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Clinical and histopathological features of familial amyloidotic polyneuropathy with transthyretin Val30Ala in a Chinese family

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ABSTRACT

Familial amyloidotic polyneuropathy (FAP) is characterized by extracellular deposition of amyloid fibrils caused by a point mutation in the transthyretin (*TTR*) gene. TTR amyloidosis is linked to a vast number of mutations with varying phenotype and tissue distribution. Several Chinese kindred with FAP type 1 have been reported in Beijing, Hong Kong, Taiwan, and elsewhere. Here, histopathological features and *TTR* gene polymorphism were analyzed by using autopsy and blood specimens from a Chinese proband of a family with FAP. This proband is a 34-year old man with FAP type 1 who developed motor, sensory and autonomic impairments with neuropathy, gastrointestinal dysfunction, and orthostatic hypotension. Genetic findings of *TTR* revealed a T to C transition in codon 30 causing the mutation TTR Ala30. This patient died of respiratory and circulatory failure 7 years after onset. Autopsy showed heavy amyloid deposition in the peripheral nerves, liver, testes, thyroid, pancreas and muscles. There was moderate deposition in the heart, kidneys, bladder, gastrointestinal tract, tongue, lung, blood vessels, and gall bladder. The spleen showed only slight deposition, and none was observed in the central nervous system. TTR amyloidosis was confirmed by immunochemical staining with a specific TTR antibody. These results indicate that the distribution of amyloid deposition, (i.e., heavy in the liver, testes and slight in the spleen), is a characteristic feature and reflects the severity of FAP with TTR Val30Ala.

1. Introduction

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disease characterized by amyloid deposition in various organs, and is caused by a point mutation in the transthyretin (*TTR*) gene. Polyneuropathy, autonomic neuropathy, vitreous opacity, cardiomyopathy and renal failure are the major manifestations. The gene encodes TTR, a tetrameric serum protein of four identical subunits of 14 kDa which is synthesized mainly in the liver and the choroid plexus of the brain. It acts as a transport protein for thyroxine and retinol, is a cryptic protease for apolipoprotein AI [1,2] and is degraded, in the rat, in the kidney, liver, muscle and skin. More than 100 amino acid substitutions of variant TTRs, including cases without pathological findings, have been reported elsewhere [3]. The most common variant of TTR in FAP is a replacement of valine by methionine at position 30, Val30Met (TTRMet30). Genetic differences lead to the heterogeneity of clinical manifestations due to the variant deposition of amyloid fibrils. The distribution of variant TTR is not necessarily the same as the region of synthesis and degradation [4].

FAP kindreds are now known to exist in many nations worldwide. In Hong Kong in 1989, several FAP families were reported with different *TTR* gene mutations [5], including the Val30Met and Val30Ala TTR types of FAP. Clinical and pathologic features of FAP type 1 are relatively clear in Europe and Japan, but few clinicopathologic or biochemical analyses for FAP using autopsy have been performed in China. Moreover, there have been no detailed systemic pathological findings, proven by autopsy, that have identified FAP with the Val30Ala TTR type.

In this report, we provide clinicopathologic features and autopsy findings of FAP with TTR Val30Ala from a proband in a Chinese family. The results indicate that the distribution of amyloid deposition, which is heavy in the liver, testes and slight in the spleen, is a characteristic feature and reflects the severity of FAP with TTR Val30 Ala.

2. Material and methods

2.1. Case presentation

The proband was a 34-year-old Chinese man who had been complaining of alternating diarrhea and constipation, and vomiting

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for 2 years following a weight loss of 20 kg. At the same time, he had noticed severe paresthesia and weakness in his legs which lead to a gait disturbance. His father, who died at 39 years of age, had also been afflicted with FAP and complained of severe diarrhea, motor, sensory and autonomic impairments with neuropathy. His maternal grandmother died suddenly with the same symptoms, age unknown but exceeding 40 years old. His sister is normal. The clinical history pointed to a genetic explanation for these symptoms (Fig. 1).

On admission, physical examination revealed hypotension (50/40 mmHg), cachexia, and cardiac enlargement. Neurologic examination showed muscle weakness with atrophy, predominantly affecting the lower extremities. Deep tendon reflexes were all absent. Severe impairment from pain and reduced light touch sensation were present, predominantly in the distal part of the four extremities. Autonomic dysfunction included abnormal gastrointestinal motility, neurogenic bladder, and postural hypotension. Electrocardiogram was always normal in several physical examinations. In the late stages of the disease, nerve conduction studies demonstrated that the velocity and compound muscle action potential amplitude of the right median peroneus and the right posterior tibial nerve could not be detected. Similarly, sensory conduction velocity and sensory action potential amplitude of the right median, ulnar and peroneus superfic nerves could also not be detected. An electromyogram showed a neurogenic pattern in the four extremities. These symptoms and examinations indicated severe polyneuropathy with autonomic failure. A biopsy of mouth mucosa confirmed amyloidosis. Approximately 7 years after the onset of symptoms, this patient died at the age of 34 years from respiratory and circulatory failure. Autopsy was performed with the informed consent of his family.

