Clinical Case Reports

Open Access

CASE REPORT

Molecular prenatal diagnosis of megalencephalic leukoencephalopathy with subcortical cysts in a child from southwest of Iran

Gholamreza Shariati[1], Mohammad Hamid[2], Alihossein Saberi[3], Behnaz Andashti[4] & Hamid Galehdari[4]

[1]Narges genetic & PND Laboratory, Kianpars, East Mihan street, Ahvaz, Iran
[2]Department of Biotechnology, Pasteur Institute, Tehran, Iran
[3]Department of Genetics, Medical School of Ahvaz Jundishapur University, Ahvaz, Iran
[4]Department of Genetics, Shahid Chamran University, Ahvaz, Iran

Correspondence
Behnaz Andashti, Department of Genetics, Shahid Chamran University, Ahvaz, Iran. Tel: 986113338965; Fax: 986113338965; E-mail: b_andashti@yahoo.com

Funding Information
No funding information provided.

Received: 17 October 2013; Revised: 26 December 2013; Accepted: 23 September 2014

***Clinical Case Reports* 2015; 3(2): 114–117**

doi: 10.1002/ccr3.168

Key Clinical Message

Megalencephalic leukoencephalopathy (MLC) is a rare neurological disorder with an autosomal recessive pattern. Clinical diagnosis was based on macrocephaly, recurrent seizure, and magnetic resonance imaging (MRI). Here we report first finding of a novel homozygous single base deletion in the *MLC1* gene in an affected Iranian child causing a premature stop codon (p.L150fs.160X).

Keywords

Iranian, leukodystrophy, *MLC1* gene, novel mutation.

Research Letter

Megalencephalic leukoencephalopathy (MLC) is a rare disorder with unknown frequency. This is the first report of molecular prenatal test for a novel mutation in the *MLC1* gene from Iran.

Van der Knaap et al. described first in 1995 a case of leukodystrophy with swelling and cysts. Their observations were based on clinical and neurobiological criteria [1]. The reason for the swollen appearance of the white matter might be the myelin vacuolation affecting the outer myelin layers [2]. However, macrocephaly and cerebral white matter abnormality is a specific neurological sign without gray matter involvement [2]. Hereby, magnetic resonance imaging (MRI) can be useful tool for differential diagnosis [2]. Brain MRI (without contrast) of our patient at age of 2.5 years old showed a diffuse myelination involving subcortical U fibers (Fig. 1).

Today, the disorder is defined as MLC with subcortical cysts associated with macrocephaly as a common sign [3]. MLC is a very rare disorder with unknown frequency, although several ethic groups show more cases [3, 4]. Molecular genetics studies show that the mutation of two *MLC1* and *HEPACAM* genes as being responsible for the disease.

Pathogenic mutations in the *HEPACAM* gene (hepatic and glial cell adhesion molecule, MIM 611642) account for ~20% of individuals with improved MLC phenotype [5]. In contrast to the *MLC1* gene with exclusive autosomal recessive inheritance, some patients show monoallelic (heterozygous) *HEPACAM* mutation with dominant inheritance [5]. These individuals have macrocephaly and mental retardation with or without autistic signs [5]. Disease causing mutations in the *MLC1* gene (MIM 605908) was found in ~75% of classic MLC patients [6]. *MLC1* appear as an oligomeric membrane protein that is exclusively expressed in brain tissue [7, 8], and localized in astrocytes junctions [7, 9]. Biologically, the MLC1 is postulated to be an ion transporter, however, its exact role is still unknown [7, 10].

