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Systemic Amyloidosis in Transgenic Mice Carrying the Human Mutant Transthyretin (Met 30) Gene

Pathological and Immunohistochemical Similarity to Human Familial Amyloidotic Polyneuropathy, Type I

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Abstract

To analyze the pathologic processes of amyloid deposition in type I familial amyloidotic polyneuropathy (FAP), mice were made transgenic by introducing the human mutant transthyretin (TTR) gene(MT-hMet 30). An inbred strain of mouse, C57 BL/6, was chosen. Transgenic mice were killed using ether anesthesia at 3-mo intervals up to 24 mo after birth. In these transgenic mice, amyloid deposition started in the gastrointestinal tract, cardiovascular system, and kidneys and extended to various other organs and tissues with advancing age. The pattern of amyloid deposition was similar to that observed in human autopsy cases of FAP, except for its absence in the choroid plexus and in the peripheral and autonomic nervous systems.

We extracted the amyloid fibrils from kidneys of these mice with a human mutant TTR gene and analyzed them immunochemically and electronmicroscopically. Deposited amyloid was shown to be composed of human mutant TTR and mouse serum amyloid P component. Amyloid fibril from transgenic mice was morphologically and immunohistochemically similar to that of human FAP.

The most striking pathologic feature of the transgenic mice was the absence of amyloid deposition in the peripheral and autonomic nervous tissues. Thus, other intrinsic factors may be involved in amyloid deposition in the nervous tissues of human FAP.

Index Entries: Molecular genetics of dominant inheritance; familial amyloidotic polyneuropathy (FAP); amyloidosis in transgenic mice; human mutant transthyretin (prealbumin) gene; amyloid fibril protein.

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Introduction

Familial amyloidotic polyneuropathy (FAP) is a heredofamilial amyloidosis transmitted by an autosomal dominant trait occurring in middle age and is characterized by sensory-dominant polyneuropathy, autonomic dysfunction, sexual impotence, cardiac conduction disturbances, and emaciation. Since its first description by Andrade in 1952 in Portugal *(1)*, this disorder has been reported in Sweden, the United States, Finland, Japan, and other countries *(2–4)*.

According to differences in clinical manifestation, the previously reported cases are classified into four clinical types: type I (Andrade type), type II (Rukavina type), type III (van Allen type), and type IV (Meretoja type). Type 1 has been reported in Portugal, Sweden, Japan, and the United States. In Japan, large family pedigrees of this disease were reported in Kumamoto and Nagano prefectures *(2,5)*.

Clinical Features and Genetics of FAP

The clinical and genetic manifestations of Japanese FAP in the Kumamoto area of 50 individuals from eight pedigrees were reported *(6)*. The inheritance pattern is an autosomal dominant with a high penetration rate and an equal sex ratio. The symptoms are first recognized when the patient is 20–45 yr of age, with a mean age of onset at 33.2 yr. Evidence of a sensory dominant mixed type peripheral neuropathy is usually manifested in the lower limbs. Dissociation of sensory impairment is common, with pain and temperature sensation being the most severely affected. The upper limbs are also frequently involved but most often only a few years after the lower limbs are affected. Autonomic nervous system involvement, such as dyshidrosis, sexual impotence, disturbance of gastrointestinal motility (alternating diarrhea and constipation), orthostatic hypotension, and urinary incontinence are frequent. The disease is progressive and death follows after about 10–20 yr. Although the Japanese FAP syndrome has no apparent genetic link to the Portuguese disease, their clinical and pathological similarities are striking *(2)*.

Recent biochemical studies have elucidated that the amyloid precursor protein is a variant transthyretin (TTR: prealbumin) with a single amino acid substitution of valine by methionine at the 30th amino acid of normal TTR *(7)*. Besides this, about 40 other variant TTRs, each with a different single amino acid substitution, are known to be major components of amyloid fibrils in FAP and in senile cardiac amyloidosis *(8)*. Thus, the classification of this disorder is possible at the gene level.

Pathologic studies of autopsy cases of FAP were reported in Portugal, Sweden, Finland, and the United States, and showed generalized amyloid deposition in various organs and tissues, particularly in the peripheral nerve tissues, heart, and kidneys. Recently we reported the pathology of FAP type I in Kumamoto, Japan *(9)*.

