**Brief report**

**SH2D1A mutations in Japanese males with severe Epstein-Barr virus–associated illnesses**

Ryo Sumazaki, Hirokazu Kanegane, Maki Osaki, Takashi Fukushima, Masahiro Tsuchida, Hiroyoshi Matsukura, Kentaro Shinozaki, Hiroshi Kimura, Akira Matsui, and Toshio Miyawaki

X-linked lymphoproliferative disease (XLP), a genetic disorder characterized by immunodeficiency to Epstein-Barr virus (EBV) infection, has been linked to mutations in the **SH2D1A** gene. To search for the occurrence of **SH2D1A** mutations in Japan, we performed genetic analysis of the **SH2D1A** gene in 40 males presenting with severe EBV-associated illnesses, including fulminant infectious mononucleosis, EBV-positive lymphoma, and severe chronic active EBV infection. **SH2D1A** mutations were detected in 10 of these 40 patients. Five of these 10 cases were sporadic. Patients with **SH2D1A** mutations displayed severe acute infectious mononucleosis with hyperimmunoglobulinemia M, hypogammaglobulinemia, and B-cell malignant lymphoma. By contrast, chronic active EBV infection was not associated with **SH2D1A** mutations. XLP survivors exhibited normal levels of circulating EBV-DNA during convalescence, suggesting that **SH2D1A** protein is not directly responsible for control of EBV replication. Thus, genetic analysis of the **SH2D1A** gene is particularly useful in the diagnosis of sporadic cases and carriers of XLP. (Blood. 2001;98:1268-1270)

**Introduction**

X-linked lymphoproliferative disease (XLP, MIM 308240) is an inherited immunodeficiency characterized by extreme vulnerability to Epstein-Barr virus (EBV).1 About two-thirds of the patients develop fatal infectious mononucleosis (IM), and others manifest a diverse phenotype that includes malignant lymphoma, dys gammaglobulinemia, aplastic anemia, vasculitis, or lymphomatoid granulation.2 Recently, an SH2-domain–encoding gene, **SH2D1A/SAP/DSHP**, was identified as the causative gene of XLP.3-5 Most, if not all, patients with XLP have **SH2D1A** gene mutations.6 This information permits direct molecular diagnosis of XLP in patients and female carriers.

The clinical diagnosis for XLP is often difficult, especially in sporadic cases, because of the diverse presentation and the lack of unique findings in XLP. For example, fatal IM in XLP can be indistinguishable clinically and histologically from sporadic forms of virus-associated hemophagocytic syndrome.7 Sumegi et al6 reported that no **SH2D1A** mutations were detected in 25 males who manifested an XLP phenotype without a family history of XLP. Thus, it is not currently clear whether molecular diagnosis of XLP is applicable to patients with a sporadic XLP phenotype.

XLP patients have been reported from North America, Europe, the Middle East, and South America but, except for one recent case report,8 not from the highly populated countries of Asia.2 To search for the possible presence of XLP in Japan, we systematically screened 40 Japanese males with severe EBV-associated illnesses. The **SH2D1A** mutations were detected in 10 cases. Notably, 5 of these 10 cases had no family history of XLP.

**Study design**

**Patients**

Forty Japanese boys with severe EBV-associated illnesses were studied. All patients, except for 2 brothers, were unrelated. Five patients, including the 2 brothers, had a family history supporting an X-linked inheritance for the XLP phenotype. The study population included 3 groups: (group 1) 18 patients who had experienced severe acute IM (ages 0-17 years); (group 2) 5 patients with EBV genome–positive lymphomas (ages 0-8 years); and (group 3) 17 patients with severe chronic active EBV infection (CAEBV) (ages 1-12 years). The cases in group 1 met the diagnostic criteria for hemophagocytic lymphohistiocytosis9 or died immediately following an acute IM-like illness. All of these patients harbored the EBV genome in tissue specimens or displayed a serologic response suggestive of primary EBV infection: antibody positive for viral capsid antigen without detectable antibodies to EBV nuclear antigen. Lymphomas were defined as EBV-positive when the tissue harbored EBV genome detected by polymerase chain reaction or by in situ hybridization for EBV-encoded small RNAs.10 This group consisted of 3 B-cell lymphomas, including one Burkitt lymphoma, one Hodgkin disease, and one nasal lymphoma. CAEBV was diagnosed according to the previously described criteria.11,12 Briefly, these patients were characterized by prolonged or recurrent IM-like symptoms.

