

CASE SERIES

Iran J Allergy Asthma Immunol
February 2014; 13(1):61-65.

Prenatal Diagnosis of Leukocyte Adhesion Deficiency Type-1 (Five Cases from Iran with Two New Mutations)

Behnaz Esmacili¹, Mohsen Ghadami², Mohammad Reza Fazlollahi¹, Shirin Niroomanesh³, Lida Atarod⁴,
Zahra Chavoshzadeh⁵, Zeinab Moradi¹, Zahra Alizadeh¹, and Zahra Pourpak^{1,6}

¹ Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

² Endocrinology and Metabolism Research Institute (EMRI), Tehran University of Medical Sciences, Tehran, Iran

³ Department of Prenatology, Zanan Hospital, Tehran University of Medical Sciences, Tehran, Iran

⁴ Department of Pediatrics, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran

⁵ Pediatric Infection Research Center, Mofid Hospital, Shaheed Beheshti University of Medical Sciences, Tehran, Iran

⁶ Department of Allergy and Clinical Immunology, Children's Medical Center,
Tehran University of Medical Sciences, Tehran, Iran

Received: 10 March 2013; Received in revised form: 20 April 2013; Accepted: 25 May 2013

ABSTRACT

Leukocyte adhesion deficiency type-1(LAD-1) is one of the autosomal recessive immunodeficiency diseases that results from mutation in integrin beta 2 (ITGB2) gene. The aim of this study was to investigate molecular prenatal diagnosis of LAD-1. Four pregnant women with five fetuses (one twin fetus) with clinical and laboratory diagnosis of LAD-1 in their previous children were studied. The chorionic villus sampling (CVS) was obtained when mothers were in 10-12th weeks of gestation.

Mutation analysis of ITGB2 gene for affected children revealed 3 missense mutations (c.382G>A, a novel mutation, c.2146G>C, and c.715G>A) and one splice site novel mutation (c.1877+2T>C). All parents were heterozygous for these mutations. Consideration of affected gene regions for five CVS samples showed two homozygotes and one heterozygote for mutant allele and two homozygotes for normal allele. Interestingly, one of the twin fetuses was affected and another was normal. Briefly, two cases of CVS samples were affected and three cases of remained CVS samples were unaffected.

This is the first report of prenatal diagnosis of LAD-1 from Iran with two new mutations that can be used for genetic and prenatal diagnosis for all patients suspected to LAD1 and can be helpful to prevent the birth of affected children with LAD-1.

This abstract was presented in the Second International Congress of Immunology, Asthma and Allergy, Tehran, Iran 2013.

Key word: CVS; LAD-1; Prenatal Diagnosis

Corresponding Author: Zahra Pourpak, MD, PhD;
Immunology, Asthma and Allergy Research Institute, Tehran

University of Medical Sciences, Tehran, Iran.Tel: (+98 21) 6691
9587, Fax: (+98 21) 6642 8995, E-mail: Pourpakz@tums.ac.ir

CASE REPORTS

Leukocyte adhesion deficiency type- 1 (LAD-1) is one of the primary immunodeficiency disorders with autosomal recessive pattern of inheritance that results from defects in ITGB2 gene which codes integrin β 2 subunit (CD18 antigen).^{1,2} LAD-1 has been described in more than 300 patients worldwide.² The length of the ITGB2 gene is over 40 kb of genomic DNA at chromosome 21q22.3 that contains 17 exons. Different types of mutations cause defects in expression of CD18 on leukocytes and sometimes affect the protein function too.³ For recognized diseases causing mutations, prenatal diagnosis can be suggested. One of prenatal options available for at risk families is chorionic villus sampling (CVS) that can be implemented during the first trimester.^{4,5}

Here, we report genetic result of five CVS samples from high risk couples for LAD-1 who had previously affected children and were registered in Iranian Primary Immunodeficiency Registry (IPIDR)⁶

