among the 60 mice, 45 who had lost body weight and were exhausted physically were dissected. For measurement of blood urea nitrogen (BUN), blood was drawn from the vena cava inferior in the 45 mice. The other 15 mice died spontaneously and were dissected within 12 h of death. As BUN controls, 12 NSY mice that were 6 months of age were used. Blood urea nitrogen was measured by the Ektakem Analyzer (Kodak, USA).

Histological studies

The tongue, rectum, heart, lung, liver, spleen and kidney were removed from all mice. The presence of amyloid in the brain, esophagus, stomach, small and large intestines, pancreas, adrenal glands and skin was examined in 10 mice.

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Figure 1 Overall appearance of a 3 month old male NSY mouse.

Light microscopy

All tissues were fixed in a Bouin's solution, embedded in paraffin and stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), alkaline Congo red method and Thioflavin T stain. Tissues were cut into 2 μm sections for HE and PAS, and 6 μm for Congo red and Thioflavin T stain. Amyloid was identified in specimens stained with alkaline Congo red from the green birefringence under a polarizing microscope.

Electron microscopy

Only the kidney tissues were examined using an electron microscope. After 5% glutaraldehyde fixation, tissue blocks were post-fixed in 2% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Epok-812 (Oken, Tokyo, Japan). The blocks were cut using a diamond knife, stained by the Reynold's method, and observed using a Hitachi electron microscope (HU-12A).

Immunohistological studies

The kidney, tongue, small intestine and adrenal gland from five mice were examined immunohistologically with anti-AApoAII and anti-AA antiserum. Immunohistological studies used the avidin-biotinylated horseradish peroxidase complex (ABC) method. Anti-AApoAII and anti-AA antiserum were obtained by the method described previously.[5]

Glucose tolerance

Glucose tolerance was measured in 29 of the 69 mice at 4–5 months of age. To test glucose tolerance, glucose in a physiological saline solution was administered intraperitoneally at a dose of 2 g/kg body weight after overnight fasting. Blood was collected from the tail vein before the administration of glucose and then 60 and 120 min afterwards. Glucose in the blood was measured using the Ektakem Analyzer. It was thought that the mice had an abnormal glucose tolerance when blood glucose at 1 h after glucose injection increased to more than 250 mg/dL.

Statistical analysis

All data are presented as mean ± s.d. Blood urea nitrogen differences between the spontaneous death NSY mice and young NSY control mice were evaluated by an unpaired Student's *t*-test.

RESULTS

Life span of NSY mice

NSY mice had a normal appearance and good development during the first year. Some mice gradually showed weakness and died from the second year. Preceding death, the mice showed a drastic decrease in activity, weight loss, skin coarseness and increased lordokyphosis of the spine. The life span of the 60 NSY mice was 618.7 ± 72.5 days.

Histological findings

Most of the NSY mice who died after 400 days showed renal amyloidosis. The renal tissues of NSY mice who died spontaneously or who were dissected because of weakness exhibited glomerular lesions. Hyaline deposits in glomeruli showed pink to red in Congo red stain under an optical microscope and showed green birefringence under a polarizing microscope (Fig. 2a, b). With Thioflavin T stain, hyaline deposits showed a white fluorescent color under a fluorescence microscope. Amyloid deposition was therefore observed in the glomeruli of the kidneys of NSY mice. Slight amyloid deposition was first observed in the mesangial regions and capillary walls, followed by the nodular deposits. The other common finding in NSY mice was cell infiltration around the large arteries of the kidneys. A hundred glomeruli of the kidney were examined and renal amyloidosis was graded as '– (nil) to 3+' severity in terms of glomerular involvement. No amyloid was found in glomeruli with grade (–) lesions. Amyloid deposits were found in some glomeruli with grade (+) lesions. Amyloid deposition was present in all glomeruli with grade (2+) lesions. All the glomeruli were completely replaced with amyloid deposits and Bowman's capsules were enlarged in glomeruli with grade (3+) lesions (Fig. 3a–d). The general appearance of the kidneys during the advanced stages was pale and somewhat deformed. In advanced cases, the tubules underwent necrosis and most

