Table 2. Characteristics of patients with mutations in *XIAP* (XLP-2)

Patient ID*	*XIAP* mutation	XIAP protein	HLH (age in years at diagnosis)	EBV at first HLH	HLH relapses (age in years at relapse)	SM (age in years at diagnosis)	Hypo-γ (age in years at diagnosis)	Chronic colitis (age in years at diagnosis)	Other manifestations (age in years at diagnosis)	Outcome (age in years)
X1.1	E99KfsX129		5	+	?	?	–	–	?	Alive, well (8)
X1.2	E99KfsX129	–	5.3	+	?	+ (5)	–	–	?	Alive, well (11)
X1.3	E99KfsX129		2.5	+	?	+ (2.5)	+	–	?	Alive, well, IVIG (14)
X1.4	E99KfsX129	–	7.8	+	+	+ (6)	–	+ (4)	Cholangitis (23)	Alive, ileitis (23)
X1.5	E99KfsX129		3	+	+	+ (3)	–	–	–	Alive, well (30)
X1.6	E99KfsX129	–	0.8	– (HHV-6+/–)	+ (EBV+)	+ (1)‡	+(10)	–	–	HSCT (11), died (11)
X1.7	ND		1.5†	?	+	+ (1.5)‡	+(42)	+ (41)	Cholangitis (41)	Died (42, colitis)
X2.1	I397FfsX414	–	1.2	+	+	+ (1)‡	–	–	–	HSCT (1.6), died (d+13, HLH)
X3.1	E118X	–	23	+	+	+ (22)	+(22)	–	–	Alive, well, IVIG (39)
X3.2	ND		0.5	?	–	?	?		–	Died (0.5, HLH)
X3.3	ND		20	+	–	?	?		–	Died (20, HLH)
X3.4	E118X	–	–	NA	–	+ (7)	–	–	–	Alive, well, SM (10)
X4.1	del. of exon 2	–	20	+	+ (21,EBV+)	+ (1)‡	–	–	–	Alive, well (28)
X4.2	del. of exon 2	–	10	?	+ (11, EBV+)	+ (6)‡	–	–	–	Alive, well (15)
X5.1	D130GfsX140	–	2.5	+	+ (3.4-3.6)	+ (1)	–	–	–	HSCT (3.6), died (4)
X5.2	ND		0.1	?	–	?	?	–	–	Died (0.1, HLH)
X5.3	ND		3.5	+	–	?	?	–	–	Died (3.5, HLH)
X6.1	R238X	–	1.7	+	+	+‡	–	–	–	Alive, recurrent HLH (3)
X7.1	I397NfsX405	–	2.7	+	+ (3.2-3.5, EBV+)	+ (2.7)	–	–	–	Alive, recurrent HLH (3.5)
X8.1	E434AfsX457	+/–	15.5	+	–	–	–	–	–	Alive, well (16)
X9.1	G466X	+/–	8†	+	–	+ (8)‡	+ (8)§	–	–	Alive, well, SM (27)
X9.2	G466X	+/–	–	NA	–	+ (21)	+ (21)§	–	–	Alive, well, SM (30)
X9.3	G466X	+/–	–	NA	–	+ (4)‡	–	+ (12)	Recurrent infections	Alive, colitis (14)
X9.4	G466X	+/–	–	NA	–	+ (22)‡	+ (10)§	–	Chronic liver failure (22)	Died (29, liver failure), IVIG,
X9.5	G466X	+/–	–	NA	–	–	–	–	–	Alive, well (39)
X9.6	ND		–	NA	–	?	?	+	?	Died (27, colitis)
X9.7	ND		–	NA	–	+‡	?	–	Recurrent infections	Died (52, pneumonia)
X10.1	T470S	+	8†	– (HSV-1+)	–	–	+ (4)§	–	Cryptococcosis (4)	Alive, well, IVIG (8)
X11.1	R381X	–	0.9†	–	NA	+ (0.6)‡	–	–	–	HSCT (1.2), died (1.4)
X11.2	ND		?	?	?	?	?	+(4)	?	Died (4, colitis)

SM indicates recurrent splenomegaly or hepatosplenomegaly; Hypo-γ, hypogammaglobulinemia; ?, unknown; HSCT, hematopoietic stem cell transplantation; ND, not done; NA, not applicable; and del., deletion.

*Patient identification: X indicates XIAP deficiency, the first number corresponds to the family and the second to the individual patient; + indicates yes or positive; –, no or negative; and +/–, weakly positive.

†Diagnosed as incomplete HLH.

‡Recurrent splenomegaly or hepatosplenomegaly associated with intermittent fever, anemia, and cytopenia.

§With recurrent respiratory infections.

relapsed in 2 of 7 SAP-deficient HLH-survivors (29%), whereas 11 of 14 XIAP-deficient HLH-survivors (79%, 3 unknown) had ≥ 1 relapse of HLH ($P = .055$).

Six of the 18 SAP-deficient patients with HLH (33%) had proven neurologic involvement with mostly (5 of 6, 83%) lethal outcome, whereas 2 of 22 of XIAP-deficient patients with HLH (9%) had neurologic involvement with less mortality (1 of 2, 50%).

EBV infection was the most-frequent identified trigger of the first HLH episode in the SAP-deficient (11 of 12, 92%, 6 unknown) and XIAP-deficient (15 of 18, 83%, 4 unknown) patients ($P = .63$) (Table 3). Only PS13.1, PX1.6, PX10.1, and PX11.1 had a first HLH episode in the absence of a proven EBV-infection, whereas the EBV status of 6 SAP-deficient patients and 4 XIAP-deficient patients is not known. PX1.6 and PX4.2 subsequently experienced an HLH-relapse with positive EBV polymerase chain reaction. In 2 patients, herpes simplex virus type 1 (HSV-1) and human herpesvirus type 6 (HHV-6) were detected in the blood by polymerase chain reaction in the course of their first HLH episode. Of note, in several XIAP-deficient patients, other viruses than EBV were tested, including cytomegalovirus, parvovirus B19, HSV, HHV-6, HHV-8, HIV, human T-cell leukemia virus, adenovirus, and varicella-zoster virus. All were negative.

Splenomegaly and incomplete forms of HLH

Recurrent splenomegaly occurring in the absence of systemic HLH and often associated with fever and cytopenia (consisting of pancytopenia, bicytopenia, thrombocytopenia, and anemia) was frequently observed in XIAP-deficient patients (20 of 23, 87%, 7 unknown), whereas it was only found in 2 of 29 SAP-deficient patients (7%, 4 unknown; $***P < .0001$; Table 3). In 8 XIAP-deficient patients, episodes of splenomegaly occurred before they developed HLH and were the first clinical sign of the disease. Overall, although 3 patients with splenomegaly up to now did not

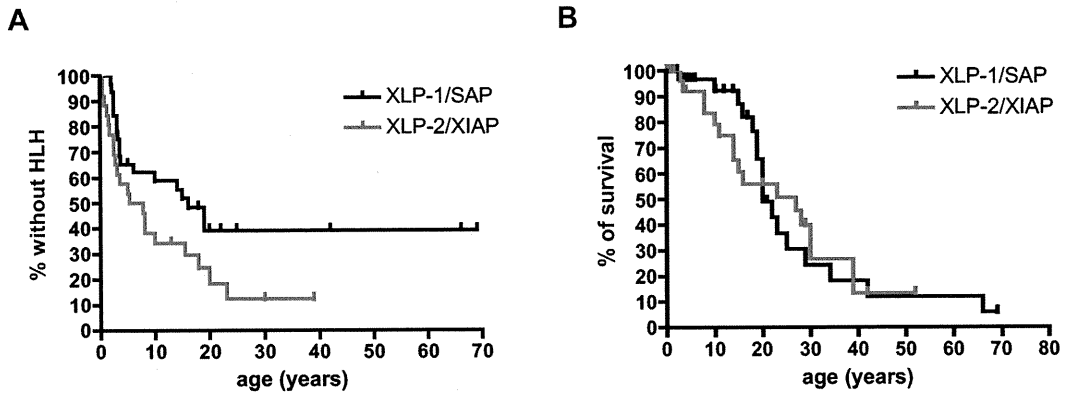

Figure 1. Comparison of HLH phenotypes and survival curves of SAP-deficient (XLP-1) and XIAP-deficient (XLP-2) patients. Kaplan-Meier survival curves were constructed on the basis of data presented in Table 1 and Table 2. Statistical analyses with log-rank tests. (A) Percentage of XLP-1/SAP and XLP-2/XIAP patients without HLH phenotype (P = .099). (B) Overall survival curves for XLP-1/SAP and XLP-2/XIAP patients (P = .948).

develop HLH, the others subsequently developed HLH within a period of time, varying from a few months to 19 years. In 2 XIAP-deficient patients, transient pancytopenia with splenomegaly was noticed after vaccinations against measles, mumps, and rubella or measles and rubella. Importantly, none of the XIAP-deficient patients developed B-cell lymphoproliferative disease.

PX4.1 underwent splenectomy at the age of 21 years, and histopathologic examination of the spleen showed reduced white pulp areas, and red pulp was extended with a mild fibrosis (supplemental Figure 2 top left). In the white pulp, most of the lymphocytes were $CD20^+$, whereas in the red pulp there was an accumulation of $CD3^+$ T cells that were mostly $CD8^+$ and cytotoxic (T-cell intracellular antigen-1^+; data not shown; supplemental Figure 2 bottom). Strikingly, features of hemophagocytosis were observed in the red pulp (supplemental Figure 2 upper right). Lymphocytes were negative for LMP-1 with very rare $EBER^+$ cells, suggesting that the infiltration was not related to EBV infection (data not shown). Altogether, these observations strongly suggest that these lymphoproliferative manifestations can be regarded as incomplete or attenuated forms of HLH.

In addition, 3 XIAP-deficient patients had liver disease (2 patients with cholangitis and 1 patient with chronic liver failure). In 2 of the patients, the cholangitis was associated with colitis, which are known to overlap.[18] For patient PX1.7, histopathologic examination of the liver showed granulomatous hepatitis in lobular areas with foci of macrophages around necrotic hepatocytes (supplemental Figure 3). Staining for LMP-1 was negative (data not shown). It is unclear whether these liver diseases should also be considered as an incomplete form of HLH.

Table 3. Comparison of XLP-1 and XLP-2 phenotypes

	SAP-/Y, n (%)	XIAP-/Y, n (%)	P*
HLH	18 of 33 (55)	22 of 29 (76)	NS
HLH relapses (/HLH-survivors)	2 of 7 (29)	11 of 14 (79)	NS
EBV at first HLH	11 of 12 (92)	15 of 18 (83)	NS
Fatal HLH	11 of 33 (33)	5 of 30 (17)	NS
Fatal HLH (/HLH patients)	11 of 18 (61)	5 of 22 (23)	.0230
Hypogammaglobulinemia	14 of 21 (67)	8 of 24 (33)	.0377
Lymphoma	10 of 33 (30)	0 of 30 (0)	.0010
Cytopenias (in the absence of full-blown HLH)	4 of 33 (12)	11 of 21 (52)	.0020
Splenomegaly (in the absence of full-blown HLH)	2 of 29 (7)	20 of 23 (87)	<.0001
Hemorrhagic colitis	0 of 33 (0)	5 of 30 (17)	.0203

*Calculated with Fisher exact tests.

Lymphoma

Ten of 33 SAP-deficient patients (30%) and none of the 30 XIAP-deficient patients developed lymphoma (Tables 1-3; supplemental Figure 1B; ***P = .001). Mean age at diagnosis of lymphoma was 15 years (range, 2-40 years). Diagnoses were non-Hodgkin lymphoma (n = 9), including EBV-positive Burkitt lymphoma (n = 6) and EBV-negative (n = 3). Lymphomas were localized in the ileocecal (n = 5), cerebral[19] (n = 1), cervical (n = 2), and spinal (n = 2) regions, and for one the origin was not known. One patient (PS1.3) had a second lymphoma at the age of 30 years, 23 years after the first one, and one patient (PS15.3) had myelodysplasia.

Dysgammaglobulinemia

Hypogammaglobulinemia was documented in 14 SAP-deficient patients (14 of 21, 67%) and in 8 XIAP-deficient patients (8 of 24, 33%) (*P = .0377) (Tables 1-3). Thirty percent (10 of 33) of SAP-deficient patients and 13% (4 of 30) of XIAP-deficient patients received intravenous immunoglobulin (IVIG) substitution (P = .1357) (supplemental Figure 1C). Interestingly, hypogammaglobulinemia was transient in 2 of the 8 XIAP-deficient patients. PX3.1 was substituted with IVIG between the age of 23 and 35 years, currently, 4 years after stopping IVIG, immunoglobulin levels remain within the normal range, and the patient does not experience recurrent respiratory infections. Two XIAP-deficient patients developed hypergammaglobulinemia, with higher than normal IgA and IgM levels in PX9.3 and elevated IgG and IgM levels in PX11.1, respectively.

Severe infections were noted in several SAP- and XIAP-deficient patients with hypogammaglobulinemia before initiation of the IVIG substitution when treated. Ten of the 14 SAP-deficient and 4 of the 8 XIAP-deficient patients had recurrent respiratory tract infections. Rare severe infections caused by *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Cryptococcus neoformans* were also observed in SAP- and XIAP-deficient patients (supplemental Table 1).

