

Severe Congenital Neutropenia or Hyper-IgM Syndrome? A Novel Mutation of CD40 Ligand in a Patient with Severe Neutropenia

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Established Facts

- Severe congenital neutropenia and CD40 ligand deficiency are two different primary immunodeficiency diseases with different underlying genetic defects.
- An elevated serum level of immunoglobulins is a common finding in severe congenital neutropenia patients, while a number of CD40 ligand deficiency patients suffer from neutropenia.

Novel Insights

- New mutation of the *CD40L* gene is associated with neutropenia.
- Patients with CD40 ligand deficiency could have normal serum levels of IgA and IgM.
- CD40 ligand deficiency should be suspected in male patients with recurrent infections and neutropenia.

Key Words

Hyper-IgM syndromes • Immunological deficiency syndromes • Infection • Neutropenia • Mutation

Abstract

Severe congenital neutropenia (SCN) and CD40 ligand deficiency (CD40LD) are two primary immunodeficiency diseases caused by different underlying genetic defects. In this

report, we present a case who clinically presented as a SCN patient, but subsequent mutation analysis of this patient was compatible with CD40LD. The patient is a 3-year-old boy, who was referred to our center because of pneumonia, oral and anal ulcers, and periodontitis. As severe consistent neutropenia and maturation arrest in the myeloid series were observed in the bone marrow, a diagnosis of SCN was made. However, no mutations were found in the *ELA2* and *HAX1* genes. As functional T cell defects were observed, we

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suspected CD40LD. DNA sequencing showed a 17-base pair deletion in the *CD40L* gene. Although the patient did not have a decreased serum level of IgA, and his serum IgM level was within the normal range, the diagnosis of CD40LD was confirmed, suggesting that CD40LD should be suspected in any male patient with recurrent infections and neutropenia.

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Introduction

Severe congenital neutropenia (SCN) is a rare primary immunodeficiency disease characterized by recurrent severe infections and persistent severe neutropenia [1–6]. CD40 ligand deficiency (CD40LD), previously known as a subgroup of Hyper-IgM syndromes, is a rare combined T and B cell immunodeficiency, characterized by recurrent severe infections, markedly decreased serum levels of IgG and IgA and normal or elevated concentrations of serum IgM [6–10]. While mutations in the gene encoding neutrophil elastase (*ELA2*) represent the underlying genetic defect in SCN with autosomal dominant and sporadic forms [1, 11, 12], *HAX1* mutations are responsible for the autosomal recessive form of SCN [13]. CD40LD is an X-linked disease caused by mutations in the *CD40L* gene located at Xq26.3–27 [8–10].

Although mutations of different genes are responsible for these two different diseases, the clinical and laboratory diagnosis of these patients is complex in a case of neutropenia and normal levels of immunoglobulins. An elevated serum level of immunoglobulins is a common finding in SCN patients [1, 4], while a number of CD40LD patients suffer from neutropenia [14–17]. In this report, we present a patient who initially was diagnosed as SCN, but where subsequent mutation analysis was compatible with CD40LD.

Case Report

The patient is a 3-year-old boy, the fourth child of a family with no history of immunodeficiency or recurrent infections (fig. 1). He has a Kurdish background and lives in a rural region of Iran. He was fully immunized without any complications and was well until the age of 6 months, when he suffered from a pneumonia that required hospitalization. He developed necrotic oral lesions at the age of 8 months, and was referred to the Children's Medical Center, the main referral center for immunodeficiency patients in Tehran, because of pneumonia, oral and anal ulcers, and periodontitis at the age of 9 months.

