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A novel *XIAP* mutation in a Japanese boy with recurrent pancytopenia and splenomegaly

X-linked lymphoproliferative syndrome (XLP) is a rare inherited primary immunodeficiency. It is clinically characterized by hemophagocytic lymphohistiocytosis (HLH), which usually develops in response to an Epstein-Barr virus (EBV) infection, dysgammaglobulinemia and malignant lymphoma. Most cases of XLP are caused by mutation in the SLAM-associated protein (SAP) or *SH2D1A*[1-3]. The X-linked inhibitor of apoptosis (XIAP) is also reported to cause XLP,[4] and at least 9 families with XIAP deficiency have been reported in Europe and the United States.[4,5] This report describes a Japanese boy with recurrent HLH and splenomegaly, and identifies a novel mutation in the *XIAP* gene.

A 3-year old boy was admitted to Kyoto Prefectural University Hospital because of fever and pancytopenia. The patient presented with splenomegaly, lymphadenopathy and pancytopenia at the age of 20 months. Epstein-Barr virus (EBV) serological tests disclosed a primary EBV infection. He was clinically diagnosed with EBV-associated HLH, and was treated with prednisolone and thereafter showed some improvement. The patient again presented with pancytopenia at the age of 23 months followed by a measles-rubella vaccination. He had frequently experienced mild pancytopenia and splenomegaly followed by infections. A physical examination at the time of admission revealed a temperature of 38.2°C, a right cervical lymphadenopathy and splenomegaly, but neither hepatomegaly nor skin eruptions. Laboratory tests showed a white blood cell count of 3.1×10^9/L with 44% neutrophils, 52% lymphocytes, 2% monocytes and 2% atypical lymphocytes, hemoglobin 10.0 g/dL, platelets of 111×10^9/L, lactate dehydrogenase 1,261 IU/L, ferritin 2,240 ng/mL (normal: <480), triglyceride 220 mg/dL (normal: <149), and soluble interleukin-2 receptor 1,700 U/mL (normal: <466). The serum immunoglobulin levels were within normal ranges (IgG; 1,362 mg/dL, IgA; 129 mg/dL, IgM; 175 mg/dL). EBV-DNA was detected in whole blood but showed low copies (48 copies/μgDNA).

The patient was clinically diagnosed with mild HLH, and he was first treated with prednisolone alone, but had only a partial response. Treatment with cyclosporine A and dexamethasone improved his condition and resulted in a decreased spleen size. The patient is scheduled to receive hematopoietic stem cell transplantation to achieve a complete remission in the near future.

Although the patient had no family history of HLH, his recurrent episodes of HLH implied that this might be caused by a genetic defect. No mutations were identified in the causative genes for familial HLH including perforin, Munc13-4 and syntaxin 11. Another possible genetic disease was XLP. The flow cytometric detection of SAP and XIAP was used to screen for XLP, as previously described.[5,6,7] The patient showed normal expression of SAP in lymphocytes, but clearly had deficient expression of XIAP, suggesting XIAP deficiency (Figure 1). Intriguingly, his mother showed bimodal expression of XIAP protein in lymphocytes, suggesting an obligate carrier. A gene analysis of *XIAP* was performed with parental informed consent, which disclosed a novel nonsense mutation (840C>T, R238X) in the patient (*data not shown*). The mother showed heterozygous alleles in this position.

The causative gene for XLP was *SAP* or *SH2D1A* (type 1)[1-3] and *XIAP* (type 2).[4] Both types of XLP can be identified by genetic analysis even in a sporadic case. However, the genetic analysis is labor-intensive and time-consuming. A flow cytometric screening for XIAP deficiency has been recently reported.[5] The present case was first screened by flow cytometry and was later confirmed by genetic analysis. The patient had 840C>T, thus resulting in R238X. Nine mutations have been identified in the *XIAP* gene (2 missense mutations, 3 nonsense mutations, one small deletion and 3 large deletions).[4,5] The nonsense mutations include Q104X, E118X and Q333X and also R238X which is a novel mutation.

Both SAP deficiency and XIAP deficiency result in XLP, but they are described as clinically indistinguishable. However, lymphoma has so far not been described and only about 50% had EBV-HLH in XIAP deficient patients.[4] In addition, patients with XIAP deficiency often have splenomegaly, unlike patients with SAP deficiency. Clinical manifestations of splenomegaly may be typical signs of XIAP deficiency, as demonstrated in the present case. EBV-HLH in SAP deficiency usually results in a fatal course, but the present case showed a mild course of EBV-HLH. EBV-HLH in XIAP deficiency may, therefore, show a milder presentation than that of SAP deficiency. To clarify the differences in the clinical picture of SAP deficiency and XIAP deficiency, a greater number of patients with XLP should be surveyed.

