A New Arg54Gly Transthyretin Gene Mutation Associated with Vitreous Amyloidosis in Chinese

Yining Shi1,*, Jing Li1, Jia Hu1, Jun Hu2, Lijun Sun1, Huijin Li1, Rui Shi1, Le Yang1, Ye Sun1, Chan Li1
1. Department of Ophthalmology, Shaanxi Provincial People’s Hospital, Xi’an 710068, China
2. Laboratory Center, Shaanxi Provincial People’s Hospital, Xi’an 710068, China

Abstract

Purpose: To analyse the hereditary features of a Chinese pedigree with familial vitreous amyloidosis in Liaoning Province, China, and to investigate the correlation between the clinical appearance of the disease and transthyretin (TTR) gene mutation, including the locus and type of TTR gene mutation.

Methods: Five patients (10 eyes) from one Chinese family were diagnosed with vitreous amyloidosis between July 1996 and April 2009. Family members were followed up subsequently, and peripheral venous blood was obtained from 13 subjects (including 2 patients, and 11 controls without clinical signs of disease). DNA samples were extracted and 4 exons of the TTR gene were amplified by polymerase chain reaction (PCR). The gene fragments were subjected to sequencing analysis. The results were analyzed with DNAMAN Windows 5.2.2.0 and Chromas sequence chart analysis software, TTR gene exons were compared between affected patients and normal controls.

Results: Family pedigree analysis revealed that patients were distributed in three generations. Male and female subjects had equal prevalence, and only one parent of affected patients had signs of disease. TTR gene exon sequencing showed that the sequence of patients was identical to that of normal individuals. No TTR gene mutations were noted in 10 unaffected family members. However, a TTR Gly–54 point mutation in the 2nd exon was detected in two patients and 1 unaffected family member (one of the patients’ daughters). Vitreous samples in 4 cases (7 eyes) showed positive Congo red staining, suggesting that these family members suffered from familial vitreous amyloidosis.

Conclusion: This pedigree affected with familial vitreous amyloidosis was characterized by autosomal dominant inheritance; a TTR Gly-54 point mutation in the 2nd exon is presumed to be the cause. This Gly-54 point mutation of the TTR gene is a novel mutation in vitreous amyloidosis. (Eye Science 2011; 26:230–238)

Keywords: familial vitreous amyloidosis; TTR gene; sequencing; point mutation

Amyloidosis refers to metabolic disorders characterized by protein deposition in tissues of single or multiple organs. Virchow (1900) defined it 100 years ago. The etiology of the disease is unknown. So far, it has been considered that its pathogenesis is by eosinophilic amorphous material from the immunoglobulin k chain and l chain, which accumulates in the organism1. The disease can also be divided into systemic amyloidosis and localized amyloidosis, according to the area affected.

Rokitansky first reported ocular amyloidosis in 1842. Amyloidosis is mostly localized in the eye. It can affect the eyeball and the ocular adnexa as vitreous; eyelids; lacrimal gland; cornea; tissues in the orbit; ciliary ganglia; and ciliary nerves. Other manifestations are pupil abnormal eye-movement disorder and glaucoma (Figure 1).

Figure 1 Amyloidosis in the eye. A: Slit-lamp photograph showing fibrillar deposits on the posterior corneal surface2. B: Extensive deposits are seen over the anterior lens surface2. C: Vitreous amyloidosis3

DOI: 10.3969/j.issn.1000-4432.2011.04.010
Corresponding author: Yining Shi, M.D., Department of Ophthalmology, Shaanxi Provincial People’s Hospital. Chief research: ocular fundus disease and optometry. Address: From the Department of Ophthalmology, Shaanxi Provincial People’s Hospital, #256 youyi xiu, Xi’an 710068, Shaanxi Province, China. Tel: 8613991817380, E-mail: shi_yi_ning@163.com
Vitreous amyloidosis is a rare ocular localized amyloidosis and is often accompanied by a family history of hereditary amyloidosis as a common type. The pathogenesis is still unclear. The inheritance mode is autosomal dominant, but has varying levels of penetration.

