Continuous Remodeling of Adult Extraocular Muscles as an Explanation for Selective Craniofacial Vulnerability in Oculopharyngeal Muscular Dystrophy

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Abstract: Oculopharyngeal muscular dystrophy (OPMD) is an inherited disorder caused by mutations of the polyadenylate binding protein nuclear 1 (PABPN1) gene. While a pathogenic hypothesis has been formulated that links the genetic and molecular abnormalities to cellular abnormalities, there is no proven explanation for the targeting of the craniofacial muscles. We propose a hypothesis that bridges this gap. It is based on the phenomenon of continuous remodeling of normal adult extraocular muscles (EOMs). Unlike the EOMs, the myonuclei of other skeletal muscles are postmitotic in the adult unless the muscles are injured. Continuous myofiber remodeling most likely requires upregulation of genes involved in cell cycling, and in protein degradation and synthesis. PABPN1 is a nuclear protein that performs the essential function of controlling polyadenylation of mRNA and the fidelity of protein synthesis. In OPMD, the ongoing production of mutant PABPN1 in muscles undergoing continuous remodeling could result in a failure of accurate production of mRNA required for the maintenance of the myocytes. Over many years, this would lead to cumulative myonuclear loss and finally to myofiber loss. This hypothesis offers an explanation for the selective involvement of extraocular muscles affected in OPMD and the onset of symptoms in adulthood.

DIFFERENCES BETWEEN CRANIOFACIAL AND OTHER SKELETAL MUSCLES

This paper presents a hypothesis regarding the predilection of oculopharyngeal muscular dystrophy (OPMD; Online Mendelian Inheritance in Man database no. 164300) (1) to involve the craniofacial muscles, a distinct allotype that includes the extraocular muscles (EOMs) (2,3). The craniofacial muscles have distinctive embryology (4); the unusual properties of the EOMs may predispose or protect them in neurogenic and myogenic diseases (5). The EOMs themselves are a heterogeneous subgroup within mammalian craniofacial muscles with distinct and unusual molecular properties. We regard EOMs and certain craniofacial muscles as conceptually interchangeable, recognizing that each specific muscle may exhibit unique characteristics at the whole muscle and molecular levels.

While the EOMs and craniofacial muscles are known to be quite similar to each other, they are distinctly different when compared with other skeletal muscles. Craniofacial muscles have more complex types of myofibers and smaller motor units than other skeletal muscles. The myosin heavy chain expression patterns of EOMs differ from those of other skeletal muscles (6). Adult EOMs express a variety of molecules, such as muscle myogenic and growth factors (7,8), which are normally downregulated in mature skeletal muscles (2, 9). Additionally, unlike adult skeletal muscles, EOMs undergo continuous addition of myonuclei (10–12). We have not yet made a thorough study of the other craniofacial muscles for evidence of continuous myonuclear addition, but our preliminary data suggest that this process occurs in the laryngeal muscles as well.

Our hypothesis for the selective involvement of craniofacial muscles in OPMD follows from our studies demonstrating that normal, uninjured, adult, mammalian EOMs undergo continuous addition and loss of myonuclei (10–12). Thus, EOM myofibers cannot be considered postmitotic cells in the usual sense. Previously, all adult skeletal muscles were considered to be postmitotic and did not undergo myonuclear addition unless they were injured or diseased. The evidence for continuous myofiber remodeling was visualized by the incorporation of bromodeoxyuridine...
into EOM satellite cells after intraperitoneal injection, followed by the incorporation of these labeled nuclei into existing myofibers (12). Subsequent studies used immunohistologic markers of myogenic activity and cell cycling to extend our observations to monkeys and humans, and they have led us to believe that continuous myonuclear addition in EOMs may be a universal phenomenon in mammals (10). In addition, we have recently found evidence of myonuclear apoptosis, which is consistent with the concept of EOM myofiber remodeling. In these studies, individual myonuclear apoptosis and apparent satellite cell apoptosis were observed in adult rabbits, as visualized by positive terminal deoxynucleotidyltransferase-mediated nick end labeling (TUNEL). Analysis of serial sections demonstrated that there was a distinct segmental localization of the activated caspase-3 immunostaining within the cytoplasm of individual myofibers (13). Because the EOMs maintain a stable ratio of nuclear/cytoplasmic volume, and a stable volume throughout adult life, a homeostatic process of myonuclear addition and loss would be required. Our experiments support the model of EOM myonuclear homeostasis, which includes the random loss and addition of individual myonuclei within existing multinucleated myofibers. Other investigators have found evidence of apoptosis in 2.2% of normal human EOM myofibers (14). Their findings in normal human EOMs support our observations. In summary, our studies have led us to conclude that EOM myofiber remodeling occurs as a result of the insertion of replacement myocytes into mature EOM, concurrent with an ongoing process of localized nuclear apoptosis and cytoplasmic remodeling affecting the myonuclear domains of the replacement myonuclei.

