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BRIEF REPORT

Novel germline TRAF3IP3 mutation in a dyad with familial acute B lymphoblastic leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common hematologic malignancy in children, representing 25% of all new cancer diagnoses. Advances in genomic sequencing have demonstrated that inherited genetic risk factors play a larger role in leukemia development than previously appreciated.

Aim: We identified a father–daughter dyad with childhood B-cell ALL and aimed to investigate whether the pair shared a gene associated with leukemia predisposition.

Methods: We performed whole exome sequencing on their leukemia and germline samples and RNA-seq on their leukemia samples.

Results: We discovered a novel germline chromosomal structural variant in chromosome 1q32.2 within the TRAF3IP3 gene. TRAF3IP3 regulates B-cell lymphopoiesis, and this mutation likely resulted in a predisposition to leukemia by causing expansion of immature B-cell precursors which are highly vulnerable to secondary somatic mutations. Based on the lack of concordance in the somatic mutational profiles between this dyad's leukemia samples, we suspect that the acquired somatic mutations rather than this germline mutation are what dictated their leukemia phenotypes, which we confirmed through RNA-seq by comparing to sporadic cases of B-cell ALL.

Conclusion: This research may have identified a novel gene involved in leukemogenesis which may also be involved in de novo cases of ALL. Additional studies are needed to further characterize this TRAF3IP3 structural variant, the co-occurring somatic mutations within these leukemia samples and their combined role in leukemogenesis.

KEYWORDS
dyad, familial ALL, familial leukemia, inherited leukemia, leukemia predisposition, pediatric acute lymphoblastic leukemia, TRAF3IP3

Abbreviations: ALL, acute lymphoblastic leukemia; GWAs, genome-wide association studies; SNPs, single nucleotide polymorphisms; B-ALL, B-cell acute lymphoblastic leukemia; COG, Children's Oncology Group; WES, whole exome sequencing; RNA-seq, RNA sequencing; SNVs, single nucleotide variants; *TRAF3IP3*, tumor necrosis factor receptor–associated factor 3-interacting protein 3; CLPs, common lymphoid progenitors; HSCs, hematopoietic stem cells; BCCA, British Columbia Cancer Agency; TARGET, Therapeutically Applicable Research to Generate Effective Treatments; GSEA, Gene Set Enrichment Analysis.

1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common hematologic malignancy in children, representing 25% of all new cancer diagnoses.[1]

Advances in genomic sequencing has demonstrated that inherited risk factors play an important role in leukemia development.[2,3] Familial ALL refers to a partially penetrant, inheritable predisposition to ALL but distinct from other well-defined, multicancer syndromes such as Li Fraumeni, Down syndrome, and ataxia telangiectasia.[4,5] Recent genome-wide association studies (GWAs) have shown that inherited genetic variants are present in a subset of familial ALL kindreds.[6] Low-penetrance germline polymorphisms have been identified which confer a modest increase in leukemia susceptibility, most of which are single nucleotide polymorphisms (SNPs) in genes encoding hematopoietic transcription factors such as 7p12.2 (*IKZF1*), 9p21.3 (*CDKN2A/CDKN2B*), 10q21.2 (*ARID5B*), 14q11.2 (*CEBPE*), 14q11.2 (*PIP4K2A*), 10p14 (*GATA3*), 10q26.13 (*LHPP*), and 12q23.1 (*ELK3*).[3,7-11] Additional genomic studies of familial ALL kindreds have identified rare germline mutations associated with strong leukemia predisposition such as *PAX5* and *ETV6*.[3,12-18] While these studies have identified some presumptive causative mutations, additional germline mutations continue to be identified, implying the full spectrum of potential lesions remains to be identified. Here, we report a novel germline microinversion in *TRAF3IP3* in a dyad with presumed familial ALL.

