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CASE REPORTS

Mild myopathic phenotype in a patient with homozygous c.416C > T mutation in *TK2* gene

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The mitochondrial DNA depletion syndrome (MDDS) is characterized by extensive phenotypic variability and is due to nuclear gene mutations resulting in reduced mtDNA copy number. Thymidine kinase 2 (TK2) mutations are well known to be associated with MDDS. Few severely affected cases carrying the c.416C > T mutation in *TK2* gene have been described so far. We describe the case of a 14months boy with the aforementioned *TK2* gene pathogenic mutation at a homozygous state, presenting with a mild clinical phenotype. In addition to severe mitochondrial pathology on muscle biopsy, there was also histochemical evidence of adenylate deaminase deficiency. Overall, this report serves to further expand the clinical spectrum of *TK2* mutations associated with MDDS.

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Conflict of interest
The Authors declare no conflict of interest

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Introduction

Mitochondrial diseases are clinically and genetically heterogenous disorders due to respiratory chain disturbance with subsequent failure of aerobic metabolism, leading to dysfunction of multiple organs, especially the highly energy dependent. The pathogenic mutations may be either in the mitochondrial DNA (mtDNA) or in the nuclear DNA (nDNA), which encode mitochondrial proteins [1]. Mitochondria are essentially the source of high-energy intermediates and are considered as the lever of cellular function. To fulfill their role, they have to constantly fuse and divide, processes that are quite complex and dependent on mtDNA replisome enzymes, nucleotides supply and balanced mitochondrial dynamics. Defects in mtDNA replication may be due to any of the aforementioned stage, resulting in usually multisystemic disorders [1,2].

Mitochondrial thymidine kinase (TK2) is the catalytic enzyme for the phosphorylation reaction of pyrimidine deoxyribonucleosides, which is the first step in the conversion of deoxynucleosides into deoxyribonucleotide triphosphates (dNTPs) (2). Therefore, *TK2*-mutations are responsible for disorders of mtDNA nucleotides supply encompassing a broad phenotypic spectrum, with myopathy and progressive external ophthalmoplegia

(PEO) being some of the most prominent clinical characteristics [2]. The clinical variability of mtDNA depletion syndrome due to *TK2*-mutations may be partly related to the different extent of enzymatic activity, which is determined by the mutant gene [3].

Herein, we describe a now 4-year-old boy, diagnosed at 14 months of age, with a mitochondrial DNA depletion syndrome (MDDS) harboring a *TK2* gene pathogenic mutation at a homozygous state with a milder phenotype than previously reported.

Case report

The index patient, a boy aged 14-months on his first admission, was firstly investigated in the pediatric department for persistently high creatine phosphokinase (CK) serum levels and mild transaminasemia, which were initially randomly detected. CK values (normal range 0-180 U/L) fluctuated from 700 U/L up to a maximum of 17.870 U/L, without any evidence of myoglobinuria.

The patient is the second child, born at term, to a healthy, non-consanguineous couple. His birth weight was 3.008 kg and he had a normal perinatal period and psychomotor development. His 4-yr old brother had an unremarkable medical history with just milk allergy. None of the patient's relatives had increased CK and there was no family history for any neuromuscular disease. Clinical examination, including muscle strength, muscle tone, tendon reflexes, cranial nerves and coordination, was normal. Although, the infant could sit and stand unsupported, he had not yet at that time achieved independent walking. With the exception of muscle enzymes, other laboratory tests, including virological markers and immunological parameters, were normal. Heart evaluation with ECG and echocardiogram did not reveal any abnormalities. Brain MRI was also normal. Genetic testing for deletions and duplications in the dystrophin gene was also normal.

A vastus lateralis muscle biopsy revealed myopathic changes with abnormal variation of muscle fiber diameter and multiple cytochromoxidase-negative (COX-negative) muscle fibers with few ragged red (trichrome Gomori staining) and/or ragged blue (succinate dehydrogenase staining), confirming the diagnosis of mitochondrial myopathy (Fig. 1). Histochemistry showed also myoadenylate deaminase deficiency (Fig. 2). Biochemical analysis of the muscle biopsy revealed a combined deficiency involving complexes 1, III and IV, pointing either to a mtDNA mutation or a defect in the mitochondrial protein synthesis due to a nuclear gene mutation.

Genomic DNA from muscle biopsy revealed an already known pathogenic mutation in the *TK2* gene in homozygosity (NM_004614.5: c.416C > T, p.Ala139Val) [4,5], which was further confirmed by Sangers analysis. Both parents were also tested and were found to be carriers of the same mutation.

