

# Novel CD40LG Mutation in Two Cousins With Immunoglobulin Class Switch Recombinant Deficiency

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**Abstract-** The hyper-immunoglobulin M (HIGM) syndrome comprises a group of rare inherited immunodeficiency disorders characterized by normal or elevated levels of serum IgM with low or absent levels of serum IgG, IgA, and IgE. Patients with this syndrome usually present with a history of recurrent infections or opportunistic infections. Here, we report two male cousins from homozygote twin mothers. The first cousin presented with no signs or symptoms other than neutropenia, which was accidentally found in a routine blood test. Immunological workup in this patient showed undetectable IgG and IgA levels and normal IgM levels. The second cousin had a history of recurrent infections, and at the time of admission, he was diagnosed with *Pneumocystis jirovecii* infection. The immunologic workup of this patient showed undetectable IgG, decreased IgA, and increased IgM level. Due to their interesting family relationship, genetic analysis was performed, which detected a novel mutation in exon 2 (c.266 del G) of the CD40 ligand gene (CD40LG).

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## Introduction

The hyper-immunoglobulin M (HIGM) syndrome consists of a group of rare inherited immunodeficiency disorders, characterized by normal or elevated serum IgM with low or absent IgG, IgA, and IgE serum levels (1). This increased IgM level is due to defects in class-switch recombination (CSR) and somatic hypermutation (SHM) (2).

Patients with HIGM usually suffer from recurrent

infections such as sinus, ear, and respiratory tract infections. Also, opportunistic infections by *pneumocystis jirovecii* are the presenting feature of this syndrome in around 40% of the cases (3).

Mutations in six different genes, encoding for CD40 ligand, CD40, nuclear factor-kB essential modulator (NEMO), activation-induced cytidine deaminase (AID), uracil-DNA glycosylase (UNG) and inhibitor of NF-B (IkB $\alpha$ ) have so far been associated to the disease (3-5).

In this study, we report 2 cases of HIGM, who are

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cousins from twin mothers. One of these cases had no signs or symptoms other than neutropenia, which was found accidentally in a routine checkup, and the other case presented with respiratory tract infection, and GI signs and symptoms compatible with food allergy and the blood tests in this patient showed eosinophilia. Further, in the genetic analysis of these patients, we found a novel mutation in the CD40 ligand gene (CD40LG).

## Case Report

### Case 1

A 2-year-old boy was referred to our center with neutropenia, which was incidentally found in routine checkups without any signs and symptoms.

Immunological workup showed undetectable IgG and IgA levels and normal IgM levels (53 mg/dL) in this case (Table 1). Therefore, a diagnosis of immunoglobulin class-switch recombination (Ig CSR) deficiency was made for this patient. In genetic analysis, *CD40LG* was sequenced, which showed a novel mutation in exon 2 (c.266 del G) of the gene (p.S89TfsX6). After HIGM syndrome diagnosis, he is under monthly immunoglobulin replacement therapy and cotrimoxazole prophylaxis, which are effective, and he is in good physical condition now.

The family history of this case revealed that he has a cousin with recurrent infections. Therefore, the cousin was also asked to be visited. They were from homozygote twin mothers.

**Table 1. Laboratory findings in case 1**

Tests	Results	Normal range
<b>Complete blood cell count</b>	WBC count / $\mu$ L	11000
	Hemoglobin g/dL	11.1
	Platelet count / $\mu$ L	251000
<b>WBC differential counts</b>	Neutrophils %	2.7
	Lymphocytes %	65.7
	Eosinophils %	4
<b>Immunoglobulin electrophoresis</b>	IgG mg/dL	undetectable
	IgM mg/dL	53
	IgA mg/dL	undetectable

### Case 2

The cousin, who was a 1-year-old boy, was hospitalized with a chief complaint of nausea and vomiting from 7 days before admission. He had a history of diarrhea at 4 months old and cutaneous rash (eczema) and failure to thrive from when he was 7 months old with an initial diagnosis of food allergy, and he was recommended to avoid milk and egg which was not effective. In his admission, according to his past medical history, physical examination finding which revealed respiratory distress and clinical findings that showed defects in chest X-ray and rise of LDH (520

U/L), *Pneumocystis jirovecii* infection was diagnosed, and therapy was started which was effective and respiratory distress was reduced. The Immunologic workup of this patient showed undetectable IgG, along with low IgA (< 0.6 mg/dL) and increased IgM level (227 mg/dL) (Table 2), which was compatible with the diagnosis of Ig CSR deficiency. In addition, the same mutation was detected in the genetic analysis of this case. Monthly immunoglobulin replacement therapy and cotrimoxazole prophylaxis were started in this patient as well after the diagnosis.

