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A study of *FHL1*, *BAG3*, *MATR3*, *PTRF* and *TCAP* in Australian muscular dystrophy patients

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Abstract

FHL1, *BAG3*, *MATR3* and *PTRF* are recently identified myopathy genes associated with phenotypes that overlap muscular dystrophy. *TCAP* is a rare reported cause of muscular dystrophy not routinely screened in most centres. We hypothesised that these genes may account for patients with undiagnosed forms of muscular dystrophy in Australia. We screened a large cohort of muscular dystrophy patients for abnormalities in *FHL1* ($n = 102$) and *TCAP* ($n = 100$) and selected patients whose clinical features overlapped the phenotypes previously described for *BAG3* ($n = 9$), *MATR3* ($n = 15$) and *PTRF* ($n = 7$). We found one *FHL1* mutation (c.311G>A, p.C104Y) in a boy with rapidly progressive muscle weakness and reducing body myopathy who was initially diagnosed with muscular dystrophy. We identified no pathogenic mutations in *BAG3*, *MATR3*, *PTRF* or *TCAP*. In conclusion, we have excluded these five genes as common causes of muscular dystrophy in Australia. Patients with reducing body myopathy may be initially diagnosed as muscular dystrophy.

Keywords: Muscular dystrophy; Diagnosis; *FHL1*; *BAG3*; *MATR3*; *PTRF*; *TCAP*

1. Introduction

The muscular dystrophies (MDs) are a genetically and phenotypically heterogeneous group of disorders defined by progressive muscle weakness and wasting, genetic aetiology and dystrophic changes on muscle pathology. They are subdivided into the dystrophinopathies, FSHD, myotonic dystrophies, distal dystrophies, congenital (CMD) and limb-girdle muscular dystrophies (LGMDs) based on clinical features. Currently, there are at least 18 genetically defined forms of LGMD identified [1] and distinguishing between the different types can be difficult [2–4]. Diagnosis relies on a combination of clinical information, muscle imaging, protein analysis (immunohistochemistry (IHC) and Western blot) and DNA sequencing. Identifying the primary mutation(s) is essential to provide accurate genetic counselling and aids clinical care. The genetic aetiology of many cases of MD is not known even after extensive investigation [4–7]. One of the likely reasons is that further genetic causes of MD are still to be identified.

There has been a number of muscle disease genes recently described including *FHL1*, *BAG3*, *MATR3* and *PTRF*. For each gene, only a handful of cases have been

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reported to date (especially for *BAG3* and *MATR3*) and as a result, the full spectrum of clinical phenotypes associated with mutation of these genes remains uncertain. The clinical and histological phenotypes described to date overlap with the MDs. We hypothesised that these genes may be responsible for some patients diagnosed with MD who lack a genetic diagnosis. We have screened well-characterised cohorts of CMD and LGMD patients in whom the known genetic causes have been largely excluded [4,8] to investigate whether these genes cause MD, in addition to the myopathies with which they have been associated so far.

Four and a half LIM domain 1 (*FHL1*) is a 32 kDa protein (*FHL1* gene on Xq26.3) that was identified as a cause of myopathy in 2008 [9]. Mutations have been associated with four overlapping clinical phenotypes: reducing body myopathy [9], scapuloperoneal muscular dystrophy [10,11], X-linked myopathy with postural muscle atrophy and generalised hypertrophy [12] and a form of Emery–Dreifuss muscular dystrophy (EDMD) [13]. The age of onset has been highly variable in the families identified to date, ranging from late infancy to adulthood [9], and both sporadic [9] and familial cases have been reported [12]. Early onset disease is usually associated with rapid disease progression. The course in later-onset disease is variable with some patients having relatively rapid progression to quadriplegia and respiratory failure and others showing only very slow decline.

Mutations in *FHL1* have been associated with a range of histological abnormalities on muscle biopsy, including dystrophic features such as increased internal nuclei, increased fibre-size variation, necrotic and regenerating fibres, fibrosis, increased adipose tissue [11,12] and histochemical reactivity for menadione staining [9]. Some patients also have intracytoplasmic inclusions which immunoreact with *FHL1* protein, desmin, ubiquitin, dystrophin, lamp1, Myo-BP-C, GRP78 [9] and phalloidin [11]. *FHL1* protein localises to the sarcomere (I-band/Z-disc) and sarcolemma in skeletal muscle but its precise roles remain uncertain. We considered *FHL1* to be an excellent candidate gene for MD, because the broad range of clinical and histological phenotypes described to date overlaps significantly with LGMD.

