

Oculopharyngeal Muscular Dystrophy – A Genetically Verified Taiwanese Family

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Background: Oculopharyngeal muscular dystrophy (OPMD) is a rare inherited muscular disorder, clinically characterized by late-onset, slowly progressive bilateral ptosis, dysphagia, and proximal limb weakness. A short polyalanine expansion in the polyadenylate binding-protein nuclear 1 (*PABPN1*) gene is a commonly reported mutation.

Methods: We studied a large family with 12 affected members who inherited a dominant trait. Drooping of eye lids and dysphagia were characteristic phenotypes starting in the sixth decade. We collected blood samples from all available familial members and 30 control subjects. They were analyzed using modified polymerase chain reaction (PCR) amplification and direct sequence analysis.

Results: The abnormally extended three GCG resulting in heterozygous (GCG)_n of *PABPN1* gene was identified in four affected and two asymptomatic carriers, but not in the 30 control individuals. The expansion of the *PABPN1* polyalanine tract which resulted from 10 to 13 alanines was further confirmed by subcloning into TOPO cloning vectors.

Conclusions: The phenotypic characteristics and genetic information confirmed our diagnosis of OPMD. We suggest that genetic intervention should be undertaken to understand the genetic epidemiology and provide counseling for carriers of OPMD in Taiwan.

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Key words: oculopharyngeal muscular dystrophy, polyadenylate binding-protein nuclear 1 (*PABPN1*) gene, polyalanine expansion

Oculopharyngeal muscular dystrophy (OPMD) is a late-onset muscular degenerative disease characterized by progressive ptosis, dysphagia and proximal limb weakness. It most often occurs in the elderly populations during the fifth or sixth decades of life. OPMD is usually inherited in a dominant pattern

with occasional recessive inheritance. In 1998, Brais et al. decoded the short GCG expansions in the polyadenylate binding-protein nuclear 1 (*PABPN1*) gene causing the specific atrophy of oculopharyngeal muscles in 144 families.⁽¹⁾ In healthy subjects, a wild-type alanine repeat stretch encodes the opening six

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alanines (GCG)₆ of this 10 alanine stretch in the protein. An expansion of heterozygous 12-17 alanine residues in the polyalanine (polyA) stretch of the PABPN1 protein are responsible for most dominant-inherited types in families, whereas only one extra alanine (GCG)₇ in the homozygous state may lead to recessive-inherited individuals.⁽¹⁾ However, it has become clear that approximately 25% of these mutations consist of (GCN) insertions or cryptic synonymous expansions that do not modify the impact on the PABPN1 protein because all four GCN triplets code for alanine.⁽²⁾ Therefore, identifying the short GCG expansions or GCA insertions have recently become the key factors for the genetic diagnosis of OPMD.

OPMD has been reported in various ethnicities,⁽³⁾ the highest prevalence being in French – Canadian kindred (1:1000)⁽¹⁾ and Bakhara Jews (~1:600).⁽⁴⁾ Genetically confirmed families or cases of OPMD have uncommonly been documented in Asian populations.⁽⁵⁻⁸⁾ In a report, Chang et al. described a 47-year-old Taiwanese woman who presented with dysphagia,⁽⁹⁾ following nasal regurgitation of fluid, nasal quality of speech and ptosis for 5 years, which is consistent with OPMD clinically, and a CT scan of the oropharynx. However, we were unable to obtain any information about the relationship between the genotype and phenotype.

To confirm the diagnosis in our familial patients clinically suggested of having OPMD, we investigated the phenotypic characters, genotypic variations, and correlations between each other in a large Taiwanese family with OPMD.

METHODS

Clinical study

A total of 12 affected members were found in this family (Fig. 1). Among the family members, 11 (4 symptomatic and 7 asymptomatic) were personally interviewed. The proband was a 71-year-old woman who first presented with drooping eyelids at 56 years of age and had subsequently received surgical reconstruction for ptosis twice. However, the drooping eyelids gradually progressed. About 5 years prior to this investigation, she developed difficulties in swallowing solid food so that she required a soft diet. Due to severe constriction of her throat, her diet was restricted to rice porridge which resulted in loss of body weight for 2 years. Through the familial survey, we further identified another 11 members who presented with similar but milder symptoms (Fig. 1). We interviewed another three affected members in the third generation and seven offspring in the fourth generation, who were available. Written informed consent was given by all participants and healthy control subjects.

