reproduce the severest subtype of ARCI: harlequin ichthyosis.[35,45] In one mouse model (*Abca12*-null mice), epidermal proliferation was not altered at E18.5 compared with wild-type mice.[43] From this finding, it is unlikely that loss of ABCA12 function directly causes proliferation of keratinocytes and leads to SCC development.

No patients with CIE or LI who developed skin malignancies have been reported to have mutations in *TGM1*, although TGM1 is thought to be the most prevalent causative gene for CIE/LI.[46,47] TGM1 encodes tranglutaminase-1, which forms the cornified envelope (CE) in the cornified layer through cross-linking of CE precursor proteins.[47] Increased proliferation in the epidermis of the *Tgm1*-null neonate skin grafted onto athymic nude mice was observed,[48] which might imply that patients with CIE/LI with *TGM1* mutations might be susceptible to skin SCC.

Netherton syndrome

Netherton syndrome (NS) is an autosomal recessive disorder characterized by trichorrhexis invaginata (bamboo hair), congenital ichthyosis and atopic diathesis.[49,50] NS is caused by mutations in *SPINK5*, which encodes the serine protease inhibitor LEKTI.[51]

Three NS cases have been reported who developed skin malignancies (Table 1).[52–54] Surprisingly, multiple SCCs (or multiple BCCs) were observed for these patients in their twenties. In one patient, epidermodysplasia verruciformis-associated human papillomavirus (HPV) DNA (HPV-19, -23, -38 and HPV-RTRX9) was preferentially detected in malignant lesions.[52] The authors speculated that impaired epidermal defence mechanisms could have promoted latent HPV DNA persistence in the patient's skin.[52] However, polymerase chain reaction amplification using HPV universal primers failed to detect HPV DNA in tumour specimens of another patient.[54] This shows that HPV infection is not always responsible for skin carcinogenesis in patients with NS at an early age. Patients with NS show recurrent infections other than HPV.[55] From the findings that several immunological abnormalities including those of memory B cells and natural killer cells are common in NS and that the patients respond well to intravenous immunoglobulin therapy,[55] it is possible to conclude that cognate and innate immunodeficiency might be associated with skin carcinogenesis in NS.

Although other serine protease inhibitors are implicated in skin carcinogenesis,[56,57] the role of LEKTI in skin cancers is unclear. NS mouse models in which LEKTI is deficient have been reported.[58–60] In one model, increased proliferation of the epidermis was observed,[59] which might underlie a susceptibility to SCC.

Miscellaneous

In each other form of ichthyosis, only a few cases have been described as having skin cancers. Ichthyosis Curth–Macklin (ICM) is a very rare form of keratinopathic ichthyosis that is characterized by massive spiky hyperkeratosis.[1,61] Mutations in the V2 domain of keratin 1 have been reported in patients with ICM. Two patients developed multiple SCC at the ages of 54 and 40 years, respectively (Table 1).[62,63] However, one patient had a history of whole-skin X-ray therapy, which might have led to the multiple skin cancers.[63]

Micropinnae, alopecia universalis, congenital ichthyosis and ectropion (MAUIE) syndrome is a syndromic form of ichthyosis that was not included in the revised nomenclature and classification of inherited ichthyoses.[1] Causative genes of MAUIE syndrome have not been reported. Two patients with MAUIE syndrome were found to have developed SCC in their twenties (Table 1).[33,64]

Epidermolytic ichthyosis (EI), formerly called bullous CIE, is a major subtype of keratinopathic ichthyosis[1] that is caused by mutations in the genes encoding keratin 1 or keratin 10 (*KRT1* or *KRT10*, respectively).[65–67] One patient with EI was reported to have multiple SCC/BCC (Table 1), although the patient had a history of whole-skin X-ray therapy.[68]

Congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome is a rare X-linked dominant disorder[69] that is caused by mutations in *NSDHL*.[70] One patient with CHILD syndrome developed SCC in the affected skin.[71]

Ichthyosis vulgaris, the most prevalent type of inherited ichthyosis, is caused by mutations in *FLG*, the gene encoding filaggrin.[72] To our knowledge, there have been no reports on the incidence of skin malignancies in ichthyosis vulgaris. Several cohort studies have reported cancer incidence in patients with atopic dermatitis (AD), in which loss-of-function mutations in *FLG* are a major predisposing factor.[73] Although many studies have confirmed that AD is associated with an increased risk of lymphoma, the estimated risk of nonmelanoma skin cancer (NMSC) in patients with AD differs among studies. Some studies reported an increased risk of NMSC in patients with AD,[74,75] whereas others demonstrated no association between NMSC and AD.[76,77] Further studies are needed to evaluate precisely the cancer risk in patients with ichthyosis vulgaris.

Future directions

Because of the limited number of patients with inherited ichthyoses, it is still almost impossible to calculate accurately the incidence of skin malignancies in these patients. However, our review of the literature shows that patients with ichthyosis can develop skin malignancies, mostly SCC, at an early age, although the literature may be biased in favour of describing only 'interesting' cases.

Generally, impaired barrier function in patients with ichthyosis might permit breech of the stratum corneum by contact chemical carcinogens. However, epithelial desquamation has been suggested as protecting against natural chemicals.[78,79] If this is true, one might guess that more rapid epidermal turnover in ichthyosis skin would be protective against, rather than contributory to, skin carcinogenesis. There are common

types of ichthyosis, such as ichthyosis vulgaris and recessive X-linked ichthyosis, which do not seem to be associated with skin cancer at a young age. On the other hand, patients with KID syndrome, ARCI and NS have been reported to develop SCC at an early age. These differences might be explained by causative genetic defects in each ichthyosis subtype.

Recent developments in bioengineering techniques have resulted in many animal models of inherited ichthyosis.[80] Experiments on ichthyosis skin carcinogenesis, including two-stage carcinogenesis assay, might provide clues to understanding the pathomechanisms underlying skin cancer in inherited ichthyosis, although neonatal lethality will prevent these experiments in several mouse models.

In the future, a worldwide registry on ichthyoses with follow-up information would be desirable towards enabling a full evaluation of skin malignancies in patients with ichthyosis. At present, routine surveillance for skin malignant changes is strongly recommended for patients with KID syndrome and inflammatory types of congenital ichthyosis such as CIE/LI and NS, even if the patients are taking systemic retinoids.

What's already known about this topic?

- There have been sporadic case reports of malignant skin tumours in patients with congenital ichthyosis.
- The frequency of skin malignancies in patients with ichthyosis is unknown.

What does this study add?

- Patients with congenital ichthyosis, especially those with KID syndrome, congenital ichthyosiform erythroderma, lamellar ichthyosis and Netherton syndrome, can develop cutaneous squamous cell carcinoma at unusually young ages.

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Frequent loss of HLA alleles associated with copy number-neutral 6pLOH in acquired aplastic anemia

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Idiopathic aplastic anemia (AA) is a common cause of acquired BM failure. Although autoimmunity to hematopoietic progenitors is thought to be responsible for its pathogenesis, little is known about the molecular basis of this autoimmunity. Here we show that a substantial proportion of AA patients harbor clonal hematopoiesis characterized by the presence of acquired copy number-neutral loss of heterozygosity (CNN-LOH) of the 6p arms (6pLOH). The 6pLOH commonly involved the HLA locus, leading to loss of one HLA haplotype. Loss of HLA-A expression from multiple lineages of leukocytes was confirmed by flow cytometry in all 6pLOH(+) cases. Surprisingly, the missing HLA-alleles in 6pLOH(+) clones were conspicuously biased to particular alleles, including HLA-A*02:01, A*02:06, A*31:01, and B*40:02. A large-scale epidemiologic study on the HLA alleles of patients with various hematologic diseases revealed that the 4 HLA alleles were over-represented in the germline of AA patients. These findings indicate that the 6pLOH(+) hematopoiesis found in AA represents "escapes" hematopoiesis from the autoimmunity, which is mediated by cytotoxic T cells that target the relevant autoantigens presented on hematopoietic progenitors through these class I HLAs. Our results provide a novel insight into the genetic basis of the pathogenesis of AA. (*Blood.* 2011;118(25):6601-6609)

Introduction

Acquired aplastic anemia (AA) is a rare condition associated with BM failure and pancytopenia.[1] A series of classic observations and experiments have unequivocally supported that the autoimmunity to hematopoietic stem/progenitor cells (HSPCs) critically underlies the pathogenesis of the BM failure in the majority of AA cases. According to the widely accepted model of immune-mediated BM failure, activated cytotoxic T cells (CTLs) that recognize an auto-antigen(s) presented on HSPCs through their class I HLA molecules have a major role in initiating the autoimmune reactions.[2-4] However, no definitive evidence exists that supports this model or the presence of such CTL repertoires. Moreover, little information is available about their target antigens or about the way by which they are recognized by effector T cells.

Another long-standing issue on AA is its close relationship with clonal hematopoiesis.[5,6] It was first suspected from an apparent overlap between AA and paroxysmal nocturnal hemoglobinuria (PNH)[7,8] and was also implicated by the frequent development of late clonal disorders in AA, such as myelodysplastic syndromes, PNH, or even acute myeloid leukemia (AML).[9-11] Clonal hematopoiesis can be explicitly demonstrated by conventional clonality assays at presentation in a substantial proportion of newly diagnosed typical AA cases.[12] Although it has been expected that the inciting autoimmune insult somehow confers selective pressures on the evolution of clonal hematopoiesis,[5] the exact mechanism for such immunologic selection or escape is still unclear.

The objectives of this study, therefore, were to characterize the clonal nature of the hematopoiesis that is maintained even under the severe autoimmune insult in AA, and to explore the genetic/immunologic mechanism that could underlie the pathogenesis of AA. To achieve these aims, we performed single nucleotide polymorphism (SNP) array-based analysis of genomic copy numbers and/or allelic imbalances in peripheral blood (PB) specimens obtained from 306 patients with AA. Initially, we found that AA patients frequently showed clonal/oligoclonal hematopoiesis that lost specific HLA alleles as a result of copy number-neutral loss of heterozygosity (CNN-LOH) of the 6p arms, which led us to further analyses of the contribution of 6pLOH(+) clones to residual hematopoiesis and a large-scale epidemiologic study on the HLA alleles that are over-represented in AA, involving a total of 6,613 transplants registered in the Japan Marrow Donor Program (JMDP).

Methods

Subjects

PB specimens from a total of 306 patients with AA were analyzed for the presence of genetic alterations using SNP arrays (see Figure 1). The clinical characteristics of these patients are summarized in Table 1 and supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Among the 306 patients, 107 were newly diagnosed and 199 were previously treated. Ninety-six patients received allogeneic BM transplantation from unrelated donors through the JMDP, and their HLA information was available from the JMDP. The other 210 were newly genotyped for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles as described elsewhere.[13] A total of 103 patients had been treated with anti-thymocyte globulin plus cyclosporine, cyclosporine alone, or anabolic steroids at the time of sampling. All patients and healthy persons provided their informed consent before sampling in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Graduate School of Medical Science, Kanazawa University and also by that of the Graduate School of Medicine, University of Tokyo.

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Table 1. Patient characteristics

	Newly diagnosed (n = 107)	Previously treated (n = 199)
Median age at diagnosis, mo (range)	64 (9-88)	24 (2-80)
Sex, male/female, no.	58/49	110/89
Severity of AA at onset, no. (%) of patients		
Severe	79 (74)	185 (93)
Nonsevere	28 (26)	14 (7)
History, mo, median (range)	19 (0.1-251)	51 (0.1-372)
Past treatment, no. (%) of patients		
ATG + CsA	—	39 (20)
CsA alone	—	51 (26)
Anabolic steroid alone	—	13 (7)
Unknown*	—	96 (48)

ATG indicates antithymocyte globulin; CsA, cyclosporine A; and —, not applicable.

*Information regarding previous therapies of 96 cases (from Japan Marrow Donor Program) was unavailable.

