Congenital muscular dystrophy due to laminin α2 (merosin) deficiency (MDC1A) in an ethnic Malay girl

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Abstract

We report the first known ethnic Malay patient with laminin alpha-2 (merosin) deficiency (MDC1A), a subtype of congenital muscular dystrophy (CMD)as a result of novel *LAMA2* gene mutations. The 21-month-old female presented with hypotonia at birth and gross motor delay of her distal lower limbs. Physical examination showed generalised hypotonia, hyporeflexia and myopathic facies but good cognitive functions. Serum creatine kinase was elevated and white matter changes were detected in the brain MRI. Muscle biopsy showed dystrophic changes with complete laminin α2 deficiency by immunohistochemistry. Mutation analysis of *LAMA2* showed compound heterozygote at exon 21, c.2888delG(p.Gly963Alafs*111) and exon 34, c.4886dupC(p.Pro1629Profs*40) leading to premature stop codon for each of the frameshift mutations. Patient review at seven years of age showed satisfactory cognitive functions despite having contractures and weakness. Genetic testing of *LAMA2* related muscular dystrophy facilitated the earlier diagnosis of MDC1A and genetic counselling for this family.

Keywords: laminin alpha-2 deficiency; merosin deficiency. *LAMA2*, Malaysia, congenital muscular dystrophy, MDC1A

INTRODUCTION

Congenital muscular dystrophy (CMD) is a group of inherited muscle diseases affecting 1:10,000 to 1:50,000 of the population. The common presentation is muscle weakness beginning at birth or early infancy, severe hypotonia, poor spontaneous movements with contractures of the large joints and scoliosis, feeding difficulties with failure to thrive, recurrent aspiration pneumonia and respiratory failure.[1] Serum creatine kinase is elevated and muscle biopsy shows dystrophy.[2] However, there is wide clinical and genetic heterogeneity and the diagnosis relies on clinical findings, brain and muscle imaging, muscle biopsy immunohistochemistry (IHC) and genetic testing. Currently recognised CMD subtypes include laminin alpha-2 (merosin) deficiency (MDC1A), collagen VI-deficient CMD (Ullrich disease), the alpha dystroglycanopathies (caused by *POMT1, POMT2, FKTN, FKRP, LARGE,* and *POMGNT1* mutations), *SEPN1*-related CMD and *LMNA*-related CMD (L-CMD).[3]

MDC1A (OMIM number 605788) is caused by mutations in the laminin alpha-2 (*LAMA2*)gene. Loss-of-function mutations had been reported in severely-affected neonates, but only missense mutations had been found in milder cases with partial LAMA2 deficiency.[4] Over 50% of MDC1A are due to complete *LAMA2* deficiency.[5,6] Brain MRI white matter abnormalities without major cognitive involvement are associated with the MDC1A subtype. We present a Malay child with MDC1A associated with novel *LAMA2*stop-codon gene mutations. There is limited information on MDC1A in the South-East Asian population, and this report extends the genotype-phenotype correlation of this condition

CASE REPORT

The patient, a one-year and 9-month old ethnic Malay girl was hypotonic since birth with gross motor delay involving the lower more than the upper limbs. There was no significant family history and she was the only child. Her parents were not related. Physical examination showed she has normal stature with generalised hypotonia,

hyporeflexia and good cognitive functions with normal developmental milestones. The distal part of the lower limbs was more affected than the proximal part. There were no dysmorphic features. She had myopathic facies but no abnormal eye movements, cranial nerve palsies or myoclonus. Visual acuity and funduscopy were normal. There were no joint contractures, calf hypertrophy or positive Gower's sign. Cardiorespiratory examination was normal.

The serum creatine kinase was 1858 IU/L. Brain MRI showed hyperintense white matter changes of the frontal, temporal, parietal and occipital lobes bilaterally with sparing of the occipital sub-cortical and cerebellar white matter. (Figure 1). The electromyogram showed evidence of myopathy. The left biceps muscle biopsy showed moderate variation in fibre size with randomly distributed atrophic but there were no hypertrophic fibres. Many fibres showed evidence of degeneration, regeneration and focal necrosis with surrounding inflammation and increased acid phosphatase activity. Moderate increase of endomysial and perimysial fibrous and adipose tissues in the fascicles were observed. There was no fibre type grouping and there were no abnormal vacuoles, cores and inclusions or ragged red, whorled and split fibres. There was only mild intermyofibrillar network disruption and lobulated fibres were absent. Immunohistochemistry (IHC) for merosin was negative. The dystrophin and sarcoglycan IHC generally showed "faint and patchy" staining, while α-dystroglycan and collagen 6 were normal. These findings were in keeping with the diagnosis of MDC1A. (Figure 2)