Features of Oculopharyngeal Muscular Dystrophy

OPMD (1) is an autosomal dominant disorder caused by a mutation in a single gene (15). It most frequently presents in the fifth or sixth decade of life and causes ptosis, chronic progressive external ophthalmoplegia, dysphagia, and weakness of the limb-girdle muscles. Weakness and wasting of the face, neck, and distal limb muscles may also occur. OPMD can be fatal because of the selective involvement of the pharyngeal muscles.

The molecular basis for OPMD is guanine-cytosine-guanine (GCG) trinucleotide/tandem repeat expansions in exon 1 of the polyadenylate binding protein, nuclear 1, PABPN1 (also termed PABP2) gene. (1) This mutation has arisen independently in many populations and has a worldwide distribution. The abnormal gene is located on chromosome 14q11.2–q13. A GCG repeat at the 5’ end of the exon encoding the N terminus of the protein is expanded from the normal number of 6 to a range between 8 and 13 repeats.

The gene expansion of this trinucleotide repeat causes expansion of the normal 10-alanine stretch to 12–17 alanines in the resultant PABPN1. Homozygous patients with autosomal dominant OPMD (GCG)8–13 have the onset of clinical manifestations at least a decade earlier than do heterozygotes, and have approximately twice as many intranuclear filament inclusions (9.4%) on electron microscopic examination of muscle biopsies (16). The (GCG)7 allele is present in about 2% of the population. For (GCG)7, homozygotes have an autosomal recessive form of OPMD. There is no exact correlation between the number of repeats and the phenotypic expression of the disease (17). Polymorphisms affecting the PABPN1 gene have also been described. These may be clinically neutral or affect the severity of the disease (18).

PABPN1 is a nuclear protein of 33 kDa whose function is to stimulate the progressive addition of poly(A) to the 3’ tail of newly transcribed mRNA. PABPN1 is a conserved protein in eukaryotic cells and has been implicated in the regulation of mRNA stability and translation critical for mRNA processing (19–24). PABPN1 is largely confined within the nucleus (25). However, perhaps important in the pathogenesis of OPMD is the fact that mammalian cells display a cell cycle-dependent cytoplasmic polyadenylation, suggesting that translational control by polyadenylation might be a general feature of mitosis (26,27). OPMD is characterized by the accumulation of mutant PABPN1 (mPABPN1) oligomers that form unique intranuclear tubulofilamentous inclusions with an outer diameter of 8.5 nm in skeletal muscle fibers (28). The conditions favoring aggregation of mPABN1 have been partially defined (29).

The Prior Hypothesis

Based on the properties of mPABPN1, several toxic gain-of-function mechanisms have been previously proposed for this protein’s role in OPMD (30) (Fig. 1). The cumulative effect of these mechanisms is to create a “nuclear trap,” sequestering proteins needed by the cytoplasm. Oligomers of mPABPN1-ala17 may accumulate and form aggregates or insoluble fibrils that are resistant to degradation by the ubiquitin-proteasome pathway and thus become cytotoxic (28,30,31). Inclusions of mPABPN1 may interfere with mRNA traffic through the nuclear pores by blocking nucleocytoplasmic transport. mPABPN1 may sequester specific proteins, such as heterogeneous nuclear ribonucleoproteins, and ski-interacting proteins that control processes essential for myogenesis and myocyte survival (32–34).

Several unresolved questions about the pathogenesis of OPMD have been posed: (1) Is the nuclear localization of the OPMD inclusions required for disease progression? (2) Does the mRNA binding capacity of the mPABN1 trap...
mRNA and interfere with its export from the nucleus? (3) Why do mutations in the ubiquitous protein PABN1 induce intranuclear inclusions mainly in muscle cells? (4) Why is OPMD an adult-onset disorder?