Analysis of genomic copy numbers and detection of 6pLOH

Genomic copy numbers, as well as allele-specific copy numbers, were analyzed by using GeneChip 500K arrays (Affymetrix) as previously described.[14,15] Briefly, genomic DNA from AA patients and normal controls were analyzed on GeneChip 500K arrays separately. After adjusting several biases introduced during experiments, signal ratios of the corresponding probes between test (patient) and controls were calculated across the genome to obtain genome-wide copy numbers. Genetic lesions, including copy number gains and losses, as well as CNN-LOHs, were first detected using a hidden Markov model-based algorithm implemented in the CNAG software.[14,15] Known copy number variations were carefully excluded by referring to the Database of Genomic Variants (www.projects.tcag.ca/variation). CNN-LOH in 6p involving the HLA locus was more specifically and sensitively detected by statistically evaluating the mean differences in allele-specific copy numbers between heterozygous SNPs on 6p (N = ~ 1400) that were telomeric from the 5′-end of the HLA-A locus (rs1655927) and all non-6p heterozygous SNPs (N = ~ 105 000) using the Mann-Whitney U test with the R package (www.r-project.org). Possible false-positive findings arising from multiple testing involving the 306 samples were evaluated by maintaining the false discovery rate under 0.01 as previously described,[16] where the microarray data of 1000 JMDP donor specimens obtained from an ongoing whole genome association study (unpublished data) were used to calculate an empiric null distribution.[17,18]

Determination of the missing HLA alleles in 6pLOH(+) clones in patients with AA

The 500K SNP data of the 1800 JMDP donor-recipient pairs (JMDP dataset), together with their HLA genotyping information, was used to generate an HLA SNP haplotype table on the GeneChip 500K platform, which contains the consensus SNPs of the 3 major haplotypes (P1, P2, and P3) in Japanese subjects[18] and the SNP sequences of all observed HLA haplotypes complementary to P1 to P3 within the JMDP set (N = 1576; data not shown). To determine the missing HLA haplotype in each 6pLOH(+) patient, those "HLA" haplotypes were first selected from the aforementioned HLA haplotype table that were compatible with the observed HLA genotypes of that patient. Among these, a candidate haplotype was selected such that it contained the minimum number of SNPs that were incompatible with the patient's genotype. For each candidate haplotype, genomic copy numbers were inferred at the heterozygous SNPs along that haplotype using the circular binary segmentation algorithm,[19,20] which divided the haplotype into one or more discrete segments with different mean copy numbers. Finally, each copy number segment was thought to be "missing," when the alternative hypothesis (Ha: $S_i \neq \overline{S}_i$, for $^{\forall}i$) was supported against the null hypothesis (H_0: $S_i = \overline{S}_i$, for $^{\forall}i$) using the Wilcoxon signed rank test with a significance level of .05, where S_i represents the allele-specific copy number at the ith heterozygous SNP site within the segment of the candidate haplotype with $\overline{S}_i$ being the corresponding value for the complementary haplotype (supplemental Figure 1). Finally, for those HLA types that appeared more than 8 times among 6pLOH(+) cases, their contribution to the observed allelic loss of HLA haplotypes was evaluated by multivariate logistic regression analysis with stepwise backward selection

Flow cytometry

Heparinized PB and BM were collected from the patients at diagnosis and/or after treatment. HLA-A expression on granulocytes, monocytes, B and T cells, and BM CD34+ cells was analyzed by flow cytometry using a FACSCanto II instrument (BD Biosciences) with the FlowJo 7.6.1 program (TreeStar). The monoclonal antibodies used for this study are provided in supplemental Table 2.

Human androgen receptor assay

The human androgen receptor gene was amplified from genomic DNA of 23 female patients, including 3 6pLOH(+) patients, as described by Ishiyama et al[21] with some modifications. Clonality was assessed using an "S value" as a marker of skewing in granulocytes and T lymphocytes.

Association of HLA types with AA

A total of 6613 patients who had received allogeneic BM transplantation through the JMDP between 1992 and 2008 were investigated to see whether the HLA alleles frequently missing in CNN-LOH in 6p with the development of AA could represent risk alleles for the development of AA. Thus, the frequencies of patients with each of the candidate risk alleles (HLA-A*31:01, B*40:02, A*02:01, and A*02:06) and those having none of these alleles were compared between 407 patients with AA and those with other hematopoietic disorders (1827 with AML, 1606 with acute lymphocytic leukemia, 1014 with chronic myeloid leukemia, 825 with myelodysplastic syndrome, 566 with non-Hodgkin lymphoma, and 368 with other hematopoietic neoplasms; supplemental Table 3) by calculating the Fisher P values in the corresponding 2 × 2 contingency tables.

Results

Genetic lesions in AA detected by SNP array analysis

After excluding known or suspected copy number variations, a total of 50 genetic lesions were identified in 46 of the 306 (15%) PB specimens of our AA case series (Table 1; Figure 1). Among these by far, the most conspicuous was the recurrent CNN-LOH involving the 6p arm, which was detected in 28 cases as a significant dissociation of allele-specific copy number graphs in 6p regions using a hidden Markov model–based algorithm implemented in the CNAG software[2,14,15] (Figure 2A-2B). Of particular interest was that all CNN-LOH in 6p commonly affected the HLA locus, causing a haploid loss of HLA alleles and uniparental HLA expression. In some cases, the breakpoint of the 6pLOH was

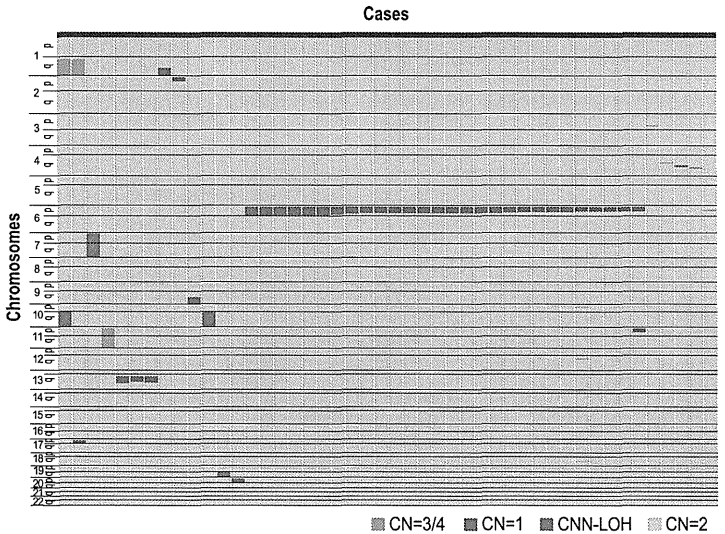

Figure 1. Copy number changes and allelic imbalances in 46 of the 306 AA cases. The copy number changes and allelic imbalances (or CNN-LOHs) in each case are summarized in the chromosomal order vertically for 46 AA cases with copy number abnormalities. Gains and losses, as well as CNN-LOHs, are shown in the indicated colors.

predicted to fall within the HLA locus (Figure 2B). These findings strongly indicated that the HLA locus was the genetic target of these 6pLOHs. Also supporting this was the finding that, in half of the cases, the dissociations in the allele-specific copy number graphs were gradually attenuated to the baseline over several mega base pair regions rather than showing a discrete breakpoint, indicating the presence of multiple 6pLOH(+) clones within a single case that had different breakpoints but still shared the same missing HLA alleles (Figure 2C). Moreover, the 6pUPDs existing only in a minor population were more sensitively detected by statistically evaluating the size of dissociation of allele-specific copy numbers in the 6p arm. With this improved statistical test, CNN-LOH in 6p was found in a total of 40 cases (13%; Figure 2D; supplemental Figure 2), where the false discovery rate was maintained at 0.01 to avoid too many false positive findings. In all 6pLOH(+) cases, substantial numbers of heterozygous SNP calls were retained within the affected regions, thus indicating that the CNN-LOHs in 6p were not constitutional but represented acquired genetic events only found in the affected subclones (Figure 1). Indeed, all 6pLOH(+) cases were shown to have "heterozygous" HLA alleles in high-resolution HLA typing of their PB (Table 2). Moreover, 6pLOH was not detected in the CD3-positive T cells in selected cases (cases 25 and 26, supplemental Figure 3). By quantitatively comparing the observed differences in allele-specific copy numbers in the 6pLOH segments with what were expected assuming 100% LOH(+) components, the 6pLOH(+) clones were estimated to account for 0.2% to 53.9% of the PB leukocytes (Table 2). The trend of the lower percentages of the 6pLOH(+) fraction in newly diagnosed patients compared with those in patients at remission was thought to reflect the fact that the former patients tended to have lower counts of granulocytes and monocytes, which were the predominant targets of 6pLOH (see supplemental Table 1).

The disease status of the 40 patients at the sampling was before treatment in 16 cases, during remission for 1 to 16 years after therapies in 15, and before BM transplantation for refractory disease in 9. All evaluable 6pLOH(+) AA cases responded to immunosuppressive therapy (IST) (23 of 23), whereas 101 of 126 evaluable cases with 6pLOH(−) responded ($P = .014$; Table 3).

Uniparental expression of HLA-A in multilineage hematopoietic cells

The genetic loss of one HLA haplotype in SNP array analysis was further confirmed by expression analysis of HLA-A in PB leukocytes using flow cytometry in 19 eligible cases with 6pLOH(+), in which the HLA-A alleles were heterozygous and fresh PB samples were available. Loss of expression of one HLA-A antigen was confirmed in all 19 6pLOH(+) cases (Figure 3A; supplemental Figure 4). The HLA-A missing cells in the PB were shown to have appeared shortly after the onset or before the initiation of treatments in 2 cases, and were confirmed to persist for 1 to 16 months (median, 6 months) in 14 patients (supplemental Table 1; supplemental Figure 5). The percentage of granulocytes lacking HLA-A antigens in the 2 patients who were responsive to IST remained almost the same during the convalescent period of 2 to 3 months (supplemental Figure 6). Importantly, uniparental expression of HLA-A alleles was detected in multiple cell lineages, including granulocytes, monocytes, B cells, and, to a lesser extent, in T cells. Moreover, uniparental HLA-A expression was demonstrated in BM $CD34^+$ cells in 5 patients whose BM samples were available for flow cytometry. All 5 patients possessed various proportions of BM $CD34^+$ cells (49.7%-71.3%), which had lost the expression of one

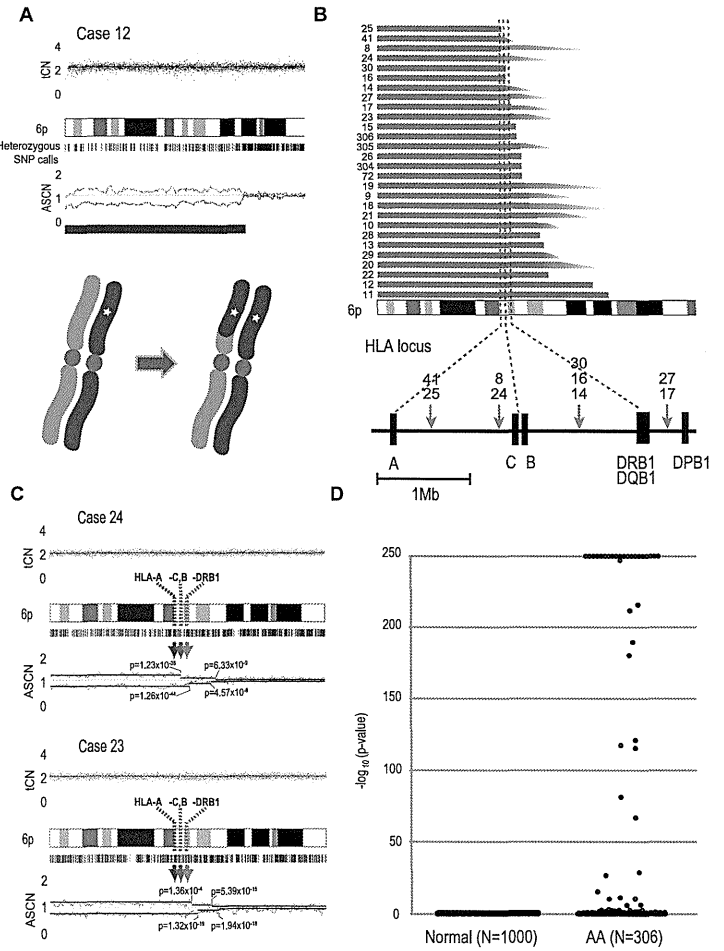

Figure 2. Acquired 6pLOHs in AA patients that target the HLA locus. (A) Typical CNAG outputs in SNP array analysis showing CNN-LOH (purple line) that appears as significant dissociation in allele-specific copy number graphs (red and green lines) from the baseline with normal total copy numbers (tCN; top panel). As a result of an allelic conversion, the affected segment causes LOH (* indicates 1; bottom panel). The "acquired" origin of these lesions is indicated by the retention of substantial numbers of heterozygous SNP calls (green bars below the chromatogram) that would otherwise mostly disappear. (B) The breakpoints of 6pLOHs found in a total of 28 AA cases, all involving the HLA locus in common. In more than half of cases (indicated by arrowheads in panel B), the exact location of the breakpoint was difficult to uniquely determine, where dissociation of the allele-specific copy number graphs continuously tapered along the 6p arm, indicating the presence of multiple 6pLOH(+) clones with common missing alleles (C). Indeed, the breakpoint containing regions are separated into multiple segments having significantly different copy numbers in the circular binary segmentation model, as indicated by solid lines with *P* values. Note that the most telomeric breakpoint is located within (case 24) or centromeric to (case 23) the HLA locus in each case. (D) A skewed distribution of the logarithm of *P* values in AA cases compared with normal persons. The *P* values were calculated in the Mann-Whitney *U* test, with which the difference in the mean allele-specific copy numbers between 6p and other chromosomal regions was evaluated (see "Analysis of genomic copy numbers and detection of 6pLOH"). A total of > 250 values are plotted as 250.