MATERIALS AND METHODS

Patients

This study included five CVS samples from four women with one previously-affected child with diagnosis of LAD-1. They were referred to the Immunology, Asthma and Allergy Research Institute (IAARI), between January 2010 and December 2012. History was taken and physical examination was done. For confirmation of the diagnosis of LAD-1 in affected children, the screening tests for primary immunodeficiency (complete blood count (CBC), Nitro Blue Tetrazolium (NBT), Total hemolytic complement activity (CH50), Immunoglobulin A (IgA), Immunoglobulin M (IgM), Immunoglobulin G (IgG), Immunoglobulin E (IgE))⁷ and flow cytometric analysis of β 2 integrin families (CD11a, CD11b, CD11c and CD18) on neutrophils were done. After genetic counseling and taking informed consent from patients' parents, 2 ml whole blood sample in EDTA was obtained from each patient and their parents and saved in the DNA bank of IAARI. DNA was isolated using high pure extraction kit (Roche, Germany). Genetic study of the affected children was verified by genetic results of parents. For finding suspected mutations in fetuses, Chorionic villus sampling (CVS) was performed at weeks 10 to 12 of gestation. Genomic

DNA of each CVS sample was isolated by specific DNA extraction kit (Qiagen, USA).

The β 2 integrin family expression was evaluated using two-color staining of polymorphonuclear (PMN) cells. Leukocytes in whole blood were incubated 30 min on ice with FITC mouse anti-Human CD11a (LFA-1), PE mouse anti-human CD11b (Mac-1), PE mouse anti-human CD11c (p150-95) and FITC mouse anti-human CD18 (integrin β 2). After incubation, Red Blood Cells (RBCs) were removed using RBC lysing solution (Becton Dickinson). The expression of markers was analyzed using FACStar plus flow cytometer (Becton Dickinson, USA) on gated PMNs. All of conjugated monoclonal antibodies were purchased from Becton Dickinson Company.

All of coding regions of the ITGB2 gene were examined by direct sequencing of genomic DNA.

This study was approved by Research Committee and Ethics Committee of IAARI.

All of fetuses were from consanguineous parents. There were not any family history of immunodeficiency, autoimmunity, malignancy, recurrent infections and abortion in their family.

Family One

This family had a 1.5 year-old girl who was born in 2008. The first complaint was delayed cord separation not until the 21st day after birth and omphalitis from 8th day of birth. The other manifestations were recurrent skin ulcers with poor healing, recurrent gastrointestinal infections (diarrhea and vomiting) with positive Ecoli in stool culture. Result of flow cytometric analysis showed a defect in CD11 and CD18 expression (Table 1). She received Imipenem, Vancomycin and Metronidazole for treatment and debridement of wounds was performed when necessary and finally, with genetic diagnosis of LAD-1, hematopoietic stem cell (HSCs) transplantation was done successfully. Her mother was referred to IAARI when she was pregnant at 10th week of gestation for prenatal diagnosis.

Family Two

This family had a two year-old boy, who was born in 2008. His umbilical cord was separated on the 9th day after birth. His symptoms started on the 27th day after birth and he was hospitalized for severe respiratory infections and otitis media (as the

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first manifestations). Result of flow cytometric analysis showed a defect in CD11 and CD18 expression (Table 1). After genetic study, he was referred to HSC transplantation and unfortunately HSC transplantation could not be done because of unavailability of any matched donors. His mother was referred to IARRI when she was pregnant at 10th week of gestation for prenatal diagnosis.

Family Three

This family had four children. The third one was a boy who was born in 1997. His symptoms started when he was 4 years old. His umbilical cord was normally separated. Recurrent and infectious ulcers without improvement were his main complaints.

He also had history of one skin graft rejection. The other symptoms were recurrent infections and otitis. HSC transplantation was done successfully. His mother was referred to IARRI when she was pregnant at 10th week of gestation for prenatal diagnosis.

