Oculopharyngeal muscular dystrophy
Phenotypic and genotypic studies in a UK population

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Summary
Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant disorder of late onset that commonly presents with ptosis and dysphagia. The genetic basis of the condition has been identified recently as a stable trinucleotide repeat expansion in exon 1 of the poly(A) binding protein 2 gene (PABP2), in which (GCG)_6 is the normal repeat length. The prevalence of OPMD is greatest in patients of French-Canadian origin. It is not clear if expansion repeat length is a reliable test in other populations. In this study, we analysed the phenotypic and genotypic characteristics of 31 patients with OPMD in the UK. Ptosis was the first reported symptom in two-thirds of the patients, and half of the subjects studied had evidence of ophthalmoplegia. All but one family had a pathological expansion in the PABP2 gene, ranging from (GCG)_8 to (GCG)_13. In contrast to the French-Canadian population, (GCG)_10 was almost as common as (GCG)_9, suggesting that the frequency of this expansion is lower than that found in the French-Canadian population. One family was negative for the expansion. Affected members presented with the classical features of OPMD, namely ptosis, dysphagia and cytoplasmic inclusions on muscle biopsy, although with some atypical features, such as early age of onset, high serum levels of creatine kinase and a profound ophthalmoplegia. This family is an example of a GCG expansion-negative oculopharyngeal syndrome requiring further genetic investigation. We conclude that PABP2 analysis is a reliable non-invasive diagnostic test for OPMD in the UK population.

Keywords: oculopharyngeal muscular dystrophy; trinucleotide repeat; UK population

Abbreviations: OPMD = oculopharyngeal muscular dystrophy; PABP2 = gene for poly(A) binding protein 2; PCR = polymerase chain reaction

Introduction
Oculopharyngeal muscular dystrophy (OPMD) is a late-onset, dominantly inherited disorder characterized by progressive ptosis, dysphagia and proximal limb weakness. It was first described in 1915 (Taylor, 1915), when it was attributed to a progressive cranial neuropathy. However, in 1962, Victor and colleagues clearly demonstrated that the primary pathology was myopathic and that it was inherited in an autosomal dominant manner (Victor et al., 1962). Muscle histology in affected individuals typically reveals abnormal variability in fibre size, an increase in endomysial fibrosis, and cytoplasmic basophilic rimmed vacuoles similar to those seen in inclusion body myositis (Coquet et al., 1990). Ultrastructural studies on muscle in OPMD have demonstrated filamentous nuclear inclusions in a minority of fibres that are not seen in other inherited or acquired muscle diseases (Tomé and Fardeau, 1980).

Until recently, the diagnosis of OPMD was made on the basis of clinical features, muscle histology and a positive family history. However, in 1998, Brais and colleagues identified a stable trinucleotide repeat expansion at the N-terminus of a poly(A) binding protein gene (PABP2) on chromosome 14 in 144 affected families (Brais et al., 1998), thereby allowing genetic diagnosis in affected individuals. The identification of trinucleotide repeat expansions in specific genes has become a common theme in inherited neurological diseases (Rosenberg, 1996). However, the
PABP2 repeat expansion in OPMD is unusual in a number of respects. First, the expansion is modest, increasing from (GCG)$_6$ in normal individuals to only (GCG)$_6$-13 in affected individuals, and the repeat length is relatively stable during meiosis (Brais et al., 1998). Furthermore, in the rare autosomal recessive form of OPMD first reported by Fried and colleagues (Fried et al., 1975), affected individuals are homozygous for the (GCG)$_7$ repeat, an allele that is found in 2% of the French-Canadian population. Brais and colleagues also speculated that the (GCG)$_7$ polymorphism may have disease-modifying effects, because a compound heterozygote with (GCG)$_7$/(GCG)$_9$ displayed a more severe disease phenotype than a sibling with (GCG)$_6$(GCG)$_6$.

Although OPMD has a world-wide distribution, its prevalence is highest in French-Canadian kindreds, and most of the families included in the study of Brais and colleagues (Brais et al., 1998) were drawn from this population. Here we report the clinical and genetic characteristics of OPMD in 31 individuals (23 families) from the south of England.

