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TO THE EDITOR:

Childhood acute myeloid leukemia shows a high level of germline predisposition

Saumya E. Samaraweera,[1,*] Paul P. S. Wang,[1,2,*] Ka Leung Li,[1,*] Debora A. Casolari,[1] Jinghua Feng,[1,2] Mark Pinese,[3,4] Kyaw Ze Ya Maung,[1] Paul Leo,[5,6] Mark Cowley,[3,4] Kelly Perkins,[7] Amanda M. Smith,[6] Jonathan Ellis,[5] Amilia Wee,[8] Devendra K. Hiwase,[8] Hamish S. Scott,[1,2,9-11] Andreas W. Schreiber,[1,2,10,12] Anna L. Brown,[1,9,10] Andrew J. Deans,[13] David M. Ross,[1,8,11] Andrew S. Moore,[6,14,15] Thomas J. Gonda,[16,17] Christopher N. Hahn,[1,9-11] and Richard J. D'Andrea,[1]

[1]Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, SA, Australia; [2]ACRF Cancer Genomics Facility, SA Pathology, Adelaide, SA, Australia; [3]Children's Cancer Institute, Lowy Cancer Centre, UNSW Sydney, NSW, Australia; [4]School of Women's and Children's Health, Faculty of Medicine, UNSW Sydney, NSW, Australia; [5]Institute of Health and Biomedical Innovation, Queensland University of Technology, QLD, Australia; [6]Diamantina Institute, The University of Queensland, Brisbane, QLD, Australia; [7]MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, United Kingdom; [8]Department of Haematology, Royal Adelaide Hospital, Adelaide, SA, Australia; [9]Department of Genetics and Molecular Pathology, SA Pathology, Adelaide, SA, Australia; [10]UniSA Clinical and Health Sciences, University of South Australia, Adelaide, SA, Australia; [11]Adelaide Medical School and [12]School of Biological Sciences, The University of Adelaide, Adelaide, SA, Australia; [13]St Vincent's Institute of Medical Research, Melbourne, VIC, Australia; [14]Oncology Service, Queensland Children's Hospital, Brisbane, QLD, Australia; [15]Child Health Research Centre, The University of Queensland, Brisbane, QLD, Australia; [16]University of South Australia Cancer Research Institute, Adelaide, SA, Australia; and [17]School of Pharmacy, University of Queensland, Brisbane, QLD, Australia

As germline variants can influence cancer patient treatment decisions, outcomes, and counseling, and as the level of genetic predisposition for sporadic childhood acute myeloid leukemia (AML) is not clearly established, we undertook a comprehensive analysis of rare germline variants in childhood AML. As childhood AML is rare,[1] to date, pan-cancer childhood cohorts have included

