COLD SPRING HARBOR
Molecular Case Studies

RESEARCH ARTICLE

Enrichment of heterozygous germline *RECQL4* loss-of-function variants in pediatric osteosarcoma

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Abstract Patients harboring germline pathogenic biallelic variants in genes involved in the recognition and repair of DNA damage are known to have a substantially increased cancer risk. Emerging evidence suggests that individuals harboring heterozygous variants in these same genes may also be at heightened, albeit lesser, risk for cancer. Herein, we sought to determine whether heterozygous variants in *RECQL4*, the gene encoding an essential DNA helicase that is defective in children with the autosomal recessive cancer-predisposing condition Rothmund–Thomson syndrome (RTS), are associated with increased risk for childhood cancer. To address this question, we interrogated germline sequence data from 4435 pediatric cancer patients at St. Jude Children's Research Hospital and 1127 from the National Cancer Institute Therapeutically Applicable Research to Generate Effective Treatment (TARGET) database and identified 24 (0.43%) who harbored loss-of-function (LOF) *RECQL4* variants, including five of 249 (2.0%) with osteosarcoma (OS). These *RECQL4* variants were significantly overrepresented in children with OS, the cancer most frequently observed in patients with RTS, as compared to 134,187 noncancer controls in the Genome Aggregation Database (gnomAD v2.1; $P = 0.00087$, odds ratio [OR] = 7.1, 95% CI, 2.9–17). Nine of the 24 (38%) individuals possessed the same c.1573delT (p.Cys525Alafs) variant located in the highly conserved DNA helicase domain, suggesting that disruption of this domain is central to oncogenesis. Altogether these data expand our understanding of the genetic factors predisposing to childhood cancer and reveal a novel association between heterozygous *RECQL4* LOF variants and development of pediatric OS.

[Supplemental material is available for this article.]

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INTRODUCTION

The RecQ like helicase 4 (*RECQL4*) gene encodes a helicase essential for repairing DNA damage and maintaining genomic stability (Lu et al. 2016). Pathogenic homozygous or compound heterozygous variants affecting *RECQL4* cause three clinically overlapping autosomal recessive (AR) disorders, namely Rothmund–Thomson syndrome (RTS), Baller–Gerold syndrome (BGS), and RAPADILINO (RAdial ray defect, PAtellar aplasia/arched or cleft PAlate, DIarrhea/Disclosed joints, LIttle size/LImb malformation, NOse slender/NOrmal intelligence) syndrome (Wang and Plon 2019). Rothmund–Thomson syndrome is the most prevalent of these syndromes, with nononcologic features including poikiloderma; sparse hair, eyelashes, and eyebrows; short stature; dental abnormalities; dysplastic or poorly formed nails; and gastrointestinal problems in infancy (Siitonen et al. 2009). BGS is the rarest of these disorders and is characterized by craniosynostosis, radial ray defects, short stature, and malformed or missing patellae (Van Maldergem et al. 2007). Finally, RAPADILINO syndrome is characterized by short stature, radial and patellar aplasia or hypoplasia, absence of thumbs, dislocation of joints, highly arched palate, infantile diarrhea, and pigmentary changes or café au lait macules (Siitonen et al. 2003). A predisposition to cancer has been reported in all three syndromes, with the highest cancer occurrence in RTS. Patients with RTS, and to a lesser degree RAPADILINO, are at greatest risk to develop osteosarcoma (OS) (Siitonen et al. 2003; Cao et al. 2017; Wang and Plon 2019), whereas patients with all three conditions are at risk for lymphoma (Van Maldergem et al. 2007; Siitonen et al. 2009).

