Oculopharyngeal Muscular Dystrophy among Bulgarian Jews: A New Cluster?

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ABSTRACT: **Background:** Oculopharyngeal muscular dystrophy (OPMD) produced by the (GCG)13 expansion mutation in the *PABPN1* gene is frequent among Uzbek Jews in Israel.

Objectives: To describe the phenotypic and genotypic features in five Bulgarian Jewish patients, from different families, with autosomal dominant OPMD.

Methods: We performed clinical follow-up, electrodiagnostic tests and mutation detection. Blood samples were obtained after informed consent and DNA was extracted; measurement of GCG repeats in both *PABPN1* alleles and sequencing of OPMD mutations were performed according to standard techniques.

Results: We identified five patients (four females), aged 58 to 71 years, with bilateral ptosis, dysphagia, dysphonia (n=3) and myopathic motor units by electromyography. In all patients we noticed proximal weakness of the upper limbs with winging scapulae in three of them. All cases shared the (GCG)13-(GCG)10 *PABPN1* genotype.

Conclusions: OPMD among Bulgarian Jews is produced by a (GCG)13 expansion, identical to the mutation in Uzbek Jews and French Canadians. In addition to the classical neurological and neuro-ophthalmological features, early shoulder girdle weakness is common in Bulgarian Jewish patients; this is an unusual feature during the early stages of OPMD produced by the same mutation in other populations. We suggest that besides the disease-producing GCG expansion, additional ethnicity-related genetic factors may influence the OPMD phenotype. OPMD is a rare disease, and the identification of five affected families in the rather small Bulgarian Jewish community in Israel probably represents a new cluster; future haplotype studies may elucidate whether a founder effect occurred.

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KEY WORDS: muscular dystrophy, oculopharyngeal, phenotype, Bulgarian Jews, genetics

Autosomal dominant oculopharyngeal muscular dystrophy is a late-onset myopathy caused by a stable trinucleotide expansion from a normal of (GCN)10 [formerly (GCG)6] to (GCN)12-17 [formerly (GCG)8-13] repeats in the first exon of the *PABPN1* gene on chromosome 14q11.1 [1]. Most of the phenotypic variation among autosomal dominant OPMD patients is related to the size of the dominant GCN expansions, the influence of a (GCN)7 expansion in the second allele, or homozygosity [1-6]. Little is known about the causes of phenotypic differences between patients with identical *PABPN1* genotype. (GCN)13 is the most frequent OPMD-producing mutation worldwide and is shared by the three largest patient clusters: French Canadians, Uzbek (Bukhara) Jews, and Hispanic New Mexicans [2,3,7]. We describe five Israeli Jewish OPMD patients of Bulgarian ancestry. Although their disease-producing mutation is (GCG)13 and the second *PABPN1* allele a normal (GCG)10, some clinical features are different from those described among Uzbek Jews who make up the large majority of OPMD patients in Israel [8].

OPMD = oculopharyngeal muscular dystrophy

PATIENTS AND METHODS

All patients were routinely followed in the departments and units of neurology, neuro-ophthalmology and otolaryngology of the participating institutions. Patients 1, 2 and 3 gave written consent for publication of their photos including identifiable portraits in medical journals.

PATIENT 1

This 66 year old female of Jewish Bulgarian ancestry was seen at the age of 60 because of ptosis, dysphonia, dysphagia and severe proximal weakness in all four limbs. Reportedly her parents had no neurological problems and died at the ages of 63 and 83. Her only sister has breast cancer but no neurological problems. A paternal cousin is now being investigated for similar muscular problems. In our patient neurological troubles began at age 52 with difficulty walking and climbing stairs. A few months later she noticed problems swallowing, nasal speech and bilateral ptosis [Figure 1A]. These symptoms progressed slowly, without fluctuations or remissions.

