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J Mol Med (2004) 82:298–307
DOI 10.1007/s00109-004-0530-7

REVIEW

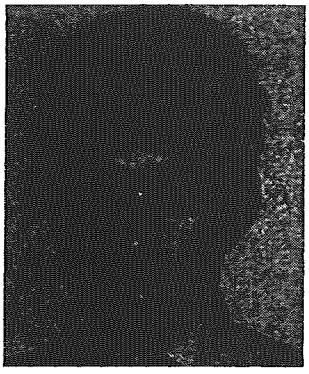
Masahisa Katsuno · Hiroaki Adachi ·
Fumiaki Tanaka · Gen Sobue

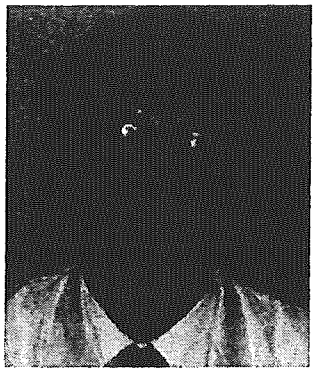
Spinal and bulbar muscular atrophy: ligand-dependent pathogenesis and therapeutic perspectives

Received: 29 September 2003 / Accepted: 13 January 2004 / Published online: 27 February 2004

Abstract Spinal and bulbar muscular atrophy (SBMA) is a late-onset motor neuron disease characterized by proximal muscle atrophy, weakness, contraction fasciculations, and bulbar involvement. SBMA exclusively affects males, while females are usually asymptomatic. The molecular basis of SBMA is the expansion of a trinucleotide CAG repeat, which encodes the polyglutamine (polyQ) tract in the first exon of the androgen receptor (AR) gene. The histopathological hallmark is the presence of nuclear inclusions containing mutant truncated ARs with expanded polyQ tracts in the residual motor neurons in the brainstem and spinal cord, as well as in some other visceral organs. The AR ligand, testosterone, accelerates AR dissociation from heat shock proteins and thus its nuclear translocation. Ligand-dependent nuclear accumulation of mutant ARs has been implicated in the pathogenesis of SBMA. Transgenic mice carrying the full-length human AR gene with an expanded polyQ tract demonstrate neuromuscular phenotypes, which are profound in males. Their SBMA-like phenotypes are rescued by castration, and aggravated by testosterone administration. Leuprorelin, an LHRH agonist that reduces testosterone release from the testis, inhibits nuclear accumulation of mutant ARs, resulting in the rescue of motor dysfunction in the male transgenic mice. However, flutamide, an androgen antagonist promoting nuclear translocation of the AR, yielded no therapeutic effect. The degradation and cleavage of the AR protein are also influenced by the ligand, contributing to the pathogenesis. Testosterone thus appears to be the key molecule in the pathogenesis of SBMA, as well as main therapeutic target of this disease.

Keywords Spinal and bulbar muscular atrophy · Polyglutamine · Androgen receptor · Ligand · Testosterone · Heat shock protein





Masahisa Katsuno is a Research Fellow in Neurology at Nagoya University Graduate School of Medicine, Nagoya, Japan. He received his M.D. and Ph.D. from Nagoya University. He has worked on therapeutic approaches for spinal and bulbar muscular atrophy.

Gen Sobue is Professor of Neurology at Nagoya University Graduate School of Medicine, Nagoya, Japan. He received his M.D. and Ph.D. from Nagoya University, and has worked at the University of Pennsylvania School of Medicine and Aichi Medical University. His research interests include the development of therapies for neurodegenerative diseases and peripheral neuropathies.

M. Katsuno · H. Adachi · F. Tanaka · G. Sobue (✉)
Department of Neurology,
Nagoya University Graduate School of Medicine,
65 Tsurumai-cho, Showa-ku, 466-8550 Nagoya, Japan
e-mail: sobueg@med.nagoya-u.ac.jp
Tel.: +81-52-7442385, Fax: +81-52-7442384

Introduction

More than a hundred years have elapsed since the first description of spinal and bulbar muscular atrophy (SBMA) [1, 2]. Early case reports [3, 4, 5, 6, 7, 8] were followed by the clinical and genetic description of 11 cases from two families by Kennedy and colleagues [9], which established the clinical entity of SBMA. Since the discovery of abnormal CAG triplet expansion in the androgen receptor (AR) gene as the cause of SBMA [10], molecular biological approaches have been undertaken to elucidate the pathogenesis of the disease and develop

approaches for its treatment. Here we review the research findings from which the ligand-dependent pathophysiology of SBMA has emerged, and discuss its therapeutic approaches.

Clinical features of SBMA

SBMA, also known as Kennedy's disease, is an inherited motor neuron disease characterized by adult-onset proximal muscle atrophy, weakness, fasciculations, and bulbar involvement [9, 11]. Fasciculations often manifest upon muscle contraction, and have been described as contraction fasciculations. The onset of weakness is usually between 30 and 50 years, but is often preceded by nonspecific symptoms such as tremor, muscle cramps and fatigue [12, 13]. Deep tendon reflex is diminished or absent, with no pathological reflex. Sensory involvement is largely restricted to that of vibration, which is affected distally in the legs [11]. Male patients occasionally demonstrate signs of androgen insensitivity such as gynecomastia, testicular atrophy, erectile dysfunction and decreased fertility [14, 15, 16], and some of these symptoms may be detected before the onset of motor symptoms. Endocrinological examinations frequently reveal partial androgen resistance with an elevated serum testosterone level [17]. Examination by electromyogram shows neurogenic abnormalities, and distal motor latencies are often prolonged in nerve conduction studies. Both the sensory nerve action potential and sensory evoked potential are reduced or absent [18, 19]. Serum creatine kinase levels are elevated in the majority of patients. Hyperlipidemia, liver dysfunction and glucose intolerance are also detected in some patients [12, 20]. SBMA exclusively affects males, and thus has been reported as an X-linked hereditary disease. The prevalence of SBMA has been estimated 1 in 40,000 in areas with where the diagnosis is efficient [21], although considerable numbers of patients may have been under-diagnosed [22, 23]. Profound facial fasciculations, bulbar signs, gynecomastia, and sensory disturbance are the main clinical features distinguishing SBMA from other motor neuron diseases, although genetic analysis is indispensable for diagnosis. Female patients are usually asymptomatic, but some express subclinical phenotypes including high amplitude motor unit potentials on electromyography [24, 25].

In the histopathology of SBMA, lower motor neurons are markedly depleted through all spinal segments and in the brainstem motor nuclei except the third, fourth and sixth cranial nerves [11, 26]. The number of nerve fibers is reduced in the ventral spinal nerve root, reflecting motor neuronopathy. Sensory neurons in the dorsal root ganglia are less severely affected, and the large myelinated fibers demonstrate a distally accentuated sensory axonopathy in the peripheral nervous system [27]. Neurons in the Onufrowicz nuclei, intermediolateral columns and Clarke's columns of the spinal cord are generally well preserved. Muscle histopathology shows both neurogenic and myogenic findings; there are groups of atrophic fibers with a number of small angular fibers, fiber type grouping, and clamps of pyknotic nuclei as well as variability in fiber size, hypertrophic fibers, scattered basophilic regenerating fibers and central nuclei.

The progression of SBMA is usually slow, but life-threatening respiratory tract infection often occurs in the advanced stage of the disease, resulting in early death in some patients [13]. No specific treatment for SBMA has been established. Testosterone has been used in some patients, although it has no effects on the progression of the disease [28, 29, 30].

Molecular pathogenesis of SBMA

The molecular basis of SBMA is the expansion of a trinucleotide CAG repeat, which encodes the polyglutamine (polyQ) tract, in the first exon of the AR gene [10] (Fig. 1). The CAG repeat within AR ranges in size from 11 to 35 repeats in normal subjects, but from 40 to 62 in SBMA patients [10, 21, 31]. Multiple founder effects have been reported in Japan, Europe and Australia [31, 32]. Expanded polyQ tracts have been found to cause several neurodegenerative diseases including SBMA, Huntington's disease (HD), several forms of spinocerebellar ataxia, and dentatorubral and pallidoluysian atrophy (DRPLA) [33, 34, 35]. These disorders, known as polyQ diseases, share salient clinical features such as anticipation [36], somatic mosaicism [37], and selective neuronal and non-neuronal involvement despite widespread expression of the mutant gene [38]. There is also an inverse correlation between the CAG repeat size and the age at onset, or the disease severity adjusted by age at examination in SBMA [36, 39], as well as in other polyQ diseases [33, 40]. These observations suggest that common mechanisms underlie the pathogenesis of polyQ diseases, although the nature of each causative protein is discrete apart from the existence of the polyglutamine stretch.

