Oculopharyngeal muscular dystrophy (OPMD) is an adult-onset autosomal dominant muscle disease with a worldwide distribution. Recent findings reveal the genetic basis of this disease to be mutations in the polyA binding–protein 2 (PABP2) gene that involve short expansions of the GCG trinucleotide repeat encoding a polyalanine tract. The underlying mechanism causing the triplet-expansion mutation in PABP2 remains to be elucidated, although the DNA slippage model is thought to be a plausible explanation of that.

Methods and Results: We analyzed PABP2 using polymerase chain reaction analysis and DNA sequencing in Japanese patients with pathologically confirmed OPMD, and found mutated (GCG)6(GCA)3GCG and (GCG)6(GCA)3(GCG)2(GCA)3GCG alleles instead of the normal (GCG)6(GCA)3GCG allele. These mutated alleles could be explained by the insertions or duplications of (GCG)3GCA (GCG)2(GCA)3 but not simple expansions of the GCG repeats. Therefore, unequal crossing-over of 2 PABP2 alleles, rather than DNA slippage, is probably the causative mechanism of OPMD mutations. All mutations that have been reported in patients with OPMD so far can be explained with the mechanism of unequal crossing-over. On the other hand, comparison of the clinical features of our patients with those of other patients in previous reports suggests that specific clinical features cannot be attributed to the length of the polyalanine tract per se.

Conclusions: The mutated alleles identified in our Japanese patients with OPMD were most likely due to duplications of (GCG)2(GCA) and (GCG)2(GCA)3 but not simple expansions of the GCG repeats. Therefore, unequal crossing-over of 2 PABP2 alleles, rather than DNA slippage, is probably the causative mechanism of OPMD mutations. All mutations that have been reported in patients with OPMD so far can be explained with the mechanism of unequal crossing-over. On the other hand, comparison of the clinical features of our patients with those of other patients in previous reports suggests that specific clinical features cannot be attributed to the length of the polyalanine tract per se.

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Subjects and Methods

Report of Cases

The first patient (patient A) was a 65-year-old man born in Hyogo Prefecture, Japan. At 53 years of age, a swallowing disturbance developed. At 60 years of age, he noticed ptosis on both sides, which gradually worsened. Results of an examination showed moderate ptosis and mild weakness of the facial and pharyngeal muscles. Extraocular muscles showed no weakness. He exhibited moderate symmetrical weakness of the proximal lower limb muscles. Results of laboratory examination showed a 6-fold increase in serum creatine kinase (CK) level (843 U/L; reference value, <141 U/L). His father had had dysphagia and dysarthria since the sixth decade of life. A sister of patient A had had dysphagia since her sixth decade of life, whereas a brother had no neuromuscular symptoms.

The second patient (patient B) was a 69-year-old woman born in Shiga Prefecture, Japan. She had no neuromuscular symptoms until 61 years of age, when she noticed ptosis on both sides. The symptoms progressed gradually, and she underwent an operation for it at 65 years of age. At 66 years of age, easy fatigability on walking, swallowing difficulty, and speech disturbance developed. Results of an examination disclosed ptosis that completely covered the pupils. Medium grade of ophthalmoplegia was noted in all directions. Eye and mouth closures were moderately impaired. She had moderate symmetrical muscle weakness of the proximal limb and neck muscles. Her serum CK level was 300 U/L. She was separated from her parents in early childhood, which hindered confirmation of parental symptoms. She had a sister with ptosis and a child without neuromuscular symptoms.

In patients A and B, results of muscle biopsy revealed the occasional occurrence of muscle fibers containing rimmed vacuoles. Results of electron microscopic studies showed intranuclear tubulofilamentous inclusions with a diameter of 8.5 nm, which is a characteristic of OPMD.

Methods

We analyzed PABP2 in both index patients and the 2 siblings of patient A after obtaining informed consent.