At seven years of age, she attended school and had appropriate cognitive function. Physical examination showed a thin child with myopathic facies, hyporeflexia and hypotonia of both upper and lower limbs. The hypotonia was more obvious over the lower rather than the upper limbs. She had generalised contractures predominantly of the
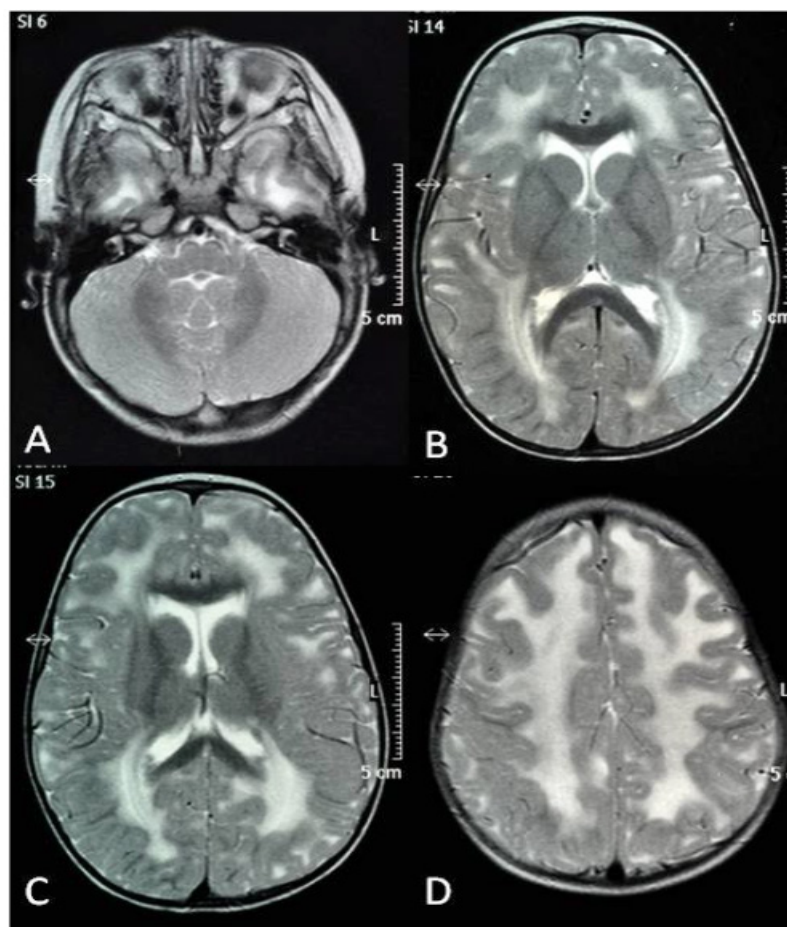

Figure 1 (A-D): Axial T2 MR images show hyperintense white matter of the frontal, temporal, parietal and occipital lobes bilaterally. The sub-cortical white matter of the occipital lobes (B) and cerebellar white matter are spared.
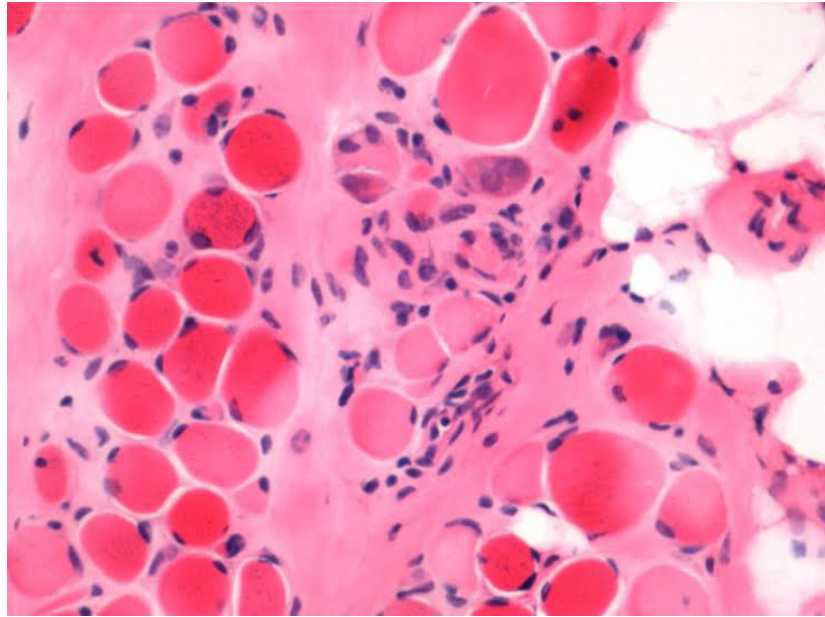
Figure 2: Muscle biopsy of the left biceps showed dystrophic muscle with fibre atrophy, hypertrophy, degeneration and regeneration, interstitial fibrosis and fat infiltration. (H&E, Magnification x20 objective)