A striking pathological hallmark of most polyQ diseases is the presence of nuclear inclusions (NIs), which have been considered relevant to the pathophysiology [33]. In SBMA patients, NIs containing the mutant,

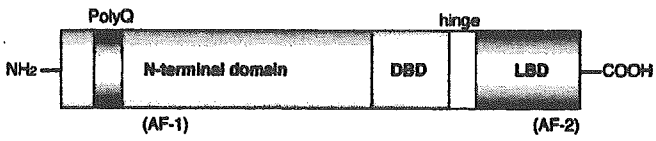


Fig. 1 Structure of the androgen receptor (AR) protein. The AR, a ligand-dependent transcriptional factor, is a member of the steroid/thyroid hormone receptor family. The AR protein consists of three major domains: an N-terminal transactivating domain, a DNA-binding domain, and a ligand-binding domain. The polyglutamine tract is located in the N-terminal domain, which possesses the major transactivating function (*AF-1*). The DNA binding domain (*DBD*) contains zinc finger structures, facilitating AR binding to DNA. The ligand binding domain (*LBD*) in the C-terminus also contains weak transactivating function (*AF-2*)

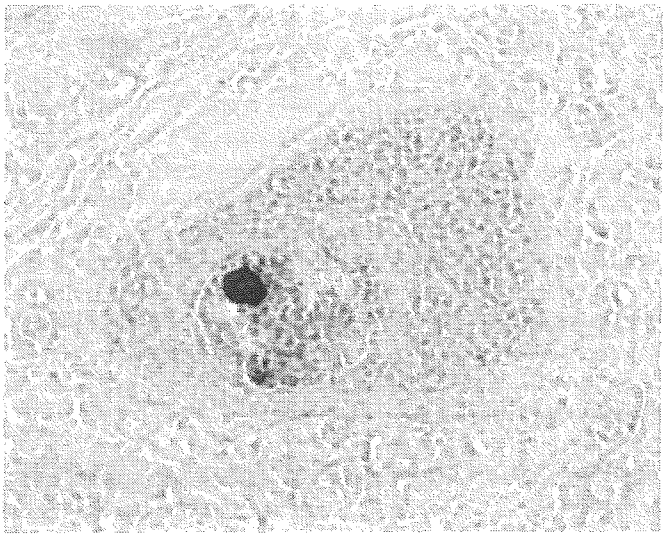


Fig. 2 Nuclear inclusion of spinal and bulbar muscular atrophy (SBMA). A residual motor neuron in the lumbar anterior horn shows a nuclear inclusion detected by an anti-polyglutamine antibody

truncated AR are detected in the residual motor neurons in the brainstem and spinal cord [41] (Fig. 2) as well as in the skin, testis and some other visceral organs [42]. These inclusions have similar epitope features detectable by antibodies that recognize a small portion of the N-terminus of the AR protein only, and they are ubiquitinated. Although considerable controversy surrounds the importance of NIs in the pathophysiology of the polyQ diseases [43], several studies have implied that the nuclear localization of the mutant protein is essential for inducing neuronal cell dysfunction and degeneration in the majority of polyQ diseases [34]. This hypothesis is supported by the fact that several transcriptional regulatory proteins are sequestrated into NIs [44, 45].

SBMA is unique among polyQ diseases in that the mutant protein, the AR, has a specific ligand, testosterone, and this ligand alters the subcellular localization of the protein by favoring its nuclear uptake. The AR is normally confined to a multi-heteromeric inactive complex in the cell cytoplasm, and translocates into the nucleus in a ligand-dependent manner [46]. This intracellular trafficking of AR and other ligand-related mechanisms appear to play important roles in the pathogenesis of SBMA.

Ligand effects in a mouse model of SBMA

In order to investigate ligand effects in SBMA, we generated transgenic mice expressing the full-length human AR containing either 24 or 97 CAG repeats under the control of a cytomegalovirus enhancer and a chicken β-actin promoter [47]. This model recapitulated not only the neurological disorder, but also the phenotypic differences with gender, which is a specific feature of SBMA. The lines with 97 CAG repeats (AR-97Q) exhibited progressive motor impairment, although no lines with 24 CAG repeats showed any phenotypes. All symptomatic lines showed small body size, short lifespan, progressive muscle atrophy and weakness, as well as reduced cage activity, all of which were pronounced and markedly accelerated in the male AR-97Q mice, but were either not observed, or far less severe, in the female AR-97Q mice, regardless of the line. The onset of motor impairment detected by the rotarod task was between 8 and 9 weeks of age in the male AR-97Q mice, and at 16 weeks or more in the females. The 50% mortality ranged from 66 to 132 days in the male AR-97Q mice, whereas the mortality of the females remained only 10–30% at more than 210 days. Western blot analysis revealed the transgenic protein smearing from the top of the gel, indicating the presence of insoluble AR fragments, in tissues such as the spinal cord, cerebrum, heart, muscle and pancreas. Although the male AR-97Q mice had more smearing protein than their female counterparts, the female AR-97Q mice had more monomeric AR protein. The nuclear fraction contained the most of smearing mutant AR protein. Diffuse nuclear staining and less frequent NIs detected by 1C2, an antibody specifically recognizing the expanded polyQ, were demonstrated in the neurons of the spinal cord, cerebrum, cerebellum, brainstem and dorsal root ganglia as well as non-neuronal tissue such as the heart, muscle and pancreas. Male AR-97Q mice showed markedly more abundant diffuse nuclear staining and NIs than females, in agreement with the symptomatic and Western blot profile differences with gender. Despite the profound sexual differences of mutant AR protein expression, there was no significant difference in the expression of the transgene mRNA between the male and female AR-97Q mice. These observations indicate that the testosterone level plays an important role in the gender-specific differences in the phenotypes, especially in post-transcriptional regulation of the mutant AR. Gender-specific phenotypes have also been demonstrated in another transgenic mouse model of SBMA carrying the full-length AR with 120 CAG repeats driven by a cytomegalovirus promoter [48].

The dramatic sexual difference of phenotypes led us to trial hormonal interventions in our mouse model. First, we castrated male AR-97Q mice in order to decrease their testosterone level. Castrated males showed profound improvement of their symptoms, histopathological findings, and nuclear localization of the mutant AR compared with sham-operated males. The body weight, motor function, and lifespan of these mice were significantly improved by castration. Western blot analysis and histopathology revealed diminished nuclear accumulation of mutant AR in the castrated males compared with the sham-operated males. Next, we administered testosterone to female AR-97Q mice. In contrast to castration of the male mice, testosterone caused a significant aggravation of symptoms, histopathological features, and nuclear localization of the mutant AR in female mice. Since the nuclear translocation of AR is testosterone-dependent,

testosterone appears to show toxic effects in the female AR-97Q mice by accelerating nuclear translocation of the mutant AR. Hence, castration of the males prevented the nuclear localization of the mutant AR by reducing the testosterone level. Nuclear localization of the mutant protein with an expanded polyQ tract is likely to be important in inducing neuronal cell dysfunction and degeneration in the majority of the polyQ diseases. Addition of a nuclear export signal to the mutant huntingtin protein eliminates aggregate formation and cell death in cell models of HD [49, 50], whereas a nuclear localization signal has the opposite effect [50]. When its nuclear localization signal is mutated, atxaxin-1, the causative protein of SCA-1, is distributed in the cytoplasm and does not cause any neurological disorders in SCA1 transgenic mice [43]. Addition of a nuclear export signal to the mouse Hprt protein containing expanded polyQ reduces NIs and delays the onset of behavioral abnormalities [51]. These findings suggest that reduction in the testosterone level improved phenotypic expression by preventing nuclear localization of the mutant AR. In support of this hypothesis, the ligand-dependent neurodegeneration has also been revealed in a fruit fly model of SBMA [52]. Alternatively, castration may enhance the protective effects of heat shock proteins, which are normally associated with the AR and dissociate upon ligand binding. Although ligand-induced neuronal dysfunction is apparent in the mouse model, testosterone administration does not worsen the symptoms of SMBA patients in preliminary clinical trials [28, 29]. This inconsistency may be explained by several reasons. The treatment duration may not be long enough to show its negative effects on disease progression. The negative effect may be saturated by the endogenous level of testosterone. The anabolic effects of androgens on the muscle may attenuate the motor symptoms induced by anterior horn cell degeneration in SBMA.

Successful treatment of AR-97Q mice by castration inspired us to trial testosterone blockade therapies, using a LHRH analogue and an AR antagonist used in the treatment of prostate cancer [53]. AR-97Q mice treated with leuprorelin, a LHRH analogue which reduces testosterone release from the testis, showed a marked amelioration of symptoms, histopathological findings, and nuclear localization of the mutant AR compared with vehicle-treated mice (Fig. 3). Leuprorelin initially increased the serum testosterone level by upregulating the LHRH receptor, but this effect was subsequently reduced to undetectable levels. Androgen blockade effects were also confirmed by reduced weights of the prostate and the seminal vesicle. The leuprorelin-treated AR-97Q mice showed significant improvements in lifespan, muscle atrophy and reduced body size as well as motor impairment as assessed by the rotarod task and cage activity. Although the negative effect on fertility was mitigated by reducing the dosage, the therapeutic effects on neuromuscular phenotypes were insufficient at a lower dose of leuprorelin. In the Western blot analysis and anti-polyglutamine immunostaining, the leuprorelin-treated male AR-97Q mice had markedly diminished levels of mutant AR in the nucleus, suggesting that leuprorelin successfully reduced nuclear AR accumulation. Testosterone, which was given from 13 weeks of age, markedly aggravated the neurological symptoms and pathological findings of the leuprorelin-treated male AR-97Q mice. Leuprorelin prevents testicular testosterone production by down-regulating LHRH receptors in the pituitary, and has extensively been used as medical castration in the therapy of prostate cancer. Leuprorelin appears to improve SBMA-related neuronal dysfunction by preventing ligand-dependent nuclear translocation of the mutant AR in the same way as castration. Given its minimal invasiveness and established safety, leuprorelin is likely to be a promising therapeutic agent for SBMA. Upon clinical trials, however, the patient's desire for fertility should be taken into account, and the appropriate therapeutic dose carefully determined.

Leuprorelin-treated AR-97Q mice showed deterioration in their body weights and rotarod task performance from the age of 8–9 weeks, when serum testosterone initially increased through the agonistic effect of leuprorelin. This change was transient and followed by a sustained amelioration together with consequent suppression of testosterone production. The footprint analyses also revealed a temporary exacerbation of motor impairment. Immunostaining of tail specimens, sampled from the same individual mouse, demonstrated an increase in the number of the muscle fibers with nuclear 1C2 staining after 4 weeks of leuprorelin administration, although this 1C2 staining was diminished after another 4 weeks of treatment. Reversibility of polyQ pathogenesis has also been demonstrated by turning off gene expression in an inducible mouse model of HD [54]. Our results, however, indicate that preventing nuclear translocation of the mutant AR is enough to reverse both the symptomatic and pathological phenotypes in our AR-97Q mice. Since the pathophysiology of AR-97Q mice is neuronal dysfunction without neuronal cell loss [47], our results indicate that polyQ pathogenesis is reversible at least in its dysfunctional stage. We need to determine the early dysfunctional period in human polyQ diseases.