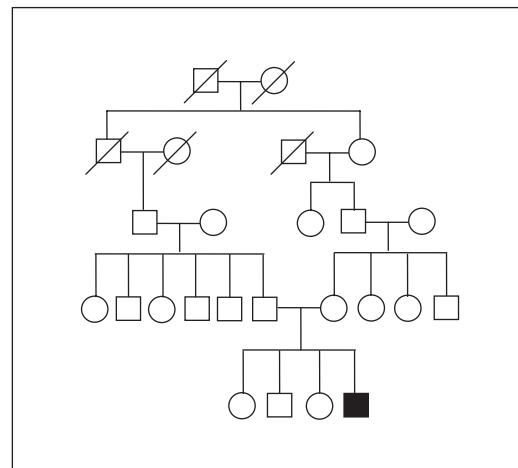
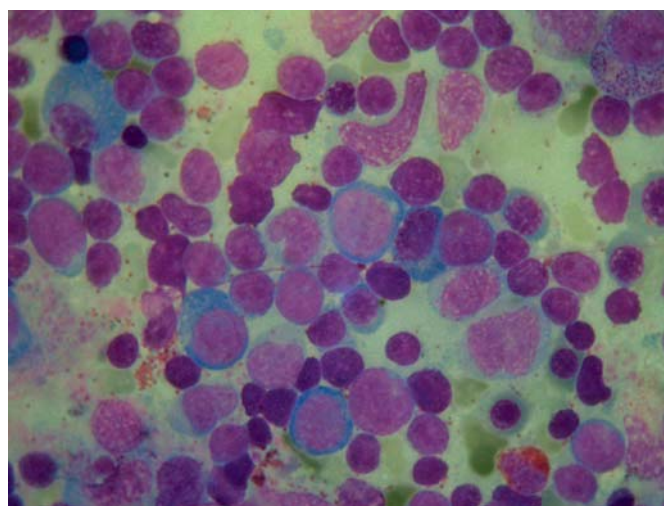


Fig. 1. Pedigree of our patient with CD40LD. Open shapes represent healthy individuals, filled shapes represent patients affected with the syndrome, and shapes with slashes represent deceased individuals. Boxes represent males, circles females.



Color version available online

Fig. 2. Bone marrow morphology of our patient with CD40LD and severe neutropenia.

Laboratory studies revealed severe neutropenia (white blood cells = 6,600 cells/mm³, polymorphonuclears = 7%, lymphocytes = 92%, monocytes = 1%) with an absolute neutrophil count (ANC) of 462 cells/mm³. Previous complete blood cell tests during the preceding 2 months also showed neutropenia. Due to consistent neutropenia, bone marrow examination was performed, which revealed a maturational arrest in the myeloid series and erythroid hyperplasia (fig. 2). A diagnosis of SCN was made for the patient. Granulocyte colony-stimulating factor (G-CSF) was started in addition to antibiotics. ANC increased to 2,697 cells/

mm³ (white blood cells = 8,700 cells/mm³, polymorphonuclears = 31%, lymphocytes = 69%).

Although clinical and laboratory findings of the patient were compatible with a diagnosis of SCN, no mutations were found in the *ELA2* and *HAX1* genes. The patient was discharged from hospital in good condition and was advised to continue GCSF therapy. However, he did not receive GCSF after discharge and subsequently neutropenia re-occurred. He was referred to our hospital again at the age of 20 months with acute diarrhea, superficial abscesses, and necrotic cutaneous lesions. At the physical examination, splenomegaly was detected. Laboratory studies revealed a moderate neutropenia (white blood cells = 6,600 cells/mm³, polymorphonuclears = 8%, lymphocytes = 84%, eosinophils = 6%, monocytes = 2%) with an ANC of 528 cells/mm³. ANC was increased to 1,740 cells/mm³ (white blood cells = 5,800 cells/mm³, polymorphonuclears = 30%, lymphocytes = 70%) after GCSF therapy. A diagnosis of SCN was still considered for this patient. However, immunological studies showed: IgG = 120 mg/dl (normal range: 656–1,350), IgA = 355 mg/dl (normal range: 86–320), IgM = 270 mg/dl (normal range: 120–320), and IgE = 2.1 IU (normal range: 0–46). Moreover, defects in cytokine production, after stimulation of T cells with PHA in vitro, were detected in this patient [IL-2: 235 pg/ml before and 238.6 pg/ml after stimulation (normal control: 159 and 5,694 pg/ml before and after stimulation, respectively); IL-4: 24.7 pg/ml before and 46.5 pg/ml after stimulation (normal control: 44.6 and 288.8 pg/ml before and after stimulation, respectively)]. Although such IgG deficiency and T cell defects may be seen in patients with combined T and B cell immunodeficiencies, this is not observed in SCN patients. In view of these findings, CD40LD was suspected; subsequently, mutation analysis of the *CD40L* gene was performed. DNA sequencing indicated a 17-base pair deletion in exon 2 of the *CD40L* gene, resulting in a frame shift after aa E61 and a premature stop codon in the extracellular domain (fig. 3).

