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

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B-cell acute lymphoblastic leukemia (B-ALL) remains one of the best genetically characterized cancers. With the introduction of single-nucleotide polymorphism (SNP)

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AUTHOR CONTRIBUTIONS

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

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, 4, 5, 6, 7] 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, 6, 7] 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.[8] 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.[9] 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, 11, 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 (SJCRH, N=265).[3, 12] The SJCRH 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 SJCRH samples when available (GeneChip Human Genome U133 Plus 2.0 Array, Affymetrix).[4, 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 $\log_2$ 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 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).

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 its 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 unexpected 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 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,[2, 4] 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, 3, 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, 3, 5, 6] 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[17] and are commonly found within genes that regulate B-cell development and differentiation, such as *EBF1*, *PAX5* and *IKZF1*.[3] 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.[5] 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.[18, 19, 20]

In summary, we observed that *VPREB1* focal deletions are common in B-ALL and occur independent of V(D)J 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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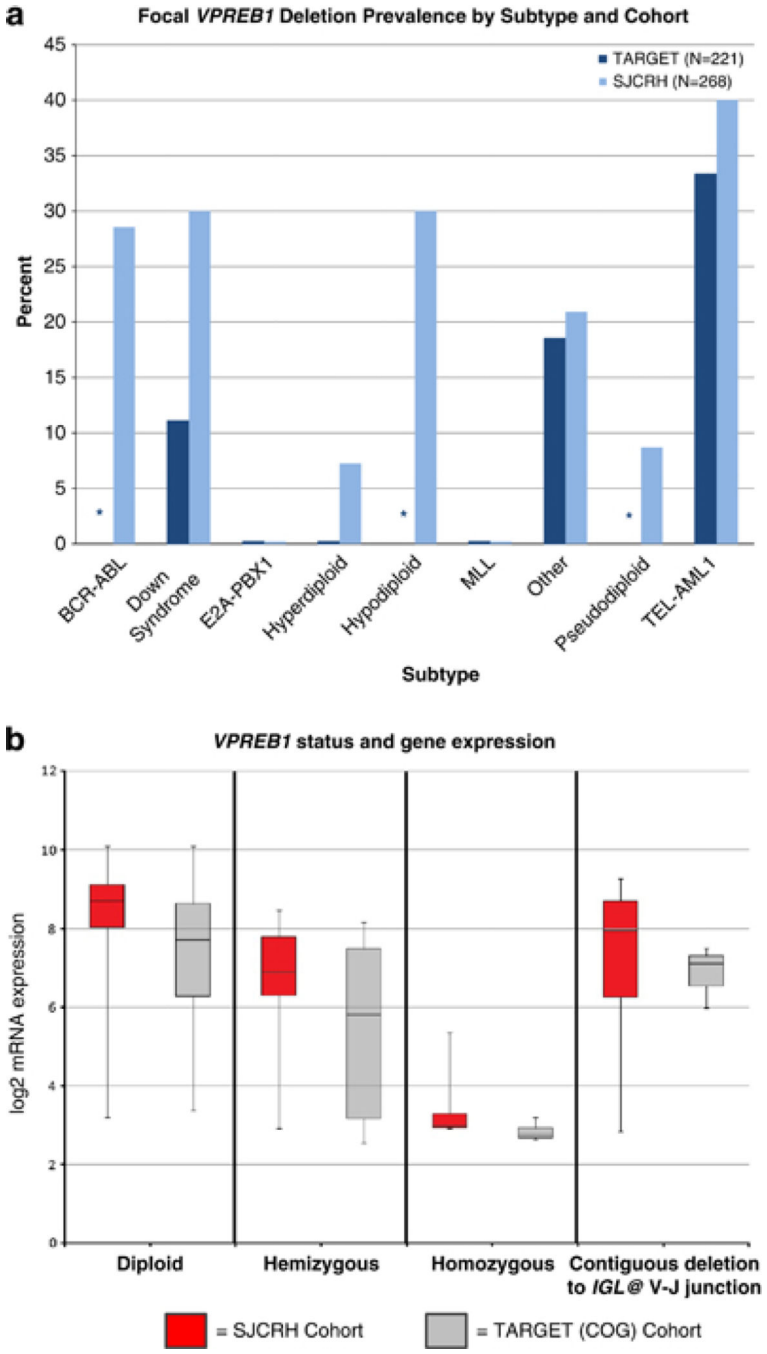

Figure 1.