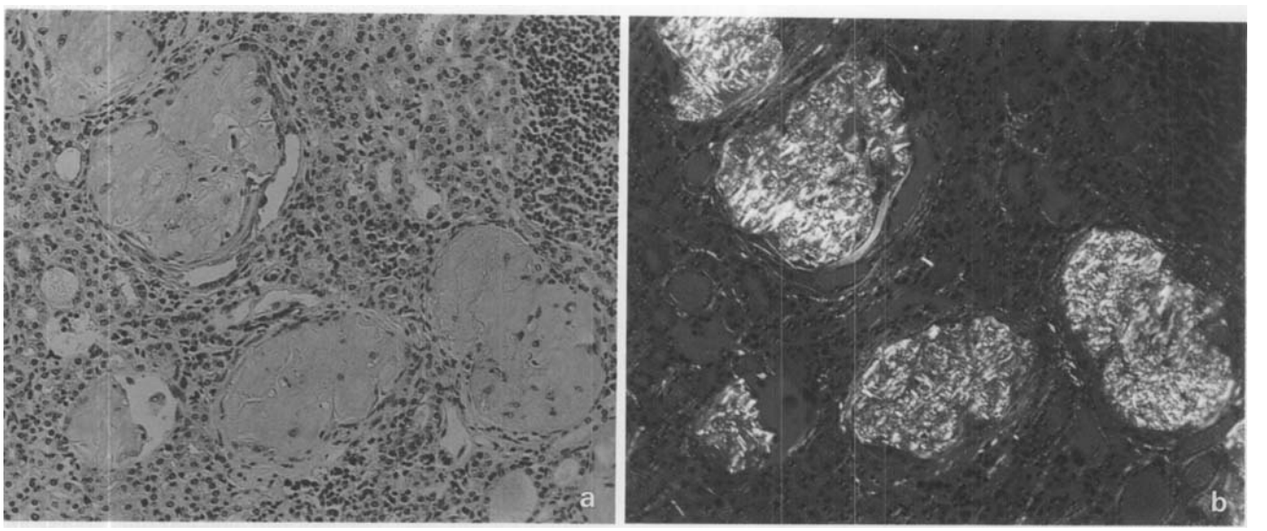

Figure 2 (**a**, **b**) Nearly all glomeruli have been totally replaced by amyloid. Green birefringence in a specimen stained with Congo red in a 539 day old male NSY mouse.