From the Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Japan; Department of Pediatrics, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Japan; Department of Pediatrics, Ibaraki Children's Hospital, Japan; and Department of Pediatrics, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Japan.

Submitted January 24, 2001; accepted April 17, 2001.

Supported by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and grants from the Ministry of Health and Welfare of Japan, the Tsukuba University Project, and the Naito Foundation.

R.S. and H.K. contributed equally to this work.

Reprints: Ryo Sumazaki, Dept of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, 305-8575, Japan; e-mail: rsuma@md.tsukuba.ac.jp.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2001 by The American Society of Hematology
for more than one year, with hepatosplenomegaly, lymphadenopathy, anemia, or cytopenia and with an unusual pattern of anti-EBV antibodies: high anti–viral capsid antigen and/or anti–early antigen, and low or absent anti-EBV nuclear antigen titers. None of these patients had an overt immunodeficient condition.

**SH2D1A mutation analysis**

Mutation analysis of the coding region in the SH2D1A gene was conducted on both complementary DNAs and genomic DNAs. Polymerase chain reaction conditions and primer sequences for amplifying the complementary DNA and the 4 exons of SH2D1A were as previously reported.4

**Results and discussion**

In a survey of 40 boys with various forms of severe EBV-associated illnesses, we identified 10 patients from 9 kindreds that harbored SH2D1A gene mutations. The characteristics of these XLP patients are summarized in Table 1.

Nonsense mutations (cases 1-3), large genomic deletion (case 4), smaller intragenic deletion (case 5), and splice site mutation (case 6) are expected to result in a truncated SH2D1A protein and, presumably, to a loss of proper SH2D1A function. All of the missense mutations (cases 7-10) are predicted to change the evolutionary conserved SH2 domain and may alter the interactions between the SH2D1A protein with the cell surface molecules SLAM4 and 2B414 on T and natural killer cells, respectively.

The nonsense mutation Arg55Stop has previously been reported as a hot spot for XLP mutations.6,15 The other 6 mutations noted here were novel and were not detected in unrelated control individuals screened from a total of 100 chromosomes. In case 4, sequencing of the complementary DNA revealed an aberrantly spliced product wherein the last 22 bases of exon 1 were deleted. In the genomic DNA, a synonymous substitution (416C>G)T was identified, suggesting that a silent mutation created the new splice site due to a high Senapathy score around the mutated codon. A similar type of mutation was previously reported in another disease.16

Five of the XLP patients (cases 3-5, 8, 10) had a negative family history suggestive for XLP. However, in those 3 sporadic cases (patients 4, 5, 10) where maternal blood was available for analysis, vertical transmission of the SH2D1A mutations was confirmed in cases 4 and 10. Until recently, XLP diagnosis rested on a family history of phenotypically affected male relatives in the maternal lineage, and XLP was considered to be extremely rare in Japan.2 The fact that half of the XLP patients in our series had no family history may explain this underestimation. Families with single affected XLP members may not be attributable to de novo mutations but to the scarcity of brothers resulting from the low birth rate in Japan. Indeed, the mutations found in the sporadic XLP cases were segregated within families. These results underscore the usefulness of a direct molecular diagnosis for XLP.

We examined the clinical features of the XLP. Nine of 10 mutations were detected in 18 patients with severe acute IM. One boy with EBV genome–positive Burkitt lymphoma also displayed a SH2D1A mutation. However, no SH2D1A mutations were detected in patients with CAEBV infection. The detection rate of SH2D1A mutation in patients with XLP phenotype varies (62%-97%) among previous reports.6,17 These proportions may be influenced by the existence of family history, family size, and each clinical symptom in the examined patients. The relatively low frequency of mutations in our series is likely due to the lack of family history for XLP in the majority of our study subjects.

