

# A boy with a novel homozygous *ZAP70* mutation presenting with a dermatological phenotype and postnatal decrease in CD8<sup>+</sup> T cells

Patients with deficiency of zeta-chain-associated protein kinase 70 (*ZAP70*) protein generally present as combined immunodeficiency (CID) with severe recurrent infections and dermatological findings during the first years of life. They also suffer from diarrhea, mainly resulting from viral agents, lymphoproliferation, and autoimmunity (autoimmune cytopenia, bullous pemphigoid, nephrotic syndrome, and adrenal insufficiency). The most striking immunological findings are severely decreased CD3<sup>+</sup>CD8<sup>+</sup> T-cell counts with decreased proliferation. The current definitive treatment of *ZAP70* deficiency is hematopoietic stem cell transplantation (HSCT).<sup>1</sup>

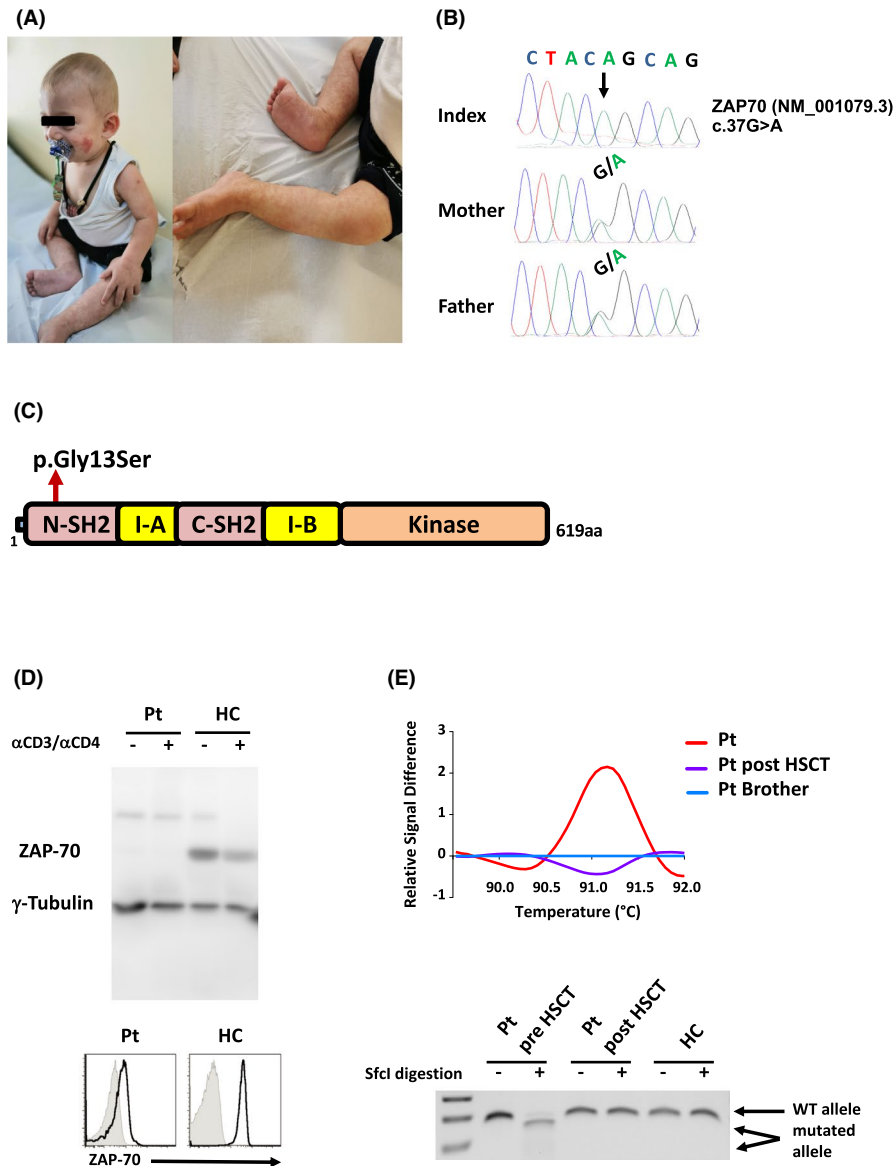
To date, 52 patients with biallelic mutations in the *ZAP70* gene have been described in the literature.<sup>1,2</sup> Herein, we report a patient with a novel missense mutation in the *ZAP70* who presented with atypical skin lesions and a rapid decrease in CD8<sup>+</sup> T-cell counts on immunological evaluations between 6 and 9 months of age. Despite undetectable *ZAP70* protein, the patient did not present severe infections in the first year of life. This description expands the spectrum of disease caused by mutations in the *ZAP70*, thus providing vigilance for early diagnosis and treatment.

