Megaconial Muscular Dystrophy with a Novel Mutation in the *CHKB* Gene

Seema Thakur[1], Arvind Kumar[1], Neerja Gupta[2], Mehar Chand Sharma[3]

[1]*Fortis Hospital, AA Block, Shalimar Bagh, New Delhi*

[2]*Genetic Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi*

[3]*Department of Pathology, All India Institute of Medical Sciences, New Delhi*

Correspondence to: Dr Seema Thakur *Email:* seematranjan@gmail.com

Abstract

Congenital muscular dystrophy is a genetically heterogenous disorder. We report two sisters with megaconial myopathy who presented with developmental delay, congenital muscular dystrophy and acanthosis with ichthyosis. About 36 cases have been described in world literature. Ours is the first case report from India. Whole exome sequencing identified a novel homozygous nonsense mutation in the *CHKB* gene.

Introduction

Congenital muscular dystrophies (CMD) are clinically and genetically heterogeneous group of inherited muscle disorders in which weakness is first apparent at birth or in infancy. The prevalence and incidence of CMD in different regions of the world are poorly known. Few studies are limited to epidemiologic figures of prevalence (Norwood et al., 2009) and a recent review estimated the overall prevalence of CMD to be 0.99/100,000 (Mah et al., 2016) which may have been an underestimate because of limited availability of diagnostic means. The relative frequency of CMD subtypes also varies in different populations.

Clinical presentation of motor delay, proximal weakness, hypotonia and learning difficulties raise the possibility of CMD. Serum creatine phosphokinase (CPK) is usually the first investigation to detect CMD. Neurophysiological studies in CMD cases may suggest myopathic pattern and muscle biopsy shows features of dystrophic changes. Further classification is based on immunohistochemistry with different antibodies e.g. merosin or alpha, beta, gamma and delta dystroglycan. With the advent of next generation sequencing (NGS)-based testing, the causative gene can be identified. CMD-causative pathogenic variants can be identified in 25%-50% of cases, underscoring the need for ongoing investigation into the genetic causes of CMD (Peat et al., 2008).

Choline kinase beta gene (*CHKB;* MIM *612395) mutations are associated with a rare recessive congenital muscular dystrophy with mental retardation called congenital megaconial type muscular dystrophy (MIM#602541). This gene codes for the choline kinase beta enzyme (EC 2.7.1.32) which catalyzes the first step of phosphatidylcholine (PC) biosynthesis. Here, we describe a novel nonsense mutation in exon 2 of the *CHKB* gene in 2 sisters with congenital megaconial muscular dystrophy.

Case Report

Two sisters (8 years and 5 years old) with intellectual disability, born to third degree consanguineous parents, were referred for evaluation.

The elder sibling (Patient 1) was born through vaginal delivery at 34 weeks gestation. There was history of delayed cry at birth. She had neonatal hyperbilirubinemia for which phototherapy was given and she was kept in the neonatal intensive care unit (NICU) for 10 days. She developed head control at 6 months of age and was able to sit by 8 months of age. However, after this she stopped acquiring new skills and developed autistic features. She never achieved standing or walking. There was no speech and no eye contact. She was not able to interact or follow any commands. There was no history of seizures and there was no cranial nerve involvement. There was a history of severe itchy skin lesions present since birth on the neck, back, axilla, antecubital regions and the abdomen.