Recent studies of eye amyloidosis have focused on transthyretin (TTR). TTR amyloidosis can involve multiple organs or tissues, but different patients have different clinical manifestations. There are some common clinical manifestations; amyloid polyneuropathy; visceral amyloidosis; leptomeningeal amyloidosis; central nervous system syndrome; and eye amyloidosis. Vitreous amyloidosis is a rare local TTR amyloidosis.

We treated five patients (10 eyes) who were from the same Chinese lineage in Liaoning Province over the past 13 years, from July 1996 to April 2009. There were two male patients (four eyes) and three female patients (six eyes). Their average condition history ranged from five to 10 years. Their ages ranged from 42 to 76, averagely aged 48.8. We performed the pars plana vitrectomy on four patients (seven eyes). The specimens were confirmed as vitreous amyloidosis by positive Congo red staining. Post-operatively, we followed up the patients and family members, and in the same year we carried out a family survey in order to draw up a genealogy. We extracted blood from 13 surviving family members and did the DNA extraction after obtaining their informed consent. We amplified the TTR exon by PCR and sequenced it; we then compared this with the control in order to detect the family’s gene mutation.

We hoped to find a clear genetic basis for mutation carriers of this disease that might predict the incidence in the next generation of this family.

Methods

This research followed the tenets of the Declaration of Helsinki and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study.

Family Members

Here was a Chinese lineage with familial vitreous amyloidosis in Liaoning Province, as shown in Figure 2, below. (No.1–7 and No.10–13 as the control group.)

![Figure 2 Map of the family lineage](image)

I: first generation, II: second generation, III: third generation, IV: fourth generation, V: fifth generation


The peripheral venous blood samples were obtained with anti-freeze by EDTA from patients; patients’ siblings; patients’ nephews; and one patient’s daughter in this family. Extracted lymphocytes were stored at −80°C until required. Table 1, below, shows the essential information of the experimental subject.

<table>
<thead>
<tr>
<th>No.</th>
<th>Family members</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>III 6</td>
<td>female</td>
<td>58</td>
<td>Patient, visit in July 2006, age of onset: 45</td>
</tr>
<tr>
<td>8</td>
<td>IV 1</td>
<td>male</td>
<td>42</td>
<td>Patient, visit in April 2009, age of onset: 42</td>
</tr>
<tr>
<td>1</td>
<td>V 1</td>
<td>male</td>
<td>10</td>
<td>Nephew of No.8, no clinical manifestation</td>
</tr>
<tr>
<td>2</td>
<td>IV 7</td>
<td>female</td>
<td>31</td>
<td>Daughter of No.9, no clinical manifestation</td>
</tr>
<tr>
<td>3</td>
<td>V 2</td>
<td>male</td>
<td>8</td>
<td>Nephew of No.8, no clinical manifestation</td>
</tr>
<tr>
<td>4</td>
<td>IV 2</td>
<td>male</td>
<td>38</td>
<td>Brother of No.8, no clinical manifestation</td>
</tr>
<tr>
<td>5</td>
<td>IV 5</td>
<td>male</td>
<td>32</td>
<td>Brother of No.8, no clinical manifestation</td>
</tr>
<tr>
<td>6</td>
<td>III 6</td>
<td>female</td>
<td>56</td>
<td>Sister of No.9, no clinical manifestation</td>
</tr>
<tr>
<td>7</td>
<td>IV 9</td>
<td>female</td>
<td>26</td>
<td>Sister of No.8, no clinical manifestation</td>
</tr>
<tr>
<td>10</td>
<td>IV 4</td>
<td>male</td>
<td>36</td>
<td>Brother of No.8, no clinical manifestation</td>
</tr>
<tr>
<td>11</td>
<td>IV 8</td>
<td>male</td>
<td>28</td>
<td>Son of No.6, no clinical manifestation</td>
</tr>
<tr>
<td>12</td>
<td>V 1</td>
<td>female</td>
<td>8</td>
<td>Daughter of No.8, no clinical manifestation</td>
</tr>
<tr>
<td>13</td>
<td>IV 6</td>
<td>male</td>
<td>34</td>
<td>Son of No.9, no clinical manifestation</td>
</tr>
</tbody>
</table>
Experimental materials