By contrast, flutamide, an AR antagonist, did not ameliorate the symptoms, pathological features, or nuclear localization of the mutant AR in the male AR-97Q mice, although there was no significant difference between flutamide and leuprorelin in terms of androgen blockade. Flutamide, the first androgen antagonist discovered, has a highly specific affinity for the AR, and competes with testosterone for binding to the receptor. It has been used for the treatment of prostate cancer, usually in association with an LHRH agonist, in order to block the action of adrenal testosterone. Although flutamide suppresses androgen-dependent transactivation, it does not reduce the plasma levels of testosterone. Furthermore, flutamide does not inhibit, but may even facilitate, the nuclear translocation of the AR [55, 56]. Flutamide also promotes nuclear translocation of mutant ARs containing expanded polyQ tracts in both cell and fly models of

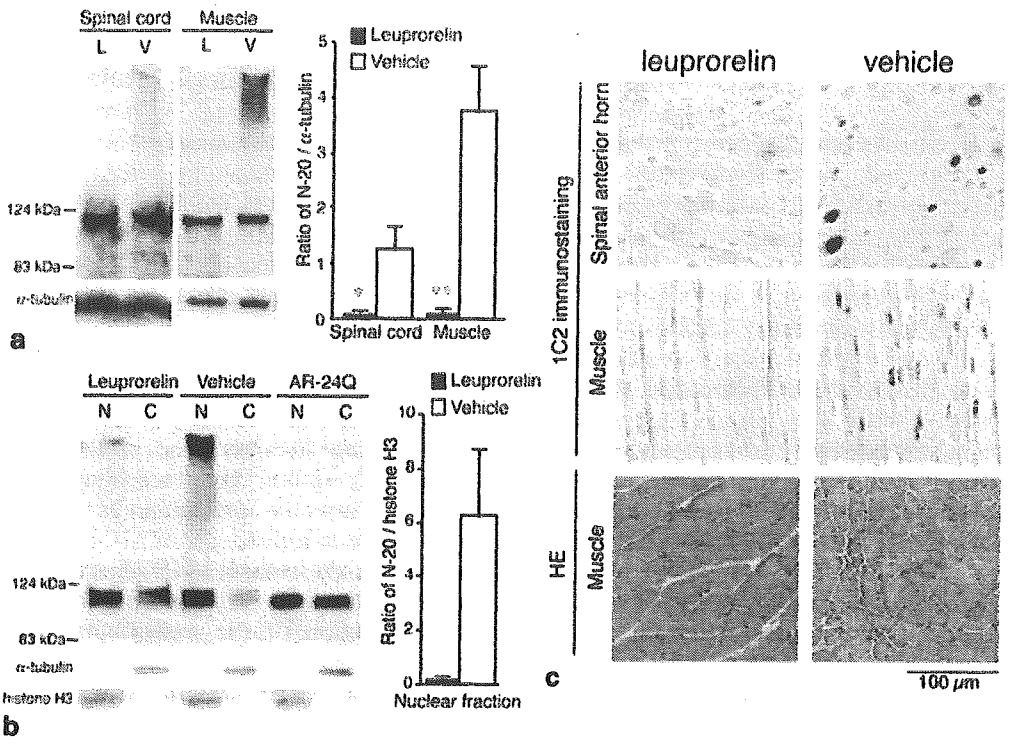


Fig. 3a–c Effects of leuprorelin on mutant AR expression and neuropathology of male AR-97Q mice. **a** Western blot analysis with an anti-AR antibody (N-20) of total homogenates from the spinal cord and muscle of the leuprorelin-treated (*L*) and vehicle-treated (*V*) male AR-97Q mice. Densitometric analysis demonstrated that leuprorelin significantly diminished the amount of mutant AR smearing from the top of the gel. (**P*=0.011, ***P*=0.015). **b** Western blot analysis (with N-20) of the nuclear (*N*) and cytoplasmic (*C*) fractions from muscle tissues of the male mice given leuprorelin (*L*) and vehicle (*V*), and a transgenic mouse carrying an AR with 24 CAG repeats (*AR-24Q*). Smearing mutant AR, which decreased with leuprorelin treatment, was contained in the nuclear fraction. In the densitometric analysis, leuprorelin significantly reduced the amount of smearing mutant AR in the nuclear fraction. (*P*=0.014). **c** Immunohistochemical studies using 1C2 showed marked differences in diffuse nuclear staining and nuclear inclusions between the leuprorelin-treated and vehicle-treated AR-97Q male mice in the spinal anterior horn and the muscle. Hematoxylin and eosin staining of the muscle in the vehicle-treated male mouse revealed apparent grouped atrophy and small angulated fibers, which were not seen in the leuprorelin-treated mice. This figure is reproduced from [53]

SBMA [52, 57]. This may be the reason why flutamide showed no therapeutic effect in our transgenic mouse model of SBMA, and hence is not likely to be a useful therapeutic agent for SBMA.

The castrated or leuprorelin-treated AR-97Q mice showed phenotypes similar to those of the female AR-97Q mice, implying that the motor impairment of SBMA patients can be reduced to the level seen in females. SBMA has been considered an X-linked disease, whereas other polyQ diseases show autosomal dominant inheritance. In fact, female SBMA patients hardly manifest clinical phenotypes, although they possess similar number of CAG repeats in the disease allele as their male siblings with SBMA [24, 25]. Indeed the lower level of mutant AR expression in females due to X-inactivation may cause the escape from phenotypic manifestations, but hormonal interventions in mouse and fly models strongly suggest that low levels of testosterone prevent the nuclear accumulation of the mutant AR, resulting in a lack of phenotypic manifestations in females. This hypothesis is supported by the finding that manifestation of symptoms is minimal, even in females homozygous for SBMA [58]. In another case report, an 85-year-old woman with 38/51 CAG repeats was also asymptomatic [59]. It would appear logical that SBMA is not an X-linked recessive inherited disease, but rather that its phenotype depends on testosterone concentration.

Ligand-dependent modifications of the AR

In addition to nuclear translocation, ligand binding modulates AR function in various ways. These ligand effects are also likely to influence the pathogenesis of SBMA (Fig. 4). Many components of the ubiquitin-proteasome and molecular chaperones are known to co-localize with polyQ-containing NIs [60, 61, 62]. These chaperones and proteasomes facilitate refolding or degradation of the mutant protein, and may play a role in protecting neuronal cells against the toxicity of the expanded polyQ tract [60, 63]. Over-expression of heat shock proteins (HSPs) decreases aggregate formation of mutant ARs and markedly prevents the cell death in a neuronal cell model of SBMA [64]. HSP70 over-expres-

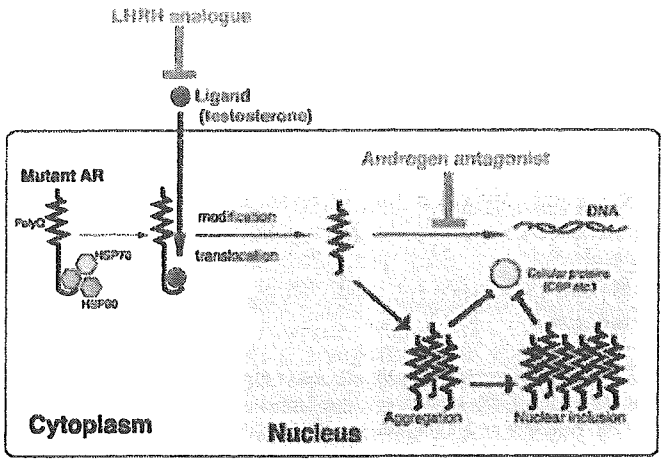


Fig. 4 Hypothetical relationship between the ligand and the mutant AR. In the absence of ligand, the mutant AR is confined to a multi-heteromeric inactive complex with heat shock proteins (*HSP*) in the cell cytoplasm. Ligand-binding facilitates its dissociation from this complex and translocation into the nucleus. Ligand also induces AR modifications such as conformational change, phosphorylation and proteolytic cleavage. In the nucleus, the mutant AR aggregates in a fashion partially dependent on transglutaminase activity, and forms nuclear inclusions as a consequence. The function of critical cellular proteins such as CREB-binding protein is inhibited by mutant the AR, resulting in transcriptional dysregulation. On the other hand, the decreased transactivation function of the mutant AR may contribute to the androgen insensitivity and neurodegeneration in SBMA. LHRH agonist, but not androgen antagonist, inhibits nuclear accumulation of mutant AR, resulting in the improvement of SBMA phenotypes in model mice. This figure is modified from [103]

sion enhances both the solubility and the degradation of mutant ARs in vitro [65]. These protective effects of the HSPs have also been reported in a wide range of polyQ disease models [60, 66, 67, 68]. Over-expression of the inducible form of human HSP70 markedly ameliorated the symptomatic and histopathological phenotypes in our transgenic mouse model, and this amelioration was correlated with the reduction in the amount of nuclear-localized mutant AR protein [69]. It should be noted that the soluble form of the mutant AR was also significantly decreased by HSP70 over-expression, suggesting that the degradation of the mutant AR may have been accelerated by over-expression of HSP70. Without ligand stimulation, the AR is associated with HSP70 and HSP90, and exists as an inactivated complex in the cell cytoplasm. Once ligand binds to the AR, the receptor is released from this complex and translocates into the nucleus. Ligand-dependent dissociation from HSPs is likely to enhance the toxic properties of mutant ARs, and thus contributes to the pathogenesis of SBMA. Supporting this hypothesis, ligand induces mutant AR aggregation even in the cytoplasm in a cell model of SBMA [70].

Proteolytic cleavage or truncation of mutant proteins appears to be of importance for the pathogenesis of polyQ diseases. In transgenic mouse models of HD and those of Machado-Joseph disease, or SCA3, the truncated protein has a particularly pronounced effect on the disease manifestation [71, 72]. NIs are detected by antibodies against an N-terminal epitope, but not by antibodies against a C-terminal epitope in SBMA [41, 42] as well as in other polyQ diseases [73]. These findings suggest that truncated polyQ-containing proteins confer the toxicity in polyQ diseases. It should be noted that a truncated mutant AR is more toxic than the full-length AR in a SBMA cell model [74]. Additionally, in vitro translated full-length AR protein with an expanded polyQ tract is cleaved by caspase-3, liberating a polyQ-containing fragment, and the susceptibility to cleavage is polyQ repeat length-dependent [75]. Thus, cleavage of polyQ-containing proteins is likely to contribute to the toxicity of polyQ tracts. Testosterone induces a conformation change in the AR, resulting in an altered propensity for proteolytic cleavage. It should be noted that the AR antagonist flutamide exerts the same effect as testosterone on mutant AR cleavage in a fly model of SBMA [52]. The activity of caspase-3 is also enhanced by phosphorylation of the AR, resulting in enhanced cytotoxicity of the polyQ tract [76]. Phosphorylation of the mutant protein is required for polyQ-induced neurodegeneration in both SCA-1 cell and fly models of SMBA [77, 78]. Phosphorylation of the AR is known to be modulated by ligand [79], indicating that ligand-dependent phosphorylation of mutant ARs would appear to contribute to the pathogenesis of SBMA. The exact role of AR phosphorylation in SBMA should be elucidated.