Through a clinical research protocol (NCT02530658), we identified a child with OS for whom germline whole-exome and whole-genome sequencing (WGS) and in-depth analysis of 156 cancer predisposition genes revealed a pathogenic alteration and a variant of uncertain significance (VUS) in *RECQL4*, with the variants present *in trans*. In light of evolving evidence that heterozygous germline variants affecting other DNA repair genes such as *ATM*, *BRCA2*, and *PALB2* confer an increased cancer risk (Thompson et al. 2005; Renwick et al. 2006; Antoniou et al. 2014; Helgason et al. 2015; Rebbeck et al. 2015; Esteban-Jurado et al. 2016; Esai Selvan et al. 2019), we questioned whether heterozygous germline *RECQL4* variants, such as the one observed in our patient, might be associated with development of pediatric cancer. To address this question, we analyzed 5562 children with cancer and identified a significant enrichment of heterozygous *RECQL4* loss-of-function (LOF) variants in patients with OS. Notably, most children carried the same *RECQL4* alteration: c.1573delT (p.Cys525Alafs), which is predicted to truncate the RECQL4 protein within its DNA helicase domain. *RECQL4* LOF variants were rarely observed in children with other cancer types, in which they were not significantly enriched compared to noncancer controls.

RESULTS

Characteristics of the Index Case

A 16-yr-old girl with previously treated OS of the left tibia developed recurrent OS of the left femur and left wrist, 5 and 8 yr after the initial diagnosis, respectively. Family history revealed a maternal first cousin with an unspecified brain tumor at 3 yr of age (Fig. 1). The maternal grandparents developed cancer after 60 yr of age, but no other family members were reported to have cancer. Germline WGS performed as part of a clinical research protocol (NCT02530658) that comprehensively analyzed 156 cancer predisposition genes (Supplemental Table 1) revealed no pathogenic or likely pathogenic (P/LP) variants in known OS genes such as *TP53*, *RB1*, the mismatch repair, and Fanconi anemia genes. However, testing did reveal two rare variants in *RECQL4*, including a frameshift c.1573delT

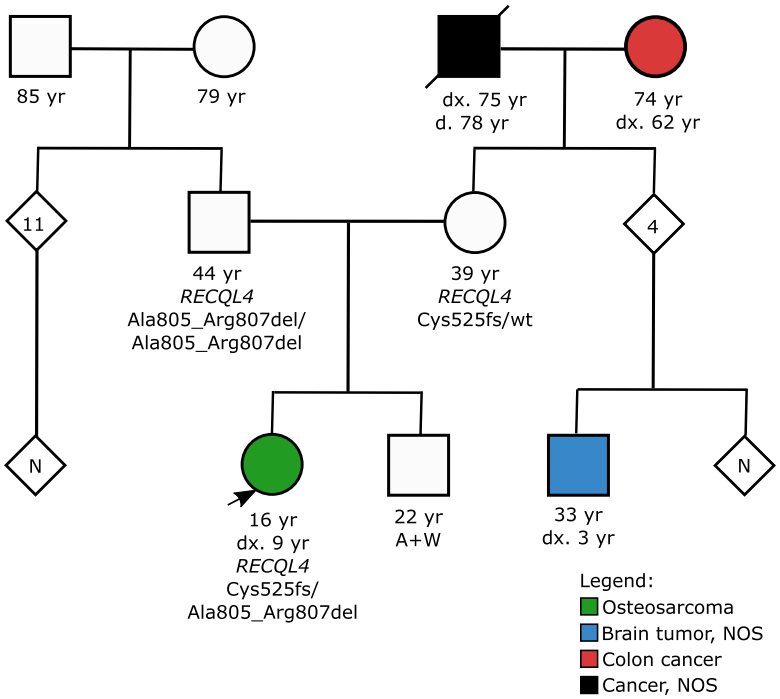

Figure 1. Three-generation pedigree of a 16-yr-old female with recurrent osteosarcoma (arrowhead). Circles and squares denote female and male family members, respectively, and shaded figures denote persons with cancer. The individuals age in years (yr), age at diagnosis (dx.), and age at death (d.) are indicated on the pedigree where applicable. (NOS) Not otherwise specified.

(p.Cys525Alafs) and an in-frame deletion of nine nucleotides that is predicted to remove three amino acids c.2412_2420del (p.Ala805_Arg807del) (Table 1). These variants were classified by a CLIA-certified laboratory as pathogenic and of uncertain significance, respectively, based on the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines for sequence variant interpretation (Richards et al. 2015).