elbow and knee joints. There were limited joint movements and motor power of MRC grade 3. She was unable to walk without support but she did not require any respiratory support.

Mutation analysis of *LAMA2* showed compound heterozygote at exon 21, c.2888delG (p.Gly963Alafs*111) and exon 34, c.4886dupC (p.Pro1629Profs*40) leading to premature stop codon for each of the frameshift mutations. (Figure 3 and 4). Since there was no similar mutations reported in the *LAMA2* mutation database and the findings were likely to be novel.[15] Parental mutation studies were not available.

DISCUSSION

The clinical findings of CMD with elevated serum creatine kinase, white matter changes in the brain MRI and the IHC finding of complete laminin α2 deficiency in muscle fibres are considered diagnostic of MDC1A.[7] While the prevalence of CMDs has been estimated between 0.7/100,000 and 2.5/100,000, the accurate prevalence of early-onset MDC1A is unknown.[8] MDC1A is being considered as the most common type of CMD all around the world, which accounts for about 30% of CMDs in European countries. The only exception would be Japan where it had been reported to account for only 6% of CMDs
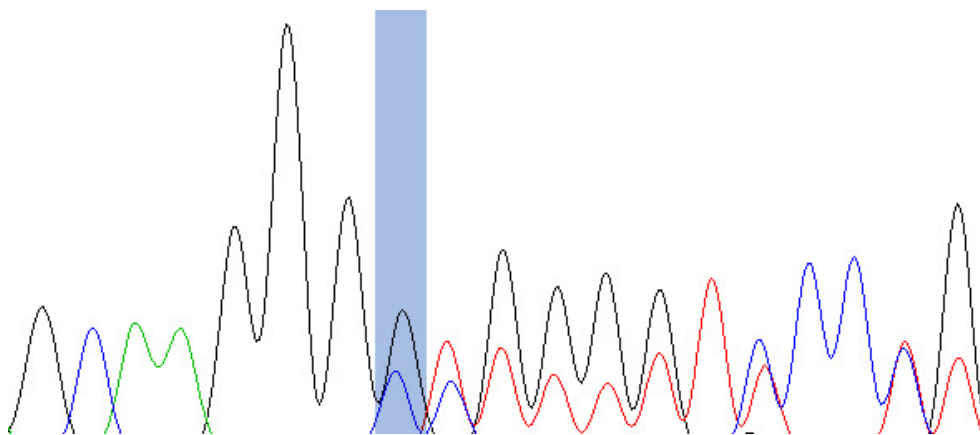