Transglutaminase has also been hypothesized to enhance polyQ toxicity [80, 81]. Mutant proteins with an expanded polyQ tract are preferential substrates for transglutaminase, which catalyzes glutamyl-lysine cross-linking, resulting in the formation of proteolysis-resistant aggregates. The AR is a substrate for transglutaminase, which induces cross-linking of mutant ARs and alters their epitope properties in vitro [82]. Transglutaminase bond formation is found in the tissues of SBMA transgenic mice, suggesting its involvement in pathogenesis. Mutant AR and transglutaminase both induce a perturbation of proteasomal function in the presence of testosterone. The degradation of the AR protein is markedly retarded by its ligands [83]. Taken together, these findings suggest that the disruption of the proteasome, which has been implicated in the pathogenesis of SBMA and other polyQ diseases [84, 85], also appears to be ligand-dependent in SBMA.

As in other polyQ diseases, a toxic gain-of-function mutation has been implicated in the pathophysiology of SBMA. Although the expansion of its polyQ tract mildly suppresses the transcriptional activities of the AR [86, 87], motor impairment has never been observed in severe testicular feminization patients lacking AR function [88] or in AR knockout mice [89]. A transgenic mouse model carrying 239 CAG repeat driven by the human AR promoter demonstrated motor impairment, suggesting that the presence of a polyQ tract is sufficient to induce the pathogenic process of SBMA [62]. Thus, the neurological impairment in SBMA is not to be attributed to the loss of AR function, a reason why testosterone shows transient

and insufficient effects when used as a therapeutic agent for SBMA [28, 29, 30]. However, recent data have demonstrated that loss of normal protein function also plays a role in the neurodegeneration in polyQ diseases. As for the AR, there are several lines of evidence in favor of ligand-induced neuroprotective effects. Exogenous administration of testosterone immediately after nerve injury impacts positively on functional recovery through actions mediated by AR [90, 91]. In a cell culture model, a mutant AR with 24 CAG repeats shows trophic effects upon ligand treatment, whereas an AR with 65 CAG repeats does not demonstrate any neuroprotection [92]. Ligand effects on AR function should be further studied using animal models of SBMA.

Therapeutic perspectives in SBMA and other polyQ diseases

As mentioned above, our recent study indicated that leuprorelin exerts a therapeutic effect in the SBMA transgenic mouse model. This approach can easily be applied to human SBMA therapy, because this drug has extensively been used for medical castration in the therapy of prostate cancer [93]. When we move on to clinical trials of hormonal therapy, it is necessary to determine clinical and laboratory markers reflecting the disease activity. Muscle strength, muscle volume and bulbar function would be reasonable parameters, although they must be quantified. Early symptoms, including hand tremor, muscle cramps or mild weakness could be the key for treatment initiation, only if the patients have no wish to have children. Although no specific ligand for the mutant protein has been revealed in other polyQ diseases, the striking therapeutic effects of leuprorelin in our SBMA mice further suggest that patients with other forms of polyQ disease can be treated by preventing the nuclear translocation of the mutant protein in question. Our studies with transgenic mice also indicate that over-expression or activation of HSP would be of therapeutic benefit in polyQ diseases. Medical approaches to enhance HSP function should be further investigated in vivo.

Besides these strategies, various molecular mechanisms are likely to be the therapeutic targets in polyQ diseases. The function of transcription factors such as CREB-binding protein (CBP) are inhibited through protein-protein interaction in mouse models and patients with polyQ diseases [94, 95]. HDAC inhibitors, which restore histone acetylation by CBP, improve transcriptional activity and ameliorate polyQ-induced neurodegeneration in a cell model of SBMA [96] as well as in a fly model of HD [97], although this is of limited therapeutic benefit in a mouse model of HD due to toxicity [98]. Protein aggregation has been shown to render polyQ-containing proteins toxic in numerous cell models of SBMA and other polyQ diseases [99, 100]. The azo-dye Congo Red inhibits oligomerization of mutant huntingtin, and ameliorates motor function and survival of in a transgenic mouse model of HD [101]. Cystamine, a transglutaminase inhibitor, has been shown to mitigate polyQ toxicity in a cell model of SBMA [82] as well as in a HD mouse model [102]. Clinical applications of these therapeutic approaches are awaited.

The ideal treatment for polyQ diseases appears to be a combination of these and other therapeutic strategies, since each approach has adverse effect and long-term treatment is unavoidable. Elucidation ofpathophysiology, high-throughput drug screening and intensive clinical trials are necessary for establishing effective therapeutic strategies.

Acknowledgements This work was supported by a Center-of-Excellence (COE) grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan, grants from the Ministry of Health, Labour and Welfare, Japan, by a grant from the Naito Foundation, and by a grant from the Kanae Foundation.

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Pathology of early- vs late-onset TTR Met30 familial amyloid polyneuropathy

H. Koike, MD, PhD; K. Misu, MD, PhD; M. Sugiura, MD; M. Iijima, MD; K. Mori, MD, PhD; M. Yamamoto, MD, PhD; N. Hattori, MD, PhD; E. Mukai, MD, PhD; Y. Ando, MD, PhD; S. Ikeda, MD, PhD; and G. Sobue, MD, PhD

Abstract—*Background:* Late-onset type I familial amyloid polyneuropathy (FAP TTR Met30) cases unrelated to endemic foci in Japan show clinical features setting them apart from early-onset cases in endemic foci. *Objective:* To compare pathologic features between the early- and late-onset types. *Methods:* Pathologic findings in FAP TTR Met30 with onset before age 50 in relation to endemic foci (11 cases) were compared with those in 11 later-onset cases unrelated to endemic foci. *Results:* Sural nerve biopsy specimens showed predominantly small-fiber loss in early-onset cases; variable fiber size distribution, axonal sprouting, and relatively preserved unmyelinated fibers characterized late-onset cases. Autopsy cases representing both groups showed amyloid deposition throughout the length of nerves and in sympathetic and sensory ganglia, but amounts were greater in early-onset cases. Amyloid deposition and neuronal cell loss were greater in sympathetic than dorsal root ganglia in early-onset cases; the opposite was true in late-onset cases. Size assessment of remaining neurons in these ganglia suggested predominant loss of small neurons in early-onset cases but loss of neurons of all sizes in late-onset cases. Transthyretin-positive, Congo red-negative amorphous material was more conspicuous in nerves from late- than early-onset cases. In extraneural sites, amyloid was more conspicuous in thyroid and kidney from early-onset cases and in heart and hypophysis from late-onset cases. In early-onset cases, cardiac amyloid deposition was prominent in the atrium and subendocardium but was conspicuous throughout the myocardium in late-onset cases. *Conclusion:* The pathology of early- and late-onset FAP TTR Met30 correlated well with differences in clinical findings.

NEUROLOGY 2004;63:129–138

Familial amyloid polyneuropathy type I (transthyretin Met30-associated familial amyloid polyneuropathy; FAP TTR Met30), in which methionine is substituted for valine at position 30 of transthyretin, is the most common type of FAP in Japan as well as in Western countries.[1-6] In Japan, cases have been particularly concentrated in two geographic areas: the village of Ogawa in Nagano Prefecture on the island of Honshu and the city of Arao in Kumamoto Prefecture on the island of Kyushu.[3,4] Although there are exceptions, typical cases of FAP TTR Met30 in these two endemic foci are characterized by early age at onset (second or third decade), a high penetrance rate, marked autonomic dysfunction, selective loss of superficial sensation including nociception and thermal sensation, atrioventricular conduction block requiring pacemaker implantation, steady progression of disease over 10 to 15 years, and presence of anticipation concerning age at onset.[3,4,7-11]

In contrast to these early-onset FAP TTR Met30 cases in endemic foci, we have reported the presence of a late-onset type of FAP TTR Met30 in a wide distribution throughout Japan.[9,10,12] Features of these cases were distinct from those of early-onset cases related to endemic foci. These differences included onset at ages over 50 years, a low penetrance rate, relatively mild autonomic dysfunction, loss of all sensory modalities rather than sensory dissociation, frequent presence of cardiomegaly, extreme male preponderance, and absence of anticipation concerning age at onset.[9,10,12] These geographic and clinical differences were confirmed in a subsequent nationwide survey.[11] Similar geographic and clinical contrasts between early- and late-onset types of FAP TTR Met30 have been reported in Portugal,[1,5,13] although not in the form of a large-scale comparative study.

The reasons for contrasting features in early- and late-onset FAP with the same mutation in the transthyretin gene have not yet been determined. In the current study, we investigated pathologic features of Japanese patients with early- and late-onset FAP TTR Met30, seeking explanations for the clinical differences.

Patients and methods. Pathologic findings were compared between consecutive patients with early- and late-onset FAP TTR Met30 who attended the Nagoya University Graduate School of Medicine for sural nerve biopsy or autopsy from 1989 to 2003. Inclusion criteria for early-onset cases were FAP TTR Met30 with an onset age under 50 years and a relationship to one of the two Japanese endemic foci within the two most recent prior genera-

From the Department of Neurology (Drs. Koike, Misu, Sugiura, Iijima, Mori, Yamamoto, Hattori, and Sobue), Nagoya University Graduate School of Medicine, Department of Neurology (Dr. Mukai), Nagoya National Hospital, Department of Laboratory Medicine (Dr. Ando), Kumamoto University School of Medicine, and Third Department of Medicine (Dr. Ikeda), Shinshu University School of Medicine, Matsumoto, Japan.

