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Case Report

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Detection of leukocyte adhesion deficiency type 1 in an infant by high-throughput targeted exome sequencing

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Abstract

Leukocyte adhesion deficiency type 1 (LAD-I) characterized by immune-deficiency and leukocytosis is rare in infant patients. A 43-day-old boy with severe leukocytosis, recurrent infections, defective wound healing and hepatosplenomegaly associated with an acquired cytomegalovirus infection. To establish the diagnosis definitively, a high-throughput targeted exome sequencing was performed, which yielded the diagnosis of LAD-I. A homozygous mutation in integrin subunit beta 2 (*ITGB2*), c.817G>A (p.G273R) was identified. Though LAD-I has been thoroughly-studied, with more than 300 detailed cases and 96 mutations in *ITGB2*, establishing a definitive diagnosis of LAD-I in infancy is challenging because of the lack of typical clinical presentations. Better understanding the molecular characterization of this disease is necessary to increase awareness and identification of infants with LAD-I.

Keywords: Immunodeficiency, leukocyte adhesion deficiency type 1, leukocytosis, *ITGB2* gene, high-throughput targeted exome sequencing

INTRODUCTION

Leukocyte adhesion deficiency type 1 (LAD-I)[1] (MIM 600065) with an occurrence of 1 in every 100,000 live births is an autosomal recessive leukocyte recruitment deficiency. It is characterized by recurrent bacterial infections, poor wound healing, and delayed umbilical cord separation. Mutations have been found in integrin subunit beta 2 (*ITGB2*) gene[2] that is located at 21q22.3 (NM_000211), which leads to the associated heterogeneous clinical spectrum of this disorder.

Since some patients with severe forms die of overwhelming infection at a young age, proper, timely diagnoses become very critical. Although the diagnosis of LAD-I is based on typical clinical presentation, combined with laboratory demonstration of leukocytosis and reduction of CD18 expression, the precise molecular characterization is required for diagnosis confirmation. Recently the use of high-throughput targeted exome sequencing (TES)[3] has resulted in faster sample turnaround time and more cost-effective analysis of the causative mutations.

In this paper, we reported a rare case of a 43-day-old boy referred to our facility for severe leukocytosis, who responded poorly to antibiotic therapy, to highlight the importance of molecular testing to definitively establish the diagnosis when LAD-I is suspected. Moreover, the early diagnosis of immunodeficiency is essential for optimal management such as hematopoietic stem cell transplantation (HSCT) and rehabilitation outcomes.

CASE REPORT

The young patient's family is of Chinese Han ethnicity; a male baby, the second neonate of unrelated parents delivered via cesarean section after an uneventful full-term pregnancy. The proband's birth weight was 3000 g. A review of the family history revealed that his older sister had passed away at the age of 3 months of sepsis with no response to various second-line and third-line antimicrobials. The patient's condition was initially observed when he suffered a fever at the age of 25 days after birth; diagnosed then by his neonatologist as systemic inflammatory response syndrome (SIRS) in a local hospital. Laboratory tests revealed leukocytosis with neutrophil predominance [83,550 (10^9/L), 70.7%] and high C reactive protein (CRP) (69 mg/L). Intravenous (IV) antibiotics therapy (vancomycin combined with meropenem) was started and maintained for 18 days, with a good clinical response.

Five days later, he was referred to our neonatal department because of hyperthermia and marked neutrophilia. Physical examination upon admission reported a well-developed infant, with head circumference of 34 cm and weighing 4140 g. Hepatosplenomegaly was noted, with liver span of 5 cm and spleen 3 cm below costal margin. The levels of serum inflammatory markers [Table 1] were abnormal, but cultures for bacteria and fungi were negative. Further laboratory investigations showed elevated aminotransferase and high cytomegalovirus (CMV) PCR titers in his urine (positive) and plasma ($3.9*10^4$ copies/mL) samples.

