Novel Mutations in *SH2D1A* Gene in X-linked Lymphoproliferative Syndrome, Diagnosed After B-Cell Non-Hodgkin Lymphoma

Svetlana O. Sharapova, PhD, Alina S. Fedorova, MD, PhD,**
Olga E. Pashchenko, MD, PhD,† Svetlana S. Vahliarskaya, MD, PhD,†
Irina E. Guryanova, Alexandr A. Migas,**
Irina V. Kondratenko, MD, PhD (DSc),†
*and Olga V. Aleinikova, MD, PhD (DSc)**

Background: X-linked lymphoproliferative disease type I (XLP I) is caused by mutations in the *SH2D1A* gene and characterized mainly by hypogammaglobulinemia and abnormal response to Epstein-Barr virus with a high predisposition to B-cell non-Hodgkin lymphoma development.

Observations: In this article, we describe the experience of 2 centers in Belarus and in Russia that follow 3 male patients who were diagnosed with XLP I after lymphoma development and treatment. Three novel mutations c.51G > C (p.E17D), c.192G > T (p.W64C), and c.53insA (p.K18KfsX67) were found in 3 males patients with XLP I. Two of them did not have any signs of immunodeficiency before B-cell non-Hodgkin lymphoma development.

Conclusions: We propose *SH2D1A* mutational screening be considered in male patients with or without hypogammaglobulinemia who received rituximab treatment for lymphoma and did not recover immunoglobulin G in a year after B-depleting therapy.

Key Words: X-linked lymphoproliferative disease type I, *SH2D1A* mutation, non-Hodgkin lymphoma, rituximab, hypogammaglobulinemia

(*J Pediatr Hematol Oncol* 2017;39:e203–e206)

Non-Hodgkin lymphoma (NHL) is the most common type of malignancies associated with primary immunodeficiency (PID) and can occur either before or after infectious complications develop.[1] Lymphoma type and frequency vary among children with different types of PID.

X-linked lymphoproliferative disease type I (XLP I) is caused by mutations in *SH2D1A* gene and may present in male patients with hypogammaglobulinemia, fulminant infectious mononucleosis or hemophagocytic lymphohistiocytosis (due to inappropriate immune response to Epstein-Barr virus [EBV] with widespread proliferation of cytotoxic T cells, EBV-infected B cells, and macrophages), as well as with lymphoma (mostly B-cell NHL [B-NHL]), aplastic anemia, vasculitis, and pulmonary lymphomatoid granulomatosis.[2] To date, >100 mutations in *SH2D1A* gene have been described.[3–5]

Lymphoma affects about 25% of XLP patients,[6] typically manifesting as EBV-associated B-mature malignancy at the ages between 5 and 8 years.[2] Rituximab is currently widely used in B-NHL treatment and can lead to a decrease in the serum immunoglobulin (Ig) level, which is usually not accompanied by severe infections. Prolonged hypogammaglobulinemia in children after rituximab-containing treatment may be a sign of PID and has to be distinguished from side effect of anti-CD20 monoclonal antibodies.[7]

In this report, we highlight 3 novel mutations in *SH2D1A* gene, identified in male pediatric patients with XLP I diagnosed after B-NHL treatment.

MATERIALS AND METHODS

Patients

Three boys were suspected and confirmed as having XLP I after B-NHL treatment due to complicated clinical course of disease and/or absence of IgG serum level recovery after rituximab-containing therapy.

For all patients, written informed consent for the performed studies was obtained from the patient's family.

Methods

Genetic and clinical data, lymphoma treatment, and immunological findings are summarized in Table 1. Histopathology was classified according to the WHO system.[8] Immunologic investigation included serum Ig levels and the number of B cells. All exons and exon/intron regions of the *SH2D1A* gene were amplified from the genomic DNA by polymerase chain reaction and sequenced in both directions using the ABI Prism BigDye Terminator Cycle sequencing kit on a GeneticAnalyzer ABI 3130 automated sequencer (Hitachi, Japan). Ensembl Genome Browser (www.ensembl.org) was used for evaluating *SH2D1A* variants (ENST00000371139). The occurrence of mutations was checked according to RAPID database (http://web16.kazusa.or.jp/rapid/mutation?pid_id = AGID_119), SH2D1A bases (http://structure.bmc.lu.se/idbase/SH2D1Abase/browser.php?content = browser), and published cases.