**Methods**

**Patient population**

Subjects were from the south of England recruited mainly through neurology, ophthalmology and ENT (swallowing clinic) departments, with subsequent confirmation of diagnosis by PABP2 gene analysis. A significant proportion of patients were already attending one of two regional muscle clinics; clinical details were available once the genetic test had confirmed the diagnosis. Details of other positive patients were obtained confidentially from the referring consultant. Because of the insidious nature of the condition, it was often only possible to approximate the date of the earliest symptom. A control population of 201 anonymous DNA samples from individuals with no known family history of OPMD was also analysed for an expansion of the (GCG) repeat. Ethical approval for the study was obtained from the Salisbury Research Ethics Committee and the Southampton and South West Hampshire Joint Research Ethics Committee.

**Genetic analysis**

Analysis of the PABP2 gene for expansion of the (GCG)$_n$ repeat sequence was performed essentially as described by Brais and colleagues (Brais et al., 1998). PCR (polymerase chain reaction) amplification was carried out in a volume of 20 µl containing 100 ng of genomic DNA (from peripheral blood), 0.5 U Taq polymerase (Hotstar Taq; Qiagen, Crawley, UK), using the manufacturer’s reaction buffer supplemented with an additional 0.5 mM MgCl$_2$ to a final concentration of 2 mM, 20% Q solution (Qiagen), 100 nM dTTP, dCTP and dATP, 50 nM dGTP, 50 nM deaza-dGTP and each primer at 0.5 µM. Primer sequences were as follows: PABPF (forward), 5’-CGCAGTGCCCCGCCTTAGA-3’; PABPR (reverse), HEX

![Fig. 1 Results of PCR fragment size analysis using an ABI 310 DNA analyser and Genotyper software](image)

The reverse primer was labelled fluorescently (HEX) at the 5’ end.

Thermocycling conditions were 94°C for 20 min followed by 35 cycles of 94°C for 1 min, 64°C for 1 min and 72°C for 1 min, and a final step of 72°C for 7 min. There is significant preferential amplification of the smaller allele, which makes detection of expanded alleles more difficult (Fig. 1). We modified the thermocycling parameters of Brais and colleagues (Brais et al., 1998) in order to minimize this effect. The PCR products were separated according to size by electrophoresis through 4% polyacrylamide on an ABI 377 automated DNA analyser (PE Biosystems, Warrington, UK). Fragment size analysis was carried out using the ABI software packages Genescan version 2.1 and Genotyper version 2.0 (PE Biosystems).

Haplotype analysis was performed on all affected individuals using the chromosome 14-specific markers D14S1041, D14S990 and D14S283 from the region of interest (Grewal et al., 1998). PCR amplification was carried out in a reaction volume of 20 µl that contained 100 ng DNA, 0.1 U Taq polymerase, dCTP, dTTP, dATP and dGTP, each at 200 nM, and each primer at 0.5 µM. One primer of each pair was labelled fluorescently. Thermocycling conditions were 20 min at 94°C followed by 32 cycles of 94°C for 30 s,
Fig. 2 Correlation between age at symptom onset and expansion repeat number. Age at onset of symptoms was dated as accurately as possible, but because of the insidious nature of the condition it is possible that actual onset may have preceded the dates given by 1 year or more. Diamonds denote siblings who were compound heterozygotes for the PABP2 locus (GCG)$_7$(GCG)$_8$.

As in the report of Brais and colleagues (Brais et al., 1998), (GCG)$_9$ was the most common repeat length (40% of families), although (GCG)$_{10}$ was detected in one-third of families (36%). There appeared to be little difference in the age at disease onset between these two groups of patients. No homozygotes for the (GCG)$_7$ polymorphism or longer repeats were detected. Four pairs of siblings were analysed, and the expansion size was the same for each sibling in all four cases. Analysis of 201 control individuals with no known family history of OPMD showed all to have two (GCG)$_8$ alleles.