On physical examination, the patient was well-developed with no dysmorphic facial features. She had relatively thin hair and eyebrows, which by the patient's account predated chemotherapy. Her height was about 2nd percentile for age and in proportion to the calculated midparental height (height = 148 cm, midparental height = 153.5 cm). Although her head circumference was small, it was in proportion to her height and weight. Approximately 12 café au lait macules were observed on the patient's trunk and buttocks ranging in size from 4 mm to 4 cm; scattered hypopigmented areas on her left thigh and

Table 1. Germline *RECQL4* variants identified in the index case

Gene	Chr	Location	Exon	HGVS DNA	HGVS protein	Variant type	dbSNP ID	Genotype	Parent of origin	Variant allele frequency	Coverage	Clinical significance in ClinVar	Comments
RECQL4	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	Rs386833845	Heterozygous	Mother	0.277	90	P	Mother is heterozygous
RECQL4	8	144,513,269	15	c.2412_2420del	p.Ala805_Arg807del	In-frame deletion	Rs766312203	Heterozygous	Father	0.308	13	VUS	Father is homozygous

trunk were also observed. No axillary freckling was observed, and none of the skin lesions were consistent with poikiloderma. Based on the clinical phenotype, the patient did not meet the diagnostic criteria for RTS (Wang and Plon 2019) by the account of two clinical geneticists who examined the patient on multiple occasions and/or reviewed multiple photographs highlighting close-up views of the face, stature, and pigmented skin lesions. The patient's parents had no clinical features of RTS and no personal history of cancer. Based on parental testing, the two variants found in the patient were determined to be on opposite chromosomes (*in trans*). This testing revealed that the patient's father was homozygous for the c.2412_2420del (p.Ala805_Arg807del) VUS. The absence of RTS features in the father provided supporting evidence that this VUS was likely benign or that it is a hypomorphic allele that does not have a phenotype when found in the homozygous state but may play some role in this patient's unusual phenotype when opposite a loss-of-function variant. The patient's mother was heterozygous for the pathogenic c.1573delT (p.Cys525Alafs) variant. The patient's brother was not reported to have any clinical features of RTS and no personal history of cancer. He did not opt to pursue genetic testing and as a result, his *RECQL4* genetic status remains unknown.

Prevalence of Heterozygous Germline *RECQL4* LOF Variants in Pediatric Cancer

Heterozygous pathogenic variants in DNA damage repair genes (e.g., *ATM*, *BRCA2*, *PALB2*) are associated with a moderate to high cancer risk (Thompson et al. 2005; Renwick et al. 2006; Antoniou et al. 2014; Helgason et al. 2015; Rebbeck et al. 2015; Esteban-Jurado et al. 2016; Esai Selvan et al. 2019), whereas compound heterozygous or homozygous variants confer syndromic presentations such as ataxia-telangiectasia (*ATM*) (Swift et al. 1987; Ahmed and Rahman 2006) and Fanconi anemia (*BRCA2*, *PALB2*) (Howlett et al. 2002; Reid et al. 2007), which confer an even greater cancer risk. Building upon this notion, we sought to determine whether heterozygous *RECQL4* LOF variants are more prevalent in pediatric oncology patients as compared to control individuals not selected for cancer. Among 4435 pediatric cancer patients at St. Jude Children's Research Hospital (Zhang et al. 2015; Wang et al. 2018) and 1127 in the National Cancer Institute TARGET database (dbGaP accession phs000218.v20.p7), we identified 24 of 5562 (0.43%) who carried heterozygous *RECQL4* LOF variants (Table 2), including five of 249 (2.0%) with OS (Fig. 2).

To determine whether these LOF variants are enriched in children with cancer, we examined their prevalence in the Genome Aggregation Database (gnomAD v2.1, noncancer; Lek et al. 2016), which spans 118,479 whole-exome and 15,708 whole-genome sequences from individuals who were not ascertained for having cancer. There were a total of 385 *RECQL4* LOF alleles in the gnomAD noncancer population after removal of the founder variant, c.1390+2delT (p.Ala420_Ala463del), which is commonly encountered in the Finnish population at a minor allele frequency of 0.4% (Lek et al. 2016) but was absent from our cohort. Low-confidence LOF variants and variants of dubious quality were also removed as detailed in the Methods. Compared to the noncancer cohort in gnomAD, we observed a significant association between heterozygous *RECQL4* LOF variants and OS ($P = 0.00087$; OR = 7.1; 95% CI, 2.9–17.0) but no association for other tumor types. One patient with retinoblastoma was noted to harbor an additional pathogenic variant in *RB1* and was excluded from subsequent analysis. Available tumor data from 12 germline *RECQL4* LOF variant positive cases did not show loss of heterozygosity for *RECQL4* or any pathogenic or likely pathogenic variants in the remaining *RECQL4* allele.