Figure 3: Sequencing results of *LAMA2* gene (exon 21) which showed c.2888delG (p.Gly963Alafs*111)
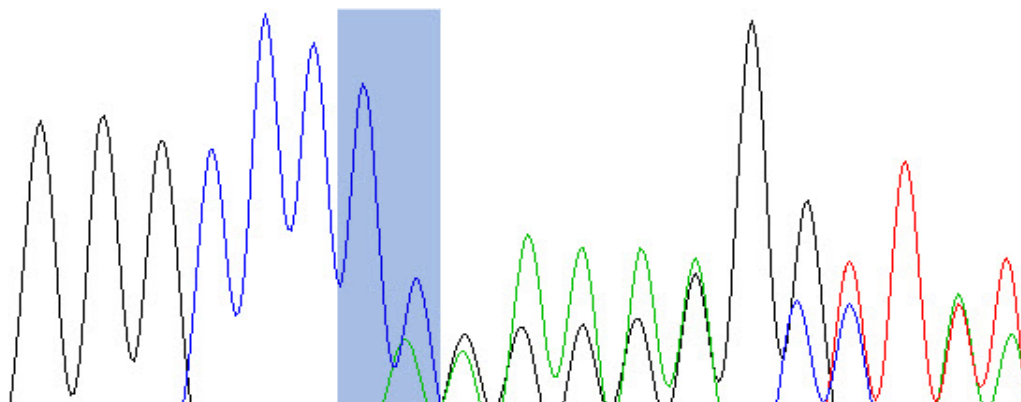
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Figure 4: Sequencing results of *LAMA2* gene (exon 34) which showed c.4886dupC (p.Pro1629Profs*40)

following Fukuyama type CMD.[9] To date, there are anecdotal case report of confirmed MDC1A in East Asia, mainly from Korea and Japan (by IHC analysis)[10,11] or confirmed by genetic and IHC analyses in two patients from China and one patient each from India and Samoa, respectively.[12-14] To the best of our knowledge, this is the first report of a patient with confirmed MDC1A based on IHC study and presence of deleterious *LAMA2* mutations in South East Asia , as well as the first Malay patient with the condition.

At birth, there is an increased in immune response leading to increased infiltration by T and B cells. Muscle biopsy done during infancy may be misinterpreted as 'infantile polymyositis'. Girgenrath found that treatment of Lama2-null mice with either minocycline or doxycycline, which inhibit apoptosis, showed clinical and pathologic improvement.[15] Muscles derived from the treated mice showed decreased inflammation, and decreased markers of apoptosis, such as Bax and caspase-3. The findings indicated that increased apoptosis is a major pathogenic mechanism in *LAMA2* deficiency. The persistence of laminin α4 and α5 leads to abnormal basal lamina causing poor myofibre regeneration in patients beyond one year of age. This leads to dystrophic picture and subsequently end-stage myopathic change. These findings were found in our patient's muscle biopsy which showed inflammatory changes and absent merosin stain on IHC. Compared to the residual merosin group, patients with absent merosin had an earlier presentation (<7days), were more likely to lack independent ambulation, or require enteral feeding and ventilatory support.[16]

The T_2-weighted brain MRI in our patient showed hyperintense white matter of the frontal, temporal, parietal and occipital lobes. These abnormalities were likely to be due to leaky basal laminar connections and increased water content and did not represent areas of demyelination and are detected in some children as early as age six months[17] and were seen in both complete and partial laminin α2 deficiency.[16] Cognitive impairment is found only in 7% of affected individuals and do not correlate with cranial MRI abnormalities.[18] Patients with intellectual disability and epilepsy were associated with bilateral occipital pachygyria[19] or dysplastic cortical changes affecting predominantly the occipital and temporal regions.[16]

In view of the lack of common mutations and limited information on genotype-phenotype correlations in the LAMA2-related muscular dystrophy at the moment, there is still a need for IHC study for MDC1A in all patients with CMD.[19,20] The clinical utility of the mutation analysis was the earlier diagnosis of MDC1A as this facilitated timely avoidance of certain anaesthetic agents such as succinylcholine during induction of anaesthesia because of risk of hyperkalaemia and cardiac conduction abnormalities.[21] Prenatal diagnosis of a foetus with CMD may be made by immunostaining of chorionic villus sample. With the availability of mutation analysis, prenatal diagnosis can be an option for some families and preimplantation genetic diagnosis can be made for families where termination of pregnancy is not an option.[22] With more information on the phenotype-genotype correlation, a more accurate genetic counselling can be offered for affected families with an autosomal recessive disorder. Mutation studies may also be useful as a confirmatory test in patients with atypical presentation, for example during early infancy where a muscle biopsy showed an inflammatory picture or cranial MRI showed

'leukodystrophic' findings or partial merosin deficiency.[23]

These is a need to increase the awareness of medical practitioners on MDC1A in this region, to facilitate early diagnosis, genetic counselling and avoidance of certain anaesthetic agents for these patients.

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