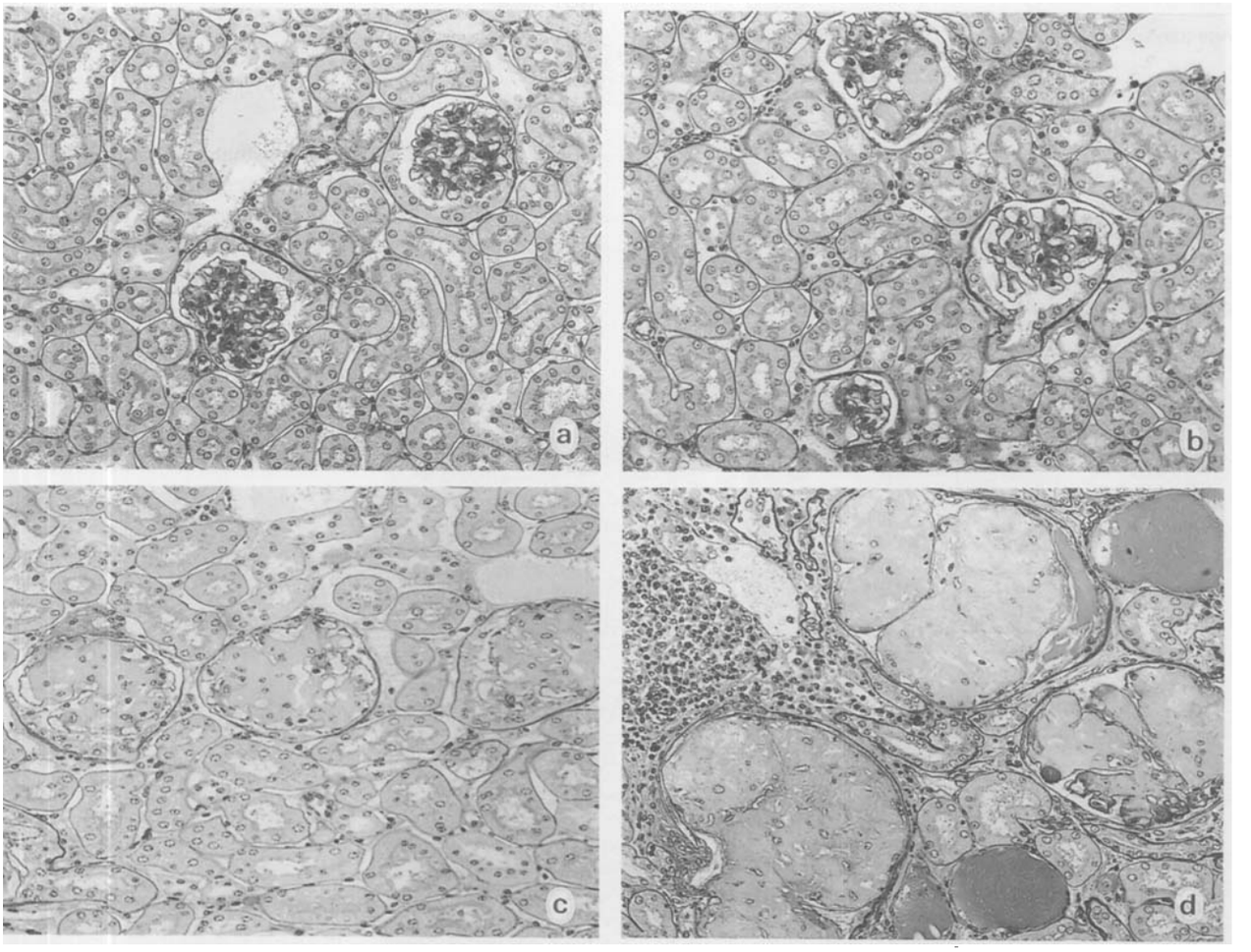

Figure 3 Different degrees of amyloid deposition in glomeruli. (**a**) No amyloid deposits were found. (**b**) Amyloid deposits were present in few glomeruli. (**c**) Amyloid deposits were observed in every glomerulus. (**d**) Large amounts of amyloid deposits were present in all glomeruli with an enlargement of the Bowman's capsules (PAS stain).

of the tubules were filled with colloid casts. The mice who lived for a long time (> 600 days), showed severe renal amyloid lesions. Two of the nine mice killed before 400 days showed only a slight amount of renal amyloid, while the other seven mice showed no amyloid (Table 1).

Ultrastructurally, massive amyloid fibrils were found to be deposited in almost all parts of the glomeruli (Fig. 4). Apart for the kidneys, amyloid deposits were found in the tongue, esophagus, stomach and small and large intestines and the rectum in approximately 90% of the mice that lived for more than 400 days. In the digestive organs, amyloid was discovered in the submucosal lesions (Fig. 5a, b). In the lungs, amyloid was deposited interstitially in the alveolar septa, gradually involving more septa (Fig. 6a, b). In the heart, amyloid was formed in the interstitial tissue of the heart muscles in the ventricles and auriculas. In the adrenal glands, amyloid was observed mainly in the zone fasciculata of the cortex. There was no infiltration in the medulla. In the liver, amyloid was located mainly in the surrounding portal venules. In the spleen, one mouse had patches of amyloid in the perifollicular legions but other mice had no amyloid lesions. The amyloidosis of the pancreas was often slight and limited to the walls of the small veins. No amyloid deposits were found in the islets of Langerhans. No amyloid lesions were observed in the brain.