Supported by grants from the Ministry of Health, Labor, and Welfare of Japan.

Received October 8, 2003. Accepted in final form February 23, 2004.

Address correspondence and reprint requests to Dr. G. Sobue, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan; e-mail: sobueg@med.nagoya-u.ac.jp

Table 1 Background and clinical features of early- and late-onset FAP TTR Met30

Case no.	Sex	Age at onset/ death, y	Duration of illness until biopsy, y	Relationship to endemic foci	Family history	Initial symptom	Sensory dissociation	Cardiac involvement		Cause of death
								Cardiomegaly	Pacemaker implantation	
Early-onset group										
1	F	28/35	—	+	+	A	+	−	+	Sudden death
2	F	37/51	—	+	+	A	+	−	+	Pneumonia
3	M	24/41	3	+	+	A	+	−	−	Pneumonia
4	M	35	2.5	+	ND*	W	+	−	−	
5	F	33	6	+	+	P	−	−	+	
6	M	35	3	+	+	P	+	+	−	
7	M	36	2	+	+	A	+	−	−	
8	M	40	1	+	+	P	+	−	−	
9	M	28	1	+	+	P	+	−	−	
10	F	34	2	+	ND*	A	+	−	+	
11	F	41	3	+	+	A	−	−	+	
Late-onset group										
12	M	64/67	2	−	−	P	+	−	−	Lung cancer
13	M	62/68	3	−	−	P	+	+	−	Heart failure
14	M	52/62	3	−	−	P	−	+	−	Heart failure
15	M	67	2	−	+	P	−	−	−	
16	M	77	0.5	−	−	P, HF	−	+	−	
17	M	56	1	−	−	W	−	+	−	
18	M	61	3	−	−	P	−	+	−	
19	M	56	3	−	−	P	−	+	−	
20	M	58	0.6	−	−	P	−	+	−	
21	M	60	1.25	−	−	A	−	+	−	
22	M	61	5	−	−	P, W	−	−	−	

Cardiomegaly was assessed at the time of first referral to the hospital. No patient belonged to the same kindred as another.

* Fathers of Cases 4 and 10 were from Ogawa Village but died of nonneurologic disease when the patients were children. Statistical significance (early-vs late-onset group) was present in the items of sex ($p < 0.05$), age at onset ($p < 0.0001$), relationship to endemic foci ($p < 0.0001$), family history ($p < 0.0001$), sensory dissociation ($p < 0.01$), cardiomegaly ($p < 0.01$), and pacemaker implantation ($p < 0.05$). Statistical analyses were performed using the χ^2 test or the Mann–Whitney U test as appropriate.

FAP = familial amyloid polyneuropathy; + = present; − = absent; A = autonomic symptoms; W = weakness in the lower legs; P = paresthesia in the legs; HF = heart failure; ND = not determined.

tions. For late-onset cases, inclusion criteria were FAP TTR Met30 with an onset age over 50 years and no relationship to the endemic foci within the two most recent prior generations. To confirm the diagnosis of FAP TTR Met30, DNA analyses for mutation of the transthyretin gene were performed in all patients as described previously.[4,14,15] Informed consent was obtained, and all aspects of the study were approved by the Ethics Committee of Nagoya University Graduate School of Medicine.

Of the 22 patients included, 11 were in the early-onset group and the other 11 belonged to the late-onset group (table 1). No patient in the study belonged to the same kindred as another. Age at onset in the early-onset group was 33.9 ± 5.4 years and in the late-onset group 61.3 ± 6.7 years. Duration from onset of neuropathy to sural nerve biopsy was 2.6 ± 1.5 years in the early-onset group and 2.2 ± 1.4 years in the late-onset group (no significant difference). Duration from onset to death in autopsy cases was 12.7 ± 5.1 years for early-onset disease but only 6.3 ± 3.5 years in late-onset cases. Clinical features in the two groups of patients agreed well with previous descriptions.[3,9,11] In the early-onset group, half of the patients initially had autonomic symptoms, and most patients manifested more profound impairment of superficial than deep sensation (i.e., sensory dissociation). Pacemaker implantation was required in five patients, and the apparent cause of death in one case was sudden cardiac arrest. In the late-onset group, on the other hand, most patients initially manifested paresthesias or weakness in the legs rather than autonomic symptoms. Sensory dissociation was infrequent, and most patients manifested cardiac hypertrophy evident by chest radiography or echocardiography as opposed to atrioventricular conduction block in the early-onset group.

Sural nerve biopsy was performed in nine of the early-onset cases and all of the late-onset cases as described previously.[16-19] Specimens were divided into two portions. The first was fixed in 2.5% glutaraldehyde in 0.125 *M* cacodylate buffer (pH 7.4). Most of this part was embedded in epoxy resin for morphometric and ultrastructural study. Density of myelinated fibers was assessed in toluidine blue-stained semithin sections using a computer-assisted image analyzer (Luzex FS; Nikon, Tokyo, Japan); densities of small and large myelinated fibers were calculated as described previously.[17-19] Clusters of two or more small myelinated fibers enclosed by one basement membrane were considered an instance of axonal sprouting.[20-22] For electron microscopic study,

Table 2 Pathologic findings in sural nerve biopsy specimens

Case no.	MF density, no./mm²				Axonal sprouting of MF, no./mm²	UMF density, no./mm²	Segmental de/ remyelination, %	Axonal degeneration, %	Amyloid deposition, %
	Large	Small	Total	Small/large					
Early onset									
3	0	0	0	—*	0	0	ND†	ND†	ND
4	13	13	26	—*	0	0	ND†	ND†	7
5	11	22	33	—*	0	216	8	25	1
6	23	0	23	—*	0	212	ND†	ND†	2
7	711	395	1,106	0.56	0	431	5	21	1
8	2,700	1,449	4,149	0.54	9	2,370	10	17	0+
9	2,015	1,515	3,530	0.75	18	3,663	6	26	0+
10	1,090	427	1,517	0.39	6	844	3	9	4
11	1,659	237	1,896	0.14	10	861	ND	ND	0+
Mean ± SD	914 ± 1,018	451 ± 608	1,364 ± 1,583	0.48 ± 0.23	5 ± 7	993 ± 1,305	6.4 ± 2.7	19.6 ± 6.9	
Controls, n = 3	3,495 ± 179	5,172 ± 528	8,666 ± 665	1.48 ± 0.11		30,104 ± 1,115	3.7 ± 5.5	0.4 ± 0.3	
Late onset									
12	79	487	566	6.16	79	2,370	17	20	0+
13	619	527	1,146	0.85	40	7,973	1	37	0
14	329	1,172	1,501	3.56	184	10,990	8	14	0+
15	66	13	79	—*	0	1,293	19	25	1
16	92	250	342	3.30	53	2,155	2	15	0+
17	461	1,831	2,292	3.97	250	7,111	13	27	1
18	0	2,423	2,423	—*	224	4,310	6	19	0
19	132	132	264	1.00	13	2,586	6	24	0+
20	514	355	869	0.69	26	1,795	4	37	0
21	1,304	1,212	2,516	0.93	105	14,438	6	26	0
22	66	277	343	4.20	40	431	ND†	ND†	1
Mean ± SD	333 ± 386	789 ± 776	1,122 ± 925	2.74 ± 1.95	92 ± 88	7,308 ± 5,417	8.2 ± 6.1	24.4 ± 8.0	
Controls, n = 4	2,891 ± 251	4,995 ± 333	7,886 ± 334	1.74 ± 0.22		29,748 ± 3,587	9.5 ± 6.2	1.9 ± 1.9	

Control values for each group were age matched. Statistical significance (early-vs late-onset group) was present in the items of small/large ($p < 0.05$), axonal sprouting of MF ($p < 0.01$), and UMF density ($p < 0.01$). Statistical analyses were performed using the Mann–Whitney *U* test.

* Populations of myelinated fibers were too small to determine the ratio.
† Teased fibers could not be obtained owing to depletion of myelinated fibers.

MF = myelinated fiber; UMF = unmyelinated fiber; ND = not determined; 0+ = <0.5%.

epoxy resin-embedded specimens were cut into ultrathin transverse sections and stained with uranyl acetate and lead citrate. To assess the density of unmyelinated fibers, electron microscopic photographs were taken at a magnification of 4,000× in a random fashion to cover the area of ultrathin sections as described previously.[17,19,21] Density of unmyelinated fibers was estimated from these electron micrographs. The remainder of the glutaraldehyde-fixed sample was processed for teased fiber study, in which microscopic observations were classified according to criteria described previously.[17,18,23,24] The second portion of the specimen was fixed in 10% formalin solution and embedded in paraffin. Sections were cut by routine methods and stained with hematoxylin and eosin, Congo red, the Klüver–Barrera method, and the Masson trichrome method. Seven sural nerve specimens were obtained from subjects with nonneurologic diseases at autopsy and examined in the same manner as age-matched control subjects (three cases for early-onset group, age 39.0 ± 7.8 years; four cases for late-onset group, age 62.3 ± 7.9 years).