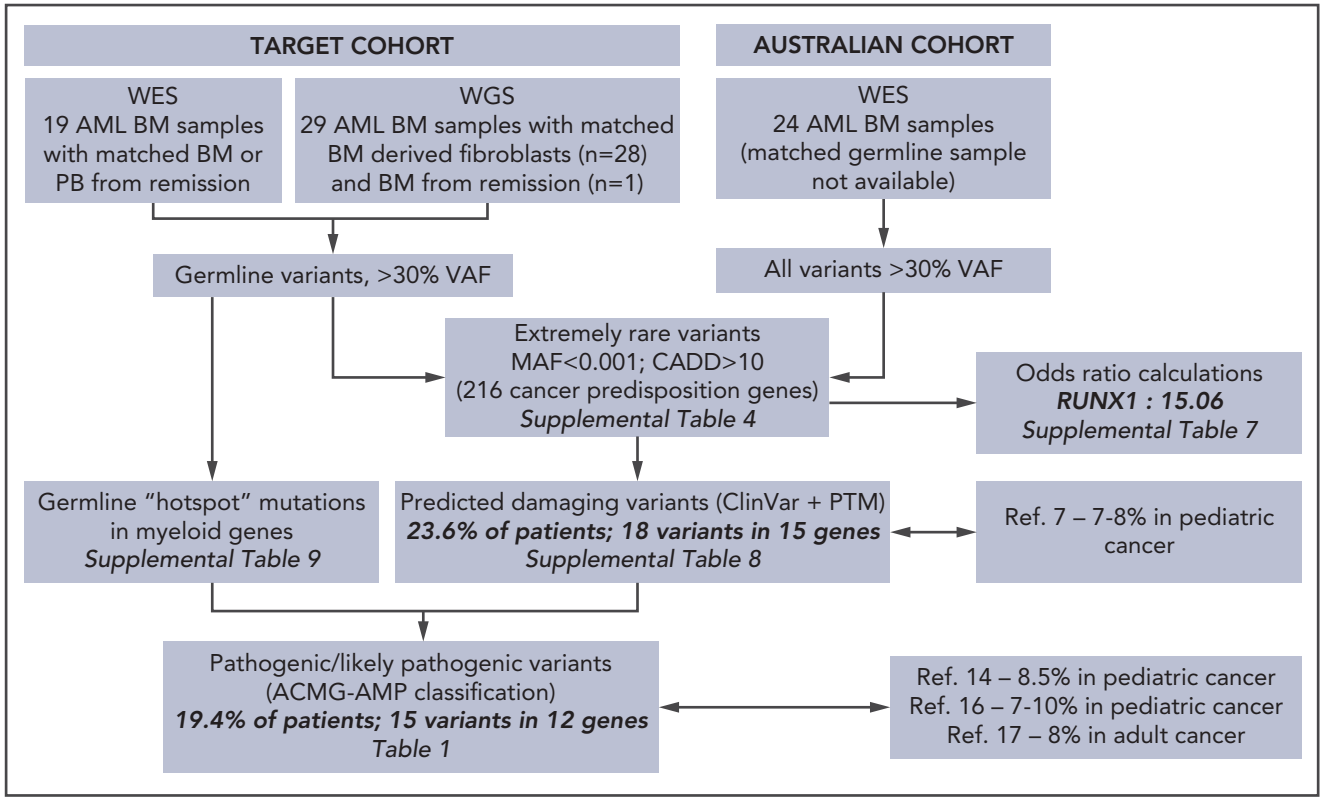

Figure 1. Germline variant analysis flowchart for TARGET and Australian childhood AML cohorts. Germline variant curation workflow for WES and WGS data from TARGET and Australian cohorts and summary of results. PTM, premature termination mutation, includes frameshift indels, stop gain, or splice acceptor/donor site variants in tumor suppressor genes. BM, bone marrow; PB, peripheral blood; WES, whole-exome sequence; WGS, whole-genome sequencing.

few AML cases, and often the germline panels used have not included key genes relevant to myeloid malignancy. We therefore combined data from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) program together with an Australian childhood AML cohort (supplemental Tables 1 and 2, available on the *Blood* Web site) to identify the rare germline variants in a large panel of cancer predisposition genes (n = 216) compiled from literature review, and including genes involved in familial hematological malignancies (HMs) and bone marrow failure syndromes (supplemental Table 3). We analyzed whole-genome sequencing and whole-exome sequence data available through the TARGET program (n = 48) (phs000218.v22.p8.c1) and whole-exome sequence data for the Australian cohort (n = 24). Given that damaging and disease-causing variants are predicted to have a low population prevalence, we identified extremely rare, potentially deleterious germline variants (variant allele frequency [VAF] > 30%; minor allele frequency [gnomAD] <0.001; combined annotation dependent depletion score >10) and classified these as shown in Figure 1. All variants passing initial filtering are listed in supplemental Table 4. The distribution of germline and somatic variants is shown in supplemental Table 5 and supplemental Figure 1. Given the small cohort size, pairwise comparisons of germline variants and clinicopathological characteristics did not reveal significant associations after applying multiple correction (supplemental Table 6).

We compared the prevalence of extremely rare variants in the combined childhood AML cohort (n = 72) to that in the Medical Genome Reference Bank[2,3] (MGRB; n = 2570) comprising individuals aged at least 70 years with no history of cardiovascular disease, dementia, or cancer. To avoid bias, rare variants in both the AML and the MGRB cohorts were selected by comparable filtering procedures, based on variant rarity in the independent gnomAD cohort. We conducted statistical tests on multiple randomized subsamples (n = 6000) of both cohorts, enabling fair testing between cohorts of different sizes. This showed a significant increase of rare alleles in childhood AML for 100% of subset comparisons ($P < .05$; Mann-Whitney *U* test; supplemental Figure 2), and this trend was maintained when analysis was restricted to the European-ancestry subset of patients (79.2% $P < .05$).

