Transthyretin-related familial amyloidotic polyneuropathy (FAP) is a fatal hereditary amyloidosis. Until 20 years ago, FAP was thought to be restricted to endemic occurrence in certain areas. However, owing to progress in biochemical and molecular genetic analyses, FAP is now believed to occur worldwide. As of today, reports of about 100 different points of single or double mutations, or a deletion in the transthyretin gene, have been published, and several different phenotypes of FAP have been documented, even for the same mutation in the transthyretin gene. We present herein the current clinicopathological, biochemical, molecular genetic, and epidemiological aspects of transthyretin-related FAP, and we introduce a new diagnostic procedure for the disease.

Among the confirmed 100 points of mutation or deletion in the TTR gene, 13...
mutations are nonpathological forms. Other abnormal TTR mutations induce FAP, which can be classified into several phenotypes such as neuropathic, oculoleptomeningeal, and cardiac (Figure 1). Several types of ATTR mutations do not cause neuropathy, although they induce other symptoms of the FAP disorder.

Neuropathic Form of FAP

Most TTR-related FAP cases are classified as the neuropathic type. Among these neuropathic FAP forms, ATTR Val30Met is the most common; its characteristics include an autonomic, sensory dominant polyneuropathy (Figure 2). Many clinicopathological studies of this FAP type have been performed. From our data on 169 FAP patients with ATTR Val30Met, the inheritance pattern is autosomal dominant with a high penetrance rate and an equal sex ratio. The symptoms are first recognized when a patient is 18 to 83 years of age, with a mean age at onset of 35.3 years. The disease is slowly progressive and reaches the terminal stage in 10.8 years. The age at onset in this type of FAP was found to show anticipation, as seen in FAP patients in other endemic areas.

Evidence of a sensorimotor peripheral neuropathy is usually found in the lower limbs. Dissociation of sensory impairment is common, with pain and temperature sensation being the most severely affected. Autonomic nervous system involvement, such as dyshidrosis, sexual impotence, disturbances of gastrointestinal tract motility (diarrhea alternating with constipation), orthostatic hypotension, and urinary disturbances, is frequent (Figure 2). Cardiac and renal dysfunction are also commonly recognized during the course of the illness and anemia is observed. Ocular involvements such as vitreous opacity, keratoconjunctivitis sicca, glaucoma, and papillary disorders are commonly seen. The initial symptoms of 117 FAP patients with ATTR Val30Met are presented in the Table and include the clinical manifestations of an autonomic sensorimotor polyneuropathy. Significant numbers of FAP patients also often complain of diarrhea, constipation, hematuria, proteinuria, decrease in visual acuity, and cardiac arrhythmias as initial symptoms. To diagnose ATTR Val30Met-type FAP, cooperation among gastroenterologists, nephrologists, ophthalmologists, and cardiologists is essential.

**Leptomeningeal Type of FAP**

Attention has recently focused on the oculoleptomeningeal form of FAP (induced by several point mutations in ATTR Val30Met). Most TTR-related FAP cases are classified as the neuropathic type. Among these neuropathic FAP forms, ATTR Val30Met is the most common; its characteristics include an autonomic, sensory dominant polyneuropathy (Figure 2). Many clinicopathological studies of this FAP type have been performed. From our data on 169 FAP patients with ATTR Val30Met, the inheritance pattern is autosomal dominant with a high penetrance rate and an equal sex ratio. The symptoms are first recognized when a patient is 18 to 83 years of age, with a mean age at onset of 35.3 years. The disease is slowly progressive and reaches the terminal stage in 10.8 years. The age at onset in this type of FAP was found to show anticipation, as seen in FAP patients in other endemic areas.
in the TTR gene), as well as on the advanced stages of ATTR Val30Met–type FAP.\textsuperscript{13,14} Cerebral amyloid angiopathy and ocular amyloidosis are common characteristic clinical features in those types of FAP.\textsuperscript{13,14} Cerebral amyloid angiopathy is characterized by amyloid deposition in the media and adventitia of medium and small arteries, arterioles, and occasionally veins of the cortex and leptomeninges.\textsuperscript{14} Typical clinical central nervous system manifestations include cerebral infarction and hemorrhage, hydrocephalus, ataxia, spastic paralysis, convulsion, and dementia.\textsuperscript{13,14} These symptoms are often found in several types of TTR-related FAP and lead to classification into (oculo)leptomeningeal amyloidosis, in which amyloid deposition is also found in vitreous bodies and other tissues of the eye.\textsuperscript{13,14} Although amyloid deposits in the meningo cerebrovascular system were thought to be the cause of those central nervous system symptoms, the precise mechanism of amyloid formation remains to be elucidated.