A 20-month-old boy, born to consanguineous parents of Kurdish descent, was admitted to the hospital due to an extensive rash covering his body, starting on the third day of life. He also had continual diarrhea, with a frequency of two times per day since birth. Local treatments and antihistamines did not improve his skin symptoms. Physical examination revealed no specific features other than erythematous papular lesions on the dorsum of arms, malar rash like lesions, and livedo reticularis formation on his legs (Figure 1A). Complete blood count showed a lymphocyte count of 4700/mm<sup>3</sup>, eosinophil of 2100/mm<sup>3</sup>, hemoglobin of 10.7 g/dl, and platelet of 988,000/mm<sup>3</sup>. Immunoglobulin levels were normal except for an IgE level of 499 mg/dl. Lymphocyte immunophenotyping revealed moderate low CD3<sup>+</sup> and CD8<sup>+</sup> T cells, accompanied by decreased naive and increased memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Levels of recent thymic emigrants were also diminished compared to healthy controls. Detailed blood test results and immunophenotyping are shown in Table 1. Skin prick test and specific IgE levels were all negative and biopsy from the skin lesions showed minimal vacuolar degenerations with focal parakeratosis. Immunohistochemical examination of the

biopsy revealed infiltrating T lymphocytes, predominantly CD4<sup>+</sup> T cells. Maternal engraftment was excluded by using short tandem repeat analysis in the patient. Viral serology for *Cytomegalovirus* (CMV), *Epstein-Barr virus* (EBV) were negative. Interestingly, a follow-up lymphocyte subset analysis at 9 months of age showed a severe decrease in CD8<sup>+</sup> T cells (1.2%). The patient's family history revealed an older sister with EBV-related B-cell lymphoproliferative disease; she died at 22 months of age, due to systemic aspergillosis leading to multiorgan failure.

Next-generation sequencing revealed a novel homozygous missense mutation in the *ZAP70* gene (c.37G>A), confirmed by Sanger sequencing. Both parents were heterozygous for the mutation. The other healthy brother had two WT alleles, as assessed by high-resolution melting analysis (Figure 1B and Figure S1A). The mutation led to a Gly13Ser alteration in the N-SH2 domain of *ZAP70* (Figure 1C) and was associated with a loss of protein expression analyzed by both Western blot and flow cytometry (Figure 1D). Furthermore, phytohemagglutinin (PHA) as well as anti-CD3/CD28-induced CD4<sup>+</sup> T-cell proliferation was defective in the patient, compared to healthy family members (Figure S1B,C). Methodological details are provided in the Supplementary data.

Although the patient presented with mild clinical features, he was considered for HSCT due to the absence of *ZAP70* protein and suspected *ZAP70* deficiency in the older sibling who died from severe fungal infection. At 9 months of age, he was transplanted using a bone marrow stem cell source without conditioning from a fully-matched male sibling. However, he failed to engraft. One month later, he received a second bone marrow transplantation from the same donor following a reduced toxicity conditioning regimen (treosulfan-10 mg/m<sup>2</sup>/day from days -5 to -3 and fludarabine-40 mg/m<sup>2</sup>/day from days -5 to -2) with mycophenolate mofetil and methotrexate prophylaxis for graft-versus-host disease (GVHD). Full donor engraftment was achieved, and the patient did not experience GVHD. Allele-specific DNA polymerase chain reaction analysis of the *ZAP70* gene showed a normal high-resolution melting analysis and restriction profile after successful HSCT (Figure 1E). Currently, the patient is doing well, 10 months after transplantation, without any complications.