(**a**) Focal *VPREB1* deletion prevalence by subtype and cohort. *VPREB1* deletions vary by specific subtype with consistently high prevalence seen in samples with *ETV6-RUNX1* (*TEL-AML1*) translocations, and a complete lack of *VPREB1* deletions seen in samples with *E2A-PBX1* and mixed-lineage leukemia (*MLL*) rearrangements. Colored asterisks (*) represent specific subtypes not present in the respective cohorts and could not be evaluated for *VPREB1* deletions. (**b**) *VPREB1* status and gene expression. *VPREB1* expression decreases in a significant manner from diploid to hemizygous to homozygous state. The far

right panel displays $\log_2$ mRNA expression for *VPREB1* in those few samples where *VPREB1* deletions were not focal and occurred as part of larger lambda light chain deletions.

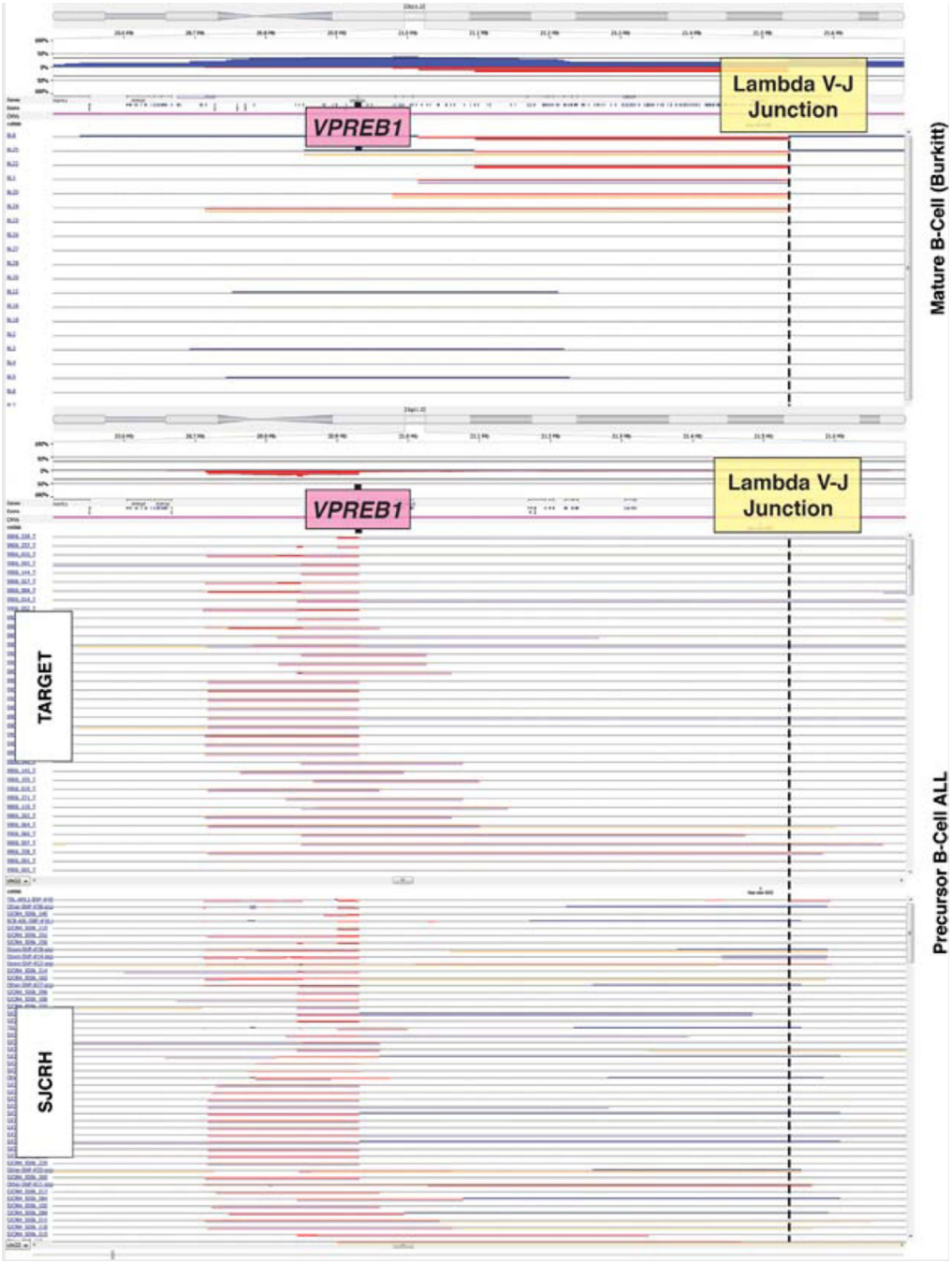

Figure 2.