**NEUROPATHOLOGICAL FEATURES**

Although various pathological reports of amyloid deposition in systemic organs have been published, the scale of the studies was small. A clinicopathological, histochecmical, immunohistochemical, and ultrastructural study of materials obtained by autopsy or biopsy in 17 patients was performed by Takahashi et al.\textsuperscript{15} In the autopsy cases, amyloid deposits were predominant in the peripheral nerve tissues, autonomic nervous system, chondroid plexus, cardiovascular system, and kidneys. Amyloid involvement in the anterior and posterior roots of the spinal cord, spinal ganglia, thyroid, and gastrointestinal tract was also frequent. In the cardiac conduction system, amyloid deposition was prominent in the sinoatrial node and in the limbs of the intraventricular bundle. In sural nerve biopsy specimens from patients with early-stage FAP, amyloid deposits were observed in the small vessel walls and the surrounding tissues. In the advanced cases, amyloid deposits were found in the subperineural and/or epineural regions. The numbers of myelinated and unmyelinated nerve fibers decreased markedly, which indicated degenerative changes in Schwann cells. Degenerative changes in the axon, myelin sheath, and Schwann cells and the decrease in collagen fibers paralleled the severity of the peripheral nerve disturbances.

Morphometric data on the number and caliber of myelinated fibers in the sural nerve biopsy specimen from patients with early-stage FAP showed a greater reduction of small-caliber fibers than of large-caliber fibers. In patients with more advanced FAP, the numbers of myelinated fibers were decreased.\textsuperscript{15}

**BIOCHEMICAL ASPECTS OF AMYLOIDOGENESIS**

It is widely believed that stabilizing tetrameric TTR, as a potential therapeutic strategy, is a prerequisite for prevention of amyloid formation, especially in ATTR Val30Met–type FAP.\textsuperscript{16} McCutchen et al\textsuperscript{17} first demonstrated this concept by using recombinant wild-type TTR and variant TTR. Alves et al\textsuperscript{18} reported that subjects possessing the TTR Thr119Met gene are asymptomatic carriers and that compound heterozygotes with ATTR Val30Met and TTR Thr119Met genes show very mild FAP symptoms or have no symptoms. These authors also showed, by means of semidenaturing isoelectric focusing, marked TTR tetrameric structural stability in patients with TTR Thr119Met or ATTR Val30Met/TTR Thr119Met genes.\textsuperscript{18} Various studies confirmed these findings, and TTR tetrameric structural stability in compound heterozygotes has now been widely accepted. In addition, Terazaki et al\textsuperscript{19} described an interesting compound heterozygote patient with late-onset FAP with ATTR Val30Met/TTR Arg104His mutation who had very mild and slowly progressive clinical symptoms, and whose tetrameric TTR stability was greater than that of the TTR from a compound heterozygote patient with ATTR Val30Met/TTR Thr119Met mutation.

These reports suggest the therapeutic possibility of stabilization of the tetrameric form of TTR. Because thyroxine is one of the most important molecules for stabilizing tetrameric TTR, thyroxine-based therapeutic drugs have been proposed. Baures et al\textsuperscript{20} also tested various nonsteroidal anti-inflammatory drugs for stabilization of the tetrameric form of TTR because the nonsteroidal anti-inflammatory drug structures resemble the structure of thyroxine and these drugs bind to TTR via a thyroxine binding site. These authors reported that flufenamic acid, a nonsteroidal anti-inflammatory drug, showed promise for tetrameric TTR stabilization. However, most nonsteroidal anti-inflammatory drugs bind to albumin in plasma, so the bioavailability of these drugs for TTR stabilization may be insufficient.

