 DOI: https://doi.org/10.5114/ceji.2021.107030

Mutation in the proline-serine-threonine phosphatase-interacting protein 1 (*PSTPIP1*) gene in a patient with acute lymphoblastic leukemia

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Abstract

Autoinflammatory syndromes are disorders characterized by recurrent or chronic inflammation caused by the dysregulation of the innate immune system. Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of overactivation of the immune system.

We present a case of a 20-month-old boy who was referred to an oncology clinic because of HLH suspicion. In the preceding time, our patient suffered from a severe form of chickenpox with prolonged fever. Tests including myelogram, cerebrospinal fluid, and magnetic resonance (MR) of the brain gave a diagnosis of acute lymphoblastic leukemia from B lymphocyte precursors, without occupying the central nervous system. To exclude inherited HLH in our patient, next-generation sequencing was performed, which revealed a heterozygous missense mutation in exon 15 of the PSTPIP1 gene (c.1213C>T, R405C). No mutations of genes associated with familial HLH syndrome were found.

Our patient may be evidence that autoinflammatory diseases caused by PSTPIP1 gene mutations are not limited to the classical pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) phenotype but may have a different clinical presentation, and the spectrum of the PSTPIP1-associated inflammatory diseases (PAID) syndrome is more extensive than previously thought.

Key words: *hemophagocytic lymphohistiocytosis, lymphoblastic leukemia, autoinflammatory syndrome.*

(Cent Eur J Immunol 2021; 46 (2): 270-274)

Introduction

Autoinflammatory syndromes are a heterogeneous group of disorders characterized by recurrent or chronic inflammation caused by dysregulation of the innate immune system. Most of these diseases are very rare. Autoinflammatory syndromes are caused by genetic mutations in molecules that take part in regulation of the innate immune response. Monogenic inheritance is common [1]. In such cases, family history is relevant.

Immune dysregulation should be suspected in patients with an unusual presentation of infections – lasting longer, more severe than usual, atypical, with prolonged fever. The occurrence of a variety of inflammatory diseases in the family is a risk factor for autoinflammatory disorders as well. The main symptoms of autoinflammatory syndromes are prolonged fever, skin inflammation, and joint involvement, and patients develop them gradually through life. Inflammatory markers are usually elevated without a source of infection [1, 2].

Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation [3, 4]. It affects mostly children but can also occur in adults. Primary HLH has an underlying genetic disorder, and secondarily occurs as a response to another condition – cancer, infection, chemotherapy. Both primary and secondary HLH can be triggered by infections. HLH should be regarded as a typical final phenotype secondary to a range of diverse underlying molecular diagnoses rather than as a distinct disease. Many other autoimmune syndromes can have a similar phenotype [5, 6].

According to guidelines of the International Histiocyte Society, a diagnosis of HLH requires at least five of the following eight criteria: fever, splenomegaly, cytopenia, hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, elevated soluble interleukin-2 receptor α (IL-2Rα),

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Submitted: 5.05.2020; Accepted: 24.07.2020

decreased natural killer (NK) cell activity, and hemophagocytosis in the bone marrow [3, 4, 6]. Details are presented in Table 1. Proper diagnosis of autoinflammatory syndromes (including HLH) is necessary not only for treatment but also to establish the prognosis. Untreated HLH is always 100% fatal. Definite diagnosis of autoinflammatory syndromes is based on genetic testing, which is not commonly available.

Table 1. Hemophagocytic lymphohistiocytosis (HLH) diagnostic criteria [6]

Five of the following eight findings
Fever ≥ 38.5°C
Splenomegaly
Bicytopenia
hemoglobin < 9 g/dl
platelets < 100,000/µl
absolute neutrophil count < 1000/µl
Hypertriglyceridemia or hypofibrinogenemia (fasting triglycerides > 265 mg/dl, fibrinogen < 150 mg/dl)
Ferritin > 500 mcg/l
Low/absent NK cell activity
Soluble CD25 elevation > 2,400 U/ml
Hemophagocytosis in bone marrow, spleen, lymph node, or liver, or a molecular diagnosis consistent with HLH