Evaluation of *RECQL4* Genotype and Association with Clinical Phenotype

To understand how germline *RECQL4* LOF variants impacted the encoded protein, we evaluated their location within the gene. Among the 24 variants identified, 17 (71%) resided

Table 2. Heterozygous germline *RECQL4* LOF variants detected in pediatric sequencing cohorts

Subject ID	Cancer diagnosis	Age at diagnosis	Ethnicity of subject	Gene	Chr	Location (hg38)	Exon	HGVS DNA	HGVS protein	Variant class	WGS variant allele frequency	WES coverage	Variant allele frequency	Coverage	dbSNP ID	Clinical significance in ClinVar	AF in gnomad noncancer v2.1	Ethnicity-specific AF in gnomAD noncancer v2.1	Cohort
SJCNS044968	Craniopharyngioma	5	NFE	*RECQL4*	8	144,516,696	5	c.423delG	p.Lys141Asnfs*39	Frameshift	0.294	34	NA	NA	-	-	NA	NA	SJCRH
SJALL016422	Acute B-lymphoblastic leukemia	NA	NFE[b]	*RECQL4*	8	144,516,254	5	c.865delG	p.Ala289Leufs*4	Frameshift	NA	NA	0.687	32	-	-	NA	NA	TARGET
SJALL019720	Acute lymphoblastic leukemia, NOS	11	NFE	*RECQL4*	8	144,516,248	5	c.871delG	p.Ala291Leufs*2	Frameshift	0.409	22	0.545	33	rs1389647533	P	4.28×10^{-06}	9.85×10^{-06}	SJCRH
SJALL015934	Acute B-lymphoblastic leukemia	NA	SAS[b]	*RECQL4*	8	144,515,890	7	c.1132-2A>G	p.Ala378_E6splice	Splice	NA	NA	0.510	249	-	-	4.29×10^{-06}	3.28×10^{-05}	TARGET
SJALL019022	Acute B-lymphoblastic leukemia	3	NFE	*RECQL4*	8	144,515,243	7	c.1391-1G>A	p.Glu464_E8splice	Splice	0.423	26	0.750	16	rs117642173	P	2.89×10^{-05}	6.59×10^{-05}	SJCRH
SJAML007029	Acute myeloid leukemia	11	NFE	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	0.567	30	0.550	20	rs386833845	P	2.43×10^{-04}	4.21×10^{-04}	SJCRH
SJOS019534	Osteosarcoma	6	NFE	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	0.355	31	0.429	28	rs386833845	P	2.43×10^{-04}	4.21×10^{-04}	SJCRH
SJCBF029	Acute myelogeneous leukemia	11	NFE	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	NA	NA	0.571	28	rs386833845	P	2.43×10^{-04}	4.21×10^{-04}	SJCRH
SJBALL030048	Acute B-lymphoblastic leukemia	3	NFE[c]	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	0.218	64	0.315	200	rs386833845	P	2.43×10^{-04}	4.21×10^{-04}	SJCRH
SJOS030129[a]	Osteosarcoma	8	AMR[c]	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	NA	NA	0.277	90	rs386833845	P	2.43×10^{-04}	1.42×10^{-04}	SJCRH
SJALL041208	Acute B-lymphoblastic leukemia	4	NFE	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	0.4	30	0.470	66	rs386833845	P	2.43×10^{-04}	2.43×10^{-04}	SJCRH
SJGCT041514	Germ cell tumor, testicular	0.3	NFE	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	0.364	44	0.473	55	rs386833845	P	2.43×10^{-04}	2.43×10^{-04}	SJCRH
SJBALL002191	Acute B-lymphoblastic leukemia	NA	NFE[b]	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	NA	NA	0.293	58	rs386833845	P	2.43×10^{-04}	2.43×10^{-04}	TARGET
SJTALL022098	Acute T-lymphoblastic leukemia	10	AMR[b]	*RECQL4*	8	144,514,983	9	c.1573delT	p.Cys525Alafs*33	Frameshift	NA	NA	0.545	11	rs386833845	P	2.43×10^{-04}	1.44×10^{-04}	TARGET
SJHL019300	Hodgkin lymphoma	7	AFR	*RECQL4*	8	144,514,363	10	c.1705-1delG	p.Ile569_E11splice	Splice	0.364	44	0.500	10	-	-	NA	NA	SJCRH
SJST030131	Spindle cell sarcoma	16	AFR[c]	*RECQL4*	8	144,514,350	11	c.1717C>T	p.Gln573*	Nonsense	0.275	58	0.25	8	-	-	3.19×10^{-05}	1.15×10^{-04}	SJCRH