HLA-A antigen; and in each case, the missing HLA-A allele was identical to that in the PB leukocytes (Figure 3B). The uniparental expression of HLA-A in case 13 was also observed in the $CD34^+$ compartment of the archived BM specimen obtained 2 years before analysis (supplemental Figure 7). Together, these findings suggested that the 6pLOH involved early HSPCs and that the 6pLOH occurred at the level of long-term repopulating stem cells.

Table 2. 6pLOH(+) AA cases and imputed allelic status of HLA alleles

UID	6pUPD(+) fraction,* %	Missing alleles						Retained alleles					
		A	B	C	DRB1	DQB1	DPB1	A	B	C	DRB1	DQB1	DPB1
19	53.9	31:01†‡	40:02†	03:04†	12:01	03:01	05:01	24:02	52:01	12:02	15:02	06:01	05:01
12	51.8	02:01†‡	40:02†	03:03	15:01	06:02	05:01	26:02	40:06	08:01	09:01	03:03	05:01
17	51.6	24:02	13:01	03:04†	12:02	03:01	04:02	24:02	52:01	12:02	15:02	06:01	09:01
304	49.3	31:01†‡	55:02	01:02	12:02	03:01	41:01	24:02	07:02	07:02	01:01	05:01	04:02
11	48.0	02:06†‡	40:02†	03:04†	15:01	06:02	ND‖	11:01	67:01	07:02	16:02	05:02	ND‖
21	46.2	31:01‡§	51:01	14:02	14:05	05:03	03:01	24:02	07:02	07:02	01:01	05:01	04:02
24	44.9	31:01†	40:02†	03:04†	11:01	03:01	02:01	24:02	40:06	08:01	09:01	03:03	05:01
26	44.3	31:01†‡§	40:01	03:04†	04:05	04:01	03:01	26:03	52:01	12:02	15:02	06:01	09:01
27	43.5	02:06†	40:02†	03:04†	04:10	04:02	02:01	11:01	52:01	12:02	15:02	06:01	09:01
10	42.1	31:01†	40:02†	03:04†	08:03	06:01	02:01	24:02	51:01	14:02	09:01	03:03	02:01
8	40.8	02:06†‡	40:02†	03:03	12:01	03:01	05:01	24:02	52:01	12:02	15:02	06:01	04:02
23	35.2	02:01†	40:02†	03:04†	09:01	03:03	02:01	24:02	54:01	01:02	04:05	04:01	04:02
25	32.1	02:06†‡			No LOH			01:01			No LOH		
9	23.5	02:06†‡	39:01	07:02	08:02	04:02	02:01	24:02	15:18	07:04	04:01	03:01	14:01
20	21.7	26:01‡	40:02†	03:03	15:01	06:02	05:01	02:18	46:01	01:02	08:03	06:01	05:01
14	21.7	31:01†‡	51:01	14:02	09:01	03:03	05:01	24:02	52:01	12:02	15:02	06:01	09:01
22	20.6	02:01†	39:01	07:02	08:03	06:01	05:01	24:02	52:01	12:02	15:02	06:01	09:01
18	17.6	02:01†‡	40:06	08:01	09:01	03:03	02:01	24:02	35:01	03:03	15:01	06:02	04:02
15	17.4	02:06†	40:06	08:01	09:01	03:03	02:01	24:02	07:02	07:02	01:01	05:01	02:01
41	15.2†	31:01†‡	35:01	03:03	09:01	03:03	03:01	26:01	39:01	07:02	08:03	06:01	05:01
28	12.8	24:02	54:01	01:02	01:01	05:01	04:02	24:02	52:01	12:02	15:02	06:01	09:01
29	11.7	31:01†	40:02†	03:04†	15:01	06:02	02:01	24:02	54:01	01:02	04:05	04:01	05:01
305	10.3	02:06†‡	40:02†	15:02	15:02	06:01	04:01	24:02	51:01	14:02	09:01	03:03	02:01
13	9.6	24:02‡	40:02†	03:04†	15:01	06:02	02:01	02:01‡	35:01	08:01	09:01	03:03	02:01
306	8.5	24:02‡	40:02†	03:04†	09:01	03:03	02:01	26:02	40:06	08:01	09:01	03:03	02:01
16	8.1	11:01	40:06	08:01		No LOH		24:02	46:01	01:02		No LOH	
30	8.0	02:06†	39:01	07:02		No LOH		24:02	40:06	08:01		No LOH	
72	5.6	02:01†	40:02†	03:04†	09:01	03:03	05:01	02:07	46:01	01:02	08:03	06:01	02:02
36	4.0	02:01†‡	ND¶	ND#	15:02	06:01	09:01	24:02	ND¶	ND#	15:02	06:01	09:01
124	3.5	24:02	40:02†	03:04†	12:01	03:01	02:01	24:02	52:01	12:02	15:02	06:01	09:01
223	2.8	31:01†‡	48:01	03:04†	09:01	03:03	05:01	02:06†	39:01	07:02	15:01	06:02	02:01
215	2.8	31:01†	51:01	14:02	08:02	04:02	04:02	03:01	44:02	05:01	13:01	06:03	05:01
181	1.3	02:06†	13:01	03:04†	12:02	03:01	05:01	24:02	52:01	12:02	15:02	06:01	09:01
97	1.0	24:02	07:02	07:02	01:01	05:01	05:01	02:01†	39:01	07:02	15:01	06:02	02:01
252	0.9	ND**	40:02†	03:04†	09:01	03:03	05:01	ND**	46:01	01:02	04:05	04:01	05:01
118	0.9	02:06‡§	40:02†	03:04†	08:02	03:02	05:01	24:02	52:01	12:02	15:02	06:01	09:01
298	0.8	24:02	40:02†	03:04†	15:01	06:02	05:01	24:02	52:01	12:02	15:02	06:01	09:01
188	0.7	24:02	52:01	12:02	15:02	06:01	09:01	02:01†	52:01	12:02	11:01	03:01	05:01
291	0.7	31:01†	51:01	14:02	15:01	06:02	02:01	24:02	40:01	03:04†	11:01	03:01	05:01
196	0.2	ND†† (A*02:06/24:02, B*35:01/51:01, C*03:03/15:02, DRB1*04:03/15:01, DQB1*03:02/06:02, DPB1*0:201/02:01)											

UID indicates unique ID.
*The percentage of 6pUPD(+) fraction is derived from total peripheral blood leukocytes that include lymphoid as well as myeloid element.
†HLA types significantly deviated to missing alleles.
‡The allelic loss was confirmed by flow cytometry.
§The missing haplotype was determined by flow cytometry.
‖DPB1*04:02/05:01.
¶B*15:18/52:01.
#C*08:01/12:02.
**A*02:01/02:07.
††Missing allele was not determined because copy number changes in these segments were not statistically significant.

Clonality of the HLA-missing granulocytes

The human androgen receptor-based clonality assays in granulocytes were performed in 3 6pLOH(+) and 20 6pLOH(−) patients, in which all 3 6pLOH(+) and 4 (20%) of the 6pLOH(−) patients showed evidence of clonality in granulocyte populations (supplemental Figure 8).

Missing HLA alleles in 6pLOH

Given that the HLA is the genetic target of 6pLOH in AA, the missing HLA alleles in 6pLOH are of particular interest because in this context they are thought to be directly involved in the presentation of the target auto-antigens to CTLs and, therefore, to be critically important in the pathogenesis of AA. We determined the missing HLA alleles in each 6pLOH(+) AA patient by the haplotype imputation of HLA alleles based on the large data of HLA haplotypes observed in the JMDP set, followed by statistical evaluation of allele-specific copy numbers along the imputed haplotypes (Figure 4). The imputed haplotypes were confirmed in 4 cases by the family studies on the HLA. The allelic status was imputed at least partially in 39 of the 40 6pLOH(+) cases. The imputed results were consistent with the patterns of uniparental expression of HLA-A in flow cytometry in 18 cases with 6pLOH (Table 2; Figure 4), except for those in case 26, in which no valid SNP haplotype

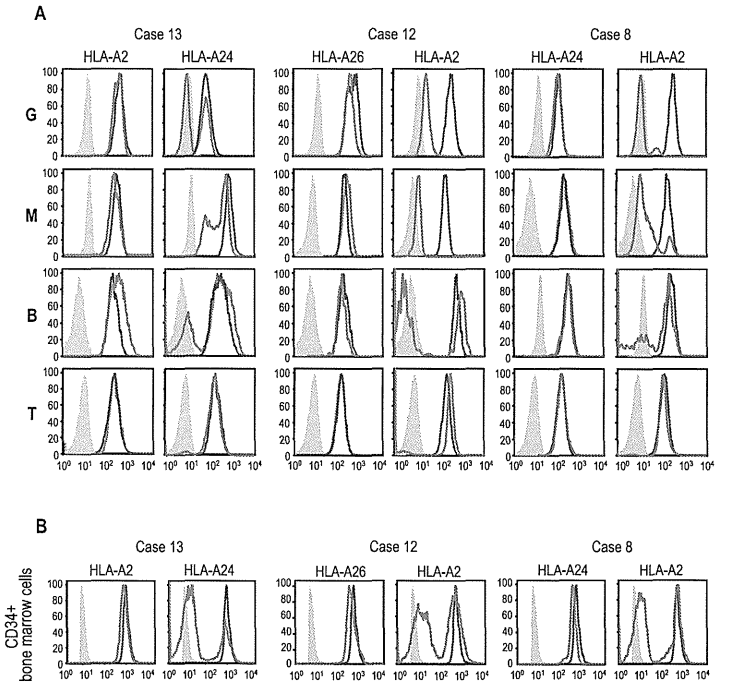

Figure 3. Uniparental expression of HLA in AA cases with CNN-LOH in 6p. Allele-specific expression of HLA-A antigens in AA specimens was examined by flow cytometry using monoclonal antibodies that specifically recognize the indicated HLA types (red lines), where leukocytes from healthy persons were used as a control (blue lines). (A-B) The uniparental expression of HLA-A antigens in PB leukocytes and BM CD34+ cells obtained from 3 AA cases with CNN-LOH in 6p. Different leukocyte compartments were separately examined, including granulocytes (G), monocytes (M), B-lymphocytes (B), and T-lymphocytes (T).

around the HLA-A locus was identified and the status of HLA-A was determined by flow cytometry. The missing HLA alleles in 6pLOH(+) AA showed a conspicuous deviation to some selected HLA alleles, including HLA-A*31:01, B*40:02, C*03:04, and, to a lesser extent, HLA-A*02:01 and A*02:06. After the effects of linkage disequilibrium between individual HLA alleles were taken into consideration by multivariate analysis, 4 HLA alleles were shown to remain as the principal determinants of the missing haplotypes, HLA-A*31:01, B*40:02, A*02:01, and A*02:06 (supplemental Table 4).

