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VPREB1 deletions occur independent of lambda light chain rearrangement in childhood acute lymphoblastic leukemia


B-cell acute lymphoblastic leukemia (B-ALL) remains one of the best genetically characterized cancers. With the introduction of single-nucleotide polymorphism (SNP) microarray technology, there has been a recent explosion in genomic investigations and discovery of recurrent alterations in B-ALL, such as PAX5, IKZF1, JAK2 and CRLF2.1 Deletions of the VPREB1 gene, a component of the surrogate light chain of the pre-B-cell receptor (pre-BCR), have been observed in childhood B-ALL,2,3 and have been suggested to result from recombination activating gene (RAG) activation and variable (joining) diversity (V(D)J) recombination based on VPREB1’s location in the immunoglobulin lambda locus (IGL@).3,4,5 In a recent study of relapsed patients with ETV6-RUNX1 translocations, focal VPREB1 deletions were presumed to be involved in leukemogenesis but were not the focus of the reported findings.5 Another study explored molecular alterations and outcome in Down syndrome B-ALL and observed VPREB1 deletions in 18% of their patients, but this deletion was again not the focus of their investigation.6 Taking advantage of publicly available microarray data sets and performing additional experiments, we found that VPREB1 deletions are not part of normal V(D)J recombination as they frequently do not involve the VJ junction nor follow the ordered model of V(D)J recombination.

We received Institutional Review Board approval to study formalin-fixed paraffin-embedded bone marrow aspirate clots from 25 B-ALL patients treated at Primary Children’s Medical Center at the University of Utah. We also obtained the leukemia and germline CEL files from the previously published Therapeutically Applicable Research to Generate Effective Treatments (TARGET) Initiative cohort of the National Cancer Institute (NCI), which included high-risk ALL patients treated on the Children’s Oncology Group (COG) P9906 trial (N = 221).4,10–12 The P9906 patients demonstrated high-risk features (older age, high white blood cell count, overt central nervous system or testicular involvement) without specific prognostic cytogenetic features (ETV6-RUNX1 translocation, trisomy of 4 and 10, BCR-ABL1 translocation, hypodiploidy). The TARGET (COG) cohort was analyzed with the Affymetrix GeneChip Human Mapping 500K SNP Array (Affymetrix, Santa Clara, CA, USA). We obtained additional data from a cohort of infant, standard and high-risk B-ALL patients treated on Total Therapies XI-XV and analyzed by investigators at St Jude Children’s Research Hospital (SICRH, N = 265).3,12 The SICRH cohort included leukemia and germline CEL files run on the Affymetrix Genome-Wide Human SNP Array 6.0 and the Affymetrix GeneChip Human Mapping 500K Array. Previously published paired gene expression data were obtained for both the TARGET and SICRH samples when available (GeneChip Human Genome U133 Plus 2.0 Array, Affymetrix).6,12 We also reviewed our published SNP array data on 27 Burkitt Lymphoma (BL) samples.13 In addition, kappa-expressing B-cells, lambda-expressing B-cells and control monocytes were separated from the whole blood of 10 healthy volunteers. DNA was isolated from the 25 Utah B-ALL samples, 11 B-ALL cell lines, 5 TARGET B-ALL samples and the healthy volunteers (RecoverAll Total Nucleic Acid Isolation Kit, Ambion, Austin, TX, USA; see Supplementary Information). SNP array data were analyzed with Nexus Copy Number 6.1 (BioDiscovery, Inc., El Segundo, CA, USA). Gene expression levels were normalized with GC-RMA methods in Partek Genomics Suite (Partek, Inc., St Louis, MO, USA) and plotted on a log, scale. Each cohort was normalized to itself, and trends of expression change by number of allele copies were tested by the method of Jonckheere-Terpstra, as well as two-way comparisons via analysis of variance and Kruskal–Wallis tests.

VPREB1 is located within the IGL@ locus among the variable immunoglobulin (ig) segments, upstream from the VJ junction. V(D)J recombination joins light chain variable (V) Ig segments with joining (J) Ig segments by deleting intervening Ig segments (light chains do not contain diversity (D) Ig segments). Therefore, physiologic VPREB1 deletions would be expected within a larger continuous deletion that contains nearby Ig segments and stretches to the 3′ border at the VJ junction. However, the majority of the VPREB1 deletions observed in the B-ALL microarray data sets were focal and did not extend to the VJ junction, and

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Using gene expression arrays paired with copy number data, VPREB1 expression differed among the diploid, hemizygous, and homozygous groups in the TARGET and SJCRH cohorts with fewer copies of VPREB1 leading to lower expression, highlighting the biological significance of these focal deletions (Figure 1b). Gene expression was significantly different between deletion groups by analysis of variance and Kruskal–Wallis ($P < 0.0001$), and test for trend across number of copies for VPREB1 expression by the Jonckheere–Terpstra test was also significant ($P < 0.0001$), as was a straight linear regression for trend. Interestingly, the few samples with physiologic VPREB1 deletions (1%) that extended contiguously to the IGL@ V-J junction had similar VPREB1 expression compared with diploid VPREB1 samples.