Seventeen autopsy and five biopsy cases of familial amyloidotic polyneuropathy were examined clinicopathologically, histochemically, immunohistochemically, and ultrastructurally. In the autopsy cases, amyloid deposits were predominant in the peripheral nerve tissues, autonomic nervous system, choroid plexus, cardiovascular system, and kidneys. Amyloid involvement in the anterior and posterior roots of the spinal cord, spinal ganglia, thyroid, and gastrointestinal tract were also frequent. In the cardiac conduction system, amyloid deposition was prominent in the sinoatrial node and in limbs of the intraventricular bundle. In the sural nerve biopsy, besides amyloid deposits, degenerative changes of nerve fibers and Schwann cells were detected ultrastructurally, and the morphometric analysis showed a marked reduction in the number of myelinated fibers that correlated with the clinical stage.

Structure and Expression of the Mouse TTR Gene

Before introducing the human mutant TTR gene into mice, we cloned mouse TTR cDNA and genomic DNA and compared the structures with those of the human prealbumin cDNA and genomic DNA *(10,11)*. The primary structure of mouse TTR deduced from the cDNA sequence shows that the overall homology of the amino acid sequences of mouse and human TTRs is 80%. We found that the substituted amino acid residues are preferentially accumulated in the outer surface of the protein, and that there is no crossreaction of mouse TTR with rabbit and swine antihuman TTR antisera *(10)*. The mouse TTR gene is separated into four exons by three introns. The numbers, the sizes of the exons, and the relative sites of the exon–intron junctions are all in complete agreement with those deter-

mined for the human gene. In addition to the exon regions, we found two highly conserved DNA regions, one in the 5'-flanking region and the other in the 3'-end region of the first intron. These DNA regions contain several consensus glucocorticoid receptor-binding sequences, and the latter also contains an enhancer sequence present in the immunoglobulin kappa-chain joining-constant intron *(11)*. RNAs hybridizing to the mouse TTR cDNA were detected as early as the 10th d of gestation, and at that time were specifically localized in endodermal cells of the visceral yolk sac, tela choroidea (the predecessor of the choroid plexus), and hepatocytes *(12)*. In the adult mice, TTR mRNA was localized in the hepatocytes and choroid plexus epithelial cells. Since we found that the exon regions and also the 5'-flanking regions of the human and mouse TTR genes are highly conserved, regulation of the TTR gene expression during development in humans and mice is probably much the same.

Transgenic Mice

Aim

To elucidate the pathologic processes of amyloid deposition in FAP, we produced transgenic mice by the introduction of the human mutant TTR gene and found systemic amyloidosis in them *(13)*. This paper describes the patterns of amyloid deposition obtained from our histochemical, immunohistochemical, and ultrastructural studies and their similarity to human FAP *(14)*.

Materials and Methods

Production of Transgenic Mice

An inbred strain of mouse, C57BL6, was chosen for DNA microinjection to minimize the possible influence of variable genetic background on amyloid deposition. A chromosomal DNA segment covering the entire sequence for the mutant TTR gene associated with type I FAP was cloned as described previously *(15)*. The 7.8-kb pair *StuI EcoRI* fragment was constructed by ligating the promoter region of the mouse metallothionein-l (MT-I) gene to the entire structural gene of the human mutant TTR gene, which includes human promoter (MT-hMet30) *(13)*.

Approximately 200 copies of these constructs (MT-hMet30) were microinjected into fertilized eggs of C57BL/6 mice according to the method described elsewhere (Fig. 1) *(16)*. Four of 12 mice derived from eggs microinjected with the MT-hMet30 gene integrated it, as revealed by Southern blot analysis. These transgenic offspring were used in the following studies and were kept in plastic cages in our laboratory according to the guidelines of the Ministry of Education. They were bred by brother × sister mating.