Over-representation of frequently missing HLAs in AA populations

Because these missing HLA alleles in 6pLOH could be involved in the pathogenesis of AA, we next tested whether these relevant HLA alleles are associated with the risk of the development of AA among the 6,613 JMDP registrants. As shown in Table 4, the 4 major missing HLA alleles, HLA-A*31:01, B*40:02, A*02:01, and A*02:06, were more frequently observed in AA cases compared with nonsignificant HLA alleles (ie, all HLA alleles other

Table 3. Response rate (CR + PR) according to the Camitta criteria

	Newly diagnosed (n = 107)		Previously treated (n = 103)	
	6pLOH(−) (n = 91), no. (%)	6pLOH(+) (n = 16), no. (%)	6pLOH(−) (n = 88), no. (%)	6pLOH(+) (n = 15), no. (%)
Immunosuppressive therapies (all)	36/49 (73)	11/11 (100)	65/77 (84)	12/12 (100)
ATG + CsA	14/19 (74)	7/7 (100)	27/33 (82)	5/5 (100)
CsA alone	22/30 (73)	4/4 (100)	38/44 (86)	7/7 (100)
Anabolic steroid alone	0/0 (0)	0/0 (0)	7/11 (64)	2/2 (100)
Unknown/not evaluable	42	5	0	1

CR indicates complete remission; PR, partial remission; ATG, antithymocyte globulin; and CsA, cyclosporine A.

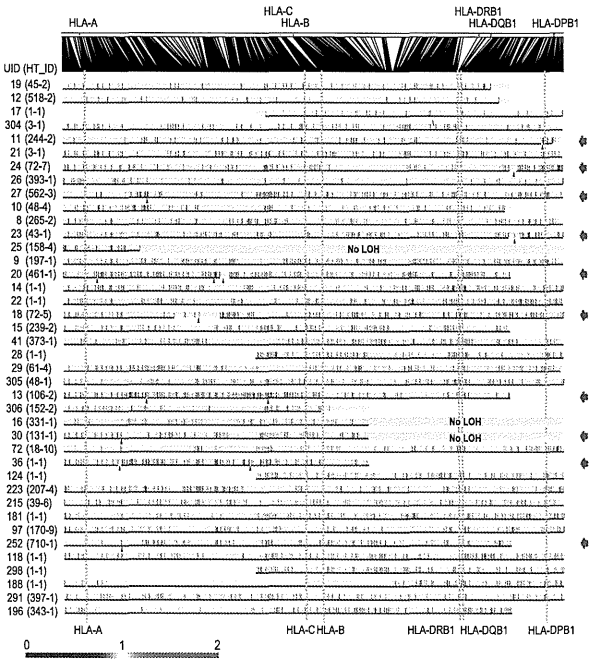

Figure 4. Imputation of missing HLA haplotypes. The observed allelic copy numbers at heterozygous SNP sites along each candidate SNP haplotype are color-coded as indicated at the bottom. Green bars showed the SNPs that are incompatible with the patient's genotype. Case IDs and haplotype ID (HT_ID) are indicated on the left. The locations of the 500K SNPs and HLA-A, C, B, DRB1, DQB1, and DPB1 are indicated in the figure. For each allele, genomic copy numbers were imputed using the circular binary segmentation algorithm. This divided each haplotype into one or more segments having discrete mean allelic copy numbers (blue arrows on the right). The positions of breakpoints are indicated by arrowheads. Finally, the mean allelic copy number of each segment was statistically compared with that of the corresponding segment on the other haplotype using the Wilcoxon signed rank test. Missing HLA haplotypes were determined based on the result of the statistic tests. Purple and blue lines indicated the retained and missing segments, respectively, whereas the allelic status was not determined statistically for those segments shown by green lines.

than these 4 alleles), where the odds ratios for the risk of the development of AA between each of these alleles and nonsignificant alleles were 1.87 (95% confidence interval [CI], 1.43-2.43) for A*02:01, 2.22 (95% CI, 1.70-2.90) for A*02:06, 1.37 (95% CI, 1.00-1.88) for A*31:01, and 1.95 (1.48-2.58) for B*40:02 (Table 4). The combined relative risk for all these alleles was 1.75 (1.42-2.17; $P = 1.3 \times 10^{-7}$).

Discussion

The origin of clonal hematopoiesis in AA is a focus of long-standing disputes, in which a profoundly reduced hematopoietic stem cell pool and/or escape from the autoimmune insults have been implicated in the evolution of the clonal hematopoiesis in AA.[5,22,23] Our findings on 6pLOH in AA provide an intriguing insight not only into the underlying mechanism of the clonal hematopoiesis in AA but also into the origin of the autoimmunity that is responsible for the pathogenesis of AA. A recent study from the United States also reported 3 cases with 6pLOH.[24] With a sensitive detection algorithm, the presence of the 6pLOH(+) components was demonstrated in as many as 13% of typical cases with AA, and the evidence from the subsequent studies strongly indicated that the HLA genes are the genetic targets of 6pLOH in AA patients. First, the HLA locus was commonly and critically involved in all 6pLOHs found in AA. Second, some AA patients carried multiple 6pLOH(+) subclones with different breakpoints, but in all cases, the 6pLOH involved the HLA locus and occurred in a manner that targeted the same parental HLA allele. Moreover, particular class I HLA alleles were over-represented among 6pLOH(+) cases and consistently found in the missing haplotypes. Finally, many of these HLA alleles were shown to be tightly

Table 4. Association of missing HLA alleles with AA in Japanese patients

Risk allele	AA (N = 407)	Other diseases (N = 6206)	Total (N = 6613)	P (χ^2 test)	Odds ratio (95% CI) (vs no risk alleles)
A*02:01	103	1173	1276	2.5×10^{-6}	1.87 (1.43–2.43)
A*02:06	100	957	1057	$< 1.0 \times 10^{-7}$	2.22 (1.70–2.90)
A*31:01	58	899	957	0.048	1.37 (1.00–1.88)
B*40:02	86	938	1024	1.8×10^{-6}	1.95 (1.48–2.58)
All risk alleles	268	3250	3518	1.3×10^{-7}	1.75 (1.42–2.17)
No risk alleles	139	2956	3095	—	—

— indicates not applicable.

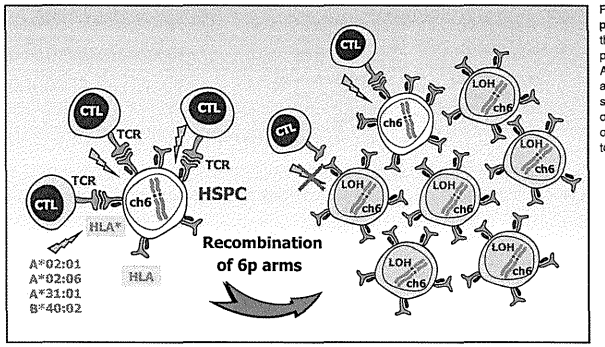

Figure 5. A proposed mechanism for escape hematopoiesis in 6pLOH(+) AA. In AA, the targets of CTLs are the HSPCs that present some auto-antigen through particular class I HLA molecules, including HLA-A*02:01, A*02:06, A*31:01, and B*40:02. In the presence of these autoimmune insults, the HSPCs that lose their expression of the antigen-presenting HLA molecule as a result of CNN-LOH in 6p would acquire a growth advantage over other HSPCs expressing the relevant HLA, leading to clonal outgrowth of the 6pLOH(+) progenies.

associated with the development of AA in Japanese patients in case-control studies using the large JMDP registry.

The conspicuous bias of the missing HLA alleles in 6pLOH to particular HLA types and the significant association of AA with those HLA types strongly suggest that the recurrent 6pLOH in AA is a phenomenon tightly related to the pathogenesis of AA rather than mere secondary event during the course of AA. Based on these observations, it is well reasoned that, in 6pLOH(+) AA cases, the autoimmunity to HSPCs is mediated by the CTLs that target the antigens presented via specific class I HLA molecules and that the 6pLOH(+) cells found in AA could be explained as escape hematopoiesis that survives the autoimmune insult by genetically deleting the relevant HLA species that are required for antigen presentation (Figure 5). These scenarios are further supported by the recent reports showing that the CNN-LOH in 6p provides a common mechanism of leukemic relapse after HLA haploidentical stem cell transplantations, in which leukemic cells that lost the mismatched HLA haplotype through CNN-LOH in 6p are thought to escape the immunologic surveillance of the engrafted donor T cells.[25,26] Importantly, it was experimentally demonstrated by immunologic assays that the 6pLOH(+) leukemic cells actually escaped GVL by CTLs, whereas 6pLOH(−) leukemic cells were effectively killed by the same CTLs. Although the immunologic targets of CTLs are different between relapse after haploidentical transplants (mismatched HLAs themselves) and AA (still unknown autoantigens presented on missing HLAs), the prominent similarities found in both cases further support that CNN-LOH in 6p confers an escape mechanism from autoreactive CTLs in AA.

In light of the above considerations, the chronologic behavior of the 6pLOH(+) components in PB is also interesting and worth discussing. Despite the assumption that 6pLOH is an effective escape mechanism from CTLs, the 6pLOH(+) stem cells were unable to repopulate the BM to cure AA, unless effective IST was applied (supplemental Figure 6). This is most probably explained by the presence of inflammatory cytokines, such as IFN-γ and TNF-α, which have also been shown to play an important role in the BM failure in AA and are thought to be responsible for the continued prevention of the 6pLOH(+) stem cells from fully expanding and reconstituting the BM (supplemental Figure 9A-B).[27,28]

When the autoimmune insults are removed after IST, no further injury of normal stem cells would occur. However, this does not necessarily mean the surviving normal stem cells can eventually outnumber the 6pLOH(+) stem cells over time. Note that, once the autoimmune insults disappear, nothing could biologically or immunologically discriminate a 6pLOH(+) stem cell from a 6pLOH(−) stem cell (supplemental Figure 9A). In particular, a 6pLOH(+) stem cell and a 6pLOH(−) stem cell will produce the same number of progeny on average and feed the same number of mature blood cells. As a consequence, once established, the predominance of 6pLOH(+) stem cells over 6pLOH(−) stem cells should be maintained, after the severely reduced hematopoietic stem cell pool has been re-expanded with removal of the inciting autoimmunity. It is also of note that the recovery of myeloid components after IST, which are affected more strongly by 6pLOH than lymphoid cells, contributes to an apparent increase in 6pLOH components in the SNP array analysis in PB (supplemental Figure 6A).

One of the most significant findings in the current study is the identification of the HLA alleles that are over-represented in the Japanese AA populations, including HLA-A*31:01, B*40:02, A*02:01, and A*02:06. All of these HLA alleles belong to class I MHCs and thus are thought to be involved in the antigen presentation to CTLs. This provides another prominent example, in which specific HLA types play a critical role in the development of a human disease, and the information about these particular HLA types provides a solid basis on which we can ultimately isolate the relevant antigens responsible for the development of AA. Of particular note, there was a previous report indicating that HLA-B*40:02 and A*02:06 were over-represented in PNH as well as AA, although the study size was much smaller than the current study.[29] Combined with our study, these findings support the hypothesis that AA and PNH are the different outcomes of the same immunologic insult[5,30] and may also provide the genetic basis of the high prevalence of AA and PNH in East Asia.[31,32]

In some AA cases, hematopoiesis could be maintained over years by the progenitors that escaped and survived the inciting autoimmune insult by deleting the target HLA through CNN-LOH in 6p. Given that the 6pLOH was detected in only 13% of our series, it is probable that other escape mechanisms may also operate to maintain hematopoiesis in AA. Indeed, clonality was clearly demonstrated in 20% of the 6pLOH(−) cases in the human androgen receptor assay study (supplemental Figure 8). In addition, our SNP array analysis also revealed a variety of clonal abnormalities in AA cases (Figure 1), although it is still open to question

whether these abnormalities actually represent the mechanism of escape hematopoiesis or were related to some neoplastic process. Further studies on the genetic basis of the escape mechanisms would contribute to our understanding of the molecular pathogenesis of AA.