2 | METHODS

We identified a father–daughter dyad with childhood B-cell ALL (B-ALL) and obtained their diagnostic leukemia bone marrow samples in addition to germline samples. A protocol was approved through the Children's Hospital of Wisconsin Institutional Review Board (IRB #915943) and the Children's Oncology Group (COG) to obtain diagnostic bone marrow specimens from the time of the patients' leukemia diagnosis from the COG bone marrow bank. For germline samples, remission marrow (daughter) and buccal swab (father) were used. Written informed consent was obtained from the patients, and their parents and buccal swabs were collected by each patient. Whole exome sequencing (WES) was performed on all specimens, and RNA

TABLE 1 Clinical characteristics of the father and daughter's leukemia cases and treatment protocols

Subject	WBC count at diagnosis (10^3/uL)	CNS Status	Cyto/FISH	Karyotype	NCI Risk Stratification	Treatment Protocol	EOI MRD
Father	54.2	CNS 1	Negative	Hyperdiploid: 54, XY, +X, +4, +6,+10, +14,+17,+21,+21	High	POG 9006	NA
Daughter	32.1	CNS 1	ETV6-RUNX1	Hyperdiploid: 56, XX, +X,+2, +4,+5, +6,+10,+14,+17,+19, +21	Average	AALL0932	Negative

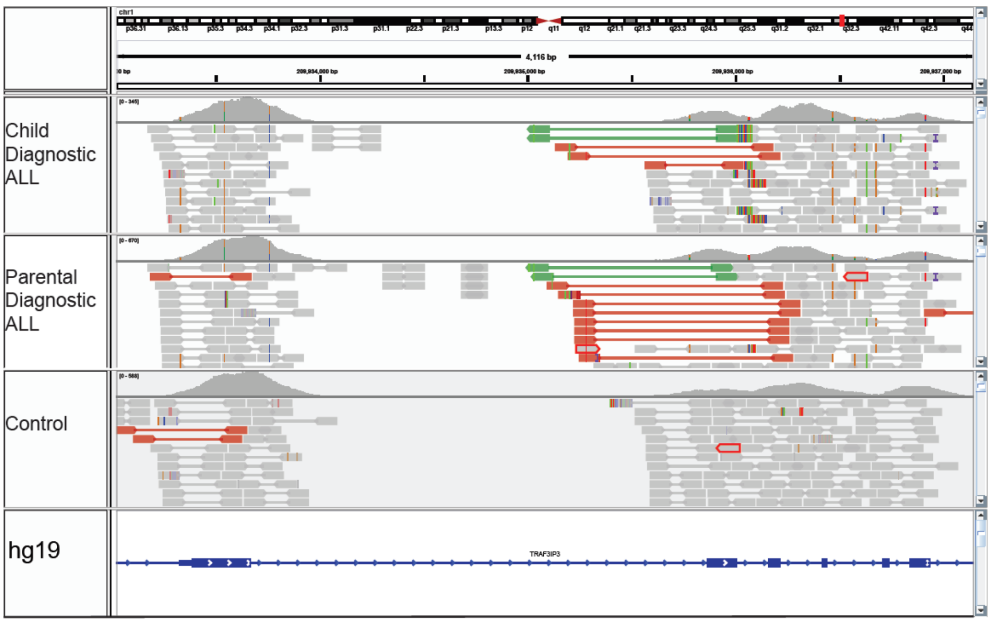

FIGURE 1 A novel microinversion at the *TRAF3IP3* locus. A visualization of the alignment of all reads from the parental diagnostic ALL, child diagnostic ALL, and control sample surrounding the *TRAF3IP3* locus (hg19). Top half of all tracks is the quantification of the number of reads which align within the given genomic interval. The lower half of each track is the alignment of the paired end (PE) reads. Individual reads are shown with boxes, and arrows indicate the direction of individual reads. The lines connecting reads is the presumed gap between the PE reads. Grey coloring indicates the reads and distance between the two closely align to the reference genome. Green reads indicate the reads are in the opposite predicted orientation, with the size of the gap indicated by a larger line connecting them. Red reads indicate the reads are in the same orientation as predicted, but with a larger than predicted gap between them

sequencing (RNA-seq) was performed on the leukemia samples. Detailed methodology is within the supplemental materials S1.