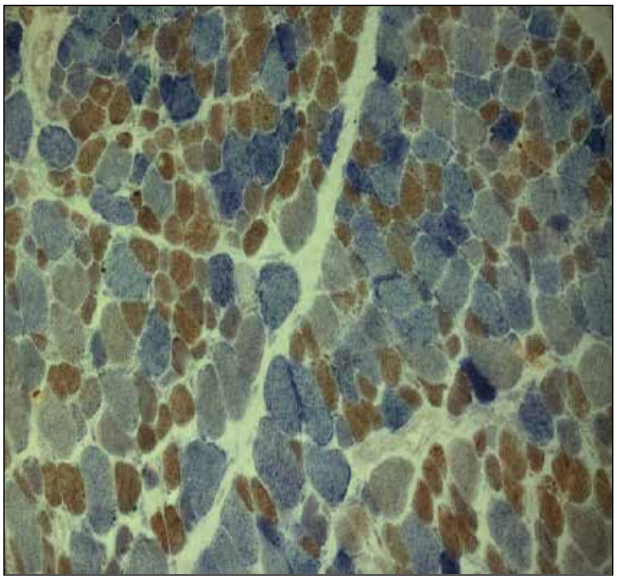

Figure 1. Transverse muscle biopsy section double stained with cytochromoxidase (COX) plus succinate dehydrogenase (SDH) – (COX-SDH x20) showing multiple COX-negative and SDH-positive fibers in blue.

Further clinical examination at a later stage showed delayed motor milestones with walking at 3 years of age. Today, at the age of 4 years, the index patient has difficulties in walking, climbing stairs and rising up from the floor. He has also a symmetrical muscle weakness of upper and lower legs (3-4 according to MRC scale in most muscle groups).

The patient's legal representative has signed written informed consent for the data to be published in a scientific journal. There was no need for ethical approval from an ethics committee, as any diagnostic step was within the routine management of such patients.

Discussion

This is a report of a toddler who presented with incidentally detected constantly high serum CK levels and was finally diagnosed with MDDS due to *TK2* mutations.

The mtDNA maintenance defects, involve, basically, either the most severe mtDNA depletion syndrome (MDDS) or the usually milder multiple mtDNA deletion syndrome. MDDS is an inherited autosomal recessive disease, caused by a reduction of mtDNA copy number and as such, it may be considered as a quantitative disturbance, with the phenotypic severity being related to the remaining normal mtDNA levels [3,6]. The clinical spectrum of the syndrome is quite diverse with variable symptoms,

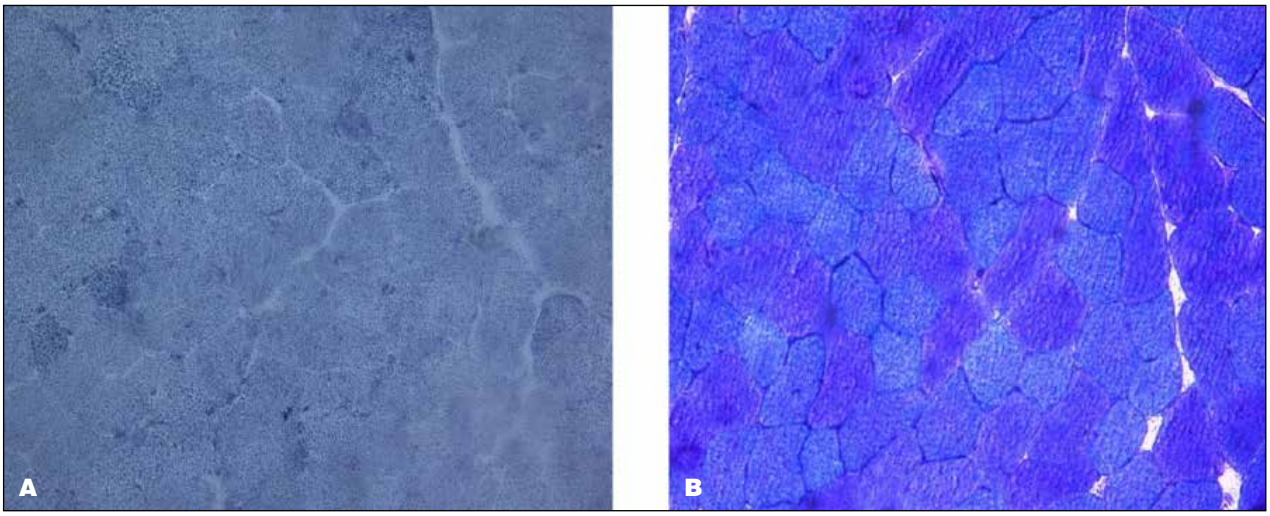

Figure 2. Myoadenylate deaminase (AMPDA x40) staining from the patient showing AMPDA deficiency (A) and from a healthy control, with type 1 muscle fibers more intensely stained than type 2 (B).

mainly related to mutations in certain genes. MDDS can be recognized by the following prevailing phenotypes: a myopathic, an encephalomyopathic, a hepatocerebral and a neurogastrointestinal [7].