Colitis

Chronic colitis with hemorrhagic diarrheas or rectal bleeding or both evoking inflammatory bowel disease was observed in 5 of 30 XIAP-patients (17%) but in none of 33 SAP-deficient patients (*P = .0203; Tables 1-3). In PX1.4, colitis initially responded to immunosuppressive treatment with corticosteroids and cyclosporine A. However, corticosteroids could not be withdrawn, and the

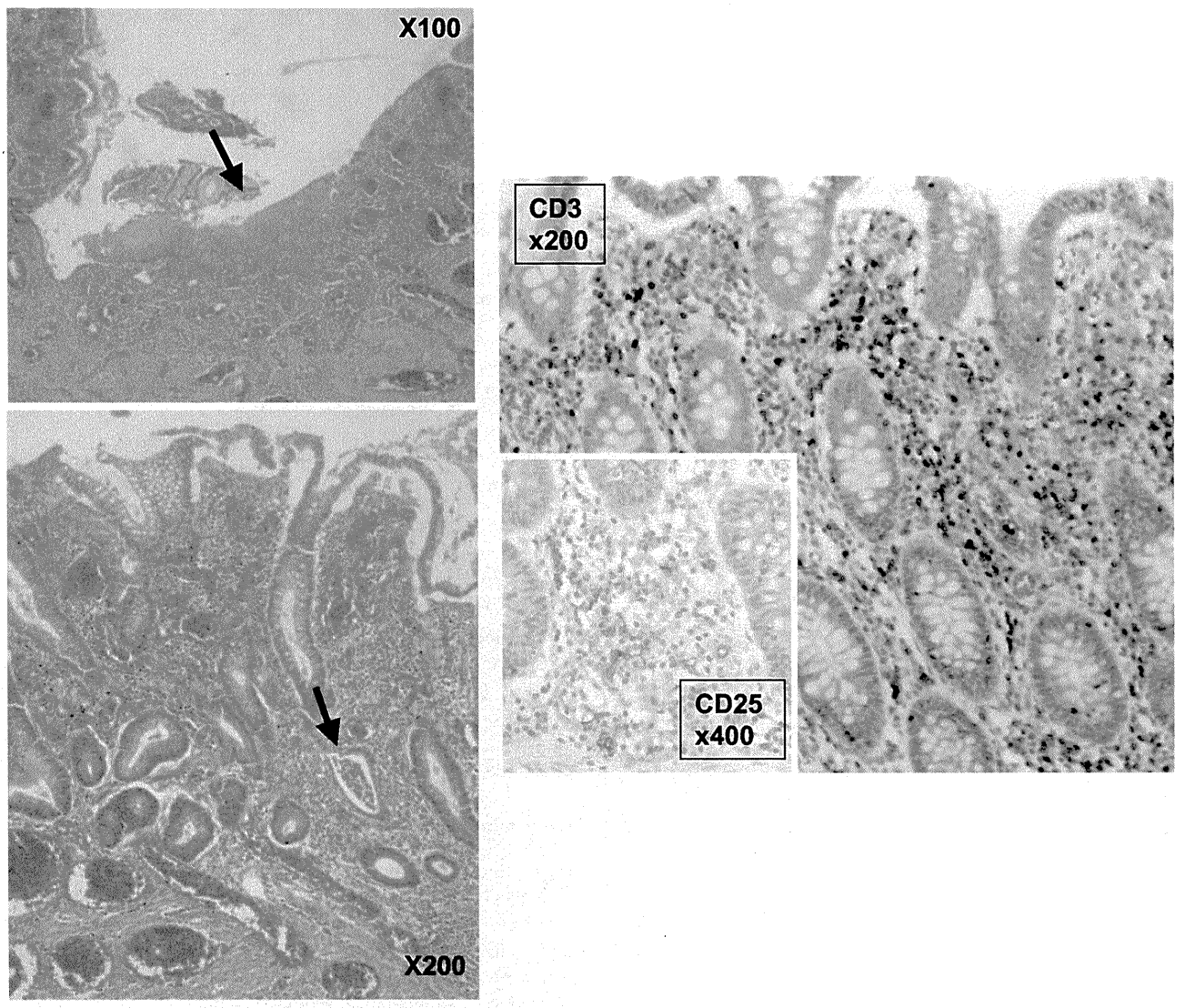

Figure 2. Histology of the large bowel of PX1.7 with XIAP deficiency. (Top left) On hematoxylin and eosin at low magnification (×100), a large ulceration is seen, indicated by an arrow. (Bottom left) Higher magnification (×200) shows a massive polymorphic inflammatory infiltrate associated with a crypt abscess (indicated by the arrow). (Central right) Immunostaining with anti-CD3 shows frequent lymphoid T cells (on the right, ×200), some of them express the activation marker CD25 (×400, inset).

addition of azathioprine could not prevent the recurrence of symptoms. Anti–tumor necrosis factor-α mAb treatment (infliximab) provided partial improvement. Recently, a colectomy was performed, but the patient now has terminal ileitis. In PX1.7, severe hemorrhagic colitis was associated with portal hypertension and massive gastroduodenal bleeding that lead to death of this patient. Patients PX9.6 and PX11.2 also suffered from chronic colitis and most probably died of intestinal hemorrhage.

Histopathologic examination of intestinal mucosa biopsy specimens was performed in 3 patients, PX1.4, PX1.7, and PX9.3. Representative images are shown in Figure 2. Hemorrhagic ulcerations of the colon associated with mononuclear infiltration consisting of lymphoid cells and plasma cells in the lamina propria were observed (Figure 2 left top). Crypt architecture was mostly preserved, except for rare crypt abscesses (Figure 2, left bottom), but frequent apoptotic crypt cells were seen (supplemental Figure 4). The lymphoid cells were mostly $CD3^+$ and $CD8^+$ with some lymphocytes expressing CD25 with numerous eosinophils (in PX1.4) (Figure 2; supplemental Figure 4). $CD20^+$ cells were rare, EBER staining was negative (not shown), and there was no granuloma formation. Microbiologic cultures were negative in all 3 cases.

Rare clinical manifestations

Rare clinical features (supplemental Table 1), each observed in 1 SAP-deficient patient, were hemolytic uremic syndrome associated with HLH, vasculitis, and arthritis. Clinical features, each observed in 1 XIAP-deficient patient, were Kawasaki syndrome and psoriasis. Additional infections in patients without hypogammaglobulinemia were caused by *Pseudomonas aeruginosa* (1 SAP-deficient patient), recurrent measles (1 XIAP-deficient patient), and HSV-(1 XIAP-deficient patient). Of note, 2 SAP-deficient patients (PS3.1 and PS3.2) had chronic gastritis.[20]

Survival and outcome

Sixteen of 33 SAP-deficient patients and 12 of 30 XIAP-deficient patients died at a mean age of 11 years (range, 2-69 years) and 16 years (range, 0.1-52 years), respectively. Survival rates did not differ between both patient groups ($P = .93$; Figure 1B), and the proportions of whom reached adulthood (age ≥ 16 years) were similar in both groups (17 of 33 SAP-deficient patients [52%] and

13 of 30 XIAP-deficient patients [43%]). Mortality was related to HLH (11 SAP- and 5 XIAP-deficient patients), lymphoma (2 SAP-deficient patients), myelodysplasia (1 SAP-deficient patient), colitis (3 XIAP-deficient patients), hepatitis (1 XIAP-deficient patient), complications of hematopoietic stem cell transplantation (2 SAP- and 4 XIAP-deficient patients), and pneumonia (1 XIAP-deficient patient). Mean age at last follow-up was 24.9 years (range, 10-66 years) for SAP-deficient patients and 17.5 years (range, 0.7- 39 years) for XIAP-deficient patients. Among the surviving 17 SAP-deficient patients, 4 are well without any treatment, 10 receive IVIG substitution, 2 are currently treated for a lymphoma, and 1 had successful hematopoietic stem cell transplantation. Among the surviving 17 XIAP-deficient patients, 10 are well without any treatment (among them 3 with splenomegaly), 2 received recently anti-CD20 antibody treatment because of EBV-related HLH, 2 are under IVIG substitution, 1 has terminal ileitis after colectomy, 1 has colitis treated with mesalazine and azathioprine, and 1 has recurrent HLH treated with cyclosporine A and dexamethasone. One XIAP-deficient and 2 SAP-deficient patients have never developed clinical signs and are considered to be asymptomatic.

Discussion

We report the first comparison of the clinical phenotypes of SAP- and XIAP-deficient patients. The present study was based on a retrospective analysis with data from medical records on 33 SAP- and 30 XIAP-deficient patients. The relatively small size of both cohorts obviously implies that data should be interpreted with caution.

The overall clinical phenotypes of the affected persons matched with the phenotypes previously reported.[2,7,9,21] In accordance to previous studies, we did not observe any genotype-phenotype correlation in the SAP-deficient patients. However, in our cohort of XIAP-deficient patients, we noticed that XIAP-deficient patients carrying non-null mutations had a tendency to be less prone to develop HLH by contrast to patients with null mutations. However, other genetic or environmental factors may contribute to the variety of phenotypes observed in XLP-1 and XLP-2.

HLH occurred both in SAP- and in XIAP-deficient patients but with more frequent neurologic involvement and fatal outcome in SAP-deficient patients than in XIAP-deficient patients. Splenomegaly often associated with cytopenia and fever was more frequent in XIAP-deficient patients than in SAP-deficient patients. Histologic analysis of one spleen showed accumulation of activated $CD8^+$ T cells and hemophagocytosis without EBV^+ cells. These symptoms probably represent incomplete forms of HLH. In addition, HLH relapses seemed to be more common in XIAP- than in SAP-deficient patients who survived HLH. Together, these findings suggest that HLH has a less severe disease course in XIAP- deficient patients than in SAP-deficient patients.

In most of the patients from both groups, the trigger of HLH was an EBV infection (> 80%); EBV may favor HLH by eliciting a potent CD8 T-cell response. It is also postulated that SAP and possibly XIAP are associated with activation pathways that are more important in triggering selective cytotoxicity toward B cells.[22-27] HLH in most hereditary conditions such as FHL, Griscelli syndrome type II, and Chediak-Higashi syndrome shares common pathophysiologic mechanisms, that is, global impaired cytotoxicity responses that lead to the inability of effector lymphocytes to kill infected cells and antigen-presenting cells.[28] In mice and humans, SAP-deficient $CD8^+$ T and NK cells exhibit defective cytotoxicity responses caused by abnormal functions of SLAM receptors.[29] This could explain the occurrence of HLH in SAP-deficient patients.[22-27] In contrast, NK-cell and T-cell cytotoxic responses appear to be preserved in XIAP-deficient patients[7,9] (C. Synaeve and S.L., unpublished data, 2009 and 2010). This might account for the lower severity of the HLH in the XIAP deficiency. Hence, the precise immune defects responsible for HLH in XIAP deficiency remain to be elucidated.

Only XIAP-deficient patients were at risk for chronic colitis with often a lethal outcome. This phenotype seems that is may be even worse than HLH, because the mortality in the group of patients with colitis (3 of 5) has a tendency to be higher than in the group with HLH (5 of 22). Histopathologic analysis of intestinal mucosal biopsy specimens showed an inflammatory process with an accumulation of activated T cells (and eosinophils in one patient) that could evoke inflammatory bowel disease. Interestingly, a recent report indicates that XIAP is involved in nucleotide-binding oligomerization domain containing 2 (NOD2) activation which is an intracellular pattern recognition receptor of the NOD-like receptor family.[30] Importantly, *NOD2* is a key susceptibility gene for Crohn disease.[31] Thus, defects in XIAP might lead to defective NOD2 responses as an additive risk factor for colitis in some of these patients. Of note, however, *NOD2* was sequenced in 2 XIAP-deficient patients with colitis, and none had the genotype shown to be a risk factor for Crohn disease (J.P. Hugot and S.L., unpublished data, June 2006).

One striking difference between XLP-1 and XLP-2 was that only SAP-deficient patients developed lymphoma, although it could not be formally excluded that XIAP-patients might develop lymphomas in the future. In SAP-deficiency, the occurrence of lymphomas may be explained by defective immunosurveillance of hematopoietic cells, resulting from alterations in SLAM receptor-mediated NK- and T-cell cytotoxicity responses,[22-24,26] but also by the proapoptotic functions that have been assigned to SAP.[32,33]

Another common finding shared by XLP-1 and XLP-2 is the hypogammaglobulinemia. Interestingly, 2 XLP-2 patients recovered from hypogammaglobulinemia, which so far seems not to be the case for XLP-1 patients. Numerous studies in mice and humans have documented that impaired antibody production found in XLP-1 resulted from a block in germinal center formation, leading to defects in the differentiation of Ig-isotype–switched memory B cells.[34-36] In most of the XIAP-deficient patients, Ig-isotype–switched memory B cells are not found to be decreased[7] (S. Siberil and S.L., unpublished data, 2008 and 2009). In XIAP deficiency, hypogammaglobunemia could be the consequence of increased activation-induced cell death of B cells, a hypothesis that needs to be tested.

In conclusion, the present comparison of the clinical features of SAP- and XIAP-deficient patients shows that SAP deficiency and XIAP deficiency share a main phenotype, that is, EBV-induced HLH. This similarity raises the possibility of a functional/molecular link between SAP and XIAP proteins. Alternatively, impairment of 2 independent pathways, both important in EBV immunity, could lead to a shared phenotype. Nevertheless, we also demonstrate that XLP-1 and XLP-2 can be distinguished on several clinical aspects, which could be helpful for diagnosis and therapeutic decisions.

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Authorship

Contribution: J.P.S. collected and analyzed the data and participated in study design, writing of the report, and patients' care; D.C. performed the immunohistochemistry experiments and analyzed histopathologic findings; F.H. participated in histopathologic analysis and writing of the report; C.L., N.L., and S.R. realized gene sequencing and protein expression tests; G.S.B. participated in data analysis; A.F. contributed to study design, data analysis, writing of the report, and patients' care; S.L. coordinated the study collected the data and contributed to sequencing, expression tests, data analysis, and wrote the report. The other authors provided and collected the clinical data on patients' status and contributed to the data analysis and patients' care.