3 | RESULTS AND DISCUSSION

The father and daughter were each diagnosed with B-cell ALL at age 3 years and successfully treated (Table 1). Key findings include both samples reporting hyperdiploidy with the daughter's leukemia also harboring an *ETV6-RUNX1* fusion. These two patients were the only ones within the extended family who had a history of hematologic malignancies. Otherwise, history and family history was negative for cytopenias, other malignancies, immune dysfunction, abnormal physical exams, frequent infections, or other relevant abnormalities, both before and after therapy. There were no identified environmental factors that were thought to have a causal association with the development of malignancy within this family. Both patients successfully completed therapy for their leukemia on COG protocols (Table 1) and are alive and healthy, father, currently, age 28 and daughter age 7.

WES revealed this dyad had no shared, known single nucleotide variants (SNVs) associated with inherited ALL (ie, *PAX5*, *ETV6*, *ARI5B*, *IKZF1*, *SH2B3*, *CEPBE*, *CDKN2A*, *PIP4K2A*, *GATA3*, or *TP53*). The dyad had only a few shared SNVs (Tables S1, S2, and S3); none of which were identified as clinically significant, which suggests the spectrum of their somatic mutations were distinctly different. Within the dyad, we identified a shared heterozygous germline structural variant in chromosome 1q32.2 corresponding to the *TRAF3IP3* gene. This microinversion was present in both leukemic and germline samples (Figures 1). This structural variant has not been previously described in ALL but represents the most likely germline defect to explain the inheritance pattern within our dyad. While we cannot exclude that another germline variant may play a role, we also must note that the two family members may both have sporadic ALL. In reviewing the GWAs literature, we were unable to identify any potentially pathogenic SNPs within the *TRAF3IP3* locus or adjacent region.[2,3,6]

TRAF3IP3 (tumor necrosis factor receptor–associated factor 3-interacting protein 3) mediates the activation of signaling pathways downstream of tumor necrosis factor family receptors[19] and overexpressed in a subset of malignancies.[20,21] In mice, TRAF3IP3 is significantly increased in $CD34^{+}$ $CD38^{-}$ $CD7^{+}$ common lymphoid progenitors (CLPs), which are known to differentiate into B-cells,[22,23] and loss of *Traf3ip3* in mice[24] causes an arrest in B-cell development, with lower numbers of both pro-B and pre-B cells in the marrow, similar to other known germline mutations in familial ALL. Given the well-described differences between mouse and human lymphopoiesis, we examined expression of *TRAF3IP3* in human B-cells, hematopoietic stem cells (HSCs), mature T-cells, and myeloid precursors (Figure 2). While *TRAF3IP3* was expressed broadly across human hematopoiesis and lymphopoiesis, there was a downregulation at the early and pro-B-cell stages, with a subsequent upregulation following the transition to a naïve B-cell. This expression pattern, while not definitive, is consistent with a loss of functional TRAF3IP3 during B-cell lymphopoiesis inducing a maturation arrest at the pro-B-cell stage. This microinversion may result in expansion of immature B-cell precursors that are highly

