Original Article

Oculopharyngeal Weakness, Hypophrenia, Deafness, and Impaired Vision: A Novel Autosomal Dominant Myopathy with Rimmed Vacuoles

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Abstract

Background: Myopathies with rimmed vacuoles are a heterogeneous group of muscle disorders with progressive muscle weakness and varied clinical manifestations but similar features in muscle biopsies. Here, we describe a novel autosomal dominant myopathy with rimmed vacuoles in a large family with 11 patients of three generations affected.

Methods: A clinical study including family history, obstetric, pediatric, and development history was recorded. Clinical examinations including physical examination, electromyography (EMG), serum creatine kinase (CK), bone X-rays, and brain magnetic resonance imaging (MRI) were performed in this family. Open muscle biopsies were performed on the proband and his mother. To find the causative gene, the whole-exome sequencing was carried out.

Results: Disease onset was from adolescence to adulthood, but the affected patients of the third generation presented an earlier onset and more severe clinical manifestations than the older generations. Clinical features were characterized as dysarthria, dysphagia, external ophthalmoplegia, limb weakness, hypophrenia, deafness, and impaired vision. However, not every patient manifested all symptoms. Serum CK was mildly elevated and EMG indicated a myopathic pattern. Brain MRI showed cerebellum and brain stem mildly atrophy. Rimmed vacuoles and inclusion bodies were observed in muscle biopsy. The whole-exome sequencing was performed, but the causative gene has not been found.

Conclusions: We reported a novel autosomal dominant myopathy with rimmed vacuoles characterized by dysarthria, dysphagia, external ophthalmoplegia, limb weakness, hypophrenia, deafness, and impaired vision, but the causative gene has not been found and needs further study.

Key words: Inclusion Body; Rimmed Vacuoles; Whole-exome Sequencing

Introduction

Rimmed vacuoles on muscle biopsy, which can be observed in a heterogeneous group of muscular disorders, are a major pathological feature or an accompanying feature. These disorders include hereditary myopathies and acquired myopathies, such as GNE myopathy, inclusion body myopathy with Paget disease of bone, frontotemporal dementia (IBMPFD),[1] oculopharyngeal muscular dystrophy (OPMD),[2] oculopharyngodistal myopathy (OPDM)[3] sporadic inclusion body myositis (s-IBM),[4] and so forth. Moreover, they have a variety of clinical manifestations but with similar pathological features. Hence, it is particularly important to make the correct diagnosis of these disorders based on the clinical manifestations, pathological findings, and the

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genetic tests. In recent years, thanks to the next generation sequencing, some novel causative genes have been found and some novel myopathies have been recognized.[5,6]

Here, we describe a Chinese family with 11 patients of three generations involved. This family manifested dysarthria, dysphagia, external ophthalmoplegia, limb weakness, hypophrenia, deafness, and visual deterioration, in an autosomal dominant inheritance. Muscle biopsy showed rimmed vacuoles and inclusion bodies in muscle fibers. The whole-exome sequencing was performed in this family.

Methods

This study was approved by the Institutional Review Board of our hospital. A clinical study including family history, obstetric, pediatric, and development history was recorded. Clinical examinations including physical examination, electromyography (EMG), serum creatine kinase (CK), bone X-ray, and brain magnetic resonance imaging (MRI) were performed.

Open muscle biopsies were performed on the proband and his mother. Muscle specimens were frozen in isopentane that was precooled in liquid nitrogen. Frozen transverse sections (8 μm) were stained with standard histochemistry and enzymohistochemistry stainings: hematoxylin and eosin (H and E staining), periodic acid-Schiff stain (PAS), oil red O, modified Gomori trichrome (MGT), nicotinamide dehydrogenase tetrazolium reductase (NADH-TR), nonspecific esterase and succinate dehydrogenase, and adenosine triphosphatase (ATPase) after incubation at pH 4.3, 4.5, and 10.9. Electron microscopic examination was performed using standard techniques.

