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Gene Diagnosis of Oculopharyngeal Muscular Dystrophy in a Chinese Family by a GeneScan Method

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This study aims to perform gene diagnosis for Chinese family patients with Oculopharyngeal muscular dystrophy (OPMD). Genomic DNAs were extracted from the pedigrees' members. Gene diagnosis was performed for these pedigrees' members by approaches, such as DNA sequencing and GeneScan. Sequence analysis and PABPN1 genotyping showed that the mutated allele in affected members of this family has nine trinucleotide repeats of GCG $(GCG)_9$, whereas the normal allele contains six trinucleotide repeats of GCG $(GCG)_6$. The above results suggest that mutated GCG repeats in PABPN1 gene may cause OPMD in this family, and PABPN1 genotyping could be used as a convenient, highly effective, and reliable gene diagnostic test for OPMD patients. J. Clin. Lab. Anal. 24:422–425, 2010. © 2010 Wiley-Liss, Inc.

Key words: oculopharyngeal muscular dystrophy; PABPN1; gene diagnosis; genescan; genotyping

INTRODUCTION

Oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant genetic disorder characterized by eyelid drooping (ptosis) and swallowing difficulty (dysphagia) (1). OPMD was largely reported in Caucasian families, especially of French-Canadian trait. Several cases in Asians were reported. The onset of OPMD is usually during the fifth or sixth decade of life, and all patients are symptomatic beyond the age of 70.

A spectrum of mutations $(GCG)_{8-13}$ $(GCA)_3$ (GCG) was found in cohorts studied throughout the world (2–4). Here, we report an OPMD case in a Chinese family with four members affected, describe their clinical details, and identify related genetic mutations in the PABPN1 gene.

MATERIALS AND METHODS

Subjects

We found an OPMD family from Fujian Province in China (Fig. 1). All seven members of the OPMD family received electrophysiological examinations (Table 1). The proband is a 78-year-old woman, who suffers from ptosis and dysphagia with solids and liquids. In neurological examinations, there are limitations for the ocular movement in all directions, especially for the extra-ocular movement. She is also noted to be having difficulty in climbing down the stairs. In this family with ten members, four members are affected (one male and three females). Three members are asymptomatic young persons (all females) but with a mutated PABPN1 gene. The other three are normal. The II:1 is a 58 year old man, who has weakness in the legs to the extent that he has difficulty in going up and down the stairs. He has a history of bilateral ptosis for 5 years and has been coughing while drinking for the past 4 years. Neurological examinations showed that his ocular movement is normal but his deep tendon reflex is reduced in four limbs. The strength of his bilateral sternocleidomastoid

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muscles is MRC grade 4 (diminished power). He also shows a duplication of right thumbs. The II:2 is a 56 year old woman who has been suffering from bilateral ptosis and has difficulty swallowing for the past 3 years. Clinical examination shows that her deep tendon reflex is reduced in the upper and lower limbs. The strength in her bilateral sternocleidomastoid muscles is MRC grade 4. The II:3 is a 53 year old woman, who has difficulty swallowing for the past 4 years. Both the II:4 and the II:5 are male, aged 48 and 42, respectively. Neither of them shows any neurological symptoms. The III:1-3 are asymptomatic of young persons and the III:4 in normal ones. Their ages, in the third generation, are all under 30 years old. With the family members' informed consent, their blood samples were collected for screening PABPN1 gene with possible expansions in the GCG repeat sequence, and genomic DNAs were extracted from the pedigrees' members.

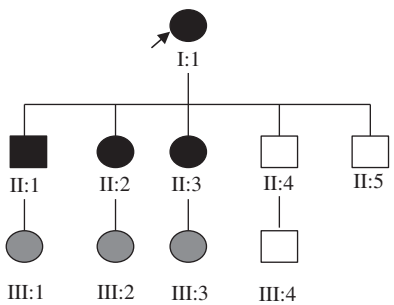

Fig. 1. The genealogical tree of a Chinese OPMD family shows a dominantly inherited disorder affecting seven family members in three generations. All affected individuals (black symbol) are heterozygous for the mutation $(GCG)_9$. Individuals of III:1, 2, 3 are asymptomatic young persons (gray symbol), who are also heterozygous for the mutation $(GCG)_9$. The arrow indicates the proband. The symbol circle and square stand for female and male, respectively.