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Authorship

Contribution: S. Ohtake, S. Ogawa, and S.N. developed the concept of the study and supervised the project; T.K., S. Ohtake, and S.N. designed the experiments; T.K., A.S.-O., Y. Sato, Y. Mori, M.K., M.S., K.H., and Y. Sasaki performed the experiments and analyzed the data; K.K. performed high-resolution HLA typing; S.M. and Y. Morishima provided the information of JMDP donor-recipient pairs (JMDP dataset); T.K., A.S.-O., S. Ogawa, and S.N. wrote the paper; and all authors approved the final version of the manuscript.

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Reduced-intensity conditioning for alternative donor hematopoietic stem cell transplantation in patients with dyskeratosis congenita

Nishio N, Takahashi Y, Ohashi H, Doisaki S, Muramatsu H, Hama A, Shimada A, Yagasaki H, Kojima S. Reduced-intensity conditioning for alternative donor hematopoietic stem cell transplantation in patients with dyskeratosis congenita.
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Abstract: DC is an inherited bone marrow failure syndrome mainly characterized by nail dystrophy, abnormal skin pigmentation, and oral leukoplakia. Bone marrow failure is the most common cause of death in patients with DC. Because previous results of HSCT with a myeloablative regimen were disappointing, we used a reduced-intensity conditioning regimen for two patients with classic DC, and one patient with cryptic DC who harbored the *TERT* mutation. Graft sources included two mismatched-related bone marrow (BM) donors and one unrelated BM donor. Successful engraftment was achieved with few regimen-related toxicities in all patients. They were alive 10, 66, and 72 months after transplantation, respectively. Long-term follow-up is crucial to determine the late effects of our conditioning regimen.

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Key words: dyskeratosis congenita – non-myeloablative hematopoietic stem cell transplantation

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DC is an inherited multisystem bone marrow failure syndrome characterized by nail dystrophy, abnormal skin pigmentation, oral leukoplakia, and cancer predisposition. Patients with DC have very short germ-line telomeres compared with normal individuals because of a defect of telomere maintenance. Until now, mutations in six genes (*DKC1*, *TERC*, *TERT*, *NOP10*, *NHP2*, and *TINF2*) involved in telomere maintenance have been identified in patients with DC (1).

Abbreviations: ATG, anti-thymocyte globulin; CMV, cytomegalovirus; DC, dyskeratosis congenita; EBV, Epstein–Barr virus; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; IST, immunosuppressive therapy; RIST, reduced-intensity stem cell transplantation; TBI, total body irradiation.

Bone marrow failure develops in 80–90% of patients with DC and is the most common cause of death, up to 60–70% (2, 3). Although androgen has been used to improve cytopenia since the 1960s, allogeneic HSCT is the only curative treatment for bone marrow failure in patients with DC. However, the outcome in previous reports has been disappointing because of unacceptable transplant-related toxicities such as severe pulmonary/liver complications especially in transplant using a myeloablative conditioning regimen or transplants from an alternative donor (3, 4).

To avoid transplant-related complications, RIST using a non-myeloablative conditioning regimen has been recently used in patients with DC, and encouraging short-term survival has been achieved. Reducing the intensity of

conditioning results in less tissue damage and decreased inflammatory cytokine release compared with myeloablative transplantation (5). However, until now, there have been only a few reports of non-myeloablative transplants, especially from an alternative donor. Here, we report our encouraging results of RIST from an alternative donor using a fludarabine-based conditioning regimen and *in vivo* T-cell depletion by ATG in three patients with DC.

Patients and methods

Case 1

Patient 1 was a 21-yr-old man with classical DC with nail dystrophy, abnormal skin pigmentation, oral leukoplakia, and bone marrow failure. He had no family history of physical or hematologic abnormalities. Nail changes began to develop in early childhood. He suffered from cytopenia and was diagnosed with aplastic anemia at the age of 9. At age 18, he was referred to our hospital and was diagnosed as having DC. He had very short telomeres – i.e., less than the first percentile for his age – although mutation analysis did not identify any mutations in *DKC1*, *TERC*, *TERT*, *NOP10*, or *TINF2*. As pancytopenia progressed, we planned HSCT from a sister who was mismatched at HLA DRB1 allele. He did not undergo HSCT before. Conditioning regimen included cyclophosphamide 750 mg/m^2/day, fludarabine 25 mg/m^2/day, and rabbit-ATG (Thymoglobulin; Genzyme, Cambridge, MA, USA) 2.5 mg/kg/day, all from days −5 to −2, and total lymphoid irradiation 3 Gy (1 fraction) on day −1. GVHD prophylaxis comprised tacrolimus (intravenous infusion of 0.02 mg/kg/day starting on day −1, with dose adjustments to maintain blood levels of 5–15 ng/dL) and short-term methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11). The administration route of tacrolimus was switched to oral after patients recovered from gastrointestinal toxicity.

Case 2

Patient 2 was a nine-yr-old girl with aplastic anemia without any physical abnormalities. At first, she was diagnosed with acquired aplastic anemia of unknown cause. However, she was identified as having a heterozygous *TERT* mutation (T726M) and very short telomeres in our retrospective study of mutation screening for telomere-related genes. She was born to healthy non-consanguineous parents and had no family history of physical or hematologic abnormalities. Subsequent screening of her family members revealed that her father had the same heterozygous *TERT* mutation (6). She was diagnosed with very severe aplastic anemia at the age of 8. She received IST with horse-ATG (Lymphoglobulin; Genzyme) 15 mg/kg/day intravenously for five days and cyclosporine because she had no HLA-matched family donor. However, the response to IST was poor, and she was still transfusion-dependent for six months after treatment. At first, she underwent HSCT from an HLA DRB1 one-allele-mismatched unrelated donor. The first conditioning regimen included cyclophosphamide 50 mg/kg/day for four days, TBI 5 Gy (two fractions), and rabbit-ATG (Thymoglobulin; Genzyme) 2.5 mg/kg for four days. Patient failed to engraft and had no autologous recovery of her bone marrow. She underwent a second transplant from an HLA B and DRB1 alleles-mismatched mother, 48 days post-transplant as salvage therapy. Conditioning regimen included fludarabine 30 mg/m^2/day and ATG 2.5 mg/kg/day from days −5 to −2, and melphalan 60 mg/m^2/day on days −2 and −1. GVHD prophylaxis was the same as for case 1.

Case 3

Patient 3 was an 18-yr-old man with classical DC with nail dystrophy, abnormal skin pigmentation, oral leukoplakia, and bone marrow failure. He had very short telomeres, and mutation analysis showed *DKC1* mutation. Nail changes began in early childhood, and pancytopenia was noted at age 13. Because pancytopenia progressed, we planned HSCT from an HLA 6/6 alleles-matched unrelated donor. He did not undergo HSCT before. Conditioning regimen included cyclophosphamide 750 mg/m^2/day, fludarabine 25 mg/m^2/day, and rabbit-ATG 2.5 mg/kg/day, all from days −5 to −2, and TBI 3 Gy (one fraction) on day −1. GVHD prophylaxis was the same as for cases 1 and 2.

Table 1 shows patient and disease characteristics. Pre-transplant cardiac, lung, or liver dysfunction was not observed in any patient except for slight elevation of liver transaminase levels in patient 2. Bone marrow examination

Table 1. Patient and disease characteristics

Patient no.	Sex	Age at diagnosis of DC	Mutation	Clinical triad	Other symptoms	Pre-transplant hematological data			Number of pre-transplant transfusions		Cytogenetics
						ANC (×10^9/L)	Hb (g/dL)	PLT (×10^9/L)	RBC	PLT	
1	Male	18	Not detected	Nail, skin, oral	Cerebellar hypoplasia, growth retardation	0.9	5.7	16	25	2	46, XY
2	Female	9	*TERT*	None	None	0.3	6	0.9	40	90	46, XX
3	Male	15	*DKC1*	Nail, skin, oral	None	0.84	7.7	19	0	2	46, XY

ANC, absolute neutrophil count; Hb, hemoglobin; PLT, platelet; RBC, red blood cell.

Table 2. Pre-transplant characteristics of donors and patients

Patient no.	Age at transplant	Donor	Donor sex	Donor age	ABO incompatibility	Source	HLA match	Mismatch locus
1	21	Sister	Female	24	Compatible	BM	5/6	DR
2	9	Mother	Female	36	Major	BM + PBSC	4/6	B, DR
3	18	UD	Female	37	Compatible	BM	6/6	–

UD, unrelated donor; BM, bone marrow; PBSC, peripheral blood stem cell.

revealed severe hypocellularity and normal karyotypes in all three patients. Table 2 shows pretransplant characteristics of donors and patients.

Supportive care

All patients received trimethoprim-sulfamethoxazole orally or inhaled pentamidine as prophylaxis against *Pneumocystis jiroveci*. Patients received standard doses of oral amphotericin B and acyclovir as fungal and viral prophylaxis. Patients received pre-emptive therapy with ganciclovir when CMV antigenemia became positive. Weekly viral studies for CMV, EBV and human herpesvirus 6 were obtained until day 90 post-transplant (7). Granulocyte colony-stimulating factor was started from day 5 to neutrophil engraftment. Acute and chronic GVHD was diagnosed and graded according to established criteria (8, 9).

Results

Transplant outcomes are shown in Table 3. Engraftment day was defined as the first of three consecutive days in which the patient had an absolute neutrophil count greater than 0.5×10^9/L. Neutrophil engraftment was achieved in all patients, although the days of platelet recovery were delayed. Analysis of short tandem repeats or fluorescent *in situ* hybridization of sex chromosomes revealed that all patients achieved >95% donor chimerism by day 100.

Engraftment syndrome developed in patient 3 and responded well to steroid therapy (10, 11). Acute GVHD did not occur in any patient, while chronic GVHD of the skin occurred in patient 3 and responded to tacrolimus therapy. Patients 1 and 2 discontinued their treatment with immunosuppressive drugs at 18 and 14 months, respectively, following transplant.

Increases in the EBV genome load were observed in patients 1 and 3. The dose of tacrolimus was decreased in patient 1, and one course of rituximab was administered in patient 3. As a result, EBV genome load decreased in both patients. Positive CMV antigenemia was seen only in patient 3. Preemptive therapy with ganciclovir was administered until the test for CMV antigenemia became negative. He did not progress to CMV disease.

To date, all three patients are alive with a follow-up of 10, 66, and 72 months, respectively. No patients have developed pulmonary or liver complications or malignancies.

Discussion

In our case reports, we report the outcome of two patients with classical DC and one patient with aplastic anemia harboring the *TERT* mutation to assess the feasibility and efficacy of a fludarabine-based non-myeloablative regimen. Our regimens are promising, as all three patients achieved complete chimerism and hematologic recovery without severe transplant-related toxicities.

Previously, results of HSCT using a myeloablative regimen for patients with DC were disappointing mainly because of pulmonary/liver complications and GVHD (12–19). Until recently, there were no survivors who received unrelated sources of stem cells (3). A high transplant-related mortality rate is considered

Table 3. Outcomes of transplantation

	Cell dose		Engraftment		GVHD				
Patient no.	NCC ($\times 10^8$)	CD34 ($\times 10^6$)	ANC (>500 μL)	PLT (>20 $\times$ 10^9/L)	Acute	Chronic	Complication	Follow-up	Outcome
1	3.45	1.73	16	31	No	No	EBV reactivation	5 yr 6 months	Alive
2	9.6	2.6	23	123	No	No	DM, enteritis	6 yr	Alive
3	0.81	N.E	19	111	No	Skin	Sepsis, engraftment syndrome, CMV antigenemia, EBV reactivation	10 months	Alive

NCC, nuclear cell count; ANC, absolute neutrophil count; PLT, platelet; N.E, not evaluated.

to be associated with impaired restorative ability of tissue damage because of defective telomere maintenance. To avoid these complications, reduced-intensity regimens have been recently used and have achieved engraftment with fewer complications in both related and unrelated settings (16, 20–24). Most recently, Dietz et al. reported encouraging results of six patients with DC who underwent HSCT using fludarabine-based non-myeloablative regimens (26). Their non-myeloablative regimen consisted of cyclophosphamide 50 mg/kg for one day, fludarabine 40 mg/m^2 for five days, and TBI 2 Gy and alemtuzumab 0.2 mg/kg for five days. Engraftment was achieved in five of six patients. Four patients are alive, three of whom were recipients of unrelated grafts. Our regimen is similar to theirs, including cyclophosphamide, fludarabine, low-dose irradiation, and ATG instead of alemtuzumab. The results of HSCT from an alternative donor for DC are shown in Table 4.