After receiving the treatment of intravenous immunoglobulin (IVIG), second-line and third-line antimicrobials in the form of cefepime, teicoplanin teiculine and cephalosporins and IV ganciclovir (10 mg/kg/d for 14 days and 5 mg/kg/d for 7 days), he was discharged after three weeks with no fever, declined white cell count and CMV titers and normal liver function. He continued to receive oral ganciclovir (5 mg/kg/d) treatment after discharge.

From then on the patient experienced recurrent upper respiratory tract infections and was admitted to our hospital with two episodes of deep infections coupled with hyperthermia and marked neutrophilia [Table 1]. At 2 months of age, a 2.5 cm × 3 cm erythematous skin area surrounding the umbilicus without pus or foul odors and tenderness to palpation around the umbilicus was noted. The ultrasound of the umbilical cord showed an infected urachus. The patient was treated with surgery debridement and resection of his urachus. At the same time, he received IV cefatriaxone, teicoplanin and ganciclovir (5 mg/kg/d). Five days later, his umbilicus had nearly returned to normal. He completed 20 days of antibiotic therapy before and after his surgery. At 4 months of age, he developed bronchopneumonia and showed neutrophilia with elevated CRP [Table 1]. Empiric antibiotic therapy was started after his admission and adjusted in form of cefmetazole, cefepime, tienam, meropenem, linezolid, fluconazole and teicoplanin based on those infection indexes. He was treated with IVIG again and IV ganciclovir (5 mg/kg/d) for 2 weeks. He recovered from his cough soon