(*Continued on next page.*)

Table 2. (*Continued*)

Subject ID	Cancer diagnosis	Age at diagnosis	Ethnicity of subject	Gene	Chr	Location (hg38)	Exon	HGVS DNA	HGVS protein	Variant class	WGS variant allele frequency	WES coverage	Variant allele frequency	Coverage	dbSNP ID	Clinical significance in ClinVar	AF in gnomad noncancer v2.1	Ethnicity-specific AF in gnomAD noncancer v2.1	Cohort
SJTALL065	Acute T-lymphoblastic leukemia	8	AFR	*RECQL4*	8	144,513,592	13	c.2178_2179insCCTGGGTC	p.Ala727Profs*119	Frameshift	NA	NA	0.2	73	-	-	NA	NA	SJCRH
SJOS040215	Osteosarcoma	NA	NFE[b]	*RECQL4*	8	144,513,412	14	c.2269C>T	p.Gln757*	Nonsense	NA	NA	0.503	153	rs137853229	P	1.24×10^{-04}	1.45×10^{-04}	TARGET
SJOS040163	Osteosarcoma	NA	NFE[b]	*RECQL4*	8	144,513,383	15	c.2296+1C>G	p.Arg766_E15splice	Splice	NA	NA	0.357	26	rs199605511	-	NA	NA	TARGET
SJNHL019456	Non-Hodgkin lymphoma	7	NFE	*RECQL4*	8	144,513,109	16	c.2492_2493delAT	p.His831Argfs*52	Frameshift	0.263	19	0.064	47	rs752729755	P	7.02×10^{-05}	7.01×10^{-05}	SJCRH
SJHL042013	Hodgkin lymphoma	18	NFE	*RECQL4*	8	144,513,109	16	c.2492_2493delAT	p.His831Argfs*52	Frameshift	0.5	34	0.400	5	rs752729755	P	7.02×10^{-05}	7.01×10^{-05}	SJCRH
SJOS040168	Osteosarcoma	NA	NFE[b]	*RECQL4*	8	144,512,846	16	c.2755+1G>A	p.Ala919_E16splice	Splice	NA	NA	0.316	19	rs373130543	-	3.16×10^{-05}	2.86×10^{-05}	TARGET
SJALL019726	Acute B-lymphoblastic leukemia	3	NFE	*RECQL4*	8	144,513,109	19	c.3073_3074delAG	p.Thr1024_Glu1025fs	Frameshift	0.44	25	0.385	39	-	-	NA	NA	SJCRH
SJRB001130	Retinoblastoma	NA	OTH	*RECQL4*	8	144,511,911	20	c.3393+2T>G	p.Arg1131_E20splice	Splice	0.6	15	NA	NA	rs557284122	-	1.52×10^{-05}	$0.00 \times 10^{+00}$	SJCRH

All ethnicities were computationally predicted unless otherwise noted. All predicted populations were consistent with self-reporting unless otherwise noted.
(LOF) Loss of function, (WGS) whole-genome sequencing, (WES) whole-exome sequencing.
[a]Index case.
[b]Self-reporting not available.
[c]Self-reported ethnicity only.