On examination, the head circumference was

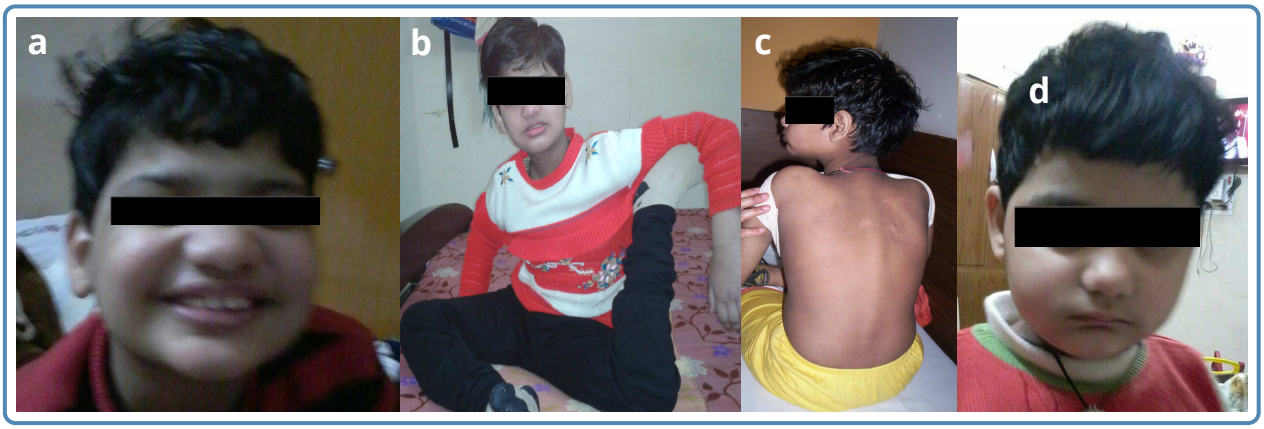

Figure 1 a) Clinical photograph of Patient 1. b) Joint laxity seen in Patient 1. c) Ichthyosis-like skin lesions in Patient 1. d) Clinical photograph of Patient 2.

45 cm (< 3rd centile for age) (Figure 1a). There was severe generalized hypotonia, hyporeflexia and joint laxity (Figure 1b). The muscle bulk was normal. Acanthosis nigricans-like lesions were present in the axilla, neck and abdomen with marked pruritus-related scarring (Figure 1c).

The younger sibling (patient 2) was born at 32 weeks gestation and had pruritic skin lesions since birth. The severity of lesions was less when compared to the elder sib (Figure 1d). She also had developmental delay along with joint laxity. Like her sister she had attained head holding at 6 months and achieved sitting at 8 months and no further milestones thereafter.

Age of death of the elder sibling was 10 years and for the younger one was 8 years. The cause of death was attributed to renal failure in both of them. Before death the elder sibling had developed aggressive behaviour whereas the younger one had become quiet for 3 months.

We investigated Patient 1 (the elder sibling) as both sibs had similar presentations but she was more severely affected of the two. Her serum CPK was 1109 IU/L. Electrophysiological study was suggestive of a myopathic pattern. Her MRI Brain showed multiple punctate perivascular and subcortical hypodensities. Mass tandem spectrometry, organic acid analysis, fundus examination, hearing evaluation and karyotype were normal. Evaluation for congenital disorders of glycosylation through transferrin isoelectric focusing was normal. Her muscle biopsy showed features suggestive of muscular dystrophy. Immunohistochemistry for dystrophin 1, 2, and 3, alpha, beta, delta and gamma sarcoglycans, merosin 80 and 300, and alfa dystroglycans was normal. Skin biopsy from the axilla showed hyperkeratosis and parakeratosis with acanthosis. Collagen VI staining of the skin done to exclude Ullrich muscular dystrophy was normal. In view of ichthyosis, steroid sulphatase gene deletion analysis was done and this was normal.

Whole exome sequencing was then done through the Illumina platform and a sequence variant was detected in exon 2 of the *CHKB* gene: c.331 C>T; p.Gln111Ter. This is a novel sequence variant which results in a stop codon. It was present in a homozygous state in both the affected sisters whereas both parents were heterozygous carriers for the same. The sequence variant is not present in the 1000 Genome, ExAC, gnomAD and also the combined VarSome Databases and it is predicted to be likely pathogenic.

Muscle biopsy was reanalysed in view of the above mutation results and cytochrome oxidase (COX) and succinate dehydrogenase (SDH) staining was done. Typical features of megaconial myopathy were seen with mega mitochondria at the periphery of the fibre, seen with COX and oxidative enzyme stains, and depletion of it at the centre of the fibre (Figures 2 & 3). This feature is specific of this disease and not seen in other mitochondrial conditions.

Discussion

Megaconial myopathy was first described in 1998 in 4 Japanese patients from three unrelated families, with mitochondrial structural changes. It is characterized by neonatal hypotonia, developmental delay and its pathological signature i.e. enlarged

mitochondria displaced to the periphery of the fibres in muscle biopsy, leaving the centre devoid of organelles (Nishino et al., 1998). The characteristic enlarged mitochondria at the periphery of the muscle fibres have given rise to the name 'megaconial myopathy'.

