

Successful allogeneic hematopoietic stem cell transplantation in a boy with X-linked inhibitor of apoptosis deficiency presenting with hemophagocytic lymphohistiocytosis: A case report

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Abstract. X-linked inhibitor of apoptosis (XIAP) deficiency, also known as X-linked lymphoproliferative syndrome type 2 (XLP2), is a rare inherited primary immunodeficiency resulting from the *XIAP* (also known as *BIRC4*) mutation. XIAP deficiency is mainly associated with familial hemophagocytic lymphohistiocytosis (HLH) phenotypes, and genetic testing is crucial in diagnosing this syndrome. Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only successful strategy for the treatment of this disease; however, a limited number of studies has been published concerning the outcomes of allogeneic HSCT in patients with XIAP deficiency. The present study reported a successful allogeneic HSCT performed to treat XIAP deficiency in a Chinese boy presenting with HLH. Polymerase chain reaction and DNA sequencing were performed to confirm the diagnosis of XIAP deficiency, and allogeneic HSCT was performed. Genetic tests revealed a two-nucleotide deletion (c.1021_1022delAA) in the patient, which was inherited from his mother, and resulted in frameshift mutation and premature stop codon (p.N341fsX348); this is considered to be a disease-causing mutation. The XIAP deficiency patient underwent allogeneic HSCT, receiving busulfan-containing reduced intensity myeloablative conditioning regimen, with a good intermediate follow-up result obtained. Therefore, genetic testing is essential to confirm the diagnosis of XIAP deficiency and detect the carrier of mutation. The present case study may promote the investigation of allogeneic HSCT in patients with XIAP deficiency.

Introduction

X-linked inhibitor of apoptosis (XIAP) deficiency, also known as X-linked lymphoproliferative syndrome type 2 (XLP2), is a rare X-linked inherited primary immunodeficiency caused by mutations in the *XIAP* (also known as *BIRC4*) gene, and mainly presents with familial hemophagocytic lymphohistiocytosis (HLH) phenotypes (1,2). The mutations of *XIAP/BIRC4* were initially demonstrated by Rigaud *et al* to be associated with XLP phenotypes (1). Since then, limited case reports and clinical information have been published, and <30 male patients have been summarized. These patients typically presented with HLH in infancy or early childhood (1-6). This disorder is characterized by splenomegaly, hypogammaglobulinemia and hemorrhagic colitis. Patients with XIAP deficiency are susceptible to Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infection (1-5); however, to the best of our knowledge, no cases of common variable immunodeficiency and lymphoma have been previously observed in patients with XIAP deficiency (7). Clinical presentation and laboratory tests facilitate the diagnosis of XIAP deficiency. Positive family history, clinical presentation (including hemorrhagic colitis, recurrent HLH, fever and hepatosplenomegaly), and laboratory tests (such as hemophagocytosis in the bone marrow, elevated ferritin levels (>500 ng/ml), cytopenia, hypertriglyceridemia and hypofibrinogenemia) facilitate the diagnosis of XIAP deficiency (8). However, genetic testing for *XIAP/BIRC4* genes is crucial for establishing a definite diagnosis (5). Although allogeneic hematopoietic stem cell transplantation (HSCT) is the only strategy for radical treatment of this condition, there is only a limited number of published studies concerning the outcomes of HSCT in patients with XIAP deficiency (6).

The current study presents the case of an XIAP deficiency patient resulting from a two-nucleotide deletion and frameshift mutation. Subsequently, successful allogeneic HSCT was performed in the patient, with good intermediate follow-up results obtained.

