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Frequent Mutations in *SH2D1A* (XLP) in Males Presenting With High-Grade Mature B-Cell Neoplasms

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Abstract

X-linked lymphoproliferative syndrome (XLP) is caused by mutations in *SH2D1A*, and is associated with overwhelming infectious mononucleosis, aplastic anemia, hypogammaglobulinemia, and B-cell lymphomas. However, the frequency of *SH2D1A* mutations in males who present with B NHL is unknown. Five cases of XLP were diagnosed among 158 males presenting with B NHL (approximately 3.2%). Four of the patients had two episodes of B NHL and one had a single episode of B NHL followed by aggressive infectious mononucleosis. Prospective screening for XLP in males with B-cell lymphoma at the time of initial diagnosis should be considered.

Keywords

B cells; EBV; lymphoma; XLP

INTRODUCTION

X-linked lymphoproliferative disease (XLP) is a sex-linked immunodeficiency syndrome estimated to occur in one of every million males born in the United States each year [1–5]. However, because the clinical signs and symptoms are highly variable, it is likely that the diagnosis of XLP is frequently missed. Fulminant infectious mononucleosis (FIM) at <5 years of age is the most common presenting finding, but an increased incidence of hypogammaglobulinemia, aplastic anemia, and B-cell lymphoma in older patients is well described. Most cases are diagnosed by 10 years of age, although some have gone undiagnosed until the 5th or 6th decade [6].

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Although XLP is estimated to increase the risk of high-grade mature B-cell lymphoma by a factor of 200 [2,6–9], the frequency of lymphoma in males with XLP has not been confirmed [10,11], neither has the frequency of *SH2D1A* mutations in males with high-grade mature B-cell lymphomas been established. Although a study of 60 Burkitt lymphoma (BL) cell lines and another of 12 BL tumor samples found no mutations in the *SH2D1A* gene [12], our institutional data suggest a relatively high frequency of *SH2D1A* mutations in males with high-grade mature B-cell lymphomas. Here we review that experience in the diagnosis and management of XLP in five males who presented with BL or diffuse large B-cell lymphoma (DLBCL; Table I).

PATIENTS AND METHODS

The registry of males with a diagnosis of high-grade mature B-cell lymphoma (either BL or DLBCL) who were first seen at our institution between 1984 and 2002 were reviewed to identify those with a clinical history suggestive of XLP. Those with a clinical history suggestive of XLP were screened for the *SH2D1A* mutation; others were not. Institutional review board approval was obtained for this retrospective study.

Southern blot analysis and/or PCR/sequencing were used for mutation detection. Briefly, 200–400 ng of genomic DNA was used to amplify each exon of *SH2D1A* in a 50- μ l reaction volume containing: 4 ng/ μ l of each forward and reverse primer, 0.64 mM dNTPs, 1 $\times$ Qiagen buffer, 1 $\times$ Q Solution, and 0.5 μ l Qiagen Taq polymerase (Qiagen, Inc., Valencia, CA) for 30 cycles with a denaturing temperature of 95°C, annealing temperature of 54°C, and extension temperature of 72°C. PCR amplicons were separated on a 2% agarose gel, and bands were purified by using a Qiaex II gel extraction kit (Qiagen, Inc.). Purified products were sequenced by using a BigDye Terminator Sequencing Kit, ver 1.1 on an ABI3100 genetic analyzer (Life Technologies, Foster City, CA). If a mutation was detected in one of the exons, the entire procedure was repeated to rule out an induced mutation resulting from enzyme infidelity during amplification.

RESULTS

Study Population

During the study period, 120 males were diagnosed with BL and 7 with DLBCL. Sixty-three males were diagnosed with large-cell lymphoma, most during earlier treatment eras when immunophenotyping was not routinely included in lymphoma evaluation at our institution. Because approximately 50% of large-cell lymphomas in children have a B-cell immunophenotype, we estimated that 31 of these 63 males were likely to have had a B-cell immunophenotype. Thus, we estimated that approximately 158 males with high-grade B-cell lymphoma (BL or DLBCL) [13] were referred to our institution between 1984 and 2002.

Clinical and Pathological Features

Among 158 males with the diagnosis of either BL or DLBCL, 5 (3.2%) were suspected to have XLP on the basis of either two separate episodes of B-cell lymphoma ($n = 4$) or B-cell lymphoma followed by FIM ($n = 1$). All five were found to have large deletions, stop codons or splice defects in *SH2D1A*. Clinical characteristics are summarized in Table I and

in the Supplementary Appendix [13–20]. The remaining 153 males were not screened for this mutation. Thus, we estimated a 3% (5 of 158) frequency of XLP among males with BL or DLBCL during the study period at our institution. If all cases of large cell lymphoma had been immunophenotyped, this result could range from 2.6% to 3.9%.