A predominantly myopathic form, associated with high CK and onset in infancy or early childhood, has been related with *TK2* mutations [7]. Typically, the children develop normally over the first months after birth, but they later lose some acquired motor milestones, usually before the second year of life. The most common presentations are hypotonia, exercise intolerance and especially proximal muscle weakness, while there may occasionally be some bulbar involvement with facial weakness, dysarthria and dysphagia [7,8]. Respiratory difficulties are also listed high among the most frequently symptoms of the disease [8]. Noteworthily, the ongoing recognition of new manifestations has expanded the phenotypic spectrum of *TK2*-related MDDS. More specifically, there may be a broader age range of the first symptoms with even a milder adult onset presentation or a multi-organ involvement, including the brain with cognitive decline, epilepsy, cerebral atrophy, cerebellar degeneration and diffuse white matter changes, the heart with cardiomyopathy, the liver with hepatomegaly etc. [8,9]. Although, the condition has no approved treatment so far, there is robust evidence that deoxynucleoside monophosphates and deoxynucleoside administration may improve the clinical outcome in both early and late onset patients and potentially mdify the natural course of the disease [10].

The patient of the present study is now a 4-yr old boy with a, so far, relatively mild pure myopathic phenotype. Among the initial diagnostic work-up tests, was the screening for deletions and duplications of the *dystrophin* gene by MLPA. After the negative results, it was not considered necessary, at that time, to perform sequencing of the gene, mainly due to a low clinical suspicion for a possible dystrophinopathy. The next diagnostic step was muscle biopsy, which revealed mitochondrial pathology with numerous COX-negative muscle fibers, few ragged red/blue fibers and myopathic changes mainly consisted of increased fiber size variation. The above-mentioned histological abnormalities, although not specific for any particular mitochondrial mutation, are amongst the most frequent features of *TK2*-related MDDS [11]. Genetic studies revealed the already described pathogenic mutation c.416C > T, p.Ala139Val mutation in homozygosity, in the *TK2* gene.

This mutation has been firstly described in homozygosity in two Greek siblings with normal early developmental milestones. The older brother run a progressively declining course after the very first years of life, rendered him, at age 5 years, wheelchair-bound with severe cognitive dysfunction, while her younger sister followed a rapid deterioration after the age of 2 years with hypotonia and inability to stand, six months later [5]. The same mutation has been already reported in a compound heterozygosity in two affected brothers with a very severe clinical phenotype. Both probands were born without any sign or symptom of a possible underlying neuromuscular disorder, but after a few months of normal early development, they showed a rapid psychomotor regression and severe respiratory failure. The older brother suffered also from aggravating epileptic seizures and died at the age of 12 years after being tracheostomized for many years [4]. In a more recent paper, there is a similar description of a young girl carrying this mutation in compound heterozygosity, who presented with

motor skill regression at the age of 13 months and died at the age of 22 months after a rapid deteriorating course [9]. Contrary to the poor outcome of the disease in all those affected children, the patient of the present study has run a more benign clinical course, so far. This discrepancy may reflect a lack of genotype-phenotype correlation, suggesting that a possible contribution of other epigenetic or environmental factors may determine the course of the disease. In any case, the existing literature based on few case reports does not permit to draw general conclusions on the underlying genetic mechanisms.

Moreover, an additional histological finding in the muscle biopsy of our patient was the absence of myoadenylate deaminase (AMPDA) activity. This is a quite common metabolic disturbance, estimated in approximately 2% of the population and can be coexist with other neuromuscular disorders. Exertional myalgia is the most frequently observed symptom, although most individuals are asymptomatic. The disorder was not genetically investigated in our patient, as it was considered coincidental, although it is difficult to determine whether AMPDA deficiency may partially contribute to the clinical picture, when combined with another metabolic disease. However, it has been shown that even a near total AMPDA deficiency does not influence exercise capacity and cellular energy charge, bringing into question a potential association to an add-on effect [12].

In conclusion, we present a paediatric patient with MDDS due to a homozygous pathogenic mutation in *TK2* gene, which has been already described in a homozygous or heterozygous state, in few severely affected patients. Interestingly, in addition to mitochondrial pathology, muscle biopsy also revealed AMPDA deficiency, which is a frequent histochemical abnormality and occasionally coexist with other metabolic disorders. We thereby expand the clinical and mutational spectrum of the *TK2*-related MDDS, although more cases are needed to investigate genotypic correlations and whether other modifiable factors may contribute to phenotypic variations and differing outcomes.

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