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Case report

Mutations in *TPM2* and congenital fibre type disproportion

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Abstract

The main diagnostic feature of congenital fibre type disproportion is that type 1 fibres are consistently smaller than type 2 fibres in the absence of other histological abnormalities. Mutations in the *TPM3*, *RYR1* and *ACTA1* genes are the most common established genetic causes. There has been one previous report of congenital fibre type disproportion due to a mutation in *TPM2*, although some atypical histological features were present. We present two cases in which novel *de novo* missense mutations in *TPM2* are associated with marked fibre size disproportion. The finding of typical histological changes of congenital fibre type disproportion in association with a p.Ser61Pro mutation confirms that *TPM2* can cause typical congenital fibre type disproportion. Although not seen on light microscopy studies, protein inclusions typical of small 'caps' were found on electron microscopy in a second patient with a p.Ala155Val mutation in *TPM2*. This case emphasises the importance of electron microscopy in patients with presumed congenital fibre type disproportion, to exclude the presence of caps, nemaline bodies or minicores, which, if present, may be very helpful in guiding genetic analysis.

Keywords: Congenital myopathy, Beta-tropomyosin; Cap myopathy; Congenital fibre type disproportion; Electron microscopy

1. Introduction

Congenital fibre type disproportion (CFTD) is an uncommon form of congenital myopathy in which consistently small type 1 (slow twitch) muscle fibres compared to type 2 (fast twitch) fibres, is the main histological abnormality [1]. When a threshold for fibre size disproportion of 35–40% is used, and other forms of congenital myopathy and neuromuscular disease are excluded, a genetic cause can be established in around half of CFTD patients [2]. The most common reported genetic causes are *TPM3* [3], *RYR1* [4] and *ACTA1* [5], and single families have been reported with mutations in *MYH7* [6] and *TPM2* [7]. Establishing the genetic basis of disease in each family is essential to provide accurate genetic counselling and prenatal diagnosis but the number of possible genetic causes (some of which likely remain undiscovered) complicates this process.

The *TPM2* gene codes for beta-tropomyosin, one of three tropomyosin isoforms that are incorporated into sarcomeres in skeletal muscle. Mutations in *TPM2* have been associated with two main phenotypes; congenital

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myopathies (nemaline myopathy, cap myopathy and CFTD) in which there is relatively stable, generalised muscle weakness from early childhood, and arthrogryposis phenotypes (distal arthrogryposis types 1 and 2A, Escobar syndrome) in which congenital contractures are the main clinical abnormality (reviewed in [8]) [7,9]. In the previous report of CFTD due to a *TPM2* mutation (Glu117Leu), some atypical histological features were present [7]. Many type 1 fibres were approaching the size of type 2 and on average, type 1 fibres were 30% smaller than type 2 fibres (by our measurements), which is below the 35–40% threshold that has been proposed for typical CFTD [10]. Nevertheless, *TPM2* is a good candidate for causing typical CFTD since a patient with cap myopathy has been described who had marked fibre size disproportion as a secondary feature [11]. In this report, we describe two patients with novel *TPM2* mutations, one who had typical histological features of CFTD, and one with marked fibre size disproportion who had cap structures only visible on electron microscopy (EM). These cases confirm that mutations in *TPM2* can cause typical CFTD and emphasise the importance of EM in the investigation of patients with suspected CFTD.

2. Methods

2.1. Genetic analysis

We sequenced the coding regions of the *TPM2* gene using methods previously described [11], from genomic DNA extracted from peripheral blood leucocytes.

3. Results

3.1. Case reports

3.1.1. Patient 1

This boy was born to a non-consanguineous couple with a family history of benign macrocephaly but no neuromuscular disease. The pregnancy was uncomplicated and he was delivered by elective Caesarean section at term. He was well in the neonatal period, except for mild laryngomalacia, and he first presented with generalised hypotonia at age 6 weeks. His development has been normal except for delayed gross motor milestones; he walked at age 23 months. At his current age of 3 years he can run, jump and climb. He has mild facial weakness, a tendency to drool, mild proximal limb weakness, and cardiac and respiratory assessments have been normal. Creatine kinase levels and nerve conduction studies were normal at age 10 weeks.

In a vastus lateralis muscle biopsy taken at age 8 months, a consistent difference in type 1 and type 2 muscle fibres size was the main abnormality on analysis of standard histological stains (Fig. 1A). On average, type 2 fibres were 54% larger in diameter than type 1 fibres. Type 1 fibres were consistently smaller than normal (mean diameter 10.6 μm; normal 15 μm), while type 2 fibres were hypertrophied (mean diameter 23.1 μm; normal 15 μm). In most fascicles type 1 fibres were predominant (~75% of fibres) but there were normal fibre-type ratios or type 2 fibre predominance in some. Around 7% of fibres were hybrid type 1/2A fibres (also called type 2C fibres). There were no protein inclusions, cores, degenerating or regenerating fibres or increase in fibrosis or internal nuclei. On EM no protein inclusions or definite structural abnormalities were seen and a clinicopathological diagnosis of CFTD was made.

3.1.2. Patient 2

This boy, who is currently 20 months old, was born to non-consanguineous Caucasian parents who had no previous family history of muscle weakness. Reduced fetal movements were noticed in the final two weeks of pregnancy and he was delivered at term by Caesarean section for failure to progress. His Apgar scores were 4, 6 and 6 at 1, 5 and 10 min respectively, and he required resuscitation for respiratory insufficiency. In the neonatal period he had severe hypotonia, a frog-like posture, generalised weakness with some anti-gravity movements, mild ptosis, a weak cry and a poor suck. He required nasal continuous positive airway pressure (CPAP) ventilation for 3 weeks and tube feeds. He slowly developed head control, began to roll over at the age of 12 months, and sat from age 15 months. At age 18 months he required treatment in intensive care for respiratory insufficiency associated with a respiratory infection and he has required nocturnal ventilatory support since then. Cardiac investigations have been normal.

In a quadriceps femoris muscle biopsy taken at age 3 months, type 1 fibre were predominant (70% of fibres) and consistently small (mean diameter 8.6 μm; normal 15 μm). Type 2 fibres were hypertrophied (mean diameter 21.3 μm; normal 15 μm), so that type 2 fibres were on average 59% larger than type 1 fibres (Fig. 1B). Four percent of fibres were type 2C fibres. On light microscopy, there were no dystrophic changes, increased internal nuclei, cores, minicores, nemaline rods, caps or protein aggregates. On EM, several fibres had subsarcolemmal areas comprised of disorganised thin-filament structures with remnant thickened Z-bands that overall resembled cap-like structures (Fig. 1C). A preliminary diagnosis of CFTD, made on light microscopy studies, was revised to cap myopathy on the basis of EM findings.

3.2. Genetic sequencing results

We identified novel heterozygous missense changes in the *TPM2* gene in both Patient 1 (c.181T>C; p.Ser61Pro) and in Patient 2 (c. 464C>T, p.Ala155Val). These changes were not found in parental genomic DNA samples, consistent with *de novo* occurrence of these *TPM2* variants in both probands. The changes were not present in the 1000 Genomes dataset (www.1000genomes.org, accessed March 2012) nor in the Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA (URL: http://