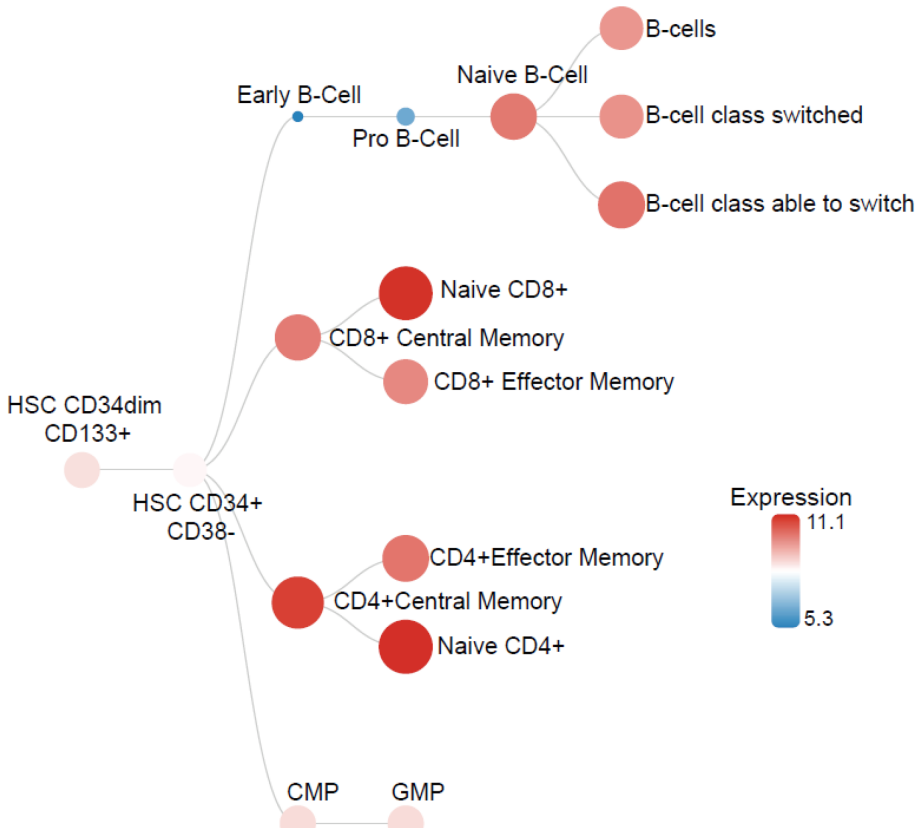

FIGURE 2 *TRAF3IP3* expression data were queried from a published dataset of sorted, man hematopoietic population and visualized using *BloodSpot*. B-cells, mature T-cells, hematopoietic stem cells (HSCs), and myeloid progenitors are included to provide context for the variability in expression of *TRAF3IP3*. CMP, common myelid progenitor; GMP, granulocyte monocyte progenitor [Correction added on 11 March 2021, after first online publication: In the original published version, the legends for Figures 2 and 3 were interchanged and have been corrected in this version.]

vulnerable to secondary somatic mutations which lead to leukemogenesis.[17,18] While we speculate that the microinversion of the *TRAF3IP3* gene in this dyad results in a nonfunctional protein, further studies are required to probe the details of how it affects TRAF3IP3 protein function. Given that the mutation is heterozygous in both germline and leukemia specimens, it may operate as a dominant negative or be haploinsufficient, but in either case would imply an autosomal-dominant inheritance pattern that does not require a loss-of-heterozygosity. Neither the father nor daughter has evidence of increased infection suggestive of a B-cell defect. Posttherapy IgG levels in the daughter have been normal, although no similar data exist for the father. This clinical information is consistent with the predicted partial penetrance of the *TRAF3IP3* structural variant. As demonstrated by the *ETV6* germline mutation literature, partial penetrance can lead to a wide range of different phenotypes within the same mutation profiles.[3] Therefore, it is possible that other family members with this mutation have subtle abnormalities in B- or T-cell function which could be missed if not specifically tested for. Other first-degree relatives of the probands have not yet undergone testing for the presence of this mutation.

Given the lack of concordance in their somatic mutational spectrum, we wondered if the two leukemia samples would exhibit a shared transcriptome, implying convergent leukemogenic pathways were altered within the dyad. In comparing the leukemia gene expression profiles identified by RNA-seq to 216 cases of sporadic B-ALL from the British Columbia Cancer Agency (BCCA) within the TARGET database (the Therapeutically Applicable Research to Generate Effective Treatments program), we discovered that these two leukemia samples did not cluster together (Figure 3 and Table S4). The father's sample clusters with sporadic cases of hyperdiploid ALL, while the daughter's sample clusters with *ETV6-RUNX1* cases (Figure 3; Table S5). It should be noted there was no evidence clinically or within our own DNA-seq and RNA-seq datasets that the father may possess an ETV6-RUNX1 fusion (data not shown). This suggests that the somatic hyperdiploidy and *ETV6-RUNX1* mutations are driving the transcriptome signature and that these two leukemia samples have no shared significant leukemogenic pathways. We reviewed the somatic mutations in the sporadic B-ALL cases within the TARGET database and found one sample with a *TRAF3IP3* mutation which clustered with the father's sample. The lack of *TRAF3IP3* mutations within the other samples is not surprising based on the heterozygosity of this mutation and fact that microinversions such as this are difficult to identify. We suspect that this germline *TRAF3IP3* mutation increased this dyad's susceptibility to leukemia development but that the somatic mutational spectrum drove the leukemia development and dictated its phenotype. Because TRAF3IP3 is involved in MEK signaling, we also compared expression in genes involved in the MEK signaling pathway between the father and daughter's sample compared to the other samples from the TARGET database. These samples did not demonstrate differential expression of any genes within the MEK pathway by Gene Set Enrichment Analysis (GSEA) though the small sample size of the familial samples may have impacted this result (Table S6). Lastly, none of the conserved SNV mutations between the father and the daughter's samples were obviously related to TRAF3IP3; however, the function of TRAF3IP3 is not fully understood, and, therefore, we cannot definitively rule this out.