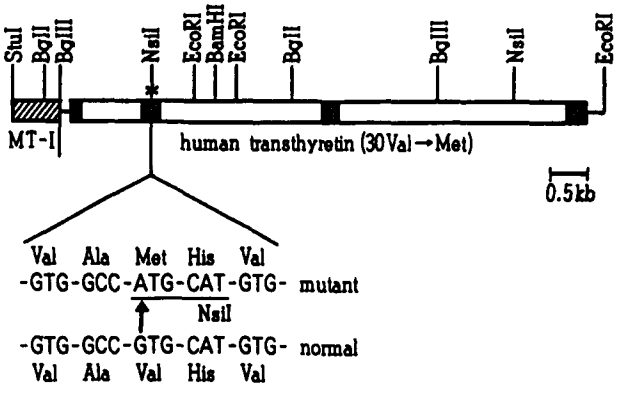

Fig. 1. Entire structural gene of the human mutant TTR gene (MT-h Met 30) used for microinjection.

DNA Isolation and Southern Blot Analysis

When the mice were 4 wk old, genomic DNAs were extracted from a piece of tail and used for Southern blot analysis to examine whether the MT-hMet30 gene had integrated into the mouse chromosome *(17)*.

Western Blot Analysis

To analyze the production of human TTR in the mice, blood was taken from each transgenic mouse before they were killed and the sera were analyzed by Western blot assay.

Preparation and Fixation of Tissues

Transgenic mice were killed using ether anesthesia at 3-mo intervals up to 24 mo after birth. At each time point, we examined two to six transgenic mice and excised the heart, kidneys, spleen, liver, lungs, pancreas, stomach, small and large intestines, urinary bladder, thryroid gland, lymph nodes, bone marrow, sciatic nerves, automonic nerves, and brain. For light microscopy, tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin sections were used for histochemistry. For immunohistochemistry, part of the tissues were fixed in periodate-lysine-paraformaldehyde (PLP) fixative for 4 h and cut by a cryostat (Bright, Huntingdon, UK). Small tissue blocks were obtained from the heart, kidneys, small intestine, and sciatic nerve, fixed in chilled 2.5% glutaralde-

hyde in 0.1 mol/L (molar) cacodylate buffer for 60 min, and submitted to electron microscopy.

Light Microscopy and Histochemistry

For light microscopy, paraffin sections were stained with hematoxylin and eosin. Semithin sections were cut from Epon-embedded blocks for electron microscopy and stained with 0.05% toluidine blue. For histochemical demonstration of amyloid, paraffin sections were stained by the Congo red method and some were treated with potassium permanganate ($KMnO_4$) before Congo red staining according to Wright's method *(18)*. To detect the emerald green birefringence emitted from amyloid deposits, the Congo red-stained paraffin sections were observed under a polarization microscope.

Immunohistochemistry

For immunohistochemical demonstration of the major components in amyloid deposits, formalin-fixed paraffin sections or PLP-fixed cryostat sections were immunostained by the avidin–biotin complex (ABC) method using polyclonal and monoclonal antibodies. The antibodies were antihuman TTR (Behringwerke, Marburg, Germany), antihuman serum amyloid A (SAA) (Dako, Santa Barbara, CA), antihuman serum amyloid P component (SAP) (Dako), antimouse SAA (supplied by Prof. S. Migita, Cancer Research Institute, Kanazawa University, Japan), and antimouse SAP (Behring Diagnostics, La Jolla, CA).

Electron Microscopy

After glutaraldehyde fixation, tissue blocks were postfixed in 2% osmium tetroxide for 60 min, dehydrated in a graded series of ethanols, and embedded in Epon812 (Oken, Tokyo, Japan). The blocks were cut by an ultratome Nova (LKB, Uppsala, Sweden), stained with uranyl acetate and lead citrate, and observed with an H-300 or 12-A electron microscope (Hitachi, Tokyo, Japan).

Results

General Appearance of Mice

Transgenic mice showed normal appearance and good development in all lines until age 12 mo. After age 15 mo, hair became coarse in two lines, but no muscular atrophy or gait disturbance was found. Southern blot analysis indicated that the copy numbers of the integrated MT-hMet30 genes varied from 2 to 30 per diploid genome. The concentrations of the human mutant TTR in the blood varied among the mice and ranged from 1 to 5 mg/dL, one tenth to one half of that in FAP patients. When the mice were killed, no inflammatory lesions, such as pulmonary abscesses or parasitic infestation, were found in any of the mice *(14)*.

Amyloid Deposition

Table 1 shows the occurrence and degree of amyloid deposition in various tissues of the transgenic mice. Amyloid deposition was first found in the small intestine, stomach, and renal glomeruli at 6 mo, and it occurred invariably in the gastrointestinal tract, kidneys, heart, and thyroid at 12 mo and thereafter.