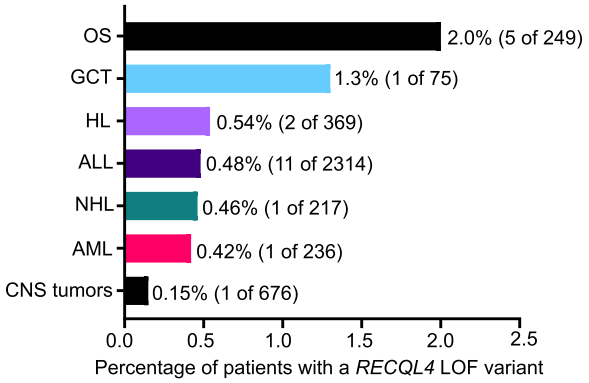

Figure 2. Frequency of heterozygous *RECQL4* LOF mutations across pediatric cancer types. (OS) osteosarcoma, (GCT) germ cell tumor, (HL) Hodgkin lymphoma, (ALL) acute lymphoblastic leukemia, (NHL) non-Hodgkin lymphoma, (AML) acute myeloid leukemia, (CNS) central nervous system, (LOF) loss-of-function.

within the helicase domain (residues 489–850; Fig. 3), which is critical to maintaining genome stability, specifically in the case of DNA repair (Chu and Hickson 2009; Croteau et al. 2012). All variants in the helicase domain are predicted to result in NMD or a disrupted protein product. Four of 24 (17%) patients harbored germline *RECQL4* variants amino-terminal to the helicase domain, all of which are predicted to cause nonsense-mediated decay (NMD). Of these, three were located in a region having sequence similarity to yeast Sld2 (the Sld2-like domain; residues 1–388), which is essential for the initiation of DNA replication, and the fourth was located in a nuclear targeting signal (residues 363–492) (Colombo et al. 2018). Three of 24 patients had variants located carboxy-terminal to the helicase domain, in exons 16 and 19, which are also predicted to cause NMD.

Notably, nine (38%) of the *RECQL4* LOF variant–positive individuals were noted to carry the same alteration, c.1573delT (p.Cys525Alafs), which was also present in our index case (Fig. 3). Patients with this variant carried diagnoses of acute lymphoblastic leukemia (ALL; $n = 4$), OS ($n = 2$), acute myeloid leukemia (AML; $n = 2$), and germ cell tumor (GCT; $n = 1$) and were of different racial and ethnic backgrounds (Table 2). Our analysis revealed that this particular variant is significantly enriched in the pediatric cancer population ($P = 0.0024$; OR = 3.3; 95% CI, 1.7–6.7) compared to the gnomAD noncancer cohort in which this variant accounts for 64 of 385 (17%) *RECQL4* LOF variants with a global allele frequency of 2.43×10^{-4}. This supports the notion that the c.1573delT (p.Cys525Alafs) alteration contributes to the pathogenesis of pediatric cancer; however, its presence in a presumably healthy population suggests that it may be a lower penetrance allele.

To investigate the possible penetrance of germline *RECQL4* LOF variants, we examined the family histories of four patients from the St. Jude cohort for whom such information was available. None of these patients had first- or second-degree relatives with early onset of cancer (defined here as cancer before 50 yr of age; Fig. 4). This further supports the idea that heterozygous *RECQL4* LOF variants function as lower penetrance alleles. These data are consistent with the literature in which there are only rare reports of relatives of RTS patients who have developed OS (Siitonen et al. 2009).

Prevalence of Germline LOF Variants in Other Genes of the RecQ Helicase Family

Because the RecQ helicase genes are highly conserved, it remained possible that other family members might also be associated with childhood cancer development. Therefore, we examined the St. Jude and TARGET cohorts for heterozygous LOF variants in *RECQL*, *BLM*, *WRN*, and *RECQL5*. Through these studies, we identified 51 children with

