Study of a Taiwanese family with oculopharyngeal muscular dystrophy

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ABSTRACT

Background: Oculopharyngeal muscular dystrophy (OPMD) is a late onset autosomal dominant muscle disorder. OPMD is caused by a short trinucleotide repeat expansion encoding an expanded polyalanine tract in the polyadenylate binding-protein nuclear 1 (PABPN1) gene. We identified and characterized a PABPN1 mutation in a Taiwanese family with OPMD.

Methods: The phenotypic and genotypic characteristics of all subjects were evaluated in a Taiwanese OPMD family. Genetic alterations in the PABPN1 gene were identified using PCR and DNA sequencing.

Results: Ten subjects with OPMD (6 symptomatic and 4 asymptomatic) within the Taiwanese family carried a novel mutation in the PABPN1 gene. The normal (GCG)6(GCA)3GCG sequence was replaced by (GCG)6(GCA)3GCG due to an insertion of (GCG)4GCA into the normal allele in the Taiwanese OPMD subjects.

Conclusions: In contrast to a single GCG expansion in most of OPMD patients in the literature, an insertion of (GCG)4GCA in the PABPN1 gene was found in the Taiwanese OPMD subjects. The identification of this mutation appears to support the molecular mechanism of unequal cross-over of two PABPN1 alleles.

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1. Introduction

Oculopharyngeal muscular dystrophy (OPMD), an adult-onset autosomal dominant genetic disorder, is characterized by slowly progressive ptosis, dysphagia, and dysphonia [1]. This disorder has a worldwide distribution [2], but reported cases are most from Caucasian families, especially French-Canadian trait [3–9]. In Asian population, OPMD patients have only been reported in the Japanese families and sporadic patients in other countries morphologically and genetically [10–16].

Genetic studies revealed a small expansion of a (GCG)9-repeat in the first exon of the polyadenylate (polyA) binding-protein nuclear 1 (PABPN1), or also known as polyA binding–protein 2 gene located on chromosome 14q11 [17,18]. The polyalanine expansion mutation may lead to aggregate with tubular fibers and is thought to confer a toxic gain-of-function on aberrant PABPN1 protein [11,19–22]. Interestingly, over-expression of normal PABPN1 gene may reduce the cytotoxicity of aberrant PABPN1 and protect cells against pro-apoptotic events [23].

Herein, we report a Taiwanese family with autosomal dominant OPMD along with genetic analysis of PABPN1. To our knowledge, this is the first report of a Taiwanese family with OPMD that also identifies the respective mutation in the PABPN1 gene.

2. Materials and methods

2.1. Patients

In this study, 10 subjects in three generations of a Taiwanese OPMD family who lived in mid-western Taiwan were studied (Fig. 1). Informed consent was obtained from all subjects who participated in this study. The proband’s father (subject I-1) died of suffocation due to difficulty swallowing at the age of 59 years. The proband’s mother died at age of ninety. All 10 members of the OPMD family received an electrophysiological examinations, and/or muscle biopsy (Table 1). The protocol was approved by the institutional review board of Chang Gung Memorial Hospital. OPMD was diagnosed according to the criteria reported by Brais et al. [17] including three major criteria 1) positive family history, 2) ptosis or previous surgical correction, and 3) dysphagia. After a detailed neurological examination, 6 of 32 individuals fulfilled the clinical criteria, and were diagnosed as symptomatic. The family pedigree shown is consistent with an autosomal dominant mode of inheritance.

Patient II-3 was a 68 year-old woman, who developed an insidious onset of bilateral eyelid dropping and double vision for 6 years and...
difficulty swallowing in her mid-forties. Neurological examination showed bilateral ptosis with mild to moderate limitation of eyeball movement, and moderate dysphagia and dysarthria. Two of her sons, patient III-7 (a 47 year-old man) and patient III-9 (a 42 year-old man) also experienced bulbar symptoms, mild bilateral ptosis and limitation of eye movement.

Patient II-4, a 67 year-old man, had suffered from speech and swallowing disturbance for more than 15 years. He had received surgical correction for bilateral ptosis at the age of 50 years, but recurrent eyelid dropping and double vision were noted. Neurological examinations showed moderate dysphagia and mild to moderate ophthalmoplegias, and ptosis in both eyes. No limb weakness was noticed. Laboratory tests revealed mild elevation of serum creatine kinase concentration (280 U/L, reference: 15–130 U/L). His 2 daughters (subjects III-10 and III-11) were normal on neurological examination.

Patient II-6, a 62 year-old man, developed slow progression of gait disturbance including climbing upstairs since 5 years ago and slurred speech and easy choking for 2–3 years. Mild ptosis in both eyes was noted at the age of 42 years. Neurological examination demonstrated dysphagia, dysarthria, ptosis and mild weakness in the shoulder and pelvic girdle muscles. Tendon reflexes were absent in the low extremities. He died of lung cancer at the age of 63 years. His son (subject III-13), a 34 year-old man, did not have specific complaints, but it was observed that the swallowing time for cold water was prolonged.