In the gastrointestinal tract, amyloid deposits occurred predominantly in the mucosa of the small intestine, particularly the terminal ileum. Amyloid deposition was marked in the lamina propria of the intestinal villi and submucosal layer, and occurred later in or around the walls of small blood vessels. Electron microscopically, amyloid deposits consisted of clusters of amyloid fibrils approx 7–10 nm wide that varied in length, findings consistent with human FAP *(9)* or other types of amyloidosis *(19)*. In the initial stage, amyloid deposits were observed in the lamina propria at the top of the intestinal villi, particularly beneath the epithelial cells. They were also found in the basal area of the lamina propria, in the muscularis mucosae, and in the submucosa. In the submucosa, amyloid deposition occurred in the connective tissue and around small blood vessels. In the advanced stage, diffuse amyloid deposition was found in the lamina propria, muscularis mucosae, submucosa, and subserosa.

Amyloid deposits first occurred in the wall of the stomach in the lamina propria of the ridge and in the submucosa, particularly the nonglandular part. In the large intestine, amyloid was deposited in the submucosa of the cecum and anal ring.

In the kidneys, amyloid deposits occurred exclusively in the glomeruli at the initial stage, later involving the renal interstitium. At age 6 mo, slight amyloid deposits first were detected in the mesangial areas of about 10% of glomeruli. At 18 mo, most of the glomeruli were affected by increased amyloid deposition. After 21 mo, nearly 100% of the glomeruli were obliterated completely by massive amyloid deposits. Ultrastructurally amyloid fibrils were first detected in the matrix around the mesangial cells or beneath the endothelial cells of glomerular capillaries. With age, the amount of amyloid fibrils increased and clusters were observed in the lamina rara interna between the basal lamina and

Table 1
Tissue Distribution of Amyloid Deposits in Transgenic Mice Carrying the MT-hMet30 Gene and Autopsy Cases of FAP Determined Histochemically

Organs	Transgenic mice: age examined (mo)								Autopsy cases of FAP
	3	6	9	12	15	18	21	24	
Brain	–	–	–	–	–	–	–	–	–
Choroid plexus	–	–	–	–	–	–	–	–	++
Sciatic nerve	–	–	–	–	–	–	–	–	+++
Heart	–	–	–	+	++	+	++	+++	+++
Lung	–	–	–	–	–	–	±	±	±
Liver	–	–	–	–	–	–	±	±	±
Spleen	–	–	–	–	–	–	±	±	±
Kidney	–	+	–	+	+	++	+++	+++	+++
Pancreas	–	–	–	–	–	–	±	±	+
Thyroid gland	–	–	–	+	+	++	++	+++	+++
Stomach	–	+	–	++	++	++	++	+++	+
Intestine	–	+	–	++	++	+++	+++	+++	+
Lymph node	–	±	–	–	–	±	±	±	±

Amyloid deposits are absent, –; limited to the wall of small vessels, ±; observed in the wall of small vessels and their surrounding regions, +; moderate in the interstitium, ++; marked in the interstitium and parenchyma, +++.
FAP, familial amyloidotic polyneuropathy.

endothelial cells. At advanced stage, clusters of amyloid fibrils were deposited massively in almost all parts of the mesangial matrix of glomeruli; the mesangial cells were swollen and the glomerular epithelial cells showed fusion of their foot processes. In the renal interstitium, amyloid deposition was found in the cortex after age 21 mo but not in the medulla. Electron microscopically, amyloid deposition was observed to be particularly dense around the renal tubules.

In the cardiovascular system, amyloid deposits were marked. At age 12 mo, slight, patchy amyloid deposition occurred in the subendocardial layer and in the superficial myocardium. Ultrastructurally clusters of amyloid fibrils in the superficial myocardium were observed initially around small blood vessels. After 18 mo, amyloid deposits coalesced and became diffuse in the subendocardial layer and superficial myocardium (Fig. 2A,B). At 24 mo, the deposition extended to the deeper areas of the myocardium. At the electron microscopic level, myocardial cells showed atrophy and degenerative changes because of massive amyloid deposits.