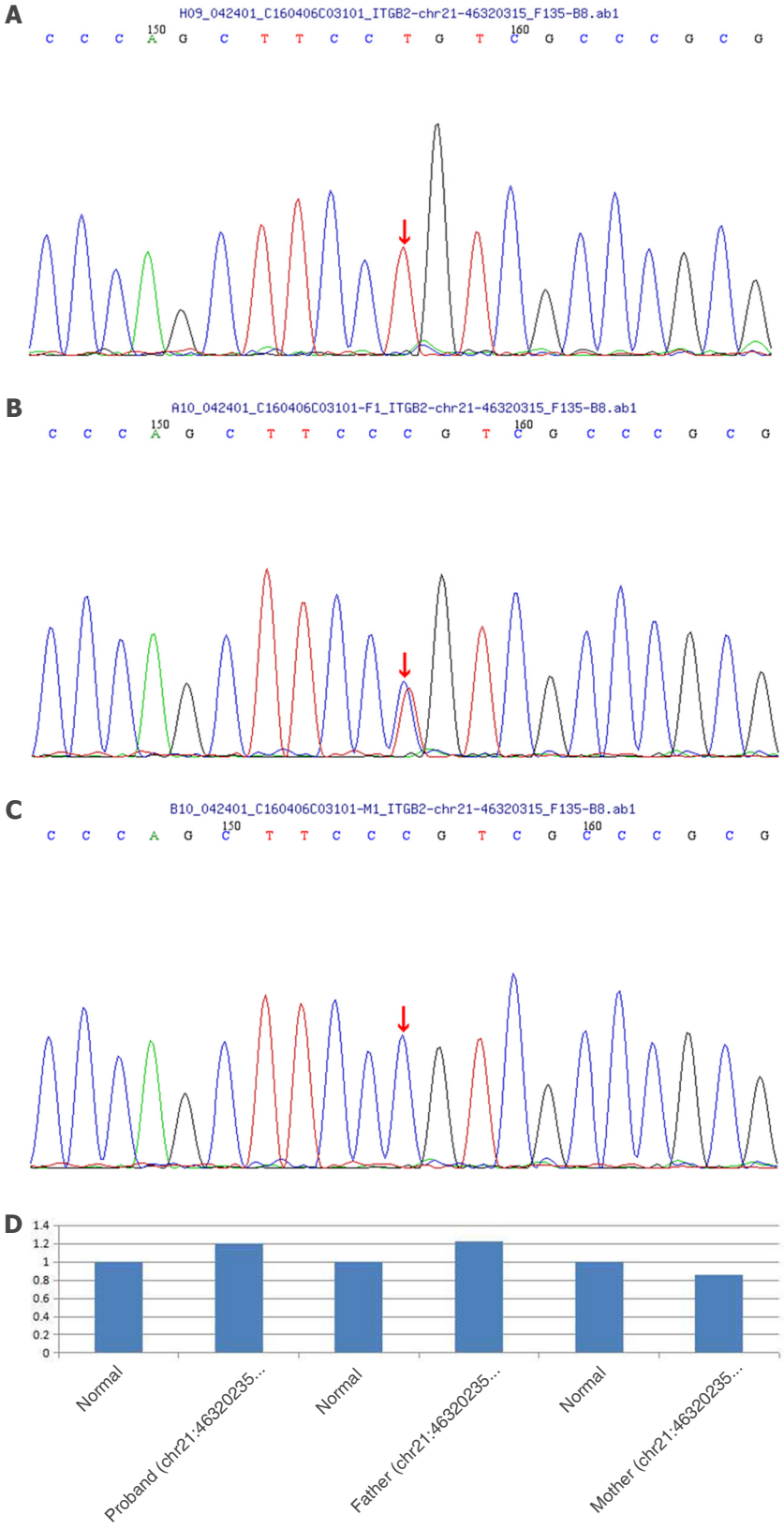

Figure 1. A: The sequencing exposes a homozygous mutation c.817G>A (p.G273R) in *ITGB2* gene in the proband; B: his father carries the mutation; C: his mother does not carry the mutation; D: normal is the control of QPCR for chr21:46320235-46320435, showing that the mother is normal

Table 1. Hematological parameters, CRP and PCT

Date	Hct (%)	Hb (g/L)	WCC (10^9/L)	PMN (%)	Ly (%)	Mo (%)	Platelets (10^9/L)	CRP (mg/L)	PCT (ng/mL)
2016/3/4	32.6	108	46.37	50.6	39.9	8.9	329	14	0.29
2016/3/6	26.2	85	26.8	47.5	38.5	12.4	282	21	
2016/3/9	30.60	101	28.3	47.5	41.8	8.7	351	< 8	
2016/3/12	25.7	85	32.28	56.7	35	7.2	396	21	
2016/3/13	28.8	96	29.22	59.2	36.4	4.2	394	9	
2016/3/15	29.6	97	24.51	62.6	30.9	5.1	445	< 8	
2016/3/18	32.2	106	29.48	59.3	32.5	7.2	457	29	
2016/3/21	28	90	15.15	41.2	49.8	7.2	429	< 8	
2016/3/22	25.1	84	16.5	47.9	43.8	7.0	427	< 8	
2016/4/1	25.1	82	22.59	54	35.1	10	361	13	0.13
2016/4/3	25.6	84	19.72	49.5	41.7	7.3	341	< 8	
2016/4/6	29.2	98	27.43	56.2	34.1	8.9	301	10	
2016/4/10	29.9	97	35.1	61.2	30	7.9	342	10	
2016/4/12	28.5	90	22.75	53.7	37	8.8	341	9	
2016/4/15	25.1	83	34.6	59.5	32.4	7.7	356	61	
2016/4/18	26.9	89	17.15	39.5	48.7	9	382	28	
2016/4/20	26.2	86	13.87	27.7	60.9	9.3	306	< 8	
2016/5/19	28.1	91	37.8	62.4	26.5	9.1	354	75	0.18
2016/5/23	30.1	101	60.35	68.2	22.2	8	403	70	
2016/5/26	27	88	41.93	66.6	23.2	6.6	371	53	
2016/5/31	27.6	93	49.65	71.5	22	5.5	406	55	
2016/6/3	27	91	57.71	78.6	14.1	7.2	430	110	0.17
2016/6/6	27.1	90	26.57	58.5	33.5	6.7	417	60	
2016/6/9	22.1	75	30.85	73.2	21.8	3.6	436	42	
2016/6/11	30.5	102	34.61	63.8	29.8	4.3	422	50	

CRP: C reactive protein; PCT: procalcitonin; Hct: haematocrit; Hb: haemoglobin; Ly: lymphocytes; Mo: monocytes; PMN: polymorphonuclear neutrophils; WCC: white cell count

after; his body temperature fluctuated from 37.5 to 38.5 ℃ and it returned to normal after 3 weeks, then the patient was discharged with liver span of 3 cm, spleen palpable 2 cm below the left costal margin.