Patient II-7, a 62 year-old woman with a history of undergoing eyelid operation 15 years ago, had difficulty swallowing for 3 years. She complained of frequent chocking while swallowing powder in her mid-thirty. On examination, ptosis and ophthalmoplegias were mild and bulbar palsy was moderate. Her daughter (subject III-14, a 40 year-old woman) and son (subject III-15) were normal on neurological examination.

Subjects II-1, and II-2 as well as their descendants were all healthy. Subject II-5 died of gastric cancer, but her daughter was normal on examination. The symptomatic subjects of the OPMD family are shown in the Fig. 1 and Table 1.

2.2. Swallowing test

All individuals of the OPMD family were requested to drink three times of 250 mL cold water (20–25 °C), with an interval of 30 min between the two tests. The time to swallow 250 mL of water was recorded. A normal reference was determined by calculating the

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**Table 1**

Clinical data of a Taiwanese family with oculopharyngeal muscular dystrophy

<table>
<thead>
<tr>
<th>Probands</th>
<th>II-3</th>
<th>II-4</th>
<th>II-6</th>
<th>II-7</th>
<th>III-7</th>
<th>III-9</th>
<th>III-13</th>
<th>III-14</th>
<th>IV-8</th>
<th>IV-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68</td>
<td>67</td>
<td>62</td>
<td>61</td>
<td>47</td>
<td>42</td>
<td>34</td>
<td>40</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
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<tr>
<td>Age of onset (years)</td>
<td>45</td>
<td>50</td>
<td>35</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>40</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Clinical manifestations</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Initial symptom</td>
<td>Dysphagia</td>
<td>Dysphagia or ptosis</td>
<td>Plos</td>
<td>Dysphagia</td>
<td>Dysphagia</td>
<td>Dysphagia, Dysphonia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dysphagia to solid food</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Time to swallow 250 mL of cold water</td>
<td>49.25**</td>
<td>25.48**</td>
<td>58.90**</td>
<td>66.30**</td>
<td>5.04</td>
<td>12.13</td>
<td>13.68**</td>
<td>13.07</td>
<td>10.43</td>
<td></td>
</tr>
<tr>
<td>Dysphonha</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Proximal limb weakness</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Nasal regurgitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Limitation of EOM</td>
<td>-2</td>
<td>-2</td>
<td>-3</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
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<tr>
<td>Gait disturbance</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CK, ref: 20-180 IU/L</td>
<td>70</td>
<td>280</td>
<td>NA</td>
<td>93</td>
<td>90</td>
<td>148</td>
<td>140</td>
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<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>SB</td>
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<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NCV/EMG</td>
<td>Normal</td>
<td>Myopathic/neurogenic</td>
<td>Myopathic</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

EOM, eye movement and 0 to −4 represent no to full limitation; CK, serum creatine kinase; EKG, electrocardiogram; NCV/EMG, nerve conduction study and electromyogram; F, female; M, male; *, surgical correction; **, prolonged swallowing time and reference (mean ± 2 SD) was 3.81–13.33 seconds; -, absent; +, mild impairment; ++, moderate impairment; NA, not available; SB, sinus bradycardia.

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average time it took for 22 unaffected subjects within this family to swallow 250 ml of cold water. The normal reference of swallowing time (mean±2 SD) was 8.62±4.7 s.

2.3. Molecular genetic analyses

Genomic DNA was extracted from peripheral blood leukocytes using the Extraction kit (Stratagene, La Jolla, CA). The PABPN1 DNA fragment flanking the (GCC)\(_n\)(GCA)\(_n\) repeat was amplified by polymerase chain reactions (PCR) using a pair of primers, forward: 5′-TCAGAGACTGGATGGAAGCTGG-3′ and backward: 5′TGGCTCTATTGTTTCAACTG-3′. Thermal cycling conditions consisted of 94 °C for 6 min followed by 50 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s with a final extension step of 72 °C for 10 min. PCR products were sequenced to determine the repeat size and sequence.

3. Results

3.1. Clinical data

Table 1 shows the clinical manifestation of the 10 family members containing an insertion of (GCC)\(_n\)GCA in the PABPN1 gene. The average swallowing time of these affected patients (32.97 s) was significantly longer than that of the unaffected subjects.

3.2. Electrophysiological studies

Electrophysiological examinations including sensory and motor nerve conduction studies, repetitive stimulation tests with a 3 Hz frequency and electromyographic studies of the muscles at face, proximal and distal extremities were performed in 6 symptomatic subjects and an asymptomatic one. The repetitive stimulation tests showed no significantly decremental responses in all subjects. The nerve conduction studies were unremarkable in all subjects. On needle electromyography, fibrillation and positive sharp waves were noted in the biceps, first dorsal interosseous and tibialis anterior muscles, decreased recruitment pattern of motor unit action potentials in frontalis and tibialis anterior muscles and early recruitment in the biceps muscles of subject II-4 and diffusely myopathic changes in subject II-6.