2.2. TTR gene analysis

A blood sample of the proband was obtained for DNA analysis from his consenting mother. After extraction of genomic DNA from lymphocytes, sequencing of the full coding region of the gene was performed. Briefly, the coding sequence of the entire *TTR* gene was amplified by polymerase chain reaction (PCR) using four sets of primers. The PCR products were purified by electrophoresis in 2% agarose gel and sequenced directly, with the same set of primers as for amplification. Permission to use this material was obtained from the local ethics committee.

2.3. Morphological study

Autopsy was performed within 3 h of death of the proband. Specimens were embedded in paraffin after formalin fixation, and sliced at 5 μm thicknesses. Serial sections were examined after hematoxylin and eosin (H&E) staining. Amyloid deposits were characterized by their congo red staining affinity and birefringence with thioflavin under polarized microscope, and by immunolabeling with polyclonal rabbit antibodies to transthyretin (Dako, Denmark). Transverse semi-thin sections were stained with toluidine blue staining. Multiple level sections of each specimen were studied to clearly delineate the distribution of amyloid deposits.

3. Results

3.1. DNA sequence analysis

Genetic findings for *TTR* revealed a T to C transition in codon 30 of exon 2 causing a mutation of TTR Ala30. Sequence analysis of other DNA stands revealed the normal sequence of the *TTR* gene, which encodes for the normal valine at codon 30 in exon 2. This indicates that the proband was heterozygous for the mutant gene.

3.2. Autopsy findings

Staining with congo red (plus green birefringence with polarized light) has been the key diagnostic criteria for FAP, although immunohistological detection with specific antibodies further narrows the diagnosis to a specific protein. In the present case, histochemical examination showed heavy amyloid deposition in the peripheral nerves, liver, testes, thyroid, pancreas and muscles. Severe neuronal loss in myelinated nerves was observed. Toluidine blue staining in transverse semithin sections displayed the loss of nerve fibers, predominately in myelinated fibers of the sciatic nerves. Immunohistochemical staining for TTR revealed extracellular amyloid deposition in the endoneurium adjacent to the perineurium, the perineurium, the epineurium and in the capillary wall and connective tissue. The biceps brachii muscle was atrophic. Heavy amyloid deposition was detected in the endoneurium of the nerves and in the capillary walls in the biceps brachii. In the pancreas, amyloid deposits were observed in the exocrine, blood vessel walls, and nerve tracts. The exocrine of the pancreas was separated into sporadic small glands by amyloid substances. In the liver, amyloidosis was more predominant in the area of the header than in the lobule. Comparison of the same sections stained with congo red under polarized light and bright light revealed complete agreement for the pattern of staining.

Other organs contained moderate amyloid deposits, including the heart, kidney, bladder, gastrointestinal tract, tongue, lung, abdominal aorta, lung and gall bladder. Moderate amyloidosis was present in a variable fraction of glandular cells of the stomach, duodenum and small intestine. Staining was performed with TTR antibody using paraffin embedded material.

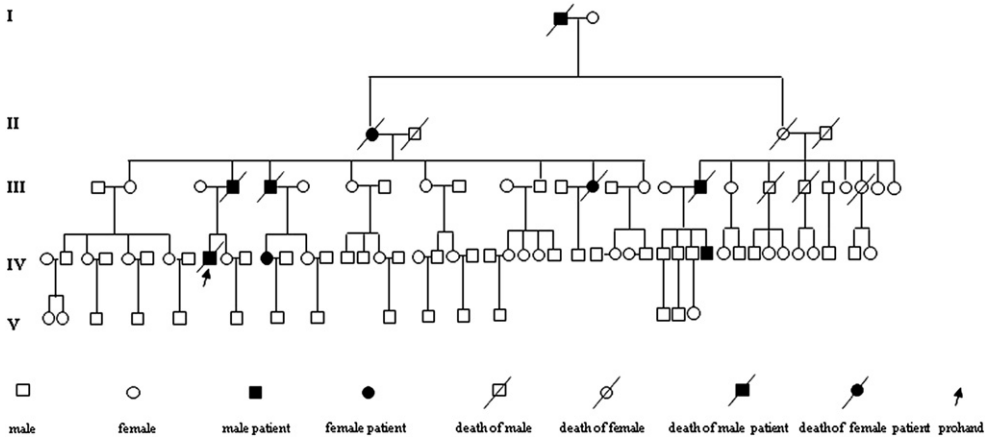

Fig. 1. The pedigree of the family with familial amyloidotic polyneuropathy amyloidogenic transthyretin (ATTR Val30Ala).