Mutations in *BAG3* are a newly identified cause of a severe myofibrillar myopathy (MFM), a genetically heterogeneous form of myopathy characterised by desmin-positive protein inclusions on muscle biopsy [14]. The Bcl-associated athanogene-3 (*BAG3*) protein, encoded by *BAG3* is highly expressed in striated muscle, where it localises to the Z-disc [15]. Only three patients have been reported to date, in a cohort study of 85 unrelated MFM patients and all three had a common dominant *de novo* change (c.626C>T; p.Pro209Leu) [14]. These patients had a severe atypical MFM clinical phenotype, characterised by onset within the first decade, severe, rapidly progressive muscle weakness and rigid spine in 2/3 cases [14]. The *BAG3* knock-out (KO) mouse has a severe fulminant myopathy with use-dependent muscle degeneration and death at day 25 from respiratory insufficiency [15]. There is already overlap between LGMD and MFMs as mutations in MYOT (myotilin) are established causes of both disorders [16]. To date, the main focus of *BAG3* research has been in MFM and we hypothesised that mutations in *BAG3* may cause other phenotypes such as MD.

Vocal cord and pharyngeal weakness with distal myopathy (VCPDM) due to mutations in *MATR3* was identified through two separate linkage studies that found the same mutation (c.C254G p.S85C) in unrelated families from Southern Tennessee and Bulgaria [17,18]. *MATR3* is a 14 exon gene on 5q31 [18] that encodes matrin 3, a highly conserved 130 kDa protein [19] that localises to the nuclear matrix in skeletal muscle [20]. VCPDM is a slowly progressive, autosomal dominant, adult-onset disease, with a distinctive clinical pattern of distal limb and bulbar weakness. Clinical presentation includes foot drop, hand weakness, swallowing difficulties, vocal cord changes and mild-moderate elevation in CK (maximum eight times normal) [17,18]. As the disease progresses, muscle involvement can become more asymmetric, and the limb girdle muscles are involved. Histopathological changes in patient muscle biopsies included rimmed vacuoles, variation in fibre size and fibre splitting. While the clinical phenotype described to date is distinctive, as the disease advances, symptoms overlap with MD and we hypothesised that *MATR3* may account for a broader spectrum of clinical and histological phenotypes that overlaps MD.

Recessive mutations in *PTRF* (17q21.2, also known as Cavin-1) have been associated with MD with generalised lipodystrophy in Japanese [21], Omani [22] and UK families [22]. A range of other clinical features have been associated, such as muscle hypertrophy, muscle mounding, metabolic complications, cardiac involvement, spinal involvement and contractures, and creatine kinase levels have ranged between 500 and 2650 μ/l [21,22]. Muscle biopsies from affected patients demonstrate chronic dystrophic changes, mislocalisation of caveolin 1, 2 and 3, [21] and reduced caveolae [21,22]. *PTRF* immunolocalises to the sarcolemmal membrane in normal muscle [21]. Mutations have been associated with absent or barely detectable *PTRF* staining [21,22] together with greatly reduced sarcolemmal and increased cytoplasmic caveolin-3 staining [21].

The *TCAP* gene was first identified as a cause of LGMD2G a decade ago, but to date mutations have only been reported in four Brazilian families, and one European case [23,24]. *TCAP* (named after titin-cap) has two exons that encode a 19 kDa protein called telethonin [25]. *TCAP* is exclusively expressed in striated muscle where it localises to the Z-disc of the sarcomere [26]. The only study to look at disease frequency estimated the incidence of LGMD2G to be 2.2% in the Brazilian general LGMD population [27]. LGMD2G is a variable autosomal recessive disease that is sometimes associated with atrophy of distal muscles or calf hypertrophy [27]. All mutations identified to date have been frameshift or nonsense mutations that are associated with absence of telethonin immunostaining in patient