Case description

A 20-month-old male patient was referred to the oncology clinic for evaluation of a possible HLH based on his laboratory test results. The patient was born at 40 weeks gestation to a healthy 34-year-old G3P3 mother, delivery by cesarean section, birth mass 3.8 kg, Apgar score of 10. The boy was vaccinated without complications until the age of 14 months according to the Polish schedule (https://szczepienia.pzh.gov.pl/en/immunization-schedule/) – BCG and hepatitis B – 24 hours after birth, 2 and 7 months, DTP 2, 3, 5 months, Hib 2, 4, and 6 months, PVC 2, 4 and 13 months, IPV 3, and 6 months, MMR 14 months. The boy was not vaccinated against VZV (it is not obligatory in Poland). He was previously diagnosed with atopic dermatitis and cows' milk allergy. The parents are unrelated, and both are healthy. The family history concerned in particular the oldest brother who is an asymptomatic carrier of the heterozygous *CFTR* gene variants (F508del, and L467F) (diagnosed through newborn screening), which he inherited one from his mother and one from the father. Our patient's mother's sister was diagnosed with Wilson's disease at the age of 25 (carrier of heterozygous H1069Q and Q1351X mutations in *ATP7B* gene), and additionally at the age of 41 with Crohn's disease. Of the five siblings of the patient's mother, Wilson's disease was also confirmed in the mother's youngest brother. Our patient and his mother are asymptomatic carriers of the H1069Q variant in the *ATP7B* gene. The boy is also an asymptomatic carrier of heterozygous variants in the *CFTR* gene, i.e., F508del and L467F. The rest of the family members, including both siblings, are healthy.

The current disease began a month earlier with enterocolitis. Five days before chickenpox, he was diagnosed with pharyngitis – treated with first-generation cephalosporin. The first hospitalization was on day 5 of fever in the course of chickenpox. The source of infection was unknown – family members were healthy, siblings and the patient himself did not attend kindergarten. On admission, physical examination revealed a rash on the trunk and swelling of hands and feet. Inflammatory markers were not clearly elevated (details are presented in Table 1). Chickenpox was severe, with many lesions and prolonged fever. VZV-DNA by PCR was found in the skin lesion scraping. He was treated with antibiotics and acyclovir for seven days. He had a fever for ten days in total. After four days without a fever, he was sent home. The day after discharge, he returned with the fever without any other symptoms. Laboratory tests on readmission are shown in Table 2. Seasonal infections such as influenza and respiratory syncytial virus (RSV) were excluded. He was empirically treated with 3rd generation cephalosporin. During the next four days, enlargement of the liver and spleen was increasing, anemia and thrombocytopenia appeared, and the level of ferritin and triglycerides had increased. HLH was suspected, and the child was referred to the oncology department.

On admission to the oncology ward, the boy was in a bad condition, he had a fever above 39°C, a rash on the trunk, swelling of the hands and feet, generalized lymphadenopathy, liver enlargement about 2 cm below the navel and spleen about 2 cm below the rib arch. Slight swelling of the upper eyelids was also found. Findings of bone marrow and lumbar puncture were not consistent with HLH. Myelogram showed 91% of cells with L1 morphology. Flow cytometric analysis revealed 86% blast CD19+, CD34+, cCD79a+, CD10+, TdT+, CD58+, CD38+, cIgM–, sIgM–, CD22+/–dim, CD20+/–. The diagnosis of acute lymphoblastic leukemia from precursors of B lymphocytes, SR groups, without the involvement of the central nervous system, was made. Hemophagocytosis in bone marrow, count per 1,000 nucleated cells, was low – 0.01%. *TEL-AML1*, *BCR-ABL*, *SIL-TAL1*, *KMT2A* rearrangements have not been observed in leukemic cells. The study with CytoScan HD Affymetrix did not confirm the deletion of the *IKZF1*, *PAX5*, *PAR1*, and *ERG* gene. However, the bi-allelic deletion of *CDKN2A* and *CDKN2B* genes was found. 24-hour leukemic cell culture showed abnormal male karyotype – 46,XY, inv(1)(p13p36.3),del(9)(p21),del(13)(?q21).