Since a thorough history, physical examination, and laboratory workup failed to identify a clear etiology of this extremely abnormal case of leukocytosis, genetic analysis was initiated to find the cause. We performed TES of the immune disease panel (designed by MyGenostics, Beijing, China) on the pedigree. A total of 232 genes associated with defects in neutrophil chemotaxis and phagocytosis were selected using a gene capture strategy with a GenCap custom exome enrichment kit (MyGenostics, Beijing, China). In *ITGB2* gene, a previously described mutation c.817G>A (p.G273R) was found in a homozygous state [Figure 1A]. This mutation co-segregated with the phenotype in the family. And a pedigree study showed that his father was a carrier of c.817G>A (p.G273R) mutation [Figure 1B], while his mother showed no variant [Figure 1C]. Furthermore the same allele was configured by qPCR to eliminate the possibility that his mother had a large fragment deletion involving this position [Figure 1D].

DISCUSSION

LAD-I is a rare immunodeficiency. HSCT remains the only curative option for these children in that conventional therapy with antibiotics and immunoglobulin transfusions only improving the symptoms, but not correcting the disease. The possibility of LAD-I should be raised with the existence of some typical clinical presentations, including delayed cord separation, recurrent severe infections from birth onward, e.g., repeated soft tissue infections, chronic ulcers of the skin and mucous membranes and strong leukocytosis, especially neutrophilia, during periods of infection. Even though flow cytometer (FCM)[4] provides a tool for its diagnosis, it is not available in many hospitals. Genetic analysis is an effective alternative for diagnosis