On examination we found marked bilateral asymmetrical ptosis, mild limitation of both vertical and horizontal gaze, slow saccadic eye movements, weakness of orbicularis oculi, severe dysphonia and dysphagia for both solids and liquids, and no gag reflex. There was 4/5 weakness of sterno-cleido-mastoids, prominent winging scapulae [Figure 2A], and 3/5 proximal weakness of upper and lower extremities except for quadriceps muscles that were completely spared. Distal muscles were stronger; however, there was 4/5 weakness of distal ulnar muscles and tibialis anterior and moderate atrophy of both first dorsal interossei [Figure 3A]. Reflexes were not elicited except for brisk patellar jerks. Plantar responses were flexor. There was no myotonia, sensory, cerebellar or autonomic dysfunction. High mental functions were normal.

Motor and sensory nerve conduction studies were normal. Electromyogram was suggestive of muscle disease without spontaneous activity or myotonic discharges. Over the years creatine phosphokinase ranged between 500 and 700 U/L (normal ≤ 175) and did not decrease after statins were stopped. Due to concern about possible overlapping pathologies a muscle biopsy was taken from the vastus lateralis; it showed myopathic features, many rimmed vacuoles and no significant ragged red fibers or other features suggestive of mitochondrial disease. Genetic screening was negative for both DM1 and DM2.

The patient required 55 seconds to drink 80 ml of cold water. Fiberoptic endoscopic evaluation of swallowing [9,10] showed pooling of saliva in the hypopharynx and aspirations. After informed consent a cricopharyngeal myotomy was performed with subsequent improvement of deglutition but no change in

Figure 1. Eyelids ptosis. Facial appearance and bilateral ptosis in patient 1 **[A]**, patient 2 **[B]** and patient 3 **[C]**

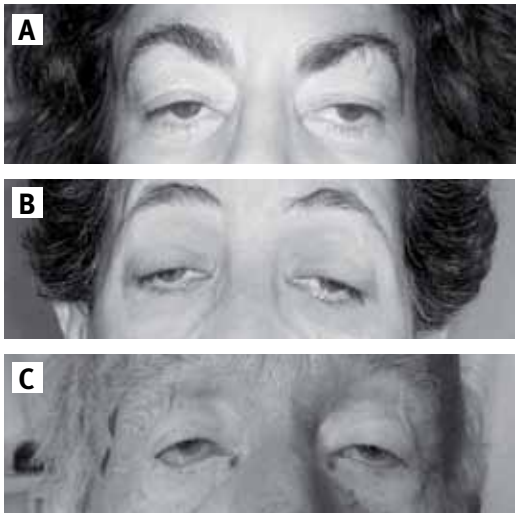


Figure 2. Scapular winging in patient 1 **[A]**, patient 2 **[B]** and patient 3 **[C]**

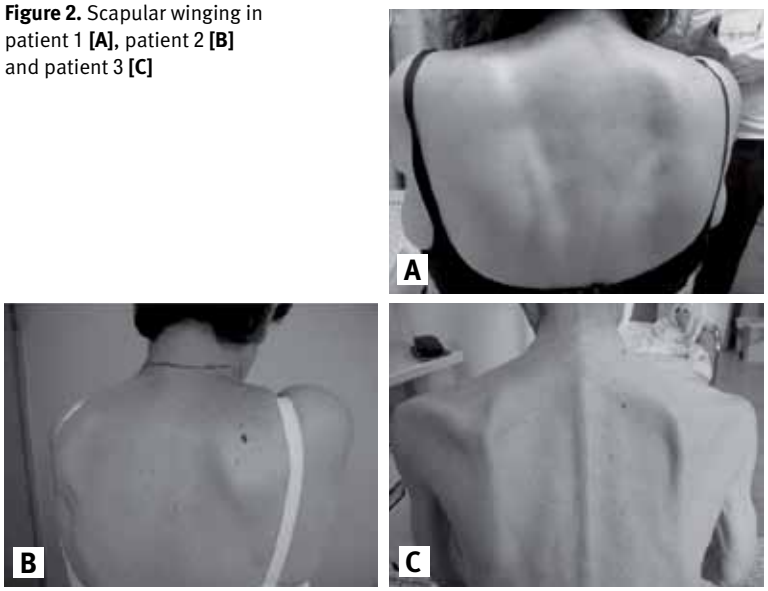
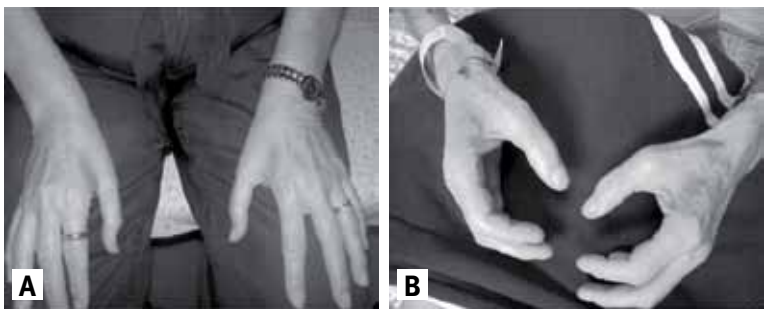