Table 1 Degrees of amyloid deposition in glomeruli in different age groups of NSY mice

Age (days)	No. mice	Degree of amyloid deposition			
		−	+	++	+++
< 400	9	7	2	0	0
401–500	17	3	5	8	1
501–600	13	1	1	4	7
601–700	26	1	3	7	15
> 701	4	0	1	2	1

−, no amyloid deposits were found; +, amyloid deposits were present segmentally in few glomeruli; ++, amyloid deposits were observed in all glomeruli; +++, large amounts of amyloid deposits were present in all glomeruli with enlargement of Bowman's capsules.

Immunohistological studies

The kidney, tongue, small intestine and adrenal glands of NSY mice were examined immunohistologically. All tissues with amyloid deposits visible with Congo red staining were stained by the ABC method with anti-AApoAII antiserum. AA amyloid deposits were not recognized. Figure 7 shows the deposition of AApoAII in the glomeruli of 617 day old NSY mice.

BUN

Blood urea nitrogen in the mice that were dissected was 71.2 ± 41.2 mg/dL (n = 43). In the control 6 month old NSY mice, BUN was 23.7 ± 5.8 mg/dL (n = 12). There was a significant difference between both groups ($P < 0.001$). In the mice that had glomerular lesions with a grade of 3+, BUN was 84.1 ± 44.2 mg/dL (n = 16).

Figure 4 Electron microscope photograph of amyloid fibrils (arrow) in the mesangium in a 690 day old male NSY mouse (×10 000).

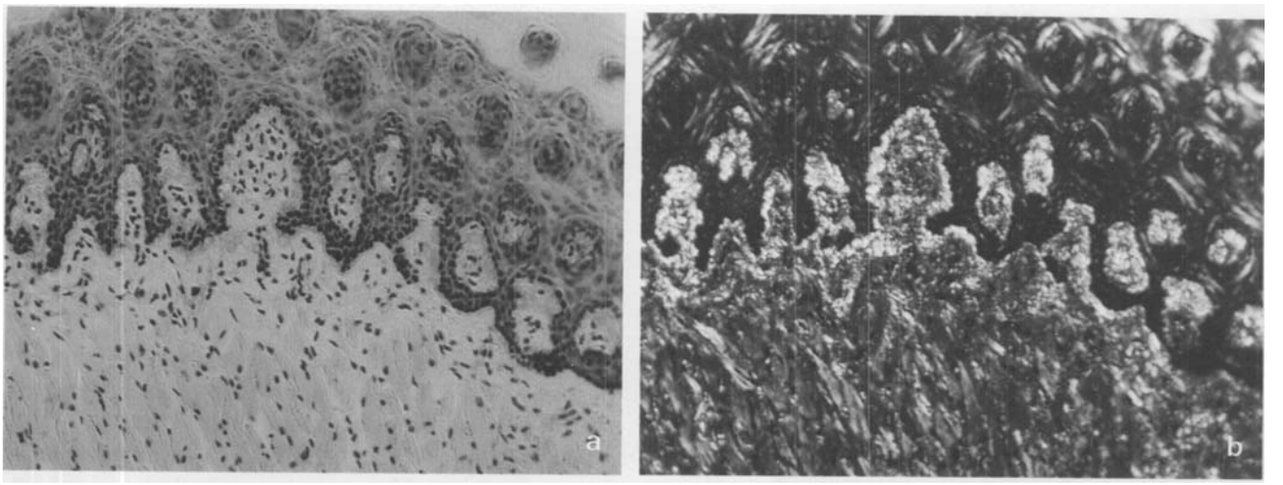

Figure 5 (**a**, **b**) Tongue of a 605 day old male NSY mouse. The amyloid appears in submucosal lesions. Congo red stain.