Methods of the DNA Sequencing Analysis

The repeat sequence of genomic DNAs was amplified by PCR using PABP-F Primer (5′-CGCAGTGCC-CCGCCTTAGA-3′) and PABP-R Primer (5′-ACAA-GATGGCGCCGCCGCCCCGGC-3′) as described by Braid et al. (5). The amplification was carried out on a Mastercycler gradient (Eppendorf, Hamburg, Germany) with an initial denaturation reaction at 94°C for 20 min, followed by a 35-cycle reaction of 94°C for 1 min, 64°C for 1 min, and 72°C for 1 min, and the final elongation reaction was at 72°C for 7 min. The PCR product was purified by 2.0% agarose gel electrophoresis stained with SYBR Green I. The 230 bp fragment was isolated using the QIAEX kit (Qiagen, Hilden, Germany) and subsequently cloned into the vector pUC19 using a pUC19-T cloning kit (TaKaRa, Shiga, Japan). The recombinant plasmid was finally transformed into *E. coli* DH5α using standard procedures (6). The amplified recombinant plasmid DNA was isolated by the alkaline lysis method (7). Both purified PCR products and the recombinant plasmids were sequenced by an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

TABLE 1. Clinical Data of a Chinese Family With Oculopharyngeal Muscular Dystrophy

Probands	I-1	II-1	II-2	II-3	III-1	III-2	III-3
Age (years)	78	58	56	53	<30	<30	<30
Sex	F	M	F	F	F	F	F
Age of onset (years)	65	53	53	49	–	–	–
Clinical manifestations							
Initial symptom	Dysphagia or ptosis	Dysphagia or ptosis	Dysphagia or ptosis	Dysphagia	–	–	–
Ptosis	++	++	++	–	–	–	–
Dysphagia to solid food	++	++	++	+	–	–	–
Time to swallow 250 ml of cold water	30.46	25.14	20.76	17.83	NA	NA	NA
Dysphonia	+	–	–	–	–	–	–
Proximal limb weakness	–	–	–	–	–	–	–
Sternocleidomastoid muscle weakness	–	+	+	–	–	–	–
Nasal regurgitation	+	+	–	–	–	–	–
Limitation of EOM	−2	0	0	0	0	0	0
Gait disturbance	–	–	–	–	–	–	–
CK, ref: 20–180 IU/l	122	83	130	56	44	51	79
EKG	Normal	Normal	Normal	Normal	Normal	Normal	Normal
NCV/EMG	Myopathic	Myopathic	Normal	Normal	Normal	Normal	Normal
Other diseases	DM	DM	DM	–	–	–	–

OM, eye movement. From 0 to −4 represents none to full limitation; CK, serum creatine kinase; EKG, electrocardiogram; NCV/EMG, nerve conduction study/electromyogram; F, female; M, male; swallowing time (sd: 2 sec); –, absent; +, mild impairment; ++, moderate impairment; NA, not available; DM, diabetes mellitus.