Main reagents
1) Biospin Whole Blood Genomic DNA Extraction Kit (Model; BSC06S1, Producer; Bioer Technology Co., Ltd).
2) Absolute ethanol (Producer; Tianjing Fu Yu Fine Chemicals Co., Ltd).
3) Physiological saline (Producer; Xi’an Jing Xi Double-crane Pharmaceutical Co., Ltd).
4) Regular agarose (Producer; Gene Co., Ltd).
5) PCR kit (Producer; TaKaRa Biotechnology [Dalian] Co., Ltd).
6) DNA Marker (Marker V) (Producer; TaKaRa Biotechnology [Dalian] Co., Ltd).
7) 6 * DNA Eletrophoresis Loading Buffer (Producer; TaKaRa Biotechnology [Dalian] Co., Ltd).
8) GoldViewTM nuclein dyes (Producer; Beijing SBS Gene Co., Ltd).

Primary apparatus:
1) Tri-Purpose Thermostatic/Electrical Thermostatic Water Chests (Model; SHHW21–420, Producer; Tianjing Taismte Instrument Co., Ltd).
2) Low-speed Micro-centrifuge (Model; TD5A, Producer; Changsha YingTai Instrument Co., Ltd).
3) High-speed Micro-centrifuge (Model; TGW16A, Producer; Changsha YingTai Instrument Co., Ltd).
4) Simple injector and tips (2.5 μl, 0.1–10 μl, 10–100 μl, 100–1000 μl) (Producer; Biohit Co., Ltd).
5) Vortex mixer (Model; QL-901, Producer; Jiangsu Hai Men Qi Lin Instrument Co., Ltd).
6) Spectrophotometer (Model; Nanodrop 2000C, Producer; Gene Co., Ltd).
7) Programmable Thermal Controller (Model; PTC-100, Producer; MJ Research, Inc).
8) Electronic balance (Model; BL-320S, Producer; Shimadzu Corporation).
9) Electrophoresis Apparatus (Model; DYY-6C, Producer; Beijing Liu Yi Instrument Co., Ltd).
10) White/Ultraviolet Transilluminator (Producer; UVP).
11)荧光 Chem Imager (Model; Multimage Light Cabinet FC2, Producer; Alpha Innotech).
12) Sequenator (Model; 3730XL, Producer; ABI).

Agarose-gel preparation
Preparation of 1% agarose gel; agarose 1g mixed with 1 * TAE electrophoresis buffer 100 ml, heated until melted in a microwave oven, cooled to approximately 50°C. GoldView 5 μl was added and mixed well. The mixture was poured on the plate and kept still for at least 30 minutes.

Experimental Procedures
Extraction of DNA from the lymphocytes*
Sample preparation; The mixture was centrifugated at 1000×g for five minutes. The frozen liquid was discarded. The mixture was re-suspended using 200 μl physiological saline and mixed well. A portion of 10 μl of PK solution was piped into the bottom of a 1.5 or 2.0 ml micro-centrifuge tube. 200 μl of the sample was added to this, then 200 μl of the Lysis B Buffer was added to it and mixed thoroughly for 5–10 seconds. It was then incubated at 56°C for 10 minutes. 200 μl of ethanol was added and mixed thoroughly for 5–10 seconds. All the mixture was transferred (in step 1.4) to the spin column. The mixture was centrifugated at 6000–8000×g for one minute. The supernate was discarded. 500 μl of the WB1 Buffer was added to the spin column. The spin column was centrifugated at 10 000×g for 30–60 seconds. The supernate was discarded.

A 700 μl Wash buffer was added to the spin column. The spin column was centrifugated at 10000×g for 30–60 seconds. The supernate was discarded. The spin column was then placed back to the tube, and then centrifugated at 13 000×g for two minutes. The spin column was then placed in a new 1.5 or 2.0 ml micro-centrifuge tube. 100 μl to 200 μl was added to the elution Buffer. It was then incubated at room temperature for one minute. The mixture was centrifugated at 13 000×g for one minute. The DNA in the collection tube was prepared for subsequent analysis.