Table 2. Summary of ITGB2 mutations in LAD-I patients

No.	Incidence	Location	Mutation	Mutation type	LAD-I phenotype*
1[11]	F	Exon 2	c.-721_58+51del833	Deletion	LAD-I^0
2[12]	F	Exon 2	c.2T>A (p.Met1Lys)	Missense	LAD-I$^-$
3[13]	-	Exon 2	c.49delC	Deletion	LAD-I^0
4[14]	F	Intron2	c.59-10C>A	Splice	LAD-I^0
5[15]	-	Exon 2	c.66-67delTC	Deletion	LAD-I^0
6[13]	-	Exon 3	c.77dupC (p.Lys27GlufsX32)	Insertion	LAD-I^0
7[16]	F	Exon 3	c.79A>T(p.Lys27X)	Nonsense	LAD-I^0
8[13]	-	Exon 3	c.106T>A (p.Cys36Ser)	Missense	LAD-I^0
9[13]	-	Exon 3	c.120delG (p.Gly42AlafsX7)	Deletion	LAD-I^0
10[13]	-	Exon 3	c.130A>C (p.Thr44Pro)	Missense	LAD-I^0
11[13]	-	Exon 4	c.184T>C (p.Cys62Arg)	Missense	-
12[13]	-	Exon 4	c.186C>A(p.Cys62X)	Nonsense	-
13[17]	S	Exon 4	c.190_200delGGCCCGGCTG	Deletion	LAD-I^0
14[11]	F	Exon 4	c.199C>T(p.Gln67X)	Nonsense	LAD-I^0
15[13]	-	Exon 4	c.268delG (p.Asp90ThrfsX13)	Deletion	LAD-I$^-$
16[18]	S	Exon 4	c.295G>A*(p.Asp77Asn)	Missense	-
17[19]	-	Exon 4	c.314T>C (p.Leu105Pro)	Missense	LAD-I$^-$
18[20]	F	Intron 4	c.328+1G>A	Splice	LAD-I^0
19[21]	S	Intron 4&Exon5	c.329-37_461del169	Deletion	LAD-I^0
20[22]	S	Intron 4	c.329-6C>A	Splice	LAD-I^0
21[22]	S	Exon 4	c.382G>T (p.Asp128Tyr)	Missense	LAD-I$^{0/-}$
22[23]	S	Exon 4	c.382G>A (p.Asp128Asn)	Missense	LAD-I^0
23[24]	S	Exon 5	c.392A>C (p.Tyr131Ser)	Missense	LAD-I^0
24[25]	F	Exon 5	c.400G>A (p.Asp134Asn)	Missense	-
25[26]	S	Exon 5	c.412T>C (p.Ser138Pro)	Missense	LAD-I$^+$
26[27]	F	Exon 5	c.446T>C(p.Leu149Pro)	Missense	LAD-I$^-$
27[13]	-	Exon 5	c.449G>A (p.Gly150Asp)	Missense	LAD-I$^+$
28[11]	F	Intron 5	c.500-12T>G	Splice	LAD-I^0
29[27]	-	Exon 5	c.505G>A(p.Gly169Arg)	Missense	LAD-I^0
30[27]	-	Exon 6	c.517T>C(p.Leu149Pro)	Missense	-
31[13]	-	Exon 6	c.520A>G(p.Lys174Glu)	Missense	LAD-I^0
32[28]	-	Exon 6	c.533C>T (p.Pro178Leu)	Missense	LAD-I^0
33[25]	F	Exon 6	c.562C>T (p.Arg188X)	Nonsense	-
34[29]	-	Exon 6	c.576dupC (p.Asn193Glnfs)	Insertion	-
35[27]	S	Exon 6	c.577G>A (p.G169R)	Missense	LAD-I^0
36[30]	-	Exon 6	c.587A>C (p.Lys196Thr)	Missense	-
37[31]	F	Exon 6	c.602delC(p.Pro201ArgfsX8)	Deletion	LAD-I^0
38[13]	-	Exon 6	c.614_615insA(p.His206AlafsX59)	Insertion	LAD-I^0
39[32]	F	Exon 6	c.605C>T (p.pro178Leu)	Missense	
40[33]	F	Exon 6	c.691G>C(p.Asp231His)	Missense	LAD-I$^+$
41[29]	-	Exon 6	c.706G>A(p.Gly236Arg)	Missense	LAD-I^0
42[13]	-	Exon 6	c.712G>A(p.Asp238Asn)	Missense	-
43[34]	S	Intron 6	c.742-14C>A	Splice	LAD-I^0
44[22]	S	Exon 6	c.715G>A(p.Ala239Thr)	Missense	LAD-I^0
45[11]	F	Exon 7	c.754T>C (p.Trp252Arg)	Missense	LAD-I^0
46[13]	-	Exon 7	c.755G>A(p.Trp252X)	Nonsense	LAD-I^0
47[13]	-	Exon 7	c.769C>T(p.Arg257Trp)	Missense	LAD-I$^{0/-}$
48[35]	S	Exon 7	c.809C>T (p.Ala270Val)	Missense	LAD-I$^{0/-}$
49[26]	S	Exon 7	c.817G>A (p.Gly273Arg)	Missense	LAD-I^0
50[22]	S	Exon 7	c.843delC(p.Asn282Thrfs)	Deletion	LAD-I^0
51[13]	-	Exon 7	c.846C>A (p.Asn282Lys)	Missense	LAD-I$^+$
52[36]	S	Exon 7	c.850G>A (p.Gly284Ser)	Missense	LAD-I^0
53[15]	-	Intron 7	c.897+1G>A	Splice	LAD-I^0
54[29]	S	Intron 7	c.891+1G>T	Splice	LAD-I^0
55[29]	S	Intron 7	c.891+1G>A	Splice	LAD-I^0
56[13]	S	Exon 8	c.905C>T (p.Pro302Leu)	Missense	LAD-I^0
57[37]	F	Exon 8	c.899A>T (p.Asp300Val)	Missense	LAD-I^0
58[13]	-	Exon 9	c.995_1004del10	Deletion	-
59[35]	-	Exon 9	c.1021G>C(p.Ala341Pro)	Missense	LAD-I$^{0/-}$
60[34]	-	Exon 9	c.1052A>G(p.Asn351Ser)	Missense	-
61[13]	-	Exon 9	c.1057_1059delinsA	Del/ins	LAD-I^0
62[29]	-	Exon 8	c.1030G>T (p.Glu344X)	Nonsense	
63[38]	F	Exon 8	c.1037_1044delinsT	Del/ins	LAD-I^0
64[13]	-	Intron 9	c.1083+5G>C	Splice	LAD-I^0
65[39]	F	Intron 9	c.1083+3G>C	Splice	LAD-I^0
66[22]	S	Exon 10	c.1143delC(p.Tyr382Thrfs)	Deletion	LAD-I$^-$
67[13]	-	Exon 10	c.1225?-1412+?del	Deletion	LAD-I^0
68[40]	F	Exon 11	c.1256_1257delAG	Deletion	LAD-I$^-$
69[13]	-	Exon 11	c.1358G>A(p.Ser453Asn)	Missense	-
70[13]	-	Exon 11	c.1388_1390delGCTinsCA	Del/ins	-
71[24]	S	Exon 11	c.1413?_1877+?del2114	Deletion	LAD-I^0