An Iranian family with a 3 years old affected child was referred to our laboratory for prenatal diagnosis of the

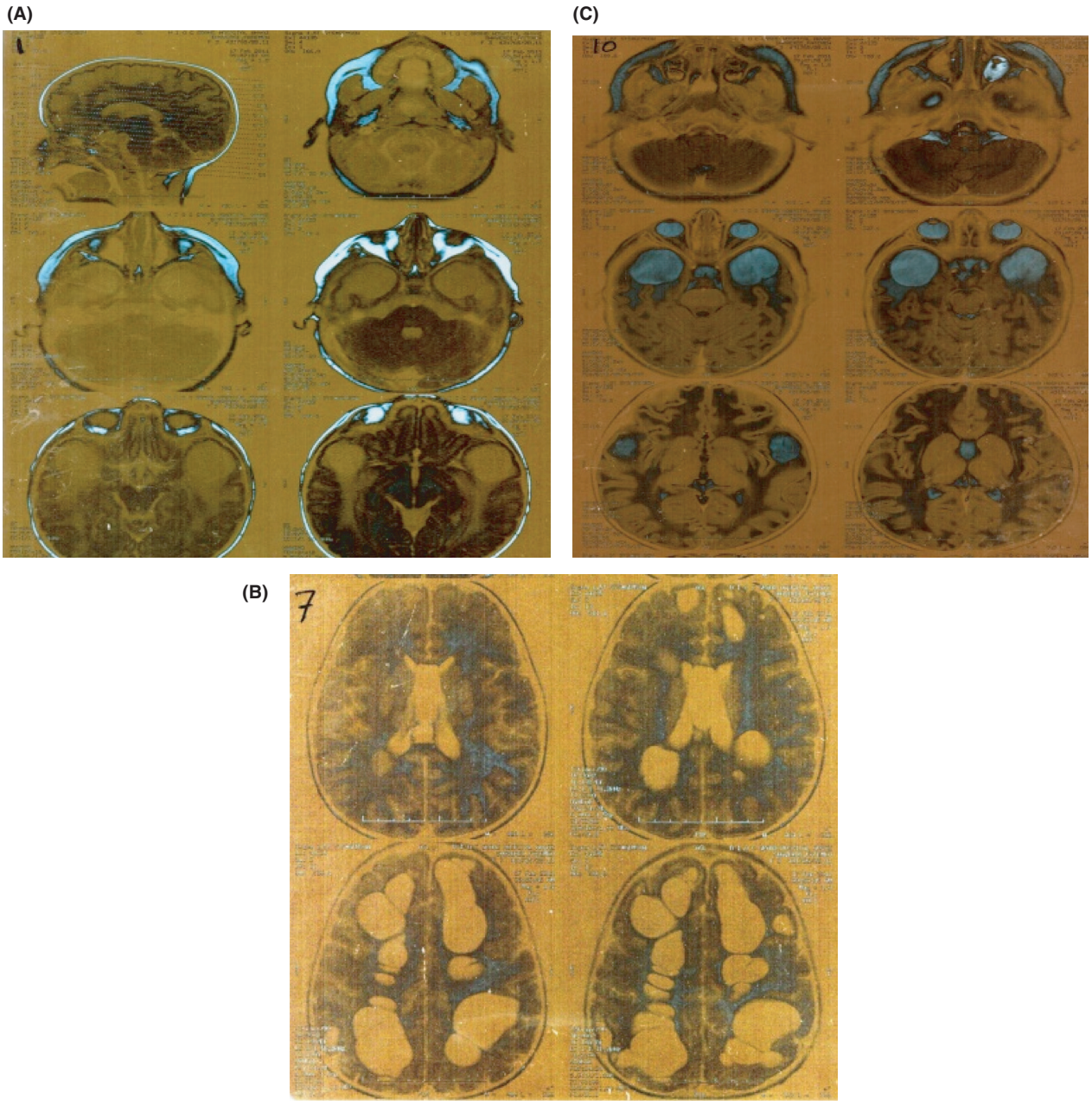

Figure 1. Brain magnetic resonance imaging (MRI) was made for the patient at the age of 2.5 showing abnormal myelination in white matter.