Molecular genetic study

Blood samples from all available familial members and 30 control subjects (IRB No. 96-1759B) were obtained in ethylenediaminetetraacetic acid (EDTA) -anticoagulant tubes. DNA was extracted using standard procedures and analyzed for the presence of polyalanine codon expansion in the *PABPN1* gene exon 1 by modified polymerase chain reaction (PCR) amplification as described previously.⁽¹⁾ PCR

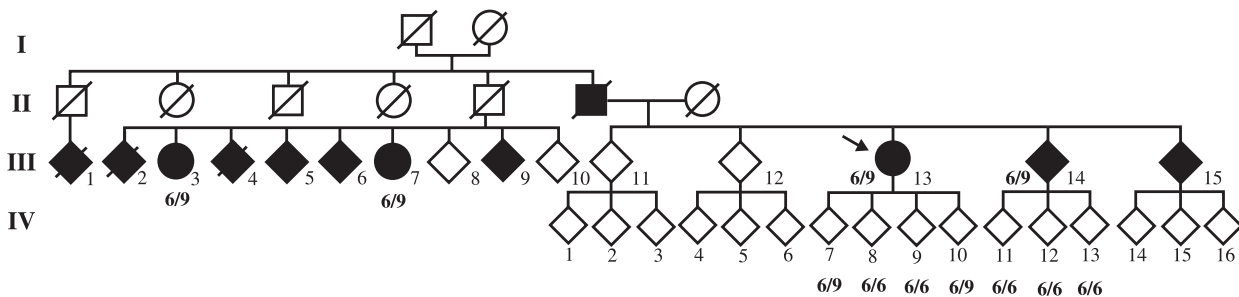


Fig. 1 Pedigree of the family. This familial tree shows the affected (black) and asymptomatic (blank) members. Normal repeat (6/6) and expanded repeat (6/9) are indicating the genetic results in the members who were also clinically examined. The results indicate a pattern of autosomal dominant inheritance. III-13 is the proband (arrow). Squares indicate male and circles female. Diagonal lines indicate deceased. Diagonals are designed for the protection of the individual's right.

was processed in a volume of 25 µl containing 100 ng of genomic DNA and 0.5 units of Taq polymerase ("FailSafe" Taq). The primer sequences were as follows: PABPF (forward), 5'-CGC AGT GCC CCG CCT TAGA-3'; PABPR (reverse), 5'-ACA AGA TGG CGC CGC CCC GGC-3'. The thermocycling conditions were 95°C for 5 minutes followed by 40 cycles of 94°C for 1 minute, 67°C for 40 seconds, and 72°C for 1 minute, and a final step of 72°C for 10 minutes. Sequence analysis was then performed on the PCR product using a DNA analyzer (ABI 3730 Genetic Analyzer). To isolate the mutant allele, the PCR product carrying the mutation was subcloned into TOPO cloning vectors (Invitrogen Corporation, Carlsbad, Calif) according to the manufacturer's instructions and the sequence verified.

RESULTS

Neurological examination of the proband showed bilateral ptosis and raised eyebrows without weakness of the extraocular muscles. The other symptoms included difficulty in tongue protrusion, nasal voice, dysphagia and atrophy of the shoulder muscles. No other limb muscle weaknesses or fasciculation were found. Serum levels of creatine kinase and myoglobin were normal. A repetitive nerve stimulation test showed no abnormally decremented

responses and needle electromyography (EMG) over facial and limb muscles did not detect abnormal neurogenic or myopathic motor unit potentials. The normal EMG findings may have resulted from mild symptoms or inadequate positions in the procedure.

The family member with the proband recalled that her father presented with drooping eyelids when he was approximately 60 years old. The other affected members also showed similar pictures at various ages of onset but no shoulder muscle atrophy (Table 1). The mean age at onset was 58.6 years (range, 52-70 years). All symptomatic relatives had received surgical reconstruction of the eyelids once or twice. Both cousins (III-2 and III-4) died of choking, however no definite diagnosis was given at the time of death.

Sequence analysis of the *PABPN1* gene on the 11 available family members showed abnormal triplet repeat expansions of GCG causing (GCG)_n in one allele of four affected (Fig. 1) and two asymptomatic carriers. The expansion of the *PABPN1* polyalanine tract resulted from 10 to 13 alanines. Fig. 2 illustrates the sequence of the family member with the proband and one healthy control subject. The expansion of the GCG of the mutated allele of the proband was further confirmed using subcloning into the TOPO cloning vectors (c.13-21 dup GCGGCGGCG; Fig. 2). In contrast, no evidence of