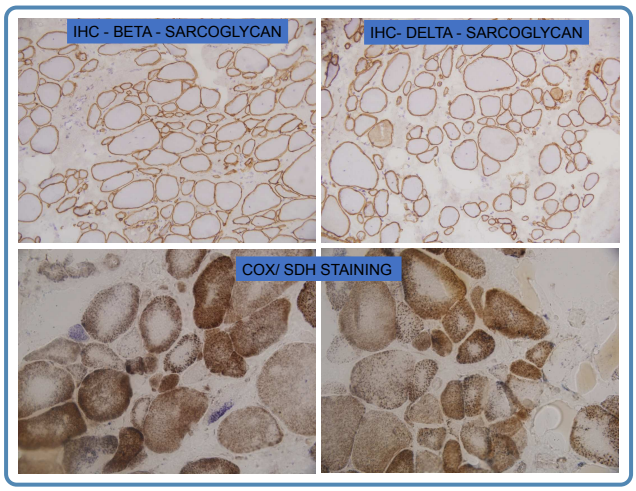

Figure 2 Images of muscle biopsy and immunohistochemical staining for beta sarcoglycan and delta sarcoglycan and COX/SDH staining in Patient 1.

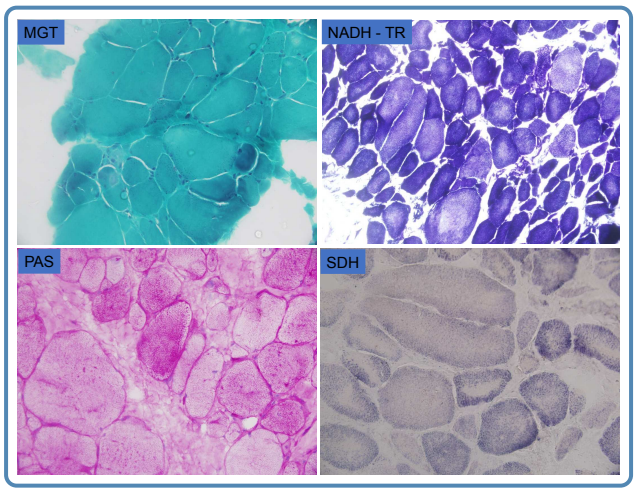

Figure 3 Modified trichrome Gomori (MGT), nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), Periodic acid Schiff (PAS) and succinate dehydrogenase (SDH) staining of muscle biopsy showing prominent mitochondria at the periphery of the muscle fibres.

About 36 patients (4 Japanese, 10 Turkish, 3 British, 1 French, 1 African-American, 16 Spanish and 1 Italian) with mutations in the *CHKB* gene have been characterized and published since then (Mitsuhashi et al., 2011; Gutierrez Rios et al., 2012; Quinlivan et al., 2013; Mitsuhashi et al., 2013; Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015; Haliloglu et al., 2015).

The age of presentation varies from 0-28 years. Both our patients presented with skin lesions since birth. Both the sisters had severe developmental delay and they were never able to walk. All patients described in literature had developmental delay. The Spanish patient described by Castro- Gago et al. (2014) had mainly motor delay. The female patient reported from Australia had intellectual disability. Two of the 15 patients described by Mitsuhashi et al. (2011) never walked. All patients in the study described by Haliloglu et al. (2015) had global developmental delay predominantly involving gross motor (n=14), and language domains (n=15). There was a delay in the age at independent walking (n=14), with one child still not able to walk at the age of 6 years 1 month. Intellectual disability was mild (n=7), moderate (n=2), or severe (n=4) in this group of Spanish patients (Haliloglu et al., 2015).

Skin changes include ichthyosis-like changes. Both the sisters in the current study had acanthosis-ichthyosis like changes which were present since birth. Skin lesions were seen mainly in the neck, axilla and abdomen. The severity was lesser in the younger sib. There was significant pruritus in both of them, more so in the elder sib. The severity was so marked that at times she had to be sedated to avoid the itching. Pruritus was lesser in the younger girl. Skin biopsy showed parakeratosis and hyperkeratosis in the elder sib. Five out of the 15 patients described by Mitsuhashi et al. (2011) had skin changes. Skin changes were not seen in the patients described from Australia and Africa (Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015). In the study by Haliloglu et al. (2015), skin changes were present in the form of diffuse ichthyosis-like changes (n=11), exfoliative and desquamative lesions mainly involving the neck, trunk, and face (n=7), hirsutism (n=1), and atopic dermatitis (n=1). The mean age at recognition of skin findings was 3 years 8 months (birth-17 yrs).