The whole-exome sequencing was only performed on the proband, his father, and his cousin because other family members refused to do the genetic tests. Genomic DNA was extracted from blood. The qualified genomic DNA samples were randomly fragmented by Covaris (Covaris, Woburn, Massachusetts, USA) and the sizes of the library fragments were mainly distributed between 250 bp and 300 bp. Then, the adapters (Agilent, Santa Clara, California, USA) were ligated to both ends of the resulting fragments. Extracted DNA was then amplified by ligation-mediated polymerase chain reaction (LM-PCR), purified, and hybridized to the Nimblegen SeqCap EZ (Roche, Basel, Switzerland). Library for enrichment, nonhybridized fragments were then washed out. Both noncaptured and captured LM-PCR products were subjected to quantitative PCR to estimate the magnitude of enrichment. Each captured library was then loaded on Hiseq2000 platform (Illumina, Santiago, California, USA), and we performed high-throughput sequencing for each captured library to ensure that each sample meets the desired average sequencing depth. Raw image files were processed by Illumina base-calling software 1.7 (Illumina, Santiago, California, USA) for base-calling with default parameters and the sequences of each individual were generated as 90 bp pair-end reads. SOAPaligner/SOAP2 was used to map reads onto the reference. While analyzing Indel, BWA was used to map reads onto the reference. Only mapped reads were used for subsequent analysis. The variants were filtered with the databases of dbSNP135 (http://www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 Genomes project (http://www.1000genomes.org/), and the YanHuang project (http://yh.genoinics.org.cn/). The software SIFT (http://sift.jcvi.orq/MutationTaster), MutationTaster (http://www.mutationtaster.org/), and PolyPhen2 (http://genetics.bwh.harvard.edu/pph 2/bqi/shtml) were used to predict the mutations' function. Variants were confirmed by Sanger sequencing.

Results

All affected patients in this family had a normal birth and normal motor development [Figure 1]. The mean age of disease onset was 17.8 years old (range: 7–42 years) [Table 1]. The mean disease duration was 19.7 years (range: 2–40 years). For I-1, the affected patient of the first generation, the age of onset was 30 years old. Moreover, the average age of onset for the second and third generation was 24 and 13.4 years old, respectively. Clinical symptoms were characterized by dysarthria, dysphagia, external ophthalmoplegia, limb weakness, hypophrenia, deafness, and visual deterioration. Among all of the 11 patients considered in this family, dysarthria was present in nine patients, dysphagia in six, external ophthalmoplegia in six, limb weakness in five, hypophrenia in four, deafness in two, and visual deterioration in one. For all of the five patients with limb weakness, proximal muscle of lower limbs was first involved. As the disease progressed, both proximal and distal muscles were involved.

The proband (III-9) was a 26-year-old male. When he was 13-year-old, he was observed intelligent deterioration. At the age of 23, he came to our hospital. External ophthalmoplegia, limb weakness, dysarthria, dysphagia, hearing loss, and visual deterioration were observed [Figure 2]. On physical examination, he had a myopathic face with whole body muscle severely atrophied but was clear in consciousness. Pupils of both eyes were equally round and of equal size. Pupillary light reflex was normal. Bilateral visual acuity was 0.15 and pallor papillae were observed. He presented bilateral ptosis and limitations in eye movements in all directions. Facial muscle weakness, dysphagia, gag reflex disappearance, and tongue movements limitation were observed. Rough audition test showed a hearing loss. Muscle strength of Medical Research Council was Grade 4 for both upper and lower limbs. Limbs were a little hypertonia. Deep tendon reflexes were symmetrically preserved. Muscle volume was reduced and joint contracture was noticed in hips, knees, feet, and hands. Babinski's sign, sensory, and coordinate movements were negative. Four years later, as the disease progressed, although he had clear consciousness, slurred speech was apparently observed, and he could only pronounce some simple words such as "eat." Remembrance, orientation force, and calculation ability could not be tested because he could not cooperate. He could only react to some simple orders such as "open