Table 1. Clinical Demography and GCG Expansion in PABPN1 Gene of the OPMD Family

Patient†	Sex	Age (years)	Age at onset (years)	Initial symptom	Age at onset (years)			GCG expansion
					Ptosis	Dysphagia	Limbs weakness	
III-2	M	74*	NA	ptosis	+	+	--	NA
III-3	F	80	70	ptosis	+(70)	+(76)	--	6/9
III-4	M	70*	NA	NA	NA	+	NA	NA
III-5	M	74	60	ptosis	+(60)	--	--	NA
III-6	F	72	NA	ptosis	+	NA	--	NA
III-7	F	70	55	ptosis	+(55)	+(61)	+(67)	6/9
III-9	M	67	NA	ptosis	+	+	--	NA
III-13	F	71	56	ptosis	+(56)	+(66)	--	6/9
III-14	F	66	52	ptosis	+(52)	+(64)	--	6/9

Abbreviations: *: Died of sudden choking; †: The same identifications as indicated in the pedigree; M: male; +: present; --: absence; NA: not available; F: female.

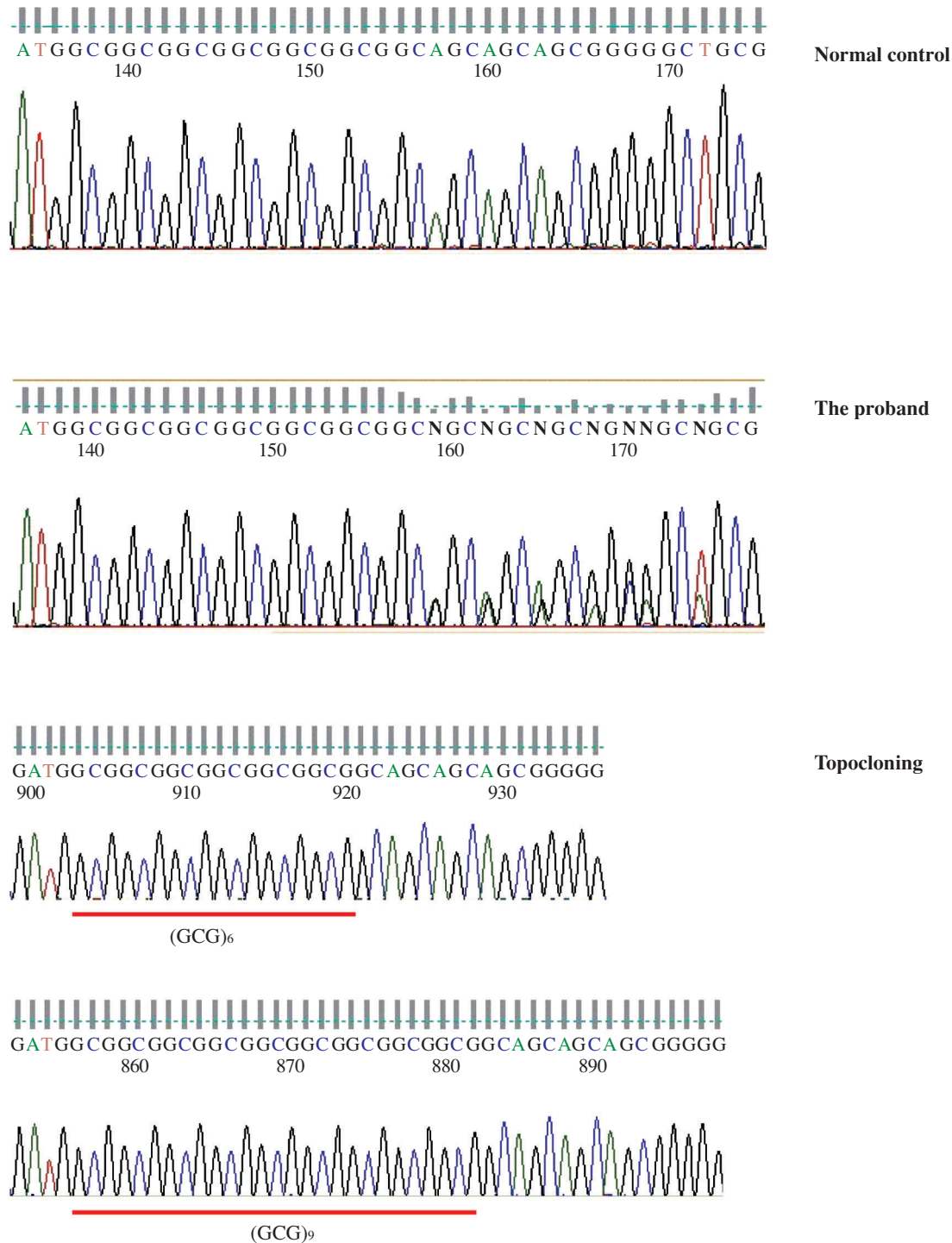


Fig. 2 Sequence analysis of genomic DNA from the proband and healthy control subjects. The heterozygous alleles (mark N and arrows) of the proband (middle panel) compare to the homozygous alleles in a healthy individual (upper panel). The lower two panels show the further analysis of the mutated allele by subcloned in Topocloning vector; one is a normal repeat (GCG)₆ and the other is an abnormal expansion (GCG)₉.

abnormal expansions, insertions, or polymorphisms was identified in the 30 healthy control subjects.