Results

Sequence analyses disclosed a 12-base pair (bp) elongation in patient A and his affected sister and a 15-bp elongation in patient B (Figure 1) within the (GCG)3(GCA), GCG normal sequence in exon 1 of PABP2. The mutated sequence detected in patient A and his sister was (GCG)6, GCA(GCG)3(GCA)GGC, and that in patient B was (GCG)6, (GCA)3(GCG)3(GCA)GGC. Both sequences could be explained by the insertions or duplications of (GCG)6, GCA and (GCG)3(GCA)3, respectively, into the normal sequence. The normal sequence in PABP2 is translated into a series of 10 alanine residues. The normal sequence in our patients increase the number of alanine residues encoded from 10 to 14 in patient A and his sister, and to 15 in patient B. All of the patients were heterozygous for the mutated and the normal alleles. The unaffected brother of patient A was homozygous for the normal allele.

In addition to the duplications, we observed in all patients and 10 Japanese controls a CG-to-GGCG change in position 1146, a G deletion in position 1215, a GG insertion in position 1229, and an ATC-to-CAT change in position 1250 (Figure 2). The latter 2 changes have previously been described in an Italian population. All 4


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Repeat expansions that involve the process of DNA replication and recombination are an important cause of repeat expansion diseases. These diseases are characterized by the presence of expanded DNA sequences, which can lead to the development of various neurological and muscular disorders. The repeat expansions in opulopharyngeal muscular dystrophy (OPMD) are expansions of the (GCG)6 repeat. These expansions are responsible for a number of hereditary neuro-muscular diseases. The pathologic repeat expansions can be explained by the slippage model, where the expanded tracts of approximately 25 to 35 perfect trinucleotide repeats are responsible for instability and expansion.

**MECHANISM OF REPEAT EXPANSIONS IN OPMD**

Except for the mutation found in Cajun patients who had a (GCG)6GCA (GCG)2GCG mutated allele, all of the PABP2 mutations reported so far in patients with OPMD were expansions of the (GCG)6 repeat. These expansions make a (GCG)6(GCA)6GCG allele from the (GCG)6(GCA)6GCG normal sequence. We found 2 novel mutations, (GCG)6GCA(GCG)3GCG and (GCG)6GCA(GCG)2GCG, in Japanese patients with OPMD.

The molecular mechanism causing the repeat expansion in PABP2 has not been determined. In general, the following 2 types of mechanisms leading to the generation of longer DNA sequences have been proposed: replication- and recombination-associated expansion. Repeat expansions that involve the process of DNA replication may originate from slipped mispairing between repeated sequences, as has been described for the slippage model. It has been shown recently that expanded triplet repeats are responsible for a number of hereditary neuro-muscular diseases. These pathologic repeat expansions can be explained by the slippage model. However, it has been proposed that tracts of approximately 25 to 35 perfect trinucleotide repeats are required for instability and expansion via slippage. The heterogeneous sequences of the mutated alleles of PABP2 detected in the Cajun patients and our Japanese patients and the fact that even the longest perfect repeat reported (13 repeats) is less than 25 repeats argue against the slippage model.

Recombination-associated repeat expansion may result from homologous recombination, which occurs in germ cells during meiosis and sometimes during mitosis. The mutations that we found suggest that the molecular mechanism resulting in generation of longer DNA sequences in PABP2 is unequal crossing-over (Figure 3), which is a kind of homologous recombination. The (GCG)6(GCA)3GCG normal allele was reported to be found in 98% and 99% of French Canadian and Japanese control chromosomes, respectively, whereas the rest of both populations carried a (GCG)6(GCA)3GCG polymorphism (Figure 3A).Unequal pairing with variable degrees of

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**Figure 1.** Mutant sequences found in Japanese patients with oculopharyngeal muscular dystrophy. Electropherograms of alleles of the polyA binding–protein 2 gene show normal allele, mutant allele detected in patient A (mutation A), and that detected in patient B (mutation B). Open circles represent GCG triplets; filled circles, GCA triplets.

**Figure 2.** Schematic representation of the exon 1 structure of the polyA binding–protein 2 gene. The horizontal line and the box indicate the 5′-untranslated region and the coding region of the exon 1, respectively. Positions of the 4 putative polymorphisms identified in the Japanese population are represented by vertical lines in the 5′-untranslated region. The filled portion of the box indicates the location of the sequence depicted in Figure 1; arrows, the positions of the polymerase chain reaction primers used in this study.