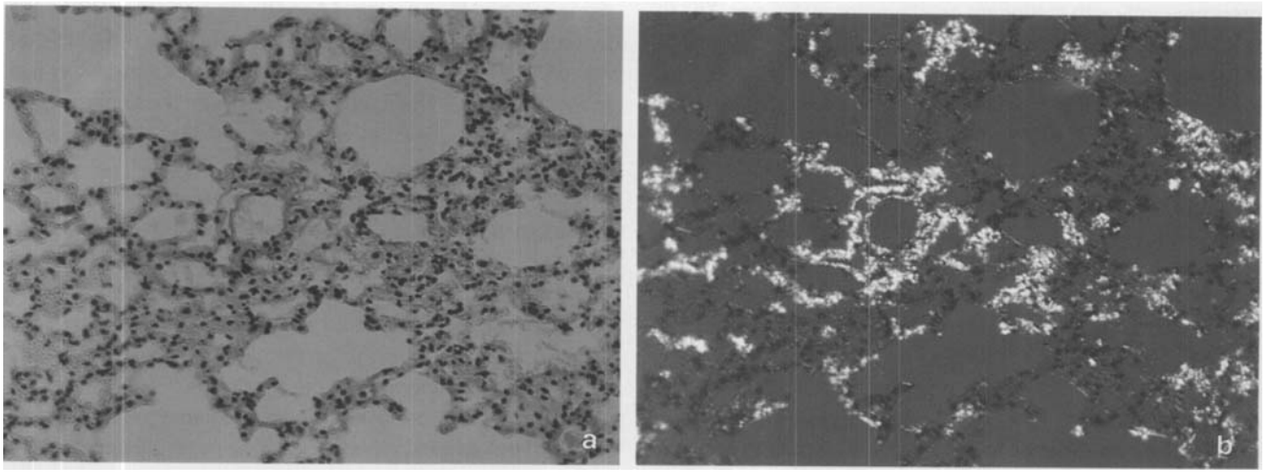

Figure 6 (**a**, **b**) Lung of a 539 day old male NSY mouse. Note the positive reaction in the alveolar septa. The amyloid appears to lie between septal cells and capillaries. (**a**) Ordinary light and (**b**) between crossed polars. Congo red stain.

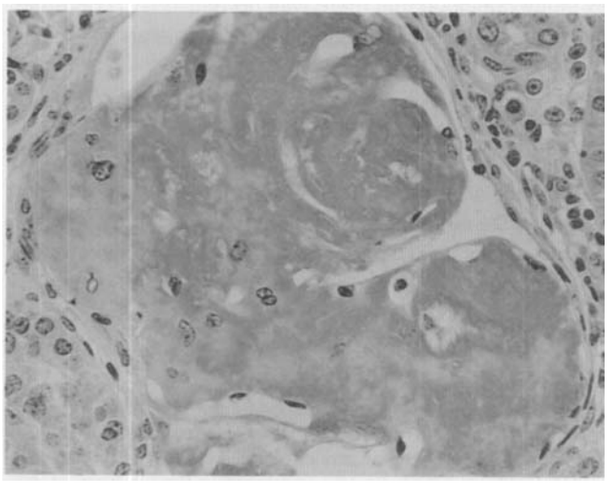

Figure 7 Deposition of AApoAII in glomeruli of 617 day old male NSY mouse (ABC method).

Glucose tolerance test in NSY mice

No relationship was found between amyloid deposits and glucose tolerance. The mice with normal glucose tolerance showed renal amyloid deposits (Table 2).

Table 2 Glucose tolerance test and renal amyloid deposition in NSY mice

Renal amyloid	No. mice	Blood glucose 60 min after injection of 2 g/kg glucose	
		< 250 mg/dL	> 250 mg/dL
–	8	4	4
+	21	7	14

–, renal amyloid deposition not present; +, renal amyloid deposition present.