The content of the collected DNA is in the standard range that was analyzed by the Nanodrop Spectrophotometer (Table 2). If the isolated DNA sample was not tested on the same day, it was frozen at −20°C.

* Note: According to the label on the bottle, add ethanol 15.68 ml to WB1 buffer and 26.95 ml to wash buffer. The PK solution should be stored at 2–
Table 2 The content of the collected DNA

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Content of DNA (ng/μl)</th>
<th>A260 10mm path/A280 10mm path</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>female</td>
<td>60</td>
<td>26.0</td>
<td>1.94</td>
</tr>
<tr>
<td>8</td>
<td>male</td>
<td>42</td>
<td>24.6</td>
<td>2.02</td>
</tr>
<tr>
<td>1</td>
<td>male</td>
<td>7</td>
<td>23.1</td>
<td>1.97</td>
</tr>
<tr>
<td>2</td>
<td>female</td>
<td>30</td>
<td>38.5</td>
<td>1.86</td>
</tr>
<tr>
<td>3</td>
<td>male</td>
<td>8</td>
<td>37.0</td>
<td>1.96</td>
</tr>
<tr>
<td>4</td>
<td>male</td>
<td>38</td>
<td>48.8</td>
<td>1.95</td>
</tr>
<tr>
<td>5</td>
<td>male</td>
<td>35</td>
<td>25.8</td>
<td>1.90</td>
</tr>
<tr>
<td>6</td>
<td>female</td>
<td>56</td>
<td>16.6</td>
<td>2.06</td>
</tr>
<tr>
<td>7</td>
<td>female</td>
<td>30</td>
<td>21.3</td>
<td>1.91</td>
</tr>
<tr>
<td>10</td>
<td>male</td>
<td>36</td>
<td>36</td>
<td>1.9</td>
</tr>
<tr>
<td>11</td>
<td>male</td>
<td>28</td>
<td>55</td>
<td>1.87</td>
</tr>
<tr>
<td>12</td>
<td>female</td>
<td>8</td>
<td>59</td>
<td>1.88</td>
</tr>
<tr>
<td>13</td>
<td>male</td>
<td>34</td>
<td>39.6</td>
<td>1.68</td>
</tr>
</tbody>
</table>

8°C.

* * Potential problems include:
1. Little or no DNA in the eluate.
2. Low amount of leukocytes in the blood sample.
3. PK Solution was not stored at 2–8°C, leading to its low activity.
4. Ethanol was not added to the WB1 buffer and Wash buffer according to the instructions before first use.

Primer design

Primer from past literatures

Primer from literature 1:

The first pair of primers (the amplification fragment included exon1 and exon2. Length of this fragment is 1,354 bp.)
Forward (1F): 5′GCAGTCAGATGGGCAGGGATA A GC3′ 506
Reverse (1R): 5′GGGCATCCTCAGAGGGCATA C TT3′ 1860

The second pair of primers (the amplification fragment includes exon3. Length of this fragment was 1350 bp.)
Forward (2F): 5′TGCTGGCAAGAGTTATCAGAGGTTA3′ 3017
Reverse (2R): 5′GAGAGCCAGATGTGTTTGTG-GAATGAAG 3′ 4367

The third pair of primers (the amplification fragment included exon4. Length of this fragment was 660 bp.)
Forward (3F): 5′AATGCTAAATCTCTAAGTCTGTGGTGGC3′ 6861
Reverse (3R): 5′GCCTTTCACAGGAATGTTTATT GTCTCT 3′ 7520

By analysis, the segment of the primer was excessively long and the mutation in exon was not found in this document because of the intron mutation, so we considered that the accuracy was questionable. Hence, it was not cited.

Primer from literature 2:
F 5′-CGGAGGCAGTCTCTGCCATCA-3′
R 5′-GCTCCCAGTGTCTACAGCA-3′

This primer was used for the reverse transcription (RT), unlike in our experiment, so it was not cited.

The design of primers in our experiment

We searched the TTR gene sequences from the NCBI gene bank, and picked up four exon sequences, then designed primers by using OLIGO software.