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References

1. Purtilo DT, Cassel C, Yang JP. Letter: fatal infectious mononucleosis in familial lymphohistiocytosis. *N Engl J Med.* 1974;291(14):736.
2. Purtilo DT, Cassel CK, Yang JP, Harper R. X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). *Lancet.* 1975;1(7913):935-940.
3. Seemayer TA, Gross TG, Egeler RM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. *Pediatr Res.* 1995;38(4):471-478.
4. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2007;48(2):124-131.
5. Sayos J, Wu C, Morra M, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature.* 1998;395(6701):462-469.
6. Coffey AJ, Brooksbank RA, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet.* 1998; 20(2):129-135.
7. Rigaud S, Fondaneche MC, Lambert N, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature.* 2006;444 (7115):110-114.
8. Zhao M, Kanegane H, Ouchi K, Imamura T, Latour S, Miyawaki T. A novel XIAP mutation in a Japanese boy with recurrent pancytopenia and splenomegaly. *Haematologica.* 2009;95(4):688-689.
9. Marsh RA, Madden L, Kitchen BJ, et al. XIAP deficiency: a unique primary immunodeficiency best classified as X-linked familial hemophagocytic lymphohistiocytosis and not as X-linked lymphoproliferative disease. *Blood.* 2010;7(116):1079-1082.
10. Gaspar HB, Sharifi R, Gilmour KC, Thrasher AJ. X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. *Br J Haematol.* 2002;119(3):585-595.
11. Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. *Immunol Rev.* 2005;203:180-199.
12. Grierson HL, Skare J, Hawk J, Pauza M, Purtilo DT. Immunoglobulin class and subclass deficiencies prior to Epstein-Barr virus infection in males with X-linked lymphoproliferative disease. *Am J Med Genet.* 1991;40(3):294-297.
13. Dutz JP, Benoit L, Wang X, et al. Lymphocytic vasculitis in X-linked lymphoproliferative disease. *Blood.* 2001;97(1):95-100.
14. Ma CS, Nichols KE, Tangye SG. Regulation of cellular and humoral immune responses by the SLAM and SAP families of molecules. *Annu Rev Immunol.* 2007;25:337-379.
15. Eckelman BP, Salvesen GS, Scott FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. *EMBO Rep.* 2006;7(10): 988-994.
16. Galban S, Duckett CS. XIAP as a ubiquitin ligase in cellular signaling. *Cell Death Differ.* 2009;17(1): 54-60.
17. Verhelst H, Van Coster R, Bockaert N, et al. Limbic encephalitis as presentation of a SAP deficiency. *Neurology.* 2007;69(2):218-219.
18. Knight C, Murray KF. Hepatobiliary associations with inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol.* 2009;3(6):681-691.
19. Hervier B, Latour S, Loussouarn D, et al. An atypical case of X-linked lymphoproliferative disease revealed as a late cerebral lymphoma. *J Neuroimmunol.* 2010;218(1-2):125-128.
20. Rougemont AL, Fournet JC, Martin SR, et al. Chronic active gastritis in X-linked lymphoproliferative disease. *Am J Surg Pathol.* 2008;32(2): 323-328.
21. Sumegi J, Huang D, Lanyi A, et al. Correlation of mutations of the SH2D1A gene and Epstein-Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. *Blood.* 2000;96(9):3118-3125.
22. Dong Z, Cruz-Munoz ME, Zhong MC, Chen R, Latour S, Veillette A. Essential function for SAP family adaptors in the surveillance of hematopoietic cells by natural killer cells. *Nat Immunol.* 2009;10(9):973-980.
23. Bloch-Queyrat C, Fondaneche MC, Chen R, et al. Regulation of natural cytotoxicity by the adaptor SAP and the Src-related kinase Fyn. *J Exp Med.* 2005;202(1):181-192.
24. Parolini S, Bottino C, Falco M, et al. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. *J Exp Med.* 2000;192(3):337-346.
25. Dupre L, Andolfi G, Tangye SG, et al. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood.* 2005;105(11):4383-4389.
26. Sharifi R, Sinclair JC, Gilmour KC, et al. SAP mediates specific cytotoxic T-cell functions in X-linked lymphoproliferative disease. *Blood.* 2004;103(10):3821-3827.
27. Hislop AD, Palendira U, Leese AM, et al. Impaired Epstein-Barr virus-specific CD8+ T cell function in X-linked lymphoproliferative disease is restricted to SLAM family positive B cell targets. *Blood. Blood.* 2010;116(17):3249-3257.
28. Fischer A, Latour S, de Saint Basile G. Genetic defects affecting lymphocyte cytotoxicity. *Curr Opin Immunol.* 2007;19(3):348-353.
29. Veillette A, Dong Z, Perez-Quintero LA, Zhong MC, Cruz-Munoz ME. Importance and mechanism of 'switch' function of SAP family adapters. *Immunol Rev.* 2009;232(1):229-239.
30. Krieg A, Correa RG, Garrison JB, et al. XIAP mediates NOD signaling via interaction with RIP2. *Proc Natl Acad Sci U S A.* 2009;106(34):14524-14529.
31. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* 2001; 411(6837):599-603.
32. Snow AL, Marsh RA, Krummey SM, et al. Restimulation-induced apoptosis of T cells is impaired in patients with X-linked lymphoproliferative disease caused by SAP deficiency. *J Clin Invest.* 2009;119(10):2976-2989.
33. Chen G, Tai AK, Lin M, Chang F, Terhorst C, Huber BT. Signaling lymphocyte activation molecule-associated protein is a negative regulator of the CD8 T cell response in mice. *J Immunol.* 2005;175(4):2212-2218.
34. Crotty S, Kersh EN, Cannons J, Schwartzberg PL, Ahmed R. SAP is required for generating long-term humoral immunity. *Nature.* 2003;421(6920):282-287.
35. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature.* 2008; 455(7214):764-769.
36. Ma CS, Hare NJ, Nichols KE, et al. Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4+ T cells. *J Clin Invest.* 2005; 115(4):1049-1059.

X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease

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X-linked lymphoproliferative disease (XLP1) is a rare immunodeficiency characterized by severe immune dysregulation and caused by mutations in the *SH2D1A*/SAP gene. Clinical manifestations are varied and include hemophagocytic lymphohistiocytosis (HLH), lymphoma and dysgammaglobulinemia, often triggered by Epstein-Barr virus infection. Historical data published before improved treatment regimens shows very poor outcome. We describe a large cohort of 91 genetically defined XLP1 patients collected from centers worldwide and report characteristics and outcome data for 43 patients receiving hematopoietic stem cell transplant (HSCT) and 48 untransplanted patients. The advent of better treatment strategies for HLH and malignancy has greatly reduced mortality for these patients, but HLH still remains the most severe feature of XLP1. Survival after allogeneic HSCT is 81.4% with good immune reconstitution in the large majority of patients and little evidence of posttransplant lymphoproliferative disease. However, survival falls to 50% in patients with HLH as a feature of disease. Untransplanted patients have an overall survival of 62.5% with the majority on immunoglobulin replacement therapy, but the outcome for those untransplanted after HLH is extremely poor (18.8%). HSCT should be undertaken in all patients with HLH, because outcome without transplant is extremely poor. The outcome of HSCT for other manifestations of XLP1 is very good, and if HSCT is not undertaken immediately, patients must be monitored closely for evidence of disease progression. (*Blood*. 2011;117(1):53-62)

Introduction

X-linked lymphoproliferative disease (XLP) is a rare primary immunodeficiency first described in 1975 by Purtilo[1] and characterized by severe immune dysregulation often after viral infection (typically with Epstein-Barr virus [EBV]). Since XLP was first

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described, our understanding of the molecular and cellular pathogenesis of the disease has greatly improved. However, clinically, it is still difficult to determine optimal management and prognosis for patients due to the variability of clinical presentation, lack of genotype-phenotype correlation, and rarity of the disease. Purtilo established an XLP registry in 1980, and by 1995 more than 270 boys had been identified in 80 kindreds.[2] To date this registry has provided the only data on clinical phenotype and prognosis for this patient group. Overall mortality in this group was 75%, with 70% of boys succumbing before 10 years of age. However, current outcomes for XLP may be very different due to the availability of unambiguous molecular diagnosis, improved viral monitoring, and the improvement in treatment regimens for disease manifestations.

XLP affects 1 to 3 million boys,[3,4] and most commonly presents in childhood or early adolescence. Presentation may be acute in the case of fulminant infectious mononucleosis (FIM)/hemophagocytic lymphohistiocytosis (HLH) or lymphoma or less aggressive with dysgammaglobulinemia or recurrent infections. Patients often manifest more than one phenotype and may progress from one phenotype to another, for example presenting with hypogammaglobulinemia and progressing to lymphoma, and different clinical features are often present in families highlighting the lack of genotype-phenotype correlation. Other rare but well-described presenting features include aplastic anemia, vasculitis, and chronic gastritis.[2,5-8] It is now known that the clinical syndrome of XLP arises from 2 different genetic defects in *SH2D1A* (XLP1, by far the most common and the focus of this report) and the *BIRC/XIAP* gene (XLP2). The gene responsible for XLP1 is the *SH2D1A* gene found on the X chromosome at position Xq25,[9-11] which encodes the cytoplasmic protein SAP (signaling lymphocyte activation molecule or SLAM-associated protein). SAP is a key regulator of normal immune function in T cells,[12-14] natural killer (NK) cells,[15-18] NKT cells,[19,20] and possibly B cells,[21] and defects in this protein lead to the varied immune defects described in XLP1 patients.[20,22] Humoral defects seen in this disease are thought to arise from impaired $CD4^+$ T-cell interaction with B cells and not an intrinsic B-cell deficit.[23]

Although it has always been presumed that EBV infection plays a crucial role in the development of clinical features in XLP1 patients, it is now clear that a proportion of boys are EBV negative at presentation and remain so. Indeed, 10% of patients have immunological abnormalities before any evidence of EBV exposure.[4,24] XLP1 is therefore a disorder of immune dysregulation rather than a disorder specifically associated with EBV infection.

Before 1994, acute management of FIM and HLH included antiviral medications, high-dose intravenous immunoglobulin (Ig), immunosuppressants, and other immune modulators such as interferon-α. These treatments proved disappointing[25] and the XLP registry data showed a survival of only 4% for boys presenting with these manifestations. Improved chemotherapy regimes for lymphoma and immunosuppressive protocols to treat HLH (including rituximab) may reduce the mortality rate for XLP1 patients and allow stabilization before hematopoietic stem cell transplant (HSCT).[26] Our report provides valuable outcome data collected since the introduction of current HLH treatment protocols, focusing on XLP1 patients with mutations in the *SH2D1A* gene.

Allogeneic HSCT remains the only curative option for XLP1 at present although large scale outcome studies are not available. Recently, Lankester et al reviewed 14 cases in the literature who had undergone HSCT and found an overall survival of 71% (10/14) with little evidence of EBV reactivation and posttransplant lymphoproliferative disease.[27] We describe here outcome data for a much larger cohort of patients transplanted since 1997.

There is no consensus on whether clinically stable XLP1 patients should undergo HSCT as the natural history of the disease is so variable, even within the same family. Treatment and management of the disease is severely hampered by the lack of data of a large cohort of patients and previously published outcome data are based on historical data, which may represent patients with conditions other than XLP1 as inclusion was based on clinical and not genetic diagnosis. Also, little recent data exist for patients who remain untransplanted. Hence, we describe a large cohort of genetically defined XLP1 patients collected from centers worldwide. The data presented will allow for better counseling of affected families regarding prognosis and management options, particularly in relation to timing of transplant.

Methods

Data collection

Questionnaires regarding patient demographics, transplant characteristics, and outcome were sent to centers worldwide identified through the European Society for Immunodeficiencies/European Bone Marrow Transplantation Registry, published case reports or centers known to perform pediatric HSCT. Retrospective analysis was performed using data collected for 91 patients from 32 centers worldwide. The number of cases from each center varied between 1 and 27 but was on average 1-2 cases. Patients included in this study were born between 1941 and 2005; 63 were born in or after 1990 (24 untransplanted patients and 39 transplanted patients). Only patients with a confirmed mutation in the *SH2D1A* gene were included in this series. Patients with mutations in other XLP-associated genes such as *XIAP/BIRC-4* were excluded, as were patients with abnormal SAP expression but no confirmed mutation in *SH2D1A*. EBV status was determined by polymerase chain reaction to avoid variable serology results in XLP1 patients and especially in those with dysgammaglobulinemia. Questionnaires offered reporting of FIM and HLH separately; thus, some centers with experience in this area reported patient data accordingly, and it is presented as such.

Data in various forms from 11 patients have been previously published[5,27-32] but standardized information was recollected in this study and added to the series.

Management of HLH and lymphoma

Patients who presented with HLH were managed predominantly in accordance with HLH 94 or HLH 2004 protocols. Additional or alternative treatment included antiviral therapy (aciclovir, ganciclovir, or foscarnet, n = 6), high-dose intravenous immunoglobulin (n = 9), immunosuppression (steroids, cyclosporine, and etoposide, n = 12), or anti-CD20 antibody (rituximab, n = 10). Intrathecal therapy was used where central nervous system involvement was suspected. Ten patients who proceeded to transplant received rituximab therapy before transplant, either as treatment for HLH or during conditioning.

Regimes for the treatment of lymphoma varied in line with appropriate national guidelines (eg, COPAD [cyclophosphamide, vincristine, prednisone, and doxorubicin]) study, Berlin-Frankfurt-Munster Group, Associazione Italiana Ematologia Oncologia Pediatrica, or United Kingdom Children's Cancer Study Group guidelines) and only occasionally involved surgical management.

Statistical analysis

Kaplan-Meier curves were used to analyze survival figures. The log rank test (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests were used to compare survival between different groups. Statistical analysis including hazard ratio calculation was performed using GraphPad Prism Version 5.00 for Windows.

Table 1. Presenting symptoms and features of XLP1 patients with associated mortality

	Incidence	Mortality
Presenting symptom		
HLH	31.9%	65.5%
FIM	7.7%	14.3%
Lymphoma	14.3%	7.7%
Dysgammaglobulinemia	22%	5%
Family history of XLP1 alone	16.5%	20%
Other	7.7%	14.3%
Features occurring at any time		
HLH	35.2%	65.6%
FIM	9.9%	22.2%
Lymphoma	24.2%	9%
Dysgammaglobulinemia	50.5%	13%
Other	15.4%	28.6%

Results

Data from 91 patients (64 pedigrees) in 32 centers worldwide were included in this report. The overall survival of XLP1 patients was 71.4% (65/91), and patients displayed a heterogeneous clinical phenotype. Due to the heterogeneity of the group, data were analyzed according to presentation with HLH, EBV status, and whether patients had received HSCT, allowing characterization of outcome after transplant.

Spectrum of XLP1 mutations

In keeping with previous publications, no genotype/phenotype correlation was evident, and the most frequently reported mutation involved the arginine residue at position 55 (exon 2) found in 11 patients from 9 different families. Detailed genetic information was available for 62 patients (50 pedigrees; supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Exon 2 had the most mutations with missense mutations accounting for the majority but nonsense, frameshift, and splice site mutations were also reported. Large gene deletions (up to 11 Mb) including those involving the whole gene were identified in 5 families. Three of these larger deletions were associated with gastrointestinal symptoms of colitis and gastritis. Such symptoms were not found in patients with other mutations apart from a patient with diarrhea as a feature (missense mutation exon 1, 62 T > C). In a further 29 patients, detailed genetic data were not supplied but a SAP/*SH2D1A* gene defect was confirmed by the documenting center.