mouth, stretch hands." Muscle weakness and wasting were so severe that he could not have independent ambulation and almost stayed in bed all the time. Deep tendon reflexes were totally disappeared but pyramidal signs were still negative. The proband's mother (II-3) was found to have mild bilateral ptosis and hypernasal voice when she was 42-year-old. The proband's brother (III-8) showed all symptoms except hearing loss and visual deterioration, the same as the proband with disease onset at 14-year-old. The proband's cousin (III-4) was found hearing loss when she was 13-year-old, and in the next 20 years, the disease progressed slowly. Finally, she presented all of the manifestations except visual deterioration. The other seven patients in this family presented one or more symptoms [Table 1]. III-8 died of asphyxia resulted from dysphagia when eating food. The death causes of I-1 and II-2 were unknown. At the follow-up, the proband (III-9) died of the complications of pulmonary infection and respiratory failure. III-4 died of the same cause as III-8.

Laboratory examination showed serum CK of the proband (III-8) was mildly elevated at 428 IU/L (normal 20–200 IU/L). EMG revealed the myopathic pattern. Needle EMG showed fibrillation potentials, positive sharp waves, low amplitude, shot duration, and polyphasic motor unit. Sensory and motor nerve conduction velocities were normal. But EMG of the proband's mother (II-3) showed nonspecific changes. Brain MRI of the proband revealed that the cerebellum and brain stem were mild atrophied. X-ray of bone was performed on the proband and his mother, but no osteolysis, osteosclerosis, or cortical thickening were found, and only joint contractures and intestinal tympanites were observed in the proband [Figure 3].

Open muscle biopsy on the right biceps brachii was performed in both the proband (III-9) and his mother (II-3). In the biopsy of the proband (III-9), the transverse sections revealed muscle fibers with variable sizes on H&E, staining. These atrophic muscle fibers could be small-round or small-angular. Some atrophic fibers had one or more rimmed vacuoles with the shapes of bar-type or oval-type. Noticeably, in these rimmed vacuoles, there were basophilic inclusion bodies. Some fibers with rimmed vacuoles displayed eosinophilic orange uniform material surrounding the vacuoles or scattered in other parts. Degeneration, necrosis, phagocytosis, and inflammation infiltration in muscle fibers were seldom observed. Nuclear internalization and fiber hypertrophy appeared in a proportion of muscle fibers. MGT-reacted sections showed no red ragged fibers (RRFs). Staining for NADH-TR displayed moth-eaten-like appearance in atrophic fibers with strong staining. ATPase reacted sections revealed no fiber grouping and the majority of the involved fibers were Type I although fibers were well differentiated. Ultrastructural examination of the proband revealed intranuclear and cytoplasmic filamentous inclusions. In the biopsy of the proband's mother (II-3), H&E, staining sections showed just a few small-round or small-angular fibers. Some individual atypical RRFs were found on MGT staining. The majority of fibers strongly reacting with NADH-TR had normal fiber structure. Type I fiber predominance was discovered in ATPase staining. No other specific changes were observed [Figure 4].

Unfortunately, the known causative genes of myopathy with rimmed vacuoles, such as *GNE*, *MYH2*, *VCP*, *PABPN1*, *TTN*, *TIA1*, and so on were excluded by the whole-exome sequencing. Meanwhile, the novel causative gene has not been found.