To determine whether focal VPREB1 deletions were observed in mature B-cells, we next examined the IGL@ deletion patterns from kappa vs lambda expression-restricted BL samples ($N = 27$), a mature B-cell malignancy that should contain normal, physiologic light chain rearrangements. As expected, whenever lambda light chain variable region gene deletions (rearrangements) occurred in BL, the lambda rearrangement was detected as a single continuous deletion with its 3’ border at the VJ junction. In one of these continuous lambda light chain deletions, VPREB1 was included within the larger deletion. In contrast to the B-ALL samples, focal VPREB1 deletions were not observed in BL ($N = 0/27$, Figure 2).

Under the ordered model of light chain rearrangement, lambda light chain rearrangement will only occur after kappa-light chain has attempted and failed to rearrange both alleles on chromosome 2p11.2.14,15 We sought to better characterize VPREB1 deletions and the kappa and lambda light chain V(D)J rearrangement pattern in B-ALL. For VPREB1 deletions to be part of physiologic light chain recombination, VPREB1 deletions would be expected to occur only in B-cells that already have attempted kappa rearrangement (as evidenced by kappa VJ junction deletions) followed by lambda rearrangement (as evidenced by lambda VJ junction deletion that extends to VPREB1 locus). Using quantitative PCR, we investigated gene copy number at loci across the lambda light chain variable gene region both upstream and downstream from VPREB1, and precisely at both kappa and lambda light chain VJ junctions. We analyzed B-ALL samples from the Utah cohort ($N = 25$), B-ALL cell lines ($N = 11$), B-ALL samples from the TARGET cohort ($N = 5$), BL samples with known kappa and lambda light chain expression status ($N = 12$), and healthy volunteer lymphocytes flow-sorted by kappa and lambda expression ($N = 10$). We also included monocytes from healthy volunteers for controls that lacked IGL@ or immunoglobulin kappa locus (IGK@) expression ($N = 10$).

Quantitative PCR demonstrated focal VPREB1 deletions in the Utah B-ALL cohort and validated focal deletions found by the SNP 500K array in the TARGET B-ALL samples. The BL samples and the healthy control samples all followed the expected pattern of only kappa rearrangement (kappa VJ deletion) in kappa-expressing mature B-cells and both kappa and lambda rearrangement (kappa and lambda VJ deletion) in the lambda-expressing mature B-cells. However, the B-ALL clinical samples and cell lines followed a disordered and unique light chain pattern of non-physiologic light chain rearrangements with abnormal combinations of kappa and lambda VJ deletions (that is, lambda without kappa rearrangement and so on, Figure 3). These findings indicate that VPREB1 deletions occur independently of normal light chain rearrangement and that light chain rearrangements in B-ALL do not follow the expected pattern of ordered rearrangement. In addition, the lack of VPREB1 deletions in lambda-sorted B-cells from healthy volunteers indicates that although VPREB1 deletions may occasionally occur within the contiguously deleted variable cassettes of the lambda light chain, these VPREB1 deletions are too rare to be detected in a heterogeneous (non-clonal) population of mature lambda-expressing B-cells. This suggests that VPREB1 deletions are uncommon in the majority of lambda-rearranged lymphocytes

thus did not follow the normal pattern of V(D)J recombination. The TARGET cohort contained 14.5% focal VPREB1 deletions that did not extend to the lambda VJ junction ($N = 32/221$, 28 hemizygous, 4 homozygous) and the SJCRH cohort contained 18% focal VPREB1 deletions ($N = 48/265$, 41 hemizygous, 7 homozygous). Only two TARGET samples (1%) and only three SJCRH samples (1%) included VPREB1 deletions located within a larger deletion extending to the lambda light chain VJ junction, consistent with normal IGL@ rearrangement and V(D)J recombination. Focal VPREB1 deletion prevalence varied by clinical subtype with ETV6-RUNX1-positive samples containing the highest frequency of deletions with a prevalence of 33–40% (Figure 1a).

![Image](image-url)
and that IGL@ rearrangements including VPREB1 would be unlikely to comprise a substantial number of B-ALL clones.

Finally, we examined the correlation of VPREB1 focal deletions with clinical outcome. In the TARGET high-risk cohort, VPREB1 focal deletions (hemizygous or homozygous) were associated with lower 5-year event-free survival (42% vs 63%, $P = 0.0029$, log-rank test) and overall survival (OS, 63% vs 81%, $P = 0.0214$, log-rank test). In the SJCRH cohort, there was no statistically significant difference in event-free survival in standard or high-risk patients (NCI-Rome criteria), but high-risk patients with the deletion tended to fail early. Fifteen-year OS was worse in the SJCRH high-risk patients (67% vs 86%, $P = 0.024$), but not the standard risk patients. These clinical findings support previous observations that VPREB1 copy number and expression loss are associated with worse outcome in childhood B-ALL, especially in high-risk patients. See Supplementary Figures 2a–d.