The principles of primer design

1. Avoiding primers self-matching internally.
2. Separating the exon and primer while designing owing to inaccuracy in the early stages of primer-replication.
3. The length of primer was 18–30 bp, 20 bp generally.

The first designed primer

The first pair of primers (the amplification fragment included exon1.)
1F 5′-3′; GAATGGATTAGCAGCGCAGTC
IR 5′-3′; CAGAGTTCAAAGTCCAGCATCAG

The second pair of primers (the amplification fragment include exon2.)
2F 5′-3′; ATTACGTCTGTGTTATACTG
2R 5′-3′; ATCCCTCAGAGGCGACTACT
The third pair of primers (the amplification fragment include exon3.)
3F 5’-3’ ; ACTCCAGCTGGTGACAA
3R 5’-3’ ; TTCATGTAAGTCAAGTCA
The fourth pair of primers (the amplification fragment included exon4.)
4F 5’-3’ ; TTAGTCAAGCTAGGTCATC
4R 5’-3’ ; GTGGTGCGATGAGCTGAATC

Why primer design was performed again?
The third and fourth exons failed frequently by adjusting the annealing temperature. The following phenomena were observed: 1) electrophoreses after PCR; there was no stripe in the gel, probably owing to the process for PCR contamination, or enzyme inactivation; 2) electrophoreses after PCR; some stripes were detected, possibly owing to inappropriate anealing temperature, or primers not specific enough, and therefore the impurity stripes were not removed. 3) electrophoreses after PCR; stripes show in coated form, possibly owing to inappropriate annealing temperature or excessive activity. 4) no stripe detected after electrophoresis; probably owing to sample-addition is not accurate or rinsing is not thorough enough during the recycling process. 5) result shows the sample is impure; combination problems led to failure because some impurities were mixed when the gel was cut down.

Other possible reasons include; 1) instrument’s precision was not accurate enough. 2) GoldViewTM was injected before the temperature was lowered to 50°C while making gel; as a result, the gel failed to be colored owing to inactivation as a result of excessively high temperature.

Redesigning the third and fourth primers
The third pair of primers (the amplification fragment included exon3.)
3F 5’-3’ ; ATGTTGTTAGTTGG
3R 5’-3’ ; TCATTCATCCCTCCTT
The fourth pair of primers (the amplification fragment included exon4.)
4F 5’-3’ ; GGTCAGTCATGTGTCATCTG
4R 5’-3’ ; AGTAAAGAAGTGGCCCTTGG

Primer synthesized
The primers were synthesized by Beijing genomics Co., Ltd., OPC, 2OD.
PCR amplification target gene exon

Deal with the primer.
The primer was shown as dry powder. The primer was centrifuged at 10 000xg for 60 seconds.
The sterilized distilled water was supplemented and mixed well. The detailed doses were stated as below (provided by the primer-synthesis company).
1F 452.3 μM 2F 477.9 μM 3F 489.4 μM 4F 428.6 μM
1R 427.4 μM 2R 497.0 μM 3R 610.4 μM 4R 381.9 μM
The mixture was centrifuged at 8 000xg for 60 seconds.

Preparation of a PCR reaction solution
Five times the Prime STAR Buffer (Mg2+plus) 2.5 μl was added in 14.0 μl sterile distilled water.
dNTP Mixture 2.0 μl (concentration of 2.5 mmol/μl) was put in this mixture, then added to the up-stream primer and downstream primer each 2.0 μl and the template DNA 2.0 μl (concentration of 100 ng/μl). Finally, the Ex Taq DNA Polymerase 0.5 μl (concentration of 2.5 U/μl) was supplemented in this mixture. The system was adjusted to 25 μl, and then centrifuged at 8 000xg for 30 seconds.

Conditions for the PCR reaction
The final reaction conditions were maintained at 94°C pre-denaturation in the last five minutes. Then, denaturation was implemented at 94°C for 30 seconds. The anneal temperature of the four exons was 57°C, 52°C, 55°C, 57°C, and these were maintained for 40 seconds, and were then extended at 72°C for one minute. After 30 cycles of the steps above, extension was performed at 72°C for 10 minutes to finish this procedure. The amplified products were confirmed to be the desired fragments by 1% agarose-gel electrophoresis (Figure 3).

