RESEARCH LETTER

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TO THE EDITOR:

EBV-positive DLBCL frequently harbors somatic mutations associated with clonal hematopoiesis of indeterminate potential

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Clonal hematopoiesis (CH) is a common aging-related phenomenon in which hematopoietic stem cells acquire somatic gene mutations.[1,2] CH of indeterminate potential (CHIP) designates a term for CH without cytopenia and dysplastic hematopoiesis.[3] Somatic variants in *DNMT3A, TET2,* and *ASXL1* (all epigenetic regulators) account for over 75% of mutations involved in CHIP.[1,4,5] They are regularly found in myeloid malignancies but much less frequently in lymphoid malignancies such as diffuse large B-cell lymphoma (DLBCL), the most common type of B-cell lymphoma.

Epstein-Barr virus (EBV)-positive DLBCL is a distinct entity in the recent World Health Organization classification of lymphoid neoplasms.[6] We identified 104 cases of EBV-positive DLBCL defined by positive expression of EBV-encoded small RNA with a cutoff of 10% tumor cells.[7] To illuminate the mutational landscape, we performed targeted RNA-seq using an Illumina TruSight RNA Pan-Cancer Panel (Genomic Testing Cooperative, Irvine, CA) with previously described methods.[8] Sequencing was successful for 99 cases, which predominantly had a nongerminal center cell-of-origin (77%, supplemental Table 1). We compared the mutational landscape in these EBV-positive cases to that in 381 EBV-negative DLBCL cases sequenced with the same panel[8] and to those revealed by DNA sequencing in our (387 EBV-negative DLBCLs)[9] and others' previous large-scale studies (Table 1) in which EBV status in DLBCL was not explicitly acknowledged.[10-12] We found that, first, *TET2, ASXL1*, and *DNMT3A*, the top 3 CHIP-related genes, were mutated in 39.4%, 19.2%, and 18.2% of EBV-positive DLBCL cases (Figure 1A), in contrast to the significantly lower 4.7%, 0.26%, and 0.52% in EBV-negative DLBCL cases overall and the 3.3%, 0%, and 0% in the EBV-negative activated B-cell–like (ABC) subset sequenced by the same targeted RNA-seq panel. In previous genomic sequencing studies, *TET2*, *ASXL1,* and *DNMT3A* mutations occurred only in 8.1%, 3.1%, 0.9% of the Schmitz et al cohort,[10] 2.24%, 0.75%, 0% of the Chapuy et al cohort,[11] 7.0%, 0%, 4.9% of the Reddy et al cohort,[12] and 8.27%, 0.52%, 3.62% of EBV-negative DLBCL cases in our DNA sequencing cohort. Second, *TP53*, the fourth most frequently mutated gene in CHIP,[2,5] were mutated in ~42% of EBV-positive

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Our targeted sequencing data are available on request with approved IRB protocol: ken.young@duke.edu.

The full-text version of this article contains a data supplement.

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Table 1. Top 10 recurrent genetic mutations by targeted RNA-seq in our EBV-positive or -negative DLBCL cases and by DNA sequencing in other DLBCL cohorts

Group	EBV-positive DLBCLs, n = 99		EBV-negative DLBCLs, n = 381		Reddy et al, 2017, n = 1001		Schmitz et al, 2018, n = 574		Chapuy et al, 2018, n = 304	
Rank	**Gene**	**%**	**Gene**	**%**	**Gene**	**%**	**Gene**	**%**	**Gene**	**%**
1	*TP53*	42.4	*KMT2D*	24.4	*KMT2D*	24.8	*KMT2D*	31.4	*KMT2D*	24.7
2	*TET2*	39.4	*MYD88*	16.8	*BCL2*	17.4	*PIM1*	27.5	*PIM1*	22.0
3	*APC*	31.3	*TP53*	16.5	*MYD88*	17.2	*MYD88*	26.8	*TP53*	21.4
4	*PTPN11*	20.2	*CARD11*	11.8	*HIST1H1E*	16.9	*TP53*	23.0	*MYD88*	18.1
5	*ASXL1*	19.2	*EZH2*	10.0	*PIM1*	16.6	*HLA-B*	21.6	*BCL2*	17.4
6	*DNMT3A*	18.2	*ACACA*	8.9	*CREBBP*	11.4	*BTG2*	18.3	*CREBBP*	16.8
7	*SMAD4*	18.2	*CD79B*	8.4	*CARD11*	11.3	*TMSB4X*	16.7	*CD79B*	14.5
8	*SOCS1*	16.2	*BCL10*	7.9	*SPEN*	10.1	*TNFAIP3*	16.7	*BTG1*	14.1
9	*ETV6*	16.2	*CD58*	6.6	*TP53*	9.9	*HLA-A*	16.0	*SGK1*	14.1
10	*STAG2*	15.1	*CREBBP*	6.0	*ARID1A*	9.7	*B2M*	15.9	*TNFRSF14*	13.8