Figure 3. Distal hand muscle atrophy. Bilateral atrophy of first dorsal interossei muscles in patient 1 **[A]** and patient 3 **[B]**



speech quality. Six years after the intervention, her swallowing is good and there are no aspirations. Her weight is stable.

PATIENT 2

This 71 year old woman has had bilateral ptosis since the age of 62 and difficulty swallowing solid food for 6 years, although even previously she was the last member of her family to finish a meal. Her father, paternal grandfather and a brother of the latter had similar problems, which began after the age of 60. A younger brother has bilateral ptosis but no swallowing problems. At the age of 46 our patient was hospitalized in another neurology department for what, in retrospect, could have been the Miller-Fisher variant of Guillain-Barre syndrome. At that time, mild bilateral scapular winging was noticed. After a partial recovery she was left with marked intention and position tremor, ataxia on index-to-nose and ankle-to-knee tests and mild wasting of distal ulnar muscles. When first seen by one of us (A.K.) 5 years ago, she also had marked bilateral ptosis [Figure 1B], mild limitation of eye adduction without diplopia, mild proximal upper limb weakness, and bilateral scapular winging [Figure 2B]. Tendon jerks were very weak or could not be elicited. There was no lower limb weakness and she was able to walk on her heels and toes. Her voice was clear, without dysphonia, and soft palate excursions were normal. She was able to drink 80 ml of cold water in 16 seconds. Motor and sensory nerve conduction studies in four limbs were normal and most motor units myopathic on EMG.

ELECTROPHYSIOLOGICAL STUDIES

Three patients had routine motor and sensory nerve conduction studies of four limbs and needle EMG sampling of proximal and distal limb muscles. These were not required in two patients with early genotype-based diagnosis and no confounding features.

Muscle biopsies were taken from vastus lateralis muscle (patient 1) and, during upper esophageal sphincter release operations, from the cricopharyngeal muscles in patients 1 and 3. They had been processed for routine histo-enzymology. Neither electron microscopy studies from these samples nor other muscle biopsies were performed because the diagnosis was already established with blood-extracted DNA studies.

EMG = electromyography

Table 1. Clinical features in five Bulgarian Jewish OPMD patients

Patient (gender)	Age (yr)	Onset	Ptosis	Dysphagia	Dysphonia	PW UL	PW LL	Scapular winging
1 (F)	66	52	+ + +	+ + +	+ + +	+ + +	+ + + QS	+ + +
2 (F)	71	60	+ + +	+ +	-	+ +	-	+ +
3 (M)	69*	59	+ + +	+ + +	+ + +	+ + +	+ +	+ +
4 (F)	70	54	BP	+ +	+	+ +	+	-
5 (F)	59	54	BP	+	-	+	-	-

*Age at death

F = female, M = male, PW = proximal weakness, UL = upper limbs, LL = lower limbs, BP = blepharoplasty, QS = quadriceps sparing, - = none, + = mild, ++ = moderate, +++ = severe

EVALUATION OF SWALLOWING AND PHONATION

Dysphagia severity was evaluated using the 80 ml ice cold water drinking test and a structured questionnaire adapted from Carter Young and Durant-Jones [11]. Patients 1 and 3 also underwent fiberoptic endoscopic evaluation of swallowing as part of their evaluation for cricopharyngeal myotomy. The endoscopy technique was similar to that described by Dziewas et al. [9] and Warnecke et al. [10], but the tasks were simplified: observation of the hypopharynx and related structures before presentation of any bolus, during and after drinking cold water and cream. Speech (articulation, speaking rate, nasal speech, hypophonia and breath-speech coordination) was evaluated using a simple global rating scale, with -0 indicating normal speech to ++++ indicating barely intelligible speech.