DNA stripe cutting and recycling
The spin column was balanced and 500 μl of the BL added (CB2). Then the mixture was centrifugated at 13 400xg for 60 seconds. The supernate was discarded. The DNA stripe was placed in a new micro-centrifuge tube and weighed at 0.3 g. PC (300 μl) was added to the micro-centrifuge tube, and incubated at 50°C for 10 minutes. The rubber block was removed from the water bath and reached room temperature when completely dissolved. The mixture was added to a spin column (CB2). Then the mix-
ture was centrifuged at 13 400×g for 60 seconds. The supernate was discarded.

PW (600 μl) was added to the spin column. Then, the mixture was centrifuged at 13 400×g for 60 seconds after setting for two minutes. The supernate was discarded, and 600 μl of the PW was added to the spin column. The spin column was centrifuged at 13 400×g for 60 seconds. The supernate was discarded. Then the mixture was centrifuged at 13 400×g for two minutes. The bleaching lotion was completely removed.

The spin column was placed in a new micro–centrifuge tube. 30 μl of the sterilized distilled water was added and the mixture hung in the air in the middle of the adsorption film, and kept at room temperature for two minutes. Then the mixture was centrifuged at 13 400×g for two minutes. The DNA solution was then collected.

Gene sequence

The products were subjected to sequencing by using sequencer ABI1000 by Beijing Genomics Co., Ltd. The self-provided primer was stored at freezing temperature. The fluorescent-labeled forward-sequence method was adopted.

Results

Characteristics of the family members

Within the family, especially the generation III and generation II, the incidence rate was close to 1 in 2. No significant difference was noted between the two genders, suggesting that the family possessed autosomal dominant inheritance. The average age of onset was older. However, the incidence in males occurred earlier than in females.

It has been known that the grandmother (II 2) of the patient, who died in her 70s did not show onset, but his grandfather (II 1) died earlier, so we couldn’t track and analyze this I generation. Assuming that the grandfather (II 1) might be a patient (the disease gene was heterozygote), but was not showing onset, since he died at a young age. The son of III 3 was not showing onset when he was an adult, while III 3 was not showing onset while alive. III 3 was the proband, and III 6 underwent the vitrectomy. The onset of the generation IV and V. IV 1 with vitreous amyloidosis in the left eye in the same year and his
eight-year-old daughter presenting the TTR gene mutation were followed up.

Comparing the sequence results

The result was analyzed with Chromas sequence chart-analysis software, and the results compared with normal human TTR gene exons. The sequence results of exons 1, 3, and 4 and normal human TTR gene exons 1, 3, and 4, were the same. Sequence results of exon2; the results among 10 family members without clinical manifestations and normal TTR gene exon2 were equivalent. Sequence results of the two patients and one family member who was not involved (V1) showed mutation at the second exon 91bp (adenine, A→guanine, G), causing the formation of No.54 amino acid R(Arg)→G(Gly) (Figure 4).

Discussion

Approximately 90% of thyroid hormone binding proteins (TTR), also known as prealbumin, is produced by the liver. The choroid plexus and the retina also produce a small fragment of the protein, and then secrete it in the cerebrospinal fluid and blood. The present research suggests that the gene mutation causes TTR tetramer dissociation, changing the protein structure and ultimately forming amyloidosis.

The TTR gene consists of four exons and three introns. TTR gene mutation in amyloidosis was detected. DNA sequence analysis revealed that 99% of the mutations occur in exons 2, 3, and 4. Currently, around 100 types of TTR mutations are related to TTR amyloidosis. Val30Met, the most common mutation as a global distribution, replaces valine by methionine. In the UK, the common genetic mutation is 60 alanine, accompanied by significant cardiac amyloidosis. Four percent of African-American gene mutation is 122-bit isoleucine (that is, Val122Ile).

