STIMULUS REPORT

blood advances

Germline variants in predisposition genes in children with Down syndrome and acute lymphoblastic leukemia

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Key Points

- Rare and pathogenic germline variants, including in *IKZF1*, contribute to acute lymphoblastic leukemia in children with Down syndrome.

Introduction

Children with Down syndrome (DS), caused by constitutive trisomy of chromosome 21, have an ~20-fold increased risk of developing acute lymphoblastic leukemia (ALL).[1] Trisomy 21 has profound effects on fetal hematopoiesis, resulting in a blockade of B-cell differentiation and an increase in hematopoietic stem cell frequency,[2] and somatic amplification of chromosome 21 occurs frequently in ALL.[3,4] Trisomy 21 clearly increases leukemia risk, yet only ~1% of children with DS will develop ALL, suggesting that additional genetic and nongenetic factors modify risk of disease.

A recent genome-wide association study (GWAS) of DS-ALL confirmed that loci associated with ALL in non-DS children also contribute to risk of ALL in children with DS.[5] Furthermore, several common alleles conferred an increased ALL risk in children with DS relative to non-DS children, including an almost fourfold risk of DS-ALL for the *CDKN2A* missense variant rs3731249. However, the role of rare, high-penetrance germline variants in predisposition genes in DS-ALL etiology has yet to be examined. Here, we performed germline whole-exome sequencing (WES) to assess the frequency of rare and likely pathogenic germline variants in 73 DS-ALL patients in the International Study of Down Syndrome Acute Leukemia.[5]

Methods

Study subjects

The study protocol was approved by the institutional review boards at the University of Southern California and New York State. Additional ethical approval was provided by the Bloodwise Childhood Leukaemia Cell Bank in the United Kingdom (REC: 16/SW/0219). All research using bloodspots was performed without the release of personal identification of any study subjects as approved by appropriate federal and state statutes. For subjects included in the Childhood Leukaemia Cell Bank, written informed consent was obtained from the parents of the participating subjects.

We obtained remission samples from 55 children (<19 years of age) with DS and ALL from the Childhood Leukaemia Cell Bank (United Kingdom) (https://cellbank.org.uk) who were enrolled in the UKALL2003 (2003 to 2011) trial.[6] Deidentified newborn dried bloodspots (DBSs) were obtained from an additional 18 children with DS-ALL, from the New York State Department of Health Newborn Screening program via linkage between the New York State Cancer Registry (to identify ALL patients) and the New York State Congenital Malformations Registry (to identify newborns with DS).[7] These subjects did not overlap with the International Study of Down Syndrome Acute Leukemia samples included in a recently published GWAS of DS-ALL.[5] DNA was extracted from remission samples or neonatal DBSs using Qiagen DNA Investigator kits, and sufficient DNA (>100 ng) for WES was obtained for 73 samples. Patient demographic data and, where available, tumor characteristic and

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clinical outcome data (limited to UK Cell Bank patients) are included in supplemental Table 1. The majority of patients (54 of 73; 74%) were non-Latino white, and the remainder were of black (N = 7), Latino (N = 5), South Asian (N = 3), mixed-race (N = 2), or unknown (N = 2) race/ethnicity. Approximately two-thirds (50 of 73, 68%) of patients were male. Mean patient age at diagnosis was 6.1 years (range, 1-18 years).

CRLF2 rearrangement was assessed by fluorescent in situ hybridization and/or multiplex ligation-dependent probe amplification[8] for 46 patients, of whom 19 (41.3%) had *CRLF2* fusions with either *P2RY8* (N = 16) or *IGH* (N = 3) (supplemental Table 1).

Identification of pathogenic/likely pathogenic variants in predisposition genes

Germline WES was performed by the sequencing service provider MedGenome, with library preparation using Agilent SureSelect Human All Exon V6 target enrichment and 150-bp paired-end sequencing at ~60× coverage using the Illumina HiSeq2500. Variant calling is described in detail in supplemental Methods. For the triploid chromosome 21, variants were called using GATK Haplotype caller in diploid mode, which appeared more accurate than triploid mode or other tools (supplemental Figure 1). Analyses were limited to variants that were predicted damaging, overlapped genes in our candidate predisposition gene list (supplemental Table 2),[9] and were rare (ie, allele frequency <0.01%) in unselected populations, as described in supplemental Methods. We followed the American College of Medical Genetics and Genomics (ACMG) guidelines for classification of variants as "pathogenic" or "likely pathogenic."[10]

Polymerase chain reaction and Sanger sequencing of a subset of variants of interest (N = 4) was performed in patients with available remission or neonatal DBS DNA to validate pathogenic/likely pathogenic variants or likely functional variants in genes of uncertain significance (primer pairs available upon request).

