Short Report

BAG3-related myofibrillar myopathy in a Chinese family


In contrast to the usual slow disease progression in myofibrillar myopathies, patients with Bag3opathy often have a rapidly progressive and more severe phenotype with a worse prognosis. We describe a Chinese patient, born to non-consanguineous parents, who first presented at age 6 with clumsy walking and difficult climbing staircase. With a history of restrictive lung disease previously diagnosed as asthma, she progressed rapidly with proximal myopathy, rigid spine and bilateral tightening of the Achilles tendons requiring surgical elongation. Hypertrophic cardiomyopathy with restrictive physiology was shown by echocardiogram. Moreover, prolonged QT interval was also noted in the patient. Family history was unremarkable yet her father was incidentally found to have prolonged QT interval. Mutation analysis with genomic DNA of the proband showed heterozygous de novo known mutation c.626C>T (p.Pro209Leu) and a germline variation c.772C>T (p.Arg258Trp) in BAG3. Her father was found to be a carrier of c.772C>T. Muscle biopsy findings were suggestive of myofibrillar myopathy on light microscopy and ultrastructural studies. To our knowledge, this is the first Chinese case of Bag3opathy so far reported.

Conflict of interest

The authors declare that there is no financial or personal potential conflict of interest in this work.

Myofibrillar myopathies are a heterogeneous group of neuromuscular disorders characterized by dissolution of myofibrils and intracellular accumulation of disease proteins, which interact with components or with chaperones of the Z-disk (1). The group of disorders show common morphological features under microscopy, yet the clinical manifestations and the molecular basis of the various subtypes can vary. Bag3opathy represents an extremely rare subgroup of myofibrillar myopathies caused by mutations in BAG3 [B-cell CLL/lymphoma 2 (BCL2)] associated athanogene-3] gene in an autosomal dominant manner (2, 3). The BAG3 gene product, expressed in skeletal and cardiac muscles and colocalizing with Z-discs, is a cyto-protective protein that regulates molecular chaperones. Although the gene was first cloned in 1999 (4), the function of the gene has not yet been fully elucidated and the disease mechanism is not completely understood (5). Animal studies showed that BAG3-deficient mice developed a fulminant myopathy characterized by myofibrillar degeneration with apoptotic features, suggesting a role for the co-chaperone gene product in the maintenance of myotube and thus mature skeletal muscle survival (6). The deficiency in human is characterized by rapidly progressive limb and axial muscle weakness, cardiomyopathy and respiratory
insufficiency. So far, seven patients from six families had been reported over the world since 2009 (2, 3), most with a young age onset, among which none of them was of Chinese ethnicity. All reported clinical cases of Bag3opathy shared a common pathogenic missense mutation c.626C>T (p.Pro209Leu) in BAG3 gene and a similar rapidly progressive clinical course. Bag3opathy is thus known to be a more severe subtype of myofibrillar myopathies with a younger age onset when compared to other subtypes caused by defects in CRYAB, DES, MYOT and ZASP (7).

In this report, we describe a Chinese patient with Bag3opathy. The proband was heterozygous for the known pathogenic c.626C>T (p.Pro209Leu) de novo mutation together with another missense variant inherited from the father. The proband had typical clinical features of Bag3opathy similar to those described in previous reports in addition to a prolonged QT interval (QTc). Harboring only the missense variation, her father did not have the classical neuromuscular manifestation of Bag3opathy apart from a prolonged QTc.

Materials and methods

The female proband was born to non-consanguineous parents of Chinese ethnicity with unremarkable antenatal, perinatal and developmental history. Her father had adult-onset deafness of the right ear of unknown cause, but otherwise there was no neuromuscular problem in the family history. The girl had symptoms suggestive of asthma in early childhood but was later confirmed to have mild restrictive lung disease on lung function test. Since the age of 6 she had been noted to have progressive clumsy walking and easy falling during exercise by the mother. At 8 years of age, she was found to have mild proximal muscle weakness of both legs and later both arms, both of Medical Research Council Grade 4. At the time of writing, her power was relatively static and she remained ambulatory at 12 years of age. Despite the relatively slow progression of her muscle weakness, she developed rapidly progressive contracture of bilateral Achilles tendons and multiple soft tissue contractures of legs which required repeated surgical elongation or soft tissue release between the age of 9 and 12 years (Fig. 1). Her range of spine movement started to decrease at the age of 11 with limited flexion and thoraco-lumbar neuromuscular scoliosis, for which surgical intervention was required. Creatine kinase was moderately elevated up to 991 IU/l (reference interval <154). Nerve conduction study showed decreased motor (tibial and peroneal) amplitudes (0.3/0.7 mV; 0.4/0.5 mV) and latencies (29/33 m/s; 28.7/34.3 m/s). Electromyography showed an increase in duration of the action potential with normal amplitude, suggestive of a neurogenic axonal disease component. With a diagnosis of probable myopathy, cardiac involvement was looked for: prolonged QTc of 0.45–0.57 s and hypertrophic cardiomyopathy with restrictive physiology were shown by electrocardiography and echocardiography, respectively. Muscle and left sural nerve biopsies were performed for pathological examination. She was initially managed as limb-girdle muscular dystrophy or Emery-Dreifuss muscular dystrophy.