MUTATION ANALYSIS

DNA samples were available from all patients. Polymerase chain reaction, amplification and measurement of the sizes and sequencing of the OPMD mutations were carried out as previously described [1].

RESULTS

All patients presented in Table 1 were heterozygotes of a (GCG)13 OPMD-producing expansion mutation in the *PABPN1* gene. There were four females and one male (patient 3). The disease started between the ages of 52 and 60 with ptosis of eyelids [Figure 1] and dysphagia; however, it is possible that proximal weakness antedated the other symptoms in patients 1 and 2. On first examination all patients had marked eyelid drooping or had already undergone blepharoplasty. Dysphagia was severe in patients 1 and 3, moderate in patients 2 and 4, and mild in patient 5. Dysphonia was severe in patients 1 and 3, correlating with dysphagia, and mild or absent in the others. Proximal weakness was present in the upper limbs in all patients and associated with scapular winging in three [Figure 2]; three patients also had mild to severe proximal weakness of lower limbs. Distal weakness and atrophy of small hand muscles was observed in two patients [Figure 3]. Two patients underwent cricopharyngeal myotomy for dysphagia with life-threatening aspirations.

DISCUSSION

We identified five OPMD patients belonging to different families from the Sofia, Plovdiv and Danubian areas of Bulgaria. All families are of Sephardic Jewish ancestry but we are not

aware of their genealogy before they reached Bulgaria. In all patients the *PABPN1* disease-producing genotype comprised the expanded (GCG)13 allele and a normal (GCG)10 allele. Clinically, the core features of OPMD – onset with bilateral ptosis and dysphagia, discrepancy between severe ptosis, and mild or absent impairment of eye movements – were present in all patients and autosomal dominant inheritance could be established in four. Proximal weakness is generally a late feature in heterozygotes for the (GCN)13 mutation. It starts more often in the lower limbs and is uncommon before the age of 70, or less than 15–20 years after disease onset [2,12,13]. In this cohort, shoulder girdle weakness was an early feature that occurred concomitantly or antedated proximal weakness of the lower limbs and was associated with scapular winging in three patients; in patient 2 scapular winging was noticed many years before the occurrence of ptosis and dysphagia. In our experience with many OPMD cases produced by the same mutation among French Canadians and Uzbek Jews, this is very unusual [12,13]. It is tempting to speculate that OPMD is more than GCN expansion and that other genetic factors in different ethnic groups may influence the phenotype.

An unusual feature in patient 1 was the sparing of the quadriceps muscles. Despite 3/5 weakness of all hip girdle muscles including iliopsoas, both quadriceps were 5/5 and patellar reflexes were bilaterally brisk in contrast to all other tendon jerks that were not elicited. This is reminiscent of the hereditary inclusion body myopathy frequent in another ethnic group, Iranian Jews, and produced by the M712T mutation in the *GNE* gene [14,15]. Since another cluster of hereditary inclusion body myopathy has been reported among Bulgarian gypsies, the possibility of OPMD and *GNE* mutations co-occurring in our patient is currently being investigated [16].

Four of the five patients also had tremor, essential in three and with both cerebellar and essential tremor features in patient 2. Because this is a common extrapyramidal disorder and our cohort is small, we cannot yet draw any conclusions about this association.

Two families with OPMD were previously reported among subjects of Bulgarian descent [17,18]. In the first, described by Mihaylova et al. [17], six individuals were affected by a (GCN)13 mutation with intrafamilial phenotypic variability; their "patient 4" also had, in addition to proximal weakness and wasting, atrophy and weakness of tibialis anterior and small hand muscles, like our patients 1, 2 and 3. The second family was Sephardic Jewish and had lived in Bulgaria for many generations before immigrating to Israel [18]. It was reported in 1993 and, at that time, the diagnosis was based on muscle biopsy, which showed on electron microscopy the typical intranuclear inclusions previously described by Tome and Fardeau [19]. We are not aware of the *PABPN1* genotype in that family.