### Table 1. Features of patients with SH2D1A mutation

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Clinical phenotype</th>
<th>Family history</th>
<th>Carrier detection by genetic diagnosis</th>
<th>SH2D1A mutation</th>
<th>Age at onset (y)</th>
<th>Dysgammaglobulinemia</th>
<th>Clinical history</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Fulminant infectious mononucleosis</td>
<td>Grandmother + brother of case 2</td>
<td>Deletion Exons 1-4</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 3: 584del(A) (frameshift)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Grandmother + brother of case 1</td>
<td>Deletion Exons 1-4</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 3: 584del(A) (frameshift)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Mother</td>
<td>Deletion Exons 1-4</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 3: 584del(A) (frameshift)</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Mother</td>
<td>Deletion Exons 1-4</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 3: 584del(A) (frameshift)</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>No carrier</td>
<td>Deletion Exons 1-4</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 3: 584del(A) (frameshift)</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Mother</td>
<td>Splice Exon 1: 416C &gt; T, Intron 1: 5’ splice site (del 22bp, frameshift)</td>
<td>ND</td>
<td>Splice</td>
<td>Exon 1: 416C &gt; T, Intron 1: 5’ splice site (del 22bp, frameshift)</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Mother Sister</td>
<td>Missense Exon 1: 396G &gt; T (Asp 33Tyr)</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 1: 396G &gt; T (Asp 33Tyr)</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>ND</td>
<td>Missense Exon 1: 378G &gt; A (Gly 27 Ser)</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 1: 378G &gt; A (Gly 27 Ser)</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>ND</td>
<td>Missense Exon 1: 321C &gt; G (His 8 Asp)</td>
<td>ND</td>
<td>Missense</td>
<td>Exon 1: 321C &gt; G (His 8 Asp)</td>
<td>3</td>
</tr>
</tbody>
</table>

ND indicates not done; PBSCT, peripheral blood stem cell transplantation; IVIG, intravenous immunoglobulins; EBV, Epstein-Barr virus; USCT, umbilical stem cell transplantation.

*This case was previously published.*
Malignant lymphomas develop in approximately 30% of XLP patients, but it is unclear whether EBV plays any role in the lymphomagenesis. Burkitt lymphoma in case 10 showed EBERs positive and t(8;14)(q24;q32), whereas Burkitt lymphoma in the brother of case 6 was EBERs negative. In addition, the latter demonstrated EBV-negative serology and negative results of EBV PCR using blood and saliva. It was recently reported that lymphoproliferative diseases occurred in EBV-uninfected XLP patients.6,17

Marked elevation of serum immunoglobulin M (IgM) and/or IgA levels or hypogammaglobulinemia was observed in all XLP cases. The onset of hypogammaglobulinemia was variable. In case 9, it developed following acute EBV infection. In case 6, it was found 4 years before fulminant IM developed. In case 10, it was noticed at the same time the EBV genome–positive lymphoma was diagnosed. These results suggest that hypogammaglobulinemia in XLP has a complex pathogenesis. By contrast, IgM elevations were noted in all cases with acute EBV infection, suggesting that hyper-IgM was related to the polyclonal activation of B cells induced by EBV. Thus, hypogammaglobulinemia and acute EBV infection with hyper-IgM were common clinical manifestations of SH2D1A mutations.

XLP patients sometimes have chronic symptoms or recurrent EBV infection.18 To assess whether active EBV replication persists in XLP patients, we quantified circulating EBV load19 in the XLP survivors and compared the results with those from patients with acute severe EBV infection (Table 2). The survivors carried normal levels of EBV-DNA copies during the convalescence phase. Recent studies have provided evidence suggesting that signal-transduction through 2B4, SH2D1A-associated receptor, was impaired in XLP patients and was indispensable for natural killer cell cytotoxicity.20,21 Our results demonstrated that XLP patients could successfully suppress EBV replication after primary EBV infection, suggesting that the SH2D1A protein function is not required for proper control of EBV replication after primary infection.22

Acknowledgments
We thank Dr Eiichi Ishii, Takeshi Shichijo, Sadao Suga, Kazumi Yamato, Katsuhide Ohta, Tadashi Matusubayashi, Masahiro Kikuchi, and Mika Makita for providing us with the specimens and clinical information on the patients and Reiko Hirochika for technical assistance. We are also indebted to Drs Tadao Arinami and Giovanna Tosato for critical discussion.

References


SH2D1A mutations in Japanese males with severe Epstein-Barr virus–associated illnesses

Ryo Sumazaki, Hirokazu Kanegane, Maki Osaki, Takashi Fukushima, Masahiro Tsuchida, Hiroyoshi Matsukura, Kentaro Shinozaki, Hiroshi Kimura, Akira Matsui and Toshio Miyawaki