DISCUSSION

This family with OPMD was diagnosed essentially on a dominantly inherited trait, the typical clinical features, and abnormal triplet GCG expansion. As far as we know, this is the first report of a Taiwanese family with OPMD confirmed genetically, although a single case has been previously described.⁽⁹⁾ Before genetic testing became available, the diagnosis of OPMD was generally made on purely clinical grounds. Later on, light and electron microscopy study results of muscle biopsy specimens provided further diagnostic confirmation by the presence of rimmed vacuoles within muscle fibers and intranuclear inclusion bodies.⁽¹⁰⁾ Since Brais et al. decoded the gene mutation of OPMD,⁽¹⁾ molecular genetic analysis of OPMD has become an important issue in the investigation this disease because of its high accuracy in spite of the unknown pathophysiology. Nowadays, it is essential for the diagnosis of OPMD in suggested cases rather than invasive muscle biopsy, which allows for testing in asymptomatic relatives of OPMD individuals.

To the best of our knowledge, rarely have patients with OPMD been recorded previously in Asia and showed variable genetic mutations, including (GCG)_n repeat or GCA insertion.⁽⁵⁻⁸⁾ The paucity of reports in Asia might be due to later onset of the disease, and the diagnosis might be masked by symptoms of other diseases of the elderly, such as other neuromuscular disorders (myasthenia gravis, mitochondriopathy such as progressive external ophthalmoplegia), eyelid muscle weakness, eyelid nerve damage, Horner's syndrome and even cosmetic problems. However, if the patient does not present with all of the cardinal symptoms, it is more difficult to confirm, which may result in underestimation.⁽¹¹⁾

Common founder mutations have been found in French-Canadians and Bukhara Jews. Researchers from the United Kingdom and Germany have indicated a large genetic heterogeneity in the populations and showed no single founder effect.^(12,13) After the pioneering investigation by the neurologist Dr. Wu in a local hospital center, several families who are inhabitants of the same area also presented with OPMD. Therefore, further studies on the possibility

of the founder effect should be very interesting.

In summary, this is a report of a genetically confirmed OPMD family with typical clinical features and heterozygous (GCG)₉ repeats of the *PABPN1* gene in Taiwan. To identify the appropriate treatments for this disease, we should raise the awareness of OPMD, estimate its prevalence and clarify the phenotype / genotype relationship in our patients in the future.

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Note: During the second review processing, a paper reporting similar results was published online. (Kuo et al., J Neurol Sci 2009;278:21-4.)

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眼咽型肌肉萎縮症——經由基因證實的一個台灣家庭

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背景： 眼咽型肌肉萎縮症 (Oculopharyngeal Muscular dystrophy) 是一種以進行性骨骼肌退化為主的罕見基因遺傳疾病。臨床症狀表現一開始多以眼皮下垂為主，經過一段時間後開始出現吞嚥困難，少部分的人會合併有近端肢體無力。發病年齡多在中年及老年期，通常約在五、六十歲，而且疾病進展速度相對緩慢。聚丙胺酸(polyalanine) 在 PABPN1 基因上的擴展是最常見的突變方式。

方法： 我們針對一個大家族來作研究。這個家族有十二個成員符合典型的眼咽型肌肉萎縮症症狀——眼瞼下垂，吞嚥困難，且多在六十歲左右發病。我們收集這個家族以及三十個對照組的血液，用聚合酶連鎖反應 (PCR) 與序列分析來進行基因研究。

結果： 這個家族中有四位出現症狀的病人與兩個無症狀的家屬在 PABPN1 基因上發生 (GCG) 數目的異常，造成 GCG₆/CGC₉ 的異合子組合，使得聚丙胺酸的數目也跟著改變。這樣的變化並沒有在三十個對照組上發現。

結論： 無論在臨床上的表現或者是基因研究上都證實眼咽型肌肉萎縮症的診斷。我們建議在未來對於在臨床上有類似症狀且對診斷有疑慮的病人，應該可以經由基因檢測來確定診斷，對於台灣地區眼咽型肌肉萎縮症的診出率也能提升而對這個疾病有更進一步的瞭解。

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關鍵詞： 眼咽型肌肉萎縮症，聚腺苷酸結合核蛋白 1 基因，聚丙胺酸擴展

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