IMMUNOLOGY: BRIEF REPORT

Diagnostic challenges for a novel SH2D1A mutation associated with X-linked lymphoproliferative disease

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Abstract
Mutations in SH2D1A, encoding the intracellular adaptor signaling lymphocyte activation molecule associated protein (SAP), are associated with X-linked lymphoproliferative disease type 1 (XLP1). We identified a novel hemizygous SH2D1A c.49G > A (p.E17K) variant in a 21-year-old patient with fatal Epstein-Barr virus infection–associated hemophagocytic lymphohistiocytosis. Cellular and biochemical assays revealed normal expression of the SAP variant protein, yet binding to phosphorylated CD244 receptor was reduced by >95%. Three healthy brothers carried the SH2D1A c.49G > A variant. Thus, data suggest that this variant represents a pathogenic mutation, but with variable expressivity. Importantly, our results highlight challenges in the clinical interpretation of SH2D1A variants and caution in using functional flow cytometry assays for the diagnosis of XLP1.

KEYWORDS
diagnostic assays, hemophagocytic lymphohistiocytosis, ITSM, NK cells, SAP, X-linked lymphoproliferative disease

1 | INTRODUCTION

X-linked lymphoproliferative disease type 1 (XLP1) is associated with hemizygous mutations in SH2D1A, encoding signaling lymphocyte activation molecule (SLAM) associated protein (SAP).1–3 XLP1 patients typically manifest with fulminant, life-threatening Epstein-Barr virus (EBV) infection fulfilling hemophagocytic lymphohistiocytosis (HLH) criteria, dysgammaglobulinemia, EBV-related lymphoproliferative disorders, or lymphoma.4,5 Hematopoietic stem cell transplantation (HSCT) represents the only curative treatment for XLP1, since survival of untransplanted patients is <20%.6

Abbreviations: EBV, Epstein-Barr virus; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; ITAM, immunoreceptor tyrosine-based activation motif; ITSM, immunoreceptor tyrosine-based switch motif; SAP, SLAM-associated protein; SLAM, signaling lymphocyte activation molecule; XLP1, X-linked lymphoproliferative disease 1.

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Expressed in T cells and NK cells, the SAP protein consists of a cytoplasmic SH2 domain that binds phosphorylated immunoreceptor tyrosine-based switch motifs (ITSM) of SLAM family receptors. EBV-infected B cells upregulate surface expression of CD48, a ligand for the SLAM family co-activation receptor 2B4 (CD244) expressed in cytotoxic T and NK cells. 2B4 signaling via SAP can trigger killing of target cells. Notably, the majority of SH2D1A mutations found in XLP1 patients are either nonsense mutations or missense mutations resulting in impaired protein stability. With respect to familial HLH, PRF1 mutations that decrease cytotoxic activity at 30°C by >95% or 80% are associated with early or later onset HLH, respectively. Furthermore, in setting of transplantation, >30% donor chimerism is sufficient to protect from HLH. The degree of loss in SAP function that may predispose to disease is not well defined.

Here, we identified and characterized a novel SH2D1A c.49G > A variant in a family with a fatal case of EBV-HLH/lymphoma, which also included three healthy hemizygous carriers. Importantly, our results highlight important challenges in the diagnosis of XLP1.

2 | RESULTS

A 21-year-old Canadian male presented initially with prolonged fever, pancytopenia, and a high EBV load, which persisted for 6 months despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy. He had no previous history of disease, despite antiviral therapy.
FIGURE 2  Expression and functional analysis of the SAP p.E17K variant. (A, B) PBMC were incubated with target cells and antibodies, as indicated. The cells were stained and analyzed by flow cytometry. Degranulation was quantified on the basis of CD107a surface expression. (A) Each dot represents PBMC from independent local controls, transported II-2, II-3, II-5 samples (hemizygous for the SH2D1A c.49G>A p.E17K variant), or transported control samples, as indicated. (B) Plot shows degranulation of gated SAP+ or SAP– NK cells in female carriers of a SH2D1A c.163C>T (p.R55X) mutation associated with XLP1. (C) 293T cells were transfected with FLAG-2B4, SAP wild-type, or SAP p.E17K variant constructs, as indicated (+). The cells were stimulated or not stimulated with sodium pervanadate (PV). Cell lysates were subjected to immunoprecipitation (IP) or whole cell lysates (WCL) probed directly by Western blotting (IB) with antibodies, as indicated. Blots are representative of three independent experiments. (D) Densitometric quantification of SAP wild-type or p.E17K variant binding to FLAG-2B4. Values represent binding relative to SAP wild-type. Data are representative of three independent experiments.