**FIGURE 1** Clinical features and functional consequence of the *ZAP70* mutation. (A) Cutaneous findings in the patient. A malar rash and erythematous papular lesions on the dorsum of arms, and livedo reticularis formation on the legs are shown. (B) Mutation segregation in the family depicting the 37G>A change. (C) Schematic diagram of the *ZAP-70* protein showing the position of the Gly13Ser missense mutation. The domain architecture of the *ZAP-70* protein consists of tandem Src homology 2 (SH2) domains, an interdomain A (I-A) between the SH2 domains, an interdomain B (I-B), and a kinase domain (D) Western blot analysis of the *ZAP-70* protein in the patient (Pt) and healthy control (HC) T cells in unstimulated and anti-CD3/anti-CD4 stimulated conditions. Tubulin staining is shown as a loading control (top). *ZAP-70* levels in CD4<sup>+</sup> T cells were almost monitored by intracellular staining with a monoclonal anti-*ZAP-70* Ab (solid lines) versus isotype controls (gray filled) and representative histograms are shown (bottom). (E) The PCR-amplified 169bp genomic product harboring the 37G>A mutation from the patient pre- and post-transplant, as well as from his brother can be distinguished by high-resolution melting analysis (HRM; top). Allele-specific restriction profile (*SfiI*) of the *ZAP70* gene in the patient before (pre) and post-HSCT as well as in a HC (bottom)

In the literature, there are three types of described mutations in *ZAP70* deficiency.<sup>1</sup> The most common is a complete loss-of-function mutation, often targeting the kinase domain. Hypomorphic mutations also exist, having been described in five patients. These patients present with less severe symptoms compared to patients with complete loss but also exhibit low levels of poorly functioning CD8<sup>+</sup> T cells.<sup>1,3</sup> Interestingly, Chan et al. described a compound heterozygosity for loss-and gain-of-function mutations, disrupting the autoinhibition of the protein, constituting a third form of mutations in *ZAP70*

deficiency. This atypical sibling pair displayed early-onset autoimmune diseases (bullous pemphigoid, colitis, hemophilia, and nephrotic syndrome) without infectious complications.<sup>4</sup> Our patient was found to have a novel homozygous missense mutation on *ZAP70*, resulting in loss of protein expression and function. The mutation is located in the N-terminal SH2 domain, and to date, there are only four described patients with mutations involving this domain.<sup>1,2</sup> Three were compound heterozygous, which included the N-SH2 and kinase domains, while the third was a single homozygous mutation found on the N-SH2

TABLE 1 Immunological evaluation of the patient before and after HSC transplantation