72[13]	-	Intron 11	c.1413-149_2080+839 del12518insAAAA	Del/ins	LAD-I^0
73[14]	-	Intron11	C.1413-396_? del27703	Deletion	LAD-I^0
74[13]	-	Exon 12	c.1421delC	Deletion	-
75[41]	F	Exon 12	c.1498delG	Deletion	LAD-I^0
76[15]	-	Exon 12	c.1590C>G(p.Tyr530X)	Nonsense	LAD-I^0
77[17]	S	Exon 12	c.1602C>A(p.Cys534X)	Nonsense	LAD-I^0
78[13]	-	Exon 12	c.1622delGins23	Del/ins	LAD-I^0
79[42]	S	Exon 12	c.1622_1657del33	Deletion	LAD-I$^-$
80[13]	-	Exon 12	c.1632C>G(p.Tyr544X)	Nonsense	LAD-I^0
81[13]	-	Intron 12	c.1658-2A>G	Splice	-
82[15]	F	Exon 13	c.1670G>C(p.Cys557Ser)	Missense	LAD-I^0
83[34]	S	Exon 13	c.1756C>T(p.arg586try)	Missense	-
84[35]	S	Exon 13	c.1768T>C(p.Cys590Arg)	Missense	-
85[35]	F	Exon 13	c.1777C>T(p.Arg593Cys)	Missense	-
86[15]	-	Exon 13	c.1834T>C(p.Cys612Arg)	Missense	LAD-I$^-$
87[22]	F	Exon 14	c.1907delA(p.Lys636Argfs)	Deletion	LAD-I^0
88[41]	S	Exon 14	c.1920delG	Deletion	LAD-I^0
89[13]	-	Exon 14	c.1943C>T(p.Pro648Leu)	Missense	-
90[43]	S	Exon 14	c.1906T>C	Missense	-
91[44]	S	Exon 15	c.2070delT	Deletion	LAD-I^0
92[13]	-	Exon 14	c.2077C>T(p.Arg693X)	Nonsense	-
93[13]	-	Intron 14	c.2080+1delG	Splice	LAD-I^0
94[12]	F	Exon 15	c.2142delT	Deletion	-
95[22]	S	Exon 15	c.2147G>C(p.Gly716Ala)	Missense	LAD-I^0
96[42]	S	Exon 15	c.2200G>T(p.Glu734X)	Nonsense	LAD-I$^-$

*Patients with severe phenotype have < 2% of CD18 expression (LAD-I^0) whereas 2%-20% (LAD-I$^-$) expression have moderate phenotype. Rarely patients may have > 20% or near normal expression of CD18 (LAD-I$^+$). F: familial; S: sporadic; -: not mentioned; del/ins: delition/insertion

confirmation in patients with LAD-I and also during prenatal diagnosis.