presence of hemophagocytosis, autopsy also revealed EBV-associated T-cell lymphoma in spleen, bone marrow, and lymph nodes. Although recommended,13 a molecular basis of lymphoma was not established. No family history of EBV-HLH was reported. Genetic screening of XLP-associated genes revealed a novel hemizygous SH2D1A c.49G>A (p.E17K) variant in the patient (Figure 1A,B). The SAP p.E17K amino acid substitution, positioned in the first α-helix of the SH2 domain (Figure 1C), was predicted “probably damaging” by PolyPhen-2 and “damaging” by SIFT, and was not found in the 1000 Genomes database. Three healthy brothers also carried the variant (Figure 1B). At the time of the initial workup, all three brothers and one sister were negative for EBV on serologic testing. The oldest brother subsequently developed infectious mononucleosis from which he recovered completely. Moreover, the second oldest brother also has seroconverted without complications. The youngest brother remained EBV seronegative. All three brothers lacked iNKT cells, indicative of SAP deficiency.14 Intriguingly, SAP expression was normal in peripheral blood T and NK cells of the hemizygous SH2D1A c.49G>A carriers (Figure 1D).

As the pathogenicity of the SH2D1A c.49G>A variant was unclear, we first used a functional assay recently described for the diagnosis of XLP1.15 Although co-engagement of 2B4 and immunoreceptor tyrosine-based activation motif coupled receptors such as NKp46 typically results in synergistic NK cell activation,16 Meazza et al reported diminished NK cell degranulation in XLP1 patients upon 2B4 and NKp46 co-engagement.15 However, NK cells from the brothers carrying the hemizygous SH2D1A c.49G>A variant displayed synergistic activation upon co-engagement of 2B4 and NKp46 (Figure 2A). To determine whether the hemizygous carriers of the
SH2D1A c.49G > A mutation in some way were atypical for XLP1 or the degranulation assay lacks sensitivity for XLP1, we also examined 2B4 and Nkp46 co-stimulation of NK cell degranulation in female carriers of a SH2D1A c.163C > T (p.R55X) mutation, which is associated with XLP1 and abolishes SAP expression. In female carriers of this mutation, approximately half of the NK cells are SAP-deficient due to X-linked inactivation. In six female carriers, 2B4 and Nkp46 co-stimulation-triggered degranulation varied among individuals and degranulation was only mildly decreased when comparing SAP− and SAP+ NK cell subsets (Figure 2B), questioning the clinical diagnostic efficacy of the posited rapid degranulation assays for the diagnosis of XLP1.15

The SAP p.E17K substitution results in a change from a negatively to a positively charged residue located outside the phospho-ITSM binding pocket of SAP. We predicted that this change could disrupt the three-pronged ITSM-recognition mechanism whereby SAP recognizes the Thr residue at position −2 of the ITSM,17 similar to other SAP mutations that disrupt this interaction.18–20 To assess our hypothesis, SAP wild-type and p.E17K variant were expressed in 293T cells. Both forms of SAP were readily expressed (Figure 2C). To test binding of SAP to SLAM family receptors, transfected cells were stimulated with sodium pervanadate to preserve phosphorylation of ectopically expressed FLAG-tagged 2B4. Following immunoprecipitation of FLAG-2B4, SAP co-immunoprecipitation was assessed (Figure 2C). As expected, neither SAP wild-type nor SAP E17K variant bound 2B4 in unstimulated 293T cells. However, SAP wild-type bound phosphorylated 2B4 in pervanadate-stimulated 293T cells, whereas the SAP E17K variant did not. Collectively, results indicated a strong impairment of SAP E17K variant binding to phosphorylated 2B4 (96 ± 2.5% relative impairment, mean ± SD; Figure 2D). Taken together, these demonstrate that SAP E17K is stable, yet unable to bind SLAM family receptors.

3 | DISCUSSION

We report a patient with fatal EBV-HLH/lymphoma carrying a novel, hemizygous SH2D1A c.49G > A variant. Highlighting diagnostic challenges for the diagnosis of XLP1, the three brothers carrying the mutation displayed normal SAP expression as well as SLAM-family receptor-mediated co-activation, yet lacked iNKT cells. Nonetheless, mutation displayed normal SAP expression as well as SLAM-family receptor expression. In female carriers of this mutation, approximately half of the NK cells are SAP-deficient due to X-linked inactivation. In six female carriers, 2B4 and Nkp46 co-stimulation-triggered degranulation varied among individuals and degranulation was only mildly decreased when comparing SAP− and SAP+ NK cell subsets (Figure 2B), questioning the clinical diagnostic efficacy of the posited rapid degranulation assays for the diagnosis of XLP1.15

An interesting remaining question is follow-up and treatment of the three male siblings, currently aged 16 to 26, carrying the SH2D1A c.49G > A mutation. During the course of the study, two brothers have seroconverted without developing HLH or lymphoma. In view of the fatal course of the index patient, consideration was given to allogeneic HSCT on all three siblings but despite many consultations worldwide, no consensus could be obtained with regard to putting three healthy young males through allogeneic HSCT. Our study of this family and studies of similar families can hopefully be informative in terms of understanding to what degree hypomorphic SH2D1A mutations predispose to HLH.

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