Assessment of *CDKN2A* missense variant rs3731249

DS-ALL patient genotypes for the low-frequency *CDKN2A* missense variant rs3731249, which was filtered out in the analysis of rare variants, were assessed separately to confirm the recently reported high frequency of the ALL-associated risk allele in DS-ALL patients.[5] For all 73 patients, rs3731249 had total read depth >20, genotype quality >20, and average genotype quality >35. Alternate allelic ratio for heterozygous carriers of the rs3731249 risk allele ranged from 0.46 to 0.62. The association between the rs3731249 risk allele and patient *CRLF2* rearrangement subtype was tested using logistic regression and assuming an allelic additive model.

Results and discussion

Germline WES was performed for the 73 DS-ALL patients at a mean read depth of ~66× (range, 26.7-114.1). Copy-number analysis (supplemental Methods) confirmed trisomy 21 status, with 1 apparent partial trisomy encompassing the DS critical region (supplemental Figure 2). Copy-number analysis did not reveal any deletions or gains encompassing known leukemia predisposition genes (data not shown). We identified 143 rare and putatively functional germline variants in cancer-related genes, including 139 missense, 3 frameshift deletions, and 1 splicing variant (supplemental Table 3). No variants of interest were found on the trisomic chromosome 21.

Variants meeting criteria for classification as "pathogenic" or "likely pathogenic" were discovered in 3 of 73 DS-ALL patients (4.1%) (Table 1). One patient (#21869) harbored a pathogenic variant p.Arg162Trp in the ALL predisposition gene *IKZF1*. Germline heterozygous variants at this codon and the adjacent codon (p.Arg162Pro and p.His163Tyr, respectively) were recently reported as pathogenic in sporadic B-cell ALL (B-ALL) patients,[11] with p.Arg162Pro in particular shown to be highly deleterious in functional assays. The p.Arg162Trp variant was previously identified in a Japanese family with inherited dysgammaglobulinemia,[12] and germline p.Arg162Leu and p.Arg162Gln variants were reported in 2 separate families with common variable immunodeficiency, in which 1 of the probands developed childhood B-ALL.[13] Thus, the *IKZF1* codon R162 appears to be a hotspot for germline variants predisposing to immunodeficiency and childhood ALL; indeed, it is the only *IKZF1* codon at which multiple amino acid changes have been reported in B-ALL patients (supplemental Figure 3).

Patient #7102 was heterozygous for the pathogenic frameshift variant p.Lys233Serfs*4 in the Nijmegen breakage syndrome (NBS) gene *NBN*, which was previously reported in families with NBS and families with hereditary breast and ovarian cancer, and is reported as pathogenic in multiple patients in ClinVar.[14-16] Although NBS is an autosomal-recessive disorder, heterozygous germline variants in *NBN* are associated with increased cancer risk, including childhood ALL.[17,18]

In patient #8725, we identified a likely pathogenic missense variant, p.Arg981Trp, in the telomere maintenance gene *RTEL1*. This variant, classed "likely pathogenic" in ClinVar, was identified in 3 unrelated patients with autosomal-recessive dyskeratosis congenita (a disease of telomere attrition), in compound heterozygosity with another *RTEL1* variant.[19] Heterozygous *RTEL1* variants are associated with bone marrow failure and myeloid neoplasms,[20] although the effects on DS-ALL risk have not been reported. Common genetic variants in the region are associated with interindividual variation in telomere length and confer risk of pediatric cancers, including ALL.[21]

In addition to these 3 patients, we identified 2 patients harboring likely functional variants in genes of uncertain significance for leukemia predisposition. One patient (#198516) harbored a heterozygous missense variant p.Arg473Gln in the leukemia fusion gene *MLLT1* (*ENL*), listed as "likely pathogenic" in ClinVar in a patient with congenital abnormalities. Translocations between *MLLT1* and *KMT2A* contribute to both acute lymphoid and myeloid leukemias.[22] Another patient (#24680) harbored a heterozygous frameshift deletion p.Gln3Serfs*80 in *FOXP1*, which encodes a B-cell transcription factor that is highly expressed in B-cell malignancies.[23] Identification of additional ALL patients with deleterious germline variants in these genes will be required to support a role for *MLLT1* and *FOXP1* in leukemia predisposition.