Treatment was consistent with the AIEOP-BFM pB ALL 2017 scheme. Chemotherapy was complicated by

Table 2. Laboratory tests results during hospitalizations. The results in bold are outside the limits of the normal value

Parameter	Value on 1st pediatric admission	Value on 2nd pediatric admission	Value on oncology admission	Normal values
Hemoglobin (g/dl)	10.4	**9.0**	**8.8**	10.1-13.0
Erythrocytes (10^6/µl)	4.03	**3.48**	**3.21**	3.9-5.10
Platelets (10^3/µl)	223	156	**55**	200-550
Leukocytes 10^3/µl	**14.3**	**28.1**	6.4	3.80-10.0
Neutrophils (10^3/µl)	**13.2**	**13.2**	**2.1**	2.0-7.0
Neutrophils (%)	**92.31**	46.98	32.81	35-55
Albumin (g/l)	36	34	**21**	35-52
CRP (mg/l)	**10.64**	**8.5**	**3.25**	0-5
Procalcitonin (ng/ml)	**0.37**	**9.96**	**4.52**	< 0.05
IgG (g/l)	5.73	ND	5.12	4.53-9.16
IgM (g/l)	1.68	ND	1.71	0.19-1.46
IgA (g/l)	0.66	ND	1.24	0.2-1.00
IgE (IU/ml)	**276.6**	ND	**281.4**	0-60
AST (U/l)	**120**	**313**	**385**	0-32
ALT (U/l)	**51**	**58**	**59**	0-33
CPK (U/l)	39	51	47	29-168
LDH (U/l)	**247**	**2583**	**3284**	125-243
Ferritin (ng/ml)	210	**18444**	**18284**	20-150
Fibrinogen (g/l)	3.1	**4.1**	**1.8**	1.80-3.05
D-dimers (mcg/ml)	0.3	**0.5**	**2.7**	< 0.4
Triglycerides (mg/dl)	90	**251**	**201**	0-150

CRP – C-reactive protein, AST – asparagine aminotransferase, ALT – alanine aminotransferase, CPK – creatine phosphokinase, LDH – lactate dehydrogenase

infection (molluscum contagiosum), hepatotoxicity, neurotoxicity, cardiotoxicity (sinus bradycardia), and endovascular clotting (Leiden mutation, as well as mutations in prothrombin, and *MTHFR* genes were excluded). On day 15, hematological remission was obtained, and on day 33, remission was confirmed by flow cytometry and molecular tests. The patient remains in full remission of leukemia, continues treatment, molluscum contagiosum lesions are still observed but to a lesser extent. To exclude congenital HLH, a genetic test (by next-generation sequencing in a range of 5227 genes included in the SureSelect Custom Constitutional Panel, Agilent Technologies) was performed. The presence of a heterozygous missense mutation in exon 15 of the *PSTPIP1* gene (c.1213C>T, R405C) and two heterozygous variants in the *CFTR* gene, i.e., F508del and L467F, was identified. Gene mutations associated with inherited HLH syndrome were not found. Neither parent carries this *PSTPIP1* variant.

Discussion

The course of the disease in our patient and the results of the tests – recurrent and prolonged fever, enlarged liver and spleen, increased anemia and thrombocytopenia, high levels of ferritin and triglycerides – suggested HLH. The patient fulfilled four out of six tested HLH-2004 criteria. However, further studies have shown leukemia and a heterozygous missense mutation in the *PSTPIP1* gene while excluding gene mutations associated with congenital HLH.

Proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1; also known as CD2 binding protein 1-*CD2BP1*) is a cytoskeletal protein within hematopoietic cells that serves as a scaffold for the binding of other cellular proteins, such as pyrin, protein tyrosine phosphatases, c-Abl, CD2, and WASP (Wiskott-Aldrich syndrome protein) [5, 6]. PSTPIP1 regulates several cellular functions, including IL-lβ release, cytoskeleton organization, cell migration, and T-cell activation cytoskeleton-associated adaptor protein that modulates T-cell activation, cytoskeletal organization, and IL-1β release [7-9].

The *PSTPIP1* gene is located on chromosome 15q24-q25.1. So far, 62 variations in the *PSTPIP1* gene have been described according to the registry of Hereditary Autoinflammatory Disorders Mutations (Infevers): 3 are pathogenic (A230T, E250Q, E250K), another five likely pathogenic and the rest are of unknown pathogenicity [9, 10]. The gene defect is inherited in a dominant manner. Depending on the mutation location within the *PSTPIP1* gene and consequent alterations in protein-protein interactions, a spectrum of autoinflammatory disorders may

result and are collectively referred to as PSTPIP1-associated inflammatory diseases (PAID). PAID encompasses pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA), pyoderma gangrenosum, acne, and hidradenitis suppurativa syndrome (PAPASH) and PSTPIP1-associated myeloid-related proteinemia inflammatory syndrome (PAMI) [11-14].