Case report

A 5.8-year-old boy, who had been experiencing abdominal distention, fever (temperature of >38.5°C) and pancytopenia

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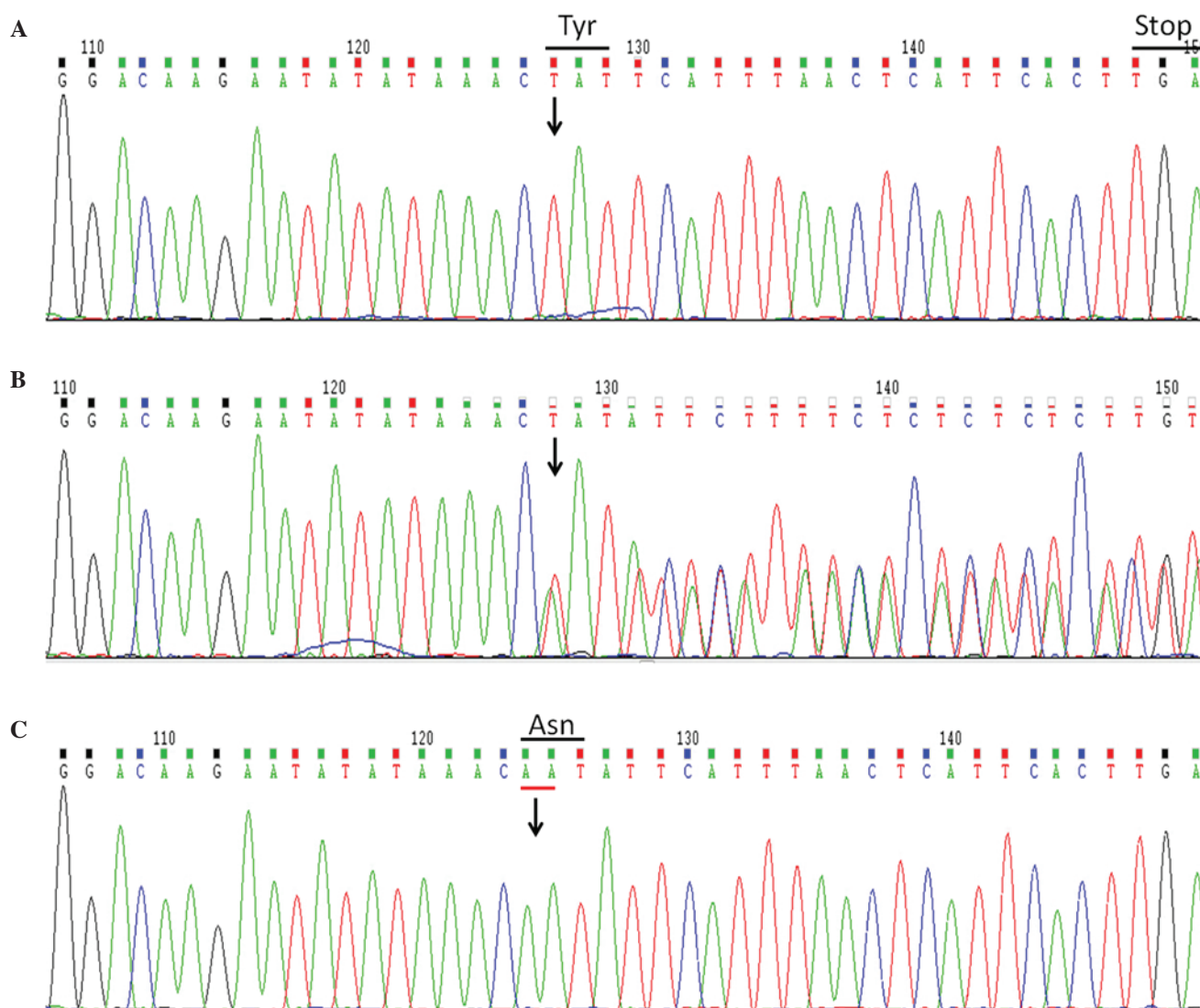


Figure 1. Sequence analysis of *XIAP* gene. Electropherograms of exon 4 of the *XIAP* gene from (A) the patient (homozygous mutation carrier), (B) his mother (heterozygous mutation carrier) and (C) his father (with normal sequence). Arrows indicate two-adenine deletion at nucleotides 1021-1022 of the coding sequence. The mutation caused a frameshift and premature stop codon (p.N341fsX348). XIAP, X-linked inhibitor of apoptosis; Tyr, tyrosine; Asn, asparagine.