Autopsy was performed in three early-onset cases and three late-onset cases. The nervous system including brain, spinal cord, ventral and dorsal roots, dorsal root ganglia from L3 to L5, and sympathetic ganglia was removed, as were the visceral organs. Tissues were fixed in 10% formalin solution, embedded in paraffin, cut, and stained as described for sural nerve specimens. In two of the early-onset and one of the late-onset cases, the median nerve from the axilla to the wrist and the sciatic/tibial nerve from the upper thigh to above the medial malleolus also were removed and fixed in 0.05 *M* phosphate buffer (pH 7.4) containing 1.5% glutaraldehyde and 3% formalin. After fixation, samples were taken every 4 cm along the nerves, embedded in paraffin or epoxy resin, cut, and stained as described for sural nerve specimens. Portions of the ventral and dorsal spinal roots were also fixed and processed in the same manner. Some of the quantitative aspects of the peripheral nervous system findings in Cases 2 and 3 were previously published.[16] Some descriptive pathologic findings in the peripheral nervous system in Cases 12 to 14 also were roughly described previously.[9]

Numbers and diameters of sympathetic ganglion neurons and

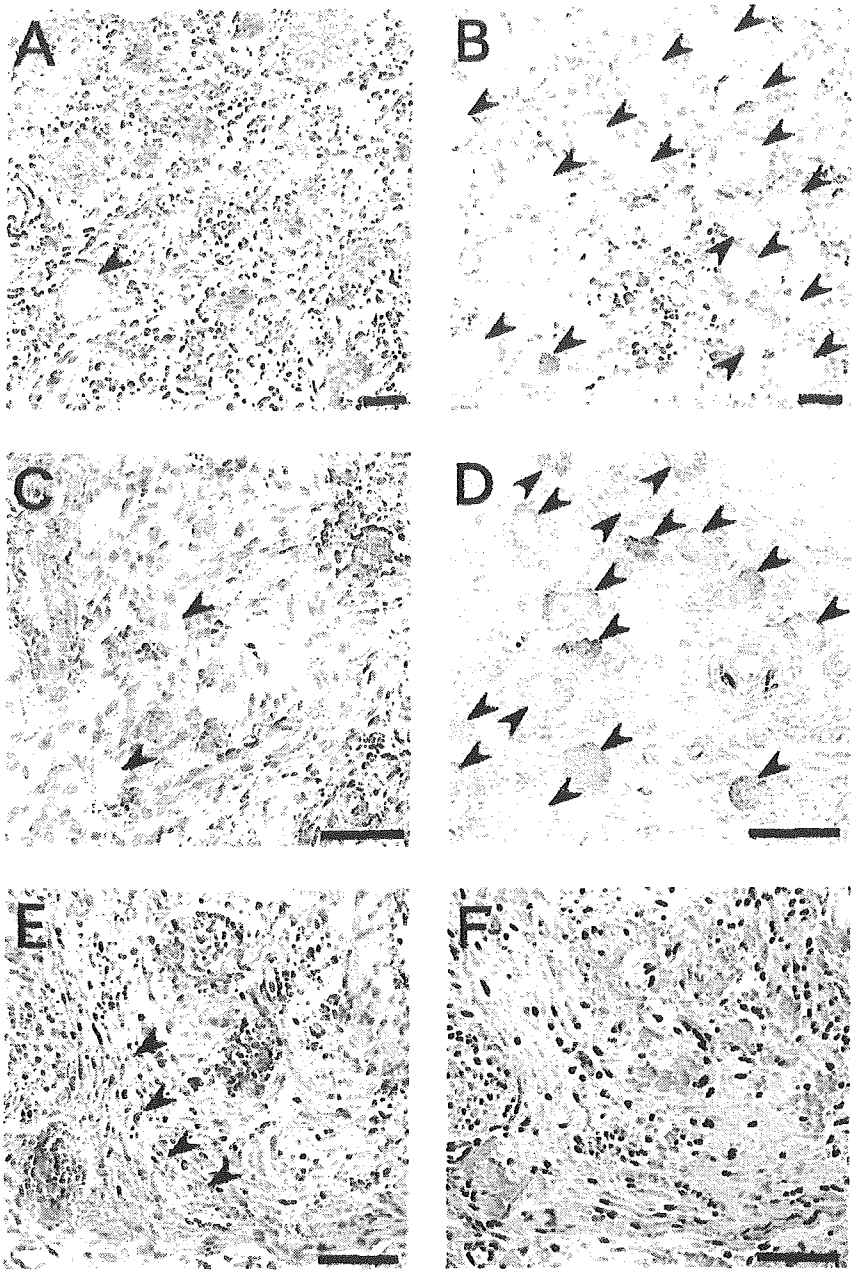

Figure 1. Representative postmortem findings in the peripheral nervous system. Amyloid deposits are identified by anti-human transthyretin antibody (A to E) or Congo red (F). (A, B) Dorsal root ganglia from early- and late-onset cases, respectively. (C, D) Sympathetic ganglia from early- and late-onset cases, respectively. In the dorsal root ganglia and sympathetic ganglia, amyloid deposition and neuronal cell loss are conspicuous in early-onset cases (A and C), whereas these are less severe in late-onset cases (B and D). Arrowheads indicate remaining neurons. (E, F) Consecutive specimens of the proximal part of sciatic nerve from a late-onset case. Amorphous material showing staining for transthyretin (E) but not with Congo red (F) is present in the subperineurial space (arrowheads). Bar = 40 μm.

dorsal root ganglion neurons were assessed using the image analyzer (Luzex FS). One hundred serial 10-μm-thick transverse sections at the middle portion of ganglia were prepared. Every tenth section was stained with the Klüver–Barrera method. Neurons showing obvious nucleoli in the sections were counted and measured to avoid split cell error. Number of neurons and area of ganglia on each section were assessed to calculate the density (neurons/mm^2). Values of neuronal cell density were expressed as means ± SD for these sections. For neuronal cell diameters, all neurons counted on 10 sections were measured. Values of neuronal cell diameter were expressed as means ± SD for these neurons. Control values for numbers and diameters of sympathetic and dorsal root ganglion neurons were obtained from four autopsy cases involving death from nonneurologic diseases.

Amounts of interstitial amyloid deposited in the parenchyma of various organs also were assessed using the Luzex FS analyzer. The proportion of area occupied by amyloid in each organ was determined as the extent of areas showing Congo red staining with emerald-green birefringence in polarized light and expressed as a percentage of the total transverse area. The proportion was assessed as the mean value for randomly selected areas covering at least 1 cm^2 of >10 sections. For nerve specimens, the proportion of area showing amyloid deposition to total endoneurial area was calculated. For the gastrointestinal tract, the proportion of area with amyloid deposition was assessed in the lamina muscularis mucosa.

Immunohistochemical assessment was performed with a peroxidase–antiperoxidase method using anti-human transthyretin antibody (Santa Cruz, CA) in consecutive deparaffinized sections.

Quantitative data, presented as means ± SD, were compared with control values. Statistical analyses were performed using the χ^2 test or the Mann–Whitney *U* test as appropriate. Values of p of <0.05 were considered to indicate significance.

Results. *Pathologic findings in sural nerve specimens.* In the early-onset cases, the density of large myelinated fibers was 914 ± 1,018 fibers/mm^2 (26% of age-matched normal control values) and that of small myelinated fibers was 451 ± 608 fibers/mm^2 (9% of age-matched normal control values), shown in table 2. Small myelinated fibers showed greater depletion than large myelinated fibers when myelinated fibers were not severely depleted overall (Cases 7 to 11). In late-onset cases, fiber size distribution

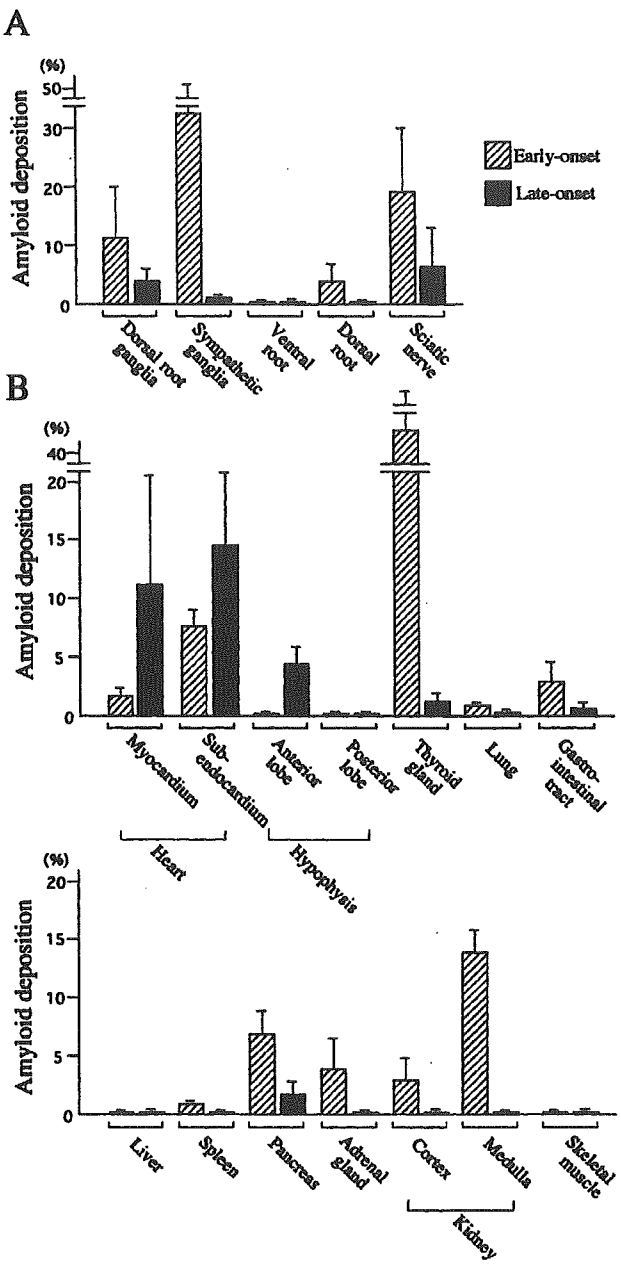

Figure 2. Amounts of interstitial amyloid deposited in the parenchyma of nervous system (A) and visceral organs (B). Hatched columns indicate mean values of early-onset cases (Cases 1 to 3), whereas filled columns indicate those of late-onset cases (Cases 12 to 14). Whiskers represent SDs. (A) Amyloid deposits in the nervous system are conspicuous in early-onset cases; these are less severe in late-onset cases. Amyloid deposition is more severe in sympathetic than dorsal root ganglia in early-onset cases, whereas the reverse pattern is seen in late-onset cases. (B) In early-onset cases, amyloid deposition is more prominent in the thyroid gland, gastrointestinal tract, pancreas, adrenal gland, and kidney than in late-onset cases. Amyloid deposition in early-onset cases is especially prominent in the thyroid gland and kidney, where deposition is scarce in late-onset cases. On the other hand, in late-onset cases, deposition is greater in the heart and anterior lobe of the hypophysis than in early-onset cases. Amyloid is scarce or absent in both groups in the posterior lobe of the pituitary gland, liver, and skeletal muscle.

of myelinated fibers, as indicated by the ratio of small to large myelinated fibers, was variable. Six cases (12, 14, 16 to 18, and 22) showed relative preservation of small myelinated fibers, whereas five cases (13, 15, 19 to 21) showed predominantly small-fiber loss as in early-onset cases. On average, density of large myelinated fibers was 333 ± 386 fibers/mm^2 (12% of age-matched normal control values) and that of small myelinated fibers was 789 ± 776 fibers/mm^2 (16% of age-matched normal control values). Axonal sprouting was scarce or absent in the early-onset group (5 ± 7/mm^2) but was relatively conspicuous in the late-onset group (92 ± 88/mm^2). Unmyelinated fibers were depleted more severely in the early- than the late-onset group (993 ± 1,305 vs 7,308 ± 5,417 fibers/mm^2; $p = 0.008$).