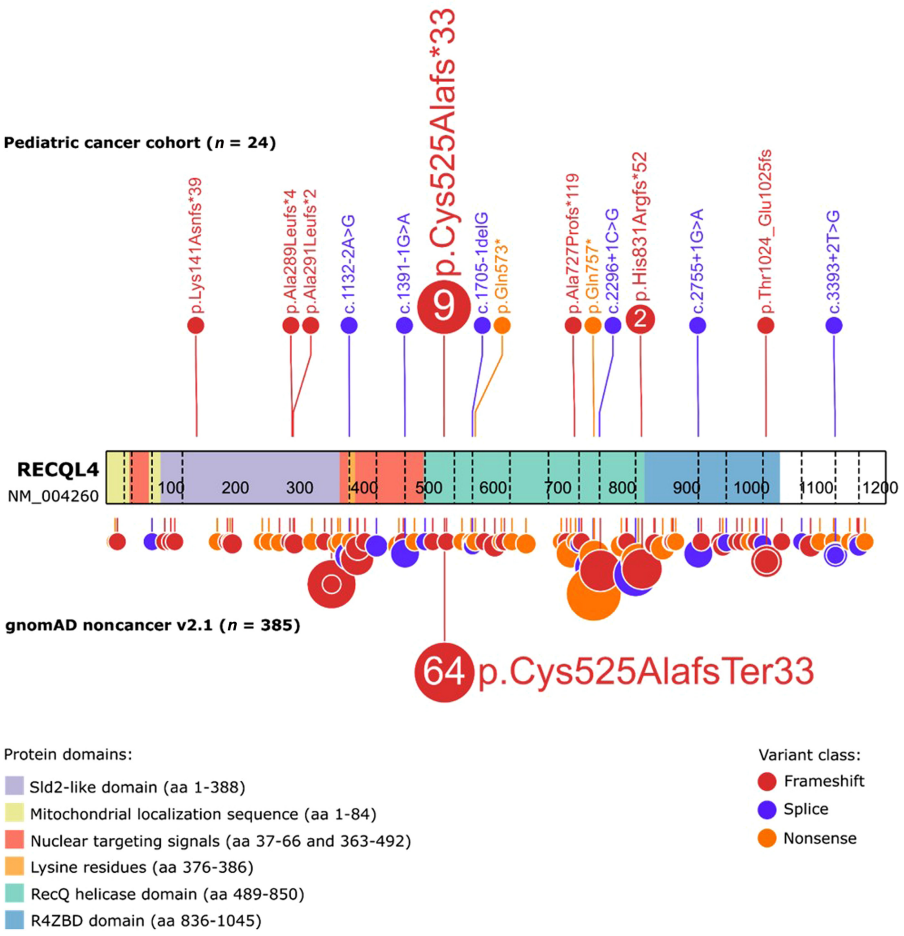

Figure 3. Distribution of the germline heterozygous *RECQL4* LOF mutations in the pediatric cancer cohort examined in this study and in gnomAD noncancer v2.1. Known domains of the RECQL4 protein are as shown.

heterozygous LOF variants in one of these genes, including 13 with variants in *RECQL*, 13 with variants in *BLM*, 15 with variants in *WRN*, and 10 with variants in *RECQL5* (Supplemental Table 2). Pan-cancer analyses did not reveal any significant associations between the presence of heterozygous LOF variants in other RecQ helicases and pediatric cancer (Table 3). However, cancer-specific analyses identified a significant association in which *RECQL* variants were present in 1.2% (3 out of 249) of OS cases ($P = 0.037$; OR = 4.2; 95% CI, 1.3–13.1). Additional nonsignificant associations of potential interest include the identification of *RECQL* variants in 1.3% (2 out of 150) of rhabdomyosarcoma cases ($P = 0.071$; OR = 4.6; 95% CI, 1.2–18.7) and *WRN* variants in 0.39% (9 out of 2314) of pediatric ALL patients ($P = 0.061$; OR = 1.9; 95% CI, 0.98–3.7).