To this list of important unresolved questions, we would add a fifth: Why are EOMs selectively targeted for presentation of the disease phenotype?

**Our Hypothesis**

The underlying theme of our hypothesis is that ongoing remodeling of the EOMs and other craniofacial muscle myofibers is associated with cell signaling or biochemical processes that are relatively upregulated in the affected muscles as compared with other uninjured and healthy adult skeletal muscles (2). This upregulation causes the affected muscles to be selectively vulnerable to the effects of the mutant gene (Fig. 2). We hypothesize that, in OPMD, the ongoing production of mPABPN1 in muscles undergoing continuous remodeling results in the generation of disabled mRNA, which causes deficient polyadenylation that cumulatively fails to support the continuous remodeling process. Defective polyadenylation may interfere with the cell cycling and differentiation required for continuous EOM remodeling (35). Over time, deficiencies in this process lead to myonuclear loss and, finally, to myofiber loss. Our hypothesis relies on the cumulative pathogenic loss-of-function of the mRNA, cell cycling, and differentiation required to sustain the processes of continuous remodeling of the muscles most affected in OPMD. In proposing this model, we recognize the possibility of multiple pathogenic mechanisms, any of which could be related to continuous myofiber remodeling, and that loss-of-function and gain-of-function pathogenic mechanisms may be additive rather than mutually exclusive.

Moreover, mPABPN1 and disabled mRNA may also react with muscle-specific proteins that are unique to EOMs or uniquely upregulated in adult EOMs, thus providing an explanation for the selectivity of this disease to EOMs. Gene array studies show a number of genes that are uniquely upregulated or downregulated in EOMs when compared with leg muscles (2,36). We propose that non-craniofacial skeletal muscles are less affected in OPMD because if they remodel, it is at a low rate, mostly as a result of exercise, injury, toxic insult, or other disease processes. This hypothesis offers an explanation for the selective involvement of the craniofacial muscles and the adult-onset of symptoms, and is compatible with the observed histopathology of OPMD.

**Rejected Hypotheses**

We rejected two other models based on continuous myofiber remodeling to explain the clinical pattern of OPMD because of lack of evidentiary support. The first rejected hypothesis was somatic mosaicism, in which the repeated division of the muscle satellite cells could result in an increasing number of GCG repeats. Somatic mosaicism is defined as the presence of a different number of trinucleotide repeats in different cells or different tissues of
an individual patient. It occurs frequently in some “long” dynamic trinucleotide repeat disorders, such as X-linked bulbospinal muscular atrophy (Kennedy disease), but not in other trinucleotide repeat disorders, including Huntington’s disease (HD) (37,38). Dynamic instability of the number of repeats has been studied in the HD mutant mouse, where the normal number of cytosine-adenine-guanine repeats is 9 to 35, and where the pathogenic number of repeats may be over 200 (39). However, the presence of a varying number of repeats in pathologic tissue obtained from different regions of a single affected human HD brain could be the result, rather than the cause, of the pathology. Somatic mosaicism has not been found in OPMD, which is considered to be a “short” trinucleotide repeat disorder (17).

A second rejected hypothesis was that of a toxic gain-of-function based on the cumulative retention of non-degraded mPABPN1 in the myofibers following each cycle of myofiber remodeling. OPMD intranuclear inclusions have been proposed as “poly(A) RNA death traps” that interfere with RNA export and thus cause muscle cell death (34). It is possible that toxic aggregates of self-associated complexes of beta-pleated-sheet complexes of the mutant protein mPABPN1-ala17 accumulate because they are not readily degraded by proteasomes (29,40). Such accumulations have been described as possibly pathogenic in a number of neurologic disorders, including HD and spinocerebellar ataxia (41,42). However, histopathologic studies do not show a clear correlation between aggregates of mPABPN1 retained in the nucleus and OPMD muscle pathology (43).

Further Considerations

The understanding of the pathogenesis and clinical presentation of genetic disorders is not always immediately evident after the identification of the mutant genes and their protein products. Structural changes that initiate the accumulation of mutant protein detected by microscopy are often identified earlier than pathogenic biochemical mechanisms. Such protein aggregates have been assumed to explain the selective targeting or sparing of specific tissues. In both HD and OPMD, the presence of abnormal accumulations was initially interpreted as supporting the concept that such accumulations cause a pathogenic, toxic gain-of-function.