The emergence of next generation sequencing technologies constituted a turning point for the advancement of our understanding of rare diseases including various primary immune-deficiencies which require a broad search for causal variants across their genetically heterogeneous spectrum[5]. And the high throughput TES for specific (known) disease-causing genes has been applied to assist with molecular diagnosis of well-defined disorders caused by a group of genes[6].

Our patient is characterized by a leukocytosis with neutrophil predominance that never reached normal level after antibiotic treatment [Table 1]. We confirmed the diagnosis by genetic testing. c.817G>A in a homozygous form was identified, which resulted in the amino acid substitution Gly273Arg residing in exon 8. This mutation shows nearly no CD18 expression on the leukocyte surface[7]. If it is, 1% of the CD18 expression in normal subjects can be defined as severely deficient patients. The condition is severe and often results in repeated infections in infants and young children, which is consistent with the clinical manifestation of this proband. His father carries heterozygotes for this mutation and his mother is normal of this allele; both parents did not give a history of consanguinity. The lack of consanguinity, as is the characteristic of LAD-I patients, indicated that autosomal recessive inheritance is less likely for this patient, although not excluded. Furthermore, the sole presence of the identified variant in his father revealed that the mutation on the other allele might emerge de novo, although this kind of probability is very low. Based on our pedigree analysis results, loss of heterozygosis[8] might be the most relevant possibility, that the embryo of the proband has undergone a change before its development, leading to two copies of its *ITGB2* gene being paternal mutations.

Another remarkable finding was the association with hepatomegaly, splenomegaly and elevated aminotransferase detected during his hospitalization. These were typical manifestations of acquired CMV infection. Though treated with ganciclovir, his CMV IgM and PCR titers never reached normal. We considered that it might be due to his impaired immunological function since CMV is an important opportunistic pathogen in immunocompromised patients[9].

Finally, a review of the literature concerning LAD-I was done by a search of Chinese Biological Medicine

Database and PubMed. The first LAD-I was described in 1984[10] with more than 300 cases and 96 pathogenic mutations [Table 2][11-44] reported, including 22 different kinds of deletion mutations, 3 insertion, 5 deletion/insertion, 11 nonsense, 12 splice and 43 missense. Mutations either lead to absent protein or expression of a truncated form of the protein usually resulting in low or no expression. Three clinical phenotypes of LAD I appear to be directly related to the level of CD18 expression on patients' leucocytes. Patients with severe phenotype have < 2% of CD18 expression (LAD-I^{0}) whereas 2%-20% (LAD-I^{-}) expression have moderate phenotype. Rarely patients may have > 20% or near normal expression of CD18 (LAD-I^{+}).

In conclusion, we presented a case of LAD-I associated with acquired CMV infection, an important opportunistic pathogen in patients who are immunocompromised. The genetic investigation helped us to identify the etiology of severe leukocytosis of the patient during his infancy. Following his discharge, further clinical course was marked by successive and progressive infections. The patient was then recommended to another hospital where HSCT is available for immunodeficiency. He was reported to be improving and thriving until now.

DECLARATIONS

Authors' contributions

Design: Zhu TW
Literature research: Hong S
Data collection and analysis: Xie LJ, Yang QN
Manuscript writing: Hong S
Manuscript editing: Zhu TW
Manuscript revision: Zhu TW

Availability of data and materials

The data were strictly obtained from medical records according to the privacy policy and ethics code of our institute.

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None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study is approved by the institutional review board of Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine. Written consent form was obtained from the patient.

Consent for publication

Not applicable.

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