Clinical manifestations of XLP1

Table 1 shows the presenting features of disease as well as features of disease manifesting throughout the course of the condition. HLH remained the most common presenting feature (39.6%), although dysgammaglobulinemia was the manifestation seen most commonly in patients during the course of the illness.

Although clinical features have remained similar to previously published data,[2] the survival associated with XLP1 is 71.4%, which is significantly improved over historical survival of 25%. The survival associated with different phenotypes has also changed significantly with mortality associated with HLH decreased from 96% to 65%, lymphoproliferative disease from 35% to 8%, and dysgammaglobulinemia from 55% to 5%.

Twenty-two patients suffered from malignant lymphoproliferative disease, with eighteen patients (81.8%) diagnosed with B-cell non-Hodgkin lymphoma mainly of the abdomen and cervical region. In 5 patients the disease was recurrent, with 1 patient experiencing a cerebral tumor. Only 1 patient was reported with cerebral T-cell lymphoma. Data on tumor histology is lacking in 3 patients.

Immunological abnormalities at diagnosis

Details of immune function were available for 57 patients, although in some cases, data were only available after the onset of disease manifestations that may have influenced immunoglobulin and lymphocyte subset levels. Immunoglobulin levels were recorded in 49 patients, and 32 of these showed varying degrees of abnormal immunoglobulin levels. Twelve children presented with neutropenia. Lymphocyte subset data were available for 47 patients; 19 showed a reduced percentage of B cells, 26 showed low NK cell numbers, and 12 had a reversed CD4:CD8 ratio.

Presentation with HLH

The mortality for patients presenting with HLH was 65.6%, with a median age at presentation of 3 years 2 months (range 8 months to 9 years). Of the 32 patients with HLH, 16 underwent transplant, of whom 8 survived (50%; Figure 1). Of those who did not receive a transplant, only 3 survived (18.8%), confirming previous reports that the prognosis for patients with HLH associated with a genetic defect is extremely poor and that HSCT is necessary.

EBV status

EBV status was documented in 79 patients showing that 51 (64.6%) were EBV positive at presentation or diagnosis (Table 2 and supplemental Figure 1). The median age of presentation in this group was 4 years (range 8 months to 40 years), and the overall mortality was 35.2% (18/51). There was no significant difference in mortality between patients with (35.2%) and without (28.6%) documented EBV infection.

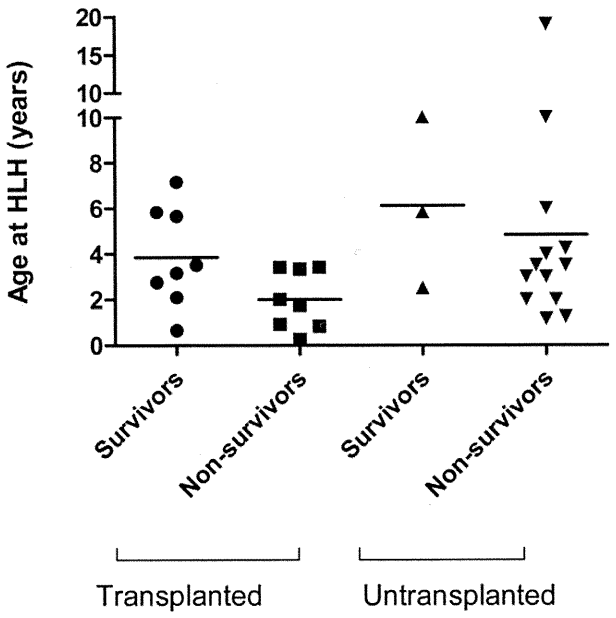

Figure 1. Outcome of patients with HLH during course of disease. Survival of patients who present with HLH—patients who remain untransplanted have a poor survival outcome with only 18.8% survival. By contrast the survival of those who undergo transplant is higher at 50%.

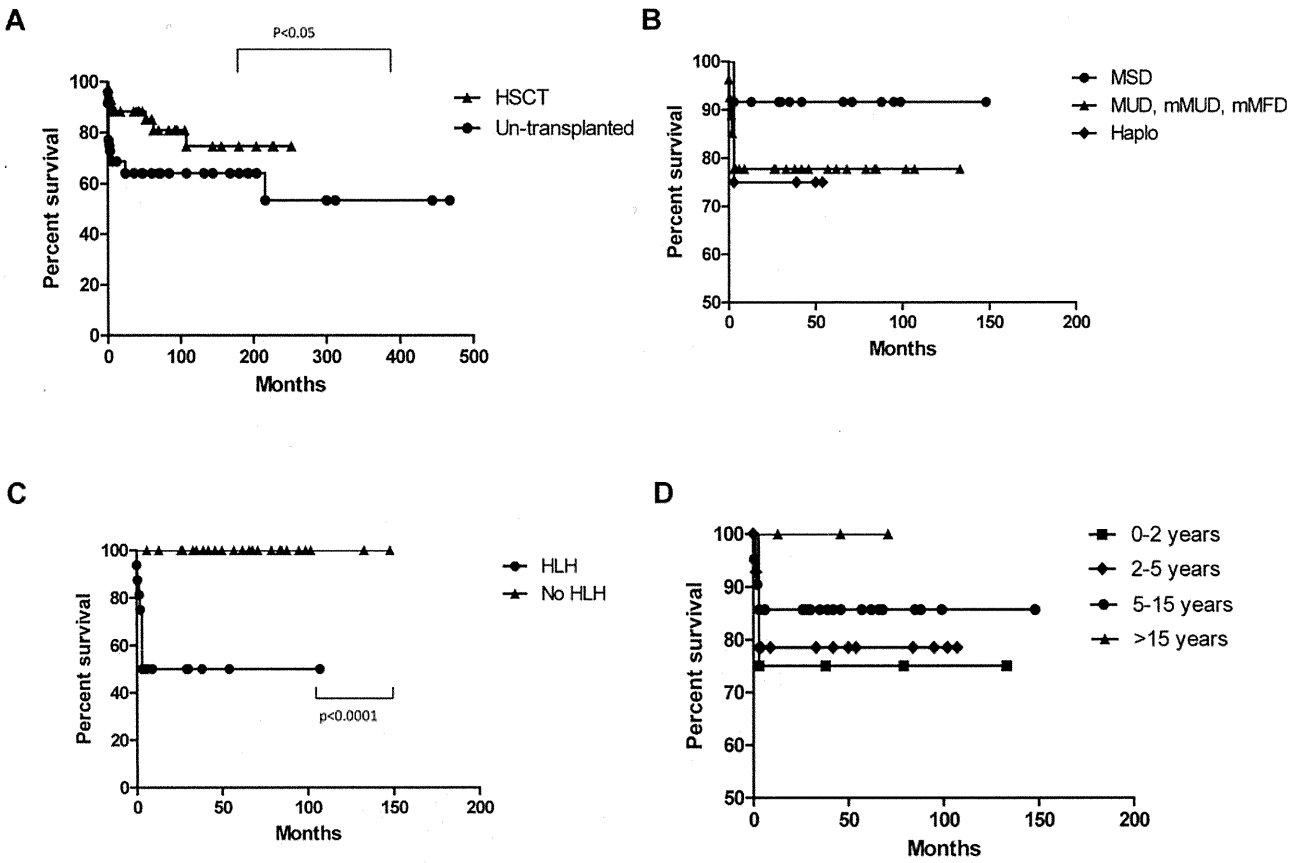

Figure 2. Survival in XLP1 related to different variables. (A) Overall survival of transplanted versus untransplanted patients. In the transplanted group this represents time from presentation and not transplant. (B) Survival according to donor source. (C) Survival after HSCT with relation to presence of HLH before transplant. (D) Survival according to age at transplant.

HLH/FIM was the most common feature in this group being seen in 35 patients (68.6%), with lymphoma present in 10 patients (19.6%), and dysgammaglobulinemia in 19 (37.2%). Nine EBV-positive patients had a family history of XLP1, and two others had a family history suggestive of an X-linked immunodeficiency. Of the 18 EBV-positive patients who died, the majority (14/18) died within 2 months of presentation due to disease progression. Three died in the early posttransplant period of infective complications and disease progression, and 1 died during treatment for lymphoma.

Twenty-eight patients were EBV negative at presentation or diagnosis. The median age of presentation for this group was 3 years (range birth to 31 years). Family history of XLP1 was the presenting feature for 12 patients, and a further 7 patients described a family history suggestive of an X-linked immunodeficiency or lymphoma. There was a higher rate of dysgammaglobulinemia (51.8%) in this group. Lymphoma was present in 7 patients. Fewer EBV negative patients presented with HLH/FIM, and this may suggest that at least for this manifestation a viral trigger is important. Information was sought on other viral infectious agents including cytomegalovirus and adenovirus, but data were not available for most patients. Other clinical features included aplastic anemia in 3 patients and vasculitis in 2 patients. The mortality for this EBV negative group was 28.6% (8/28); 3 patients died shortly after presentation before HSCT with central nervous system vasculitis (2) and HLH with enterococcal sepsis (1). One patient died 11 years after presentation following a complex course, and a further 4 patients died in the early posttransplant period (described in Table 5).

Table 2. Characteristics of EBV-positive and EBV-negative XLP1 patients

	EBV positive (64.6%, n = 51)	EBV negative (35.4%, n = 28)
Median age at presentation	4 y (8 mo-40 y)	3 y (0-31 y)
Family history of XLP1	17.6%	42.9%
HLH	51%	21.4%
FIM	17.6%	
Lymphoma	19.6%	25%
Dysgammaglobulinemia	37.2%	51.8%
Mortality	35.2%	28.6%
Median age at death	3 y 6 mo (14 mo-21 y)	5 y 11 mo (20 mo-31 y)

HSCT for XLP1

HSCT was undertaken in 22 centers (range of patients/center: 1-7) between 1997 and 2009 (Table 3). Forty-six transplants were performed on 43 patients, and the median age at transplant was 6.25 years (range 8 months to 19 years); 1 patient who had undergone a haploidentical transplant received a $CD34^+$ selected boost 1 year after initial transplant. One patient received an allogeneic HSCT to treat lymphoma before a diagnosis of XLP1 was established. Most patients received bone marrow or peripheral blood stem cells, and only 2 patients received umbilical cord HSCT. Donor grafts were from human leukocyte antigen-matched family donors in 14 cases, mismatched family donors or matched unrelated grafts in 28 cases, and haploidentical donors in 4 cases. Half of the transplant procedures (23/46) were performed using myeloablative conditioning regimes including combinations of

Table 3. Characteristics of XLP1 patients receiving allogeneic HSCT

	Percentage	Number	1-y survival	HR	95% CI	*P*
XLP1 features						
Previous HLH	37.2%	16/43	50%	23.93	5.31-108.0	< .0001
Previous NHL	27.9%	12/43	74.2%	0.23	0.05-1.06	.06
Previous dysgammaglobulinemia	46.5%	20/43	80%	1.2	0.29-4.96	.77
EBV$^+$	51.2%	21/41	75%	1.37	0.36-5.3	.65
Age at HSCT	Mean 7 y (8 mo to 19 y 7 mo)					
0-2 y	9.3%	4/43	75%	5.75	0.11-302.1	.38
2-5 y	34.9%	15/43	78.6%	3.61	0.18-71.76	.40
5-15 y	48.8%	21/43	85.7%	3.16	0.11-90.83	.50
> 15 y	7%	3/43	100%			
Year of HSCT						
< 2000	7.0%	3/43	66.7%			
2000-2005	37.2%	16/43	87.5%			
2005-2009	55.8%	24/43	79.2%			
Donor Type						
MSD, MFD	30.4%	14/46	91.77%			
MUD, mMFD, mMUD	60.9%	28/46	77.8%	0.42	0.08-2.07	.27
Haplo	8.7%	4/46	75%	0.24	0.01-6.58	.4
Source						
Bone marrow	58.5%	24/41*	82.6%			
Peripheral blood	36.6%	15/41*	92.9%			
Umbilical cord	4.9%	2/41*	50%			
Conditioning						
MA	50%	23/46	82.9%			
NMA	50%	23/46	78.9%	1.25	0.30-5.2	.77
Serotherapy	30.4%	14/46				
GVHD	50%	19/38				
Grade 1	18.4%	7/38				
Grade 2-3	26.3%	10/38				
Grade 4	5.3%	2/38				
Chronic	5.3%	2/38				
Chimerism						
Full (> 98%)	92%	35/38	100%			
Mixed	8%	3/38	88.8%	2.98	0.06-151.0	.59
Replacement IVIg	20%	7/35†				
Alive	81.4%	35/43				
Follow up	6 wk to 148 mo					

*Data missing on 5 transplants, 1 died during conditioning.

†Three patients < 1 year after transplant.

CI indicates confidence interval; HR, hazard ratio; MSD, matched sibling donor; MFD, matched family donor; MUD, matched unrelated donor; mMFD, mismatched family donor; mMUD, mismatched unrelated donor; Haplo, haploidentical transplant; MA, myeloablative; and NMA, nonmyeloablative.

busulfan 12-20 mg/kg, cyclophosphamide 50-200 mg/kg, and total body irradiation 5-12 Gy. The other half of procedures used nonmyeloablative conditioning regimes consisting of fludarabine (30 mg/kg), melphalan (70-140 mg/kg), busulphan (4-12 mg/kg), or total body irradiation (3-5 Gy). Twenty-six patients received additional serotherapy with alemtuzumab, anti-thymocyte globulin, anti-CD3 antibody, and anti-CD20 antibody (rituximab). Graft-versus-host disease (GVHD) prophylaxis regimes differed between centers, but mostly involved combinations of cyclosporin with methotrexate, mycophenolate mofetil, steroids, and tacrolimus. T-cell depletion of the graft was used in 1 case.

Outcome for XLP1 patients who received allogeneic HSCT was good with 81.4% surviving the procedure (35/43) with a median follow up of 52 months. The majority of these patients (28/35 survivors) required no ongoing immunoglobulin replacement therapy. Tables 3 and 4 highlight details of transplanted patients, and Figure 2 describes survival according to several factors.

