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Case Report

Megaconial Congenital Muscular Dystrophy Due to a Mutation in CHKB Gene: Two Cases in China

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Abstract

Megaconial congenital muscular dystrophy (MCMD) is a rare autosomal recessive disorder characterized by muscle weakness and intellectual disability attributed to CHKB mutations, which result in mitochondrial structural and functional abnormalities. We report two Chinese patients with Megaconial CMD. Patient 1 presented limb weakness and retardant intelligence from childhood. He was attacked by a cerebral embolism and was found to have dilated heart disease when he was 35 years old. A homozygous nonsense mutation (c.940C>T, p.Arg314Cys) was identified in CHKB gene. Light microscopic (LM) and electron microscopic (EM) examination on muscle fibres showed enlarged and peripherally displaced mitochondria. Patient 2 was a 15-month-old boy when he visited. He was found to be floppy at birth. He has limb weakness and show delay motor development as he grows. Muscle MR showed sparse muscle and fatty deposits in muscle in bilateral upper arms, bilateral thighs. A muscle biopsy showed basophilic particles gathered around muscle fibres. Histochemical staining for oxidative enzymes (SDH and COX) showed enhanced staining of large particles. Electron microscopy showed the fibres presented mitochondria enlarged in length and diameter. A c.598del (p.Gln200Argfs*11) homozygous mutation in CHKB gene was found. His parents are heterozygous carrier at this locus. This is the first report of Han Chinese patients with Megaconial CMD in China Mainland, and a new mutation was found.

Keywords: Megaconial Congenital Muscular Dystrophy; Choline Kinase Beta; Enlarged Mitochondria

Abbreviations: MDCMC: Muscular Dystrophy, Congenital, Megaconial Type; CHKB: Choline Kinase Beta; LM: Light Microscopic; EM: Electron Microscopic; CK: Creatine Kinase; HGMD: The Human Gene Mutation Database; MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes; ESP: Exome Sequencing Project; NADH-TR: Nicotinamide Adenine Dinucleotide Tetrazolium Reductase; ATP: Adenosine Triphosphate; SDH: Succinate Dehydrogenase; COX: Cytochrome Oxidase

Introduction

Megaconial congenital muscular dystrophy (CMD) is an autosomal recessive disorder characterized by muscle weakness and intellectual disability. Since the first case of a young boy presenting proximal muscle weakness with an abnormal distribution of giant mitochondria in type II muscle fibers was reported in 1981, several cases of similar muscle pathological findings have been disclosed [1,2]. In 2006, a spontaneous mutant mouse with a neonatal onset of autosomal recessive muscular dystrophy (rmd) due to loss-of-function mutation in choline kinase β (Chkb) [3], interestingly had the same pathological signature in muscle fibers with human victims. In 2011 researchers screened four Japanese patients described in 1998 and 11 other patients from Turkish and British and identified homozygous or compound heterozygous mutations in CHKB in all of the patients [2,4]. So far, forty patients of Megaconial CMD have been reported, most from Turkey and Japan, and some from West Europe, Australia, India and China [5-13], with mutations in the gene encoding choline kinase beta (CHKB) verified in all these cases. Mutations in this gene result in decreased choline kinase activity and declined levels of phosphatidylcholine [3]. Altered phospholipid composition in muscular mitochondrial membrane may lead to impaired mitochondrial structure and function. Clinical phenotypes include hypotonia in the neonatal/infantile period, proximal muscle weakness, muscle atrophy, mental retardation, ichthyosis and seizures, and some patients may have autistic and behavioural problems. Approximately half of patients develop dilated cardiomyopathy, which is the main cause of death. Light microscopic (LM) and electron microscopic (EM) examination on muscle fibers reveal enlarged and peripherally displaced mitochondria, acknowledging the original denomination of the syndrome as "megaconial myopathy [14]." Here we report two cases of Megaconial CMD in China, one with a homozygous CHKB gene mutation, which had never been reported.

