Two Different MLC1 Variants Compounded with a Common Variant S93L in Japanese Patients of Megalencephalic Leukoencephalopathy with Subcortical Cysts

TWMUJ 4: 94-97, 2020

Case Report

Two Different *MLC1* Variants Compounded with a Common Variant S93L in Japanese Patients of Megalencephalic Leukoencephalopathy with Subcortical Cysts

Keiko Yamamoto-Shimojima,[1] **Yasuhiro Kimoto,**[2] **Yoshiyuki Watanabe,**[3] and **Toshiyuki Yamamoto**[1]

[1]Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan
[2]Department of Pediatrics, Miyazaki University, Miyazaki, Japan
[3]Department of Pediatrics, Dokkyo Medical University, Tochigi, Japan

(Accepted August 5, 2020)
(Advance Publication by J-STAGE September 25, 2020)

Megalencephalic leukoencephalopathy with subcortical cysts (MLC; MIM#604004) is a rare congenital disorder of the cerebral white matter. Most MLC patients have *MLC1* mutations which are inherited in an autosomal recessive manner. Since there is no established biomarker for MLC, genetic testing is necessary for a final diagnosis. We identified compound heterozygous mutations in two different Japanese patients having MLC. Both patients showed a common mutation of c.278C>T (p.Ser93Leu) in a heterozygous status. In addition, two different mutations (c.337_353delinsG and c.423+1G>A) were identified in the homologous alleles of the patients, respectively. Although these two variants were first identified in the Japanese population, they have since been reported in other Asian countries and might be founder mutations of the gene in Asian population.

Key Words: Sanger sequencing, leukodystrophy, macrocephaly

Introduction

Megalencephalic leukoencephalopathy with subcortical cysts (MLC; MIM#604004) is a congenital disorder of the cerebral white matter.[1] Patients with MLC typically show megalencepahly since the neonatal period. Thus, patients with MLC are often referred to the hospital by a health checkup system for neonates and are radiologically examined. If the patients exhibit cystic-like findings in the subcortical white matter, presence of MLC would be strongly suggested. White matter involvement and clinical severity often display step-wise progression. Most patients with MLC have *MLC1* mutations which are inherited in an autosomal recessive manner.[2] Since there is no biomarker for MLC, genetic testing is necessary for a final diagnosis. Previously, many genetic mutations have been reported for MLC.[3] Here, we report on two additional patients with MLC.

Case Reports

Patient 1 is a 3-year-old Japanese boy, who was born at 39 weeks of gestation, with a birth occipitofrontal circumference (OFC) of 36.0 cm (> 97th centile), indicating

Corresponding Author: Toshiyuki Yamamoto, Institute of Medical Genetics, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. yamamoto.toshiyuki@twmu.ac.jp