next child, as yet unborn. Clinical diagnosis was based on macrocephaly and recurrent seizure. MRI of patient showed white matter dystrophy with several subcortical cysts. The mother was pregnant in the 6th week. With informed consent, genomic DNA from whole blood and chorionic villous sample was extracted with routine salting out method. PCR primers to amplify exons and flanking intron sequences were designed by Primer3out software program according to the gene accession number NT_019197. Primer sequences and PCR condition are given in Table 1. To exclude maternal cell contamination, standard VNTR (variable number tandem repeat) and STR (Short tandem repeat) markers were used for parent's samples and CVS (Chorionic villus sample). Bidirectional sequencing was performed with big dye terminator V3.1 cycle sequencing kit using an Applied Biosystem 3130 genetic analyzer (ABI Newyork USA).

As it has been illustrated in figure 2 a novel homozygous single base deletion at codon 150 was found in the affected child causing a premature stop codon

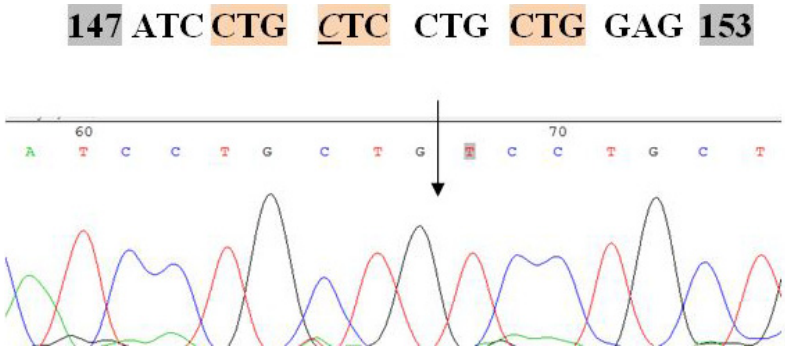

Figure 2. Partial sequence of the megalencephalic leukoencephalopathy 1 (*MLC1*) gene shows homozygous deletion of a cytosine at codon 150 that has been detected in affected child.

Table 1. Primer sequences of the coding exons and flanking intron sequences with appropriate PCR product length and annealing temperatures.

Exon	Sequence	Tm (C)	Size (bp)
X2-F	AAGTTGCCGATGGATGTTGT	60.38	168
X2-R	tttgaagaagaaaatgagcacttg	59.93	
X3-F	gtttcctcagagtggccaaa	60.23	336
X3-R	gtcaccagagggaccagatg	60.53	
X4-F	ctggaagcgcaaatgttaga	59.06	199
X4-R	acactgtctgtcagcccctc	60.32	
X5-F	gaatggcctgaagtgtggtt	59.97	227
X5-R	ctgtgggtgtcaggcgtc	61.38	
X6-F	gtccggtggacgctgaag	62.89	229
X6-R	cctggggtgatgcctctg	62.71	
X7-F	gcagtgctgagtccctgtg	60.63	203
X7-R	acgtgacgtttaatccagcc	60.00	
X8-F	ctccacttccttatgagccg	59.83	251
X8-R	tgaatgcaccaagactgagc	59.99	
X9-F	ttggaattcgacttcttcgac	59.30	192
X9-R	caccaagggagggctagg	60.60	
X10-F	aaaaggcagaggtttcagca	59.99	324
X10-R	agagcaccacatgtctggg	59.68	
X11-F	gagggagctttggtctcctg	61.30	277
X11-R	ccactcacctccccagtg	60.08	
X12-F	tggccctggtgaagtaacac	60.95	457
X12-R	TGAGAGAGGCAGGAAGAGGA	60.21	

Exon 1 is noncoding. The same primers were used for direct sequencing of PCR products.

(p.L150fs.160X). Consanguine parents were heterozygous for the mentioned change. To see whether this mutation is pathogenic, we checked this point mutation in 35 healthy controls. None of 35 normal genomes showed this mutation. After molecular confirmation of the disease, we performed molecular prenatal diagnosis in the 11th week of pregnancy on DNA extracted from CVS. The fetus was heterozygous and also a carrier for the novel deletion. We also advised the family to continue the pregnancy.

