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Frequency and phenotype of patients carrying *TPM2* and *TPM3* gene mutations in a cohort of 94 patients with congenital myopathy

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Abstract

Congenital myopathies are difficult to classify correctly through molecular testing due to the size and heterogeneity of the genes involved. Therefore, the prevalence of the various genetic causes of congenital myopathies is largely unknown. In our cohort of 94 patients with congenital myopathy, two related female patients and two sporadic, male patients were found to carry mutations in the tropomyosin 2 (*TPM2*) and tropomyosin 3 (*TPM3*) genes, respectively. This indicates a low (4.3%) frequency of *TPM2* and *TPM3* mutations as a cause of congenital myopathy. Compared to previously described patients carrying the same mutations as found in our study (c.503G > A, and c.502C > T in *TPM3*, and c.415_417delGAG in *TPM2*), clinical presentation and muscle morphological findings differed in our patients. Differences included variation in distribution of muscle weakness, presence of scoliosis and ptosis, physical performance and joint contractures. The variation in clinical profiles emphasizes the phenotypic heterogeneity. However, common features were also present, such as onset of symptoms in infancy or childhood, musculoskeletal deformities and normal or low plasma levels of creatine kinase.

One patient had nemaline myopathy and fiber size disproportion, while three patients had congenital fiber type disproportion (CFTD) on muscle biopsies. *TPM2*-related CFTD has only been described in two cases, indicating that mutations in *TPM2* are rare causes of CFTD.

Keywords: *TPM2*; *TPM3*; Congenital myopathy; Congenital fiber type disproportion; Nemaline myopathy

1. Introduction

Congenital myopathy (CM) is a collective term for a heterogeneous class of muscle diseases. They vary clinically, histologically and genetically, but share a number of common features such as early onset of symptoms, slow disease progression, occurrence of musculoskeletal abnormalities, low or normal levels of plasma creatine kinase (CK) and characteristic morphological features on muscle biopsy that originally helped to classify the conditions [1,2].

CMs have been difficult to classify correctly through molecular testing, because of the size and heterogeneity of the genes involved. As a result, the prevalence of the various genetic causes of congenital myopathies is largely unknown [1,3].

Mutations in the tropomyosin (*TPM*) genes are uncommon causes of CMs. The *TPM* genes *TPM2* and *TPM3* are predominantly expressed in human skeletal

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muscle, where both genes are expressed in slow (type I) fibers, while *TPM2* is also found, to a lesser extent, in fast (type II) muscle fibers [4,5]. Mutations in *TPM2* and *TPM3* have previously been associated with nemaline myopathy [1], congenital fiber type disproportion (CFTD) [6,7] and cap disease [5,8].

Mutations in these genes are generally uncommon. A total of 14 mutations in *TPM2* and 16 in *TPM3* have been associated with various types of CM [9]. However, generally the mutations were not described in a larger setting in which the frequency of *TPM* gene mutations as cause of CM could be ascertained. The purpose of this investigation was therefore to determine the frequency of *TPM2* and *TPM3* mutations in a cohort of 94 patients with CM in Denmark, and to describe the clinical and laboratory findings in patients identified with these gene mutations.

2. Case report

All with a diagnosis of congenital myopathy and 5 years or older at referral to us, were invited to participate in the study. This included also patients with onset of symptoms before the age of 5 years. A 5-year limit was chosen owing to validity limitations of the functional tests, which were carried out. This included: manual muscle test (Medical Research Council scale 0–5) [10], motor function measure (MFM) [11] and EK scale [12]. 107 patients, suspected of having congenital myopathy, were originally included in the study, but 13 were excluded as they did not fulfil diagnostic criteria for a congenital myopathy based on clinical features and muscle histology. Thus, subjects with a phenotype deemed incompatible with a diagnosis of congenital myopathy based on adult onset, fast progression, CK more than 500 or dystrophic features on muscle biopsy as a main morphological finding, were excluded. In addition, one subject with a longstanding severe hypothyroidism was also excluded, because it could interfere with the interpretation of the myopathy.

The 94 subjects who fulfilled the inclusion criteria for a congenital myopathy came from 75 families. Inclusion criteria included (1) slow progression, (2) CK below 500, (3) at least one musculoskeletal abnormality, (4) onset before age 20, and (5) a muscle biopsy showing either characteristic changes or at least myopathic, but not dystrophic features. The participants were divided into groups according to the most specific muscle histology in the family. Cores, rods and centronuclear (CNK) findings were considered the main pathologies, and fiber type disproportion (FSD), type I dominance or myopathic changes with fiber size variation and/or central nuclei less specific. Twelve families (16%) had unspecific myopathic biopsies, 24 families (32%) had type I fiber dominance or CFTD histology, 9 families (12%) had rod pathology, 11 had cores (14.7%), 14 CNK (18.7%), 3 families (4%) both rods and cores. In the remaining 2 families (2.7%), the pathology report was unavailable, but the referring clinicians described the biopsies as myopathic.

Amongst the 94 included patients, 10 had a confirmed genetic diagnosis with mutations in *ACTA1*, *RYR1*, *DNM2* and *NEB* genes on entry into the study. Therefore, 84 patients were genetically unclassified, in whom genetic analysis for *TPM2* and *TPM3* mutations were performed in all. The genetic analysis of the large cohort is in progress. For the larger genes, including *NEB*, a next generation sequencing approach is currently in use to identify the genetic profile of our cohort. However, this analysis will yet continue in several months before the final results can be reported.

2.1. Mutation analysis

DNA was isolated from a 6 ml EDTA blood sample using a standard desalting procedure.

All coding exons of *TPM2* and *TPM3*, including flanking intronic sequences, were PCR amplified and sequenced directly using standard techniques. Identified mutations were confirmed in a new PCR and sequencing reaction.

Three mutations were identified. Patients 1 and 2 were heterozygous for c.502C > T; p.R168C and c.503G > A; p.R168H in *TPM3*, respectively, whereas patient 3 was heterozygous for c.415_417delGAG; p.E139del in *TPM2*. All three mutations have previously been associated with CM [5,6,8,13–19]. Parental analysis documented that the three mutations were *de novo* in all probands. Patient 3 had passed the *TPM2* mutation onto her child (patient 4). Thus, 3 of the 75 families studied carried *TPM* mutations (4%).

2.2. Clinical presentation

Main clinical signs and symptoms in the four affected patients are summarized in Table 1.

Common symptoms and clinical findings included a moderately reduced pulmonary vital capacity, musculoskeletal abnormalities such as elongated face and high-arched palate (Fig. 1), slim build, early onset of disease with hypotonia and delayed motor milestones, no overt progression of disease, and in some cases even clear improvement in childhood, normal or low CKs, and a preferentially diffuse, moderate weakness of limbs and trunk. Generally, all four patients were characterized with joint hypermobility, but mild Achilles tendon contractures were noted in patients 2 and 4.

2.3. Heart examination and MRI

All patients had a cardiac evaluation including electrocardiogram (ECG), echocardiography and 48-h Holter-monitoring. Patient 3 had short runs of non-sustained ventricular tachycardia on Holter-monitoring, but the patient was asymptomatic and ECG and