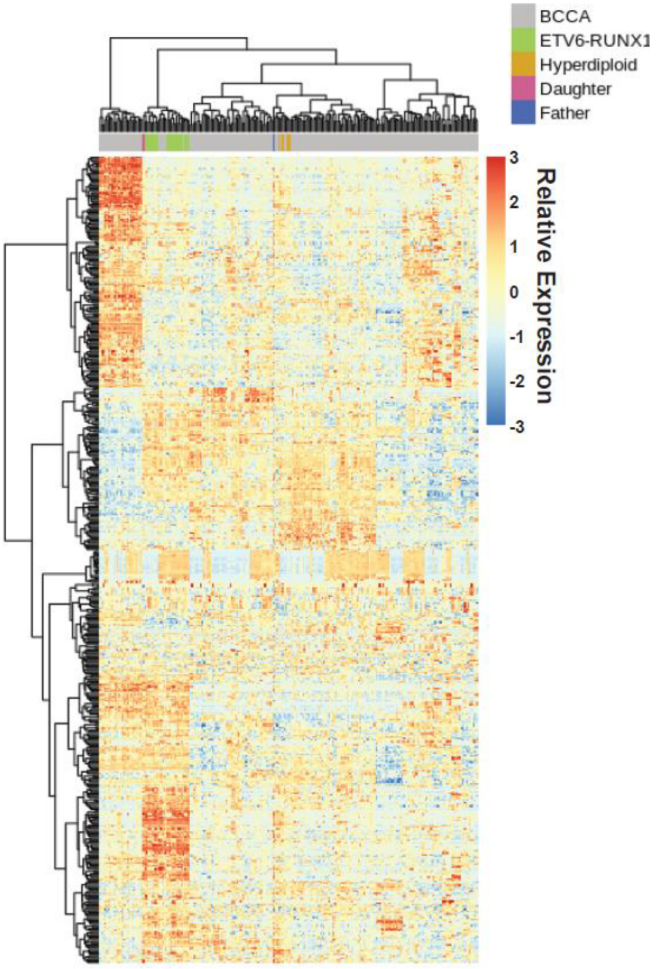

FIGURE 3 RNA-seq indicates additional mutations, rather than germline alterations, drive transcriptomic signature. RNA-seq analysis of the father–daughter pair compared to the BCCA database of sporadic B-ALL (grey) cases via unsupervised hierarchical clustering. Leukemia samples of the father (blue) and daughter (pink) are indicated. Other cases come from the TARGET database of B-ALL, with ETV6-RUNX1 (green) or hyperdiploid (orange) cases indicated. Other cases are shown in grey. Relative expression on a Log_2 axis is indicated using a red:blue color scheme

This research may have identified a novel gene involved in leukemogenesis which may also be involved in de novo cases of ALL. Additional studies are needed to further characterize this *TRAF3IP3* structural variant, the co-occurring somatic mutations within these leukemia samples, and their combined role in leukemogenesis.

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CONFLICTS OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

AUTHOR CONTRIBUTIONS

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, L.P., J.B., M.J.B., S.R., J.B., M.J.B., S.R.; *Investigation*, L.P.; *Formal Analysis*, R.B., Q.F., K.P.; *Data Curation*, R.B., Q.F., K.P.; *Resources*, L.P.; *Writing - Original Draft*, L.P.; *Writing - Review & Editing*, R.B., Q.F., J.B., M.J.B., S.R.; *Visualization*, L.P., S.R.; *Supervision*, S.R.; *Funding Acquisition*, L.P., S.R.

ETHICAL STATEMENT

A protocol was approved through the Children's Hospital of Wisconsin Institutional Review Board (IRB #915943) and the Children's Oncology Group (COG). Written informed consent was obtained from the father and from both parents for the daughter.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding authors. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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