It is still unclear whether HSCT can prolong the overall survival of patients with DC. Dietz et al. combined 18 cases who had undergone RIST in the literature with their six cases and calculated an overall survival rate of 65%, which was similar to another historical cohort that included both myeloablative and non-myeloablative transplants reported by Alter et al. (4). However, the follow-up periods in non-myeloablative transplants seem to be shorter than in myeloablative transplants. Although bone marrow failure is the most common cause of death in patients with DC, pulmonary fibrosis is another common cause of death (27). Alter et al. reviewed 65 patients who had received HSCT until 2008 (4). According to the review, nine of 30 deaths after HSCT were because of pulmonary fibrosis, suggesting that the high rate of this lung complication might originate from the natural history of DC. A prospective long-term follow-up study is necessary to clarify whether HSCT procedures, including conditioning agents and allogeneic immune responses to recipient's organ such as the lungs and liver, affect the natural course of DC.

Fludarabine is a potent immunosuppressive and less myeloablative agent, which has been used successfully in RIST for aplastic anemia (28) and other bone marrow failure syndromes

Table 4. Summary of HSCT from an alternative donor for dyskeratosis congenita

Patient	Age/sex	Donor source	HLA	Conditioning regimen	Outcome	Complication	References
1	23/M	MUD BM	6/6	CY 120 mg/kg and TBI 12 Gy	Death	Disseminated candidasis	Langston et al. (16)
2	20/M	MUD BM	6/6	CY 120 mg/kg and TBI 12 Gy	Death	Disseminated candidasis	Langston et al. (16)
3	29/M	MUD BM	6/6	CY 200 mg/kg and TBI 6 Gy	Death	Rejection Died of respiratory failure after 2nd BMT	Dokal et al. (17)
4	3/M	MUD	6/6	CY 120 mg/kg, Flu 180 mg/m^2 and ATG 160 mg/kg	Alive >15 months		Dror et al. (24)
5	8/F	MUD	6/6	CY 120 mg/kg, Flu 180 mg/m^2 and ATG 160 mg/kg	Alive >16 months	EBV reactivation	Dror et al. (24)
6	15/M	MUD	6/6	CY 120 mg/kg, Flu 180 mg/m^2 and ATG 160 mg/kg	Death	Cardio-respiratory arrest on day 0 Diffuse capillaritis	Brazzola et al. (25)
7	24/M	MMUD dUCB	4/6 4/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Death	Sepsis outside of hospital	Dietz et al. (26)
8	5/F	MUD BM	6/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Alive >40 months		Dietz et al. (26)
9	2/M	MUD BM	8/8	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Death	Adenoviral sepsis	Dietz et al. (26)
10	18/F	MMUD dUCB	4/6 5/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Alive >12 months	Acute GVHD grade IV (gut)	Dietz et al. (26)
11	25/M	MMUD dUCB	4/6 5/6	CY 50 mg/kg, Flu 200 mg/m^2, TBI 2 Gy and Alem 1 mg/kg	Alive >12 months		Dietz et al. (26)
12	21/M	MMRD BM	5/6	CY 3 g/m^2, Flu 100 mg/m^2, TLI 3 Gy and ATG 10 mg/kg	Alive >5 yr	EBV reactivation	This report
13	9/F	MMRD BM+PBSC	4/6	MEL 120 mg/m^2, Flu 120 mg/m^2 and ATG 10 mg/kg	Alive >6 yr	DM, enteritis	This report
14	18/M	MUD	6/6	CY 3 g/m^2, Flu 100 mg/m^2, TBI 3 Gy and ATG 10 mg/kg	Alive >10 months	Sepsis, engraftment syndrome, EBV reactivation	This report

MUD, matched unrelated donor; MMUD, mismatched unrelated donor; MMRD, mismatched related donor; BM, bone marrow; PBSC, peripheral blood stem cell; dUCB, double unrelated cord blood; CY, cyclophosphamide; Flu, fludarabine; Alem, alemtuzumab; MEL, melphalan; BMT, bone marrow transplantation; DM, diabetes mellitus.

such as Fanconi anemia (29), Shwachman-Diamond syndrome (30), and Diamond-Blackfan anemia (31). In this study, fludarabine seemed to be well tolerated in patients with DC who achieved engraftment even after transplant from an alternative donor.

Reduction in the dose of cyclophosphamide may contribute to a decrease in transplant-related toxicity. We administered cyclophosphamide at a total dose of 3000 mg/m^2, which was a tolerable dose for our patients. In several reports, the total dose was reduced to 40–50 mg/kg with durable engraftment. However, one patient who received 50 mg/kg cyclophosphamide and an unrelated double-cord graft (one set of 4/6 and 4/6 HLA match) developed primary graft failure (26). The appropriate dose of cyclophosphamide remains undetermined.

The dose of irradiation is another important issue to achieve engraftment without increasing toxicities. Because patients with DC possess chromosomal instability, they are suspected to show increased radiosensitivity. In fact, a full-dose TBI regimen resulted in unacceptable toxicities in previous reports (16). From our experience as well as other reports, inclusion of low-dose TBI may contribute to achieve durable engraftment without undesirable complications.

Dietz et al. tried to provide a natural pulmonary compensation by delivering irradiation side-to-side, instead of anterior-to-posterior, with the patient in a seated position and the arms resting at the side of the thoracic cage (26). In our institute, patients are in a supine position with the arms at the side of the thoracic cage during TBI, which is delivered side-to-side. Our method also can provide for pulmonary compensation. In addition to the dose of irradiation, the method of irradiation may be important to assess the true effects on lungs in patients with DC.

GVHD prophylaxis is another important issue for successful transplant from an alternative donor. *In vivo* T-cell depletion can reduce the risk of GVHD in HSCT for bone marrow failure syndrome (32). Our conditioning regimen included rabbit-ATG for the purpose of *in vivo* T-cell depletion to prevent severe acute GVHD. Acute GVHD did not occur in any patient in our series, even in the patient who received both bone marrow and peripheral blood from an HLA haploidentical donor. Finke et al. reported the outcome of patients with hematologic malignancies who underwent HSCT from unrelated donors using a regimen containing rabbit-ATG (33). The cumulative incidence of grade II-IV acute GVHD and chronic GVHD for HLA-mismatched transplantation was 20% and 44%, respectively, which were equal to 21% and 43% for HLA-matched transplants. The authors concluded that a single-antigen mismatch might not compromise the outcome after HSCT from an unrelated donor when ATG is used in addition to standard GVHD prophylaxis.

In conclusion, our study indicated that RIST can provide successful engraftment with few complications in patients with DC, even in transplants from an alternative donor. Long-term follow-up is crucial to monitor the late effects of conditioning agents and allogeneic immune responses to the recipient's organs, such as the lungs and liver. Given these encouraging results, we believe that RIST should be explored further.

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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 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.

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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.

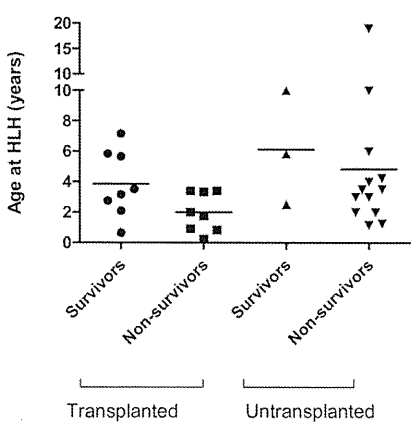

Figure 1. Outcome of patients with HLH during course of disease. Survival of patients who present with HLH—patients who remain untransplanted have a poor survival outcome with only 18.8% survival. By contrast the survival of those who undergo transplant is higher at 50%.

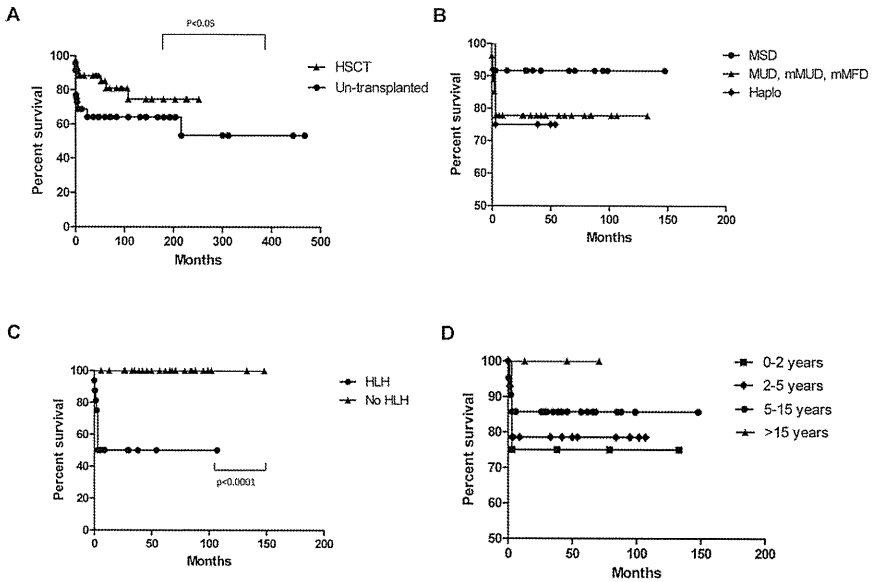

Figure 2. Survival in XLP1 related to different variables. (A) Overall survival of transplanted versus untransplanted patients. In the transplanted group this represents time from presentation and not transplant. (B) Survival according to donor source. (C) Survival after HSCT with relation to presence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

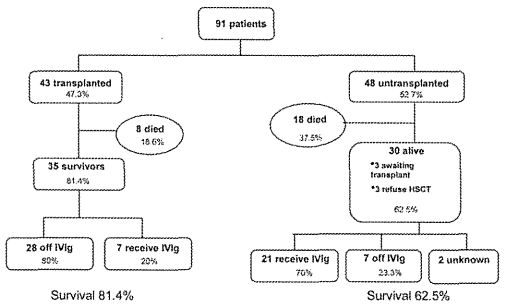

Figure 3. Outcome of patients with SAP/*SH2D1A* mutations.

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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Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP deficiency) versus type 2 (XLP-2/XIAP deficiency)

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X-linked lymphoproliferative syndromes (XLP) are primary immunodeficiencies characterized by a particular vulnerability toward Epstein-Barr virus infection, frequently resulting in hemophagocytic lymphohistiocytosis (HLH). XLP type 1 (XLP-1) is caused by mutations in the gene *SH2D1A* (also named *SAP*), whereas mutations in the gene *XIAP* underlie XLP type 2 (XLP-2). Here, a comparison of the clinical phenotypes associated with XLP-1 and XLP-2 was performed in cohorts of 33 and 30 patients, respectively. HLH (XLP-1, 55%; XLP-2, 76%) and hypogammaglobulinemia (XLP-1, 67%; XLP-2, 33%) occurred in both groups. Epstein-Barr virus infection in XLP-1 and XLP-2 was the common trigger of HLH (XLP-1, 92%; XLP-2, 83%). Survival rates and mean ages at the first HLH episode did not differ for both groups, but HLH was more severe with lethal outcome in XLP-1 (XLP-1, 61%; XLP-2, 23%). Although only XLP-1 patients developed lymphomas (30%), XLP-2 patients (17%) had chronic hemorrhagic colitis as documented by histopathology. Recurrent splenomegaly often associated with cytopenia and fever was preferentially observed in XLP-2 (XLP-1, 7%; XLP-2, 87%) and probably represents minimal forms of HLH as documented by histopathology. This first phenotypic comparison of XLP subtypes should help to improve the diagnosis and the care of patients with XLP conditions. (*Blood*. 2011;117(5):1522-1529)

Introduction

X-linked lymphoproliferative syndrome (XLP) is a rare immunodeficiency condition characterized by an extreme vulnerability to Epstein-Barr virus (EBV) infection, frequently resulting in hemophagocytic lymphohistiocytosis (HLH) or virus-associated hemophagocytic syndrome (VAHS).[1-3] HLH is caused by overwhelming T-cell and macrophage activation, leading to fever, splenomegaly, cytopenia, hypofibrinogenemia, or hypertriglyceridemia, hyperferritinemia, and hemophagocytosis.[4]

