ONLINE MUTATION REPORT

Oculopharyngeal muscular dystrophy: a point mutation which mimics the effect of the *PABPN1* gene triplet repeat expansion mutation

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Background: Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant late onset neuromuscular disease characterised by proximal muscle weakness, ptosis, and swallowing difficulty. The only causative mutation described to date is a triplet repeat expansion consisting of two to seven additional base triplets in a repeat sequence in exon 1 of the polyadenine binding protein nuclear 1 (*PABPN1*) gene. This results in an increase in length of a polyalanine tract in the *PABPN1* protein from 10 to 12–17 residues.

Objective: Description of another mutation in a case of OPMD.

Methods: Sequence analysis of exon 1 of the *PABPN1* gene was undertaken on 202 patients referred for a possible diagnosis of OPMD but negative for the triplet repeat expansion mutation.

Results: A case was identified with typical symptoms of OPMD, negative for the repeat expansion mutation but with a missense mutation in *PABPN1* close to the 3' end of the normal polyalanine codon repeat sequence.

Conclusions: The single base mutation changes a glycine codon to an alanine codon and results in an increase in the number of contiguous polyalanine codons. This mimics the effect of the common triplet repeat expansion mutation and represents a previously undescribed mechanism of mutation.

Oculopharyngeal muscular dystrophy (OPMD) is a late onset dominantly inherited condition that usually presents with dysphagia and ptosis, and later may be accompanied by progressive proximal limb weakness. The underlying cause of this disease is an expansion of a trinucleotide repeat sequence normally encoding 10 contiguous alanine codons in exon 1 of the polyadenine binding protein nuclear 1 gene (*PABPN1*, also known as *PABP2*) on chromosome 14q11.¹ Most published reports describe the extra triplet repeats as pure GCGs; however, some studies have identified GCA interspersions within the expanded GCG sequence.^{2–4} Expansion mutations cause an increase in the number of alanine residues in the *PABPN1* protein and are associated with the deposition of characteristic nuclear filament inclusions in skeletal muscle fibres. Brais *et al*¹ found the expanded alleles to have an additional two to seven alanine codons in the dominantly inherited condition and also described a recessive allele consisting of a single additional GCG triplet.

We have previously described the analysis of exon 1 of the *PABPN1* gene in 288 patients referred for a possible diagnosis of OPMD, 86 of which had an expansion mutation.² Here we describe a case negative for the expansion mutation but with

a point mutation close to the 3' end of the polyalanine codon repeat sequence.

METHODS

Subjects

Patient A is a woman of NW European origin and was referred with OPMD at the age of 65 for genetic analysis. Ptosis developed at age 61, followed by dysphagia. Her eye movements were normal and peripheral muscles were of normal strength. Creatine kinase levels were normal at 122 IU/l (normal <180). No biopsy was available for analysis of muscle histology. Her mother developed ptosis at age 70 followed by severe dysphagia requiring placement of a percutaneous gastrostomy. Her maternal grandfather and three maternal half siblings (two male, one female) also developed ptosis in later life (fig 1). No samples were available from relatives of the proband.

Samples from a further 202 patients referred for a suspected diagnosis of OPMD and negative for the classical OPMD repeat expansion mutation were also analysed, together with 80 control samples of NW European origin with no known family history of OPMD. The family pedigree is shown in fig 1.

Molecular analysis

Samples were referred as blood in EDTA anticoagulant and DNA was extracted using standard procedures.⁵ Samples were analysed for the presence of the *PABPN1* gene exon 1 polyalanine codon expansion by polymerase chain reaction (PCR) amplification, as described previously.^{1,2,6} DNA sequence analysis was carried out by amplification of exon 1 of the *PABPN1* gene by PCR in a volume of 20 µl containing 50 ng of genomic DNA and 0.5 units Taq polymerase ("Failsafe" Taq, Cambio, Dry Drayton, Cambridgeshire, UK), using the manufacturer's recommended reaction buffer

Figure 1 Family pedigree. III.1 = proband; II.2 developed mild ptosis at age 70 with severe dysphagia; I.2, II.3, II.4 and II.5 developed ptosis in later life.