Discussion

Here, we described a family of autosomal dominant myopathy with rimmed vacuole, characterized by hypophrenia, dysarthria, dysphagia, external ophthalmoplegia, limb weakness, deafness, and visual deterioration. The disease onset was from adolescence to adulthood but the third generation developed clinical symptoms at an earlier age, progressed more rapidly, and had more severe clinical symptoms than the older generations. This raised the possibility of clinical heterogeneity and genetic anticipation. The most prominent histopathologic features were rimmed vacuoles and basophilic inclusion bodies in the muscle biopsy of the proband. In addition, there was no lymphocytic infiltration observed. According to the genetic background, progressive muscle weakness and pathologic phenotype, this disease should be considered as a type of hereditary IBM. The

Table 1: Clinical symptoms in 11 affected patients in this family

Items	I-1	II-2	II-3	II-5	III-4	III-5	III-6	III-7	III-8	III-9 (proband)	III-13
Sex	Male	Female	Female	Male	Female	Female	Male	Male	Male	Male	Female
Age (years)	70 (died)	56 (died)	52	49	30	27	24	20	24 (died)	26	22 (died)
Age onset (years)	30	20	42	10	13	20	7	7	14	13	20
Disease duration (years)	40	36	10	39	17	7	17	13	10	13	2
Symptoms											
Hypophrenia	+	–	–	–	+	–	–	–	+	+	–
External ophthalmoplegia	–	–	+	+	+	–	+	–	+	+	–
Limb weakness	–	+	–	+	+	–	–	–	+	+	–
Dysarthria	+	+	+	+	+	+	–	+	+	+	+
Dysphagia	+	+	–	+	+	–	–	–	+	+	+
Hearing loss	–	–	–	–	+	–	–	–	–	+	–
Visual deterioration	–	–	–	–	–	–	–	–	–	+	–

+: Present; –: Not present.

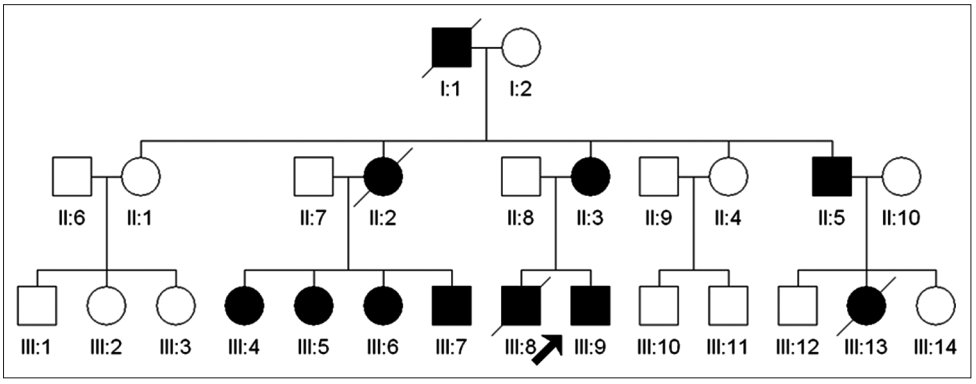

Figure 1: Pedigree of this affected family. Pedigree of the family: □unaffected male; ○unaffected female; ■affected male; ●affected female; ↗the proband.

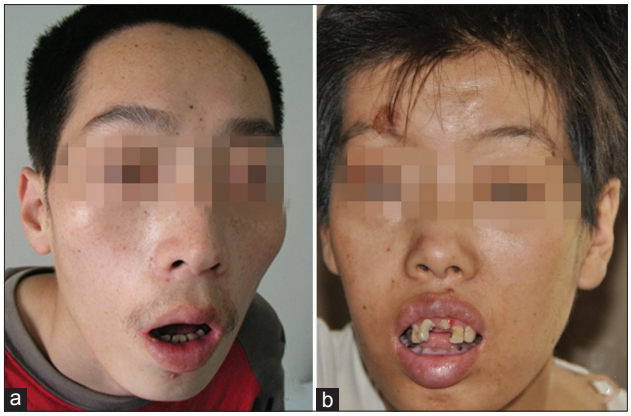

Figure 2: Photos of the proband and his cousin. The proband (a) and his cousin (b) show myopathic faces and external ophthalmoplegia.

most frequently observed symptom was ocular pharyngeal muscle weakness. However, not every patient manifested all of the symptoms, so this disease was probably incomplete penetrance. Laboratory examination showed CK was mildly elevated, and brain MRI of the proband revealed cerebellum and brain stem seemed mildly atrophied. The EMG result and muscle pathology of the proband were much worse than his mother, so these may be related to the severity of the disease.

