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CASE REPORT

Introduction of Identical Twins with a Mutation in the *STK4* Gene Showing Clinical Manifestations Associated with Mutations at Different Ages: A Case Report

Farhad Abolnezhadian[1], Sara Iranparast[2,3*], Fatemeh Ahmadpour[3,4]

[1]Department of Pediatrics, Abuzar Children's Hospital, [2]Student Research Committee, [3]Department of Immunology, [4]Department of Clinical Biochemistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

ABSTRACT

Combined immunodeficiencies (CIDs) are a heterogeneous group of disorders characterized by various gene mutations. The mutations in the STK4 (Serine Threonine Kinase 4) gene, which has a role in the regulation of apoptosis and proliferation, can be a cause of immunodeficiency. In the current paper, we reported a case of identical twin brothers with a novel STK4 mutation, one of whom showed clinical manifestations associated with this mutation with a delay of two years. The mutation in the STK4 gene was identified employing Whole Exome Sequencing (WES), and we described the probable reasons for this delay. We found that the STK4 genetic defect caused almost the same clinical symptoms of immunodeficiency in the twin brothers. Meanwhile, the severity of the disease was higher in one of them, which may be due to extra genetic defect in LRBA, and likely differences in the percentage of B lymphocyte population and CD4+/ CD8+ state.

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Keywords: Combined Immunodeficiency, Identical Twin, *LRBA* Defect, *STK4*, Symptoms

*Corresponding author: Dr. Sara Iranparast, Department of Immunology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, e-mail: Sara.Iranparast@yahoo.com

INTRODUCTION

Monogenic disorders of the immune system have revealed prominent insights into various signaling pathways (1). Despite the considerable progress in this field, several disorders are approximately unknown. Identifying new genetic mutations in patients with immunodeficiency syndromes might shed light to novel insights into the basic mechanisms of the human immune system. STK4 (Serine/Threonine Kinase 4) deficiency is a novel human primary immunodeficiency syndrome. STK4 with a two-year delay is located in 20q13.12 and encodes a 63-kD protein that is known as MST1 (Macrophage-stimulating protein-1), and a cytoplasmic kinase, which acts upstream of the stress-induced mitogen-activated protein kinase (MAPK) cascade. STK4 has a crucial role in the regulation of apoptosis and proliferation (2) even though the role of STK4 in cell death is still controversial (3,4). Moreover, it is an important element in the process of 'inside-out' signaling in murine lymphocytes, which is critical for lymphocyte polarity (5) and the formation of the immune synapse (6). STK4 mutation-associated diseases include T-Cell Immunodeficiency, recurrent infections, autoimmunity with or without cardiac malformations, hyper-IgE recurrent infection syndrome, and autosomal dominant (7,8). Mutation in the STK4 can lead to certain clinical complications including recurrent bacterial infections such as meningitis, cellulitis, osteomyelitis, pneumonia, and viral infections. Furthermore, in some cases, recurrent oral ulcers have been observed to be consistent with the clinical diagnosis of Herpes simplex virus (9). In this paper, we reported a case of meningitis infection associated with the STK4 gene mutation, which was presented in the serum along with some changes in the level of immune cells and inflammatory compounds. However, the suffering twin brothers also showed the symptoms of the disease with about a 2-year delay. In this study, due to the similarity of the genome in identical twins, the possible causes of the delay in STK4 gene expression were investigated. We also described the first identical twin patients with a mutation of STK4 (NM_006282: c.360+5G>A), one of whom has manifested the mutation with a two-year delay.