Case 1

A 35-year-old Han Chinese male has been admitted to hospital for progressive fatigue in both lower limbs for 32 years. His parents are close relatives and the kinship relationship is shown in Figure 1. He only started speaking when he was 3 years old, and his reaction and comprehension were poorer than his peers. He was also found his progressive weakness in both lower limbs and difficulty to jump at the same period. Muscle atrophy in his lower back and buttocks has been noticed when he was 10 years old. Two months ago, he was hospitalized in a local hospital with "cerebral embolism" and then the dilated cardiomyopathy has been diagnosed. Physical examination revealed disability in comprehension, memory, computational power and orientation. Other discoveries included unclear speech and duck steps, which is also called waddling gait, due to amyotrophy in the trunk and limbs, with muscle strength of grade 4 either proximally or distally, and scattered fish scale rash all through the neck and back (Figure 1). Electromyography showed myogenic damage. Cardiac ultrasound suggested enlargement of the left atrial and ventricle with generally reduced wall motion, consistent with previous diagnosis of dilated cardiomyopathy. Laboratory results indicated elevation of creatine kinase(CK) as 973U/L. Gene detection: no mutations in MELAS gene have been detected in hot spots including m3243A, m3250T, m3252A, and m3271T sites, neither any known pathogenic mutations have been detected in the DMD gene assay, SMN1 gene or DMPK gene. Homozygous missense mutations in CHKB gene: c.940C>T, p.Arg314Cys, which had not been reported before, has been disclosed by the next generation targeted sequencing while a c.940C>T heterozygous mutation has been discovered in his father. His mother cannot be detected the gene because she passed away. Muscle biopsy was carried on to find some atrophied muscle fibers, and most of the type 2 muscle fibers and some type 1 fibers were stained with abnormal granules. Some muscle fibers have been lightly stained in the centre with NADH staining, with a large amount of aggregation of basophilic particles around with MGT staining (Figure 2). Electron microscopy showed accumulation of significantly expanded mitochondria beneath the membrane of muscle fibers (Figure 2).

Case 2

Case 2 was a 15-month-old boy born from non-consanguineous Chinese parents without relevant family history after a normal pregnancy and delivery. Since the birth, he was found that his limbs were floppy, and he could not raise his head at 2 months of age. He could sit but unstable with the assistance at 10 months of age. At the age of 40 weeks, he was tested on the Gesell scale to assess his developmental function and found that his development was completely backward (Table 1). On examination at 15 months of age, he could neither stand up independently nor raise his head when lying prone. He had normal serum creatine kinase level of 190U/L. Serum somatotropin, organic acid and serum carnitine metabolism combination, blood coagulation function, electrolytes, thyroid function, liver and kidney function, and 25-hydroxyvitamin D were normal. Head MRI, conventional electroencephalogram, spine/extremities X-ray, and chromosome

examination showed no abnormalities. Muscle MRI showed sparse muscle and fatty deposits in bilateral forearms, bilateral upper arms, bilateral thighs, and the indicated bilateral hip muscle groups (Figure 4). A muscle biopsy gastrocnemius muscle showed increased muscle fiber size variability. Modified Gomori trichrome (mGT) staining showed that basophilic particles gathered around muscle fibers. Histochemical staining for oxidative enzymes (SDH and COX) showed enhanced staining of large particles, and a few COX- negative and SDH-negative fibers were also detected. Electron microscopy showed that the fibers presented mitochondria enlarged in length and diameter with "megaconial" appearance (Figure 3). Then whole-exome sequencing revealed a homozygous mutation, c.598del (p.Gln200fs) in CHKB gene, which has previously been reported before. Both parents are heterozygous mutations at this locus.

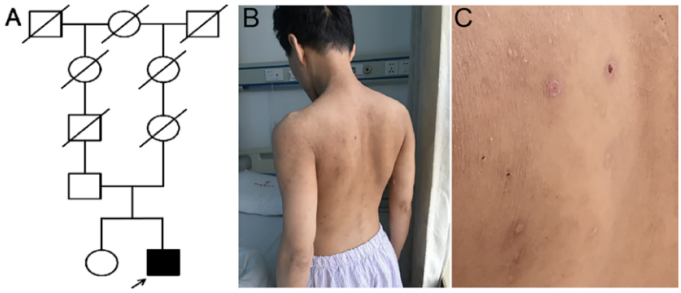

Figure 1: A. The family diagram of the patient 1. The proband's parents are close relatives. B.C. Ichthyosis of the patient in case 1. The patient was covered with ichthyosis all over his neck and back.

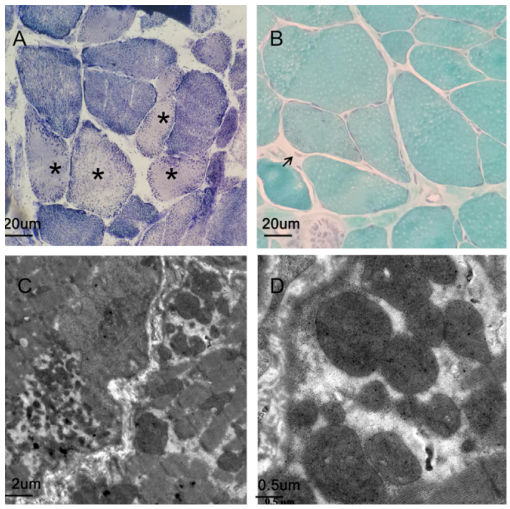

Figure 2: A. NADH staining shows the absence of mitochondria in the central areas of the myofibers (asterisks). B.MGT staining shows a large amount of aggregation of basophilic particles around the myofibers (arrows), suggesting the presence of mitochondria. C.D. Electron microscopy showed accumulation of significantly expanded mitochondria beneath the membrane of muscle fibers. (The scale bar represents 0.5um.).

