Mutation Analysis of Oculopharyngeal Muscular Dystrophy in Hispanic American Families

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Background: Oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant muscular dystrophy characterized by progressive ptosis, swallowing difficulties, and proximal limb weakness. Recently, the genetic basis of this disease has been characterized by mutations in the PABP2 gene that involve short expansions of the trinucleotide repeat GCG.

Objectives: To independently confirm the presence and study the meiotic stability of the GCG expansion mutations in a distinct ethnic population with OPMD.

Settings: Hospital and university research laboratories in Los Angeles, Calif.

Subjects and Methods: Three unrelated families of Hispanic American descent were identified in whom OPMD was transmitted in an autosomal dominant pattern. All of these families can trace affected ancestors to the southwestern United States or to the bordering states of Mexico. In these families, 14 persons with OPMD were identified and studied.

Results: Our results confirm that in these families, expansion mutations characterized by a gain of 3 GCG repeats in the wild-type allele result in an abnormal nucleotide length of 9 GCG repeats in the PABP2 gene. In these families, these mutations are associated with the OPMD phenotype. The identical repeat mutation ([GCG]9) is found in all affected members of these unrelated families and shows relative meiotic stability.

Conclusions: These results support and extend our study of haplotype analysis and suggest that a founder effect may have occurred for OPMD in this Hispanic American population.

Arch Neurol. 1999;56:1378-1381

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant disorder characterized by slowly progressive ptosis, dysphagia, and proximal limb weakness.1 This condition was initially genetically mapped2 to chromosome 14q11.2-q13 in French Canadian families in whom prevalence of the disease is the highest reported anywhere in the world. Since this initial report, mutations in the putative disease gene, poly(adenylate)-binding protein 2 gene (PABP2), have been described. These mutations involve an expansion of the trinucleotide repeat GCG from a wild-type repeat length of 6 repeats—(GCG)6—to an abnormal repeat length of (GCG)8-13.3

In the United States, in addition to patients of French Canadian and Scottish descent, patients of Hispanic descent living in or originating from the southwestern states of Arizona, New Mexico, Colorado, and California have manifested the disease.1,4-5 This gene has been previously genetically mapped6 to a 1-million-base-pair region on chromosome 14q11.2-q13 in 3 unrelated families of Hispanic American descent with OPMD. We report the results of our mutation analysis of the PABP2 gene in these 3 families.

RESULTS

Every patient clinically diagnosed as having OPMD had a GCG expansion mutation (Figure 2). All unaffected persons from all 3 families were homozygous and carry the (GCG)6 allele, and all affected persons are heterozygous and carry an expanded mutant (GCG)7 allele. Four of the 11 persons classified as at risk but not affected also carry the disease genotype, and they are all younger than 40 years. Because this disease has an age-dependent penetrance, they are most likely presymptomatic.

Of the 14 clinically affected persons whose disease was genetically confirmed, 10 had sought medical attention...
**PATIENTS AND METHODS**

**PELDiGES AND FAMILY MEMBERS**

Patients were recruited with procedures and informed consents approved by the University of Southern California, Los Angeles, Institutional Review Board. All family members agreeing to participate were examined by a neurologist (R.P.G.) and an ophthalmologist (R.K.G.). In addition, at least 1 affected member in each family was subjected to electrophysiologic studies and a muscle biopsy, the results of which were consistent with a diagnosis of OPMD. These families demonstrate autosomal dominant transmission with vertical and male-to-male transmission, and all families can trace an affected ancestor to the southwestern United States or the contiguous areas of northern Mexico (Figure 1). In these 3 families, at least 19 persons are known to have suffered from or to currently be diagnosed as having OPMD.

In addition to the neurologic examination, all at-risk persons were assessed for swallowing function by drinking ice-cold water. By previously published criteria, the ice-water swallowing time was abnormal if the person took more than 7 seconds to swallow 80 mL of ice water, and the palpebral fissure distance was abnormal if it was less than 8 mm. These values were reported to differentiate between affected persons and control subjects with a sensitivity of 87% and a specificity of 77%. Consequently, before genetic analysis, all at-risk persons were coded as follows: 1 = affected: unequivocal neurologic signs consistent with OPMD or an abnormal ice-water swallowing test result and reduced palpebral fissure; 2 = possibly affected: an abnormal ice-water swallowing test result, reduced palpebral fissure, or equivocal neurologic signs; and 3 = at risk but not affected. Relatives who were deceased were diagnosed as “affected” or “unaffected” on the basis of descriptions of and 50°C. The allele sizes were defined by the peak or 0.5-mm wide short-plate gels (about 11.5 cm long) consisting of 6% acrylamide, urea (7 mol/L), and 0.6 X TBE (Tris-borate-ethylenediaminetetraacetic acid) (ALF Ready Mix Gel; Pharmacia Biotech Inc, Piscataway, NJ); 0.5-mm wide short-gels (about 11.5 cm long) using the ALF Fragment Manager (Pharmacia Biotech Inc). About 1.2 µL of the PCR product and 0.6 fmol of each internal molecular weight standard in 42% formamide was denatured at 95°C for 2 minutes before loading and then subjected to electrophoresis at 600 V and 50°C. The allele sizes were defined by the peak or peaks with the greatest area (Figure 2).