Myopathy with rimmed vacuoles is a heterogeneous group of muscle disorders that present progressive muscle weakness with similar features on the muscle biopsy but have variable clinical manifestations.[7,8] For example, GNE myopathy mainly affects distal limb muscle and shows distal muscle weakness and wasting, especially in tibial anterior muscle but quadriceps relatively sparing caused by GNE mutation.[9,10] GNE myopathy rarely manifests with external ophthalmoplegia or pharyngeal muscle weakness. Moreover, GNE myopathy is an autosomal recessive disorder. IBMPFD is characterized by variable expression of slow, progressive muscle weakness, Paget's disease of bone with abnormal bone homeostasis, and frontotemporal dementia with early behavioral or language changes caused by valosin-containing protein gene (*VCP*).[1,11] However, bone X-ray in the proband and his mother showed no osteolysis, osteosclerosis or cortical thickening, and no mutations were found in *VCP* gene. Hence, IBMPFD was excluded.[12] OPMD mainly manifested by limb weakness and oculopharyngeal muscle weakness caused by abnormal (GCG) n expansions of the first exon in *PABPN1* gene.[13,14] The disease onset of OPMD is usually more than 50-year-old and the disease seldom manifests deafness or visual impairment. The genetic test showed this family had no mutations in *PABPN1* gene. OPDM is a rare, clinicopathologically distinct muscular disease with both autosomal dominant and autosomal recessive inheritance, but the pathogenic gene of OPDM is still unknown. OPDM is characterized by external ophthalmoplegia, pharyngeal muscle weakness, and distal muscle weakness.[15] Although, there are many similarities between the symptoms of OPDM and those presented in this family, OPDM is characterized by distal muscle weakness, and this family showed both proximal and distal muscle weakness. Moreover, OPDM rarely presents deafness or visual impairment. As far as we know, myopathy with rimmed vacuoles characterized by limb weakness, oculopharyngeal weakness, hypophrenia, deafness, and impaired vision have not been reported. In 2006, Gambelli *et al.* reported an Italy family that presented autosomal dominant muscular disorder characterized by cerebrocortical and cerebellar atrophy, progressive cognitive impairment, autonomic dysfunction and myopathy with rimmed vacuoles, and intranuclear filamentous inclusions, but the causative gene has not been found.[16] However, this Italy family had no oculopharyngeal muscle involved, either our family presented abnormal pupils. Hence, the family we reported here may be a novel type of myopathy with rimmed vacuoles.

The whole-exome-sequencing was performed on the proband, his cousin, and his father, but we have not found the causative gene. Moreover, other patients in the family refused to do the genetic examination and the proband and his cousin died during the follow-up, so it is hard to do gene linkage analysis. Meanwhile, there are some limitations in the whole-exome-sequencing. For example, the whole-exome-sequencing can only detect the information of the exomes and cannot detect the large structure variation in the genome. It also cannot detect the repeat regions of exon sequence at the end of the chromosomes or the regulatory sequences in the introns. So far, we have not found any other similar families or sporadic case. These all make it more difficult to find the causative gene.

