Biochemical Characterization of Familial Amyloidotic Polyneuropathy in Various Districts of Japan

Masayuki Tanaka, Masamitsu Nakazato, Teruyuki Kurihara, Shigeru Matsukura, Kenji Kangawa* and Hisayuki Matsuo*

The purpose of this study is to disclose the molecular basis of type I familial amyloidotic polyneuropathy (FAP) in Japan. Amyloid fibril protein of type I FAP consists of a variant transthyretin (also called prealbumin) with one amino acid substitution of methionine-for-valine at position 30. This variant transthyretin is present in the serum as a precursor protein of amyloid. A radioimmunoassay (RIA) has been established to detect the variant transthyretin. All the 94 patients with FAP who originate from various districts in Japan have the variant transthyretin, but any one of 78 healthy adults of families with FAP do not have it. Half of the symptom-free children of FAP patients have the variant transthyretin even before clinical manifestations appear. The RIA is widely applicable for early diagnosis. The methionine-for-valine substitution is due to a base change from guanine to adenine at the first letter of the valine codon at position 30. Type I FAP in Japan is considered to be a molecular disorder of transthyretin. Since the age of onset ranges from twenties to forties, genetic counseling is recommended to prevent the transmission of this intractable disorder to the next generation.

Key Words: Familial amyloidotic polyneuropathy (FAP), Transthyretin, Radioimmunoassay (RIA), Gene abnormality, Molecular disease, Early diagnosis

Familial amyloidotic polyneuropathy (FAP) is an inherited systemic amyloidosis with autosomal dominant inheritance. There are four types of FAP1). Type I FAP (Portuguese type2)) usually starts between twenties and forties with dissociated sensory disturbance in the lower extremities and autonomic dysfunction such as light-headedness, alternating diarrhea and constipation, dysuria, and impotence. As the disease progresses, the sensory disturbance of pain and temperature advances to affect the trunk and the upper extremities as well, and then motor neuropathy follows. From the previous autopsy reports of Japanese Type I FAP, systemic amyloid deposition occurs especially in the heart, kidneys, spleen, and the thyroid gland. The patients usually expire from cardiac or renal failure in 10 to 15 years after the onset of this disorder.

We have reported that amyloid fibril protein isolated from Japanese patients with FAP consists of a variant form of transthyretin (also called prealbumin3). The variant transthyretin contains a methionine-for-valine substitution at position 30 of 127 amino acid residues. This variant transthyretin has been found in Portuguese6,7 and Swedish descent in USA5,6 as well. Transthyretin is composed of four identical subunits, and it plays a role in the transport of thyroxine and retinol (vitamin A). The variant transthyretin is circulating in the serum as a precursor protein of amyloid5. Presence of the variant transthyretin in the serum leads us to devise a new diagnostic method

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for FAP. Diagnosis of FAP is suspected by clinical manifestations and positive family history, and definite diagnosis is made by biopsy.

Since the variant transthyretin differs from normal one by only one amino acid substitution, it is impossible to differentiate the two by using an antiserum against the whole molecule of the variant. We have established a radioimmunoassay (RIA) based on a nonapeptide (position 22-30) of the variant transthyretin. This method can serve as a diagnostic method for FAP. The RIA is also applicable for identifying symptom-free carriers of FAP.

Araki and Kito described two conglomerations of FAP families in Kumamoto and Nagano Prefectures in Japan, respectively. Recently, FAP has been reported from many districts in Japan away from these two districts. The purpose of this study is to clarify the biochemical nature and molecular basis of FAP in various areas in addition to the original two districts.

MATERIALS AND METHODS

Subjects

The subjects studied here consist of 94 patients with FAP aged from 26-70 yrs (44.1 ± 11.8; mean ± SD), 78 healthy adults of families with FAP aged from 29-81 yrs (46.2 ± 12.4), and 37 children of FAP patients aged from 7-21 yrs (16.1 ± 3.5). Among them 125 subjects originate from Arao City, Kumamoto, 55 from Ogawa Village, 11 from Miyata Village, 4 from Iiyama, and 1 from Azumi Village, Nagano, 6 from Saitama, 5 from Mie, 1 from Yamanashi, and 1 from Kamo, Hiroshima. The cases originating from Saitama, Mie, Yamanashi, and Hiroshima have no familial relation to those of two major conglomerations of Japanese FAP families.