The parents were largely asymptomatic without any neuromuscular manifestation. However, on family screening, her father was noted to have a persistently prolonged QTc of 0.45–0.49 s on electrocardiography. Her mother on the other hand had a normal QTc.

Genomic DNA was extracted from peripheral whole blood of the proband and the parents using a QIAamp blood kit (Qiagen, Hilden, Germany). Informed consent was obtained. For the proband, all coding exons with the flanking intronic regions of BAG3, LMNA, CAV3, EMD, CAPN and FKRP gene were amplified [primer sequences and polymerase chain reaction (PCR) conditions available upon request]. PCR products were purified by ExoSAP-IT (Roche Affymetrix, Santa Clara, CA) and sequenced using BigDyeDeoxy™ terminator cycle sequencing reagents (Applied Biosystems). The sequencing fragments were separated by capillary electrophoresis and detected on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). All mutations and unknown variations were confirmed by bidirectional sequencing. The corresponding exons of the parents were sequenced after the identification of mutations and unknown variations in the proband.

Fig. 1. Clinical photos showing the rapidly progressive contracture of right Achilles Tendon, whereas the left side was released 1 year prior to the photo taking. Left: posterior view; right: lateral view.
Pathogenicity prediction for the unreported variation was performed using Polyphen (8) and Sorting Tolerant from Intolerant (SIFT) (9) in silico systems. Sequence alignment among species was performed with CLUSTALW (European Molecular Biology Laboratory, Heidelberg, Germany) (10).

Results

Morphological study

Muscle biopsy showed atrophic fibers, focal myofibrillar disorganization and type 1 fiber predominance. Ultrastructurally, there was sarcoplasmic accumulation of electron-dense granulofilamentous materials (Fig. 2) and myofibrillar degeneration with minicores. An increase in free glycogen content was noted. On left sural nerve biopsy, axonopathy in some large myelinated fibers and occasional giant axons with thin myelin sheaths were observed.

Mutation analysis

By direct sequencing the proband was found to be heterozygous for a missense mutation BAG3 NM_004281.3:c.626C>T (NP_004272.2:p.Pro209Leu), the only known pathogenic mutation so far reported to cause Bag3opathy (2, 3), as well as a non-synonymous variation BAG3 NM_004281.3:c.772C>T (NP_004272.2:p.Arg258Trp). The mother was found to have wild-type sequences for BAG3 and her father was a carrier of c.772C>T (p.Arg258Trp). The known disease-causing mutation c.626C>T (p.Pro209Leu) was not detected in either parent.

The variation c.772C>T (p.Arg258Trp) was located in a highly conserved region of the gene (Fig. 3). Two copies were detected among 286 unaffected ethnic-matched chromosomes, giving an allele frequency of 0.007. In addition, in silico characterization also supported c.772C>T (p.Arg258Trp) to be a pathogenic mutation (Polyphen: position-specific independent counts score difference 2.458, probably damaging; SIFT: score 0: damaging/intolerable amino acid change). Mutation analysis of LMNA, CAV3, EMD, CAPN and FKRP were all negative for the proband.

Discussion

The BAG family is an ubiquitous family of chaperone regulators which are involved in diverse functions ranging from apoptosis to tumorigenesis and distinguished by a common conserved region known as the BAG domain (4, 11). The BAG proteins bind to heat shock protein (Hsp) 70 and regulate the activity of the chaperone complex (4). BAG3 protein is cleaved during apoptosis, while the cleavage is inhibited by different caspase inhibitors (12). Over-expression of BAG3 was observed in malignancies including pancreatic cancer, leukemias and many epithelial carcinomas (13–16). It was shown that over-expression of the gene in tumors promotes survival through the nuclear factor-kappa B pathway and BAG3 gene could be a potential target for anticancer therapies (17). BAG3 is also involved in a number of activities including proteasomal degradation of ubiquitinated proteins (18) and the modulation of human immunodeficiency virus type 1 gene transcription in infected cells (19).