Family Four

This family had one affected boy and one healthy boy. The affected child was born in 2003 and died after transplantation. His manifestations were delayed umbilical cord separation as the first symptom, skin infections and osteomyelitis. His mother was referred to IARRI for molecular investigation for twin fetuses at 10th week of gestation.

Table 1. Laboratory findings and characterization of CD11/CD18 markers on neutrophils of affected children.

Test	Patient 1	Patient 2	Patient 3	Patient 4
	<2 yr	<2 yr	6-10 yr	2-6 yr
WBC*	28300	38800	26300	14300
Normal range	6000-17000	6000-17000	4500-13500	5000-15500
ANC*	10000	25000	18000	7500
Normal range	1500-8500	1500-8500	1500-8500	1500-8500
CH50	140	102	152	-
Normal range	70-160	70-160	70-160	
IgA**	158	38	366	10
Normal range	14-123	14-123	45-236	25-154
IgG**	1254	968	2793	283
Normal range	424-1051	424-1051	608-1572	463-1236
IgM**	259	260	260	42
Normal range	48-168	48-168	52-242	43-196
IgE**	24	126	9.6	2.7
Normal range	0.3-29	0.3-29	0.9-57	1-68
NBT	100%	100%	100%	100%
Normal range	>90%	>90%	>90%	>90%
CD11a%	11	3.5	1	11
Normal range	>90%	>90%	>90%	>90%
CD11b%	1.5	3	12	58
Normal range	>90%	>90%	>90%	>90%
CD11c%	5	0	5	18
Normal range	>90%	>90%	>90%	>90%
CD18%	40	10	10	8
Normal range	>90%	>90%	>90%	>90%
Genetic Results	p.Asp128Asn	p.Gly716Arg	c.1877+2T>C	p.Ala239Thr

*: Normal Range from Chapter 14, Ahsan S, Noether J. Hematology. In: Tschudy MM, Arcara KM, editors. The Harriet Lane Handbook. USA: Elsevier Mosby, 2010: 322-353.

** : Normal Range from Chapter 15, Kim W. Immunology and Allergy. In: Tschudy MM, Arcara KM, editors. The Harriet Lane Handbook. USA: Elsevier Mosby, 2010: 354-369 (IgG, IgA, IgM mg/dl and IgE unit/ml).

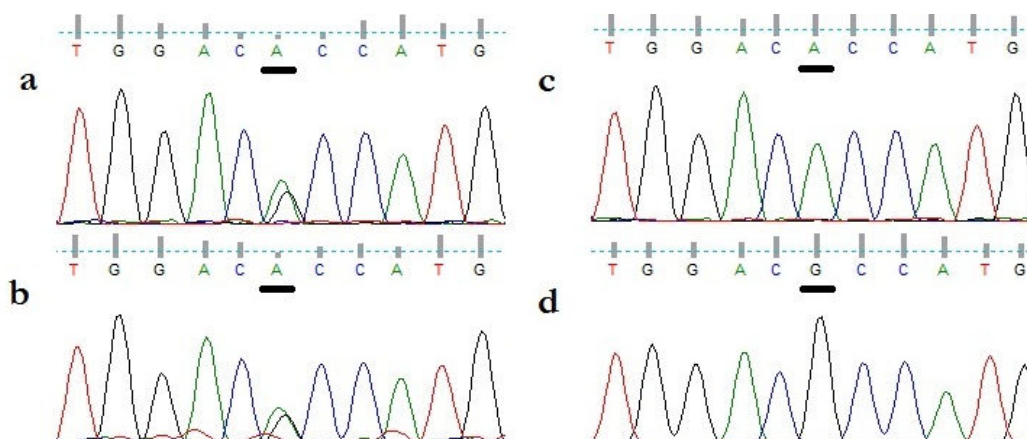


Figure 1. Sequence analysis of c.715G>A mutation in family 4, **a.** mother of twins **b.** father of twins **c.** affected case (one of the twins) **d.** non-affected case (the other one of twins)

Laboratory Findings

A significant increase in WBC and Absolute neutrophil count (ANC) was found in affected children in all four families and all of them had defects in CD11 and/or CD18 markers.