3.3. Muscle biopsy

Muscle biopsy was obtained from the vastus lateralis muscles of subject II-3. Specimens of the muscle biopsy were processed for histological and ultrastructural studies. Using light microscopy analyses, muscle pathology showed mild variability in fiber size and rare fibers containing rimmed vacuoles. Electron microscopy studies showed neither paracrystalline mitochondrial inclusions nor intranuclear filamentous inclusions in the at least 200 examined cells.

3.4. Characterization of a PABPN1 mutation with trinucleotide expansion

Bloods from members of the 3-generation OPMD family were obtained for genetic analysis of the PABPN1 gene. The DNA fragment of the mini-repeat in the first exon of the PABPN1 gene on chromosome 14 was amplified by PCR. A fragment of slightly longer length (256 bp) than the expected 241 bp was observed on the gel in subject II-3, II-4, II-6, II-7, III-7, III-9, III-13, III-14, IV-8, and IV-10. The data indicated a pathologically expanded allele on one chromosome 14 along with the normal allele on the other one. Unaffected members did not have such expanded alleles.

3.5. Sequence analysis of the exon 1 of the PABPN1 gene of affected subjects

To determine the size and sequence of the mutation, direct sequencing was performed from the expanded allele after obtaining the band from the running gel. Compared to healthy control subjects, a mutant allele with the insertion of (GCC)\(_n\)GCA was found as demonstrated in 6 symptomatic patients with OPMD and 4 asymptomatic carriers (III-13, III-14, IV-8, and IV-10).

4. Discussion

The diagnosis for OPMD in this family was based on the clinical manifestations, pathological findings and confirmed by molecular genetic analysis. Inheritance is autosomal dominant with complete penetrance, but clinical manifestation in a dominant OPMD usually occurs in middle age. The clinical data indicate a dominant inheritance in the OPMD family studied herein, despite 4 asymptomatic subjects (subjects III-13, III-14, IV-8, and IV-10) with a PABPN1 gene mutation. The latter subjects may be too young to fully exhibit all OPMD specific symptoms. In muscle pathology of patients with heterozygotes for OPMD, the frequency of tubulo-filamentous inclusions and rimmed vacuoles varies from 0% to 5%. It is dependent on different sites of biopsied muscles [8,21,22]. The intranuclear inclusions are usually absent in severe atrophied muscles [8,21]. It was difficult to find the rimmed vacuoles and intranuclear tubulo-filamentous inclusions in the muscle specimen of patient II-3. To explain our pathologic findings, the result might be due to the specimen was obtained from normal muscle strength and electromyographic studies. Otherwise, it is necessary that the examined muscle fibers should be adequate for detecting intranuclear inclusions on ultrastructural studies.

The main genetic finding of this Taiwanese OPMD family was a heterogeneous trinucleotide expansion being caused by an insertion of (GCC)\(_n\)GCA in PABPN1. Instead of expansion of the GCC repeat from 6 to 8–13, a mutated allele (GCC)\(_n\)(GCA)(GCC)\(_n\)(GCA)GCC was identified in this study as compared to (GCC)\(_n\)(GCA)GCC in healthy subjects. Given that both the GCC and GCA codons are translated into alanine residues, transcription will cause an expansion of the alanine stretch (changed from 10 to 15 alanines per protein as compared to normal PABPN1 protein). Similar mutations causing a differential elongation of the alanine stretch have been reported [12,24,25]. We therefore proposed that because of the similar alteration at the protein level, a comparable phenotype is applied to subjects examined in this study. However, all affected subjects showed variability in age of onset after fourth decade, initial symptoms, disease course, and electrophysiological findings and one subject carrying this mutation was still asymptomatic in the beginning of her forty. Indeed, it was proposed that the specific clinical features might not be necessarily attributed to the length of the polyalanine tract of PABPN1 [12].

This mutation can be helpful to further understand the molecular mechanism underlying OPMD. A repeat-length variation in the polyalanine tract of PABPN1, involving polymerase slippage during DNA replication [26], is generally considered as a possible explanation for the mutation-disease correlation. Polyalanine expansion, it is though by a crossing-over developed between two homologous but mispaired normal alleles [25,27]. However, recent evidence strongly suggested that other mechanisms than the crossing-over theory might result in polyalanine expansions [28]. The mutated alleles identified in these Taiwanese OPMD subjects were most likely due to the insertion of (GCC)\(_n\)GCA into the normal allele. This type of triplet repeat mutation in the PABPN1 gene has been identified in English OPMD families [28], but not in the Asian population. Similarly, mutated PABPN1 alleles caused by insertion of (GCC)\(_n\)GCA and (GCC)\(_n\)(GCA)\(_n\) have been found in Japanese OPMD patients [12] and GCC(GCA)\(_n\) in a Korean patient [15]. It is notable that both mutations found in the Taiwanese family and the Japanese OPMD patients were of successively as well as meiotically stable in nature. Unequal crossing-over of two PABPN1 alleles, rather than DNA slippage, was postulated by the results of this study. Further
studies are required to pinpoint the influence of an expanded polyalanine tract in \textit{PABPN1} on the malfunctioning of \textit{PABPN1} protein and OPMD symptom initiation.

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**References**