Received for publication April 1, 2016; accepted February 1, 2017.
From the *Research Department, Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Minsk Region, Belarus; and †Department of Clinical Immunology, Russian Clinical Children's Hospital, Moscow, Russia.
The work was supported by grants of the Ministry of Health of Belarus. The authors are responsible for the contents of this publication.
The authors declare no conflict of interest.
Reprints: Svetlana O. Sharapova, PhD, Immunology Laboratory, Research Department, Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Settlement of Borovliani, Minsk Region 223053, Belarus (e-mail: sharapovasv@gmail.com).

For interpretation of novel missense variants, sorting intolerant from tolerant (SIFT) and polymorphism phenotyping (PolyPhen-2) were used.

CASE REPORTS

Patient 1

A 6-year-old Slavic boy was transferred from another hospital 1 week after the laparotomy with resection of the ileum and ileostomy imposition was performed. He had a 2-week history of abdominal pain accompanied by dysuria and constipation. Acute intestinal obstruction was the reason for emergency surgery, and mesentery tumor was revealed and resected. He experienced night sweats and significant weight loss. A computed tomographic scan of the abdomen and pelvis showed tumor mass in hypogastry, $7.5 \times 4.0 \times 8.0$ cm, spreading to the bladder and the abdominal wall, and multiple enlarged mesenteric and ileac lymph nodes up to 4 cm in diameter. Bone marrow aspirate and cerebrospinal fluid were negative for lymphoma. The resected tumor was histologically determined to be Burkitt lymphoma. The tumor cells were strongly positive for LCA, CD20, and CD79 and negative for T lymphocyte markers (CD3 and TdT) as well as for EBV by immunohistochemistry. The boy was treated within the NHL-BFM-95 protocol with 4 chemotherapy cycles at full dosage, and tolerated well (Table 1). The patient received 3 additional doses of intravenous rituximab 375 mg/m^2 once weekly after the second course of chemotherapy because of a long interruption in treatment due to the reconstructive bowel surgery.

TABLE 1. Genetic, Immunologic, and Clinical Characteristic of the Patients

	Patient 1	Patient 2	Patient 3
Mutation in *SH2D1A*	p.E17D c.51G > C	p.W64C c.192G > T	p.K18KfsX67 c.53insA
Age at lymphoma diagnosis (y)	6.0	14.5	6.8
Lymphoma type/ stage/ involved sites	Burkitt/III/ mesentery, jejunum, abdominal lymph nodes, bladder, abdominal wall	DLBCL/IV/ mediastinum, soft tissue of the left thigh, the anterior chest wall and bone marrow	DLBCL/I, not resected/right testicle
Treatment protocol, number of rituximab infusion	NHL-BFM 95, TG2, R2, full dosage, + 3 dosage of rituximab	B-NHL-M2004, high risk group, full dosage, 4 dosage of rituximab	NHL-BFM 90, TG2, R2, full dosage, + 3 dosage of rituximab
B cell recovery after rituximab	In 9 mo CD19$^+$ = 15.9% (860 cells/μL)	No data	In 12 mo CD19$^+$ = 29.5% (406 cells/μL)
IgG (g/L)	0.0	0.33	0.12
IgM (g/L)	0.33	0.17	0.01
IgA (g/L)	0.01	0.06	0.01
Family history	First pregnancy, the only child	First pregnancy, first delivery, parents are cousins	First pregnancy, first delivery
Outcome	Alive, 14 y old	Died at the age of 19 y	Died at the age of 8.3 y

B-NHL indicates B-cell non-Hodgkin lymphoma; DLBL, diffuse large B-cell lymphoma; Ig, immunoglobulin; NHL, non-Hodgkin lymphoma.

Absence of Ig serum level recovery within 9 months after the end of treatment (Table 1), while the level of CD19$^+$ cells became normal, and a considerable decrease in IgD-switched memory B cells 0.3% (normal, 4% to 20%), were the reason to perform genetic investigations, and XLP I was confirmed. The child's parents refused alloHSCT. The patient receives monthly intravenous Ig and has no evidence of lymphoma or severe viral infections after a follow-up of 8 years.

We detected a novel missense mutation in exon 1 of *SH2D1A* gene resulting in substitution of a glutamine to asparagine at amino acid position 17. The mutation has not been reported previously in the literature[11,12] and according to RAPID database and SH2D1Abases. This mutation was predicted to be pathogenic by prediction programs (PholyPhen-2, score of damaging = 0.99, SIFT = 0).