As >200 patients are needed to conduct Burden testing,[4] we performed odds ratio (OR) analysis for genes in which variants were observed in ≥4 of 72 patients with AML (5.5%; 10 genes). For the MGRB cohort, we assumed 1 extremely rare variant per gene per individual. This revealed increased odds of rare variants in *RUNX1* occurring in childhood AML (OR, 15.06; $P = .0004$; supplemental Table 7). As the MGRB cohort is 97% non-Finnish European,[3] we also performed a subanalysis with only European ancestry patients with AML after first confirming no high-degree patient relatedness, which may skew these results. This analysis provided strong evidence that ancestry is not driving the increased odds of rare *RUNX1* variants (n = 47; OR, 23.81; $P \leq .0001$). Of the 4 patients in our cohort with rare variants in *RUNX1*, 2 from the TARGET cohort harbored pathogenic mutations linked in the ClinGen resource to familial platelet disorder with associated myeloid malignancy; however, as we did not have access to family history, we cannot provide definitive evidence for familial cancer risk. The other 2 patients harbored extremely rare *RUNX1*

Table 1. Classification of pathogenicity for 21 germline variants using ACMG-AMP guidelines

Patient ID	Cohort	Ancestry	Genomic location	HGNC_ symbol	AML VAF, germline VAF	dbSNP ID	Variant	ACMG-AMP classification	Description of classification
PATABB	TARGET	European	11:108199929	ATM	0.38, 0.48	rs28904921	p.Val2424Gly/ c.7271T>G	Likely pathogenic	PS3 moderate, PM1 moderate, PP3 supporting, PP5 strong, BS1 strong
PASRTP	TARGET	American	17:59760675	BRIP1	0.44, 0.47	rs730881646	p.Met1244fs/ c.3730_3731delAT	Likely benign	BS1 strong
PATJMY	TARGET	European	17:8138449	CTC1	0.54, 0.5	rs748852501	p.Glu454fs/ c.1360delG	VUS	PVS1 very strong, BS1 supporting
PAMYMA	TARGET	European	5:34945846	DNAJC21	0.42, 0.54	rs369359554	p.Arg388*/ c.1162C>T	VUS	PVS1 very strong, BS1 supporting
PARBTV	TARGET	European	2:25457242	DNMT3A	0.46, 0.52	rs147001633	p.Arg882His/ c.2645G>A	Likely pathogenic	PM1 moderate, PM5 moderate, PS3 moderate, PP2 supporting, PP3 supporting, PP5 supporting, BS1 supporting.
PARZIA	TARGET	Asian	2:25468174	DNMT3A	0.56, 0.31	rs149738328	p.Asn501Ser/ c.1502A>G	Likely benign	PM1 moderate, PP2 supporting, BS1 strong, BP4 supporting, BP6 supporting
PAMXZY	TARGET	European	17:17119708	FLCN	0.4, 0.4	rs80338682	p.His429fs/ c.1285delC	Pathogenic	PVS1 very strong, PP3 supporting, PP5 strong, BS1 supporting
PASCGC	TARGET	Asian	13:20763351	GJB2	0.46, 0.51	rs397516874	p.Gln124*/c.370C>T	Pathogenic	PVS1 strong, PP3 supporting, PP5 strong, BS1 supporting
PARZIA	TARGET	Asian	13:20763529	GJB2	0.64, 0.41	rs750188782	p.Gly59fs/ c.176_191delGC TGCAAGAACGTGTG	Pathogenic	PVS1 strong, PP3 supporting, PP5 strong, BS1 strong
13.3	Australian	European	7:151947936	KMT2C	0.65, NA	NA	c.1735 + 2T>C	Pathogenic	PVS1 very strong, PM2 moderate, PP3 supporting
19.3	Australian	Asian	17:29553477	NF1	0.45, NA	rs587781807	p.Ile679fs/ c.2033dupC	Pathogenic	PVS1 very strong, PP3 supporting, PP5 supporting, BS1 supporting,
19.3	Australian	Asian	17:29676260	NF1	0.41, NA	NA	p.Tyr2438fs/ c.7313dupA	Pathogenic	PVS1 very strong, PM2 moderate
PASSLT	TARGET	American	5:131915553	RAD50	0.47, 0.59	NA	c.552-1G>A	Pathogenic	PVS1 very strong, PM2 supporting, PP3 supporting, PP5 supporting

See supplemental Methods for more details.
NA, not available; VUS, variant of uncertain significance.