Serum Level of the Variant Transthyretin

The serum (5 µl) was treated by cyanogen bromide and trypsin, and then submitted to the RIA procedure as previously described.

Serum Level of Total Transthyretin

The serum (5 µl) was applied to a single radial immunodiffusion plate for transthyretin determination (Behringwerke AG).

RESULTS

Table 1 summarizes serum levels of the variant and total transthyretin in subjects. All the 94 FAP patients had the variant transthyretin in the serum, ranging from 1.60 to 19.50 mg/dl with a mean of 9.45 ± 3.31 (SD) mg/dl. Their ages of onset were 20 to 65 yrs with a mean of 39.2 ± 11.7 yrs. Duration of illness ranged from 1 to 17 yrs with a mean of 5.1 ± 3.7 yrs. There was no significant difference in serum levels of the variant transthyretin between males and females, whose mean values were 9.57 and 9.31 mg/dl, respectively. The variant transthyretin was not present in 100 normal individuals, nor in 78 healthy adults of families with FAP.

Serum levels of total transthyretin in FAP patients ranged from 7.9 to 40 mg/dl, which were significantly reduced as compared with normal individuals (P < 0.001). There were, however, no correlations of the levels of the variant transthyretin or total transthyretin, with ages of onset and duration of illness.

The results of 37 children of 13 FAP patients are summarized in Table 1. Seventeen children had the variant transthyretin. Their serum levels ranged from 6.34 to 14.21 mg/dl with a mean of 10.90 ± 2.65 mg/dl. Other 20 children did not have the

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number</th>
<th>Age</th>
<th>Age of onset</th>
<th>Duration of illness</th>
<th>Transthyretin Variant</th>
<th>Transthyretin Total</th>
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</thead>
<tbody>
<tr>
<td>FAP patients</td>
<td>94</td>
<td>26-70</td>
<td>39.2 ± 11.7</td>
<td>5.1 ± 3.7</td>
<td>9.45 ± 3.31</td>
<td>22.9 ± 7.2</td>
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<td>healthy adults of families with FAP</td>
<td>78</td>
<td>29-81</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>31.1 ± 5.6</td>
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<tr>
<td>children of FAP patients</td>
<td>17</td>
<td>7-20</td>
<td>--</td>
<td>--</td>
<td>10.90 ± 2.65</td>
<td>28.3 ± 3.3</td>
</tr>
<tr>
<td>normal individuals</td>
<td>20</td>
<td>8-21</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>32.1 ± 5.2</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.

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variant transthyretin. There was no significant difference in the serum levels of total transthyretin between the two groups of children.

Table 2 shows the results of the RIA of FAP patients in various districts. FAP patients in all the districts studied had the variant transthyretin with methionine-for-valine substitution at position 30, whereas healthy adults of the families did not have it.

**DISCUSSION**

The variant transthyretin is present in all the 94 FAP patients who originate from various districts in Japan. Their geographic distributions are represented in Fig. 1. Sporadic cases in Saitama, Mie, Yamanashi, and Hiroshima have the same variant transthyretin as those from the two major conglomerations. Nucleotide sequence analysis on the transthyretin gene may clarify whether these sporadic cases have any familial relationship to the two major conglomerations of FAP.

FAP patients in Iiyama City (FAP Iiyama type) are characterized by cerebellar signs of dysarthria, ataxic gait and pyramidal tract sign in addition to typical clinical features of type I FAP. Signs of central nervous system (CNS) involvement start in the early phase of the disease. In FAP Iiyama type, signs of FAP and CNS manifestations always coexist in the same patients. In typical type I FAP other than Iiyama type, CNS involvement is not present. The RIA study reveals that three patients with FAP Iiyama type have the same abnormality in transthyretin as that of typical type I