Laboratory findings and CD marker analysis of four patients are shown in table 1.

Mutation Analysis Results

For patient 1, a missense mutation c.382G>A at exon 5 was found that changed p. Asp128Asn. Her parents were heterozygous at this position. The fetal genotype on CVS sample was considered for affected genomic region. Results showed homozygote mutation alleles that verified the affected status. For patient 2, a novel missense mutation c.2146G>C at exon 15 was found which changed Gly716Arg in ITGB2 protein. His parents were carriers. The genetic testing on the CVS sample demonstrated only one mutant allele (heterozygote) that indicated the unaffected status of fetus. For patient 3, a novel splice site mutation c.1877+2T>C was detected at intron 13 of ITGB2 gene. The parents were carriers. The affected region was investigated for CVS sample that revealed existing of normal allele. For patient 4, a missense mutation c.715G>A at exon 6 was found that changed p. Ala239Thr in ITGB2 protein. The parents were heterozygous. Investigation of affected region in CVS samples of twin fetuses revealed one homozygote for normal allele and one homozygote for mutant allele. (Figure 1)

DISCUSSION

IAARI has followed the diagnosis and treatment of LAD-1 patients from March 2001^{8, 9} and has started the investigation of molecular basis of the LAD-1 since 2010 and prenatal diagnosis for high risk families for LAD-1 has been performed parallel to this project.

Previously Parvaneh et al.¹⁰ described clinical presentation and genetic results of 11 Iranian patients of LAD-1 in 2010; this is the first report of prenatal diagnosis with two new mutations from Iran.

Over 60 different mutations for ITGB2 in LAD-1 have been reported up to now (bioinf.uta.fi/ITGB2base). Heterogeneity in the CD18 mutations among LAD-1 patient's results in variance in clinical features of this disorder.¹ Our findings showed two new mutations in the ITGB2 gene.

The extracellular domain of the CD18 was divided into four regions: PIS (plexin, semphorin and integrin), highly conserved region (HCR) (may suppose a "I like domain") a mid- region that links the HCR to forth region that is named CCR (the cysteine rich region).¹ High percentage of $\beta 2$ integrin mutations occur within extracellular domain of the CD18 that is a highly conserved domain and is coded by exons 5-9.³

One of the mutations (p.Asp128Asn) that we found was located in exon five (VWFA domain). This mutation has been reported by Matsuura et al. in two Japanese patients with severe form of LAD-1 in 1992.¹¹ Considering the law of the medical genetic ethics in Iran,¹² mother was referred to forensics for guidance

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about abortion. We found another mutation in exon 15 in family two. Follow up of this case confirmed the healthy status of baby after birth. The third mutation targeted the splice donor site of intron 13. Studies have shown that this region was important for gene expression. Follow up of this case confirmed the healthy status of baby after birth. One of the twins in family four had mutation in exon 6. After finding the mutation the mother was referred for guidance about abortion the affected one.

Mutation analysis of parents revealed that all couples were heterozygotes. Investigation of the affected region for five fetuses demonstrated affected status for CVS sample from family one and unaffected status for two fetuses from families two and three, for family four our results revealed one affected and one unaffected status for CVS samples.

Understanding of underlying mutation in ITGB2 is very important for genetic counseling and DNA based prenatal diagnosis. Evaluation of the fetal genotype by mutation based DNA analysis during the first trimester of gestation can reduce the concern and undetermined condition of the parents.⁴

ACKNOWLEDGEMENTS

The authors would like to appreciate Ms Parisa Dashti and Mr. Mohsen Badalzadeh for technical assistance. This study was supported by Immunology, Asthma and Allergy Research Institute in Tehran University of Medical Sciences.

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