Reported frequencies of VPREB1 deletions in B-ALL range from 26 to 37.5%.²⁻⁴,⁷,⁸,¹⁶ Similar to BTG1 deletions, VPREB1 deletions occur at higher frequencies among patients with specific B-ALL subtypes including ETV6–RUNX1 (43–68%), BCR–ABL1 (40%) and BCR–ABL1-like by gene expression profile (34%)²⁻⁴,⁷,⁸,¹⁶ Although the TARGET P9906 cohort contained fewer VPREB1 deletions overall (15.5%, 34/221) that cohort included only three patients with ETV6–RUNX1, and BCR–ABL1 translocations were excluded entirely.
Copy number alterations affect multiple cellular pathways in B-ALL and are commonly found within genes that regulate B-cell development and differentiation, such as EBF1, PAX5 and IKZF1. Consistent with this pattern, we observed in B-ALL that focal deletions are common in VPREB1, an essential gene in B-cell development and differentiation due to its role as part of the surrogate light chain in the pre-BCR. Nearly 10% of samples in each cohort contained a focal deletion of VPREB1 as the only deleted B-cell developmental gene, perhaps describing a new mechanism of B-ALL development caused by loss of this critical gene in B-cell maturation. VPREB1 loss could contribute to leukemogenesis as a result of failure to form a viable surrogate light chain in the pre-BCR, which has been demonstrated in both mice and humans to block the pro- to pre-B-cell transition in the bone marrow with a decrease in circulating mature B-cells.

In summary, we observed that VPREB1 focal deletions are common in B-ALL and occur independent of VDJ light chain recombination. Focal deletions of VPREB1 correlate with decreased expression levels and high-risk patients with focal deletions tend to have poorer OS. VPREB1 has a central role in B-cell development as part of the pre-BCR and we believe VPREB1 represents an excellent candidate for further study of B-ALL leukemogenesis and clinical outcome.

**CONFLICT OF INTEREST**

JDS is a scientific consultant for Affymetrix, Inc. The remaining authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

JDS was supported by American Society of Hematology (ASH) Scholar Award, CureSearch Foundation Award, Cancer and Control Population Sciences Pilot Award from Huntsman Cancer Institute, and is the Edward B Clark, MD Chair in Pediatric Research. DSM was supported by Albert Einstein School of Medicine Student Exchange Program. SPH is the Ergen Family Chair in Pediatric Cancer. CGM is Pew Scholar in the Biomedical Sciences and a St Baldrick’s Scholar. MM is a Scholar of the Leukemia and Lymphoma Society and a Senior Investigator of the Wellcome Trust. This work was also supported by grants to the COG including the COG Chair’s grant (CA98543) and a supplement to support the TARGET Project, U10 CA98413 (COG Statistical Center) and U24 CA114766 (COG Specimen Banking), and the American Lebanese Syrian Associated Charities of St Jude Children’s Research Hospital.

**AUTHOR CONTRIBUTIONS**

DSM, JD, CCM, MM, NM, NT, JKF, RCH, SPH, JY, PB, CGM, RR, and JDS wrote the paper. DSM, JD, CCM, MSJ, JY, RR, and JDS designed and performed research. MM and SPH contributed reagents, cell lines and/or samples. CLW, RCH, SPH, JY, CGM contributed genomic data.

**Figure 3.** Disordered light-chain rearrangement in B-cell precursor ALL. Real-time quantitative PCR (qPCR) results are displayed for both kappa and lambda light chain V-J junctions. Deletions in V-J regions suggest attempted, but not necessarily successful, rearrangement of respective light chain. Also displayed are qPCR results from VPREB1 and flanking upstream and downstream regions in IGL@ as mapped to specific letters in the chart. Although some of the B-ALL samples and cell lines followed the expected sequence of light chain rearrangement (i.e., kappa followed by lambda), many of these samples followed a disordered pattern indicating abnormal light chain rearrangement in B-ALL clones.
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Bilateral corneal infections associated with topical steroid therapy prophylaxis for cytarabine arabinoside-induced keratoconjunctivitis


Cytarabine arabinoside is a commonly used chemotherapy agent in the treatment of acute myeloid leukemia (AML). One of the reported side effects is cytarabine-associated keratoconjunctivitis, which occurs in up to 40–100% of patients.1 As such, it is exceedingly common for patients to be commenced on prophylactic steroid eye drops. We present a case of bilateral microbial keratitis in a patient commenced on topical steroid prophylaxis for cytarabine-associated keratoconjunctivitis. After resolution of the infection, the patient was left with permanently reduced visual acuity.