doi: 10.24488/twmuj.2020007

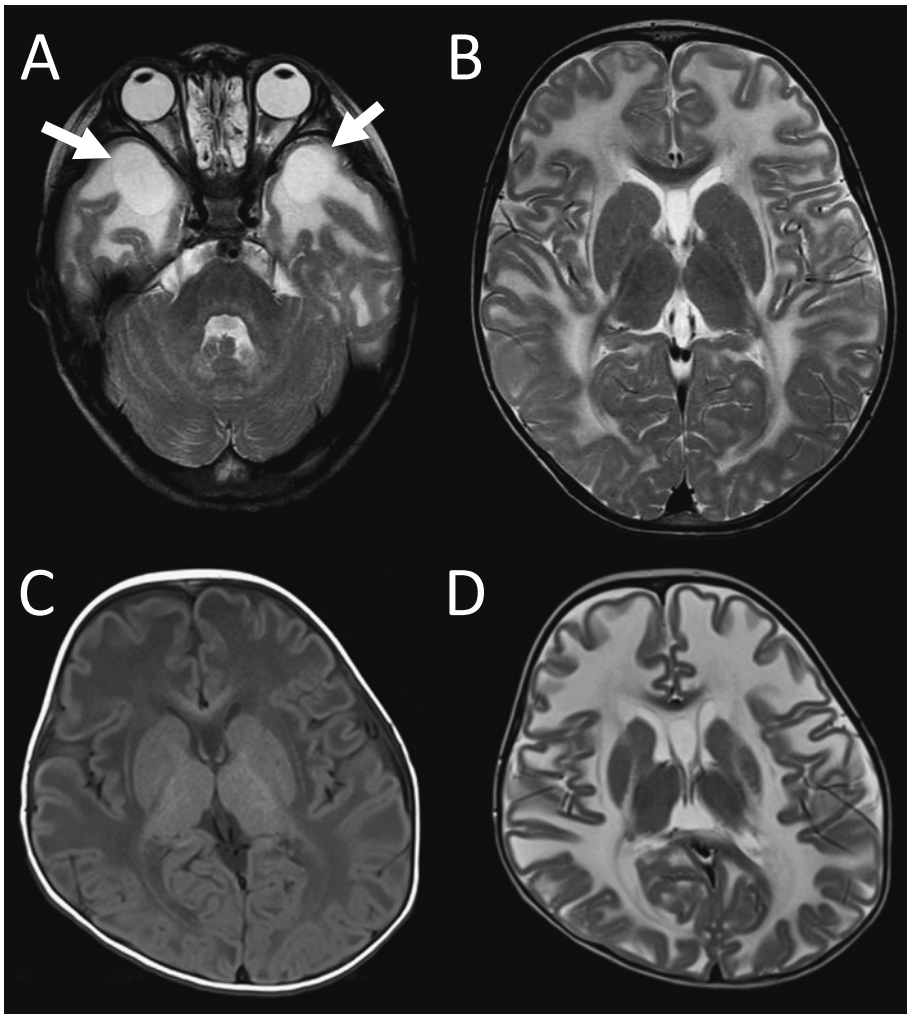

Figure 1 Results of MRI examination. Patient 1 shows cystic-like findings in the subcortical white matter (**A**; white arrows) and diffuse white matter involvement (**B**). Patient 2 shows diffuse T1-low (**C**) and T2-hypointensities (**D**) in the white matter.

macrocephaly. The patient is the only child of his healthy parents who were non-consanguineous. His family history is no-remarkable. His OFC was measured to be 48.0 cm (> 97th centile) during a health checkup at 5 months, and macrocephaly became remarkable. Then, he was referred to the hospital, and brain magnetic resonance imaging (MRI) displayed a high T2 intensity in the white matter, in association with suspected cystic findings in the subcortical region, suggesting the presence of MLC. At present, he shows mildly delayed motor development, probably due to macrocephaly with an OFC of 57.3 cm (> 97th centile). Brain MRI clearly demonstrated typical MLC findings (**Figure 1A and B**).

Patient 2 is a 14-month-old Japanese girl, who was born at 36 weeks of gestation, with a birth weight of 2,435 g. The patient is the only child of her healthy parents who were non-consanguineous. There is no-remarkable family history. During early infancy, the patient showed normal development; however, she demonstrated mildly delayed gross motor development. At 10 months, she could not sit upright and macrocephaly was also noted. Then, she was referred to the hospital and her brain MRI displayed a diffuse white matter abnormality (**Figure 1C and D**).

Molecular Diagnosis

Since MLC was suspected in both the patients, genetic diagnosis was performed. This study was approved by the institutional ethics committee (#338C) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the families, and pe-

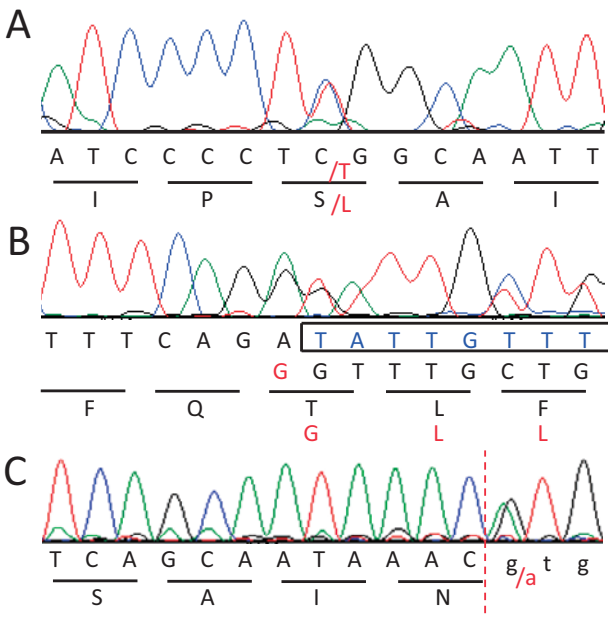

Figure 2 Electropherograms of Sanger sequencing. The c.278C>T is commonly observed (**A**). Both c.337_353delinsG (**B**) and c.423+1G>A (**C**) are shown as heterozygous.

ripheral blood samples were obtained from the patients and their parents.

