

Letter to the Editor

Epstein-Barr virus-independent diffuse large B-cell lymphoma in DNA ligase 4 deficiency

To the Editor:

DNA repair disorders predispose the development of primary immunodeficiency and lymphoma. Epstein-Barr virus (EBV)-associated B-cell lymphomas and precursor T-cell lymphoblastic leukemia/lymphomas are described in ligase 4-deficient (LIG4) patients, presenting with variable T- and B-cell immunodeficiency, radiosensitivity, microcephaly, developmental delay, and cytopenias.¹ We report on one of the first patients, 411BR, who carried a homozygous 833G>A/R278H mutation and 2 cooperating homozygous N-terminal single nucleotide polymorphisms.² Despite remaining well for many years, albeit with profound learning difficulties, persistent anemia, thrombocytopenia and lymphopenia, bronchiectasis, and combined immunodeficiency treated with immunoglobulin replacement (see Table E1 in this article's Online Repository at www.jacionline.org), he represented at 23 years of age with rapidly enlarging cervical lymphadenopathy. EBV was negative in blood by PCR. Biopsy revealed diffuse large B-cell lymphoma (DLBCL). Computerized

tomography with contrast showed bilateral nasopharyngeal masses with extensive cervical lymphadenopathy (Ann Arbor stage IIA disease). Because of radiosensitivity and cytopenias, dexamethasone, rituximab, and low-dose cyclophosphamide were given, with 1 dose of lomustine, an alkylating nitrosourea compound. The absence of a suitable donor precluded allogeneic hematopoietic stem cell transplantation and the patient died.

Right cervical nodule biopsy showed a diffuse infiltrate of large atypical lymphoid cells with vesicular nuclei, 1 or more prominent nucleoli, and scanty cytoplasm. Numerous mitotic figures were present, and there was prominent single cell death. Immunohistochemistry showed the large atypical cells to be B cells expressing CD20, CD79a, CD10, GCET1, and Bcl6, but not MUM1/IRF4, consistent with germinal center B-cell differentiation (Fig 1). Staining for Bcl2, CD5, and cyclin D1 was negative, as was *in situ* hybridization for EBV (Epstein-Barr virus-encoded small RNA). Unusually, the atypical cells expressed IgD, but not IgM, IgA, or IgG. Almost all were positive for Ki67; a minority showed nuclear p53 positivity, and a similar number expressed the p53 target p21(waf1/cip1), providing no evidence for *TP53* gene mutation. There was a sparse background population of