Patient 2

A 6-year-old boy was admitted to the Immunologic Department with suspected PID. He was the first and the only child of consanguineous (second cousins) healthy parents of white origin. Two male cousins of the patient's mother died in infancy of infections (Fig. 1). The patient suffered from recurrent purulent conjunctivitis since the age of 5 months. Diarrhea, recurrent purulent otitis, and respiratory infections appeared in the second year of life after the completion of breastfeeding. Chronic pulmonary infection poorly controlled by antibiotics and accompanied by repeated febrile episodes and chronic diarrhea were the reasons to investigate the immunologic status. Agammaglobulinemia was revealed while the B cell level was 15% (normal, 4% to 20%) (Table 1). At that time, the patient was recognized as having common variable immunodeficiency (CVID), and continuous intravenous Ig replacement therapy with antibiotic prophylaxis was started. The boy had progressive growth retardation. At the age of 12 years, his height was 129 cm and his weight was 22 kg.

At the age of 14 years, a painless mass of about 2.0×2.5 cm in size appeared on the front right side of the chest wall. A tumor biopsy was performed, and the diagnosis of diffuse large B-cell lymphoma was established. Immunohistologic staining showed malignant cells positive for LCA and CD20 and negative for CD21 and CD30. The patient had stage IV disease according to the St Jude staging system with abdominal lymph nodes, mediastinum, soft tissues of the left thigh, the anterior chest wall, and bone marrow involvement. He was treated according to the B-NHL-M2004 protocol, and received 6 courses of chemotherapy similar to the NHL-BFM-95 (group R4) regimen combined with rituximab given at the dose of 375 mg/m^2 on day 0 in cycles 1 to 4. The dose of methotrexate was reduced to 1 g/m^2 in the first 2 courses. Complete remission was achieved and maintained throughout the duration of observation.

Despite supportive treatment, chronic bronchopulmonary disease was progressing, pneumofibrosis and chronic respiratory failure of the 3-d degree developed and caused his death at the age of 19 years.

Genetic investigation was performed after the patient's death, and XLP I was diagnosed postmortem. A second unpublished mutation was detected in exon 2, c.192G > T (p.W64C). All previously described prediction programs showed pathogenicity (PholyPhen-2, score of damaging = 0.98, SIFT = 0). Different nucleotide changes at the position c.192G > A resulting in p.W64X[9] and nearest changes c.191G > A (p.W64X)[3] have been reported previously.

Patient 3

A 6-year-old boy from the Russian Far East, who had neither relevant family history, no serious infections except frequent respiratory infections of the upper airway since the age of 2 years, was diagnosed with DLBL of the right testicle and was treated with 4 chemotherapy cycles within the NHL-BFM-90 protocol, added with 3 doses of rituximab. A year after the diagnosis of lymphoma, he was hospitalized in a poor condition with right pneumothorax and fever not responding to antibiotic therapy. Lung biopsy revealed unspecific interstitional pneumonia with massive eosinophilic