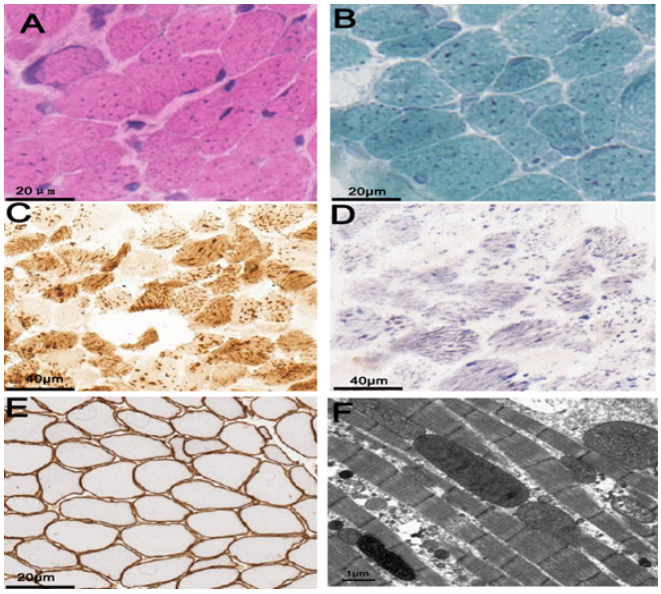

Figure 3: A. Muscle biopsy gastrocnemius muscle showed increased muscle fiber size variability. B. Modified Gomori trichrome (mGT) staining showed that basophilic particles gathered around muscle fibers. C.D.Histochemical staining for oxidative enzymes (SDH and COX) showed enhanced staining of large particles, and a few COX- negative and SDH-negative fibers were detected. E. Dystrophin staining showed no reduction in muscle fibers and the absence of membrane endocytic vesicles. F. Electron microscopy showed that the fibers presented mitochondria enlarged in length and diameter with "megaconial" appearance.

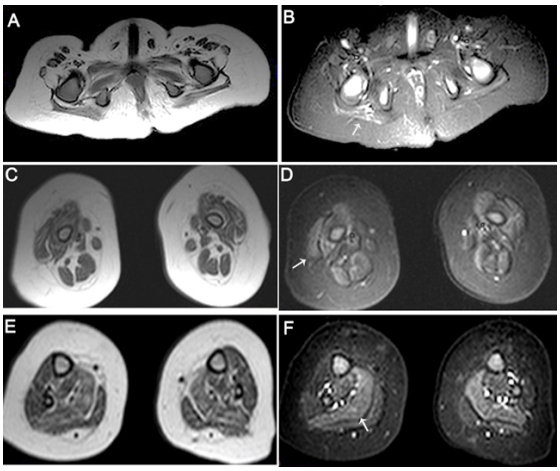

Figure 4: Muscle MRI showed high signal in the fat suppression sequence of the gluteus maximus, quadriceps and gastrocnemius muscles bilaterally (white arrow), suggesting the presence of muscle edema.

Gesell test(40 weeks)	Adaptability	Fine motor behavior	Large motor behavior	Language behavior	Personal social behavior
Developmental quotient	70.6	73.7	31.6	73.7	53.4
Developmental Age(week)	30.6	32	13.7	32	23.2
Developmental quotient (DQ): normal range, DQ>75; mild delay, 75≥DQ>55; moderate delay, 55≥DQ>35; severe cases, 35≥DQ>25; extremely severe cases, 25≥DQ.					

Table 1: Geselle developmental scales of the patient in case 2.