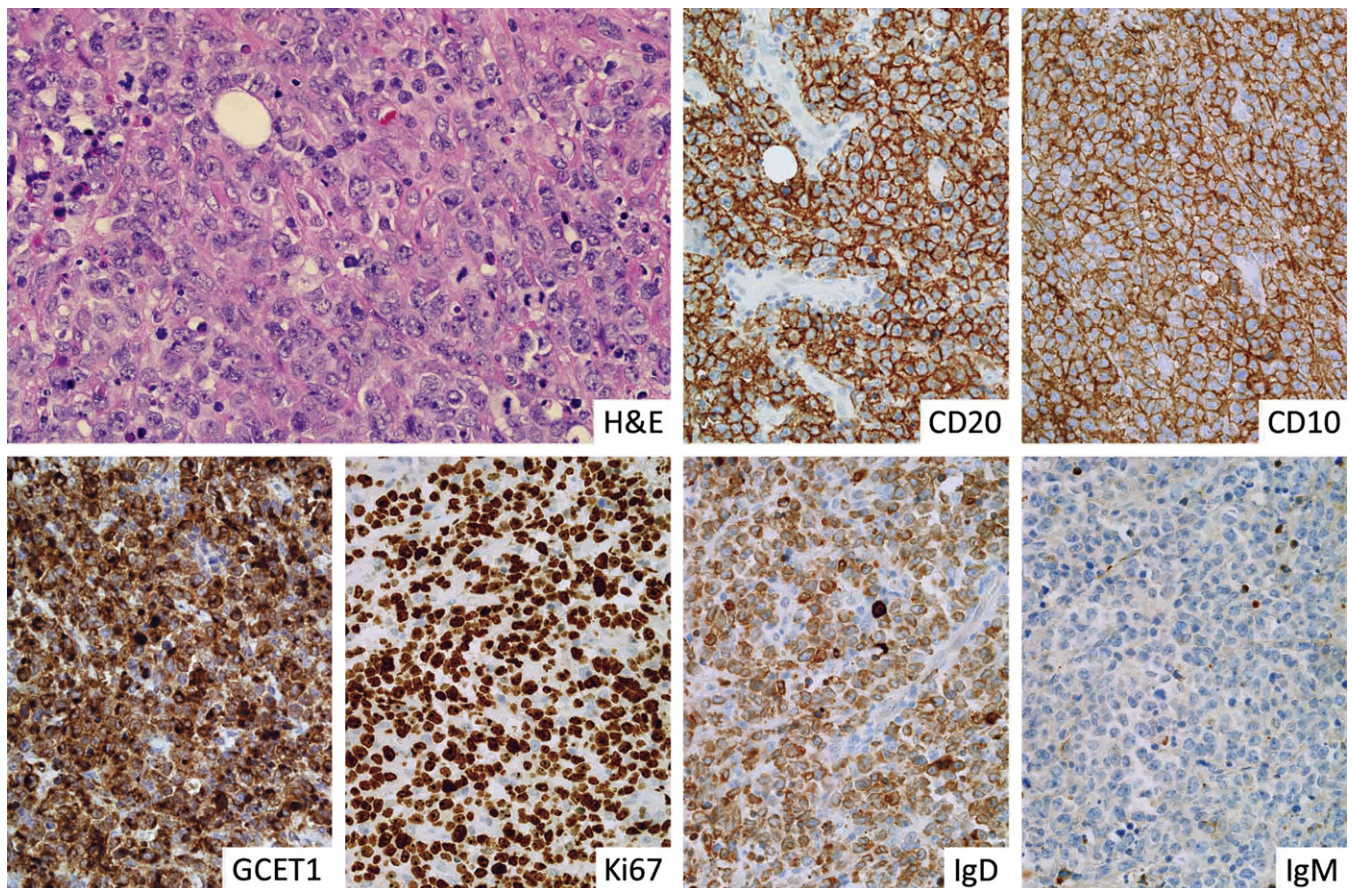


FIG 1. Diffuse large B-cell lymphoma, not otherwise specified. *Upper left:* Diffuse large atypical lymphoid cells with a high mitotic rate and abundant single cell death (hematoxylin and eosin stain). *Remaining panels:* Immunohistochemical staining showing neoplastic cells expressing CD20, CD10, and GCET1, consistent with a germinal center B-cell phenotype, and IgD but not IgM. The Ki67-positive proliferative fraction was greater than 95% of neoplastic cells.

small reactive T cells and macrophages; staining for CD21 showed no underlying follicular dendritic cell meshworks. The immunohistologic features were indicative of a nonspecific DLBCL. Interphase fluorescence *in situ* hybridization showed no translocations involving *IGH*, *IGK*, *IGL*, *BCL2*, *BCL6*, or *MYC* but revealed 3 copies of *IGL* in 65% of the nuclei assessed, possibly reflecting a clone of cells with trisomy 22. Array-based comparative genomic hybridization and fluorescence *in situ* hybridization identified numerous copy number changes (seen in other aggressive B-cell lymphomas) including trisomy of chromosomes 5, 6, 7, 12, 20, 21, 22, and X; gains of 1cen-q41, 2p16.1-p15, and 17q21.31-qter; and loss of 1p21.1-cen and *CDKN2A* at 9p21. Immunohistochemical staining for gamma-H2AX as a marker of DNA double-strand breaks showed diffuse, moderately strong positivity (also seen in some of the control DLBCL samples stained simultaneously); reactive lymphocytes were virtually negative.

LIG4 deficiency is associated with increased incidence of hematologic malignancies, described in at least 6 of 15 characterized patients. Two presented with precursor T-cell lymphoblastic leukemia/lymphoma (T-ALL/LBL).^{1,3} One (180BR) carried the same homozygous hypomorphic missense mutation (R278H) identified in 411BR. Mice homozygous for R278H or another hypomorphic mutation in the proximal adenylation domain similarly developed thymic precursor T-cell lymphomas.^{1,4,5} One adult patient had uncharacterized "myelodysplasia,"² and 4 developed B-cell lymphoproliferation during the course of their disease. In 2 nontransplanted children, one with a severe combined immunodeficiency phenotype, these were EBV-associated, clonal, DLBCLs.¹ A third child with severe combined immunodeficiency developed fatal EBV-associated lymphoproliferative syndrome 50 days after allogeneic hematopoietic stem cell transplantation.¹ The contribution of LIG4 deficiency to the development of lymphoproliferation by impairing immunity to EBV, rather than promoting oncogenic genomic changes through impaired DNA damage repair, is unclear. Nevertheless, a shared pathogenic mechanism is possible as all 3 patients harbored compound heterozygote hypomorphic mutations within the proximal adenylation domain, together with a truncating null K424FS mutation. The DLBCL reported herein differed from those described above in being EBV independent and presenting at an older age.

Classical nonhomologous end joining (NHEJ) deficiency in *Lig4*^{-/-} or *Xrcc4*^{-/-} murine B cells is associated with the development of chromosomal translocations of relevance to human B-cell lymphogenesis. Mice lacking *p53* and *Lig4* or *XRCC4* develop pro-B-cell lymphomas associated with RAG-induced *IgH* *JH-c-Myc* translocations.⁶ In mature murine B cells, deficiency in classical NHEJ is associated with a marked increase in activation-induced cytidine deaminase-induced *IgH* translocations, including *IgH-c-Myc* translocations, during class switch recombination.⁷ In each case, the translocations are mediated by 1 or more alternate-end joining pathways normally largely suppressed by competent classical NHEJ.⁷ We examined the lymphoma in this study for translocations of *IG* genes and the most common *IG* translocation partners (*BCL2*, *BCL6*, and *MYC*) but did not detect such events. The possibility that deficient classical NHEJ directly contributed to the pathogenesis of the lymphoma remains as classical NHEJ deficiency in murine B cells also results in an increase in non-*IgH* translocations. *Lig4* haploinsufficiency on a murine *cdkn2a*^{-/-} background results in tumors bearing clonal amplifications and deletions,

including some syntenic to regions altered in our patient (eg, +2p16.1-p15 harboring the oncogene *REL*).^{7,8}