[hemoglobin level, 81 g/l (normal range, 110-146 g/l); platelets, $80 \times 10^9/l$ (normal range, $100-450 \times 10^9/l$); neutrophils, $0.78 \times 10^9/l$ (normal range, $0.88-5.7 \times 10^9/l$) with 14% atypical lymphocytes] for 14 days was admitted to the Department of Pediatric Hematology and Oncology, West China Second University Hospital of Sichuan University (Chengdu, China) in February 2013. Physical examination showed that the patient's spleen was 6 cm below the left costal margin in the mid-clavicular line, with a soft and sharp margin, whereas the liver was 7 cm below the right costal margin in the mid-clavicular line. The patient also presented with hypertriglyceridemia (fasting triglyceride level, 4.11 mmol/l; normal range, <2.83 mmol/l) and hypofibrinogenemia (fibrinogen level, 125 mg/dl; normal range, 200-400 mg/dl). Laboratory tests, including Wright's staining of bone marrow smears, revealed hemophagocytosis in the bone marrow, with a ferritin level of >16,500 ng/ml. EBV DNA and CMV DNA serological tests, as well as blood culture, were negative. A bone marrow smear indicated the presence of hemophagocytosis. Therefore, based on the aforementioned findings, the patient was clinically diagnosed with HLH.

Familial HLH associated genetic testing (including the following genes: *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *XIAP*, *SH2D1A*, *Rab27a*, *AP3B1* and *LYST*) was performed on the patient subsequent to obtaining written informed consent from his parents and the approbation of the Ethics Committee of West China Second University Hospital. Genomic DNA was extracted from peripheral blood using a commercially available kit according to the manufacturer's protocol (Tiangen Biotech, Co., Ltd., China). The quality and quantity of the extracted DNA samples were determined by UV spectrophotometry. Mutation of XIAP was detected by polymerase chain reaction (PCR) analysis. Specific primers were used for exon 3 of XIAP: Forward, 5'-ACTGAAAAG CAAGTTAATGG-3' and reverse, 5'-ACTGTGAATATCAC ATGAAG-3'. The total reaction volume of 25 μ l contained 150 ng DNA, 12.5 μ l PCR buffer (2X; Tiangen Biotech Co., Ltd.) and 1 μ l of specific primers (final PCR concentration 0.4 μ M). Amplifications were performed using a programmable PCR thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the following thermal cycling

parameters: 5 min at 94°C, 30 cycles of 30 sec at 94°C, 30 sec at 60°C, 1 min at 72°C, followed by final extension for 7 min at 72°C. Each PCR included a negative and positive control. The 309 bp PCR products were separated by 2.5% agarose gel electrophoresis and subsequently sequenced to investigate the mutation of XIAP. The results indicated a two-nucleotide deletion in *BIRC4* gene (c.1021_1022delAA), which resulted in a frameshift mutation and premature stop codon (p.N341fsX348), while the XIAP protein lost 156 amino acids (Fig. 1). Subsequently, *BIRC4* genetic testing was performed on the patient's parents following informed consent. The mother was found to be a heterozygote carrier of the mutation; thus, the mutation was considered to be disease causing, and the patient was confirmed with XIAP deficiency. Next, the patient was treated with dexamethasone alone (initial dose of 10 mg/m², which was subsequently tapered) for 3 months. The treatment improved the general condition of the patient, and resulted in a decreased spleen size and complete remission of HLH, which was demonstrated by a reduction in ferritin levels to within the normal range, and the recovery of blood cell count and fibrinogen levels to within the normal range.