Parameters	Age (6 months)	Age (9 months)	Reference values	At 10 <sup>th</sup> month of HSCT	Reference values
Leukocyte count (/mm <sup>3</sup> )	13,600	17,000	6800–17,500	7700	5500–16,500
Absolute lymphocyte count (/mm <sup>3</sup> )	4700	6100	3740–10,500	3100	2963–11,346
Absolute neutrophil count (/mm <sup>3</sup> )	5000	7800	1100–6800	3800	1200–9150
Absolute monocyte count (/mm <sup>3</sup> )	1700 (†)	1500 (†)	0–600	600	0–600
Absolute eosinophil count (/mm <sup>3</sup> )	2100 (†)	2330 (†)	0–300	160	0–300
IgG (mg/dl)	759	1230 (on IVIG)	304–1231	1182	605–1430
IgA (mg/dl)	91	167	7–123	111	30–307
IgM (mg/dl)	99	145	32–263	67	66–228
IgE (IU/ml)	273 (†)	–	1.1–10.2	13.3	0–60
Specific antibody titers					
Anti-Hbs IgG (mIU/ml)	0.06 (↓)	–	0–10	–	0–10
Anti-measles IgG (IU/L)	2.23	–	>1.1	–	>1.1
Anti-mumps IgG (AI)	1.0 (↓)	–	>1.1	–	>1.1
Anti-rubella IgG (IU/ml)	0 (↓)	–	≥15	–	≥15
Lymphocyte subsets					
CD3 <sup>+</sup> T cell (%)	42.3 (↓)	46.6 (↓)	51–85.3	61.3	51.7–84.1
CD3 <sup>+</sup> T cell, count (/mm <sup>3</sup> )	1987 (↓)	2840	2340–7300	1890	1882–7372
CD3 <sup>+</sup> CD4 <sup>+</sup> T (%)	36.3	44.2	29.7–63.6	21.7	29.2–58
CD3 <sup>+</sup> CD4 <sup>+</sup> T count (/mm <sup>3</sup> )	1700	2696	1479–5050	672	1211–4696
CD3 <sup>+</sup> CD8 <sup>+</sup> T (%)	6.1 (↓)	1.2 (↓)	11.5–33.7	37.2	14–31
CD3 <sup>+</sup> CD8 <sup>+</sup> T count (/mm <sup>3</sup> )	287 (↓)	73 (↓)	584–2617	1147	567–2494
CD19 <sup>+</sup> B cell (%)	37.4	28.5	7.9–53.6	27.9	13–37
CD19 <sup>+</sup> B cell, count (/mm <sup>3</sup> )	1757	1738	450–3743	868	526–3126
CD16 <sup>+</sup> 56 <sup>+</sup> NK cell (%)	5	–	2.3–17.2	6.9	2–26
CD16 <sup>+</sup> 56 <sup>+</sup> NK cell count (/mm <sup>3</sup> )	235	–	163–894	214	105–1461
Naive B cell (%)	73 (↓)	–	77–99	88.8	69–97
NS B cell (%)	10.7	–	3–13	4.1	3.6–19
CS B cell (%)	12	–	0.8–12	1.3	2–17
CD21 <sup>low</sup> CD38 <sup>low</sup> activated B (%)	2.9	–	0.7–6.9	7.4	0.9–9
CD3 <sup>+</sup> TCR <sup>α/β</sup> cells (%)	81.2 (↓)	–	90–99.2	88.3	87–98
CD3 <sup>+</sup> TCR <sup>γ/δ</sup> cells (%)	9.1	–	2–10	4.8	2–14
Recent thymic emigrants (%)	12.8 (↓)	–	63–90	54.9	60–83
CD4 <sup>+</sup> CD45RA <sup>+</sup> CCR7 <sup>+</sup> T cell (%)	17.9 (↓)	–	62–98	54.4	52–97
CD4 <sup>+</sup> CD45RA <sup>-</sup> CCR7 <sup>+</sup> T cell (%)	21.8	–	2.6–54	15.8	8–38
CD4 <sup>+</sup> CD45RA <sup>-</sup> CCR7 <sup>-</sup> T cell (%)	52.5 (†)	–	0.1–13	25.3	0.3–11
CD4 <sup>+</sup> CD45RA <sup>+</sup> CCR7 <sup>-</sup> T cell (%)	7.7	–	0.1–76.3	4.5	0.2–55
CD8 <sup>+</sup> CD45RA <sup>+</sup> CCR7 <sup>+</sup> T cell (%)	1.25 (↓)	–	34–91.5	12.3	26.3–90
CD8 <sup>+</sup> CD45RA <sup>-</sup> CCR7 <sup>+</sup> T cell (%)	24.6 (†)	–	0.4–15.5	1.9	0.9–8.1
CD8 <sup>+</sup> CD45RA <sup>-</sup> CCR7 <sup>-</sup> T cell (%)	68.3 (†)	–	1–68	42.8	3–40
CD8 <sup>+</sup> CD45RA <sup>+</sup> CCR7 <sup>-</sup> T cell (%)	5.8 (↓)	–	9.6–70	42.9	13–69
T-cell proliferation (anti-CD3/CD28; %)	–	Undetectable	50–65	62	50–65