The present lymphoma is unusual among DLBCLs in expressing IgD but not IgM. While a minority of germinal center-derived nodular lymphocyte predominant Hodgkin lymphomas and hairy cell leukemias, and occasional plasma cell myelomas, show a similar IgD-only phenotype, there was no evidence of these in the present patient. Approximately 1% to 3% of human B cells adopt an IgD⁺ IgM⁻ phenotype, probably as a result of homologous recombination involving nonclassical switch sequences within the cm-cd intron. These cells display restricted *VH* gene usage, undergo heavy somatic hypermutation and clonal expansion within germinal centers, and frequently produce autoreactive antibodies.⁹ The relationship of the lymphoma described herein to this B-cell subset, and the potential role of the reduced and altered class-switch recombination resulting from *LIG4* mutations in its pathogenesis, remains unknown.

Early diagnosis is critical for the optimum care of these patients. Detailed histopathologic examination of these tumors is important to increase the awareness of malignancy in primary immunodeficiencies with NHEJ machinery defects, and may inform on the role of the NHEJ complex, and the development of novel therapies. Efficacy of tumorigenic risk reduction through the provision of immunocompetent T cells and the removal of potentially oncogenic lymphoid precursors following hematopoietic stem cell transplantation, and subsequent potential long-term effects from conditioning regimens, needs careful evaluation.

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REFERENCES

- Slatter MA, Gennery AR. Primary immunodeficiencies associated with DNA-repair disorders. *Expert Rev Mol Med* 2010;18:12.e9.
- O'Driscoll M, Cerosaletti KM, Girard PM, Dai Y, Stumm M, Kysela B, et al. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell* 2001;8:1175-85.
- Plowman PN, Bridges BA, Arlett CF, Hinney A, Kingston JE. An instance of clinical radiation morbidity and cellular radiosensitivity, not associated with ataxia-telangiectasia. *Br J Radiol* 1990;63:624-8.
- Nijnik A, Dawson S, Crockford TL, Woodbine L, Visetnoi S, Bennett S, et al. Impaired lymphocyte development and antibody class switching and increased malignancy in a murine model of DNA ligase IV syndrome. *J Clin Invest* 2009;119:1696-705.
- Rucci F, Notarangelo LD, Fazeli A, Patrizi L, Hickernell T, Paganini T, et al. Homozygous DNA ligase IV R278H mutation in mice leads to leaky SCID and

- represents a model for human LIG4 syndrome. *Proc Natl Acad Sci U S A* 2010; 107:3024-9.
6. Zhu C, Mills KD, Ferguson DO, Lee C, Manis J, Fleming J, et al. Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations. *Cell* 2002;109:811-21.
 7. Boboila C, Jankovic M, Yan CT, Wang JH, Wesemann DR, Zhang T, et al. Alternative end-joining catalyzes robust IgH locus deletions and translocations in the combined absence of ligase 4 and Ku70. *Proc Natl Acad Sci U S A* 2010;107: 3034-9.
 8. Sharpless NE, Ferguson DO, O'Hagan RC, Castrillon DH, Lee C, Farazi PA, et al. Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions. *Mol Cell* 2001;8: 1187-96.
 9. Koelsch K, Zheng NY, Zhang Q, Duty A, Helms C, Mathias MD, et al. Mature B cells class switched to IgD are autoreactive in healthy individuals. *J Clin Invest* 2007;117:1558-65.

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TABLE E1. Immunologic and hematologic results prior to chemotherapy treatment

	Age (y)				
	14	15	18	20	23
IgG* (g/L)	13.3	17.3	10.9	19.2	10.8
IgA (g/L)	0.85	0.81	0.67	0.73	0.31
IgM (g/L)	0.41	0.46	0.45	0.55	0.23
CD3 cells/ μ L	216	265	297	324	109
CD4 cells/ μ L	58	73	69	93	63
CD8 cells/ μ L	136	180	192	195	35
CD16/56 cells/ μ L	94	101	132	148	85
CD19 cells/ μ L	3	<1%	<1%	0	0
Hb (g/dL)	15.7	10.6	9.7	12.1	8.0
WCC $\times 10^9$ /L	1.5	2.1	1.8	2.2	1.81
N $\times 10^9$ /L	1.00	1.37	1.04	1.33	1.14
L $\times 10^9$ /L	0.3	0.38	0.43	0.48	0.34
Plt $\times 10^9$ /L	40	55	48	53	45

Values in boldface are below the normal age-related range.

*Denotes value on immunoglobulin replacement.