In this report, we describe a new case of BAG3-related myofibrillar myopathy. To our knowledge, this is the first Chinese family reported, which shows that the de novo mutagenesis event of the hotspot c.626C>T (p.Pro209Leu) can recur in different ethnic groups and cases of Bag3opathy should therefore present across the world. In particular, two different heterozygous mutations were identified in this family. The known pathogenic

![Fig. 2. Electron microscopic examination of muscle biopsy showed sarcoplasmic accumulation of electron-dense granulofilamentous material.](image1)

![Fig. 3. ClustalW sequence alignment of BAG3 from different species showed that residue 258 was conserved among species.](image2)
mutation c.626C>T (p.Pro209Leu) was detected in all patients described previously and absent in their unaffected family members (apart from a father harboring mosaicism) (2, 3). Thus we believe that the phenotype of our proband was mainly attributed to the known de novo mutation, which was reported to cause rapidly progressive limb and axial muscle weakness, rigid spine, cardiomyopathy and respiratory insufficiency. The histological findings were characteristic as well. The pathogenicity of c.626C>T (p.Pro209Leu) was also supported by that the residue is situated in a conserved Ile–Pro–Val motif which plays an important role in the interaction between BAG3 and the Hsps (20).

The structural observations of the muscle biopsy of our proband were consistent with the features of myofibrillar myopathy. The subtle differences in light microscopy findings are usually insufficient to distinguish between myofibrillar myopathy subgroups (21). The observations in ultrastructural examination in BAG3-related myofibrillar myopathy, particularly accumulations of electron-dense granulofilamentous material, were similar to those seen in desminopathies and alpha B-crystallinopathies (22). The presence of filamentous bundles with floccular thin filamentous accumulations, characteristics of ZASPopathies, and the presence of filamentous bundles and tubulofilamentous inclusions in the sarcoplasm and nucleus, features in myotilinopathies, were rarely seen in Bag3opathies. Peripheral nerve involvement, featuring axonal neuropathy with giant axons in nerve biopsies, seemed to be characteristic of BAG3-related myofibrillar myopathy. Similar findings were evident in the three cases reported by Odgerel et al. (3).

In this family, the father did not have any neuromuscular manifestation as in classical Bag3opathy patients and only have an asymptomatic yet persistent and significant prolonged QTc. He only carried c.772C>T (p.Arg258Trp), a non-synonymous change in a conserved codon, which was predicted to be an intolerable amino acid change and damaging by in silico analysis with an allele frequency <1%. The non-synonymous change was recently submitted to the NCBI database in May 2010 with the status of an unvalidated variation in the pilot data release for the 1000 Genomes Project, with one copy detected in the batch of 120 chromosomes (allele frequency 0.008). When prolonged QTc is relatively common and can be caused by the additive effects of multiple genetic susceptibilities (23), this variant may contribute to the pathogenesis. It has been proposed that other myofibrillar myopathies like desminopathy could also contribute to prolonged QTc (24). This cardiac involvement caused by a defective BAG3 would actually be grouped under the term of desmin-related cardiomyopathy (25). The function of the involved residue p.Arg258 was, however, largely unpredictable when it was not situated on any known structural domains known to bind the Hsps (26). The resultant effect of a change of the polar basic arginine to a hydrophobic residue was also unknown.

We postulate that the subtype alone does not define the phenotypic severity of the myofibrillar myopathy, which could also be affected by the nature of the genetic mutation; the age onset and clinical course probably should not be used to depict the genes to be studied when the diagnosis of myofibrillar myopathy is evident on microscopy. Our study was limited by the lack of functional study to support the pathogenicity of the new genetic variant. The probable pathogenicity of the genetic variation was based on the genotype–phenotype correlation, the allele frequency, and the in silico characterization of the non-synonymous amino acid change. On the other hand, when the clinical phenotype was mild and consisted of only cardiac involvement, the variant is predicted to cause a slight change of the protein function and highly sophisticated functional studies might be required to characterize this change.

References
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