

Two cases with megalencephalic leukoencephalopathy with subcortical cysts and *MLC1* mutations in the Turkish population

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SUMMARY: Yiş U, Scheper GC, Uran N, Ünalp A, Çakmakçı H, Hız-Kurul S, Dirik E, van der Knaap MS. Two cases with megalencephalic leukoencephalopathy with subcortical cysts and *MLC1* mutations in the Turkish population. *Turk J Pediatr* 2010; 52: 179-183.

Megalencephalic leukoencephalopathy with subcortical cysts is a rare leukodystrophy that is characterized by macrocephaly and a slowly progressive clinical course. It is one of the most commonly reported leukoencephalopathies in Turkey. Mutations in the *MLC1* gene are the main cause of the disease. We report two patients with megalencephalic leukoencephalopathy with subcortical cysts with confirmed mutations in the *MLC1* gene. The mutation in the second patient was novel. We also review identified mutations in the Turkish population.

Key words: megalencephalic leukoencephalopathy, subcortical cysts, *MLC1* gene, Turkish population.

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is an autosomal recessive neurological disorder in children. Neurologic findings are initially normal or near normal despite megalencephaly and brain magnetic resonance imaging (MRI) of white matter involvement. The neurologic progression is generally slow and intellectual functioning is usually preserved for a long time despite loss of motor milestones and development of cerebellar ataxia¹. The cerebral hemispheric white matter appears diffusely swollen whereas gray matter structures are preserved. The presence of subcortical cysts in the anterior temporal and frontoparietal region is typical for the disease². Singhal et al.³ described the disorder in 18 patients with megalencephalic leukodystrophy from India at a meeting in Japan in 1991. In 1995, van der Knaap et al.⁴ described a syndrome of cerebral leukoencephalopathy and megalencephaly with infantile onset in eight children. Screening for inborn errors, especially those that cause either megalencephaly, white matter disease, or both,

was negative. A distinguishing feature of the present disorder was the apparently severe abnormality of the cerebral white matter as demonstrated by MRI, which contrasted with the remarkably slow course of functional deterioration. In Turkey, Topçu et al.⁵ reported the clinical and radiological findings of 12 patients in 1998. In 2000, Topçu et al.⁶ showed that the *MLC1* gene is located on chromosome 22qtel and subsequently, the *MLC1* gene was identified by Leegwater et al.⁷. The function of the *MLC1* protein is unknown.

Here, we report clinical and radiological findings of two patients with MLC in whom different mutations were identified in the *MLC1* gene. We also review the mutations of the *MLC1* gene that were identified in the Turkish population.

Case Reports

The first patient was a seven-year-old boy who was admitted for the investigation of ataxia, epilepsy and macrocephaly. He was the third child of consanguineous parents

and his elder sisters were healthy. He was born at term with normal weight, length and head circumference. Development of motor milestones was normal, but there was a delay in mental milestones. His social interaction was poor and his language development was delayed. Abnormal enlargement of the head developed from the age of six months to the age of two years. He walked without support at 18 months of age but had mild ataxia. At three years of age, he began to have generalized seizures that were easily controlled with carbamazepine treatment. A cranial computed tomography at this age revealed enlarged lateral ventricles and mild widening of the subarachnoid spaces.

Neurological examination at seven years of age showed macrocephaly with a head circumference of 62 cm (above 98th percentile). Deep tendon reflexes were hyperactive and there was a mild spasticity in his legs. He also had mild ataxia. The remainder of the neurological and physical examinations did not reveal abnormalities.

Cranial MRI examination revealed diffuse increased T2 signal of white matter and subcortical cysts in the frontal and temporal lobes (Fig. 1). Central white matter structures, including the corpus callosum and internal capsule, were spared. Ventricular enlargement was also seen. Diffusion-weighted imaging showed increased diffusion of the affected white matter.

The clinical and radiological findings suggested MLC, and the patient was demonstrated to be homozygous for a c.353C>G / p.Thr118Arg mutation in the *MLC1* gene. The same mutation was not found in >200 control chromosomes, making it unlikely to be a benign polymorphism. The parents and healthy siblings were heterozygous for the mutation.