XLP belongs to the group of familial hemophagocytic lymphohistiocytosis (FHL) as originally proposed by Purtilo et al.[1] In the original description, the term "lymphoproliferative disease" in the Duncan kindred[1] was used for benign or malignant lymphoproliferation but also for the diffuse organ "infiltrates composed of lymphocytes, plasma cells, and histiocytes, some containing erythrocytes," describing histologic features of HLH. Thus, the term "X-linked lymphoproliferative disease or syndrome" used thereafter to name this condition refers not only to malignant lymphomas but also to HLH. Two genetic causes are responsible for XLP. XLP type 1 (XLP-1) is caused by hemizygous mutations in the gene *SH2D1A* encoding the signaling lymphocyte activation molecule (SLAM)–associated protein (SAP) (MIM no. 308240).[5,6] Hemizygous mutations in the gene encoding the X-linked inhibitor of apoptosis protein (XIAP; also termed *BIRC4*; MIM no. 300635) have been discovered in a cohort of patients with clinical XLP without any identified mutations in *SH2D1A* and normal SAP protein expression.[7] Thus, mutations in *XIAP* define the XLP type 2 (XLP-2). These findings were confirmed by the identification of additional patients with XIAP deficiency.[8,9] After EBV infection in most (but not all) cases, patients bearing mutations in *SH2D1A* (hereafter denoted SAP-deficient patients) may experience variable manifestations such as fulminant infectious mononucleosis corresponding pathophysiologically to HLH, malignant lymphoma, and hypogammaglobulinemia.[2,10,11] Less common findings are dysgammaglobulinemia, bone marrow hypoplasia, especially aplastic anemia, and lymphocytic vasculitis.[12,13] However, although HLH is almost always triggered by EBV, the other manifestations can be present even in SAP-deficient patients who have never encountered EBV.[2,3,10,11] The clinical features of the 12 patients with mutations in *XIAP* (hereafter denoted XIAP-deficient patients) initially described, slightly differed from the features described above. In some XIAP-deficient patients, splenomegaly was noticed as the first clinical symptom, and chronic colitis occurred during the disease course in 2 patients.[7]

The gene product affected in XLP-1 patients, SAP, is a small SH2-containing adaptor protein that is expressed in T, natural killer (NK), and invariant NKT (iNKT) cells.[5,14] SAP binds with high affinity and specificity to tyrosine-based motifs located in the cytoplasmic domains of the transmembrane receptors of the SLAM family. SAP couples SLAM family receptors to downstream signaling pathways and thereby enables SLAM receptors to mediate an array of activating or regulatory signals. In SAP-deficient humans and mice, multiple cellular defects have been documented, including altered $CD8^+$ T- and NK-cell cytotoxicity responses, $CD4^+$ T helper cell cytokine production and function, block of CD1d-restricted iNKT-cell development, defective antibody production associated with reduced numbers of switched memory B cells and defects in germinal center formation.[11,14] Studies of SAP-deficient humans and mice support the notion that the immune dysfunctions seen in SAP-deficiency are mostly caused by alterations in the signal transduction of SLAM family receptors.

The XLP-2 gene product, XIAP, belongs to the family of inhibitor of apoptosis proteins and is well known to be a potent physiologic inhibitor of caspases 3, 7, and 9.[15] XIAP is ubiquitously expressed.[7] In addition to its antiapoptotic role, XIAP is also involved in multiple signaling pathways, including copper metabolism, activation of the nuclear factor κB and the mitogen-activated protein kinases pathways and the transforming growth factor-β–receptor and bone morphogenetic protein–receptor signal transduction.[16] In XIAP-deficient patients, lymphocytes are characterized by an increased susceptibility to apoptosis in response to CD95 and tumor necrosis factor receptor–related apoptosis-inducing ligand receptor stimulation as well as enhanced activation-induced cell death.[7] XIAP-deficient patients also display low but detectable numbers of iNKT cells in blood although a recent study indicated that they can have normal numbers of iNKT cells.[9] NK cell–mediated cytotoxicity is apparently normal in XIAP-deficient patients.[7,9]

Our knowledge of the immune dysfunctions underlying the clinical manifestations in SAP-deficient patients has been largely improved in the past decade. However, this is not the case for XIAP-deficient patients. A better characterization of the clinical similarities and the differences between XLP-1 and XLP-2 could provide hints for a better understanding of the pathogenesis of these conditions and, furthermore, improve diagnostic and therapeutic procedures for these patients. Therefore, we performed a retrospective analysis of the clinical features observed in cohorts of 33 SAP- and 30 XIAP-deficient patients.

Methods

Patients and diagnosis

We performed a retrospective analysis of the clinical and laboratory features of SAP- and XIAP-deficient patients in whom confirmative molecular diagnosis had been performed at the Necker Children's Hospital. Patient conditions were diagnosed as XLP-1 and XLP-2 on the basis of molecular results or on the basis of clinical features when disease had been molecularly proven in male relatives on the mother's side (supplemental Methods and Results, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Patients and families provided informed consent for genetic and immunologic studies in accordance to the 1975 Declaration of Helsinki, and the study was approved by the local ethics regulations (Necker-Enfants Malades Ethical Board Committee).

Protein expression

Expression of SAP and XIAP was analyzed by Western blotting or flow cytometry or both after intracellular staining in phytohemagglutinin-induced T-cell blasts or peripheral blood mononuclear cells or both as described.[7] The monoclonal antibody (mAb) anti-SAP was kindly provided by Dr A. Veillette, IRCM, Montréal. Intracellular SAP was stained by fluorescein isothiocyanate– or phycoerythrin-coupled anti-SAP mAb and XIAP detected with noncoupled anti-XIAP mouse mAb (clone 48; BD Biosciences) revealed with fluorescein isothiocyanate–coupled anti–mouse antibodies (Jackson ImmunoResearch Laboratories Inc) after cell permeabilization with Perm 2 (BD Biosciences).

Histology and immunohistochemistry

All diagnostic specimens were fixed in 10% buffered formalin and stained with hematoxylin and eosin, Giemsa, or trichrome dyes (for the liver). Immunohistochemistry was performed on fixed tissues with a peroxidase-based method (Dako). Antibodies used were raised against CD20, CD3, CD8, and latent membrane protein 1 (LMP-1) (Dako); CD25 (Novocastra); and T-cell intracellular antigen-1 (Immunotech). EVB-encoded RNA (EBER) was probed on some specimen with the use of in situ hybridization technique. Slides were observed using a Leica DM LB microscope with ×20, ×40, and ×100 objectives and a 10× eyepiece. Acquisition of images was with IM50 software (Leica Microsystems). All slides were analyzed by the same pathologist (D.C.), and an independent review was also performed (F.H.).

Clinical assessment

The patients' clinical events and laboratory features were assessed retrospectively by retrieval of data from medical records.

Statistical analysis

The statistical analyses were performed with Fisher exact tests or log-rank tests (for comparison of survival curves) with the use of the PRISM software (GraphPad Software Inc).

Results

XLP-1 was diagnosed in 33 patients from 19 families, and mutations of *SH2D1A* were found in 18 families, and XLP-2 was

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Table 1. Characteristics of patients with mutations in *SH2D1A/SAP* (XLP-1)

Patient ID*	*SH2D1A/SAP* mutation	SAP 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)	Lymphoma (age in years at diagnosis)	Other manifestations (age in years at diagnosis)	Outcome (age in years)
S1.1	E67G		–	NA		–	–	13	–	Alive, well (19)
S1.2	E67G	–	3	+	+ (25)	–	+ (26)	34	–	Alive, under lymphoma treatment (34)
S1.3	E67G		15	?	–	–	–	7, 30	–	Alive, under lymphoma treatment (30)
S1.4	E67G		–	NA		–	+ (4)†	–	–	Alive, well, IVIG (10)
S2.1	I96X	–	4	?	–	?	?	–	–	Died (4, HLH)
S3.1	del. of exons 1-4		–	NA		–	+†	–	Chronic gastritis,	Alive, well, IVIG (20)
S3.2	del. of exons 1-4		–	NA		–	+†	–	IM (2), chronic gastritis	Alive, well, IVIG (20)
S4.1	R55X		–	NA		–	–	40	–	Alive, well (42)
S4.2	ND		6	+	–	–	–	–	–	Died (6, HLH)
S5.1	del. of exon 2		3.7	+	–	?	?	–	–	Died (3.7, HLH)
S5.2	ND		–	NA		–	?	5	–	Died (5, lymphoma)
S6.1	del. of exon 1		2.2	+	–	–	?	–	–	Died (2.2, HLH)
S7.1	R55X	–	2.5	+	–	–	?	–	Recurrent infections	HSCT (2.7), alive (11)
S8.1	X129RfsX141	–	2.4	+	+ (9)	–	+ (3)†	–	–	First HSCT (9); second HSCT (10); died (10.2)
S8.2	ND		2	+	–	–	?	–	–	Died (2, HLH)
S9.1	C42Y	+/–	–	NA		–	–	2	–	Alive (18)
S9.2	C42Y		–	NA		–	+ (1)†	–	–	Alive, well, IVIG (16)
S10.1	R55Q		14	?	–	?	?	–	–	Died (14, HLH)
S11.1	X129R fsX141	–	–	NA		–	+	–	–	Alive, well, N+T, IVIG (22)
S11.2	X129R fsX141		–	NA		?	?	–	Recurrent pneumonia	Alive, well (66)
S11.3	X129R fsX141		–	NA		–	+	–	–	Alive, well, IVIG (15)
S11.4	X129R fsX141		–	NA		–	+ (9)	7	–	Alive, well, IVIG (19)
S12.1	del. of exon 3	–	19	+	–	–	+ (10)†	11	T (22)	Alive, T, IVIG (23)
S12.2	del. of exon 3		19	?	–	–	+ (19)†	20	–	Died (21, lymphoma)
S13.1	N82FfsX103	ND	10§	–	+ (12, EBV+)	+ (9)‡	?	–	–	Died (12, HLH)
S14.1	del. of exons 1-4		3.5	+	–	–	–	–	HUS (3.5)	Died (3.6, HLH)
S15.1	A22P	–	–	NA		–	+ (13)†	–	–	Alive, well, IVIG (25)
S15.2	ND		3.6	?	–	–	?	–	–	Died (3.6, HLH)
S15.3	ND		–	NA		+ (45)‡	?	–	–	Died (69, myelodysplasia)
S16.1	del. of exons 2-4	–	3.1	+	–	–	?	–	–	Died (3.1, HLH)
S17.1	M1T	–	–	NA	–	–	+ (4)†	–	IM (2.4)	Alive, N+T, IVIG (20)
S18.1	No mutation	–	16§	?	–	–	+ (15)†	9	–	Died (17, HLH)
S19.1	del. of exons 1-4	–	3.3	+		–	–	–	Hypopigmented hair	HSCT (3.7), died (3.8)

SM indicates recurrent splenomegaly or hepatosplenomegaly; Hypo-γ, hypogammaglobulinemia; NA, not applicable; del., deletion; ?, unknown; IM, infectious mononucleosis; ND, not done; HSCT, hematopoietic stem cell transplantation; N, neutropenia; T, thrombocytopenia; and HUS, hemolytic uremic syndrome.

*Patient identification: S indicates SAP-deficiency, the first number corresponds to the family and the second to the individual patient.

†With recurrent respiratory infections; + indicates yes or positive; –, no or negative.

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

§Diagnosed as incomplete HLH.

diagnosed in 30 patients from 11 families (Tables 1 and 2). In one patient (PS18.1), no mutation in *SH2D1A* was found; however, no SAP protein expression was detected.[17] Six and 7 mutations in *SH2D1A* and *XIAP* were novel and not reported, respectively (supplemental Methods and Results).

Clinical manifestations included HLH, splenomegaly and incomplete forms of HLH, lymphoma, dysgammaglobulinemia, colitis, and rare clinical manifestations.