One family was found to be negative for the mutation. Members of this family had an atypical form of OPMD characterized by early symptom onset in the second or third decade, profound ophthalmoplegia and elevated serum levels of creatine kinase (324–1076 IU; normal range 25–170). Muscle biopsy revealed basophilic rimmed vacuoles with no ragged red fibres or cytochrome oxidase-negative fibres that would suggest a mitochondrial myopathy. Mutation analysis for the mitochondrial 11778, 14484 and 3460 Leber’s optic neuropathy, 8344 MERRF (myoclonus, epilepsy and ragged red fibres) and 3243 MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) mutations was negative. In the two affected family members, the remainder of the PABP2 gene was analysed by single-strand conformation polymorphism for mutations other than the (GCG) repeat amplification. No other mutation was identified, although this technique does not identify all potential mutations. It is also possible that a large expansion of the PABP2 gene would not be detected by conventional PCR because of preferential amplification of the smaller allele. However, analysis by Southern blotting of the PCR products and hybridization with a chemiluminescent (GCC) oligomer probe did not reveal an unusually large (GCC) expansion. Linkage analysis carried out on the five available members of this family did not exclude linkage to chromosome 14q, but too few meioses were analysed to provide positive evidence of linkage to chromosome 14q (data not shown).

Haplotype analysis of all those with a GCC expansion revealed at least four different haplotypes segregating with the GCC expansion (data not shown). The parents of the patients were not available for analysis, so it was not possible to assign a specific haplotype to the expanded allele. However, the spread of allele sizes allowed us to discern at least four different haplotypes segregating with the expansion.

Discussion

All of our patients with genetically proven classical OPMD displayed the typical features of ptosis and dysphagia, though proximal limb weakness was a variable finding. Although others have reported that dysphagia is rarely present at presentation (Murphy and Drachman, 1968), it was the first reported symptom in 23% of the patients in our series. In the majority of patients (61%), ptosis was severe enough to
warrant corrective surgery, whereas oesophageal dilatation or insertion of a percutaneous gastrostomy feeding tube was only required in 22%. It is also worthy of note that half of the patients were noted to have a moderate or severe restriction of ocular movement in at least one direction of gaze at the time of presentation. This sign is not often commented on, yet one of the three cases described in the original paper of Victor and colleagues was noted to have a ‘. . . moderately severe impairment of ocular movement in all directions of gaze . . . ’ (Victor et al., 1962). Murphy and Drachman also recorded extra-ocular muscle weakness in a proportion of subjects, although their data were incomplete (Murphy and Drachman, 1968). Our data suggest that a degree of external ophthalmoplegia is a relatively common finding in OPMD, at least in the UK population.

This study provides confirmation that the detection of expanded (GCG) repeat lengths in the PABP2 gene is an appropriate diagnostic test for OPMD in a population of patients from the south of England. However, our results differ from those reported in previous studies in two respects. First, the spread of expansion repeat lengths was evenly distributed between (GCG)9 and (GCG)10, both of which were detected in approximately one-third of the families involved in the study. In contrast, Brais and colleagues found that nearly 70% of their sample population, drawn from 15 countries, had nine repeats (Brais et al., 1998). Although Brais and colleagues did not provide a breakdown correlating country of origin with repeat length, they noted that the (GCG)9 mutation was detected in all French-Canadian families with OPMD, suggesting a strong founder effect in this population. A report of mutation analysis in three Hispanic American families (Grewal et al., 1999) also demonstrated the predominance of the (GCG)9 allele, although the numbers are too small to permit any comments on common origin.

The distribution of repeat lengths in our population suggests that it is unlikely that there is a common founder. This hypothesis was supported by the haplotype analysis of all of the positive subjects. At least four different haplotypes were identified, indicating separate origins of the (GCG) expansions. This is likely to be an underestimate of the actual number of distinct haplotypes, because in most cases parents were not available for analysis and the assignment of alleles to a specific chromosome was thus not possible. A similar, smaller study of expansion repeat lengths in OPMD in an Italian population (Mirabella et al., 2000) also demonstrated a more diverse distribution of mutations than that reported in the study of Brais and colleagues (Brais et al., 1998), mirroring our findings in the UK.