This method has been used to assess mutation frequencies of disease alleles in trinucleotide-repeat disorders. It has a sensitivity of detecting changes of more than 1 base pair in the size of a PCR product and therefore can reliably distinguish a change of 1 triplet repeat. As a further control of the analysis, a different method was used to confirm the results. All specimens were subjected to PCR using a primer radiolabeled with sulfur (data not shown). The results of the analysis of trinucleotide-repeat size and stability using these 2 different methods were consistent.

**MUTATION ANALYSIS**

Genomic DNA was extracted from blood specimens, as previously described, and subjected to polymerase chain reaction (PCR) to amplify the portion of exon 1 containing the GCG expansion mutation. The PCR was performed in a total volume of 50 µL containing DNA, 20 ng; Tris hydrochloride (pH 8.3), 100 mM; 100 µmol/L each of deoxyctydine triphosphate, deoxyadenosine triphosphate, deoxycytidine triphosphate, and deoxyguanosine triphosphate; 3% (volume per volume) dimethyl sulfoxide; and Taq polymerase, 1 U. The primers used in these experiments have been previously described: 5’-GGCGATGCGGGCGCTTAGA-3’ and 5’-ACAAGAATGGCGCGCGCGCGCGC3’.

Four persons who were clinically diagnosed as affected before genetic testing had not sought medical attention despite having unequivocal neurologic signs consistent with OPMD. Subsequently, all of these persons were genetically confirmed to carry the (GCG)9 mutation.

Our results confirm the association of the GCG expansion with the OPMD disease phenotype in this ethnic group. The size of this expansion mutation, (GCG)$_9$, is the most commonly observed in 99 of 144 families from a variety of ethnic groups. The next most frequent expansion mutations were (GCG)$_{10}$ and (GCG)$_{11}$, occurring in 19 and 16 families, respectively. In all affected persons in our families, only the (GCG)$_9$ was observed, suggesting a common affected ancestor.

**COMMENT**

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In French Canadian families with OPMD, there is evidence of relative meiotic stability of this trinucleotide repeat. In 8 meiotic transmissions observed in these 3 families, there is no evidence of any GCG repeat instability. The relative stability of this GCG repeat is in contrast to many of the trinucleotide-repeat disorders where significant meiotic instability is observed. A previous study revealed the presence of a conserved haplotype in affected members from all 3 families. Furthermore, despite considerable effort, we found no evidence that any of these 3 families are directly related to each other. Affected ancestors, however, all came from the same geographic region of the southwestern United States and Mexico. The haplotype data suggest that in this population, similar to findings for OPMD in French Canadians, there is a founder effect for this disease. The presence of the identical-repeat expansion mutation in all affected members provides further support for this founder effect and is consistent with the relative meiotic stability of this (GCG)9 expansion.

Anticipation is a common feature of the trinucleotide-repeat disorders and correlates with increases in the triplet-repeat number as mutant alleles are transmitted through successive generations. Although the number of parent-child pairs in this study was limited, the available evidence of an earlier onset of disease in affected children of only 2.4 years suggests that anticipation may not be an important feature of this disease. The presence of the identical-repeat expansion mutation in all affected members provides further support for this founder effect and is consistent with the relative meiotic stability of this (GCG)9 expansion.

The major presenting clinical symptoms in our genetically proven patients were either ocular (usually bilateral ptosis) or pharyngeal (dysphagia). These presenting features are consistent with those reported from patients of various ethnic origins with OPMD. The clinical data show, however, that there are substantial delays before the diagnosis of OPMD is made. These delays may be due in part to the subtle and slowly progressive nature of the neurologic symptoms so that months or years may elapse before the patients seek medical attention. Once medical attention is sought, delays may occur because OPMD is a relatively uncommon disease.

The discovery of the genetic basis of this disease represents an important advance and will facilitate genetic counseling. It provides another test that can complement muscle biopsy as a method of confirming the diagnosis of OPMD. This may be particularly useful in atypical patients or in persons who have the typical signs and symptoms of OPMD, but a good family history is not available. The discovery also provides a first step toward understanding the pathogenesis of this disease.
Accepted for publication January 5, 1999.

This work was supported in part by grant K12AG00521 from the National Institutes of Health, Bethesda, Md (Dr R. P. Grewal), and by personal funds (Drs R. P. Grewal and R. K. Grewal).

We thank Viliam Furdick, MD, for bringing some patients to our attention. We acknowledge the cooperation and assistance of all participating families.

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REFERENCES