VPREB1 deletions are not part of V(D)J recombination. Deleted regions within *IGL@* in lambda-expressing mature B-cells from Burkitt Lymphoma (BL) samples are displayed on the top panel. Individuals are represented horizontally and red bars equal deletions, with thicker bars indicating homozygous deletions (blue bars represent gains). Note that 100% of deletions in BL samples extend contiguously to lambda V-J junction, including the single sample with deleted *VPREB1* locus. The bottom panels display the samples with *VPREB1* deletions in the TARGET and SJCRH cohorts, respectively. Note that in B-ALL, as opposed

to BL, *VPREB1* deletions are almost entirely focal in nature and rarely extend downstream to the lambda V-J junction.

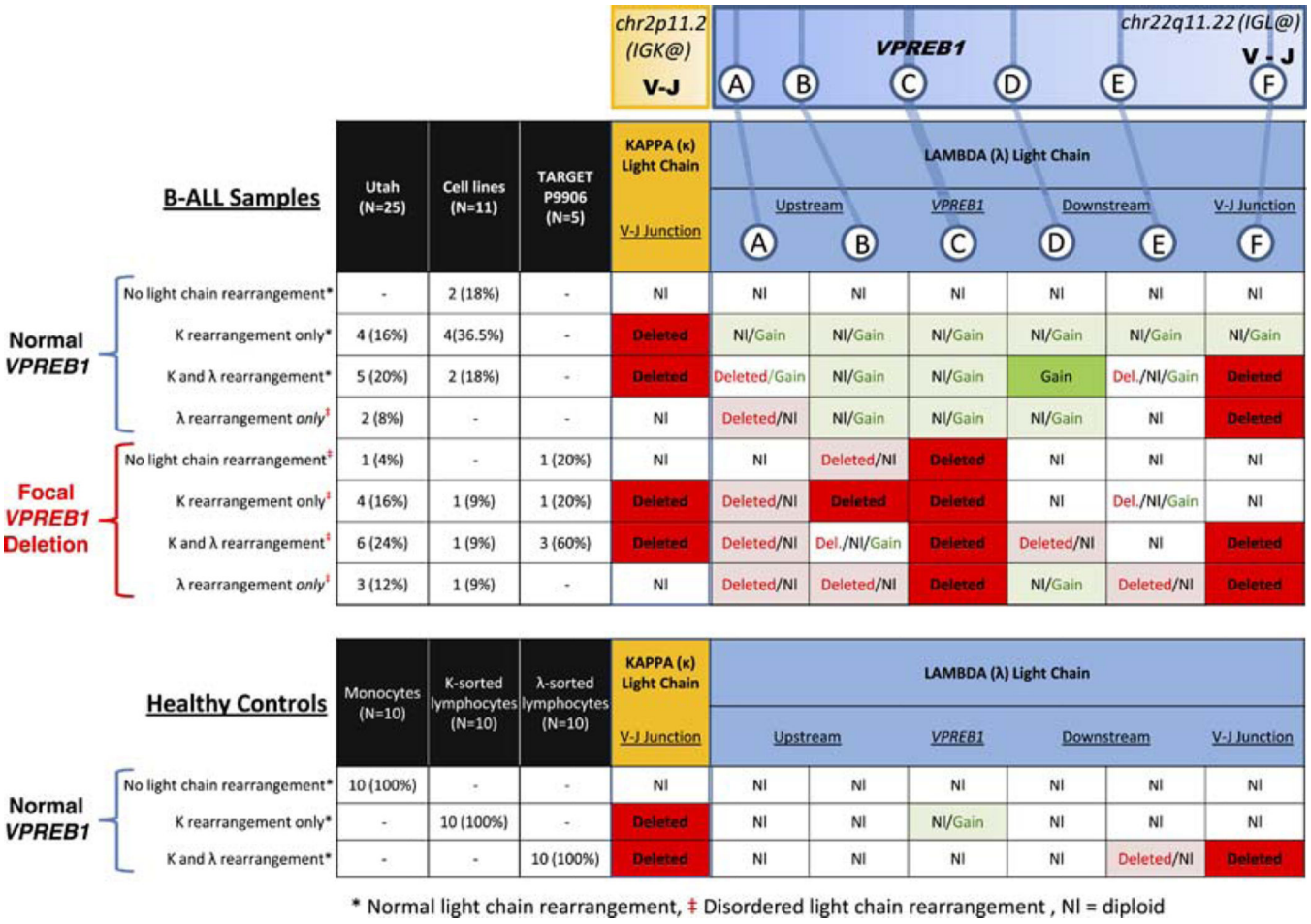

Figure 3.

Disordered light-chain rearrangement in B-cell precursor ALL. Real-time quantitative PCR (qPCR) results are displayed for both kappa and lamda 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.