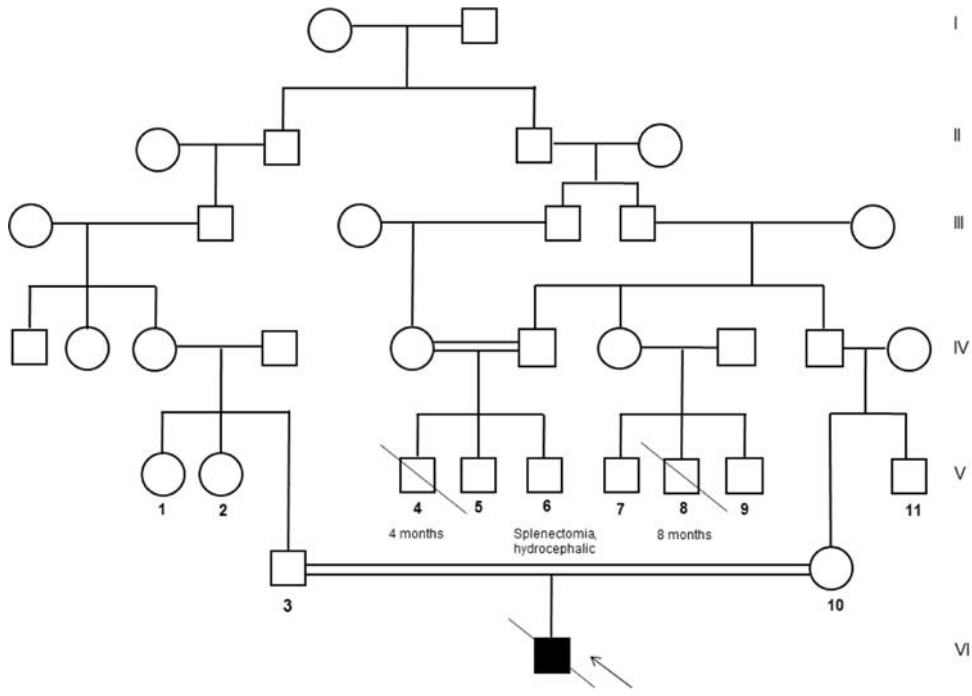

FIGURE 1. Family tree of patient 2. A pedigree was constructed based on the index case patient 2. Generations are designated by Roman numerals (I to VI)), and individuals of generation V are designated by Arabic numerals (1 to 11); The symbols used are proband (arrow), male (box), female (circle), deceased (line through), affected male (black fill), unknown (no fill), consanguinity (double lining). Pedigree showing the consanguinity in 2 generations in the family (IV and VI). Two male cousins of the patient's mother (4 and 8) died in infancy due to infections and cousin 6 was hydrocephalic. Other relatives were not checked for mutation carrier because of postmortem genetic investigation of the proband.

infiltration and proliferation of alveolocytes. Respiratory insufficiency progressed over a week despite escalation of antibiotic treatment and antifungal therapy. Bronchoalveolar lavage was checked for possible viral (PCR including CMV/EBV), bacterial, pneumocystic and fungal causes, but no causative organism was identified.

Pleural fluid analysis did not reveal lymphoma cells but demonstrated a cytosis of 426 cells/mm^3 (leukocytes [lymphocytes 82%] and erythrocytes) and an extremely high protein level = 43 g/L (normal, 0.15 to 0.45 g/L). Immunologic investigation showed agammaglobulinemia coupled with an increased B cells CD19 + = 29.5% (Table 1). The rapidly deteriorating clinical condition, the lack of response to broad-spectrum antimicrobial therapy, and the inability to make a quick differential diagnosis between pulmonary vasculitis, lymphoma and immune pneumonitis, were the reasons to start immunosuppressive treatment. The patient received pulse therapy with methylprednisolone and a single dose of cyclophosphamide 1000 mg/m^2, but without response. A few days later, the patient died due to progression of multiple organ failure. The autopsy revealed diffuse fibrosing alveolitis of unknown etiology with the formation of fibrosis and emphysema of the mediastinum soft tissue without evidence of lymphoma.

The diagnosis of XLP was established postmortem. Sequencing of genomic DNA detected an insertion c.53insA resulting in a stop codon after a 67 nucleotides.

DISCUSSION

XLP is associated with a high predisposition to lymphoid malignancies.[2] Some research groups carried out mutational screening of *SH2D1A* gene among DNA samples of male patients with B-NHL.[10,11] Sandlund et al described 5 cases of XLP diagnosed among 158 male patients (approximately, 3.2%) presenting with B-NHL.[10]

In this article, we present case reports of 3 male pediatric patients who were diagnosed as having XLP I after treatment of B-NHL. In one of them (patient 1), immunodeficiency was suspected due to the absence of Igs despite normal B cell counts more than 1 year after B-depleting therapy was stopped. Patient 2 was misdiagnosed as having CVID before malignancy had developed. XLP 1 in this patient was suspected postmortem because of low Igs level and lymphoma. In patient 3, XLP was suspected, when he developed severe nonmalignant lung interstitial disease, a year after lymphoma treatment.

Patients 1 and 3 in our report are very similar in their clinical presentation. They had no serious infections before lymphoma diagnosis and developed malignancy at the same age (Table 1). Their serum Ig levels were not investigated before lymphoma development. Similarities between patients 1 and 3 may be explained by the close mutation position (Table 1). However, patient 1 had missense mutation, which was not described previously. It could be the reason for the milder clinical presentation and survival without severe infections for 7 years before lymphoma development and (in patient 1) for 7 years after lymphoma treatment only with Ig substitution.

