Supplemental data:

METHODS

Patients and data collection

We systematically searched the PubMed database (NCBI) for patients with XR-EDA-ID who were hemizygous for hypomorphic *IKBKG* mutations. We then sought reports of patients fitting these criteria who had undergone HSCT, for further analysis[1,8,9,11,13-27,29,30,32,40-42]. Additional data were obtained from the corresponding authors, via a detailed questionnaire. We also collected unpublished cases from around the world, by asking physicians known to offer HSCT to PID patients to complete the same questionnaire, after sending broadly based e-mail requests. Clinical and laboratory data were collected retrospectively for the patients, from their birth until January 2017, or death if they died before this date. The demographic and clinical data collected before HSCT included ethnicity, country of residence, age at symptom onset, viral, bacterial and fungal infections (age at infection, pathogen identification and outcome), autoimmune manifestations (cytopenia, organ-specific lesions), rash or eczema, failure to thrive, colitis and gastrointestinal symptoms, signs of EDA, osteopetrosis, and lymphedema. Laboratory data collected before HSCT included IKBKG exon sequence, serum immunoglobulin (IgG, A and M) levels, specific antibody levels post-immunization, $CD4^+$ T-cell, $CD8^+$ T-cell, B-cell, and NK-cell counts, T-lymphocyte proliferation assays (in response to mitogens and recall antigens) in all patients, and NK cell activity in some patients.

Conditioning regimen (drugs, doses and toxicity) were reviewed and classified as:

- Classical myeloablative regimens (MAC) with cyclophosphamide, busulfan and anti-T-cell globulins (ATG).
- Reduced intensity conditioning regimens (RIC) based on fludarabine with busulfan (total dose < 8mg/kg) or melphalan and ATG or anti-CD52 monoclonal Abs.

Conditioning regimen associating fludarabine and total body irradiation (total dose 2 grays) was also classified as RIC.

HSCT source (bone marrow, peripheral blood or cord blood stem cells), donor type (matched related, matched unrelated, or haploidentical donor), number of $CD34^+$ cells/kg or mononuclear cells (MNC)/kg and graft versus host disease (GVHD) prophylaxis were reviewed. For patients for whom the donor was a sister or a mother, her *IKBKG* genotype was collected, and in the case of carriers, information was requested about X inactivation in leukocytes and clinical status. Various elements were recorded after engraftment, including the number of days taken to reach an absolute polymorphonuclear neutrophil count (ANC) above 500/mm^3, symptoms/evidence of GVHD, leukocyte chimerism at least 60 days post-HSCT or at the most recent follow-up visit, and clinical outcome (alive or dead, infections, colitis, inflammation, EDA, and other potential manifestations of EDA-ID). The use of unconditioned stem cells for infusion from the same donor (boost infusions) and second transplantations were also recorded. The symptoms and signs of heterozygous mothers and female carrier donors were also reviewed (signs of IP, dental abnormalities, infectious and autoimmune diseases).

Statistical analysis

Descriptive statistics are presented for the biological and clinical characteristics of the patients. Categorical variables are described in terms of frequencies and rates. Numerical variables are described in terms of means, standard deviations, 95% confidence intervals, median and range. Quantitative data were analyzed in one-tailed Mann-Whitney tests. Frequencies were analyzed in two-tailed Fisher's exact tests. Survival curves were estimated by the Kaplan Meier method, and, when necessary, curves were compared in log-rank tests. For all statistical tests, *p* values below 0.05 were considered significant. Statistical analyses were performed with Prism 5.0b (GraphPad Software, Inc.).

RESULTS

Hemizygous *IKBKG* mutation and functional NF-kB assays

The mutations were not compared with each other, in terms of deleteriousness. Thirteen different missense hemizygous mutations were identified. One of these mutations, D113N (P22), was predicted not to be deleterious by Polyphen2 and SIFT software. However, functional assays revealed impaired NK cell cytotoxicity, low levels of T-cell proliferation and impaired NF-κB activation after TCR ligation[8,9]. Moreover, a female patient heterozygous for this mutation had already been reported with an IP phenotype and skewed X inactivation[44].