**Figure 3.** Schematic representation of GCG and GCA repeats found in the polyA binding–protein 2 (PABP2) gene. Open circles represent GCG triplets; filled circles, GCA triplets; Xs, possible points of the DNA crossing-over; and underlines, duplicated nucleotides. A, Normal and polymorphic alleles of PABP2 found in healthy individuals consist of alanine-encoding 10- and 11-triplet repeats, respectively. B-E, All of the reported mutant PABP2 alleles can be generated by unequal crossing-over.

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**COMMENT**
overlap can generate each of the (GCG)$_{12}$ (GCA)$_3$ GCG mutant alleles by crossing-over of the 2 (GCG)$_4$ (GCA)$_3$ GCG normal alleles (Figure 3B). At most, a (GCG)$_{14}$ (GCA)$_3$ GCG allele can be derived by unequal crossing-over of the (GCG)$_2$ (GCA)$_3$ GCG normal allele and the (GCG)$_3$ (GCA)$_3$ GCG polymorphic allele. This mechanism can also explain the (GCG)$_2$ (GCA)$_3$ (GCG)$_3$ GCG mutation reported in Cajun patients (Figure 3C) and the (GCG)$_2$ (GCA)$_3$ (GCG)$_2$ (GCA)$_3$ GCG (Figure 3D) and (GCG)$_2$ (GCA)$_3$ (GCG)$_3$ (GCA)$_3$ GCG mutations found in our patients (Figure 3E). Since the (GCG)$_{6,10}$ (GCA)$_3$ GCG pathologic expansions in PABP2 are reported to be stable with no variation among family members and between different tissues as blood and skeletal muscle in the same individual, we suspect that unequal crossing-over occurred once at meiosis in an ancestor of each patient with OPMD. Similar expansions of cryptic repeats composed of mixed synonymous codons causing a polyalanine expansion in the homeobox D13 (HOXD13) protein has been found to cause synpolydactyly. The HOXD13 mutation has been explained by unequal crossing-over.

GENOTYPE/PHENOTYPE RELATIONSHIP IN OPMD

The muscle degeneration seen in OPMD may be a consequence of the toxic effects of the aggregates caused by the expanded polyalanine tracts, or of the altered properties of the mutated PABP2 protein. In both cases, the length of the polyalanine tract may be a key determinant of the effect. The (GCG)$_2$ (GCA)$_3$ GCG sequence in the normal PABP2 gene is translated into 10 alanine residues in the protein. The mutated alleles in our patients encode 14 and 15 alanine residues instead of 10. In that case, the number of alanine residues in our patients are equivalent to those reported to be generated by the (GCG)$_{10}$ (GCA)$_3$ GCG and (GCG)$_{11}$ (GCA)$_3$ GCG expanded alleles, respectively.

All of our patients were heterozygous for the mutations. To our knowledge, only 1 Japanese family and 2 Italian families heterozygous for the (GCG)$_2$ (GCA)$_3$ GCG allele and 2 Japanese families heterozygous for the (GCG)$_{11}$ (GCA)$_3$ GCG allele have been described clinically and genetically. According to the reports, dysphagia with moderately increased CK levels and proximal myopathy of the lower legs initially developed in the Japanese patients heterozygous for the (GCG)$_2$ (GCA)$_3$ GCG allele. Ptois first developed in the 2 Japanese families heterozygous for the (GCG)$_{11}$ (GCA)$_3$ GCG allele, whereas their CK levels were generally within normal limits or only mildly increased. These clinicogenetic correlations are compatible with those of our patients, especially with respect to the initial symptoms and CK levels. However, both Italian families heterozygous for the (GCG)$_2$ (GCA)$_3$ GCG allele presented with ptoias as the first symptom, with later development of dysphagia and severe weakness of limb and pelvic girdle muscles. Therefore, the specific clinical feature perhaps cannot be attributed to the length of the polyalanine tract of PABP2 per se. Although these observations suggest that the pathologic characteristics of OPMD may be the result of nucleotide configuration, intrapopulational similarities of phenotype among the Japanese and among the Italian patients are more likely to due to the genetic background of each race. Further case accumulation is needed to clarify the relationship between genotype and phenotype in OPMD.

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REFERENCES