PAPA syndrome is known to be caused by A230T, E250Q, and E256G variants of the *PSTPIP1* gene. Mutations located in region 15q24-q25.1 are believed to create dysfunction in the innate immune response that leads to chronic low-level inflammation in the body presenting as PAPASH (E277D) or PAMI [15-17]. In patients with PAID, variable expressiveness of disease is noted. These autoinflammatory syndromes have little in common, but in all gene variants, the skin is affected, with skin inflammation appearing in a variety of manifestations. The clinical picture presented by our patient resembles PAMI. PAMI, also known as hypercalprotectinemia and hyperzincemia (Hz/Hc), is a rare inborn error of zinc metabolism characterized by recurrent infections, hepatosplenomegaly, anemia and chronic systemic inflammation in the presence of high plasma concentrations of zinc and calprotectin [18]. Patients typically present dermal ulcers or other cutaneous manifestations and arthralgia. Myeloid-related protein 8 (MRP) and MRP 14 are ligands of Toll-like receptor four and are highly expressed in granulocytes, monocytes and keratinocytes. Marked dysregulation of myeloid-related protein 8/14 (MRP 8/14) metabolism is associated with the accumulation of zinc caused by the zinc-binding capacities of MRP 8/14. Zinc levels are increased in patients with Hz/Hc but not in patients with PAPA syndrome, familiar Mediterranean fever, or systemic juvenile idiopathic arthritis. Usually, inflammation induces a reduction in plasma zinc concentrations because of redistribution in the cellular compartment [19]. This could be the reason that in our patient the zinc and calprotectin levels were not elevated.

Mutation detected in our patient was previously described in a patient with PASH syndrome [20]. *In silico* analysis used for computed estimation of the potential pathogenesis of this missense mutation proved that it is the first PAPA-associated mutation that has been found in the SH3 domain *PSTPIP1* [8]. The authors also proved that the PSTPIP1-R405C mutant had significantly impaired interaction with WASP and demonstrated that the mutation is pathogenic in a patient with an aggressive form of PG [8].

The majority of previously described patients with mutations in the *PSTPIP1* gene diagnosed with PAPA or PAPASH were adults who developed symptoms later in life. In contrast, patients with PAMI described by Holzinger *et al*. developed symptoms in early childhood; the median age in the study group was 13 months [9].

The described patient suffered from severe varicella with prolonged fever, which is unusual for an immunocompetent patient with sporadic exposure to the virus. More severe cases are experiences after household exposure to the varicella virus. The source of the varicella virus was unknown; family members were healthy. It is known that the depletion of CD4 T cells results in higher viral loads, prolonged viremia, and more severe varicella. Severe herpes infections are particularly characteristic of primary immune deficiency and can become the most notable feature in a patient. Our patient also developed severe molluscum contagiosum infection from the very first day of chemotherapy. Similar to herpes virus susceptibility, molluscum can be seen with nearly any T cell defect. The *PSTPIP1* gene encodes a cytoskeletal protein that binds to CD2, an effector of T cell activation and adhesion, downregulating CD2-triggered adhesion [8]. Janssen *et al*. proposed that mutations in the *PSTPIP1* gene cause defects in T-cell differentiation, by defective control of F-actin polymerization [15].

Our patient had an abnormal immune response to viral infection with primarily severe inflammation. Severe molluscum contagiosum can be seen in patients with Wiskott-Aldrich syndrome, and PSTPIP1 protein is a cytoskeletal protein within hematopoietic cells that serves as a scaffold for the binding of WASP [8].

The described course of the disease supports a role for *PSTPIP1* mutations in immunodeficiency. Autoinflammatory diseases due to *PSTPIP1* mutations are not restricted to the classical PAPA phenotype but might present with other distinct clinical features [9, 21]. With variation present between disease presentations from case to case, it is possible that the spectrum of the PAID syndrome is broader than currently thought. There is also the possibility that the patient had a prolonged infection because of developing ALL, and HLH-like symptoms were caused by cancer. The detected mutation has not affected his clinical course so far but may cause significant symptoms later in life. However, his clinical course was different from other oncology patients.

Further research is needed, which may uncover the full picture of PAID spectrum diseases.

The authors obtained written informed consent from the patient's legal representatives for the publication.

The authors declare no conflict of interest.

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