**Table 2. Laboratory findings in case 2**

Tests	Results	Normal range
<b>Complete blood cell count</b>	WBC count / $\mu$ L	18000
	Hemoglobin g/dL	11.6
	Platelet count / $\mu$ L	280000
<b>WBC differential counts</b>	Neutrophils %	3
	Lymphocytes %	86
	Eosinophils %	6
<b>Immunoglobulin electrophoresis</b>	IgG mg/dL	undetectable
	IgM mg/dL	227
	IgA mg/dL	<0.6

## Discussion

HIGM, also known as immunoglobulin class switch recombination (CSR) deficiency, characterized by recurrent infections, decreased serum immunoglobulin IgG, IgA, and IgE levels and normal to increased IgM levels (6). Our first case which was the asymptomatic case, had no history of recurrent infections and IgM serum level was normal according to his age, but he had decreased serum level of IgG, IgA, and IgE. while the second case which was the symptomatic case, had recurrent infections and elevated IgM serum level with decreased IgG, IgA, and IgE serum level,

Additionally, it has been shown that gastrointestinal infections, recurrent rhino-sinusitis, and pneumonitis are common in the early infancy of patients with HIGM syndrome, and based on a study, the pathogen responsible for pneumonitis in more than 50% of patients, is *Pneumocystis jiroveci* (7-9). In our study, the symptomatic case had gastrointestinal symptoms during infancy and pneumonitis with *Pneumocystis jiroveci* at the time of admission.

In some other studies, sclerosing cholangitis, stomatitis, and also increased risk for hepatocellular carcinoma, bile duct carcinoma, and pancreatic cancer in HIGM cases are reported (10-12). However, our cases did not show any signs or symptoms compatible with these diseases.

In laboratory studies on HIGM, neutropenia is a common finding, and it has been reported that it may cause oral ulcers (10,11,13). In our study, neutropenia was detected in the CBC of both patients while their parents did not give us any history of oral ulcers.

Our second case, which was the symptomatic patient, had a cutaneous rash, gastrointestinal complaints such as diarrhea, and in further investigations, a *Pneumocystis jiroveci* pneumonia. These findings plus neutropenia in his CBC led us to do immunological studies for him, and it was compatible with HIGM syndrome. However, the vague point in his laboratory data was eosinophilia in the CBC. We were suspicious of food allergy, but eosinophilia did not disappear after a tight control on his food regimen. After IVIG therapy, eosinophilia was lowered, but it was not disappeared. We could not find unexplained eosinophilia in HIGM cases in other studies. So, it could be a new point, and also it might be a clue for further investigations.

Our first case had no complaints. One reason for that to happen is maybe he had not enough time to show symptoms, he was found by a CBC during a routine

checkup in which he had neutropenia. Because of his family history (recurrent infections in his cousin), more immunological studies were done on him, and HIGM was diagnosed. After IVIG therapy, the neutropenia was cured, and he is healthy with no complaint, and his blood tests are normal now.

Because of their unique representations and interesting family relationship between these two patients, we checked them genetically for the causative mutation, which showed a deletion in exon 2 (codon 266) of the CD40LG (p.S89TfsX6).

The interaction between CD40 on B cells and CD40L on T cells have an important role in CSR and SHM. Therefore, mutations in CD40LG lead to impaired T cell function, CSR, and germinal center formation. In addition, the Granulocyte-colony stimulating factor (GCSF) synthesis is stimulated in the bone marrow by the interaction between CD40L on T cells and CD40 on stromal cells, which results in the release of mature neutrophils into the circulation. Thus, CD40LG mutations can cause neutropenia as well (14), which was observed in our patients.

Immunoglobulin replacement therapy is the main treatment for these patients. Additionally, GCSF could be effective in cases with severe neutropenia, and the prevention of opportunistic infections such as *Pneumocystis jiroveci* is also important (15).

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