Intravenous immunoglobulin replacement therapy was started in addition to GCSF and prophylactic antibiotics (trimethoprim-sulfamethoxazole). The patient responded to this therapy with ANC rising to 1,679 cells/mm³ (white blood cells = 7,300 cells/mm³, polymorphonuclears = 23%, lymphocytes = 69%, monocytes = 6%). He is clinically well and has not experienced any major infectious complications after starting the new protocol of treatment.

Discussion

CD40LD is an X-linked form of Hyper-IgM syndromes characterized by recurrent opportunistic infections and decreased serum levels of IgG and IgA with normal or elevated concentrations of the serum IgM level [6–10]. In this report, we present a case with consistent neutropenia who was clinically diagnosed as SCN, but where molecular studies indicated a diagnosis of CD40LD. While hematological abnormalities usually occur in association with neutropenia in SCN patients [1, 2], eosinophilia and monocytosis were not documented in this case, which is similar to other SCN patients in Iran [5]. Although the patient did not have a decreased se-

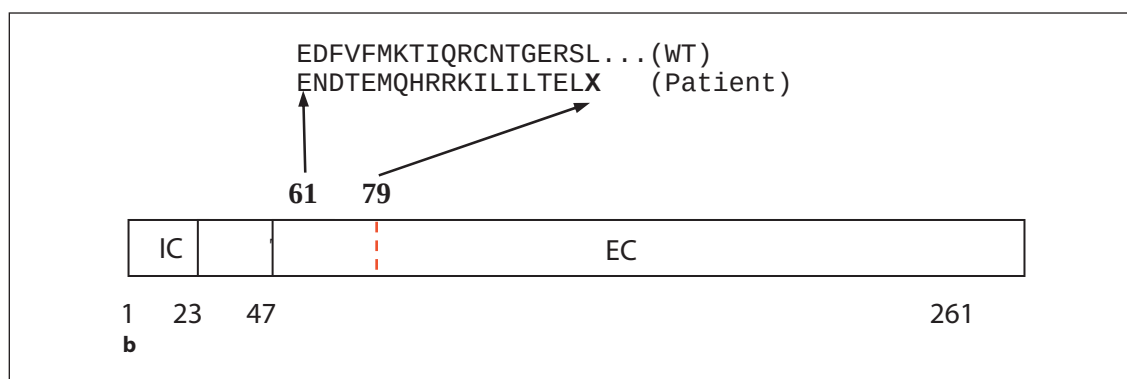
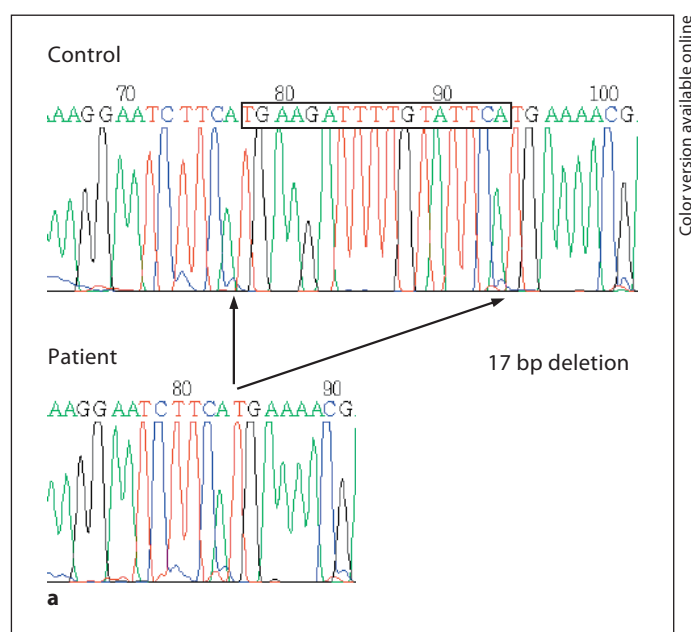