In the vascular system, initial amyloid deposits were observed electron microscopically around blood capillaries or venules. Around the blood capillaries, deposits were observed beneath the basal lamina, extending to the perivascular region. Amyloid deposition in the arterial wall occurred mostly in the advanced stage, particularly in adipose tissue and gastrointestinal tract. Vascular amyloid deposition was also found in the salivary glands, testes, lungs, and liver at age 15 mo, and later in the pancreas, skeletal muscles, and splenic trabecules.

In the thyroid gland, amyloid deposits occurred around the interfollicular blood capillaries at 12 mo, increased with age, and became prominent at 24 mo. In the advanced stage, the thyroid follicles were compressed by marked interstitial amyloid deposition (Fig. 2C,D).

No amyloid deposition was detected in any transgenic mouse, in the brain, choroid plexus, peripheral nerves, or in the hematopoietic tissues such as the bone marrow, spleen, liver, or lymph nodes. In the nontransgenic mice, no amyloid deposition was detected in any tissue examined up to age 24 mo.

Histochemical and Immunohistochemical Features of Amyloid Deposits and Localization of Antihuman TTR in the Transgenic Mice

Amyloid deposits in the transgenic mice were stained with Congo red, showed resistance to treatment $KMnO_4$ by Wright's method, and emitted an

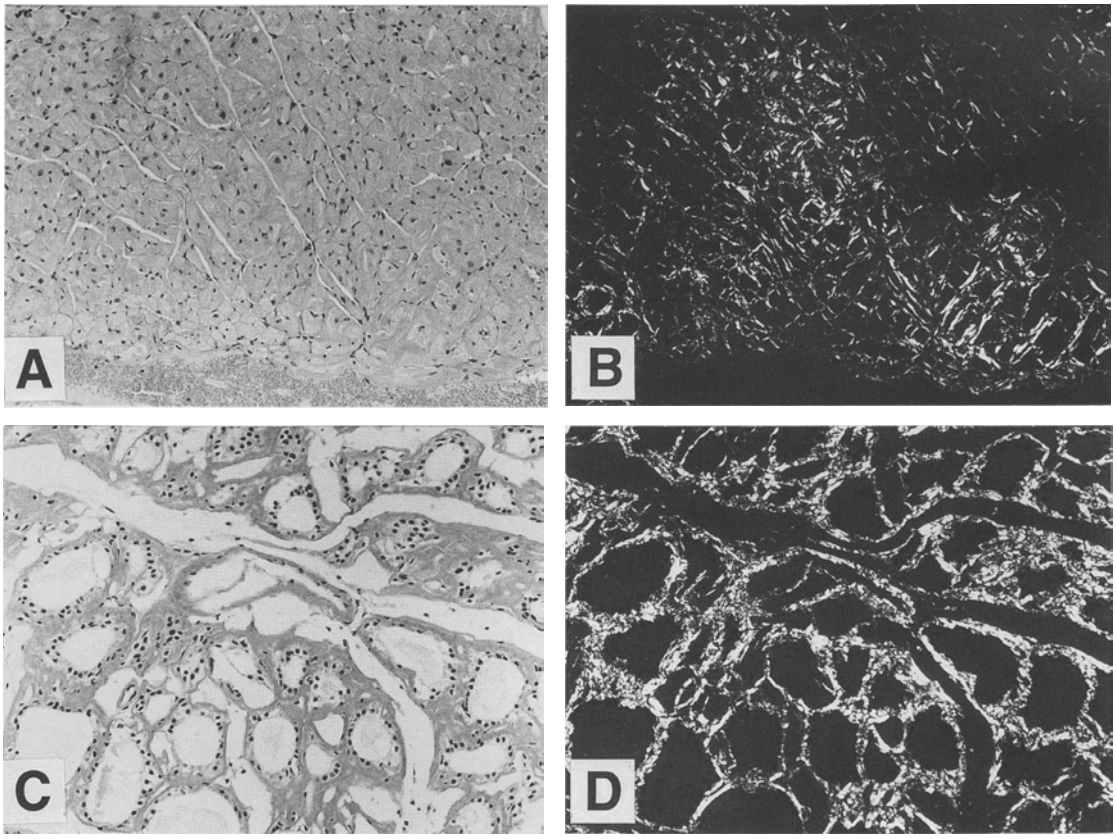

Fig. 2. Light microscopic changes of principal organs in the transgenic mouse carrying the MT-h Met 30 gene. **(A, B):** Amyloid deposits of the heart at 18 mo of age. Amyloid deposits were marked around the myocard fibers (×30). **(C, D):** Amyloid deposits of the thyroid gland at 24 mo of age. Amyloid deposits were marked in the interstitium (×25). A and C, Congo red and hematoxylin; B and D, identical area viewed under polarizing light.

emerald-green birefringence under polarized light. By the ABC method, the amyloid deposits reacted with antihuman TTR and antimouse SAP antisera, but not with antimouse SAA, antihuman AA, or antihuman SAP antisera.