Discussion

We present one adult and one juvenile patient with MCMD, respectively. The combination of symptoms in case 1 is myopathy, mental decline, and ichthyosis and dilated cardiomyopathy, which is a typical symptom group of MCMD. It is worth noting that the patient first developed cerebral infarction, then the dilated cardiomyopathy was discovered, which is presumed to be the mechanism of cerebral embolism. Anticoagulant therapy is necessary for this patient. In both cases, there were typical pathological changes of MCMD, including large granules of MGT and typical giant mitochondrial morphology shown on electron microscopy. In case 1, there was a loss of central mitochondria in muscle fibers, which was a manifestation of mitochondrial depletion. In case 2, typical COX staining and coarse SDH positive particles were observed, and some fibers were absent, which was also a manifestation of heterogeneous mitochondrial aggregation in MCMD. Currently, the specificity of CHKB gene detection in patients with an abnormal distribution of giant mitochondria in muscle fibers has been reported to be 100%. CHKB mutations would lead to abnormal conformation of β-choline kinase, which may disturb synthesis of phosphatidylcholine, resulting in dysfunction of respiratory chain and yield ATP synthesis. Deficiency of ATP would be compensated by activating alternative pathway to produce energy and the significant expansion of mitochondria is exclusive in Megaconial CMD [15,16]. The enlarged mitochondria are usually located at the periphery of the muscle fiber, and the mitochondria in the centre of the muscle fiber are exhausted. A variety of CHKB gene mutations have been reported, most of which are homozygous. A small number of mutations reported are complicated heterozygous mutations, including nonsense, deletion, missense, and shear mutation in either exon regions or shear regions [17]. Patient 1 has presented a totally new mutation, CHKB gene c.940C>T, p.Arg314Cys. This is a variation that has not been previously reported. It is not easy to determine whether a new variation is pathogenic. According to the ACMG guidelines for the interpretation of gene mutations, this variation is not reported in HGMD database, nor in ESP and Thousand Genome databases. This is the PM1 evidence in the ACMG guidelines. There were few benign variants in the previously reports in the CHKB gene, and a new mistranslation mutation in this gene may be considered as PP2 evidence. Moreover, this mutation may be harmful for its location in the conserved sequence of genes analysed by the current biological data software, which is a PP3 evidence. The clinical symptoms of patient 1, especially the realization of giant mitochondria on pathological findings, are highly compatible with MCMD disease. Taken together, it can be said that there is one moderate evidence and three supporting evidences. It does not meet the criteria for possible pathogenic variants according to ACMG guidelines (1PM +>4PP). Nevertheless, the patient's specific clinical symptoms and unique pathological changes are highly consistent with MCMD, and homozygous or complex heterozygous mutations in the CHKB gene have been found in all of the previous 40 typical cases of giant mitochondria. Therefore, we still believe that this site is a possible causative site for MCMD, but this requires future case reports or protein functional verification. The mutation c.598del (p.Gln200fs) in case 2 has been previously reported only in two Chinese MCMD patients from different lineages in Hong Kong [18]. Previous literature on CHKB mutations with loss of function supports the pathogenicity of this mutation. Case 2 is from Guangdong Province, China, and most of the Chinese in Hong Kong are also from Guangdong, suggesting that the mutation may have a founder effect in the Guangdong-Hong Kong region of China, but more population information is needed. Case 2 may have some clinical features that have not been shown due to younger age. His Gesell score showed an overall lag in his development in social adjustment (mild backwardness), major movements (severe backwardness), fine movements (mild backwardness), language (mild backwardness), and personal sociability (moderate backwardness). The most striking backwardness of major movements was consistent with the main manifestations of MCMD muscle damage, and the development of children's personal social skills may be related to mental retardation, which reminds us that we should pay attention to the observation and training of children's psychosocial development. The majority of patients had a slight increase in muscle enzymes, and case 2 showed a normal range of CK, probably at a relatively early stage with the patient, but his muscle magnetic resonance findings had shown extensive systemic muscle damage in the trunk, upper and lower limbs. There are few magnetic resonance reports on MCMD in the past. Extensive and varying degrees of fatty infiltration of both thigh and calf muscles was reported previously

[19]. Patients may have relatively retained adductor longus (AL) and extensor digitorum longus (EDL) muscle, including the MR characteristics of the two patients in Hong Kong, China [18]. Case 2 showed an increased STIR signal in both thighs and legs, suggesting the presence of edema, which was inconsistent with previous MR reports, probably because the patient was young, the muscle was still in an early period of damage and fat infiltration was not obvious.

Conclusions

We report two cases of megaconial congenital muscular dystrophy in China. Complete clinical data, muscle pathology and genetic testing results are all important for timely and accurate diagnosis for rare neuromuscular diseases. It is necessary to monitor muscle and cardiac function in MCMD patients, and to pay attention to the increased risk of cerebrovascular events caused by dilated heart disease. In addition to muscle function testing training, intervention is necessary for comprehensive psychosocial function assessment and training of young patients.

Ethical Publication Statement: The study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University in China (equivalent to an Institutional Review Board).Written informed consent was obtained from the patient.

Disclosure of Conflicts of Interest: None of the authors has any conflict of interest to disclose.

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