The second patient was an eight-year-old girl admitted for the investigation of increasing gait disturbance. She was the first child of healthy consanguineous parents. She had one sibling who was completely healthy. She was born at term with normal weight, length and head circumference. There was abnormal head enlargement within the first year of life. There was mild delay in motor and mental milestones. She walked without support at 2.5 years of age, but from four years of age,

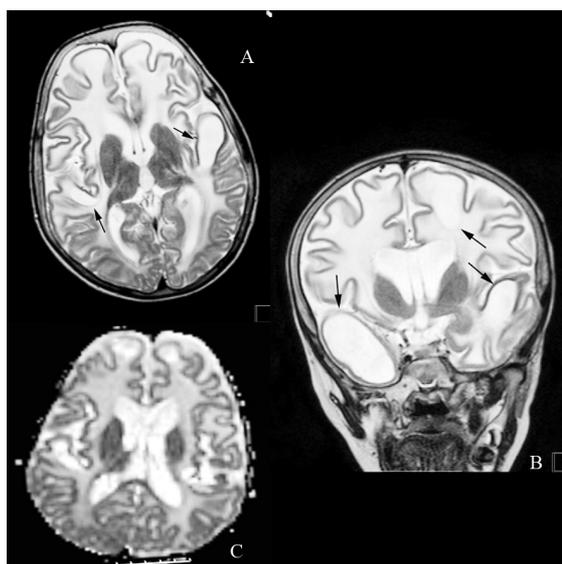


Fig. 1: T2-weighted (TR/TE: 2200/90 msec) axial (a) and coronal (b) images demonstrate high signal white matter and subcortical cysts (arrows) in the frontal and temporal lobes. Diffusion weighted imaging-ADC (apparent diffusion coefficient) map (c) shows increased diffusion in the white matter and subcortical cysts.

the gait slowly deteriorated and she began to walk on her toes.

Neurological examination at eight years of age showed macrocephaly with a head circumference of 62 cm (above 98th percentile). Deep tendon reflexes were hyperactive and there was spasticity in her legs. The remainder of the neurological and physical examinations did not reveal abnormalities.

On cranial MRI, there was increased T2 signal in the cerebral white matter, sparing the corpus callosum and internal capsule. Subcortical cysts were seen only in the bilateral temporal lobes (Fig. 2). No significant ventricular enlargement was seen. Diffusion-weighted imaging showed increased diffusion of the affected white matter. Metabolic investigations including serum and cerebrospinal fluid lactate and pyruvate, serum ammonia, urine organic acids, and lysosomal enzyme analysis revealed no abnormalities. The slow disease progression despite severe MRI findings suggested MLC. The patient was shown to be homozygous for a c.821C>T / p.Thr274Ile mutation in the *MLC1* gene. The same mutation was not found in >200 control chromosomes, making it unlikely to be a benign polymorphism. The parents and healthy sibling were heterozygous for the mutation.

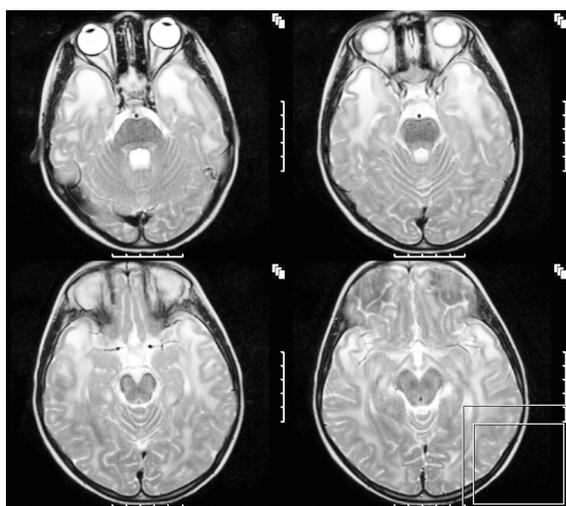


Fig. 2: T2-weighted (TR/TE: 2200/90 msec) consecutive axial images show high signal white matter and subcortical cysts in the temporal lobes.

Discussion

Megalencephalic leukoencephalopathy is an autosomal recessive disorder. It is one of the most commonly reported leukodystrophies in Turkey. In 1998, Topçu et al.⁵ reported the clinical and radiological findings of 12 patients from Turkey. Five were affected siblings and all patients had consanguineous parents. Half of these patients had a severe form and the other half had a milder variant of the disease. Twenty-five percent of the patients had epilepsy but patients' responses to antiepileptic drugs were satisfactory. Although the seizures were not an important feature of the disease, some patients had status epilepticus and epileptic encephalopathy with bilateral continuous spike waves during slow wave sleep. The clinical findings of our cases were mild. They had mild ataxia and spasticity despite severe MRI findings; the seizures of the first patient were easily controlled with appropriate antiepileptic drug.