DLBCL cases (compared to the 16.5% frequency in overall EBV-negative DLBCL cases and the 13.3% in EBV-negative ABC subset, supplemental Figure 1A-B), representing the highest *TP53* mutation rate in hematologic cancer. Most (88.2%) *TP53* mutations occurred in the DNA binding domain and were predominantly missense mutations (88.3%), whereas 62.5% of mutations in other p53 domains were nonsense mutations (supplemental Table 2). p53 inactivation and the expression of oncogenes from EBV in aging hematopoietic stem cells or lymphoid progenitor cells could have facilitated DLBCL pathogenesis. Third, *MYD88* mutations occurred in only 4 patients with EBV-positive DLBCL (only 1 had the L265P mutation). The mutation rate (4%) was significantly lower than the expected ~20% frequencies in overall DLBCL (Table 1) and 34% in our ABC subset of EBV-negative cases (supplemental Figure 1B). Finally, the landscape of genetic drivers in EBV-positive DLBCL was also different from that of other EBV-associated cancers, including Burkitt lymphoma,[13] extranodal NK/T-cell lymphoma,[14] EBV-positive PTLD, nasopharyngeal carcinoma,[15,16] and gastric cancer.[17,18] Despite the lacking of normal tissues and blood or bone marrow samples, these data suggest that somatic mutations in CHIP genes, including *TP53*, *TET2, ASXL1*, and *DNMT3A,* may play roles in EBV-mediated DLBCL pathogenesis. This retrospective study was conducted following data collection protocols involving no more than minimal risk to subjects with a waiver of the written consent requirement approved by the institutional review boards of Duke University and each participating institution.

Niroula et al[5] have classified CHIP variants into myeloid-CHIP (M-CHIP, 56 genes) and lymphoid-CHIP (L-CHIP, 235 genes) based on their recurrence in myeloid and lymphoid malignancies, and demonstrated that M-CHIP vs L-CHIP carriers have stark differences in the incidence of myeloid vs lymphoid neoplasm development. The 3 most frequent CHIP, *DNMT3A*, *TET2*, and *ASXL1* mutated in 87% of individuals with M-CHIP and 73% of all CHIP individuals,[5] are M-CHIP and frequent driver mutations in myelodysplastic syndrome, acute myeloid leukemia, and myeloproliferative neoplasms.[3,21,22] Some less frequent CHIP variants are lymphoid driver mutations and proposed as L-CHIP,[5] including *KMT2D, SPEN*, *ARID1A*, and *MYD88* frequently mutated in DLBCL.[10-12] Yet, both *TET2* and *DNMT3A* are frequently mutated in some lymphoid neoplasms of T-cell lineage including angioimmunoblastic T-cell lymphoma/peripheral T-cell lymphoma of follicular T-helper cell origin,[23] and both *TET2* and *ASXL1* mutation frequencies are high in plasmablastic lymphoma.[24] Among the top 10 mutated genes in our EBV-positive cases (Figure 1A), 7 are M-CHIP genes (*TP53*, *TET2*, *PTPN11*, *ASXL1*, *DNMT3A*, *ETV6*, and *STAG2*) in addition to 1 L-CHIP (*SOCS1*). In contrast, 8 of the 12 most frequently mutated genes in EBV-negative DLBCL in supplemental Figure 1 are L-CHIP genes in addition to 3 M-CHIP (*TP53*, *EZH2*, and *CREBBP*).[5] EBV-positive DLBCL may represent the first identified B-cell lymphoma subtype overwhelmingly associated with M-CHIP and high frequencies of variants in all the 3 most common CHIP driver genes, crossing the line for the lineage of incident malignancies drawn between M-CHIP and L-CHIP drivers.