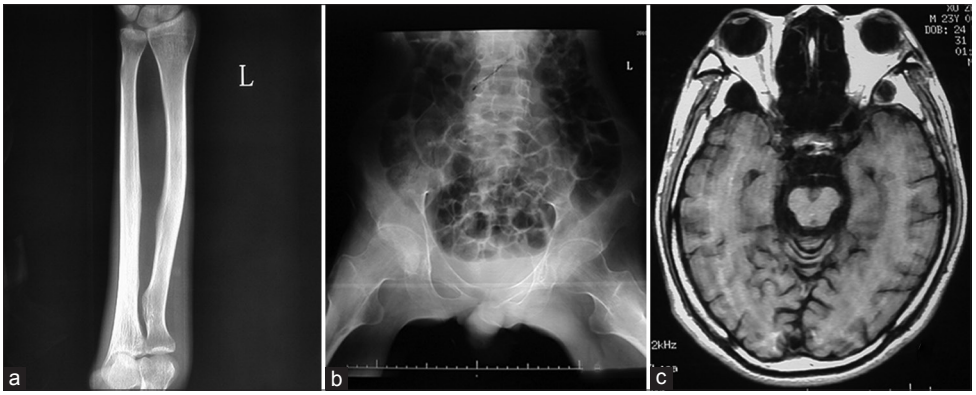

Figure 3: Bone X-ray and brain MRI of the proband. Bone X-ray of the proband shows no osteolysis, osteosclerosis or cortical thickening (a and b). Brain MRI presents cerebellum and brain stem atrophy (c). MRI: Magnetic resonance imaging.

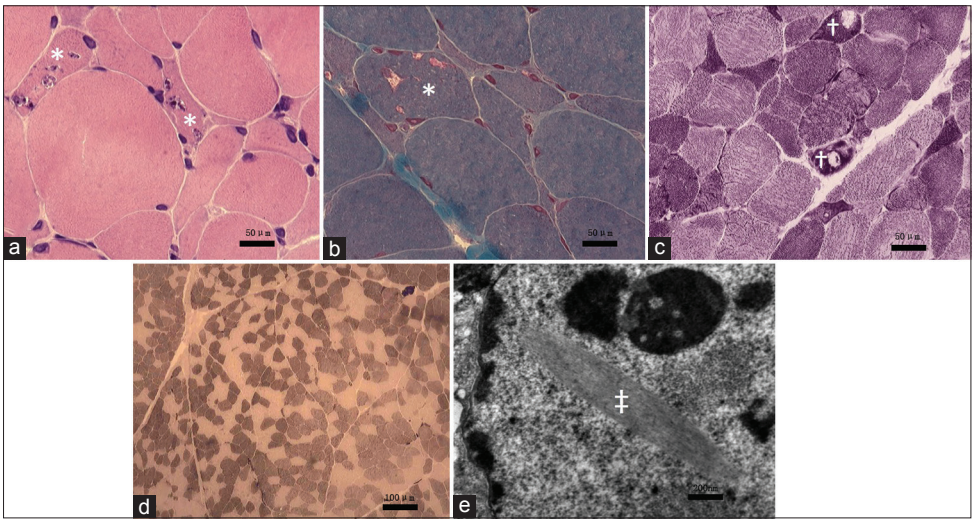

Figure 4: Muscle sections of the proband. H and E staining shows rimmed vacuoles (*) with basophilic royal blue particles in atrophic fibers (a, original magnification ×200).MGT staining reveals red inclusions (*) in rimmed vacuoles (b, original magnification ×200). Staining for NADH-TR displayed moth-eaten-like appearance (†) in atrophic fibers with strong staining (c, original magnification ×200). ATPase (pH 4.5) reacted sections revealed fibers well differentiated (d, original magnification ×100). Intranuclear tubulofilamentous inclusions (‡) are observed in an electron microscope (e, original magnification ×50,000). MGT: Modified Gomori trichrome; NADH-TR: Nicotinamide dehydrogenase tetrazolium reductase; ATPase: Adenosine triphosphatase.

We conclude that this familial disorder may present a novel myopathy with the rimmed vacuole. This investigation highlights the existence of different types of myopathies with rimmed vacuoles. Moreover, this family expands the clinical phenotype with ocular pharyngeal muscle weakness, unusually severe central nervous system (CNS) involvement and intra-familial variability of muscle phenotype. We suspect that the patients in this family should have mutations in a gene encoding a protein that is critical for both ocular pharyngeal muscle and CNS. Further molecular investigations are underway to localize and clone this gene and it will be important for us to understand the genetic background and the pathogenesis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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