First, the common mutation, NM_139202(MLC1_v001):c.278C>T (p.Ser93Leu), in exon 4 was confirmed by PCR Sanger sequencing according to a previous report.[3] Since both the patients displayed the mutation in a heterozygous status (**Figure 2A**), other exons were subsequently analyzed. NM_139202(MLC1_v001):c.337_353delinsG (p.Ile113Glyfs*4) in exon 5 and NM_139202(MLC1_v001):c.423+1G>A in intron 6 were identified in patient 1 and patient 2, respectively (**Figure 2B and C**).

In patient 1, the p.Ser93Leu and the p.Ile113Glyfs*4 were inherited from the mother and the father, respectively. In patient 2, the p.Ser93Leu and the c.423+1G>A were inherited from the father and the mother, respectively. Therefore, both the patients showed *MLC1* mutations as a compound heterozygous pattern.

Discussion

The p.Ser93Leu mutation was first identified in 2003.[4] Subsequently, this mutation has been recognized as a common mutation among Japanese patients with MLC.[5] Indeed, in our laboratory, all previous patients with MLC displayed p.Ser93Leu in at least one allele.[3] Thus, we firstly check the existence of p.Ser93Leu in patients with suspected MLC.

In this study, we initially identified p.Ser93Leu in two patients. Subsequently, we analyzed all exons with confidence. As expected, two different mutations were identified in both the patients in combination with p.Ser93Leu. Thus, this supported the fact that our strategy was reasonable.

The mutation, c.337_353delinsG (p.Ile113Glyfs*4), identified in patient 1 and his father, is related to a frameshift alteration. Thus, loss-of-function was suspected as a consequence. This mutation has only been reported in Korea.[6] The mutation, c.423+1G>A, identified in patient 2 and her mother, was located on the splice donor site and hence is considered to cause a splicing abnormality. This splicing mutation has only been reported in Iran.[7] Although these two mutations are the first to be found in Japanese population, it is extremely rare that the same mutations occur independently in the same population. Therefore, these mutations are considered to be founder mutations in Asian population.

Generally, clinical features of the patients with MLC are variable and there is no clear genotype-phenotype correlation in MLC. Thus, it is hard to predict future prognosis or severity of patients with MLC from their genotypes.[3]

Anyway, since both the parents in the two families were carriers of *MLC1* mutations, these two couples have recurrent risk in future pregnancies. Therefore, genetic information identified in this study would be beneficial for genetic counseling and future prenatal diagnosis.

Acknowledgements

We would like to express our gratitude to the patients and their families for their cooperation.

Conflicts of Interest: There is no conflicts of interest for any of the authors.

References

1. van der Knaap MS, Barth PG, Stroink H et al: Leukoencephalopathy with swelling and a discrepantly mild clinical course in eight children. Ann Neurol 37: 324−334, 1995
2. Leegwater PA, Yuan BQ, van der Steen J et al: Mutations of *MLC1* (*KIAA0027*), encoding a putative membrane protein, cause megalencephalic leukoencephalopathy with subcortical cysts. Am J Hum Genet 68: 831−838, 2001
3. Shimada S, Shimojima K, Masuda T et al: *MLC1* mutations in Japanese patients with megalencephalic leukoencephalopathy with subcortical cysts. Hum Genome Var 1: 14019, 2014
4. Saijo H, Nakayama H, Ezoe T et al: A case of megalencephalic leukoencephalopathy with subcortical cysts (van der Knaap disease): molecular genetic study. Brain Dev 25: 362−366, 2003
5. Tsujino S, Kanazawa N, Yoneyama H et al: A common mutation and a novel mutation in Japanese patients with van der Knaap disease. J Hum Genet 48: 605−608, 2003
6. Choi SA, Kim SY, Yoon J et al: A Unique Mutational Spectrum of *MLC1* in Korean Patients With Megalencephalic Leukoencephalopathy With Subcortical Cysts: p.Ala275Asp Founder Mutation and Maternal Uniparental Disomy of Chromosome 22. Ann Lab Med 37: 516−521, 2017
7. Kariminejad A, Rajaee A, Ashrafi MR et al: Eight novel mutations in *MLC1* from 18 Iranian patients with megalencephalic leukoencephalopathy with subcortical cysts. Eur J Med Genet 58: 71−74, 2015