DISCUSSION

Several authors have described many amyloid distribution patterns in the organs of spontaneous or experimental amyloidosis mice. In experimental amyloid mice induced with a single injection of Freund's complete adjuvant containing additional heat-killed *M. butyricum*, DeLellis *et al.* reported that amyloid appeared in the spleen, liver, intestines, lymph nodes, heart, tongue and subsequently decreased or disappeared.[6] Williams reported that amyloidosis was induced by serial casein injections in the spleen and kidney of rabbits and that lesser degrees of amyloid infiltration were completely removed by the reticulo-endothelial system in the spleen.[7]

In spontaneous amyloid mice, Zschiesche classified spontaneous amyloid disease in the inbred mice strains AB/Jena and A/J Han Jena according to two patterns of distribution and pathogenesis. Type 1 was classified as being associated predominantly with malignant or inflammatory lesions. These lesions preferably exhibited subintimal amyloid deposits in the small blood vessels of the spleen, liver and kidneys and, in addition, exhibited perfollicular splenic and glomerular renal amyloid localization. In Type 2, interstitial renal, sinusoidal liver and red pulp splenic amyloid deposits predominated and had no underlying disease.[8] In SJL/J mice that developed spontaneous reticulum cell tumors and serum M components, amyloid deposits were prominent in the perifollicular zones of the spleen and in the lobular areas of the liver.[9] In the LLC mice reported by Chai in 1976 which were characterized by reticular cell hyperplasia and a low leukocyte number, amyloid deposits were found in the spleen, liver, kidneys and adrenal glands.[10,11] In aging obese-hyperglycemic mice reported by Naeser *et al.* in 1977, large amounts of amyloid were usually seen in the adrenal glands, liver, spleen and kidneys.[12] In the senescence accelerated mouse (SAM) reported by Takeda *et al.* in 1981, a high incidence of amyloid deposits was observed in the liver, spleen, kidneys, gastrointestinal tract, lungs, heart, gonads, pancreas, salivary glands, adrenal glands, thyroid, skin epineurium and blood vessels, and the amyloid lesions were positive for AApoAII (originally called AS_{SAM}).[13,14]

Amyloid deposition in the liver, spleen and kidneys has been reported in almost all the mice with spontaneous or experimental amyloidosis.[6-12] The histological findings observed in NSY mice do not completely fit with the findings for the other mice. In NSY mice, amyloid deposits were dominant in the kidneys but were slight in the liver and spleen. Amyloid deposits were observed in the tongue, esophagus, stomach, small duodenum, rectum, heart, lungs and adrenal glands. The most dominant amyloid deposition in NSY mice was observed in the glomerulus of the kidneys.

In the kidneys, the area of the amyloid deposition differed between NSY mice and the other mice. Dunn reported amyloid deposits in the intertubular interstitial tissues of the papilla of kidneys in strain A mice.[15] In the kidneys of LLC mice, the amyloid deposits were located in the interstitial tissue space of both the cortex and the medulla.[10] West *et al.* reported that renal amyloid deposition was observed in the tips of papillae of A/Sn mice.[16] Renal amyloid deposition in SAM was frequent and prominent in the papillae and the interstitial tissues around the thin portions of Henle's loops.[17] The most striking amyloid deposits in NSY mice were found in the glomeruli and, to a slight extent, in other parts of the kidneys.

It is not clear why there is a difference in the amyloid deposition in the kidneys between the experimental mice and the other spontaneous mice.

A unique amyloid fibril AApoAII that was distinguishable from murine protein AA in secondary amyloidosis was isolated from the liver of SAMP-P_1 mice by Matsumura *et al.*[5,18] The same amyloid fibril protein AApoAII was evident in NSY mice by immunohistological methods. Higuchi *et al.* reported that AApoAII deposition was significantly age-dependent in five strains of mice.[19] The relationship between the findings from the kidneys and life span would suggest that amyloid deposition was related to aging of the mice.

NSY mice have an abnormal glucose tolerance that is related to body weight. Naeser reported the deposition of amyloid in the juxtamedullary cortical zone in the adrenal glands of aged and obese-hyperglycemic mice. At the same time, he emphasized that adrenal cortical enlargement may have contributed to the insulin resistance already at an early stage in the development of the syndrome.[20] NSY mice also showed amyloid deposition in the juxtamedullary cortical zones of the adrenal glands. However hyperglycemic states were observed in NSY mice when they were 4 months old. At 4 months, amyloid deposition was not found. The authors of this study conclude that there was no relationship between amyloid deposition and abnormal glucose tolerance.

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