The patient remained in full remission of HLH at the time of HSCT. A reduced intensity myeloablative conditioning (MAC) regimen was performed at the West China Second University Hospital in October 2013. The transplantation procedures were performed according to the institutional standard practices. The conditioning regimens prior to HSCT consisted of 1 mg/kg busulfan (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) for 4 days (between days -8 and -5), 60 mg/kg cyclophosphamide (Heng Rui Medicine Co., Ltd., Jiangsu, China) for 1 day (on day -3), 5 mg/kg antithymocyte globulin (Fresenius Biotech GmbH, Gräfelfing, Germany) for 3 days (between days -3 and -1) and 8 mg/kg etoposide (Heng Rui Medicine Co., Ltd.) for 1 day (on day -4). Next, the patient received fully matched unrelated peripheral blood stem cells based on typing 10 human leukocyte antigens (HLAs), including HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1, with 6.9x10⁶/kg CD34⁺ cells and 4.7x10⁸/kg mononuclear cells. Furthermore, he received: Graft-versus-host disease (GVHD) prophylaxis, which included 3-6 mg/kg oral cyclosporin A (North China Pharmaceutical Group Corp., Shijiazhuang, China) for 6 months and 15 mg/kg oral mycophenolate mofetil (Roche Pharmaceuticals Ltd., Shanghai, China) for 3 months; other routine transplantation care, which included antimicrobial prophylaxis with 100 mg/day oral mebendazole (Xian Janssen Pharmaceutical Ltd., Xi'an, China) for 3 days, 50 mg/day oral fluconazole (Pfizer, Inc., New York, NY, USA) for ~2 months, 5 mg/kg twice daily ganciclovir (North China Pharmaceutical Group Corp.) by intravenous infusion for 10 days, 75 mg/kg twice daily mezlocillin sodium and sulbactam sodium (Hainan General Sanyang Pharmaceutical Co., Ltd., Haikou, China); hepatic venoocclusive disease (VOD) prophylaxis with alprostadil, which included 10 µg/day prostaglandin E1 (Beijing Tide Pharmaceutical Co., Ltd., Beijing, China) by intravenous infusion for 35 days; intravenous immunoglobulin replacement (400 mg/kg once a week; Rongsheng Pharmaceutical Co., Ltd., Chengdu, China); and fluid and nutrition supplementation per institutional standard practices. The patient suffered from drug-associated

enteritis while receiving the preparative regimen. In addition, he developed engraftment syndrome at day +6 after HSCT, CMV viremia at day +26, autoimmune hemolytic anemia at day +35, hemorrhagic cystitis at day +42; however, no acute GVHD, VOD, pulmonary hemorrhage, bacteremia, fungal infection and cardiac toxicity were reported. The patient developed complete donor chimerism (>95% host-derived cells in the whole blood) at day +20. The recipient's blood type converted from group AB to the donor's blood type, group B. The patient received cyclosporin A, mycophenolate mofetil, and methylprednisolone for ~6, 3 and 6 months respectively for GVHD prophylaxis following HSCT. At an intermediate follow-up performed 528 days after HSCT, the patient was alive and remained free of disease. At the latest follow-up performed in April 2016, the patient remained free of HLH, exhibited normal cellular immunity and had successfully withdrawn from all therapeutic agents for GVHD, VOD and antimicrobial prophylaxis.

Discussion

XIAP deficiency, also known as XLP2, is a rare X-linked inherited primary immunodeficiency resulting from *XIAP/BIRC4* mutations (1), and is mainly associated with familial HLH phenotypes (8). The present study reported the case of a patient presenting with typical clinical features of HLH, including fever, hepatosplenomegaly, pancytopenia, hypertriglyceridemia, coagulopathy with hypofibrinogenemia, hemophagocytosis in the bone marrow and elevated levels of ferritin. Although the current patient presented negative results in EBV serological tests, EBV infection remains one of the most frequent pathogens detected in HLH patients. Other symptoms of XLP, such as hemorrhagic colitis, have been reported in patients with XIAP deficiency, but malignant B-cell lymphoma has not been reported (1-3,5). In addition, recurrent splenomegaly frequently occurs in XIAP deficiency, and it may occur even in the absence of systemic HLH (3,8).