Moreover, we have noted that CHIP mutations are frequently found in another DLBCL population. Lee et al[25] identified 6 genes that are more significantly altered in DLBCL with African ancestry than DLBCL with European ancestry: *ATM, MGA, SETD2, TET2, KMT2C*, and *DNMT3A*; all 6 genes had somatic alterations in over 10% of DLBCL with African ancestry. These results were obtained from an unsupervised model-based Admixture global ancestry analysis of the Reddy et al cohort.[12] It is striking that all 6 genes more frequently mutated in DLBCL with African ancestry are CHIP genes: *TET2* and *DNMT3A* are among the 3 dominant CHIP genes, *SETD2* is among the top 25 M-CHIP genes, and *ATM, MGA,* and *KMT2C* are among the top 11 L-CHIP genes.[5] Of note, *ATM* is the most commonly altered gene in DLBCL with African ancestry (22%, compared to 7.75% in DLBCL with European ancestry, 13.1% in our EBV-positive DLBCL cases, 4.65% in our EBV-negative DLBCLs, and 3.5% and 2.2% in two previously reported large DLBCL cohorts[10,11], respectively; Table 1). A single nucleotide polymorphism in the *TET2* locus (rs144418061, not detected in our EBV-positive DLBCL cases) exclusively present in individuals with African ancestry confers an over 2-fold increased risk of CHIP.[4] It is unclear whether this variant is associated with an increased risk of subsequent DLBCL. Nonetheless, the reported findings implicate that CHIP may play a more profound role in the pathogenesis of DLBCL with African ancestry than DLBCL with European ancestry.

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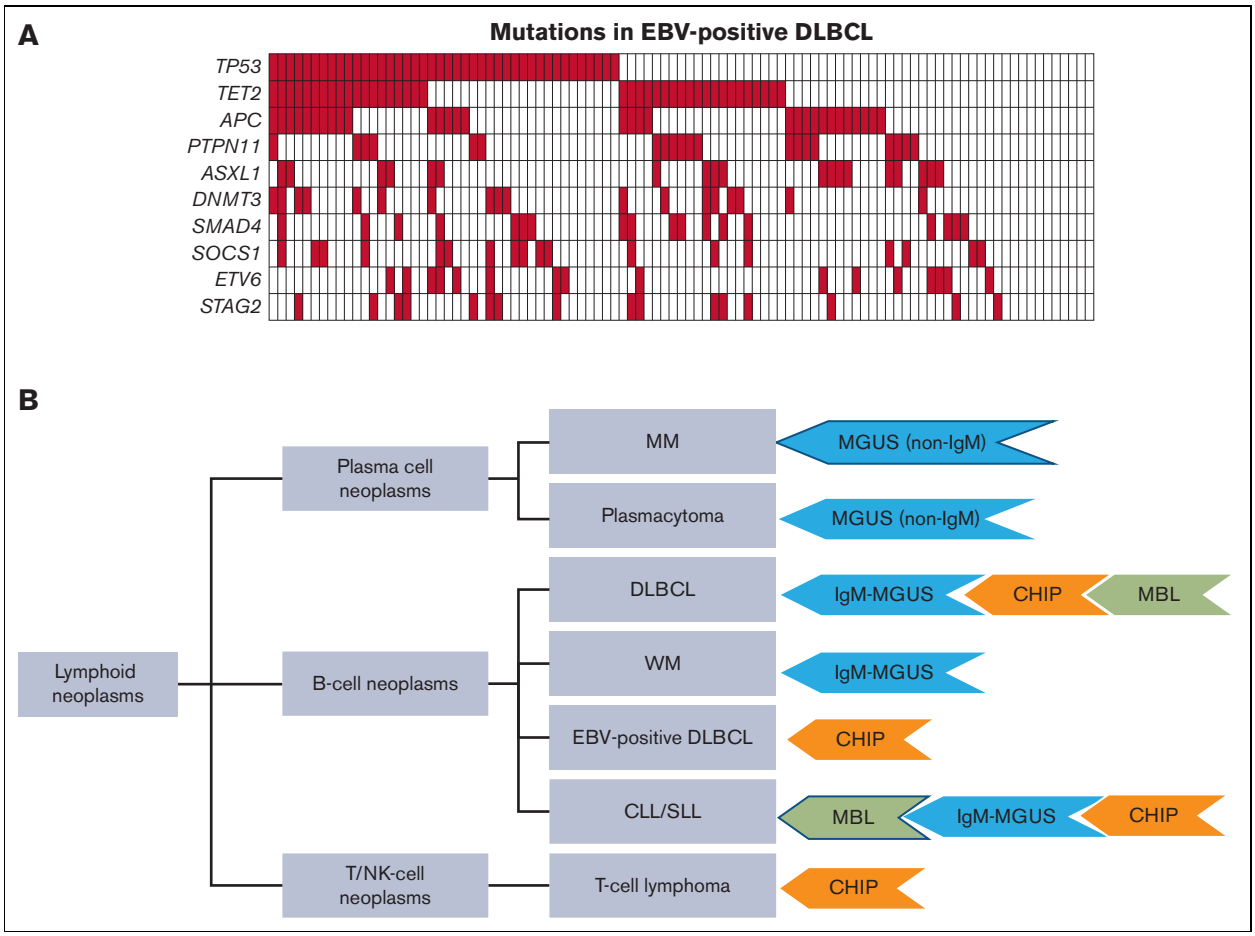