Functional assays specifically testing the NF-κB pathway were performed in 17 patients (P3, 5-11, 15, 16, 18, 19, 22, 24, 26-28). TNF-α production by peripheral blood mononuclear cells (PBMCs) after stimulation with TLR ligands (mostly LPS) was impaired in 13 of the 14 patients tested, as was IκBα degradation (6/6). IκBα degradation was assessed in fibroblasts (P4 and P5), PBMCs (P3 and P15), EBV-B cells (P28) or lymphocytes (P19), after stimulation with TNF-α, anti-CD40 antibody or PHA[1,24,27,29,45]. PBMCs from P16 displayed impaired TCR signaling, but no defect of TLR signaling[8,9]. The secretion of IFN-γ secretion by PBMCs after TNF-α stimulation, lymphocyte proliferation after CD40L stimulation and DNA binding by NF-κB were also found to be impaired in the patients in whom they were tested (6/6, 3/3 and 3/3 patients tested, respectively). The binding of NF-κB to DNA was assessed by electrophoretic mobility shift assays on fibroblasts (P8), preparations enriched in B cells (P5) and EBV-transformed B cells (EBV-B cells) (P28)[1,11,27]. No functional assays were performed for 12 children, three of whom carried previously described mutations.

Supplemental legends:

Supplemental Figure 1: Survival of NEMO-deficient patients after HSCT

S1a: Survival after HSCT, for patients with (dotted line) and without (black line) GVHD after HSCT. **S1b:** Survival after HSCT, by age. The black line corresponds to patients under the age of two years, and the dotted line corresponds to patients over the age of two years. **S1c.** Survival after HSCT, for patients with (dotted line) and without (black line) viral infection before HSCT. **S1d.** Survival after HSCT, for patients with (dotted line) and without (black line) fungal infection before HSCT. **S1e.** Survival after HSCT, for patients with (dotted line) and without (black line *Pneumocystis* infection before HSCT. **S1f.** Survival after HSCT for patients with mutations affecting the NEMO protein zinc finger domain (black line) and other patients (dotted line).

Discussion (Supplemental Figure): Acute GVHD had no effect on survival rate (survival of 83.2% at 108 months for children with GVHD, versus 76.9% at 104 months for children without GVHD, p=0.69) (**Supplemental Figure S1a**). Age at transplantation also did not seem to influence survival: 87.5% at 108 months for children under the age of two years at HSCT and 74.4% at 108 months for older children (p=0.69) (**Supplemental Figure S1b**). There was no significant difference if the cutoff age was set at five years (survival rate: 77.2% at 108 months for children under the age of five years at HSCT and 77.8% at 104 months for older children; p=0.69, data not shown)[47,48]. The children who died were older at HSCT than the surviving patients, but this difference was not statistically significant (mean ages at HSCT of 6.88 and 4.27 years, respectively, p=0.25). Infection with viruses or fungi before HSCT had no effect on survival after this procedure (survival of 72.2% at 104 months for infected

children, versus 75% at 108 months for uninfected children, p=0.82, for viruses; 57% vs. 79%, respectively at 108 months, p=0.17, for fungi; and 83.3% at 104 months vs. 72% at 108 months, respectively, p=0.90, for *Pneumocystis*) **(Supplemental Figure 1d-e)**.

Supplemental Table 1: Comparison of the characteristics of NEMO-deficient patients between our cohort and that of Hanson *et al*[5].

	Patients of this series	**Patients of Hanson's series**	***p* value**
Mutation affecting the NEMO ZF domain	62% (18/29)	53% (38/72)	0.51
EDA	89% (24/27)	77% (40/52)	0.24
O	21% (6/28)	7.5% (5/65)	0.08
L	18% (5/28)	10% (6/65)	0.29
Pyogenic infections	92% (25/27)	86% (45/52)	0.71
Mycobacterial infection	57% (16/28)	44% (23/52)	0.35
Chronic viral infection	57% (16/28)	19% (11/52)	**0.0025*****
Inflammatory symptoms	89% (24/27)	23% (15/61)	**<10^{-4}*****
IBD	60% (15/25)	21% (13/61)	**0.0009*****
IV IgG replacement	89% (25/28)	50% (29/58)	**0.0003*****
Prophylaxis against *Pneumocystis*	79% (22/28)	19% (11/58)	**<10^{-4}*****

EDA: ectodermal anhidrotic dysplasia; O: osteopetrosis; L: lymphedema; IBD: inflammatory bowel disease; prophylaxis against *Pneumocystis* is based on trimethoprim-sulfamethoxazole.

Supplemental figure S1