Recent studies have suggested that certain metal ions affect amyloidogenesis in several types of amyloidosis. In FAP, metal ions may influence the stability of the tetrameric form of TTR. Ando\textsuperscript{21} therefore investigated whether various metal ions (eg, Zn\textsuperscript{2+}, Cu\textsuperscript{2+}, Ca\textsuperscript{2+}, Fe\textsuperscript{3+}, Al\textsuperscript{3+}, and Cr\textsuperscript{3+}) affect amyloidogenesis of wild-type TTR and ATTR. Among the metal ions, Cr\textsuperscript{3+} increased the tetrameric stability of both wild-type TTR and ATTR Val30Met purified from healthy subjects and homozygote FAP patients, respectively, and suppressed TTR amyloidogenesis induced by low pH levels in a concentration-dependent manner. In contrast, Al\textsuperscript{3+} decreased TTR tetrameric stability and induced TTR amyloidogenesis in a concentration-dependent manner. These findings indicate that Cr\textsuperscript{3+} and Al\textsuperscript{3+} act as a suppressor and an inducer, respectively, of TTR amyloidogenesis, although in vivo evaluation of the effects is needed. An ingredient in health foods, Cr\textsuperscript{3+} is widely used throughout the world, so administration of this metal ion to patients with FAP would present no problems.

**MOLECULAR GENETICS**

The human TTR gene was localized at 18p11.1-q12.3, and its structure was first determined by Tsuzuki et al.\textsuperscript{22} Complementary DNA coding for the human TTR gene was cloned from a complementary DNA library prepared from human liver; genetic analysis used the restriction enzyme NsiI. In 100 abnormal TTR genes, 0, 36, 40, and 24 points
of mutation were detected in exons 1, 2, 3, and 4, respectively; a deletion in the TTR gene also occurred.

The main sites of production of TTR confirmed by in situ hybridization methods are the liver, retinal pigment epithelium, choroid plexus of the brain, and visceral yolk sac endoderm.23

Clinical Genetic Testing

For the screening of FAP, we first performed mass spectrometric analysis (Figure 3) with a system for serum and cerebrospinal fluid that uses methods such as electron spray ionization–mass spectrometry and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry. With this system, the presence or absence of mutations in the TTR gene can be determined. A shift in the molecular mass caused by substitution of amino acids can be detected as a peak that differs from those identifying the wild-type TTR gene.

Another method, in addition to polymerase chain reaction and restriction endonuclease analysis, has recently been used for screening in patients who have a family history or typical clinical manifestations or who come from an area where FAP is prevalent. By means of the LightCycler method (Roche Diagnostics, Basel, Switzerland),24 we can detect the presence or absence of a TTR mutation in 1 hour.

Detection of ATTR Val30Met via the LightCycler. LightCycler technology can detect mutations quickly and accurately by using fluorescent hybridization probes and melting curves.25 For example, an anchor probe (5′-TGTGGCCGTTGCTGT-3′-fluorescein isothiocyanate, in which the underlined nucleotide is the normal nucleotide) and a sensor probe (LC Red 640-5′-CAGAAAGGCTGCTGATGACACCTGGGAGCCATTTGCCTCTGGG-3′-OH) were used to detect ATTR Val30Met. Because a single mismatch can significantly reduce the melting temperature of the oligonucleotide, the melting temperature is reduced when the amplified gene encodes ATTR Val30Met. Therefore, it is possible with the use of melting curve analysis to discriminate among a healthy subject, an FAP ATTR Val30Met homozygote, and an FAP ATTR Val30Met heterozygote (Figure 3A).

Nonisotopic Single-Strand Conformational Polymorphism. Single-strand conformational polymorphism (SSCP) was applied with samples with molecular mass shift in TTR to determine the exon in which a mutation was present.26 However, a conventional type of SSCP requires a radioisotope to perform the procedure. Therefore, to avoid the need for a radioisotope, we recently developed SSCP analysis with capillary electrophoresis27 in which a forward primer labeled with Cy5 and a microchip (iChip 12; Hitachi Chemical Co, Ltd, Tokyo, Japan) filled with a separation gel for SSCP (i-S gel 3; Hitachi Chemical Co, Ltd) are used. An example of SSCP analysis with capillary electrophoresis of an FAP ATTR Tyr114Cys heterozygote is presented herein. As shown in Figure 3B, an additional peak is observed when the amplified gene encodes for ATTR Tyr114Cys.

Other Diagnostic Methods

Ando et al28 presented a histochemical method of diagnosis of ATTR Val30Met by using the hair of FAP patients and monoclonal antibody supplied by Paulo M. P. Costa, PhD, Centro de Estudos de Paramiloidose, Porto, Portugal. Also, Ando et al28 demonstrated abundant ab-
normal ocular vessels in FAP patients withATTR Val30Met, a finding that has diagnostic value.