The disease is caused by mutations in the *MLC1* gene, which is located on chromosome 22q13.33. The gene product is the MLC1 protein. It is a plasma-membrane protein, the function of which is unknown. It is located in distal astrocytic processes in perivascular, subependymal and subpial regions in the blood-brain and cerebrospinal fluid-brain barriers, in astrocyte-astrocyte contacts, and in Bergmann glia, but not in oligodendrocytes

and microglia⁸⁻¹¹. It shows some similarity with proteins of voltage-gated potassium channel. It is suggested that the MLC1 protein may be involved in the transport of ions and/or other molecules across the blood-brain barrier and cerebrospinal fluid interfaces¹². There is a close relationship between MLC1 and the dystrophin-associated glycoprotein complex; the dystrophin-associated glycoprotein complex regulates the organization and function of the MLC1 protein in astrocyte membranes^{9,10}.

A recent study, which focused on the molecular pathogenesis of MLC, showed that most *MLC1* mutants are mainly retained in the endoplasmic reticulum. Endoplasmic reticulum retention is a consequence of the misfolding of the mutant enzyme. Mutations could also impede the correct oligomerization, which may be required for endoplasmic reticulum exit. On the other hand, mutant proteins are also targeted to lysosomes for degradation after their internalization from the plasma membrane. Alternatively, mutations could simply disrupt protein structure¹².

The mutations of the *MLC1* gene are distributed along the whole gene, and splice-site mutations, nonsense mutations, missense mutations, deletions, and insertions have all been described¹³. In 20-30% of the patients with the typical findings of the disease, no mutations can be found in the *MLC1* gene. This may be due to performing standard mutation analysis or there may be other genes causing the disease. Fifty different mutations have been identified in the *MLC1* gene, and the most common are missense mutations¹³. In Turkey, missense mutations are also observed more than other types of mutations (Table I). The mutations in our patients were also missense mutations. The mutation in the first patient was described by Leegwater in 2001⁷. This was patient was also Turkish. As far as we know, the missense mutation in our second patient has not been described before. There is no evident genotype-phenotype correlation in MLC¹³. Another interesting finding is that patients with no identified mutations in the *MLC1* gene may share similar clinical findings with the patients with proven mutations¹³. On the other hand, clinical phenotypes may be different in patients with identical mutations¹³. These findings suggest that there may be other

Table I. MLC1 Mutations in the Turkish Population

Mutation type	Nucleotide change	Effect	Exon	Reference
Insertion or deletion	c.110_111insGC	p.Gly38ArgfsX20	2	Ilja Boor et al. ¹³
	c.449_455del	p.Leu150ArgfsX8	6	Leegwater et al. ⁷
	c.908_918delinsGCA	p.Val303GlyfsX95	11	Leegwater et al. ⁷ , Rubie et al. ¹⁵
Missense	c.249G>T	p.Leu83Phe	3	Leegwater et al. ¹⁶ , Patrono et al. ¹⁷
	c.278C>T	p.Ser93Leu	4	Leegwater et al. ⁷ Saijo et al. ¹⁸ , Tsujino et al. ¹⁹
	c.353C>G	p.Thr118Arg	5	Leegwater et al. ⁷
	c.353C>T	p.Thr118Met	5	Leegwater et al. ¹⁶
	c.422A>G	p.Asn141Ser	5	Leegwater et al. ¹⁶
	c.736A>C	p.Ser246Arg	9	Leegwater et al. ¹⁶
	c.821C>T /	p.Thr274Ile	10	This paper
	Nonsense splice site	c.423+6T>G	Splice defect	Intron 5
c.895-2A>G		Splice defect	Intron 10	Leegwater et al. ⁷

environmental and genetic factors that modify the severity of the disease.

Magnetic resonance imaging of the brain is an important diagnostic tool in MLC. Neuroimaging studies show signal abnormality of almost all cerebral hemispheric white matter. Central white matter structures, including the corpus callosum and internal capsule, are relatively spared. The cerebral white matter is swollen, leading to broadening of gyri. A characteristic feature of the disease is the presence of subcortical cysts in the anterior temporal and frontoparietal regions. These cysts are already present early in the disease, and may increase in size or number over the disease course. There is mild cerebellar atrophy, and the cerebellar white matter is less affected than the cerebral white matter. Diffusion studies show increased diffusion in the affected white matter. Magnetic resonance spectroscopy of the affected white matter is remarkable for low metabolite levels, particularly N-acetyl aspartate (NAA) levels¹⁴. Our patients had MRI abnormalities typical for MLC.

In conclusion, MLC is a slowly progressive disease despite severe cerebral white matter involvement and subcortical cysts. It is one of the most reported leukoencephalopathies in Turkey. Molecular genetic analysis of the *MLC1* gene should be performed for genetic counselling and prenatal molecular genetic diagnosis in subsequent pregnancies.

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