Fig. 3. DNA sequencing of *CD40L* gene: a 17-base pair deletion in exon 2 of *CD40L* gene, resulting in a frame-shift after aa E61 and a premature stop codon in the extracellular domain.

rum level of IgA, and his serum IgM level was also within the normal range, the diagnosis of CD40LD was confirmed by mutational analysis. Decreased serum levels of IgG, IgA, and IgE with normal to elevated IgM are usually expected in CD40LD, as CD40-CD40L is necessary for T cells to induce B cells to undergo class switching from IgM to IgG, IgA, and IgE production [15]; however, there are occasional patients with a normal or even elevated level of serum IgA [16].

He did not have any history of infections with opportunistic microorganisms such as *Pneumocystis jiroveci*, which is common in CD40LD patients [17]. Neutropenia is a common finding in CD40LD, and may be seen in more than half of the affected patients [2, 16, 17]. It can be episodic, cyclic, or chronic and plays an important role for secondary complications [14, 17–19]. Although the underlying pathophysiology of this association with CD40LD is still unclear, it may result from autoantibodies against neutrophils, a defective myeloid differentiation causing maturational arrest [19, 20] or a defect in the release of mature granulocytes from the marrow to the peripheral blood [21]. Attempts to find antineutrophil antibodies have, however, been unsuccessful to date [20, 21].

The interaction between CD40L on T cells and CD40 on B cells is essential for class switch recombination and somatic hypermutation. Mutations in the CD40L gene prevent class switch recombination and germinal center formation and affect T cell function [15, 22]. The interaction between CD40 on stromal cells and CD40L on T cells in the bone marrow may also stimulate synthesis of GCSF, which subsequently induces the release of mature neutrophils from the marrow pool to the peripheral blood; therefore, defects of CD40L may reduce local GCSF production, resulting in severe neutropenia in patients with CD40LD [21]. GCSF therapy in combination

with intravenous immunoglobulin, especially in patients with low serum levels of IgG, could increase the number of neutrophils, reduce the number of infections and hospitalization periods, and improve the prognosis and quality of life [19, 21].

Severe and recurrent infections must always initiate a search for an immunodeficiency syndrome, as a delay in diagnosis may result in chronic infection, irreversible end-organ damage or even death of the patient. Early diagnosis and appropriate management remain the keys to prevent such complications [5]. Due to advances in immunological and molecular diagnostic techniques, a definitive diagnosis of most primary immunodeficiency diseases can now be easily obtained. Functional studies such as T cell proliferation assays and evaluation of CD40L on stimulated T cells could also suggest a diagnosis in a case of suspected CD40LD [23], while subsequent mutation analysis of specific genes can confirm this diagnosis.

There are several primary immunodeficiency diseases, which are associated with neutropenia, including SCN, CD40L, Shwachman-Diamond syndrome, Chediak-Higashi syndrome, WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome, etc. [1, 2, 14]. Thus, it is recommended that, in addition to SCN, other immunodeficiency diseases associated with neutropenia should be considered in neutropenic patients with recurrent infections and further screening tests, including immunoglobulin levels, should be performed.

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