Figure 1. Mutation landscape in EBV-positive DLBCL and summary of putative premalignancies to lymphoid neoplasms. (A) Mutation distribution plots for the top 10 mutated genes by RNA-seq in 99 patients with EBV-positive DLBCL. (B) Schematic illustration of the 3 premalignant conditions as potential precursors to lymphoid neoplasms. CHIP are regularly found in myeloid malignancies but much less frequently in lymphoid malignancies. Our study showed high frequencies of CHIP gene mutations in EBV-positive DLBCL. Virtually all multiple myeloma (MM) and chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) are preceded by MGUS and MBL, respectively[19] (highlighted by solid outlines in the figure). There are 2 types of MGUS. The lymphoid type, secreting IgM, may progress to Waldenström macroglobulinemia (WM), DLBCL (most likely the ABC subtype with *MYD88* mutation), occasionally CLL/SLL, or amyloid light-chain amyloidosis. The nonimmunoglobulin M (IgM) type (secreting IgG, IgA, Ig light chain only, IgD, or IgE) may progress to MM, plasmacytoma, or AL amyloidosis.[20] MBL may progress to CLL/SLL, marginal zone B-cell lymphoma, or mantle cell lymphoma, and the former 2 can transform into DLBCL. L-CHIP carriers have a higher risk of CLL/SLL and other lymphoid malignancies than individuals without L-CHIP.[5] Therefore, all 3 types of precursors can precede DLBCL and CLL/SLL.

CHIP is now recognized as a potential hematologic premalignancy condition, along with monoclonal gammopathy of undetermined significance (MGUS) and monoclonal B-cell lymphocytosis (MBL). MBL and MGUS represent expansions of lymphoid lineage-committed cells (postgerminal center B cells or memory B cells), whereas CHIP involves hematopoietic stem cells or less mature progenitor cells.[3] By definition, CHIP excludes MGUS and MBL,[3] yet an individual with CH could have elevated monoclonal paraprotein (MGUS) or clonal B-cell populations (MBL). Like MGUS and MBL, most patients with CHIP will never develop an overt neoplasm. The rate of progression from CHIP to hematologic cancer appears to be 0.5% to 1% per year, similar to MBL and MGUS.[3] As shown in Figure 1B, all 3 types of putative precursors have the potential to precede DLBCL and chronic lymphocytic leukemia/small lymphocytic lymphoma. In summary, different from other DLBCL subtypes, EBV-positive DLBCL and DLBCL with African ancestry have high frequencies of CHIP variants. CHIP can be a precursor state for a broader range of hematologic cancer, including both myeloid and lymphoid neoplasms. However, myeloid or lymphoid malignancies do not have an obligate CHIP precursor. The findings in our study may help understand the lymphomagenesis of EBV-positive DLBCL and DLBCL with African ancestry and have therapeutic implications.

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Contribution: Y.L. and K.H.Y. designed the study; Z.Y.X-M. and K.H.Y. performed the experiments and analyzed the data; J.A., A.R.S., G.B., A.T., C.V., S.Z., K.D., Z.P., M.X., W.T., Y.Z., E.D.H., F.B.H., H.G., J.H.vK., J.N.W., M.P., A.J.M.F., M.B.M., M.A.P., Y.W., M.Z., and K.H.Y contributed patient samples and clinical data; L.Y., Z.Y.X-M., and K.H.Y. interpreted the data together with all coauthors, and L.Y., Z.Y.X-M., and K.H.Y. wrote the manuscript with contributions and approval from all authors.

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