Table 1. (continued)

Patient ID	Cohort	Ancestry	Genomic location	HGNC_ symbol	AML VAF, germline VAF	dbSNP ID	Variant	ACMG-AMP classification	Description of classification
PATELT	TARGET	European	21:36171704	RUNX1	0.53, 0.49	rs121912499	p.Tyr287*/c.861C>A	Pathogenic	Classified as pathogenic by the ClinGen Myeloid Malignancy Variant Curation Expert Panel
PAKKBK	TARGET	European	21:36252865	RUNX1	0.47, 0.49	rs1060499616	p.Arg166Gln/ c.497G>A	Pathogenic	Classified as pathogenic by the ClinGen Myeloid Malignancy Variant Curation Expert Panel
PARXYR	TARGET	European	16:3639742	SLX4	0.59, 0.56	rs763914156	p.Arg1299fs/ c.3895_3896del AG	VUS	PVS1 very strong, BS1 supporting
14.3	Australian	European	15:38643503	SPRED1	0.35, NA	rs1057518683	p.Arg325*/c.973C>T	Likely pathogenic	PVS1 strong, PM2 moderate, PP3 supporting, PP5 supporting
PATELT	TARGET	European	4:106197552	TET2	0.33, 0.42	rs200971953	p.Pro1983Leu/ c.5948C>T	VUS	PP3 supporting, BS1 strong
24.3	Australian	European	17:7579689	TP53	0.92, NA	NA	p.Val31fs/ c.90_96 + 10delCGT TCTGGTAAGGACAA	Pathogenic	PVS1 very strong PM2 moderate, PP3 supporting
5.3	Australian	European	11:32417942	WT1	0.36, NA	NA	p.Val371fs/ c.1109_1110insC	Pathogenic	PVS1 very strong PM2 moderate, PP3 supporting
PASYEJ	TARGET	European	7:152357810	XRCC2	0.45, 0.5	rs730882048	p.Phe32fs/c.96delT	Pathogenic	PVS1 very strong, PM2 moderate, PP3 supporting, PP5 supporting

See supplemental Methods for more details.
NA, not available; VUS, variant of uncertain significance.

missense variants, including the previously reported pathogenic variant, p.Asp198Asn.[5]

We next applied additional stringent filtering to determine predicted damaging germline predisposition variants. First, we considered variants that are (1) annotated as pathogenic or likely pathogenic in the ClinVar[6] database, or (2) frameshift indels, stop gain, or splice acceptor/donor site variants in tumor suppressor genes, which are likely to result in loss of protein function. As matched nontumor material was not available for the Australian cohort, we have used caution for variants from this cohort and excluded those that have been recurrently reported as somatic in HM (see supplemental Methods). Overall, 17 patients were identified (24%; 21% of the Australian cohort and 25% of the TARGET cohort) with a total of 18 variants across 15 genes (supplemental Table 8). This frequency is substantially higher than that reported with cancer predisposition gene sets and similar variant classification criteria in a pan-cancer childhood cohort (7% to 8%[7]; Figure 1) and adult AML (9%[8]). The frequency of predicted damaging germline predisposition variants in childhood AML may be higher than that estimated, as we identified a further 3 germline variants in the TARGET cohort (supplemental Table 9) by analyzing mutation hotspots in additional genes that are somatically mutated in myeloid malignancies (supplemental Table 10), including a heterozygous mutation affecting *DNMT3A* Arg882 (patient PARBTV; germline specimen bone marrow–derived fibroblasts); such mutations are associated with Tatton-Brown-Rahman syndrome[9,10] for which an adolescent case of AML has been reported.[11]