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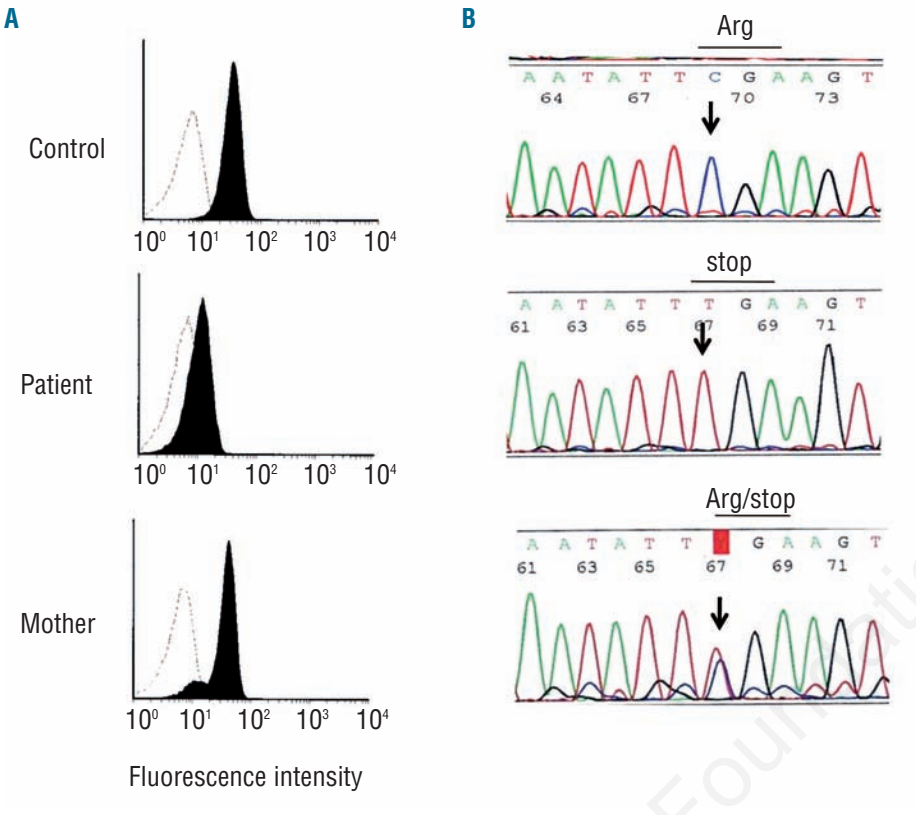

Figure 1. (A) XIAP expression in lymphocytes. Flow cytometric detection of XIAP in lymphocytes from the patient, his mother and the control using anti-XIAP antibodies (clone 48, BD Biosciences, San Jose, CA, USA). Filled histograms represent XIAP staining, while open histograms represent the isotype control antibody. (B) Sequence analysis of XIAP gene. Electropherograms of exon 1 of the *XIAP* gene from the patient, his mother and the control. Arrows indicate the substitution of cytosine to thymine at nucleotide 840. The patient possessed a nonsense mutation, R238X. A heterozygous mutation was detected in the mother.

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Mutations of the Shwachman-Bodian-Diamond syndrome gene in patients presenting with refractory cytopenia – do we have to screen?

Children diagnosed with acquired hypocellular myelodysplastic syndrome (MDS), such as refractory cytopenia (RC), share clinical features with patients suffering from inherited bone marrow failure (IBMF). The Shwachman-Diamond syndrome (SDS; OMIM #260400) is an autosomal recessive disorder associated with bone marrow failure, pancreatic exocrine insufficiency, short stature and liver abnormalities. Other symptoms, such as eczematous lesions, oral disease, cognitive/behavioral problems, immune dysfunction or urinary tract anomalies, may occur.[1] In addition, SDS predisposes to the development of leukemia.[1] Mutations in the Shwachman-Bodian-Diamond Syndrome gene (*SBDS*) are found in approximately 90% of SDS patients.[2] Studies in yeast suggest an important role of the SBDS protein in RNA metabolism.[1]

In children with suspected RC or IBMF, meticulous clinical examination is important because RC and IBMF cannot be distinguished by hematologic or morphological features alone. Underlying congenital disorders may be missed specifically in cases of hypocellular RC and normal karyotype. Our group recently reported in this journal 2 patients with germline mutations of the human Telomerase RNA Component (*TERC*) gene among 80 children with hypocellular RC.[3] Here we hypothesized