Abbreviations: OPMD, oculopharyngeal muscular dystrophy

ATG GCG GCG GCG GCG GCG GCG GCA GCA GCA GCG GG→CG GCT GCG GGC GGT CGG GGC TCC GGG CCG GGG CGG CGG CGC
 M A A A A A A A A A A G→A A A G G A G S G P G A A A

Figure 2 Coding sequence of the first 25 codons of the *PABPN1* gene indicating the c.35G→C base change and the predicted Gly12Ala amino acid change identified in patient A

(buffer J). Primer sequences were as follows: PABPF (forward), 5'-CGC AGT GCC CCG CCT TAGA-3'; PABPR (reverse), 5'-ACA AGA TGG CGC CGC CGC CCC GGC-3'.

Thermocycling conditions were 94°C for 12 minutes followed by 25 cycles of 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30 seconds, and a final step of 72°C for seven minutes.

Sequencing analysis in both forward and reverse directions was then undertaken on the 242 base pair PCR product using ABI Big Dye Terminator cycle sequencing chemistry version 1.1 (PE Biosystems, Norwalk, Connecticut, USA) and an ABI 3100 DNA analyser.

RESULTS

A total of 202 samples referred for OPMD but with no evidence of the *PABPN1* exon 1 expansion mutation were sequenced. One of these, patient A, was found to have a point mutation in exon 1 of the *PABPN1* gene (fig 2). The mutation was a c.35G→C base change resulting in a glycine to alanine substitution at amino acid position 12 (p.Gly12Ala). [The nomenclature follows the Human Genome Variation Society guidelines, web site 'http://www.hgvs.org/', using DNA sequence accession number ENSE00001099642].

No evidence of this mutation was found in 367 other cases which included the remaining 201 triplet repeat expansion negative OPMD referrals, 80 control samples and 86 triplet repeat expansion positive OPMD samples previously sequenced.²

The coding sequence for the first 25 codons is shown in fig 2.

DISCUSSION

Until now the triplet repeat expansion in exon 1 was the only known *PABPN1* mutation causative of OPMD. However, the point mutation described here is having a phenotypic affect comparable to that of the common OPMD repeat expansion mutation.

Patient A has late onset dysphagia and ptosis symptoms typical of OPMD, and the family history was strongly suggestive of autosomal dominant inheritance (fig 1). She is heterozygous for a base change in the *PABPN1* gene altering a glycine residue which is conserved in bovine, rat, and human homologues. This is unlikely to be a neutral polymorphism because of its absence in the general population, the fact that the site is conserved in other species, and the nature of the base change. The G→C substitution changes a glycine GGG codon, situated immediately 3' to the normal 10 alanine codon repeat sequence, to an alanine GCG codon (fig 2). Immediately 3' of this are a further two alanine codons. This base substitution without gene expansion therefore generates a contiguous sequence of 13 alanine codons, which is causative of disease in the common triplet repeat expansion mutation. Compared with OPMD patients with the 13 alanine residue expansion mutation, patient A does not have the amino acid sequence Gly-Ala-Ala immediately 3' of the repeat sequence; however, this appears

not to have any great affect on the course of the disease, and her clinical symptoms were consistent with OPMD but somewhat milder, with later onset than that of the (GCN)₁₃ expansion mutation. In the context of a clinical diagnosis of OPMD and the presence of a point mutation in *PABPN1*, a muscle biopsy was thought to be clinically unjustified and was not done.

In summary we have identified a point mutation in the *PABPN1* gene in a patient with OPMD and illustrated a novel mutational mechanism not previously documented. However, this may be unique to OPMD because of the small size of the *PABPN1* gene polyalanine tract, the nature of the surrounding sequence, and the small increase in size necessary to cause disease.

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