These pathogenic/likely pathogenic germline variants and likely functional variants in genes of uncertain significance (ie, *MLLT1* and *FOXP1*) were validated via polymerase chain reaction and Sanger sequencing (supplemental Figures 3 and 4), except for the *MLLT1* variant as germline material from patient #198516 was exhausted; however, visual inspection in the Integrative Genomics Viewer provided strong confirmatory evidence (supplemental Figure 5). There was no prior history of acute myeloid leukemia in any of these 5 DS-ALL patients, with ALL their primary cancer diagnosis.

Table 1. Pathogenic/likely pathogenic germline variants in 3 of 73 DS-ALL patients in the International Study of Down Syndrome Acute Leukemia

Pt ID	Gene	Chromosome position (hg38)	Ref	Alt	Classification	Amino acid change	CADD scores*	Population allele frequency†	COSMIC somatic hotspot‡	ClinVar codon pathogenic (allele ID)
21869	*IKZF1*	7:50382602	C	T	Missense	p.Arg162Trp	31	0	Yes (4 × R162W)	Pathogenic (226597 + 226598)
7102	*NBN*	8:89971173	CTGTT	C	Frameshift deletion	p.Lys233Serfs*4	33	2.04E-05	—	Pathogenic (133335)
8725	*RTEL1*	20:63693160	C	T	Missense	p.Arg981Trp	32	3.27E-05	—	Likely pathogenic (51189)

—, not present; Alt, alternative allele; CADD, combined annotation-dependent depletion; COSMIC, Catalogue of Somatic Mutations in Cancer; ID, identifier; Pt, patient; Ref, reference allele.

*CADD Phred-scaled scores derived from the GRCh38-v1.5 update.

†Population variant allele frequency presented is the highest value in either the Genome Aggregation Database (gnomAD) or in the Trans-Omics for Precision Medicine (TOPMed) program.

‡Somatic hotspot if codon is mutated in at least 2 unique tumor samples in the COSMIC database.

Finally, in a recent GWAS of DS-ALL, low-frequency *CDKN2A* missense variant rs3731249 (Genome Aggregation Database [gnomAD] risk allele frequency = 0.02), a known risk locus for non-DS ALL,[24] demonstrated a significantly higher risk allele frequency in DS-ALL (~8%) than non-DS ALL patients (~4%).[5] WES enabled assessment of this coding variant, revealing a high risk allele frequency (9.6%) in our independent set of 73 DS-ALL patients (supplemental Table 1). Among 46 patients with available *CRLF2*-rearrangement data, 19 (41.3%) were *CRLF2*-fusion positive, reflecting the higher proportion of *CRLF2* rearrangement known to occur in DS-ALL than in non-DS ALL.[25] We found a significantly higher rs3731249 risk allele frequency in *CRLF2*-fusion patients (21.1%) than in *CRLF2*-null patients (3.7%) ($P = .0099$; odds ratio = 6.68; 95% confidence interval, 1.26-35.5), an association that remained when limiting to non-Latino whites (odds ratio = 7.42; 95% confidence interval, 1.30-42.3), suggesting possible cooperation between germline and somatic alterations in this leukemia subtype. We did not test association of rs3731249 with other cytogenetic subtypes that are common in non-DS ALL, for example, *ETV6-RUNX1* fusion and high hyperdiploidy, due to their low frequency among our DS-ALL patients (supplemental Table 1), as previously observed in DS-ALL.[26]

To our knowledge, this is the first exome-wide assessment of rare germline variation in predisposition genes in DS-ALL; the analysis revealed ~4% of patients with a likely pathogenic variant, a frequency similar to that previously reported for non-DS leukemia.[9] The frequency of such variants in individuals with DS who did not develop ALL is unknown; therefore, sequencing large numbers of individuals with DS, with and without DS-ALL, is warranted to further understand the contribution of rare germline variants to ALL predisposition in this vulnerable population. This would provide estimates on the effect of specific variants, or genes harboring variants, on risk of developing DS-ALL, in addition to the already ~20-fold increased ALL risk due to trisomy 21.[1]

A recent GWAS demonstrated that common variants modify risk of DS-ALL[5]; here, we show that rare germline variants may also cooperate with trisomy 21 in DS leukemogenesis. Our findings support that children with DS-ALL should not be excluded from initiatives to assess germline predisposition genes in ALL patients.

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Authorship

Contribution: A.J.d.S. and A.R. conceived and designed the study; A.J.d.S. performed the experiments; P.W., A.J.d.S., I.S.M., K.M.W., and J.L.W. analyzed the data; A.R., I.R., A.V.M., and A.V. provided patient samples and data; and A.J.d.S. drafted the manuscript; and all authors edited and approved the manuscript.

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