CASE REPORT

An 8-year-old boy was referred to the department of infectious diseases of Abuzar hospital due to fever, headache, and drowsiness and with a diagnosis of meningitis. Since this patient had been hospitalized several times for recurrent infections, immunological consultation was done for him. The studied patient (patient 1) and his identical twin brother (patient 2) were the third and fourth children's first-degree cousin's parents, who had been frequently hospitalized in other centers. Given the pedigree of the patients, the uncle and aunt died, due to fungal infection and undetermined cause, respectively [as revealed in Figure 1]. He (patient 1) was healthy before the first admission, and had no particular problems, and had age-appropriate growth and development. During the first admission, lymphopenia, with a reduction in CD4+ T cells, was diagnosed for patient 1 (Table 1). One month later, the patient was admitted to the hospital with complaints of fever and oral lesions, diagnosed with gingivosotemitis. Therefore, he was treated with imipenem and acyclovir. Primarily, around the age of 8, right knee septic arthritis was diagnosed for patient 1; the pneumococcus observed in the smear and culture of the synovial fluid of the right knee confirmed arthritis. The patient was subsequently treated with ampicillin, clindamycin, and ceftriaxone antibiotics for 21 days.

Table 1. The results of flow cytometry investigation and the CBC test performed for both patients1 and 2.

CD Markers	Patient1	Patient2	Unit	Normal Range
CD3	87 (4035)	72 (1504)	% of Lymph (Absolut Count)	30-78 (1391-3618)
CD4	18 (835)	31 (648)	% of Lymph (Absolut Count)	22-58 (1021-2691)
CD8	68 (3154)	38 (794)	% of Lymph (Absolut Count)	10-37 (464-1716)
CD4/CD8	0.26	0.81	_	1-4
CD19	2 (92.7)	16 (334)	% of Lymph (Absolut Count)	9-38 (418-1763)
CD56, CD16	5 (232)	1(20)	% of Lymph (Absolut Count)	3-15 (139-694), 5-19 (232-880)
CD20	2 (92.7)	16(334)	% of Lymph (Absolut Count)	7.1 - 23.8 (163 - 600)
W.B.C	29000	7300	/ml	4000-10000
PMN	92	60.4	% of the total	40-80
Lymphocyte	7	26.8	% of the total	20-45

During this period, the laboratory data showed high ESR, CRP, and WBC (with high PMN frequency). Repeatedly, about 7 months later, the patient spent a period of 4-day hospitalization, due to right knee septic arthritis.

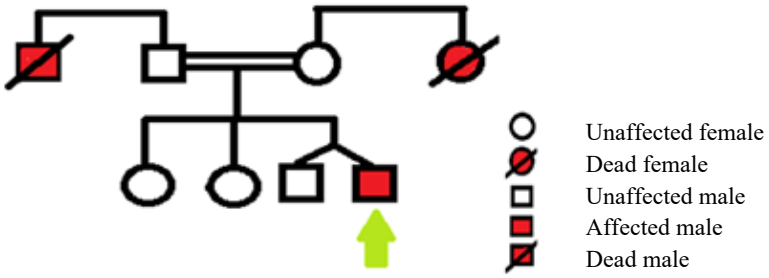

Figure 1. The pedigree of the patient's family, which shows the history of his family in terms of inheritance of the new mutation associated with meningitis.

Following 10 months, in the third hospitalization period, the patient referred to a center complaining of long-term headache, fever, drowsiness, and meningitis; therefore, he was treated with ceftriaxone and vancomycin antibiotics. According to the Lumbar Puncture examination, the diagnosis of meningitis was confirmed. Investigation of CSF, which is represented in Table 2.B, approved meningitis. The results of CSF culture were reported to be negative after the treatment (Table 2.B). This patient was suspected of Rt. knee septic arthritis and was treated with cefazolin since his blood was observed his synovial fluid with cell count of 200000 and dominant population of PMN (approximately 90%). During this course of treatment, ESR=91, CRP=3+, and an increase in complement proteins such as C3 and C4 were reported (Table 2.A).

Table 2. The results of the patient's (1) laboratory findings in the blood sample (A) and the results of CSF culture of the patient (1) during the last hospitalization (B).