### Table 2. RIA analysis on FAP patients and their family members from various districts of Japan

<table>
<thead>
<tr>
<th>District</th>
<th>Number (M:F)**</th>
<th>Met30(+)</th>
<th>Met30(-)**</th>
<th>Number (M:F)**</th>
<th>Met30(+)</th>
<th>Met30(-)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogawa</td>
<td>50 (28:22)</td>
<td>40 (22:18)</td>
<td>10 (6:4)</td>
<td>5 (1:4)</td>
<td>2 (1:1)</td>
<td>3 (0:3)</td>
</tr>
<tr>
<td>Miyata</td>
<td>10 (5:5)</td>
<td>3 (2:1)</td>
<td>7 (3:4)</td>
<td>1 (0:1)</td>
<td>1 (0:1)</td>
<td>0</td>
</tr>
<tr>
<td>Iiyama</td>
<td>4 (4:0)</td>
<td>3 (3:0)</td>
<td>1 (1:0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azumi</td>
<td></td>
<td>1 (1:0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saitama</td>
<td>4 (0:4)</td>
<td>2 (0:2)</td>
<td>2 (0:2)</td>
<td>2 (0:2)</td>
<td>0</td>
<td>2 (0:2)</td>
</tr>
<tr>
<td>Mie</td>
<td>5 (1:4)</td>
<td>4 (1:3)</td>
<td>1 (0:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yamanashi</td>
<td>Mie (M:F)***</td>
<td>1 (1:0)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kamo</td>
<td></td>
<td>1 (1:0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Met30(+)**: variant transthyretin with methionine-for-valine substitution at position 30
Met30(-)**: normal transthyretin, (M:F)****: (Male:Female)
FAP. Their serum levels are 8.86, 10.91, and 12.95 mg/dl, respectively, which are not significantly different from those of typical type I FAP not including Iiyama type (9.40 ± 3.30 mg/dl). It is probable that FAP Iiyama type may be accompanied by hereditary spinocerebellar degeneration (SCD). However, the probability of coincidence is very low since both disorders are present in all the patients with FAP Iiyama type, and in addition, the prevalence of SCD (1—7/100,000) is low. The possibility remains that the variant transthyretin may be deposited as amyloid fibrils in the CNS, or a genetic mutation associated with SCD is located very near to the transthyretin gene.

The nucleotide sequence analysis of transthyretin gene from a Japanese patient with FAP17 in comparison to normal human transthyretin18 has revealed that the methionine-for-valine substitution is caused by a base change from guanine (G) to adenine (A) at the first letter of the valine codon at position 30. The base change yields new restriction sites for Nsil (ATGCAT) and Ball (TGGCCA)19-21. Using Southern blot analysis, a mutated transthyretin gene can be detected by identifying extra DNA bands after digestion of DNA with Nsil and/or Ball. Sasaki et al. have demonstrated that all the 7 FAP patients from Arao, 21 from Ogawa Village, 1 in Miyata Village, and 1 in Hiroshima have the same mutated transthyretin gene. They have also shown that none of the normal individuals, nor healthy adults of families with FAP have the mutation17. The results obtained by the RIA and DNA analysis are perfectly consistent, and the biochemical and genetic abnormalities in transthyretin are completely linked to clinical diagnosis of type I FAP. Type I FAP is considered to be a molecular disorder of transthyretin. Patients have the mutation heterozygously, indicating that the presence of normal and the variant transthyretin in their serum is due to an expression of two allelic genes.

Studies on children of FAP patients have revealed that the variant transthyretin is present in the half. This incidence is compatible to the autosomal dominant inheritance of this disorder. Children having the genetic abnormality will most likely develop the disease in the future, and therefore, they have to be closely observed.

Since the usual age of onset of FAP is 25 to 40 years and the disease is transmitted to the next generation with high penetrance rate, early diagnosis for this intractable disorder has been desired. We believe that the RIA and DNA analysis are clinically applicable for this purpose. The RIA is preferable as a wide screening test, because it is simple and quick, and it requires only 5 μl of the serum sample from each subject. Genetic advice based on these diagnostic methods is helpful for families with FAP, since premarital diagnosis can be done even before clinical manifestations appear. The results of the diagnostic tests have to be explained to the patients with essential humanity at an appropriate time when they are ready to accept the results, since we have no any causal therapy as of today. Nevertheless, the transmission of this disorder to the next generation has to be avoided as much as possible by early diagnosis and appropriate genetic counseling.

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REFERENCES