Marked hypotonia and joint laxity were present in both sisters. Hypotonia was noted in 9 out of the 15 patients described by Mitsuhashi et al. (2011). Hypotonia was also seen in patients of

Australian and African origin (Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015).

Both our patients had raised serum CPK (Patient 1 - 1109 IU/ L, Patient 2- 405 IU/ L). Serum CPK was raised in all patients described by Mitsuhashi et al. (2011) and in 12 out of 15 of the Spanish patients described by Haliloglu et al (2015).

Our patient showed changes typical of muscular dystrophy which include variation in muscle fibre size. IHC for dystrophin 1, 2 and 3 and alpha, beta, gamma and delta sarcoglycans was normal. COX and SDH staining were suggestive of mitochondrial abnormalities. All affected individuals in the Mitsuhashi series exhibited nonspecific dystrophic features; Gomori trichrome, NADH-TR, SDH, and COX staining showed prominent mitochondria at the periphery as well as central areas devoid of mitochondria. The typical muscle dystrophic changes and typical mitochondrial structural changes were present in all muscle biopsy cases of Haliloglu et al. (2015); enlarged mitochondria were easily identified by Gomori trichrome, SDH, and COX stains.

The *CHKB* gene consists of 11 exons. Mutations reported so far include nonsense, frame shift, missense, nonframeshift deletions, and splice-site mutations, in exons 1, 4, 5, 6, 7, 8, 9, and 11 (Mitsuhashi et al., 2011; Gutierrez Rios et al., 2012; Quinlivan et al., 2013; Mitsuhashi et al., 2013; Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015; Haliloglu et al., 2015). Eight out of 36 patients have been reported to have mutation in exon 5, 8/36 in exon 8 and 6/36 in exon 9. The c.677+1 G>A is the commonest mutation reported and has been found in 6/36 patients. The homozygous nonsense pathogenic variant in exon 2 found in our patients is a novel variant. We have not done functional studies to confirm the pathogenicity of this variant but the segregation pattern in the family and clinical and immunohistological phenotype in the patient are supportive of the diagnosis.

Conclusions

We hereby report the first case of megaconial myopathy in two sisters from India with a novel homozygous mutation in the *CHKB* gene. The skin manifestations such as ichthyosis and acanthosis nigricans along with joint laxity in a case of congenital muscular dystrophy are major clues towards this diagnosis.

Acknowledgments

We would like to acknowledge Prof Carsten Bonneman for helping with the Whole exome sequencing for these patients.

References

1. Cabrera-Serrano M, et al. Novel CHKB mutation expands the megaconial muscular dystrophy phenotype. Muscle Nerve 2015; 51:140-143.
2. Castro-Gago M, et al. Exome sequencing identifies a CHKB mutation in Spanish patient with Megaconial Congenital Muscular Dystrophy and mtDNA depletion. Eur J Paediatr Neurol 2014; 18: 796-800.
3. Gutierrez Rios P, et al. Congenital megaconial myopathy due to a novel defect in the choline kinase Beta gene. Arch Neurol 2012; 69: 657-661.
4. Haliloglu G, et al. Clinical characteristics of megaconial congenital muscular dystrophy due to choline kinase beta gene defects in a series of 15 patients. J Inherit Metab Disease 2015; 38: 1099-1108.
5. Mah JK, et al. A systematic review and meta-analysis on the epidemiology of the muscular dystrophies. Can J Neurol Sci 2016; 43:163-177.
6. Mitsuhashi S, et al. A congenital muscular dystrophy with mitochondrial structural abnormalities caused by defective *de novo* phosphatidylcholine biosynthesis. Am J Hum Genet 2011; 88: 845-851.
7. Mitsuhashi S, Nishino I. Megaconial congenital muscular dystrophy due to loss-of-function mutations in choline kinase beta. Curr Opin Neurol 2013; 26: 536-543.
8. Nishino I, et al. A new congenital muscular dystrophy with mitochondrial structural abnormalities. Muscle Nerve 1998; 21: 40-47.
9. Norwood FL, et al. Prevalence of genetic muscle disease in Northern England: In-depth analysis of a muscle clinic population. Brain 2009; 132: 3175-3186.
10. Peat RA, et al. Diagnosis and etiology of congenital muscular dystrophy. Neurology 2008;71: 312-321.
11. Quinlivan R, et al. Muscular dystrophy with large mitochondria associated with mutations in the CHKB gene in three British patients: extending the clinical and pathological phenotype. Neuromuscul Disord 2013; 23: 549-556.