In the transgenic mice, liver cells, ductal epithelia of the salivary glands, pancreatic exocrine cells, epithelial cells of renal tubules at the proximal convolution, myocardial cells, and part of the skeletal muscle cells showed immunoreactivity with antihuman TTR, but the epithelial cells of the choroid plexus were negative. The nontransgenic C57BL/6 mice demonstrated no immunoreactivity with antihuman TTR in any kind of cell.

We extracted amyloid fibrils (AF) from kidneys of transgenic mice with the human variant TTR gene and analyzed it by immunochemical and morphological methods *(20)*. The soluble substance, isolated from the kidneys of transgenic mice, was similar to that seen in patients with FAP under electron microscopy. On immunoblotting, the AF protein reacted with antihuman TTR and antimouse SAP, whereas it did not react with antimouse SAA. This result indicated that the AF of transgenic mouse kidney was immunochemically and morphologically similar to that of FAP.

Discussion

In this paper we demonstrated clearly that hMet30 can be deposited as amyloid fibrils in transgenic mice carrying the human mutant TTR gene (MT-hMet30). Amyloid deposition occurred predominantly in the intestinal mucosa, renal glomeruli, myocardium, small vascular walls, and thyroid. With age, amyloid deposition became marked and was found in various other organs and tissues, except nervous tissues. The possibility of the development of age-associated amyloid deposition in

transgenic mice can be ruled out for the reasons described below.

Senile amyloidosis is known to occur spontaneously in various strains of mice, including C57BL mice, and to involve the spleen, liver, heart, kidneys, or gastrointestinal tract *(21)*. In senile amyloidosis, renal amyloid deposition occurs mainly in the renal papilla or interstitium, and glomerular involvement is slight or absent. In senile amyloidosis of C57BL mice, amyloid deposition was reported to occur in the renal papilla in 50% of the animals at age 18 mo and to involve the spleen, particularly around the white splenic pulp; however glomerular amyloid involvement is slight *(22)*. By contrast, in the transgenic mice amyloid deposition was prominent in the renal glomeruli but slight or absent in the spleen or liver. Compared to the development of spontaneous senile amyloidosis in mice *(23)*, the amyloid deposition occurs at least 6 mo earlier in transgenic mice. Another definitive difference involves amyloid precursor protein. The amyloid precursor protein in human senile amyloidosis is TTR *(24,25)*; its nature has not been determined in mice. Non-AA or apoprotein A II (Pro5-Gln) *(26)* have been mentioned. In transgenic mice, however, we demonstrated clearly that the amyloid precursor protein is human variant TTR. In the following section, four subjects will be discussed in relation to the pathologic similarity and dissimilarity between the transgenic mice and human FAP autopsy cases.

First the pathologic changes of the transgenic mice correlate well with those reported in previous pathologic studies of FAP *(27)*. The major sites and pattern of amyloid deposition in the transgenic mice are similar to these human autopsy cases, except for the peripheral and autonomic nervous tissues. In the mice, cardiac amyloid deposition initially occurs in the subendocardial layer and in the superficial areas of myocardium, and then it extends deeper into the cardiac wall. This pattern is similar to that in the heart of human cases confirmed by our pathologic study. In the kidneys of transgenic mice, amyloid deposition first occurred in the mesangial areas of the glomeruli. The number of involved glomeruli and the grade of glomerular involvement increased with age and amyloid deposition was found around the renal tubules in advanced age. These findings are also almost consistent with those reported in autopsy cases of FAP type I. Amyloid deposition in the thyroid gland of transgenic mice is prominent and its pattern closely resembles that of human cases of FAP *(9)*.