Note: Abnormal values are indicated in the parenthesis

Abbreviations: CS: Class switched, IVIG: Intravenous immunoglobulin, NS: Non-class switched, (-): Not done.

domain. Dermatitis was described in all of these patients, but apart from our report, other patients also presented respiratory and gastrointestinal infections.<sup>2,5-7</sup>

The majority of described patients with ZAP70 mutations had a clinical diagnosis of CID. Furthermore, some patients were also reported to have EBV-related lymphoproliferative disease, autoimmunity (mostly autoimmune cytopenia), hemophagocytic lymphohistiocytosis, and lymphoma.<sup>1</sup> The cutaneous lesions in patients with ZAP-70 deficiency have consisted of an erythematous urticarial rash, bullous pemphigoid, eczema perineal ulcers, and widespread xerosis. Notably, in previously reported patients, these dermatological problems have been accompanied by more prominent clinical findings such as respiratory tract and gastrointestinal infections.<sup>1</sup> Similar to our case, one infection-naïve patient showing eczematous skin lesions with infiltrating CD4<sup>+</sup> T cells has been described in the literature.<sup>5</sup> The survival and infiltration of CD4<sup>+</sup> T cells may be attributed to an increased expression of the ZAP-70 related Syk protein tyrosine kinase, leading to an abnormal activation and differentiation of CD4<sup>+</sup> T lymphocytes.<sup>5,8</sup>

Immunological findings of our patient were compatible with the findings of reported patients showing eosinophilia and elevated IgE levels, resembling Omenn syndrome.<sup>5,9</sup> A unique feature of our patient was the decrease in the percentage of CD8<sup>+</sup> T cells in the 2<sup>nd</sup> flow cytometry analysis, reflecting a loss of CD8<sup>+</sup> T cells during the postnatal period (between 6 and 9 months of age). This phenomenon has not been described in other reported patients. However, normal to low newborn T-cell receptor excision circle levels in the same patient were reported previously, indicating that the immunological findings of disease can exacerbate over time.<sup>1</sup> The other possibility of decrease CD8<sup>+</sup> T cells can be explained by a potential loss of maternal T cells with age,<sup>10</sup> but this was excluded in our patients. These data strongly suggest that serial measurements of CD8<sup>+</sup> T cells in the setting of clinical suspicion of ZAP70 deficiency would be helpful for appropriate diagnosis.

In conclusion, ZAP70 deficiency is characterized by a broad spectrum of clinical presentations. Even in patients with subtle clinical findings, progressive reduction in CD8<sup>+</sup> T-cell counts should evoke the possibility of ZAP70 deficiency, improving early diagnosis and promoting a better outcome for these patients.

## KEYWORDS

dermatitis, inborn errors of immunity, transplantation, ZAP70

## CONFLICT OF INTEREST

None to declare.

## AUTHOR CONTRIBUTIONS

**Royal Babayeva:** Formal analysis (equal); Investigation (equal); Resources (equal); Writing – original draft (lead). **Cedric Mongellaz:** Investigation (equal); Methodology (equal). **Ibrahim Serhat Karakus:** Data curation (equal); Resources (equal); Writing – original draft (equal). **Murat Cansever:** Resources (equal). **Sevgi Bilgic Eltan:** Resources (equal). **Mehmet Cihangir Catak:** Investigation (equal);

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## ETHICAL APPROVAL

The study was approved by the Ethics Committee of Marmara University, School of Medicine.

## CONSENT TO PARTICIPATE

Informed consent for participation was obtained from the family.

## CONSENT FOR PUBLICATION

Informed publication consent was obtained from the family.

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## PEER REVIEW

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## DATA AVAILABILITY STATEMENT

The data generated during the study are included in this published article and its supplementary file.

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