The second unexpected finding concerned the (GCG)7 polymorphism. This is reported to have a background frequency of 2% in the French-Canadian population. We failed to detect it in 201 normal individuals, suggesting that the frequency in the UK population is smaller, perhaps <0.5%. In view of this, it is not surprising that no autosomal recessive homozygotes were detected in our study.

We have shown that longer repeat expansions tend to result in earlier disease onset in OPMD, although this effect is less striking than that seen with other trinucleotide repeat disorders. Such analyses are complicated by the insidious nature of the condition, making accurate dating of disease onset difficult, though the small range of repeat lengths and the stability of the expansion is also likely to limit the range of disease severity. Thus, the predominance of the (GCG)9 and (GCG)10 mutations is likely to contribute to the homogeneity of the disease phenotype. Our data also add support to the observation that the polymorphism (GCG)7 may have a disease-modifying effect, in that the compound heterozygote sibling pair presented much earlier than the patient with the (GCG)6(GCG)8 phenotype, although the fact that they were not related makes it difficult to draw direct comparisons. It was reported recently that compound heterozygotes were relatively common in the Italian population (Mirabella et al., 2000), yet these subjects did not appear to have early-onset disease. Further data are required to clarify the role of (GCG)7 in modifying the disease phenotype.

One family presenting with the typical features of OPMD, namely ptosis, dysphagia and cytoplasmic inclusions on muscle histology, was found to be negative for the PABP2 repeat expansion. However, affected members of this family did display atypical features, such as early age of onset, profound external ophthalmoplegia and high serum creatine kinase levels. The possibility that these patients had a mitochondrial myopathy was considered, but was felt to be unlikely in view of the absence of cytochrome oxidase-negative and ragged red fibres upon histological analysis and the absence of the common mitochondrial mutations. There are isolated case reports in the literature of patients presenting with progressive ptosis and dysphagia with variable additional features. Rose and colleagues described a Greek sibling pair with a progressive dystrophy presenting in adolescence that led rapidly to respiratory failure (Rose et al., 1997). Like our family, they had elevated serum creatine kinase levels and muscle biopsies showed dystrophic changes with basophilic rimmed vacuoles. Electron microscopy revealed filamentous intranuclear inclusions similar to those that have been described previously in autosomal dominant and recessive OPMD (Schröder et al., 1995). It is possible that these cases were a further example of autosomal recessive OPMD, though the results of mutation analysis have not been reported.

There are two reports of a total of five families with an autosomal dominant ‘oculopharyngodistal myopathy’ characterized by distal limb weakness in association with ocular and bulbar dysfunction (Satoyoshi and Kinoshita, 1977; Scrimgeour and Mastaglia, 1984). Like the family reported here, these patients had a profound ophthalmoplegia, although proximal limb muscle strength was relatively preserved. Muscle histology in all of these cases showed marked dystrophic changes but no basophilic rimmed vacuoles. Some of the cases reported by Mirabella and colleagues (Mirabella et al., 2000) more closely resembled
our family. Five of their 16 patients with the OPMD phenotype were negative for the \textit{PABP2} repeat expansion. Although clinical details of these patients are brief, the condition manifested itself at or around the age of 40 years in all cases. All the patients had basophilic rimmed vacuoles on muscle histology, though filamentous nuclear inclusions were not detected. Taken with the results on our family, these data suggest that there may be a number of rare oculopharyngeal syndromes the genetic basis of which is unclear. In our (GCG) expansion-negative family, single-strand conformation polymorphism analysis showed that a different mutation in the \textit{PABP2} gene was unlikely to be responsible for the phenotype. However, insufficient information was obtained from linkage analysis to include or exclude linkage to chromosome 14q11.2-q13 definitively.

In summary, we have shown that expansion repeat length in the \textit{PABP2} gene is a reliable test for oculopharyngeal muscular dystrophy in an English population. We have also highlighted the fact that there is a rare group of myopathic conditions that share certain features with OPMD but remain uncharacterized at the genetic level.

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References


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