Sixteen patients were diagnosed with HLH before transplant and 12 patients had some form of lymphoproliferative disease (lymphoma). Only 51.2% of the cohort had documented evidence of EBV infection (by polymerase chain reaction) with survival rates in EBV$^+$ patients similar to those without EBV infection (75% vs 80%). Most patients experienced some delay from first symptoms to diagnosis (average delay 2 years 7 months) but once a diagnosis of XLP1 was established time to transplant was generally less than 1 year. Median age at transplant was 6.25 years with a range of 8 months to 19 years.

Univariate analysis was performed to identify the major risk factors for survival after HSCT. The most important risk factor was prior HLH, which significantly decreased the survival outcome to 50%. A previous diagnosis of lymphoma had a near significant effect, but other variables were not shown to have a significant effect including importantly, previous evidence of EBV infection, the age at transplant, donor type, or the conditioning regime. It is also important to note that only patients who had HLH at some point before or during transplant died. Conversely, all patients without HLH (n = 27) survived the transplant procedure.

Half of the patients underwent a nonmyeloablative conditioning regime before HSCT and this did not impact on survival (nonmyeloablative vs myeloablative, 78.9% vs 82.9%) or long-term chimerism. More than 90% of patients achieved full donor chimerism, and

Table 4. Details of XLP1 patients surviving allogeneic HSCT

Year of HSCT	EBV	HLH	Age at HSCT	Donor	Conditioning/serotherapy/ graft manipulation	GVHD prophylaxis	GVHD	Chimerism	Follow up (mo)	Ig
1997	NK		7 y	MSD	Cy, TBI	MTX, CSA	1 S*	100%	148	
1998			1 y	MUD	Bu, Cy, ATG	MTX, CSA, P	1 S	100%	133	
2000	+		4 y	MUD	Bu, Cy, Campath	CSA	2 S	100%	102	
2000	+	Yes	3 y	mMUD	Bu, Cy	MTX, CSA	2 S, L	100%	107	
2001			4 y	MUD	Flu, Melph, ATG, TBI	MMF, CSA		100%	102	
2001	+		10 y	MSD	Bu, Cy, VP-16 (NHL)	MTX, CSA	2-3 GI	100%	99	
2001			4 y	MSD	Bu, Cy	CSA	2 S	100%	95	
2002			13 y	MSD	Thio, Flu, ATG	CSA		100%	88	
2002	+		7 y	MUD	Bu, Cy, ATG	MTX, CSA	1 S	100%	85	
2002			3 y	MUD	Bu, Cy, ATG	MTX, CSA		100%	84	
2003			8 mo	mMUD	Flu, Melph, ATG, TBI	TAC, MTX, P	S	100%	79	
2003			19 y	MSD	Thio, Flu, ATG	CSA		100%	71	
2003			11 y	mMUD	Flu, Melph, ATG	MMF, CSA		100%	68	
2004	+		5 y	MSD	Bu, Cy	MTX, CSA		20% PBMC	66	
2004	+		12 y	mMFD	Flu, Melph, Campath, 34+	MMF, CSA	4 S, L*	100%	62	Y
2004	+		8 y	mMUD	Flu, Melph, Campath	CSA	2-3 S, GI	100%	57	
2005	+	Yes	2 y	Haplo	Bu, Cy, ATG, 34+	CSA		100%	54	Y
2005			2 y	Haplo	Bu, Cy, ATG, 34+, top up 1 year	CSA		88% PBMC 97% M	50	Y
2005			12 y	mMUD	Flu, Melph, Campath, 34+	MMF, CSA	3 S	100%	46	
2005			18 y	MUD		NK		NK	46	NK
2006			5 y	MSD	Bu, Cy	CSA	1 G, 3 S	100%	42	
2006	NK		2 y	MUD	Bu, Flu, Campath	MTX		100%	42	
2006	+		7 y	Haplo	Flu, Melph, Thio, OKT3, ATG			100%, 75% CD3	39	
2006	+	Yes	1 y	MUD	Flu, Melph, Ritux	CSA	1 S	5%	38	
2006	+		11 y	MSD	Bu, Cy, ATG	MTX, CSA		100%	35	
2006			4 y	MUD	Bu, Cy, Campath	MMF, CSA	1 S	100%	33	
2007	+	Yes	6 y	MSD	Bu, Cy	MTX, CSA		99%	30	
2007	+	Yes	7 y	MSD	Bu, Cy	CSA	3 S, L, GI	100%	29	
2007	NK		7 y	MUD	Flu, Melph, TBI	TAC, MTX		98%	27	
2007			7 y	MSD/mMUD	Bu, Cy	CSA	2 S, GI	100%	26	
2008			17 y	MFD	Flu, Melp, Campath	MMF, CSA		100%	13	
2008		Yes	3 y	MUD	Bu, Flu	TAC, MTX		100%	9	Y
2009	+		7 y	MUD	Bu, Cy			100%	6	Y
2009	+	Yes	6 y	mMUD	Flu, Melph, Campath	CSA, MMF	1 S	100%	5	Y
2009	+	Yes	3 y	MUD	Thio, Cy, ATG	CSA, P		100%	4	Y

*Chronic GVHD.

PBMC indicates peripheral blood mononuclear cell; Flu, fludarabine; Melph, melphalan; 34+, CD34+ stem cell infusion; Bu, busulfan; Cy, cyclophosphamide; Thio, thiotepa; TBI, total body irradiation; CSA, cyclosporin A; MMF, mycophenalate mofetil; MTX, methotrexate; P, prednisolone; TAC, tacrolimus; S, skin; GI, gastrointestinal; L, lung; and Ig, replacement immunoglobulin.

those with a mixed or falling chimerism remained well with 1 patient still receiving replacement immunoglobulin.

Data were also collected on common posttransplant complications such as GVHD, infectious complications and toxicity attributable to chemotherapy. Half of the patients (50%) suffered from some form of GVHD; the majority of cases were grade 1-3 affecting the skin, liver, and gut. Two patients suffered grade 4 disease (of skin and liver), and 1 of these children died. Only 2 patients went on to develop chronic GVHD (see Table 3). One patient experienced both veno-occlusive disease and renal toxicity due to conditioning (busulfan, cyclophosphamide, and antithymocyte globulin), and this patient succumbed shortly after a haploidentical transplant.

In 3 patients with mixed chimerism in peripheral blood mononuclear cells, this remained stable in all but 1 patient, in whom it fell from 92% to 5%. However, this patient remains well 3 years posttransplant and does not require replacement immunoglobulin therapy. From this series, there is little evidence of viral reactivation posttransplant. Thirty-five patients are alive with 5 suffering some long-term effects including EBV viremia (managed with rituximab), bronchiectasis, autoimmune disease, chronic psoriasis, and neutropenia.

Eight patients did not survive after HSCT (see Table 5). Seven patients who died presented with HLH before HSCT (4/7 EBV+) compared with 8 of 35 survivors, but HLH was a feature of disease in all 8 nonsurvivors. The majority of nonsurvivors were ≤ 3 years old (5/8), and conditioning regime did not appear to play a role as 5/8 patients received a full myeloablative regime. The main cause of death in this group was sepsis, but disease progression accounted for 2 deaths. The 2 children dying with disease progression went into transplant with active disease; 1 died during conditioning and the other 3 days after HSCT. One further patient died 3 weeks after HSCT (7 months after presentation) from veno-occlusive disease (VOD), multiorgan failure, and renal toxicity attributable to chemotherapy. The remaining 5 patients died of sepsis (2 pseudomonal sepsis, 1 parainfluenza III infection, 1 with disseminated adenoviral infection, and 1 with EBV and fungal infection) within 3 months of HSCT.

Untransplanted patients

Data were available for 48 patients who did not receive HSCT (Table 6); 30 are alive, 4 of whom are actively awaiting transplant, and 3 who refused HSCT. One patient had received an autologous HSCT before diagnosis with XLP1, and this patient's data were

Table 5. Details of XLP1 patients not surviving allogeneic HSCT

EBV	HLH	Age at HSCT, y	Year of HSCT	Donor	Conditioning/serotherapy/ graft manipulation	GVHD prophylaxis	GVHD	Chimerism	Cause of death
+	Yes	2	2005	MMFD	Flu, TBI	N/A			Died during conditioning 6 wk from presentation
+	Yes	3	2003	MUD	Bu, Flu, Campath, Rituximab	CSA			Died 3 d after HSCT disease progression
	Yes	6	2005	MMFD	Bu, TBI	MMF. MTX, P			Died 14 d after HSCT MDR pseudomonal sepsis
+	Yes	3	2009	Haplo	Bu, Cy, ATG	TCD			Died 3 wk after HSCT VOD, MOF, renal toxicity
+ (after HSCT)	Yes	5	2008	mMUD (cord plus PBSC 4 months later)	Bu, Flu, ATG then Flu, TBI	TAC, P		100%	Died 2 mo after second HSCT EBV, fungal, and ?PCP sepsis
	Yes	3	1998	MSD × 2	Flu, Melph	CSA, P		100%	Died 3 mo after HSCT *Pseudomonas* sepsis
+	Yes	12	2003	MUD	Bu, Cy, Flu, Campath		4 S	100%	Died 3 mo after HSCT disseminated adenovirus
	Yes	1	2007	MUD	Flu, Melph, ATG, 34^+	CSA	2-3 S, L	100%	Died 3 mo after HSCT paraflu III sepsis

PBSC indicates peripheral blood stem cell; Flu, fludarabine; Melph, melphalan; 34^+, $CD34^+$ stem cell infusion; Bu, busulphan; Cy, cyclophosphamide; Thio, thiotepa; TBI, total body irradiation; CSA, cyclosporine A; MMF, mycophenalate mofetil; MTX, methotrexate; P, prednisolone; TAC, tacrolimus; TCD, T-cell depletion; S, skin; L, lung; VOD, veno-occlusive disease; MOF, mullti-organ failure; MDR, multidrug resistant; and PCP, *Pneumocystis jiroveci*.

analyzed as though untransplanted. Less detailed information was available for this set of patients compared with those receiving HSCT. This may be because some patients died before EBV status and immune function could be established and any first symptoms may not have been recognized as a manifestation of XLP1. From data available, median age at presentation was 5 years, and delay in diagnosis ranged from a few weeks to 32 years.

Presentation was highly variable but as expected included HLH/FIM, dysgammaglobulinemia, and recurrent infection. More unusual presentations included 1 patient with central nervous system vasculitis, intracranial hemorrhage and myocardial fibrosis, and peripheral eosinophilia. The course of XLP1, both temporal and clinical, was extremely variable without any apparent correlation to family history or genetic mutation.

Table 6. Characteristics of XLP1 patients not receiving HSCT

		Number
Age at first symptom	8 y 8 mo (6 mo-40 y)	
Age at death	7.5 y (1-31 y)	
Time from presentation to death	17.3 mo (1 NK) 9 d-18 y	
Time from first symptom (in those patients alive)	12 y (1 NK) 1-39 y	
Presenting symptom		
HLH	31.3%	15/48
FIM	10.4%	5/48
Lymphoma	16.7%	8/48
Dysgammaglobulinemia	29.2%	14/48
Other	12.5%	6/48
Features		
HLH	33.3%	16/48
FIM	12.5%	6/48
Lymphoma	20.1%	10/48
Dysgammaglobulinemia	56.3%	27/48
Gut	8.3%	4/48
Other	14.6%	7/48
EBV status		
EBV^+	66.6%	32/48
EBV^-	14.6%	7/48
Unknown	18.8%	9/48
Mortality	37.5%	18/48 (4 EBV^-)
Associated with HLH	81.3%	13/16
Associated with FIM	33.3%	2/6
Associated with lymphoma	20%	2/10 1 had previous HLH and died during chemotherapy; 1 had recurrent lymphoma and many other problems
Immunoglobulin replacement		
Yes	70%	21/30
No	23.3%	7/30
Unknown	6.7%	2/30

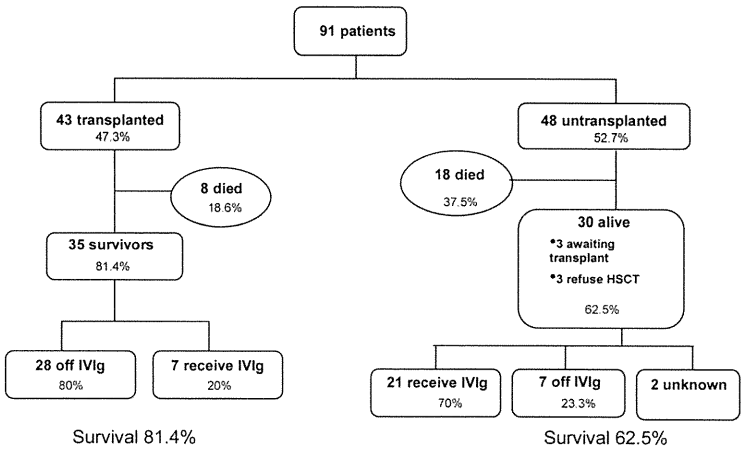

Figure 3. Outcome of patients with SAP/*SH2D1A* mutations.

As with transplanted patients the significant mortality associated with HLH is evident in untransplanted patients (81.3%). Presentation or manifestation of HLH (n = 15 and 16, respectively) was associated with a rapid decline and death within 6 weeks, especially in patients less than 5 years of age. Of the 48 patients, 32 did not have manifestations of HLH, and in this group 5 died, thereby giving a survival of 84.4% with a mean follow-up in this group of 11.6 years. For those untransplanted patients who survive, 70% received replacement immunoglobulin therapy, with few suffering from long-term complications. Only 5 patients have recorded complications, including 1 with recurrent infection, 1 with neutropenia, 1 with bronchiectasis, and 2 boys with gastrointestinal disease and growth delay.

Supplemental Table 2 compares the demographics between transplanted and untransplanted patients. No significant differences were seen between the 2 populations other than mortality, which was twice as high in the untransplanted cohort ($P < .05$). Age of death was lower in transplanted patients and may reflect the more severe course that may have led to the need for HSCT.