(A)			(B)			
Test	**Results**	**Normal Range**	**Factors**	**Results (in admission time)**	**Results (after the treatment)**	**Normal Values**
C3	232 mg/dL	88-150 mg/dL	**Protein**	130 mg/dl	27 mg/dl	15-45
C4	82.6 mg/dL	12-32 mg/dL	**Glucose**	48 mg/dl	40 mg/dl	60-80
CH50	94%	70-150%	**RBC**	1700/ml	395/ml	<1
NBT	96%	100%	**WBC**	Many	5/ml	0-5
DHR	240%	50- 200%	**PMN**	90%	5%	2-5%

The antibodies titer and inflammatory characteristics of the patient were also modified (according to Table 3). Thus, the consultation for immunodeficiency was performed after recurrent infections and the mutation in the STK4 gene was identified with Whole Exome Sequencing (WES).

Table 3. The results of the antibodies titration and evaluation of the liver enzymes, which were done for patient 1 and his twin brother (as patient 2).

Class of Antibody	Patient (1)	Patient(2)	Normal Range
IgA	988 mg/dl	543 mg/dl	20-100 mg/dl
IgM	47 mg/dl	33 mg/dl	19-146 mg/dl
IgG	2827 mg/dl	1759 mg/dl	453-916 mg/dl
IgE	195.7 mg/dl	175.4 mg/dl	1.03-161.3 mg/dl
HIV Abs	Negative	Negative	Negative
AST	6.7	19 IU/L	<37 IU/L
ALT	157	59 IU/L	<41 IU/L
RBC	5.01×10^{12}	5.84×10^{12}	$3.5\text{-}5.50 \times 10^{12}$ /L
HGB	10.6 g/dl	11.6 g/dl	12-16 g/dl
MCV	68.46 fL	64.2 fL	80-100 fL
MCH	21.16 Pg	19.9 Pg	27-37 Pg
MCHC	30.9 g/dL	30.9 g/dL	31-37 g/dl
Hb-A	88.7 g/dL	90.2 g/dL	96.5-98.5 g/dL
Hb-A2	7.5 g/dL	6.5 g/dL	1.5-3.5 g/dl
Hb-F	3.8 g/dL	3.3 g/dL	2≥ g/dl

At the last time, the patient referred to the clinic, while STK4 deficiency and pneumonia were diagnosed for him. Furthermore, skin manifestation such as herpes were observed (Figure 2). Additionally, the Flat Wart examination was observed at his face, forehead, and neck. A “complete exome sequencing” test was performed for the patient, the STK4 gene mutation was confirmed at his gene sequences. The diseases associated with STK4 include T-cell immunodeficiency, recurrent infections, autoimmunity with or without cardiac malformations, hyper-IgE recurrent infection syndrome, and autosomal dominant.

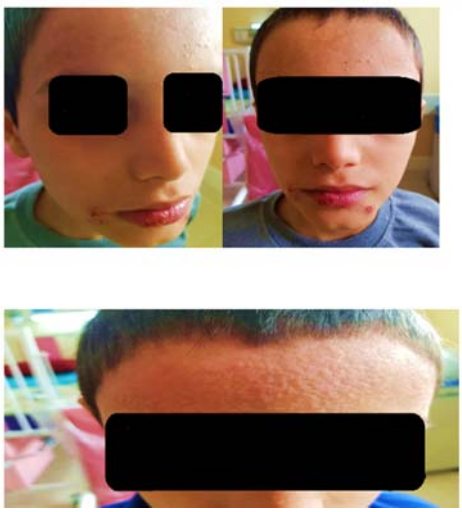

Figure 2. The herpes simplex virus manifestation observed in the patient under study.