Currently, HD pathogenesis is being reevaluated and loss-of-function mechanisms are being proposed (41,42, 44–46). Toxic gain-of-function and loss-of-function pathogenic mechanisms may ultimately prove to be complimentary even though they may be initially presented as mutually exclusive. Intranuclear inclusions notwithstanding, OPMD lacks structurally identifiable abnormalities, such as aggregates of misfolded undegraded proteins, that are quantitatively correlated with the expression of the disease at the organismal level (43). This finding tends to shift the balance of competing hypotheses toward loss-of-function hypotheses.

Thus, it is reasonable to propose that mPABPN1 may fail to support the mRNA production required to maintain continuous EOM myofiber remodeling. Patients with OPMD have one normal copy and one mutant copy of the PABPN1 gene and therefore produce normal, wild-type PABPN1 and mutant PABPN1. The major known function of PABPN1 is to influence the production of normal mRNA (18,20,24). The continuous EOM remodeling process is reflected at the RNA level in the expression profiles of the EOMs as compared with skeletal muscle from the leg. Genes related to muscle growth, development, and regeneration are upregulated in EOMs (2). The production of mRNA, some proportion of which consists of disabled mRNA, could lead to cumulative loss of affected myofibers. Also, because polyadenylation is important in the regulation of the cell cycle, abnormalities in this process could interfere with the process of EOM myonuclear addition and integration in the affected muscles. Failure of normal withdrawal from the cell cycle and impaired differentiation have been shown to be pathogenic factors in myotonic dystrophy (47).

mPABPN1 may also react with unique EOM and craniofacial muscle-specific proteins that are not expressed in other skeletal muscles. As noted, gene array studies show that there are a number of genes uniquely upregulated or downregulated in EOM when compared with leg muscles. It is important to note that gene array data from whole EOMs include a mixture of activated muscle satellite cells and myoblasts, in addition to resident connective tissues, nerves, neuromuscular junctions, and blood vessels (2). Therefore, these reports reflect contributions from a mixture of tissues and make interpretation of the results more ambiguous.

Our hypothesis bridges the gap between a specific genetic defect partially understood at the molecular and cellular level (Fig. 1), and the clinical presentation at the organismal level (Fig. 2), where there is presently no understanding as to the selective susceptibility of the EOMs and certain craniopharyngeal muscles. The greatest problem with our hypothesis is its failure to explain the low-grade involvement of the proximal limb muscles if it is assumed that normal appendicular skeletal muscles do not undergo continuous myonuclear replacement. We speculate that proximal limb muscle may undergo non-exercise–induced myonuclear replacement at a undetectable level or at specific regions of the muscles not yet studied, such as the tendinous insertions. We might also invoke some exercise/injury-induced myonuclear addition to explain the
lesser involvement of the proximal appendicular muscles compared with greater abnormalities of the craniofacial muscles. Further studies of normal adult mammalian EOMs, craniofacial muscles, and noncraniofacial skeletal muscles will be needed to determine the relative concentration of PABPN1-specific mRNA, the rate of mRNA turnover, and the ratios of mPABPN1 and wild-type PABPN1 present in the sarcoplasm compared to other proteins such as cyclophilin, which has been demonstrated to be stable during aging. It would also be useful to compare the activity of the major pathways for protein degradation, such as the ubiquitin-proteasome pathway, in skeletal muscles from various regions of the body. One would predict higher endogenous activity in muscles with higher protein turnover. It has been speculated that the accumulation of mPABPN1–ala17 in older individuals explains the delayed clinical onset, given that older cells have a diminished capability to induce the production of heat shock proteins (31).

The selective targeting and sparing of the EOMs and craniofacial muscles in various human myopathies suggest a need for continued study of the biology of continuous remodeling specific to these muscles. Such studies would be valuable in targeting therapies, not only to ameliorate OPMD, but also to facilitate therapies such as myocyte replacement in disorders like Duchenne muscular dystrophy.

REFERENCES

8. McLoon LK, Peters E, Wirtschafter JD. Adult extraocular muscles express HSPG, Syndecan and growth factor receptor molecules that are normally down-regulated in adult skeletal muscle. *Invest Ophthalmo* n *V* *is* Sci 1999;40:5408.