Discussion

This report summarizes data on 91 patients from 64 families worldwide with a genetic diagnosis of XLP1 and provides information on outcome with and without allogeneic HSCT using current treatment protocols (summarized in Figure 3). This report is the first large-scale analysis of XLP1 patients since the report by the XLP1 registry in 1995 and has for the first time gathered patients who have confirmed SAP/*SH2D1A* mutations. Therefore this report represents a genetically homogeneous cohort and avoids possible phenotypic variability through inclusion of other patients with genetic defects such as *XIAP/BIRC* 4 mutations.

The clinical features of the disease are similar to those reported by the XLP1 Registry, with HLH and FIM remaining the most common and most lethal complication. With the advent of more accessible genetic screening and mutation analysis confirming the diagnosis, more patients have been diagnosed early on the basis of family history and increased awareness of the disease has also led to patients being diagnosed after presentation with immune dysregulation and more unusual presenting features such as vasculitis.

A diagnosis of XLP1 is still a difficult one to make, and it is possible that some patients mistakenly fall under the umbrella of common variable immunodeficiency, although previous genetic screening studies suggest that the incidence of XLP1 patients in common variable immunodeficiency cohorts is low.[33] It is also possible that there are older individuals who present in adulthood and have not been identified and included in this study, and this may result in a bias in the method of data collection as the majority of centers approached to contribute data were specialist pediatric centers. For example, a recent case report describes a 41-year-old man who presented with an EBV-induced central nervous system B-cell lymphoma and absent B cells.[34] The oldest surviving patient from this cohort presented at the age of 7 years with recurrent infections and hypogammaglobulinemia, but remains well without transplant and is receiving replacement immunoglobulin therapy at 46 years of age.

The prognosis for XLP1 has greatly improved since 1995, when Seemayer et al[2] reported an overall survival of 25% survival with 71.4% of patients in this cohort alive at the time of data analysis. Indeed, the mortality in untransplanted patients was lower than we expected, with 62.5% surviving, including 3 boys who presented with HLH, but the mortality in this group secondary to HLH remains high at 81.3%. It is also interesting to note that a considerable mortality of 28.6% is seen in EBV-negative patients who do not receive HSCT and is related to HLH, sepsis, and vasculitis, suggesting that underlying immunological abnormalities in XLP1, and not only EBV-driven disease, can be fatal. Few complications from recurrent infection and immune dysregulation were reported, suggesting that early diagnosis and good supportive care with replacement immunoglobulin and prophylactic antibiotics can improve the outcome for untransplanted patients. Although over 60% of patients survive without HSCT, it will be important to follow patients carefully, since there is the potential for more severe manifestations to arise, and the options for transplant should be explored.

The mortality associated with the different clinical phenotypes has changed over time, with an improved survival for both HLH (34.5% vs 4%) and lymphoma (91% vs 35%).[2] This most likely reflects improved treatment strategies for both HLH (especially the use of agreed protocols such as HLH 94[35] and 2004[36]) and malignancy. Although these figures represent survival with either HLH or lymphoma as features of XLP1 at any stage, they are very similar to the survival seen if patients present with these features (44.5% and 92% for HLH/FIM and lymphoma, respectively). A mortality of 13% in patients who exhibit dysgammaglobulinemia is associated with HLH, infection, vasculitis, and hemorrhage and highlights that although clinically this phenotype may be milder, it is not an innocuous phenotype, and progression to further fatal symptoms is not uncommon.

The outcome data following allogeneic HSCT from this report is encouraging. The outcome data presented is the largest ever

gathered and shows that approximately 80% of patients survive the procedure with complete cellular and humoral reconstitution in the large majority of cases. In this series, there is little evidence of problematic EBV reactivation adversely affecting transplant outcome and no increased incidence of long-term complicating features such as autoimmunity in comparison to transplant for other conditions.[37,38] Although donor chimerism in the majority of patients was complete, even low level chimerism in 2 patients with 5% and 20% donor chimerism was associated with good immune recovery. Conversely however, when the patients who required ongoing immunoglobulin support are analyzed, all but 1 have 100% donor engraftment. Further detailed lineage-specific analysis and study of T- and B-cell function in these patients is necessary to determine why humoral function has not been established. The availability of a fully matched donor is associated with an improved survival outcome (approximately 92%), although with the present low numbers this is not statistically significant. Haploidentical grafts show a good outcome in this cohort, but the numbers are extremely low (only 4 transplants performed), and therefore this information needs to be interpreted with caution.

The most important factor affecting survival after transplant is a manifestation of HLH, which significantly reduces survival to 50%. Indeed all 8 patients who died had a complication of HLH at some point in their clinical course. This may reflect the effects of HLH itself or HLH chemotherapy and immunosuppression on the transplant process, including increased organ related toxicity and increased susceptibility to pathogens. In comparison to data reported on cohorts of patients undergoing transplant for HLH associated with other gene defects (eg, perforin and munc 13-4)[39-41] it appears that the outcome for HLH associated with XLP1 is worse and may relate to the multiple immune deficits associated with SAP deficiency. By contrast all XLP1 patients who had no HLH manifestations (n = 27) survived the HSCT procedure.

These data may now allow more informed recommendations to be made regarding transplantation in XLP1. It is clear from this report that HLH in XLP1 has a very poor prognosis if left untransplanted. Therefore any individual with HLH as a manifestation of XLP1 should undergo allogeneic HSCT.

For patients who are newly diagnosed because of a family history but with no clinical features or for those who present with manifestations other than HLH/FIM, the decision to transplant a relatively well child has been more challenging. An important observation from this report is that all patients (n = 27) who went into transplant without prior HLH survived the procedure in comparison to 84.4% survival for those who are untransplanted and have not manifested with HLH. Since progression to HLH without transplant may occur at a later stage, there is a strong argument to transplant all individuals with a diagnosis of XLP1.

However, there is a counter argument to such a recommendation. As with other immunodeficiencies, the data collected and presented here may not give a complete picture of the natural course of XLP1 and is a historical cohort study conducted before the advent of recent improved therapies. Further, milder patients may also remain undiagnosed having been labeled with a diagnosis of common variable immunodeficiency. It is also the case that HLH is most often seen in younger patients (median age of presentation 3.2 years) and older individuals are less likely to manifest with HLH. There may also be reluctance on the part of families and physicians to undertake a transplant in a well child given that, even in the best-case scenario, there will be a certain mortality associated with any allogeneic transplant procedure.

A more pragmatic recommendation would be to undertake transplant in all patients presenting or manifesting with HLH. Similarly for newly diagnosed or young children without any HLH, if a well-matched donor is available, HSCT should be undertaken, since a manifestation of HLH may be catastrophic or may severely compromise transplant outcome. For older individuals, we would still recommend that HSCT be undertaken, but this decision to transplant should be based on available donor status, wellbeing of the patient, and the attitude of family and physician to the risk of transplant. If HSCT is not undertaken immediately, it is recommended that a donor source is identified and that all patients are followed very carefully in case of disease progression and onset of other manifestations, at which point HSCT could be performed rapidly.

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Authorship

Contribution: C.B. designed the research, collected and acquired data, analyzed the data, and wrote the manuscript; H.B.G. assumed overall responsibility for the research, oversaw analysis, and revised the manuscript; and all authors contributed clinical data and reviewed the manuscript before submission.

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For a complete list of Inborn Errors Working Party participants, please see the supplemental Appendix.

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References

1. Purtilo DT, Cassel CK, Yang JP, Harper R. X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). *Lancet.* 1975;1(7913):935-940.
2. Seemayer TA, Gross TG, Egeler RM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. *Pediatr Res.* 1995;38(4):471-478.
3. Purtilo DT, Grierson HL. Methods of detection of new families with X-linked lymphoproliferative disease. *Cancer Genet Cytogenet.* 1991;51(2):143-153.
4. Sumegi J, Huang D, Lanyi A, et al. Correlation of mutations of the SH2D1A gene and Epstein-Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. *Blood.* 2000;96(9):3118-3125.
5. Rougemont AL, Fournet JC, Martin SR, et al. Chronic active gastritis in X-linked lymphoproliferative disease. *Am J Surg Pathol.* 2008;32(2):323-328.
6. Talaat KR, Rothman JA, Cohen JI, et al. Lymphocytic vasculitis involving the central nervous system occurs in patients with X-linked lymphoproliferative disease in the absence of Epstein-Barr virus infection. *Pediatr Blood Cancer.* 2009;53(6):1120-1123.
7. Loeffel S, Chang CH, Heyn R, et al. Necrotizing lymphoid vasculitis in X-linked lymphoproliferative syndrome. *Arch Pathol Lab Med.* 1985;109(6):546-550.
8. Kanegane H, Ito Y, Ohshima K, et al. X-linked lymphoproliferative syndrome presenting with systemic lymphocytic vasculitis. *Am J Hematol.* 2005;78(2):130-133.

9. Sayos J, Wu C, Morra M, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature.* 1998;395(6701):462-469.
10. Coffey AJ, Brooksbank RA, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet.* 1998; 20(2):129-135.
11. Nichols KE, Harkin DP, Levitz S, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A.* 1998;95(23):13765-13770.
12. Okano M, Gross TG. Epstein-Barr virus-associated hemophagocytic syndrome and fatal infectious mononucleosis. *Am J Hematol.* 1996; 53(2):111-115.
13. Dupre L, Andolfi G, Tangye SG, et al. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood.* 2005;105(11):4383-4389.
14. Sharifi R, Sinclair JC, Gilmour KC, et al. SAP mediates specific cytotoxic T-cell functions in X-linked lymphoproliferative disease. *Blood.* 2004;103(10):3821-3827.
15. Benoit L, Wang X, Pabst HF, Dutz J, Tan R. Defective NK cell activation in X-linked lymphoproliferative disease. *J Immunol.* 2000;165(7): 3549-3553.
16. Nakajima H, Cella M, Bouchon A, et al. Patients with X-linked lymphoproliferative disease have a defect in 2B4 receptor-mediated NK cell cytotoxicity. *Eur J Immunol.* 2000;30(11):3309-3318.
17. Tangye SG, Phillips JH, Lanier LL, Nichols KE. Functional requirement for SAP in 2B4-mediated activation of human natural killer cells as revealed by the X-linked lymphoproliferative syndrome. *J Immunol.* 2000;165(6):2932-2936.
18. Bottino C, Augugliaro R, Castriconi R, et al. Analysis of the molecular mechanism involved in 2B4-mediated NK cell activation: evidence that human 2B4 is physically and functionally associated with the linker for activation of T cells. *Eur J Immunol.* 2000;30(12):3718-3722.
19. Nichols KE, Hom J, Gong SY, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med.* 2005;11(3):340-345.
20. Chung B, Aoukaty A, Dutz J, Terhorst C, Tan R. Signaling lymphocytic activation molecule-associated protein controls NKT cell functions. *J Immunol.* 2005;174(6):3153-3157.
21. Ma CS, Pittaluga S, Avery DT, et al. Selective generation of functional somatically mutated IgM+CD27+, but not Ig isotype-switched, memory B cells in X-linked lymphoproliferative disease. *J Clin Invest.* 2006;116(2):322-333.
22. Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. *Immunol Rev.* 2005;203:180-199.
23. Ma CS, Hare NJ, Nichols KE, et al. Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4+ T cells. *J Clin Invest.* 2005; 115(4):1049-1059.
24. Gilmour KC, Cranston T, Jones A, et al. Diagnosis of X-linked lymphoproliferative disease by analysis of SLAM-associated protein expression. *Eur J Immunol.* 2000;30(6):1691-1697.
25. Okano M, Pirruccello SJ, Grierson HL, et al. Immunovirological studies of fatal infectious mononucleosis in a patient with X-linked lymphoproliferative syndrome treated with intravenous immunoglobulin and interferon-α. *Clin Immunol Immunopathol.* 1990;54(3):410-418.
26. Trottestam H, Beutel K, Meeths M, et al. Treatment of the X-linked lymphoproliferative, Griscelli and Chediak-Higashi syndromes by HLH directed therapy. *Pediatr Blood Cancer.* 2009;52(2):268-272.
27. Lankester AC, Visser LF, Hartwig NG, et al. Allogeneic stem cell transplantation in X-linked lymphoproliferative disease: two cases in one family and review of the literature. *Bone Marrow Transplant.* 2005;36(2):99-105.
28. Strahm B, Rittweiler K, Duffner U, et al. Recurrent B-cell non-Hodgkin's lymphoma in two brothers with X-linked lymphoproliferative disease without evidence for Epstein-Barr virus infection. *Br J Haematol.* 2000;108(2):377-382.
29. Arkwright PD, Makin G, Will AM, et al. X linked lymphoproliferative disease in a United Kingdom family. *Arch Dis Child.* 1998;79(1):52-55.
30. Amrolia P, Gaspar HB, Hassan A, et al. Non-myeloablative stem cell transplantation for congenital immunodeficiencies. *Blood.* 2000;96(4): 1239-1246.
31. Hugle B, Suchowerskyj P, Hellebrand H, et al. Persistent hypogammaglobulinemia following mononucleosis in boys is highly suggestive of X-linked lymphoproliferative disease - report of three cases. *J. Clin Immunol.* 2004;24(5):515-522.
32. Nistala K, Gilmour KC, Cranston T, et al. X-linked lymphoproliferative disease: three atypical cases. *Clin Exp Immunol.* 2001;126(1):126-130.
33. Eastwood D, Gilmour KC, Nistala K, et al. Prevalence of SAP gene defects in male patients diagnosed with common variable immunodeficiency. *Clin Exp Immunol.* 2004;137(3):584-588.
34. Hervier B, Latour S, Loussouarn D, et al. An atypical case of X-linked lymphoproliferative disease revealed as a late cerebral lymphoma. *J Neuroimmunol.* 2009;218(1-2):125-128.
35. Henter JI, Arico M, Egeler RM, et al. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. HLH study Group of the Histiocyte Society. *Med Pediatr Oncol.* 1997;28(5):342-347.
36. Henter JI, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2007;48(2):124-131.
37. O'Brien TA, Eastlund T, Peters C, et al. Autoimmune haemolytic anaemia complicating haematopoietic cell transplantation in paediatric patients: high incidence and significant mortality in unrelated donor transplants for non-malignant diseases. *Br J Haematol.* 2004;127(1):67-75.
38. Sonici E, Bennato B, Bertoni E, et al. Autoimmunity after BMT in primary immunodeficiency diseases: single centre report of 184 children [abstract]. 36th Annual Meeting of the European Group for Blood and Marrow Transplantation; 2010.
39. Cooper N, Rao K, Gilmour K, et al. Stem cell transplantation with reduced-intensity conditioning for hemophagocytic lymphohistiocytosis. *Blood.* 2006;107(3):1233-1236.
40. Yoon HS, Im HJ, Moon HN, et al. The outcome of hematopoietic stem cell transplantation in Korean children with hemophagocytic lymphohistiocytosis. *Pediatr Transplant.* 2010;20(2):438-45.
41. Ohga S, Kudo K, Ishii E, et al. Hematopoietic stem cell transplantation for familial hemophagocytic lymphohistiocytosis and Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis in Japan. *Pediatr Blood Cancer.* 2010;54(2):299-306.