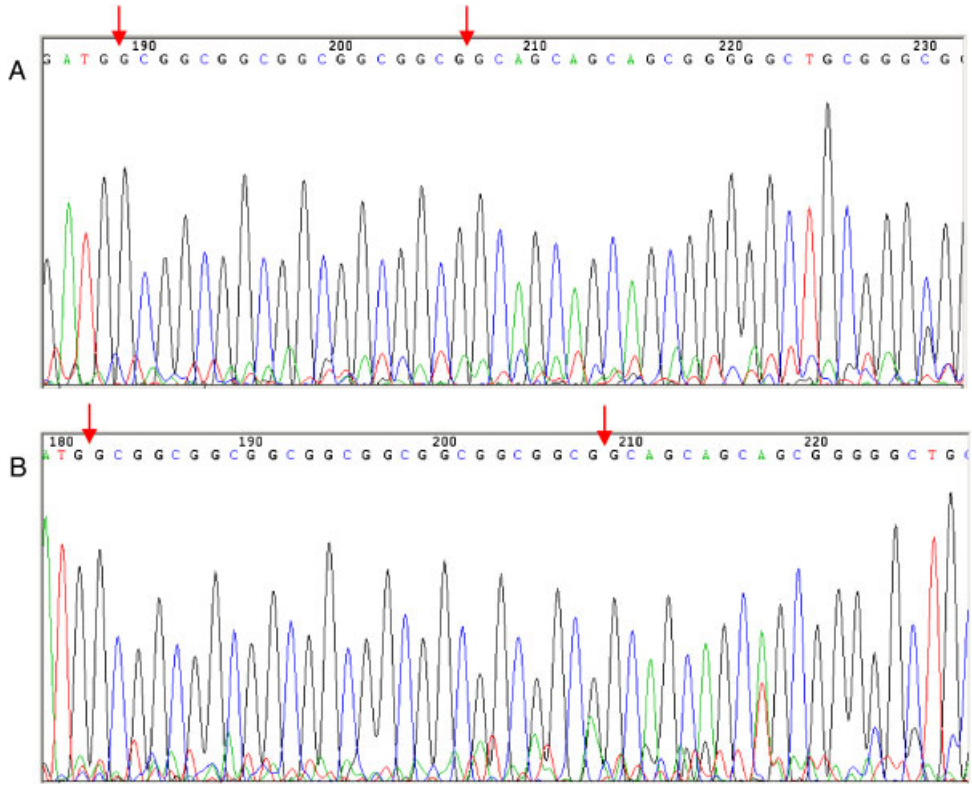

Fig. 2. Sequencing of PABPN1 genes from both unaffected persons and from OPMD patients. In Panel **A**, the arrow indicates the normal allele with 6-GCG repeats in PABPN1 gene. In Panel **B**, the arrow shows the expanded allele with 9-GCG repeats in PABPN1 gene.

Methods of the GeneScan Analysis

A GeneScan analysis was applied in the OPMD diagnosis. DNA samples from OPMD patients and normal persons were analyzed by PCR amplification, essentially as described above, but the reverse primer was labeled with fluorescent HEX (hexachlorofluorescein) at its 5′ end. The PCR products were separated according to their sizes by capillary electrophoresis on an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems). Loading cocktails included a 9.5 ml Hi-DiTM Formamide (Applied Biosystems) and a 0.5 ml ILS600 ladder (Promega, Madison, WI). The electrokinetic injection was carried out at 3 kV for 5 sec and the running voltage is 15 kV. The standard running conditions include a 36 cm capillary array, a 3,130 POP-4TM polymer and 1 × Genetic Analyzer Buffer with EDTA (all from Applied Biosystems). Running files were analyzed by the GeneMapper 3.2 software with a 50 RFU analysis threshold.

RESULTS

DNA Sequencing Analysis

The purified PCR products and recombinant plasmids were sequenced by an Applied Biosystems 3130 Genetic Analyzer. In DNA samples of I:1, II:1-3, and III:1-3, sequencing analysis showed that all of them have one allele of PABPN1 gene with nine repeats of GCG-trinucleotide and the other allele with only six repeats. But II:4, II:5, and III:4 have six repeats of GCG-trinucleotide in both alleles of PABPN1 gene (Fig. 2).

GeneScan Analysis

Results obtained by the GeneScan analysis were in accord with DNA sequencing analysis (Fig. 3). The allele of PABPN1 gene at this locus has two copies of $(GCG)_6$ with a 18 bp sequence. But the allele of the mutated PABPN1 gene at this locus has one copy of the $(GCG)_6$ with a 18 bp sequence and one copy of the $(GCG)_9$ with a 27 bp sequence. The difference between the allele $(GCG)_6$ and $(GCG)_9$ is 9 bp sequence.