Due to the compassion of their compatriots, Bulgarian Jews were largely spared during the Holocaust. Since families remained together it has been possible to gather information about their ancestry and health status in past generations. This, together with haplotype DNA studies, should elucidate whether the presence of five OPMD families in the relatively small community of Bulgarian Jews in Israel is a coincidence or if this indicates a new founder effect.

OPMD is uncommon and under-diagnosed worldwide [20,21]. As in other uncommon inherited diseases [22,23], being aware of populations with a high prevalence (clusters) may guide the clinician in providing the correct diagnosis and spare the patient useless time-consuming investigations and stress.

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Capsule

Cold-inducible RNA-binding protein (CIRP) triggers inflammatory responses in hemorrhagic shock and sepsis

A systemic inflammatory response is observed in patients undergoing hemorrhagic shock and sepsis. Qiang et al. report increased levels of cold-inducible RNA-binding protein (CIRP) in the blood of individuals admitted to the surgical intensive care unit with hemorrhagic shock. In animal models of hemorrhage and sepsis, CIRP is upregulated in the heart and liver and released into the circulation. In macrophages under hypoxic stress, CIRP translocates from the nucleus to the cytosol and is released. Recombinant CIRP stimulates the release of tumor necrosis factor-alpha (TNFα) and HMGB1 from macrophages and induces inflammatory responses and causes tissue injury when injected in vivo. Hemorrhage-induced TNFα and HMGB1 release and lethality were reduced in CIRP-deficient mice. Blockade of CIRP using antisera to CIRP attenuated inflammatory cytokine release and mortality after hemorrhage and sepsis. The activity of extracellular CIRP is mediated through the Toll-like receptor 4 (TLR4)-myeloid differentiation factor 2 (MD2) complex. Surface plasmon resonance analysis indicated that CIRP binds to the TLR4-MD2 complex, as well as to TLR4 and MD2 individually. In particular, human CIRP amino acid residues 106–125 bind to MD2 with high affinity. Thus, CIRP is a damage-associated molecular pattern molecule that promotes inflammatory responses in shock and sepsis.

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Capsule

Genetic identification of a neural circuit that suppresses appetite

Appetite suppression occurs after a meal and in conditions when it is unfavorable to eat, such as during illness or exposure to toxins. A brain region proposed to play a role in appetite suppression is the parabrachial nucleus, a heterogeneous population of neurons surrounding the superior cerebellar peduncle in the brainstem. The parabrachial nucleus is thought to mediate the suppression of appetite induced by the anorectic hormones amylin and cholecystokinin, as well as by lithium chloride and lipopolysaccharide, compounds that mimic the effects of toxic foods and bacterial infections, respectively. Hyperactivity of the parabrachial nucleus is also thought to cause starvation after ablation of orexigenic agouti-related peptide neurons in adult mice. However, the identities of neurons in the parabrachial nucleus that regulate feeding are unknown, as are the functionally relevant downstream projections. Carter et al. identified calcitonin gene-related peptide-expressing neurons in the outer external lateral subdivision of the parabrachial nucleus that project to the laterocapsular division of the central nucleus of the amygdala as forming a functionally important circuit for suppressing appetite. Using genetically encoded anatomic, optogenetic and pharmacogenetic tools, the authors demonstrate that activation of these neurons projecting to the central nucleus of the amygdala suppresses appetite. In contrast, inhibition of these neurons increases food intake in circumstances when mice do not normally eat and prevents starvation in adult mice whose agouti-related peptide neurons are ablated. Taken together, these data demonstrate that this neural circuit from the parabrachial nucleus to the central nucleus of the amygdala mediates appetite suppression in conditions when it is unfavorable to eat. This neural circuit may provide targets for therapeutic intervention to overcome or promote appetite.

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