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Typical age of onset/years</th>
<th>Neuropathy 0–4+</th>
<th>Cardiac 0–4+</th>
<th>Intestinal 0–4+</th>
<th>Eye vitreous yes+/no= Rare</th>
<th>Leptomeninges yes+/no= Rare</th>
<th>Renal yes+/no=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val30Met</td>
<td>30–55 (Portugal, Japan)</td>
<td>3–4</td>
<td>2–3</td>
<td>2–4</td>
<td>±</td>
<td>Rare</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>55–60 (Sweden)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu58His</td>
<td>50–60</td>
<td>2–3</td>
<td>3–4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thr60Ala</td>
<td>60–65</td>
<td>1–2</td>
<td>4</td>
<td>0–4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ser77Tyr</td>
<td>50–60</td>
<td>3–4</td>
<td>3–4</td>
<td>2–3</td>
<td>–</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>Ile84Leu</td>
<td>45–55</td>
<td>2–3</td>
<td>4</td>
<td>2–3</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Val1122Ile</td>
<td>60–65</td>
<td>1–2</td>
<td>4</td>
<td>1–1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

From: “Genetics; Clinical Implications of TTR Amyloidosis.”
In the Japanese population, Val30Met mutation occurs most commonly. The serine 44 mutation reported by Murakami and 71 of the alanine mutations reported by Zambarkaj are associated with the occurrence of vitreous amyloidosis. Solano also reported a vitreous amyloidosis case associated with point mutations of the Asp18Glu. Among those mutations, Liepnieks, and Benson considered that Ile84Ser is closely related with vitreous amyloidosis. Benson gave part of the common types of TTR mutations as follows; although the Val30Met TTR amyloidosis is the most common mutation type, the clinical manifestations involve multiple neural and cardiac diseases (Table 3).

<table>
<thead>
<tr>
<th>City</th>
<th>Mutation point</th>
<th>Mutation point</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhongshan</td>
<td>Gly38Asp, exon3, Glycine to Arginine</td>
<td>GGC to CGC</td>
<td></td>
</tr>
<tr>
<td>Guizhou</td>
<td>Gly103Arg, exon3, Glycine to Arginine</td>
<td>GGC to CGC</td>
<td></td>
</tr>
<tr>
<td>Shaanxi</td>
<td>Arg54Gly, exon2, Arginine to Glycine</td>
<td>CAG to CGG</td>
<td></td>
</tr>
</tbody>
</table>

Chinese scholars have previously reported one case of point mutation (Gly38Asp) in the 83 amino acids of exon3, from GGC mutation to CGC (from glycine into arginine). Another case in a genealogy was reported by Guizhou Medical College Hospital, where its point mutation was observed in the 103 codon of exon3, an amino acid glycine mutated into arginine.

Chinese, not all the TTR gene mutations cause diseases. Ala74His, His90Asn, Pro102Arg, Arg104Cys, Thr119Asn, etc., are considered as non-pathogenic mutations, and may be considered normal gene polymorphism in different ethnic populations.

Fewer than 20 cases of vitreous amyloidosis have been reported worldwide. In this research, firstly reported by Shi in China, a novel mutation, TTR gene Gly-54 point mutation in exon2 was a new gene in the vitreous amyloidosis, which was first found in the world, and differed from previous reports. It is hypothesized that this mutation may be the nosogenesis of vitreous amyloidosis in this Chinese genealogy.

In this experiment, two patients and one girl with no clinical symptoms (one of the fifth generation) have TTR gene Gly-54 point mutation. The girl is the daughter of a male patient in the fourth generation, which is consistent with the autosomal dominant characteristics of this disease. Hence, we regard the TTR gene Gly-54 point mutation as the point that induces vitreous amyloidosis in this family. One of the features of this disease is that the affected girl is only eight years old, so we can follow up this girl in the next 20 years or longer. According to previous results, morbidity was approximately one in two, so we presume that another member of this family presents gene mutation. Next, we will collect DNA samples from the remaining members and observe the pathogenicity of Gly-54 mutation. If other member(s) has/have gene mutation without disease onset, the occurrence of this disease may be properly predicted.

References

References