Brief report

CBL mutation in chronic myelomonocytic leukemia secondary to familial platelet disorder with propensity to develop acute myeloid leukemia (FPD/AML)

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Familial platelet disorder with a propensity to develop acute myeloid leukemia (FPD/AML) is a rare autosomal dominant disease characterized by thrombocytopenia, abnormal platelet function, and a propensity to develop myelodysplastic syndrome (MDS) and AML. So far, > 20 affected families have been reported. Recently, a second *RUNX1* alteration has been reported; however, no additional molecular abnormalities have been found so far. We identified an acquired *CBL* mutation and 11q-acquired uniparental disomy (11q-aUPD) in a patient with chronic myelomonocytic leukemia (CMML) secondary to FPD with *RUNX1* mutation but not in the same patient during refractory cytopenia. This finding suggests that alterations of the *CBL* gene and *RUNX1* gene may cooperate in the pathogenesis of CMML in patients with FPD/AML. The presence of *CBL* mutations and 11q-aUPD was an important "second hit" that could be an indicator of leukemic transformation of MDS or AML in patients with FPD/AML. (*Blood*. 2012; 119(11):2612-2614)

Introduction

Familial platelet disorder with a propensity to develop acute myeloid leukemia (FPD/AML) is a rare autosomal dominant disease characterized by thrombocytopenia, abnormal platelet function, and a propensity to develop myelodysplastic syndrome (MDS) and AML.[1,2] Since Song et al reported haploinsufficiency of the *RUNX1/CBFA2* gene,[3] more than 20 affected families have been reported.[4-8] Notably, various types of mono-allelic mutations of the *RUNX1* gene have been found in patients with AML secondary to FPD.[3,7-9] *RUNX1*, which is a key regulator of definitive hematopoiesis and myeloid differentiation, is also commonly involved in sporadic cases of MDS and AML, by translocations in AML[10] and by point mutations in AML[11,12] and MDS.[13] Recently, a second *RUNX1* alteration has been reported[8]; however, no additional molecular abnormalities have been found so far.

In this regard, recent reports of somatic mutations of the *CBL* proto-oncogene in myeloid neoplasms are intriguing because these *CBL* mutations have been shown to result in aberrant tyrosine kinase signaling, which would also lead to the activation of RAS signaling pathways. So far, we and others have reported that *CBL* mutations occurred in a variety of myeloid neoplasms, including de novo AML,[14,15] MDS,[16,17] and myeloproliferative neoplasm,[16,17] especially in chronic myelomonocytic leukemia (CMML)[16,17] and juvenile myelomonocytic leukemia.[18] The importance of *CBL* mutations for leukemogenesis has substantially increased, which prompted us to search for possible *CBL* mutations in this pedigree.

Here, we reported that *CBL* mutation developed at the time of diagnosis of CMML, but not during refractory cytopenia, in a Japanese patient with FPD/AML harboring a *RUNX1* mutation.

Methods

RUNX1 mutation analysis

DNA and RNA were extracted from peripheral blood (PB) of the proband, her sister, and their mother after obtaining informed consent. We performed mutation analysis of the *RUNX1* gene by PCR followed by direct sequencing with the use of an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). For further confirmation of deletion mutations, the PCR products were subcloned with the use of a TOPO TA Cloning Kit (Invitrogen) and then sequenced. Mutations were screened from exons 1-8 of the *RUNX1* gene.

CBL mutation analysis

Because *CBL* mutations thus far reported almost exclusively involved exons 8-9 that encode Linker/RING finger domains, we confined our mutation analysis to these exons, which were subjected to direct sequencing. Because the frequency of 11q-acquired uniparental disomy (11q-aUPD) was reported as ~ 85%-90% in *CBL* mutations, we also analyzed the sample with Affymetrix GeneChip 250K *Nsp*I.[17-19] Genome-wide detection of copy number abnormalities or allelic imbalances was performed with CNAG/AsCNAR Version 3.0 software (http://www.genome.umin.jp), which enabled sensitive detection of copy number neutral loss of heterozygosity (or aUPD).[19] In addition, we examined mutations of the following genes in the proband as previously reported: *FLT3, KIT, RAS, JAK2, PTPN11, ASXL1, IDH1/2,* and *MPL*.[20-22] The study adhered to the principles of the Helsinki Declaration and was conducted under the regulations enacted by the Ethics Board of Gunma Children's Medical Center.

Results and discussion

The proband (III-2), who was the second child of nonconsanguineous parents, underwent an 8-year follow-up of mild to moderate

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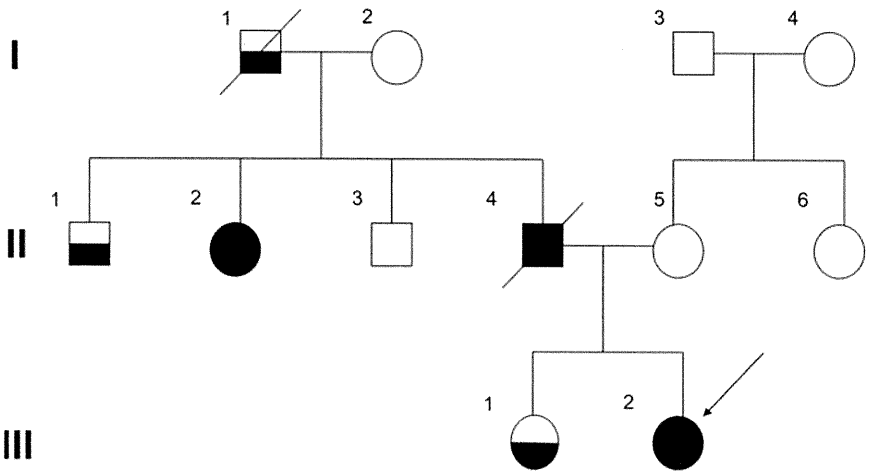

Figure 1. The family pedigree. Squares indicate males and circles indicate females. Open symbols represent unaffected persons, half-filled symbols represent persons affected by thrombocytopenia, and closed symbols represent persons affected by FPD who developed MDS/AML. The proband (III-2) is indicated by an arrow.

thrombocytopenia (50-80 × $10^3/\mu L$), and at that age of 10 years, her condition was diagnosed as refractory cytopenia. Cytogenetic analysis found a normal karyotype, and FISH showed neither monosomy 7 nor trisomy 8. The proband had been closely observed without any therapy for 2 years and 9 months because she did not require transfusion and her disease remained stable; however, at the age of 12 years, leukocytosis and monocytosis developed and she became dependent on platelet transfusions. Finally, the disease evolved to CMML, and allogeneic bone marrow (BM) transplantation from an unrelated donor was performed. During the entire course, the number of blast cells in PB was constantly < 2%, and no additional symptoms were observed, such as hepatosplenomegaly. Her elder sister (III-1) was also followed for 10 years with mild thrombocytopenia; however, the morphologic findings of PB or BM were not compatible with myeloproliferative neoplasms.[17] Because her platelet count has been gradually decreasing, allogeneic BM transplantation is being considered. Although her father (II-4) developed MDS at the age of 41 and died 2 years later, her paternal aunt (II-2) developed MDS at the age of 49 and has remained in complete remission for 11 years after successful allogeneic cord blood transplantation. Her paternal grandfather (I-1) and uncle (II-1) also had a history of thrombocytopenia (Figure 1). Direct sequencing analysis of *RUNX1* found a one-base deletion of adenine at position 2364 within exon 7, resulting in a frameshift mutation that corresponded to AML1b transcript in the proband and her sister (Figure 2A). This resulted in a frameshift after amino acid change G262GfsX21. This mutation was not detected in their mother. All these data suggested that her paternal grandfather (I-1), uncle (II-1), aunt (II-2), and her father (II-4) were considered to have FPD/AML, carrying the same *RUNX1* mutation.

Although no *CBL* mutations were found in the proband sample of refractory cytopenia before development of CMML, homozygous mutation of the *CBL*, which was located in the splice acceptor site of intron 8 (Figure 2B), was identified in the proband sample in the CMML. We also found 11q-aUPD (Figure 2C) in the proband sample, confirming a strong association of *CBL* mutations with 11q-aUPD, as previously described[16-18]; however, no mutations of any other genes, including *FLT3*, *KIT*, *RAS*, *JAK2*, *PTPN11*, *ASXL1*, *IDH1/2,* and *MPL*, were found and no additional somatic *RUNX1* alterations. No *CBL* mutations were found in her sister's sample at this time.

Inherited *RUNX1* mutations were clustered in the N-terminal region in exons 3-5, which affect the runt homology domain. Mutations in the C-terminal region, detected in the present pedigree, have been reported less frequently so far and are considered to affect the transactivation domain (Figure 2D).

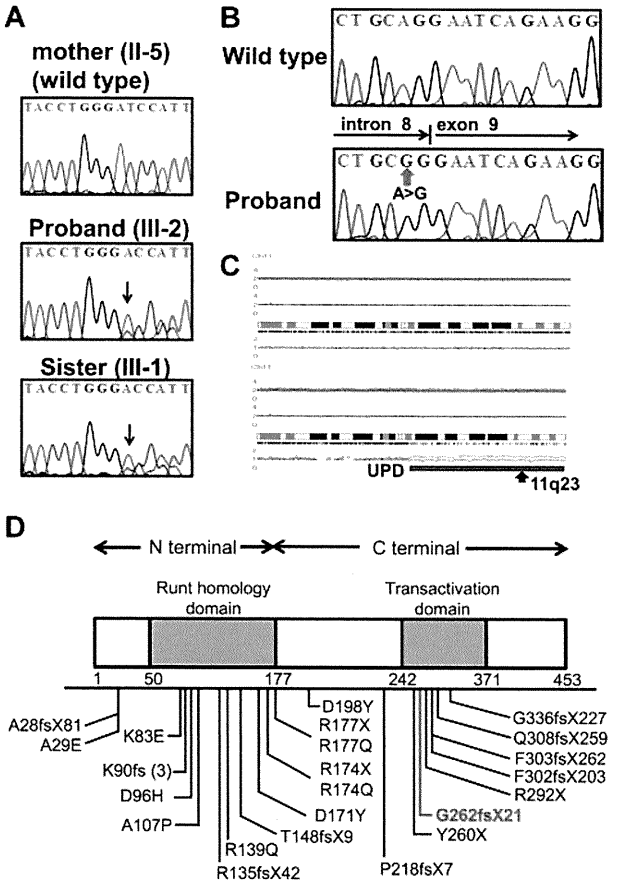

Figure 2. Mutation analysis of *RUNX1* and *CBL* genes in the pedigree. (A) Direct sequencing analysis of affected patients (III-1, III-2) and an unaffected family member (II-5) is shown. Arrow indicates a one-base deletion of adenine. (B) Mutated *CBL* is shown in the proband. (C) Identification of acquired uniparental disomy of 11q in the proband. Total copy number (tCN; red plot) is shown above the cytoband, and the results of allele-specific copy number analysis with anonymous references (AsCNAR) plots are shown below the cytoband. Larger allele is presented by a red line, and the smaller allele is presented by a blue line. Allele-specific analysis showed 11q-aUPD (blue line), which contained the *CBL* region (arrow). (D) Schematic representation of wild-type and mutated *RUNX1*. The affected *RUNX1* is truncated at the C terminus of the transactivation domain (TAD). Part of TAD is lacking in this proband (red line).

It has been postulated that disruption of the *RUNX1* gene is not sufficient to cause AML, as previously reported with monoallelic and biallelic inactivation of *Runx1* in mice[23,24] and in mice carrying the knocked-in *Runx1-Eto* chimeric gene. These data indicate that a second-hit mutation in addition to the dysfunction of *RUNX1* is required for the development of AML. Minelli et al postulated that the mutations seen in FPD cases have a mutation effect that induces additional genetic abnormalities and promotes progression to hematologic malignancies.[25]

Marked associations between chromosome translocation and gene mutations have been reported: *KIT* mutation in core binding leukemia, t(8;21)/*AML1-ETO* and inv(16)(p13q22)/*CBFβ-MYH11*, *FLT3*-ITD in leukemia with t(15;17)/*PML-RARα*, or with t(6;9)/*DEK-CAN*. We consider that it is important to find an association to administer clinically relevant treatment. In addition to the germline *RUNX1* mutation, we identified an acquired *CBL* mutation in the proband and assumed it to be a second hit mutation by which FPD evolved into CMML. To our knowledge, this is the first patient with FPD/AML in whom *CBL* mutation has developed. This finding suggests that alterations of the *CBL* gene and *RUNX1* could cooperate in the pathogenesis of CMML or AML in patients with FPD/AML. The presence of 11q-aUPD provided evidence that loss of the wild-type copy of *CBL* with duplication of the mutant copy was an important second hit that could be an indicator of leukemic transformation in patients with FPD/AML.