Amyloid deposition was scarce or absent in most cases in both groups, but relatively conspicuous deposition was observed in two of the early-onset cases (4 and 10). In both groups, amyloid was found in the endoneurium, both with and without relationship to small vessels.

Postmortem findings in the nervous system. Central nervous parenchyma was essentially intact in both groups, except that a small-cell carcinoma of the lung had metastasized to the cerebellum in Case 12, as previously described.[9,18] In the spinal cord, motor neurons as well as neurons in the Clarke columns were well preserved, and minimal to moderate myelinated fiber loss was observed in the posterior columns in both groups. Central chromatolysis was observed in spinal motor neurons in both groups. Amyloid deposits were not found in spinal cord parenchyma in any case.

In the dorsal root ganglia, amyloid deposits and neuronal cell loss were conspicuous in early-onset cases; these were less severe in late-onset cases (figures 1, A and B, and 2A). The mean diameter of remaining dorsal root ganglion neurons was larger in early-onset cases as compared with normal controls, suggesting predominant loss of small neurons (table 3). Mean neuronal cell diameters in late-onset cases were not notably different from those in normal controls, suggesting loss of neurons of all sizes. In the sympathetic ganglia, amyloid deposition and neuronal cell loss were very prominent in early-onset cases but less so in late-onset cases (see figures 1, C and D, and 2A). Size-selective neuronal cell loss was likely to be observed as in dorsal root ganglia (see table 3). Amyloid deposition and neuronal cell loss were more severe in sympathetic than dorsal root ganglia in early-onset cases, whereas the reverse pattern was seen in late-onset cases (see figure 2A and table 3). In the ventral spinal root, amyloid deposition was not apparent or only minimally present in both groups; likewise, myelinated fiber loss also was mild or not apparent in both groups. In the dorsal spinal root, amyloid deposition and myelinated fiber loss were moderately conspicuous in all early-onset cases but absent or minimal in late-onset cases. In sciatic and tibial nerves, amyloid deposition was more prominent in early-onset cases than the late-onset case (figure 3A). However, the late-onset case showed considerable myelinated fiber loss despite relative paucity of amyloid deposition (see figure 3B). In the median nerve, amyloid deposition also was more severe in early-onset cases than in the late-onset case, whereas myelinated fiber loss was more severe in the late-onset case than in early-onset cases. Amorphous material showing staining for transthyretin but not with Congo red was

Table 3 Neuronal cell loss and diameter in sympathetic and sensory ganglia

Case no.	Dorsal root ganglia		Sympathetic ganglia	
	Neuronal cell density, no./mm^2	Diameter of neurons in dorsal root ganglia, μm	Neuronal cell density, no./mm^2	Diameter of neurons in sympathetic ganglia, μm
Early-onset group				
1	4.0 ± 0.9	54.3 ± 8.6	4.2 ± 2.2	24.3 ± 3.8
2	1.9 ± 1.2	53.7 ± 8.8	4.0 ± 1.6	23.7 ± 4.2
3	2.6 ± 1.0	56.0 ± 9.0	1.6 ± 1.4	24.5 ± 3.3
Late-onset group				
12	7.5 ± 1.6	46.2 ± 11.4	46.2 ± 7.4	22.9 ± 4.6
13	4.8 ± 1.2	45.4 ± 9.1	44.8 ± 6.2	19.8 ± 4.6
14	5.8 ± 1.3	49.9 ± 11.4	ND	ND
Controls, n = 4	10.2 ± 3.2	48.3 ± 16.3	60.4 ± 6.6	20.0 ± 4.2

Values of neuronal cell density were expressed as means ± SD for densities on 10 sections as described in Patients and Methods. Values of neuronal cell diameter were expressed as means ± SD for all neurons counted on 10 sections. Control values were obtained from four autopsied cases.

ND = not determined.

abundant in the subperineurial space of the nerve trunk in the late-onset case (see figure 1, E and F), being less plentiful in early-onset cases.

Pathologic findings in visceral organs. In early-onset cases, amyloid deposition was more prominent in the thyroid gland, gastrointestinal tract, pancreas, adrenal gland, and kidney than in late-onset cases (figures 2B and 4). Amyloid deposition in early-onset cases was especially prominent in the thyroid gland and kidney, where deposition was scarce in late-onset cases. On the other hand, in late-onset cases, deposition was greater in the heart and the anterior pituitary lobe than in early-onset cases. Amyloid was scarce or absent in both groups in the posterior lobe of the pituitary gland, liver, and skeletal muscle. The heart weighed 420, 440, and 450 g in early-onset cases, whereas in late-onset patients dying of heart failure, it was 690 and 700 g. In a late-onset patient who died of lung cancer and had relatively short clinical duration of amyloid neuropathy, the heart weight was 380 g. In early-onset cases, cardiac amyloid deposition was prominent in the atrium and the subendocardial region. In the myocardium, amyloid was observed mainly in relation to walls of vessels, particularly arterioles. In the subendocardial layer, myocardial cells showed atrophy, degeneration, and eventual cell loss, producing a histologic picture of amyloid rings (see figure 4A). Among late-onset cases, amyloid was prominent throughout myocardium in two cases (13 and 14), whereas amyloid rings or atrophy of the myocardium was not apparent in any case (see figure 4B). The anterior lobe of the hypophysis showed scarce or no amyloid deposition in early-onset cases, but marked parenchymal deposition was observed in late-onset cases (see figure 4, C and D).

Discussion. In this study, we compared pathologic features of early-onset FAP TTR Met30 cases from endemic foci with those of late-onset cases from non-endemic areas. In anecdotal report of pathologic findings in FAP TTR Met30 patients presenting beyond age 50,[25] the distribution of amyloid deposition differed slightly from findings in the current study. Differences may be attributable to inclusion of late-onset cases from endemic foci in that report; these patients show clinical features similar to early-onset cases in endemic foci.[11] The current study demonstrated that pathologic features of the two groups differed, as has been shown for clinical features.[9-11]

The characteristic finding in early-onset cases was predominant loss of small fibers, including unmyelinated fibers; this agrees with previous reports.[26,27] On the other hand, fiber loss patterns in our late-onset cases were variable; half of the cases showed predominantly small-fiber loss as in early-onset cases, whereas others showed relative preservation of small myelinated fibers. As a whole, the total number of myelinated fibers was more severely reduced in late- than early-onset cases. This difference correlated well with the prominent sensory dissociation noted in early-onset cases in contrast to impairment of all modalities in late-onset cases.[3,9,11] The finding that amyloid deposition and neuronal cell loss were more severe in sympathetic than sensory ganglia in early-onset cases—with the reverse pattern seen in late-onset cases—also correlated with the severity of autonomic symptoms.[3,7-9,11] Furthermore, preferential loss of small neurons in the sensory ganglia in early-onset cases and loss of neurons of all sizes in late-onset cases, as suggested by the mean diameter of remaining neurons, corresponded to clinical differences in sensory involvement. Amyloid deposition and atrophy of myocardial cells in the atrium and subendocardial layer of the myocardium, where the cardiac conduction system is located, explains a more frequent occurrence of cardiac conduction abnormalities and need for pacemaker implantation in early- than late-onset cases.[3,11] On the other hand, diffuse deposition of amyloid with ventricular wall thickening agrees well with frequent observations of cardiac hypertrophy and occur-