DISCUSSION

We described an OPMD family found in Fujian Province, China. The diagnosis by genotyping applied in this family shows a heterozygous abnormal expansion of GCG trinucleotide repeats in PABPN1 gene, which is one of the most commonly recognized gene related to OPMD (5,8). OPMD is rarely reported in Asians, especially in Chinese populations. Goh et al. reported a

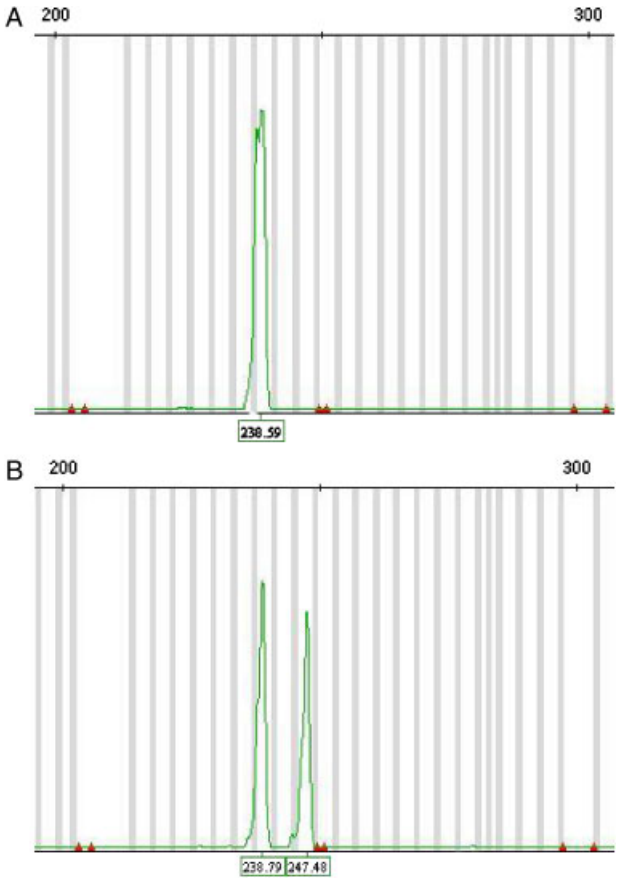

Fig. 3. PABPN1 genotyping by a GeneScan analysis. The number beneath each peak is the estimated size (bp) of DNA fragments. Panel **A** shows that an unaffected individual has 6-GCG repeats in both alleles in his PABPN1 gene. Panel **B** shows that the PABPN1 gene from an affected individual has one allele with 6-GCG repeats and one allele with an expanded 9-GCG repeats.

Malaysian family of Chinese descent with autosomal dominant OPMD (9). Kuo et al. found an insertion of $(GCG)_4$ GCA in the PABPN1 gene in the Taiwanese OPMD subjects (10). Although a few OPMD patients in mainland China have been reported, few cases have been genetically confirmed. Liu et al. reported the first OPMD family confirmed genetically in mainland China (11). They found the abnormal expansions of $(GCG)_8$ and $(GCG)_{10}$ repeats in the PABPN1 gene using DNA sequencing in three OPMD families (11). Here, we report another OPMD family in mainland China. The abnormal expansion of $(GCG)_9$ repeats is found in one allele of their PABPN1 gene.

The paucity of reported cases suggests that OPMD is an uncommon disorder in Chinese populations. However, the condition could be underrecognized as the symptoms of this disorder appear in later life and may even not be noticed in the initial stages, just as those found in III: 1, 2, 3 of this family. Therefore, medical attentions could be never paid to these cases since they lack of symptoms. This report highlights the existence of this form of muscular dystrophy in China.

It is estimated that 99% of patients diagnosed with severe, autosomal dominant OPMD actually carry a pathogenic PABPN1-triplet repeat expansion (12). Therefore, expansion analysis of GCG-triplet repeats in PABPN1 gene is a reliable gene diagnostic test for OPMD. It only takes about 5 hr to determine the normal and the expanded alleles of GCG repeats by a complete GeneScan analysis, which is faster and more convenient than using DNA sequencing assay.

In summary, we report a Chinese OPMD family confirmed genetically. Our results suggest that PABPN1 genotyping could be used as a convenient, highly effective, and reliable gene diagnostic test for OPMD patients.

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