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Authorship

Contribution: Y.H. and C.O. designed the study; A.M., C.O., and D.H. provided critical reagents and samples; N.S., M.P., A.S.-O., and C.M. performed the experiments; H.A. and S.O. supervised the work; N.S. and M.P. analyzed the results; N.S. and D.H. constructed the figures; N.S. and Y.H. wrote the paper; and all the authors critically reviewed and revised the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

1. Dowton SB, Beardsley D, Jamison D, Blattner S, Li FP. Studies of a familial platelet disorder. *Blood.* 1985;65(3):557-563.
2. Ho CY, Otterud B, Legare RD, et al. Linkage of a familial platelet disorder with a propensity to develop myeloid malignancies to human chromosome 21q22.1-22.2. *Blood.* 1996;87(12):5218-5224.
3. Song WJ, Sullivan MG, Legare RD, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet.* 1999;23(2):166-175.
4. Buijs A, Poddighe P, van Wijk R, et al. A novel CBFA2 single-nucleotide mutation in familial platelet disorder with propensity to develop myeloid malignancies. *Blood.* 2001;98(9):2856-2858.
5. Michaud J, Wu F, Osato M, et al. In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. *Blood.* 2002;99(4):1364-1372.
6. Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood.* 2008;112(12):4639-4645.
7. Kirito K, Sakoe K, Shinoda D, Takiyama Y, Kaushansky K, Komatsu N. A novel RUNX1 mutation in familial platelet disorder with propensity to develop myeloid malignancies. *Haematologica.* 2008;93(1):155-156.
8. Preudhomme C, Renneville A, Bourdon V, et al. High frequency of RUNX1 biallelic alteration in acute myeloid leukemia secondary to familial platelet disorder. *Blood.* 2009;113(22):5583-5587.
9. Heller PG, Glembotsky AC, Gandhi MJ, et al. Low Mpl receptor expression in a pedigree with familial platelet disorder with predisposition to acute myelogenous leukemia and a novel AML1 mutation. *Blood.* 2005;105(12):4664-4670.
10. Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, Ohki M. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. *Proc Natl Acad Sci U S A.* 1991;88(23):10431-10434.
11. Osato M, Asou N, Abdalla E, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. *Blood.* 1999;93(6):1817-1824.
12. Taketani T, Taki T, Takita J, et al. AML1/RUNX1 mutations are infrequent, but related to AML-M0, acquired trisomy 21, and leukemic transformation in pediatric hematologic malignancies. *Genes Chromosomes Cancer.* 2003;38(1):1-7.
13. Harada H, Harada Y, Niimi H, et al. High incidence of somatic mutations in the AML1/RUNX1 gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. *Blood.* 2004;103(6):2316-2324.
14. Sargin B, Choudhary C, Crosetto N, et al. Flt3-dependent transformation by inactivating c-Cbl mutations in AML. *Blood.* 2007;110(3):1004-1012.
15. Caligiuri MA, Briesewitz R, Yu J, et al. Novel c-CBL and CBL-b ubiquitin ligase mutations in human acute myeloid leukemia. *Blood.* 2007;110(3):1022-1024.
16. Dunbar AJ, Gondek LP, O'Keefe CL, et al. 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. *Cancer Res.* 2008;68(24):10349-10357.
17. Sanada M, Suzuki T, Shih LY, et al. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature.* 2009;460(7257):904-908.
18. Shiba N, Kato M, Park MJ, et al. CBL mutations in juvenile myelomonocytic leukemia and pediatric myelodysplastic syndrome. *Leukemia.* 2010;24(5):1090-1092.
19. Nannya Y, Sanada M, Nakazaki K, et al. A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res.* 2005;65(14):6071-6079.
20. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood.* 2006;108(10):3472-3476.
21. Rocquain J, Carbuccia N, Trouplin V, et al. Combined mutations of ASXL1, CBL, FLT3, IDH1, IDH2, JAK2, KRAS, NPM1, NRAS, RUNX1, TET2 and WT1 genes in myelodysplastic syndromes and acute myeloid leukemias. *BMC Cancer.* 2010;10:401.
22. Tartaglia M, Niemeyer CM, Fragale A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. *Nat Genet.* 2003;34(2):148-150.
23. Sun W, Downing JR. Haploinsufficiency of AML1 results in a decrease in the number of LTR-HSCs while simultaneously inducing an increase in more mature progenitors. *Blood.* 2004;104(12):3565-3572.
24. Ichikawa M, Asai T, Saito T, et al. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. *Nat Med.* 2004;10(3):299-304.
25. Minelli A, Maserati E, Rossi G, et al. Familial platelet disorder with propensity to acute myelogenous leukemia: genetic heterogeneity and progression to leukemia via acquisition of clonal chromosome anomalies. *Genes Chromosomes Cancer.* 2004;40(3):165-171.

CBL mutations in infant acute lymphoblastic leukaemia

Infant acute lymphoblastic leukaemia (ALL) is relatively rare, occurring in approximately 2·5–5% of cases of childhood ALL (Biondi *et al*, 2000). Infant ALLs are much more likely to present with high leucocyte counts, hepatosplenomegaly and overt central nervous system (CNS) diseases (Taki *et al*, 1996). T cell phenotype is much less common in infants, while myeloid antigen co-expression and the absence of CD10 expression are more frequent in infants than in older children with ALL. When molecular techniques [such as fluorescence *in situ* hybridization (FISH) or Southern blot analysis] are used in addition to karyotype, *MLL* gene rearrangements (*MLL*-R) are found in 70–80% of infant ALL compared with only 2–4% of older children with ALL (Taki *et al*, 1996; Biondi *et al*, 2000). Thus, infant ALL appears to be biologically distinct from the disease in older children (more than 1 year old).

In this regard, recent reports of somatic mutations of the *CBL* proto-oncogene in myeloid neoplasms are intriguing, because these *CBL* mutations were shown to result in aberrant tyrosine kinase signalling, which also leads to activation of RAS signalling pathways. So far, we and others have reported that *CBL* mutations occur in a variety of myeloid neoplasms, including *de novo* acute myeloid leukaemia (AML) (Caligiuri *et al*, 2007), myelodysplastic syndrome (MDS), and myeloproliferative neoplasm, especially in chronic myelomonocytic leukaemia (CMML) (Sanada *et al*, 2009), and juvenile myelomonocytic leukaemia (JMML) (Shiba *et al*, 2010). The importance of *CBL* mutations regarding leukaemogenesis is substantially increased. Recently, we found *CBL* mutation in therapy-related AML with *MLL*-R (Shiba *et al*, 2011). Interestingly, the *MLL-CBL* fusion gene has been reported in a *de novo* AML case (Fu *et al*, 2003), and this prompted us to search for possible *CBL* mutations in infant ALL with *MLL-R*.

Because *CBL* mutations thus far reported were almost all clustered within exons 8–9 that encode Linker/RING finger domains (Caligiuri *et al*, 2007; Sanada *et al*, 2009; Shiba *et al*, 2010), we confined our mutation analysis to these exons, in which polymerase chain reaction-amplified exons 8–9 were subjected to direct sequencing using an ABI PRISM 310 Genetic Analyser (Applied Biosystems, Branchburg, NJ, USA). The study adhered to the principles of the Helsinki Declaration, and was conducted under the regulations enacted by the Ethics Board of Gunma Children's Medical Centre.

CBL gene analysis was performed in 41 infant ALL patients in which *MLL*-R was found in 33 patients (80·5%), including 15 patients with t(4;11)(q21;q23), 4 with t(9;11)(p22;q23) and 5 with t(11;19)(q23;p13.3). Median age at diagnosis was 4·7 months (range, 0–12 months). We also performed *CBL* gene mutation analysis in 28 B cell precursor (BCP)-ALL patients (age range, 1–14 years).

Heterozygous mutations of the *CBL* gene were identified in 2 (4·9%) of 41 infant ALL patients, but not in older children with BCP-ALL. These were located in exon 8 (Fig 1). One patient was a 3-month-old female with t(4;11)(q21;q23) and the other patient was a 6-month-old male with t(11;19)(q23;p13.3). They were registered and treated on two Japanese infant leukaemia protocols, MLL96 and MLL98 respectively (Isoyama *et al*, 2002; Kosaka *et al*, 2004). Although strong association between *CBL* mutations and 11q-acquired uniparental disomy (aUPD) has been reported (Sanada *et al*, 2009), we did not perform the single nucleotide polymorphism array analysis due to lack of DNA.

MLL-R are more frequent in younger infants; up to 90% of infant ALL less than 6 months old at diagnosis have detectable *MLL*-R compared with 30–50% of infant ALL aged 6–12 months (Taki *et al*, 1996). *MLL*-R ALL has a characteristic gene expression profile that significantly differs from that of non-*MLL*-R BCP-ALL and of AML, confirming that *MLL*-R ALL is a biologically unique leukaemia subtype. Thus, the distinctive presenting features and clinical behaviour of infant

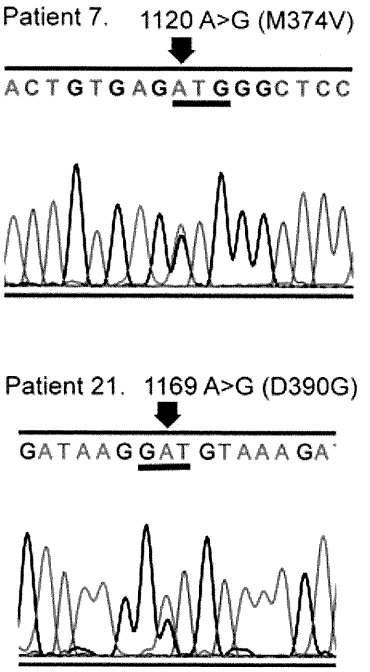

Fig 1. Identification of *CBL* mutations. Heterozygous mutations of the *CBL* gene were identified in Patients 7 and 21.

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ALL appear to be primarily due to the high frequency of *MLL*-R in this age group. However, outcome data comparing infant and non-infant patients with *MLL*-R suggest that there may be other factors which impact the prognosis of infant ALL. Both of the patients with *CBL* mutations were diagnosed before 6 months of age. In our previous report, all of three cases with *CBL* mutation developed JMML before 4 months of age (Shiba *et al*, 2010). These data suggested that *CBL* mutation may have a strong association with very early onset disease. *CBL* mutations have been reported as germline mutations in JMML (Niemeyer *et al*, 2010). Unfortunately, we could not investigate whether the mutations in our cases were germline mutations or not, because somatic cells were not available.

CBL mutations have been found in approximately 5% of 2000 samples from patients with myeloid neoplasms, including AML transformed from MDS. Gene aberrations in addition to *MLL*-R have rarely been reported in infant ALL. No reports of ALL with *CBL* mutations have so far been reported, suggesting that the pathogenesis of infant ALL is different from paediatric or adult ALL. To our knowledge, this is the first report of infant ALL patients with 11q23 translocation/*MLL*-R and *CBL* mutations. The present study suggests that alterations of *CBL* gene and *MLL*-R may cooperatively play a pathogenic role in the development of infant ALL with *MLL*-R.

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Author's contributions

TT and YH designed the study. JT, MH, TK, MS and EI provided critical reagents and samples. NS and MP performed the experiments. EI, HA and SO supervised the work. NS and MP analysed the results. NS, TT, and YH wrote the paper and all the authors critically reviewed and revised it.

Conflict of interest

The authors declare no conflict of interest.

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References

Biondi, A., Cimino, G., Pieters, R. & Pui, C.H. (2000) Biological and therapeutic aspects of infant leukemia. *Blood*, **96**, 24–33.

Caligiuri, M.A., Briesewitz, R., Yu, J., Wang, L., Wei, M., Arnoczky, K.J., Marburger, T.B., Wen, J., Perrotti, D., Bloomfield, C.D. & Whitman, S.P. (2007) Novel c-CBL and CBL-b ubiquitin ligase mutations in human acute myeloid leukemia. *Blood*, **110**, 1022–1024.

Fu, J.F., Hsu, J.J., Tang, T.C. & Shih, L.Y. (2003) Identification of CBL, a protooncogene at 11q23.3, as a novel MLL fusion partner in a patient with de novo acute myeloid leukemia. *Genes Chromosomes Cancer*, **37**, 214–219.

Isoyama, K., Eguchi, M., Hibi, S., Kinukawa, N., Ohkawa, H., Kawasaki, H., Kosaka, Y., Oda, T., Oda, M., Okamura, T., Nishimura, S., Hayashi, Y., Mori, T., Imaizumi, M., Mizutani, S., Tsukimoto, I., Kamada, N. & Ishii, E. (2002) Risk-directed treatment of infant acute lymphoblastic leukaemia based on early assessment of MLL gene status: results of the Japan Infant Leukaemia Study (MLL96). *British Journal of Haematology*, **118**, 999–1010.

Kosaka, Y., Koh, K., Kinukawa, N., Wakazono, Y., Isoyama, K., Oda, T., Hayashi, Y., Ohta, S., Moritake, H., Oda, M., Nagatoshi, Y., Kigasawa, H., Ishida, Y., Ohara, A., Hanada, R., Sako, M., Sato, T., Mizutani, S., Horibe, K. & Ishii, E. (2004) Infant acute lymphoblastic leukemia with MLL gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation. *Blood*, **104**, 3527–3534.

Niemeyer, C.M., Kang, M.W., Shin, D.H., Furlan, I., Erlacher, M., Bunin, N.J., Bunda, S., Finklestein, J.Z., Sakamoto, K.M., Gorr, T.A., Mehta, P., Schmid, I., Kropshofer, G., Corbacioglu, S., Lang, P.J., Klein, C., Schlegel, P.G., Heinzmann, A., Schneider, M., Starý, J., van, den., Heuvel-Eibrink, M.M., Hasle, H., Locatelli, F., Sakai, D., Archambeault, S., Chen, L., Russell, R.C., Sybingco, S.S., Ohh, M., Braun, B.S., Flotho, C. & Loh, M.L. (2010) Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. *Nature Genetics*, **42**, 794–800.

Sanada, M., Suzuki, T., Shih, L.Y., Otsu, M., Kato, M., Yamazaki, S., Tamura, A., Honda, H., Sakata-Yanagimoto, M., Kumano, K., Oda, H., Yamagata, T., Takita, J., Gotoh, N., Nakazaki, K., Kawamata, N., Onodera, M., Nobuyoshi, M., Hayashi, Y., Harada, H., Kurokawa, M., Chiba, S., Mori, H., Ozawa, K., Omine, M., Hirai, H., Nakauchi, H., Koeffler, H.P. & Ogawa, S. (2009) Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature*, **460**, 904–908.

Shiba, N., Kato, M., Park, M.J., Sanada, M., Ito, E., Fukushima, K., Sako, M., Arakawa, H., Ogawa, S. & Hayashi, Y.. (2010) CBL mutations in