The patient underwent Whole Exome Sequencing (WES) for a definite diagnosis. Current novel homozygous mutation in the STK4 gene (NM_006282: c.360+5G>A) is an intronic mutation which is involved in splicing. Sanger sequencing confirmed that the patient and his affected brother are homozygous and his parents are carrier heterozygous for this mutation whereas his healthy sister is homozygous wild type (Figure 2). This test was performed in order to identify the most relevant mutations that might explain the disease in this patient. This mutation is consistent with the patient’s clinical presentation, which was confirmed in both patients and parents with Sanger sequencing WES sequence (Figure 3). On the other hand, the ratio of CD4: CD8 and B cell level reduced in the patient, initially suspected of being CD4+ T cell lymphopenia and burton syndrome, respectively. Additionally, the titration of IgA, IgG, and IgE antibodies increased significantly, all of which could be attributed to the gene mutation of STK4. Moreover, the other homozygote mutation, located in the intronic LRBA (LPS Responsive Beige-Like Anchor) gene (NM_001199282, c.1755+54G>A), was observed in gene sequencing, which it did not relate to the clinical manifestation of the disease. LRBA gene involves in splicing and its gene mutation leads to certain disorders, including recurrent infections (specifically respiratory infections), lymphadenopathy, hypogammaglobinemia, defect in B-cell differentiation, autoimmune disorders, granuloma formation, and neutropenia (in some patients). In general, the presentation and phenotype are highly variable, even within families.

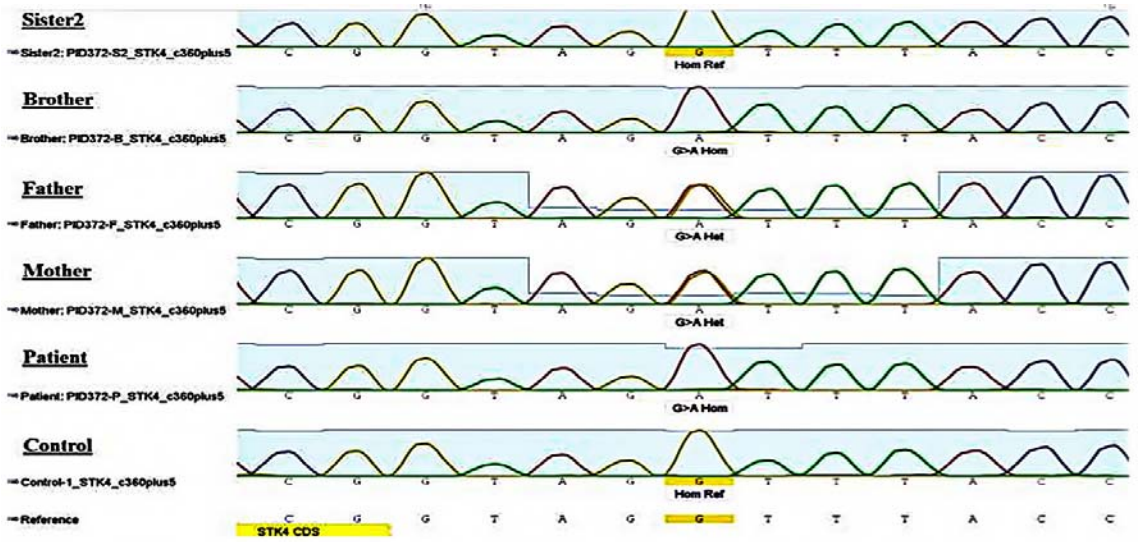

Figure 3. A novel mutation in the STK4 gene (NM-006282: c.360+5G>A) related to immunodeficiency disorder was found, which leads to a stop-gain mutation and ultimately, protein dysfunction. Sanger sequencing confirmed that the patient is homozygous and the parents (father and mother) were both heterozygous carriers for this mutation. ("[*]" is the symbol of the termination of the sequence).