This is the first molecular diagnosis report of the MLC in southwest of Iran that extend the mutation spectrum of the *MLC1* gene. Because of the involvement of second gene in the pathogenicity of MLC disorder and because of our finding, we suggest considering the *MLC1* gene as the first choice for molecular screening of patients, at least for Iran and the Middle East. Systematic screening of high-risk pregnancies of some inherited disorders such as alpha and beta thalassemia have been done successfully for two decades in Iran. But other abnormalities are not followed, consequently. We showed here the feasibility of prenatal diagnosis of some difficult cases in relatively short time, like the present case.

Conflict of Interest

None declared.

References

1. van der Knaap, M. S., P. G. Barth, H. Stroink, van Nieuwenhuizen, O., W.F., Arts, et al. 1995. Leukoencephalopathy with swelling and a discrepantly mild clinical course in eight children. Ann. Neurol. 37:324–334. [PubMed: 7695231]
2. van der Knaap, M. S., P. G. Barth, G. F. Vrensen, and J. Valk. 1996. Histopathology of an infantile-onset spongiform leukoencephalopathy with a discrepantly mild clinical course. Acta Neuropathol. 92:206–212. [PubMed: 8841668]
3. Leegwater, P. A., P. K. Boor, B. Q. Yuan, J. van der Steen, A Visser, A. A. Konst, et al. 2002. Identification of novel mutations in MLC1 responsible for megalencephalic leukoencephalopathy with subcortical cysts. Hum. Genet. 110:279–283. PubMed: [11935341]
4. Montagna, G., O. Teijido, E. Eymard-Pierre, K Muraki, B. Cohen, A. Loizzo, et al. 2006. Vacuolating megalencephalic leukoencephalopathy with subcortical cysts: functional

studies of novel variants in MLC1. Hum. Mutat. 27:292. [PubMed: 16470554]
5. López-Hernández, T., M. C. Ridder, M. Montolio, X. Capdevila-Nortes, E. Polder, S. Sirisi, et al. 2011. Mutant GlialCAM causes megalencephalic leukoencephalopathy with subcortical cysts, benign familial macrocephaly, and macrocephaly with retardation and autism. Am. J. Hum. Genet. 88:422–432. PMC free article: [PMC3071909] [PubMed: 21419380]
6. Ilja Boor, P. K., K. de Groot, V. Mejaski-Bosnjak, C. Brenner, M.S. van der Knaap , G.C. Scheper, J. C. Pronk, et al. 2006. Megalencephalic leukoencephalopathy with subcortical cysts: an update and extended mutation analysis of MLC1. Hum. Mutat. 27:505–512. [PubMed: 16652334]
7. Teijido, O., A. Martínez, M. Pusch, A. Zorzano, E. Soriano, J. A. Del Río, et al. 2004. Localization and functional analyses of the MLC1 protein involved in megalencephalic leukoencephalopathy with subcortical cysts. Hum. Mol. Genet. 13:2581–2594. [PubMed: 15367490]
8. Boor, P. K. I., K. de Groot, Q. Waisfisz, W. Kamphorst, C.B. Oudejans, J.M. Powers, et al. 2005. MLC1: a novel protein in distal astroglial processes. J. Neuropathol. Exp. Neurol. 64:412–419. [PubMed: 15892299]
9. Teijido, O., R. Casaroli-Marano, T. Kharkovets, F. Aguado, A. Zorzano, M. Palacín, et al. 2007. Expression patterns of MLC1 protein in the central and peripheral nervous systems. Neurobiol. Dis. 26:532–545. [PubMed: 17434314]
10. Kaganovich, M., A. Peretz, M. Ritsner, U. Bening Abu-Shach, B. Attali, R. Navon, et al. 2004. Is the WKL1 gene associated with schizophrenia? Am. J. Med. Genet. B. Neuropsychiatr. Genet. 125B:31–37. [PubMed: 14755440]