DISCUSSION

The 3% frequency of *SH2D1A* mutations that we observed in males who presented with high-grade mature B-cell lymphomas during the 18-year study period was surprisingly high. Only males with a clinical picture suggestive of XLP were screened. The long delay (11 years in one of our patients) that may separate the initial lymphoma diagnosis and the development of FIM or second lymphoma suggests that others in our study population may be at risk. These data highlight the need to consider XLP in any male who develops recurrent lymphoma beyond 1-year from the time of diagnosis; it may not simply be a case of late relapse in an otherwise normal healthy patient. The XLP status of a male with a late B-NHL relapse may influence future management plans. In the setting of a late relapse, HSCT is considered in males with XLP, whereas HSCT may not be necessary in a patient lacking the XLP mutation.

The types of mutations observed in patients with XLP have been extensively studied. In one review of XLP families, 34 of 35 had members who carried mutations in the *SH2D1A* gene [8]. The most common abnormality was deletion, including complete loss of the *SH2D1A* gene and intragenic deletions resulting in frameshifts. Other abnormalities included single-base changes leading to splice-site, nonsense, and mis-sense mutations. In our study, the range of abnormalities included deletion of exon 1, an A to G substitution at the –2 position of the splice acceptor site for intron 1, a G to A single base substitution in codon 64 (Trp64stop), and deletion of the entire *SH2D1A* gene; these findings are consistent with other reports [8].

The results of our study have important therapeutic implications and suggest that prospective screening for *SH2D1A* mutations should be considered for males who present with a mature B-cell lymphoma. The only curative therapy for XLP is allogeneic HSCT [5,15]. Patients with XLP are at increased, ongoing risk of FIM and high-grade B-cell NHL (BL and DLBCL). Therefore, allogeneic HSCT should be considered as early as possible. If an *SH2D1A* mutation is identified by prospective screening, the patient could be considered for allogeneic HSCT immediately after completing primary lymphoma therapy, rather than after the development of a second B-cell lymphoma or FIM. This would result in a reduction in both the amount of pre-HSCT chemotherapy and the associated risk of HSCT-related complications. Such a consideration is particularly important in this set of patients, who are already at increased risk of HSCT-related morbidity and mortality. In this regard, a reduced intensity HSCT was successful and well tolerated in patient number 5, and may be a consideration for other XLP patients. The early diagnosis of XLP would also allow earlier identification and treatment of affected male siblings and relatives (maternal cousins or uncles) with XLP. If shown to have an *SH2D1A* mutation before developing B-cell lymphoma or FIM, these individuals could be considered for HSCT, with reduced risk of associated complications.

In summary, *SH2D1A* mutations were identified in approximately 3% of males with B-cell NHL. Screening for *SH2D1A* gene mutations should be a consideration for males at the time of initial diagnosis of either BL or DLBCL.

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TABLE 1

Clinical Characteristics of Five Patients With XLP

Patient	Initial diagnosis	Treatment	Outcome	SH2D1A mutation	Relapse diagnosis	Treatment	Outcome	XLP sibling (yes/no)	Diagnosis
1	DLBCL (EBNA-)	CHOP 6MP/MTX	CR for 4 years	Gene deletion	DLBCL	CHOP, 6MP/MTX, and HLA-matched sibling HSCT	CCR 14 years	No	n/a
2	BL	Total B	CR for 11 years	Exon 1 deletion	FIM	Supportive, rituximab, and haploHSCT	Expired: liver failure EBV in CNS	Probable	NHL
3	BL	LMB89	CR for 4 years	A to G -2 splice acceptor site for intron 1	BL	LMB89 and haploHSCT	Expired post HSCT, respiratory failure	Yes	n/a
4	DLBCL (EBER-)	LMB89	CR for 3 years	G to A in codon 64 (Trp64stop)	DLBCL (EBER-)	LMB89 and unrelated donorHSCT	Expired post HSCT, pneumonitis	No	n/a
5	BL (EBER-)	LMB-89	CR for 6 years	Gene deletion	BL	LMB96 and unrelated donorHSCT	CCR 1 year post HSCT	No	n/a

The histological diagnoses included non-Hodgkin lymphoma (NHL), Burkitt lymphoma (BL), DLBCL, and fatal infectious mononucleosis (FIM); in some cases this result was not applicable (n/a).

The responses to therapy included complete response (CR) and continuous complete remission (CCR). Chemotherapy included 6-MP (6-mercaptopurine) and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). EBV studies included Epstein-Barr virus-encoded small RNA (EBER) and Epstein-Barr nuclear antigen (EBNA).