**EPIDEMIOLOGY**

The presence of patients with TTR-related FAP has been confirmed in more than 30 countries, with ATTR Val30Met verified in patients in more than 15 countries6 (Figure 4). Only ATTR Val30Met–type FAP has large foci in the world, although the reason for this is not known. Holmgren et al30 performed an epidemiological study in the northern part of Sweden and estimated that the number of ATTR Val30Met gene carriers in a total population of 500,000 in the area was approximately 7500, although the penetrance of the mutation was as low as about 2%. By 1994, 1233 FAP patients with ATTR Val30Met from 489 pedigrees had received a diagnosis at Centro de Estudos de Paramidoidose in Porto, Portugal.30 So far, more than 1500 FAP patients have been registered in Portugal (M. J. M. Saraiva, PhD, oral communication, September 2003). In Japan, more than 350 FAP patients were found in the following 2 endemic foci: Arao city in the Kumamoto prefecture and Ogawa village in the Nagano prefecture. In addition, 44 FAP kindreds with ATTR Val30Met were traced. They were genealogically independent and were geographically scattered throughout Japan.

**ORIGIN OF FAP ATTR Val30Met**

As already described, in the 1960s, large foci of patients were found in Japan and Sweden,3,6 in addition to Portugal. These 3 countries are geographically distant, and a consanguineous relationship between foci has not been identified. The issue of whether there is a common origin in the foci for a mutant allele has not been resolved. Furthermore, Continho31 hypothesized that a mutant allele in the Portuguese kindred could be a single origin of the mutation for FAP foci throughout the world, including Japan, Europe, North and South America, and Africa. This hypothesis was based only on well-known historical relations and has not been scientifically tested. Ohmori et al32 compared haplotypes in several foci of patients with FAP and discovered that a common founder could be conceivable for Japanese and Portuguese patients and for Portuguese and Spanish patients but not for Swedish and other patients. Additional studies of genotypes and phenotypes are needed.

**LIVER TRANSPLANTATION**

For treatment of FAP, liver transplantation has been reported to halt the progression of clinical manifestations.33 According to data in the Familial Amyloidotic Polyneuropathy World Transplant Registry, 54 centers in 16 countries have performed orthotopic liver transplantation (OLT) for FAP. During 2003, approximately 60 OLTs were performed worldwide. During the past decade, 539 patients have undergone 579 OLTs. Survival of the patients has been excellent (overall 5-year survival of 77%) and comparable to the survival for OLT performed for other chronic liver disorders, but a longer follow-up is needed to compare the outcome after OLT with the natural course of the disease. The main cause of death was related to cardiac difficulties (39%).34

The use of sequential liver transplantation with resected livers from patients with FAP started in Portugal; more than 50 patients have received livers from FAP pa-

---

Figure 4. Distribution of familial amyloidotic polyneuropathy (FAP) in the world. Locations of foci of patients with FAP amyloidogenic mutated transthyretin Val30Met described in previous reports and obtained from personal communications are presented. The size of the circles is related to the number of patients at each location.

©2005 American Medical Association. All rights reserved.
tients.  Although no patients have started to show the clinical symptoms of FAP, careful neurological follow-up examinations should be continued.

In conclusion, TTR-related amyloidosis is not an insignificant disease, and many more affected patients would likely be found if more careful and precise investigations were performed. The collaboration of neurologists, gastroenterologists, ophthalmologists, and cardiologists is needed to make a definitive diagnosis.

Accepted for Publication: June 27, 2004.
Correspondence: Yukio Ando, MD, Department of Diagnostic Medicine, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-0811, Japan (yukio@kaiju.med.kumamoto-u.ac.jp).
Author Contributions: Study concept and design: Ando. Acquisition of data: Ando and Nakamura. Analysis and interpretation of data: Ando, Nakamura, and Araki. Drafting of the manuscript: Ando and Nakamura. Critical revision of the manuscript for important intellectual content: Araki. Statistical analysis: Nakamura. Obtained funding: Ando. Administrative, technical, and material support: Ando and Nakamura. Study supervision: Araki.

Funding/Support: This study was supported by grants from the Amyloidosis Research Committee, the Pathogenesis and Therapy of Hereditary Neuropathy Research Committee for Surveys and Research on Specific Diseases from the Ministry of Health and Welfare of Japan, Charitable Trust Clinical Pathology Research Foundation of Japan, and Research for the Future Program Grant and Grants-in-Aid for Scientific Research (B) 15390275 and (B) 20253742 from the Ministry of Education, Science, Sports and Culture of Japan, Tokyo, Japan.

REFERENCES