Close position but another type of mutation (insertion) in the *SH2D1A* gene in patient 3 was likely to be the reason for severe pulmonary complication after lymphoma treatment. This complication was not identified during the disease course, one of the supposed versions was pulmonary vasculitis. Vasculitis in XLP patients was described in literature,[5] but to confirm the diagnosis, it is necessary to perform immunohistologic staining, which was not performed for patient 3.

Patient 2 presented symptoms in early childhood and fulfilled the diagnostic criteria for CVID at the age of 6 years. However, conventional Ig replacement therapy and antibiotics were unsuccessful, and diffuse large B-cell lymphoma developed at the age of 14 years. To our knowledge, the patient's mutation p.W64C has not been reported in literature.[3–5] Family history may help identify a link with X chromosome inheritance for many male patients with immunodeficiency, but some XLP cases can be occurred de novo.[6] Family tree of patient 2 (Fig. 1) was really intriguing, but does not have signs of X-linked disease inheritance. Two male relatives died at the age of 4 and 8 months, in the families of grandfather's sister and brother (consanguineous marriage). Such complicated family history did not allow the exclusion of additional genetic abnormalities in this family except mutation in *SH2D1A* gene, which were described earlier.[12]

Dysgammaglobulinaemia and lymphoma are 2 common manifestations in XLP I, which occur in EBV-positive as well as EBV-negative patients.[2,6] Laboratory data of all our patients did not reveal the presence of EBV infection in clinical manifestations of the disease. Previously healthy male patients developed EBV-negative lymphoma, had no evidence of infectious mononucleosis and absence of IgG EBV throughout the period of observation. Up to one-third of XLP patients with lymphoma are EBV-seronegative, indicating that mechanisms other than malignant transformation of EBV-infected B cells, such as defective antitumor immunosurveillance, contribute to lymphomagenesis.[2]

We propose *SH2D1A* mutational screening be considered in male patients with or without hypogammaglobulinemia who received rituximab treatment for lymphoma and did not recover IgG a year after B-depleting therapy.

ACKNOWLEDGMENTS

The authors sincerely thank all the patients and their family members who made this study possible and the referring doctors for their help.

REFERENCES

1. Rezaei N, Hedayat M, Aghamohammadi A, et al. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol*. 2011;127:1329–1341.
2. Rezaei N, Mahmoudi E, Aghamohammadi A, et al. X-linked lymphoproliferative syndrome: a genetic condition typified by the triad of infection, immunodeficiency and lymphoma. *Br J Haematol*. 2011;152:13–30.
3. Overwater E, Smulders Y, Van Der Burg M, et al. 17-year-old boy with XLP de novo mutation. *Eur J Pediatr*. 2014;173:1695–1698.
4. Tóth B, Soltész B, Gyimesi E, et al. Severe XLP phenotype caused by a novel intronic mutation in the SH2D1A Gene. *J Clin Immunol*. 2015;35:26–31.
5. Gray PE, O'Brien TA, Wagle M, et al. Cerebral vasculitis in X-linked lymphoproliferative disease cured by matched unrelated cord blood transplant. *J Clin Immunol*. 2015;35:604–609.
6. Tangye SG. XLP: clinical features and molecular etiology due to mutations in SH2D1A encoding SAP. *J Clin Immunol*. 2014;34:772–779.
7. Rastetter W, Molina A, White CA. Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. *Annu Rev Med*. 2004;55:477–503.
8. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th ed. Lyon, France: IARC Press; 2008.
9. Sumegi J, Huang D, Lanyi A, et al. Correlation of mutations of the SH2D1A gene and Epsteinbarr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. *Blood*. 2000;96:3118–3125.
10. Sandlund JT, Shurtleff SA, Onciu M, et al. Frequent mutations in SH2D1A (XLP) in males presenting with high-grade mature B-cell neoplasms. *Pediatr Blood Cancer*. 2013;60:E85–E87.
11. Koochakzadeh L, Hosseinverdi S, Hedayat M, et al. Study of SH2D1A gene mutation in paediatric patients with B-cell lymphoma. *Allergol Immunopathol (Madr)*. 2015;43:568–570.
12. Rigaud S, Lopez-Granados E, Sibéril S, et al. Human X-linked variable immunodeficiency caused by a hypomorphic mutation in XIAP in association with a rare polymorphism in CD40LG. *Blood*. 2011;118:252–261.