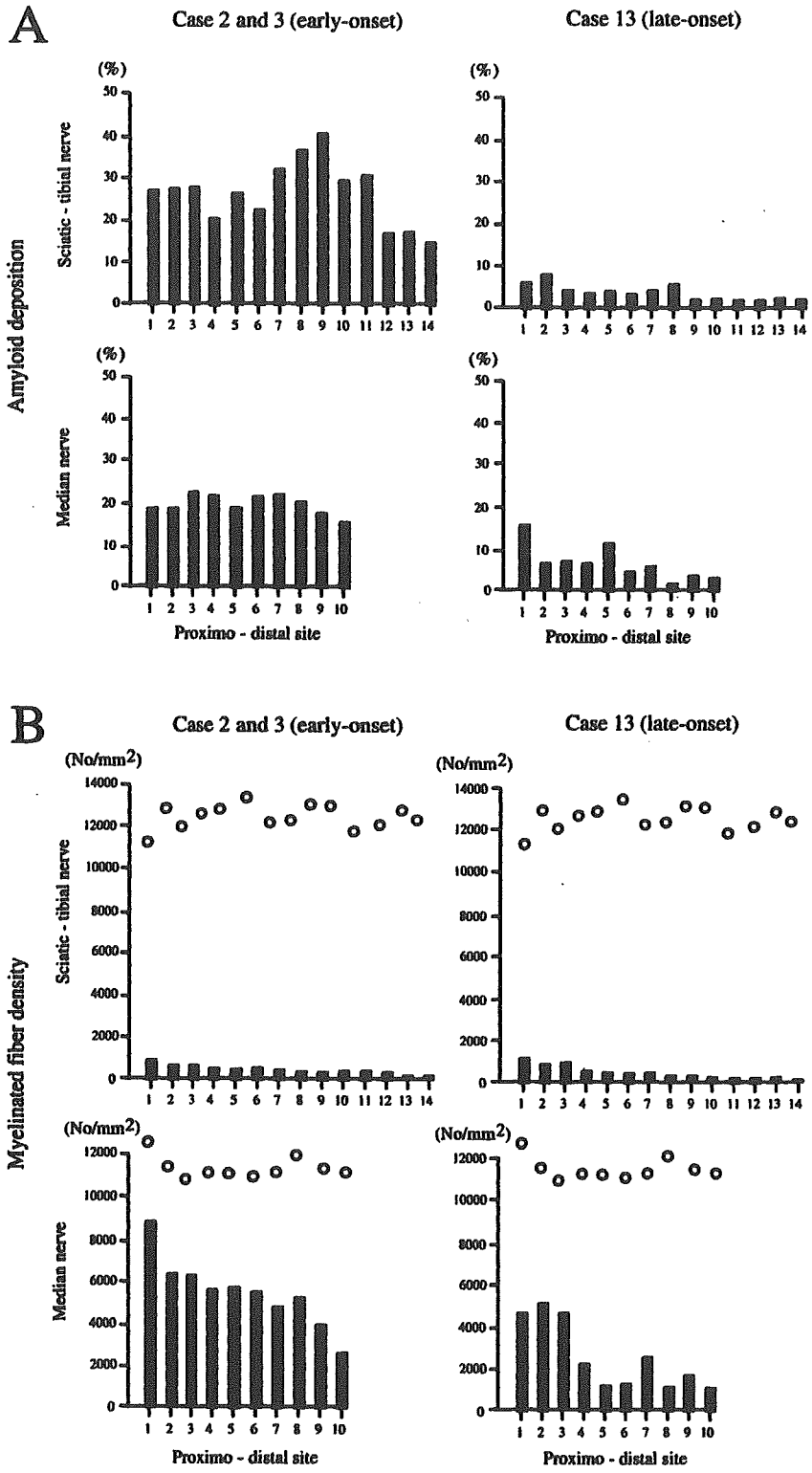

Figure 3. Proportion of amyloid deposition in the endoneurium (A) and density of myelinated fibers (B) in consecutive portions of the sciatic/tibial and median nerves from early-onset cases (Cases 2 and 3) and late-onset case (Case 13). Values for early-onset cases are represented as the means of Cases 2 and 3. Open circles represent normal control values obtained from a subject with nonneurologic disease. In sciatic and tibial nerves, amyloid deposition is more prominent in early-onset cases than in the late-onset case. However, the late-onset case showed considerable myelinated fiber loss despite relative paucity of amyloid deposition. In the median nerve, amyloid deposition also is more severe in early-onset cases than in the late-onset case, whereas myelinated fiber loss is more severe in the late-onset case than in early-onset cases.

rence of heart failure in late-onset cases.[9] Thus, differences in clinical features reported between early- and late-onset FAP TTR Met30 corresponded well to pathologic differences.

A question remains as to why the severity and distribution pattern of amyloid deposition differ between early- and late-onset cases. A longer interval from onset of neuropathic symptoms to autopsy in the early-onset group may explain some of the pathologic differences. The peripheral nervous system, thyroid gland, gastrointestinal tract, pancreas, adrenal gland, and kidney showed more severe amyloid deposition in the early-onset group, consistent with a longer duration of illness. However, the heart and hypophysis showed more prominent deposition in the late-onset group, which had a shorter clinical disease duration. The diffuse amyloid deposition observed in the ventricular myocardium in late-onset cases is

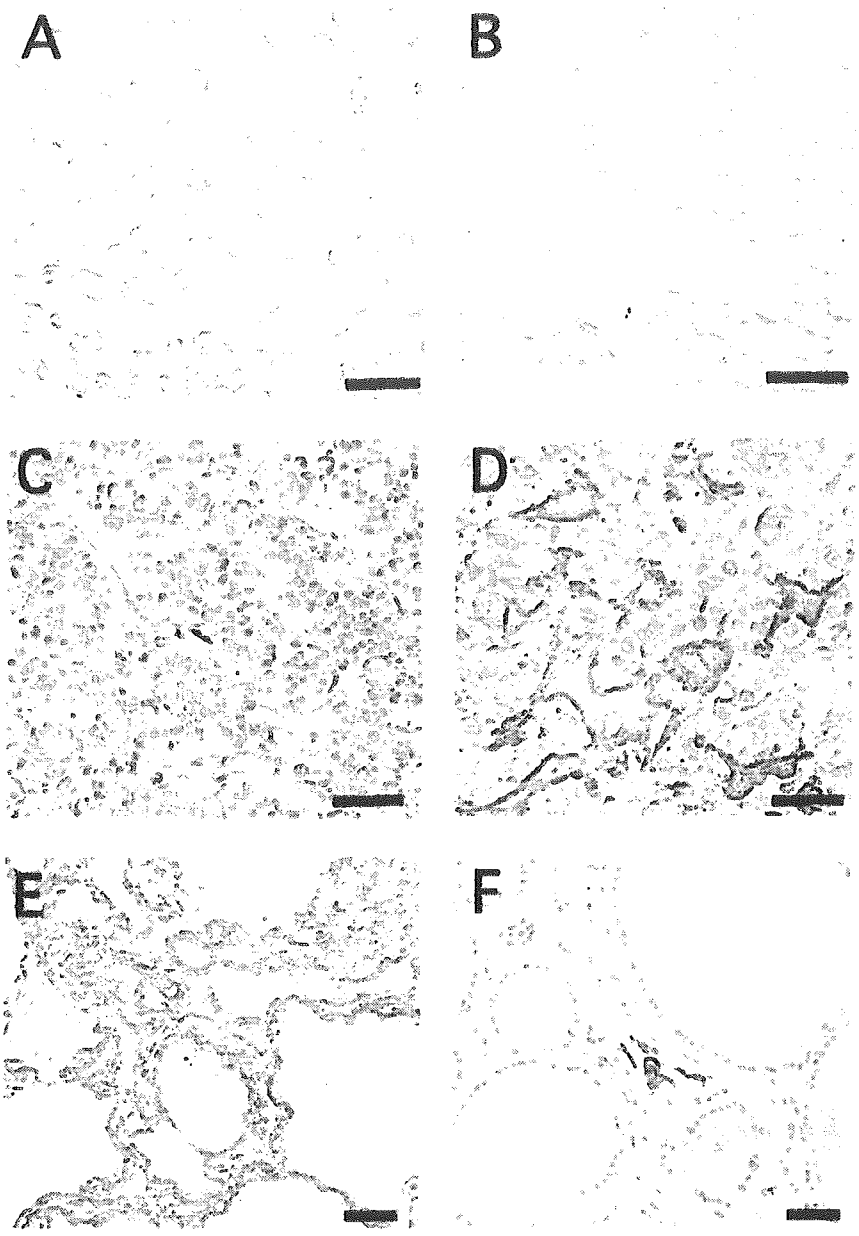

Figure 4. Representative pathologic findings in the visceral organs from early-onset cases (left) and late-onset cases (right). Amyloid deposits are identified by anti-human transthyretin antibody. (A, B) The heart. In the subendocardial layer of an early-onset case (A), myocardial cells show atrophy and amyloid rings are present. In late-onset cases, amyloid is prominent throughout myocardium (B). Amyloid rings or atrophy of the myocardium is not apparent as in (A). (C, D) Anterior lobe of the pituitary gland. Amyloid deposition is scarce in early-onset cases (C), whereas it is conspicuous in late-onset cases (D). (E, F) The thyroid gland. Amyloid deposition was especially prominent in early-onset cases (E), but it was scarce in late-onset cases (F). Bar = 40 μm.

similar to that of senile cardiac amyloidosis with deposition of normal transthyretin protein.[28] Age-related accumulation of amyloid in the interstitium of the anterior lobe of the pituitary gland also has been reported.[29-31] These observations suggest that age-dependent changes in the microenvironment of interstitial tissues in various organs determine the severity and distribution pattern of amyloid deposition in each group. For example, properties of extracellular matrix components including proteoglycans and glycosaminoglycans, which may become components of amyloid,[32] show organ-specific changes with age.[33,34] Interestingly, the posterior lobe of the hypophysis, the liver, and skeletal muscle did not show detectable amyloid deposition in cases with either age at onset. Mechanisms of underlying organ-specific, age-related amyloid deposition still need to be elucidated, but age at onset itself may influence some of the organ-specific amyloid deposition patterns.

Another question remaining is why size dependence of axonal and neuronal cell loss differed between early- and late-onset cases. Even after taking axonal sprouting into account, small myelinated and unmyelinated fibers seemed relatively well preserved in many late-onset cases. Although the pathogenesis of peripheral neuropathy in amyloidosis has not yet been clarified, possibilities might include ischemia from obliteration or dysfunction of small vessels supplying nerves,[35,36] nerve fiber compression or infiltration by amyloid deposits,[26,37] or toxic effects of amyloid precursors.[38-40] Experiments in animals as well as human studies suggest that the nonfibrillar form of transthyretin is present in tissues and exerts cytotoxicity, including oxidative stress,[40] before the congophilic fibrillar form of amyloid can be seen.[38,41]

The concept that a nonfibrillar precursor of amyloid can exert toxic effects can be extended to other pathologic conditions where amyloid is considered pathogenetically important, including Alzheimer disease.[42-45] In our study, amyloid was not deposited in late-onset cases until patients had become elderly, considering that amyloid deposition was scarce or absent in sural nerve biopsy specimens. Accumulation of amyloid may progress rapidly once initiated. Abundant nerve fiber regeneration despite scarce amyloid deposition was observed in most sural nerve biopsy specimens from our late-onset cases, indicating that axons had been degenerating long before amyloid deposition occurred in these cases. At autopsy, less amyloid deposition but severe myelinated fiber loss in nerve trunks from a late-onset case in comparison with early-onset cases support this view. Furthermore, amorphous material staining with anti-transthyretin antibody but not with Congo red, indicating presence of the nonfibrillar amyloid precursor, was more abundant in the late-onset case. Taken together, these observations support cytotoxicity from nonfibrillar transthyretin affecting all sizes of axons and neurons prior to deposition of congophilic amyloid in late-onset cases. In early-onset cases, amyloid deposition was likely to have been severe even in early stages, judging from amounts of amyloid observed. Nerve fiber compression by amyloid deposits could cause predominantly small-fiber loss, as previously suggested.[26] Differential duration of exposure to a toxic amyloid precursor may result in pathologically different modes of axonal and neuronal cell loss.

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