Gene testing is essential for the diagnosis of XIAP deficiency, particularly in patients who present an atypical phenotype or have no positive family history. *XIAP* is located in Xq25, adjacent to the *SH2D1A* gene, and comprises 6 exons (9). XIAP protein, as an anti-apoptotic molecule, consists of 497 amino acids and contains three baculovirus IAP repeat domains and one RING domain; these bind to caspases 3, 7, and 9 together with flanking residues, thereby inhibiting the proteolytic activity of caspases 3, 7, and 9 (10). To date, at least two small deletions and three large deletions of *XIAP* have been identified, resulting in frameshifts and premature stop codons (6,11). In particular, a deletion of cytidine 291 (291delC) resulted in a frameshift leading to a stop codon at position 387 (G99K/X129) (1). A deletion of 2,606 nucleotides encompassing exon 2 (1), and deletions of exons 1-5 or exon 6 resulted in the expression of XIAP decreased significantly (2). More specifically, 2 brothers from Japan have been reported to have the same two-nucleotide deletion (c.1021_1022delAA) in exon 3 as that reported in the present case, but their mother was not a carrier of an *XIAP* mutation (6). In the present study, a direct sequencing method was used to identify not only mutations in the Chinese boy, but also heterozygous mutation in his mother. This mutation resulted in a frameshift and premature

termination (p.N341fsX348). In addition, other *XIAP* mutations have been reported in previous studies, including exonic nonsense mutations, missense mutations, large deletions and gross deletion exons (6,11).

Although allogeneic HSCT is the only strategy for the complete treatment of XIAP deficiency, limited studies have been published concerning the outcomes of HSCT in patients with this syndrome. As reported in an international survey (11), 19 patients with XIAP deficiency received HSCT. Among them, 8 patients received MAC regimens, while 11 patients received reduced intensity conditioning (RIC) regimens (consisting of fludarabine, alemtuzumab and melphalan) (11). The comparison between the two groups revealed that the conditioning regimens affected the HSCT outcome. There was a high incidence of conditioning-associated toxicity among MAC regimen-treated patients, with a higher mortality observed in these patients compared with patients receiving the RIC regimens (11). The main causes of mortality included pulmonary hemorrhage, VOD and multiple organ failure (MOF). Therefore, it is possible that the loss of XIAP and its antiapoptotic functions contributes to the high incidence of toxicity observed in patients receiving the MAC regimen (11).

Although it is suggested that RIC regimens should be administered to patients with XIAP deficiency, this regimen was not selected in the present study, due to inability to obtain alemtuzumab (a CD52 monoclonal antibody) in our hospital. Given the lack of alemtuzumab, the current patient underwent HSCT with reduced intensity MAC regimen (including etoposide, which may contribute to controlling HLH) and still presented good intermediate follow-up results. Furthermore, the results of the current study indicated that the full-matched HLA donor (12), the effective control of HLH activity prior to HSCT (13) and the successful prevention of complications (such as VOD, pulmonary hemorrhage and infections) may also contribute to the success of HSCT in patients with XIAP deficiency in developing countries, where alemtuzumab cannot be obtained.

In conclusion, the current study detected a two-base deletion mutation of *XIAP/BIRC4* in a Chinese family, and successfully performed allogeneic HSCT in the patient. Identifying *XIAP* mutations is important for the diagnosis of affected families, and more evidence is required prior to making transplantation decisions. Thus, conditioning regimens should be administered with caution in patients with an available matched stem cell donor. In addition, where possible, efforts should be made to ensure HLH remission prior to HSCT and successful prevention of complications following HSCT in these patients.

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