HLH

The mean age at first episode of HLH was 7.35 years (range, 2.0-19.0 years) in SAP-deficient and 6.5 years (range, 0.1-23.0 years) in XIAP-deficient patients ($P = .89$). The occurrence of HLH in SAP-deficient (18 of 33, 55%) and in XIAP-deficient (22 of 29, 76%, one unknown) patients did not differ significantly ($P = .112$) (Figure 1A; Table 3). XIAP-deficient patients with null mutations (families X1 to X7 and X11) more frequently developed HLH (19 of 20, 95%) compared with XIAP-deficient patients expressing non-null mutations (families X8, X9, and X10; 3 of 9, 33%; $^{**}P = .0011$; supplemental Figure 1A).

Overall, 11 of the 33 SAP-deficient patients (33%) and 5 of 30 the XIAP-deficient patients (17%) succumbed to HLH ($P = .1563$). Among patients with HLH, HLH-associated lethality was significantly higher in SAP-deficient patients (11 of 18, 61%) than in XIAP-deficient patients (5 of 22, 23%) ($^{*}P = .0230$). HLH

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

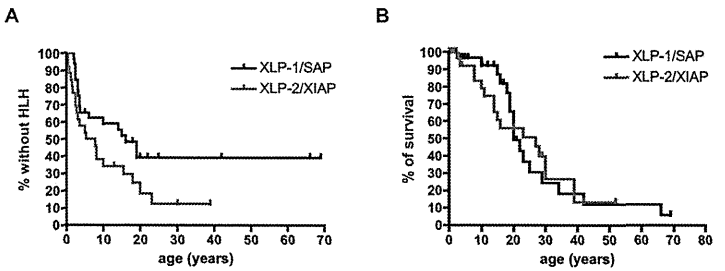

Figure 1. Comparison of HLH phenotypes and survival curves of SAP-deficient (XLP-1) and XIAP-deficient (XLP-2) patients. Kaplan-Meier survival curves were constructed on the basis of data presented in Table 1 and Table 2. Statistical analyses with log-rank tests. (A) Percentage of XLP-1/SAP and XLP-2/XIAP 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

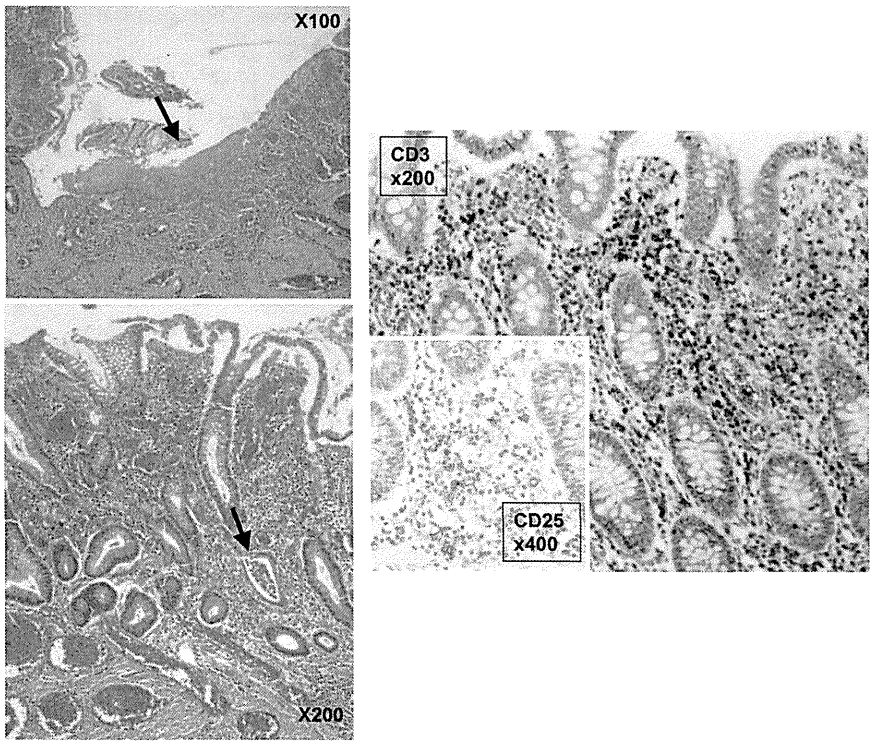

Figure 2. Histology of the large bowel of PX1.7 with XIAP deficiency. (Top left) On hematoxylin and eosin at low magnification (×100), a large ulceration is seen, indicated by an arrow. (Bottom left) Higher magnification (×200) shows a massive polymorphic inflammatory infiltrate associated with a crypt 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.I., 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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Correspondence

Possible modifier effects of keratin 17 gene mutation on keratitis–ichthyosis–deafness syndrome

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Madam, Keratitis–ichthyosis–deafness (KID) syndrome (OMIM 148210, 242150) is a rare type of ectodermal dysplasia caused by mutations in the gap junction protein beta-2 gene (*GJB2*)[1] or beta-6 gene (*GJB6*).[2] On the other hand, mutations in genes encoding keratin 6a, 6b, 16 and 17 (*KRT6A*, *KRT6B*, *KRT16* and *KRT17*) are known to cause pachyonychia congenita (PC; OMIM 16720, 17210). PC and KID syndrome share similar symptoms, such as palmoplantar hyperkeratosis and onychodystrophy. This study reports a Japanese patient with atypical KID syndrome with the combined heterozygous mutations of a recurrent mutation in GJB2 and a novel mutation in the V1 region of KRT17.

The proband was a 40-year-old Japanese woman. She was the child of healthy, nonconsanguineous parents. From childhood, she had shown diffuse mutilating palmoplantar hyperkeratosis (Fig. 1a), nail dystrophy (Fig. 1b), hypotrichosis, sensorineural hearing loss, and vascularized keratitis. Periorificial hyperkeratosis was not seen. From these findings, the diagnosis of KID syndrome was made. She had had recurrent bacterial and fungal skin infections. In her twenties, painful tumours appeared on her lower limbs. In her thirties, tumours on both buttocks developed to take on a papilloma-like appearance (Fig. 1c). Etretinate with topical or systemic antibiotics and antifungal agents did not alleviate her symptoms. Skin abrasion was repeatedly conducted on the tumours. Histopathology of the lesions revealed epidermal pseudocarcinomatous hyperplasia with dilation of vessels in papillary and reticular dermis accompanied by mixed immune cell infiltrates, excluding the involvement of squamous cell carcinoma (Fig. 1d). Vacuolated keratinocytes, suggesting human papillomavirus infection, were not detected.

Genomic DNA extracted from peripheral blood was used as a template for polymerase chain reaction (PCR) amplification. Direct sequencing of *GJB2*, *GJB6*, *KRT6A*, *KRT6B*, *KRT16* and *KRT17* was performed as described elsewhere.[3–5] The medical ethical committee of Hokkaido University approved all the described studies. The study was conducted according to the Declaration of Helsinki Principles. The proband gave her written informed consent.

Mutation analysis of the proband's genomic DNA revealed a c.148G>A transition (p.Asp50Asn) in GJB2 (Fig. 2a), which is the most prevalent mutation in patients with KID syndrome.[1] Furthermore, the proband was found to be heterozygous for a c.177C>A transversion (p.Ser59Arg) in KRT17 (Fig. 2b). Restriction enzyme digestion of the PCR products by PvuII was carried out to confirm the c.177C>A in KRT17 (Fig. 2c). The c.177C>A in KRT17 was novel and was not detected in 200 alleles from 100 normal Japanese individuals. Mutation screening on the proband's parents could not be performed because the father was not alive and the mother did not consent. Keratin 17 (K17) immunohistochemistry on skin samples from several different sites revealed K17 expression in whole epidermis although its expression level did not vary between nonlesional and lesional skin specimens (data not shown).

As the clinical manifestations of the proband were atypical and more severe than those of other patients with KID syndrome – as evidenced, for example, by diffuse mutilating palmoplantar hyperkeratosis and recurrent granulation tissue formation on the buttock – we hypothesized that mutations in other genes might have affected the proband's phenotype through modifier effects. Modifier genes are defined as genes that affect the phenotypic expression of another gene, and several studies have demonstrated that modifier genes are involved in manifestations of inherited disorders.[6] *KRT6A*, *KRT6B*, *KRT16* and *KRT17*, the causative genes of PC, which affects the nails and the palmoplantar area, were selected as candidates for modifier gene investigation in our case, although we cannot exclude the possibility that there are some other genes which modify KID syndrome phenotype.

Most of the keratin mutations are within the helix boundary motifs, which are crucial for keratin monomers to form dimers and subsequent keratin networks.[7] The KRT17 mutation found in the proband was located not within the helix boundary motifs but in the V1 region of K17 (Fig. 2d). In other keratin genes, such as KRT5 and KRT16, some mutations have been reported within the V1 region, and the phenotypes resulting from these mutations are milder than those resulting from the mutations within the helix boundary motifs.[7] The V1 regions of keratin intermediate filament have glycine loops[8] and it has been suggested that these structures modulate flexibility and other unknown physical attributes of keratin filaments by interacting with similar structures in loricrin.[9] Ser^{59} is located within a highly conserved segment composed of the glycine loop in K17 (Fig. 2e). p.Ser59Arg in K17 is predicted to be probably damaging by PolyPhen-2, with a score of 0·893.[10]

Based on these findings, it is conceivable that the p.Ser59-Arg variant in K17 has a modifying effect on the pathogenic

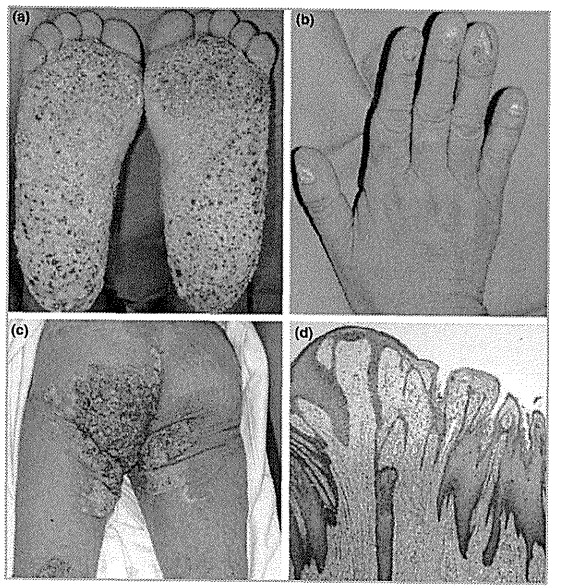

Fig 1. Clinical features of the proband. (a) Numerous erosive papules are coalesced into a hyperkeratotic plaque on the proband's soles. (b) Nail dystrophy is seen in the fingers. (c) A tumour is observed on the left buttock. Scars after skin abrasion are seen on the dorsal aspects of the thigh and on the right buttock. (d) Specimens from the tumour show pseudocarcinomatous hyperplasia of the epidermis. Dilated vessels with monocytic infiltrates are seen in the dermis (haematoxylin and eosin; original magnification × 100).

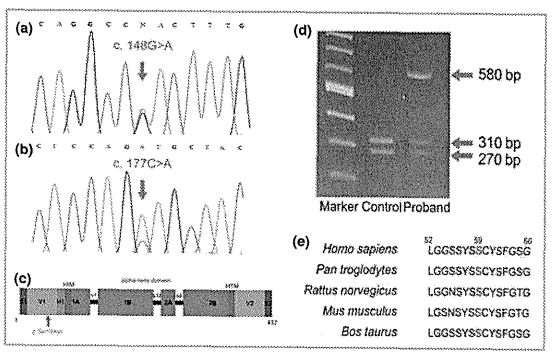

Fig 2. Mutation analysis. (a) The proband was heterozygous for a c.148G>A transition (p.Asp50Asn) mutation in GJB2 (arrow). (b) c.177C>A (p.Ser59Arg) in KRT17 was detected in the proband's genomic DNA (arrow). (c) PvuII restriction enzyme digestion of the polymerase chain reaction (PCR) products from genomic DNA of the proband and a normal control. c.177C>A resulted in the loss of a site for PvuII. PvuII restriction enzyme digestion of the PCR products from a normal controls reveals 270- and 310-bp bands. In contrast, 270-, 310- and 580-bp bands were detected in the proband, suggesting that she was heterozygous for c.177C>A. (d) A schematic of the structure of keratin 17. Note that Ser^{59} is located at the V1 region of the keratin molecule (arrow). HIM, helix initiation motif; HTM, helix termination motif. (e) Keratin 17 amino acid sequence alignment shows the level of conservation in diverse species of the amino acid Ser59 (red characters).