Brief report

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

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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 hyperimmunoglobulin 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)

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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, dysgammaglobulinemia, 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 al[6] 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 lymphoma (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 lymphohistiocytosis[9] 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.[11-13] Briefly, these patients were characterized by prolonged or recurrent IM-like symptoms

for more than one year, with hepatosplenomegaly, lymphoadenopathy, 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.

SH2DIA mutation analysis

Mutation analysis of the coding region in the *SH2DIA* 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 *SH2DIA* 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 evolutionarily conserved SH2 domain and may alter the interactions between the SH2D1A protein with the cell surface molecules SLAM [4] and 2B4 [14] 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>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

Clinical phenotypes		Carrier detection by genetic diagnosis	SH2D1A mutation		Age at onset (y)	Dysgammaglobulinemia	Clinical history
Case no.	Family history		Type	Nucleotide/(protein)		IgG/IgA/IgM	
Fulminant infectious mononucleosis							
1	+ brother of case 2	Grandmother Mother	Nonsense	Exon 2: 462C > T (Arg 55Stop)	2	1190/555/2010	Died after 19 days
2	+ brother of case 1	Grandmother Mother	Nonsense	Exon 2: 462C > T (Arg 55Stop)	2	1661/756/1296	Died after 20 days
3	−	ND	Nonsense	Exon 2: 462C > T (Arg 55Stop)	3	1502/126/3604	Died after 18 days
*4	−	Mother	Deletion	Exons 1-4	1	1570/543/1200	Died after 21 days
5	−	No carrier	Deletion	Exon 3: 584del(A) (frameshift)	1	1548/827/700	Died after 34 days
6	+ 1 brother with EBV-negative lymphoma	Mother	Splice	Exon 1: 416 C > T Intron 1: 5′ splice site (del 22bp, frameshift)	2	938/635/926	Died after 21 days
7	+ 1 brother with hypogammaglobulinemia and lymphoma	Mother Sister	Missense	Exon 1: 396G > T (Asp 33Tyr)	8	hypogammaglobulinemia since 4 years old	In remission after chemotherapy followed by PBSCT
8	−	ND	Missense	Exon 1: 378G > A (Gly 27 Ser)	1	1002/283/322	Died after about 1 month
9	+ 1 brother with hypogammaglobulinemia	ND	Missense	Exon 1: 321C > G (His 8 Asp)	3	1280/214/638 → hypogammaglobulinemia	Stable at 17 years old under IVIG
EBV-associated lymphoma							
10	−	Mother	Missense	Exon 2: 445G > T (Gly 49 Val)	3	51/4/24	In remission after chemotherapy USCT planned

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.[8]

Table 2. Epstein-Barr virus loads in serum from patients with X-linked lymphoproliferative disease

Case no.	Clinical phenotype	Time from disease onset	Copy number of EBV (copies/mL of serum)
Acute periods			
1	Fulminant IM	10 days	5.3×10^6
2	Fulminant IM	18 days	3.2×10^5
3	Fulminant IM	18 days	1.3×10^5
5	Fulminant IM	34 days	3.0×10^4
Convalescence periods			
9	Fulminant IM	14 years	$< 2.0 \times 10^2$
10	EBV-associated Burkitt lymphoma (under chemotherapy)	4 months	$< 2.0 \times 10^2$
		7 months	$< 2.0 \times 10^2$

IM indicates infectious mononucleosis. Other abbreviations are explained in Table 1.

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 load[19] 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]

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