Immunologic findings are also variable and may include decreased B cells, hypogammaglobinemia, and deficiency of CD4+ T regulatory (Treg) cells. The patient's sister had no copies of the mutation and was completely healthy. This mutation is inherited in the form of an autosomal recessive (AR). Interestingly, even though the patient and his twin brother were genetically identical twins, the clinical manifestations were observed at different ages, with a two-year delay. Interestingly, the twin brother (patient 2) referred to the department of infection at the age of 10, due to pneumonia with some symptoms such as alternative fevers, vomiting, and non-bloody diarrhea. He was then under treatment with ampicillin and ceftriaxone. Considering the STK4 immuno-defiencicy in his twin brother, he was consulted to survey immunological defects. At the second hospitalization, he referred to the immunology clinic complaining of pain and swelling in the ankle of the left foot. Subsequently, he spent the third and fourth admission periods due to septic arthritis and pneumonia, respectively, and received a determined antibiotic-pattern including ampicillin, clindamycin, and ceftriaxone. A fungal infection was observed in his forehead. Therefore, he was admitted for several periods for recurrent lung infections. Moreover, the ratio of TCD4: TCD8+, and also cell counts decreased (Table 1). Furthermore, vitamin D deficiency was diagnosed in the patient (2). The examination of the peripheral blood of both patients (2) revealed thalassemia conflict (Table 3). Eventually, this patient was discharged with a good general condition. Interestingly, he also suffered from Flat Wart on the face, forehead, and neck during the next examination. What is important in this study is the probable reasons for the approximately 2-year gap in identical twins with the same environmental condition.

DISCUSSION

In the current research, we evaluated twin brothers with homozygous STK4 mutations. Sanger sequencing for STK4 deficiency reported a novel mutation (NM_006282: c.360+5G>A). STK4 deficiency has recently been described as a rare type of combined immunodeficiency. Recurrent bacterial infections, fungal and viral cutaneous, mildly elevated IgE, high IgA and IgG levels, autoimmune cytopenias with eosinophilia, immunological findings, and moderate anemia are associated with this disease, which were reported in this study on two patients, although the clinical symptoms appeared after 2 years in patient 2. In the current study, we observed recurrent bacterial infections, including meningitis and pneumonia in both patients, which are commonly manifested in most patients with STK4 deficiency (7). Erythematous skin lesions and eczema were reported in the other STK4 deficient patients (10). In line with previous studies, the skin lesions related to herpes simplex virus was observed in patient 1 (Figure 2). Nehme *et al.* reported autoimmune hemolytic anemia (AHA) in a patient with STK4 deficiency (10). In our study, moderate anemia was found in both patients (Table 3). Both our patients had mild increased IgE found in three STK4 deficient patients in other studies (9-11). Lymphopenia, transient neutropenia, and eosinophilia, which are common characteristics of STK4 deficiency, were found in these patients. Other immunological findings showed an obvious reduction in the B cell population in patient 1 compared with patient 2, who had a normal rate of B cell. Additionally, the CD4/CD8 ratio in patient 2 was higher compared with that in patient 1 (Table 1). As mentioned in previous studies, inflammation and reduced number of lymphocytes, particularly T cells, are observed in all the patients with STK4 deficiency (7). In general, patient 1 manifested severe clinical symptoms, infections, immunological indexes, and also delay in the response to treatment compared with his twin brother (patient 2). This might be due to extra genetic defect in LRBA, and likely differences in the percentage of B lymphocyte population and CD4+/ CD8+ state. However, patient 2 did not have mutation for LRBA gene when he was being investigated. Therefore, LRBA defect may result in the occurrence of his symptoms two years earlier than his brother. The LRBA protein is encoded by the LRBA gene. Its expression is induced in B cells and macrophages by bacterial lipopolysaccharides (LPS). The encoded protein is associated with protein A and may be involved in leading intracellular vesicles to activate receptor complexes, which facilitates the secretion and/or membrane deposition of immune effector molecules. The defects in this gene are associated with the disorder common variable immunodeficiency- 8 with autoimmunity. In conclusion, we identified the effects of the STK4 genetic defect on the twin brothers who demonstrated almost the same clinical symptoms associated with immune deficiency. Yet the severity of the disease was higher in one of the twins, which may be due to another genetic defect, LRBA defect, and likely differences in the percentage of B lymphocyte population and CD4+/ CD8+ state. In this study, we found that in identical twins with the same pattern of gene mutation and the same environmental conditions, it is possible to not observe simultaneous clinical manifestations, in view of the existence of other gene disorders, different immune status, and so forth.

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