On the other hand, in the gastrointestinal tract of transgenic mice, amyloid deposition is more prominent than in the human autopsy cases of FAP and occurs predominantly in the mucosa of the terminal ileum, in the gastric submucosa at the ridge of the nonglandular part, and around the anal ring. In the mouse, the stomach is divided into two parts, the left nonglandular side and the right glandular side, by a U-shaped ridge. This ridge is formed by a thickened lamina propria. Unexpectedly, amyloid deposition is more prominent on the nonglandular side, including the ridge, than on the glandular side. A similar pattern of amyloid deposition was observed around the anal ring. It was more prominent under the squamous epithelium than the glandular epithelium. It is interesting to note that such a peculiar pattern of amyloid involvement is also found in spontaneous senile amyloidosis of mice *(23)*. These results suggest that the difference of amyloid deposition in the alimentary tract between transgenic mice and FAP patients is caused by the difference in anatomic structure and that microenvironment, including the fine anatomic structure or local blood flow, are involved in the amyloid deposition.

One important question is how the amyloid deposition initiates and proceeds in the early stages of FAP. So far there is no clear data on this subject, partly because detailed pathologic tissue analysis is possible only at autopsy. By that time a large amount of amyloid usually has accumulated in many tissues, as discussed earlier. The main target tissues for amyloid deposition in FAP patients are not liver or choroid plexus where the hMet30 gene is expressed. This suggests that hMet30 is transported via blood, CSF, or lymphatic circulation into tissues to deposit as a major amyloid component in loco. Transgenic mice presented here made it possible to analyze the initial stage of amyloid deposition. As expected, amyloid deposition began around blood capillaries. From this point of view, it is reasonable that amyloid deposition is most prominent in tissues with a rich blood supply, such as kidneys, heart, and thyroid glands. Although the MT-hMet30 gene is expressed in a variety of tissues, the distribution pattern of amyloid deposition is not related to the tissue specificity of the MT-hMet30 gene expression *(28)*. This finding is consistent with the notion that amyloid fibrils are derived from the blood.

Although the most striking pathologic feature of FAP is amyloid deposition in the peripheral and

autonomic nervous tissues, no amyloid deposition was observed in the nervous tissues of the transgenic mice examined up to age 24 mo. In all autopsy cases of FAP, we observed marked amyloid deposition in the choroid plexus and substantiated the histochemical localization of human TTR in the choroid plexus epithelial cells *(9)*. The choroid plexus and liver are considered the major sites of human TTR production *(29)*. If we consider the fact that the peripheral nerve is open-ended with respect to the subarachnoid space *(30)*, we can speculate that the production of human variant TTR by the choroid plexus epithelia is related to amyloid deposition in the peripheral nerve tissues. In the transgenic mice, however, we demonstrated immunohistochemically little or no production of human TTR in the epithelial cells of the choroid plexus. This could be the reason for the absence of amyloid deposition there and in the peripheral and autonomic nervous system in transgenic mice. A second possibility is the low-level expression of the hMet30 gene in these transgenic mice. To provide more definite evidence for this speculation, we are making mice transgenic that produce a high level of hMet30 in the epithelial cells of the choroid plexus. A third possibility lies in the characteristic metabolic features of the mouse itself, where exact metabolic turnover is not known. Interestingly, the degenerative changes in the peripheral nerve tissues before amyloid deposition were reported in FAP patients *(9)*. Thus, another intrinsic factor(s) may be involved in amyloid deposition in the nervous tissues of FAP.

Little is known about how a mutation actually leads to expression of pathologic conditions. What goes on between expression of the variant gene that gives a structurally abnormal protein and development of the corresponding disease? Many biochemical processes are involved in generating the conditions that cause pathology. Recently, the structure of a variant TTR has been determined by X-ray crystallography at 2.3 A resolution. Comparison to the known structure of the normal TTR tetramer shows that the bulkier methinine residue 30, which lies between the nearly orthogonal beta sheets of the dimer, results in the sheets being displaced an average of 0.4 A. Such global changes may affect the metabolic properties and the tendency toward polymerization of the mutant protein *(31)*.

The present study on the transgenic mice carrying the human mutant TTR (Met30) gene may prove to be an important step in showing how point mutations in TTR structure can have far reaching effects on amyloid formation.

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