Finally, we assessed the 18 rare variants identified by our analysis above (supplemental Table 8), and the 3 additional hotspot variants from the TARGET cohort (supplemental Table 9), for pathogenicity using the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) criteria.[12] Fifteen variants in 14 patients (19.4%; 21% of the Australian cohort and 19% of the TARGET cohort) were classified as pathogenic or likely pathogenic using these criteria (Table 1). *RUNX1* mutations may be particularly important for sporadic childhood AML, as 2 patients harbored pathogenic mutations (p.Arg166Gln and p.Tyr287*) that would fit into the recently defined World Health Organization category "myeloid neoplasms with germline predisposition."[13] The overall frequency of predisposition variants when ACMG-AMP criteria are used is considerably higher than that reported in other childhood cancer studies (7% to 10% overall[14-16]) and for most adult cancers (8% overall[17]; Figure 1), although in a similar range to that reported recently for high-risk pediatric cancers (16.2%)[18] and specific childhood tumors.[16,19] We acknowledge that analyses of the frequency of predicted and classified pathogenic variants across different cohorts are not directly comparable, as differences or improvements in variant calling may contribute to the observed elevated frequency.[18] Interestingly, apart from *RUNX1*, we did not identify pathogenic or likely pathogenic variants in several established familial myeloid malignancy genes (*GATA2*, *DDX41*, *SAMD9*, *SAMD9L*, *ETV6*, or *CEBPA*), or in genes associated with familial childhood syndromes that confer risk of HM (*TP53*, *SBDS*, RAS pathway genes). A pathogenic variant of interest was detected in *XRCC2/FANCU*, associated with Fanconi anemia, which confers a highly elevated risk of HM in early life.[20] Some predisposition genes for which we detected predicted pathogenic germline variants in TARGET patients have not previously been associated with HM (eg, *GJB2* and *FLCN*).

The key finding from this study is that the frequency of rare germline cancer predisposition variants in newly diagnosed childhood AML is higher than suggested previously,[21] indicating a clinically significant risk in an important fraction of patients. The detection of such a germline mutation at diagnosis is an important consideration for stem cell donor selection, and genetic counseling for the family and patient. Further studies in larger, independent cohorts with detailed family history are now warranted to clarify the role of germline predisposition variants in childhood AML.

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Authorship

Contribution: S.E.S. conducted the analyses, interpreted results, and wrote the manuscript; P.P.S.W. conducted bioinformatic and statistical analyses of variants; K.L.L. analyzed and interpreted variant distributions; D.A.C. supervised research, interpreted results, managed resources, and contributed to manuscript drafts; J.F. performed bioinformatic analysis of next-generation sequencing data for germline variants; M.P. provided data and interpreted results; K.Z.Y.M. analyzed gene variants and contributed to manuscript drafts; P.L. performed bioinformatics analyses; M.C. provided analysis tools and interpreted results; K.P. analyzed variants and interpreted data; A.M.S. provided clinical samples and information; J.E. performed bioinformatic analyses; A.W. and D.K.H. provided methodology for variant classification; H.S.S. contributed to interpretation of data and manuscript revisions; A.W.S. supervised bioinformatic analyses; A.L.B. analyzed variants and interpreted data; A.J.D. provided advice on bone marrow failure syndromes, interpreted data, and provided manuscript revisions; D.M.R. interpreted data and provided critical review and revision of the manuscript; A.S.M. provided clinical material and patient information, interpreted data, and acquired funding; T.J.G. interpreted data and acquired funding; C.N.H. coordinated the variant analysis and interpreted data; S.E.S., P.P.S.W., T.J.G., C.N.H., and R.J.D. wrote the paper; R.J.D. and T.J.G. conceived the study; and R.J.D. supervised the research, acquired funding, and wrote the manuscript.

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ORCID profiles: S.E.S., 0000-0002-9619-0624; K.L.L., 0000-0001-5674-4621; M.C., 0000-0002-9519-5714; A.M.S., 0000-0002-8716-6745; H.S.S., 0000-0002-5813-631X; A.W.S., 0000-0002-9081-3405; A.L.B., 0000-0002-9023-0138; D.M.R., 0000-0001-7171-2935; C.N.H., 0000-0001-5105-2554; R.J.D'A., 0000-0002-4915-4612.

Correspondence: Richard D'Andrea, Centre for Cancer Biology, University of South Australia, GPO Box 2471, Adelaide, SA 5001, Australia; e-mail: richard.dandrea@unisa.edu.au.

Footnotes

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*S.E.S., P.P.S.W., and K.L.L. contributed equally to this study.

The sequence data for the Australian cohort will be deposited at the European Nucleotide Archive, which is hosted by the European Bioinformatics Institute. Access to the Pediatric Cancer Research in National Cancer Institute TARGET (dbGAP Study phs000218) dataset is restricted by the National Institutes of Health and was used with approval (project ID #19648). Other data and resources are available from the corresponding author upon reasonable request.

The online version of this article contains a data supplement.

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