Population Admixture of the Childhood Cancer Cohort Compared to the gnomAD Cohort

To determine whether the enrichment of germline *RECQL4* or *RECQL* variants might be due to differences in the ethnic composition of pediatric cancer versus gnomAD noncancer cohorts, we compared the population admixtures of these two groups. Here, the ethnicity of individuals in the pediatric cancer cohort was computationally predicted (Supplemental

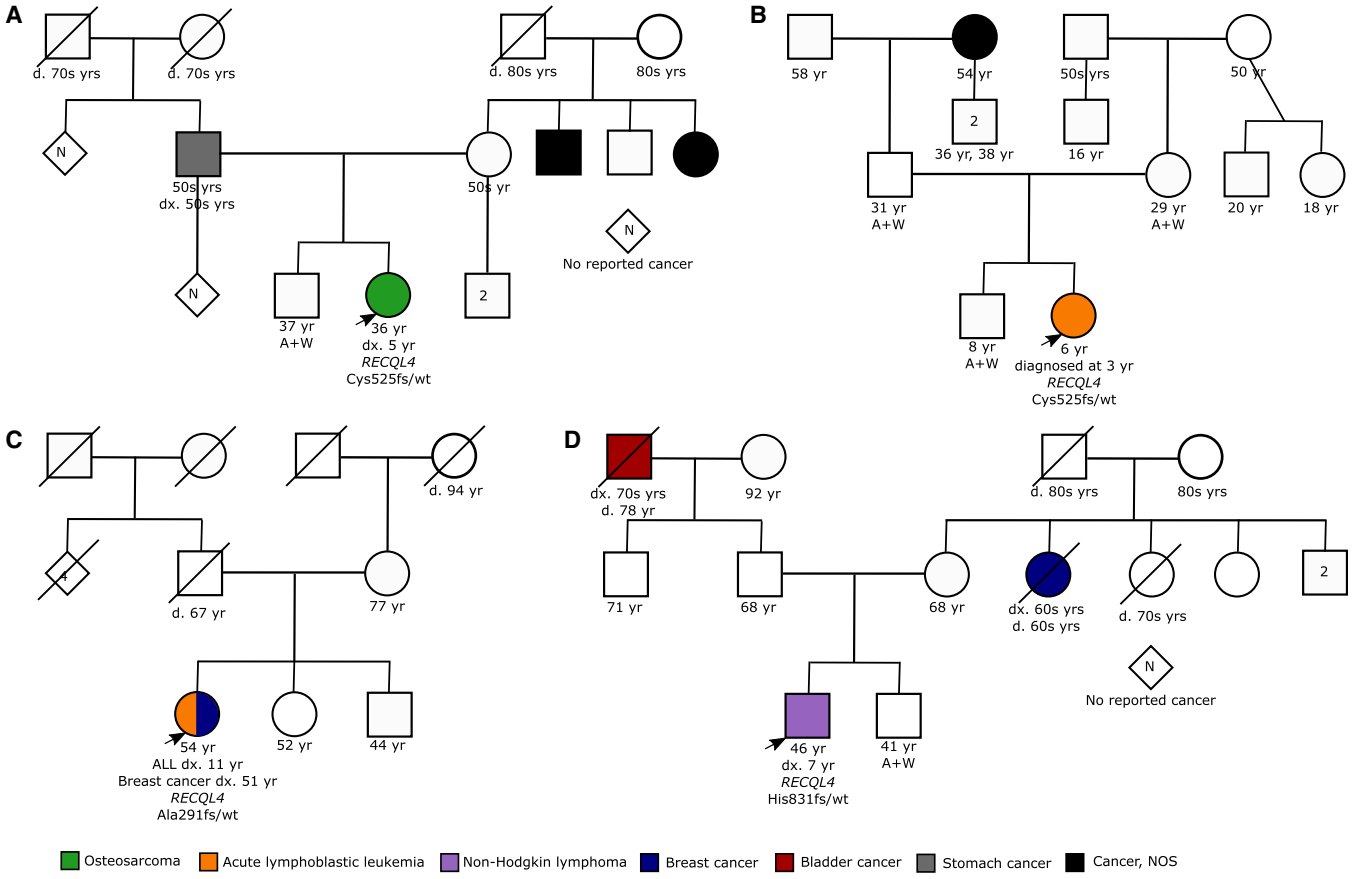

Figure 4. Pedigrees of families with *RECQL4* LOF mutations.

Fig. 1). As shown in Supplemental Table 3, the population admixture in the pediatric cancer cohort is not equivalent to that found in gnomAD noncancer version 2.1. Nevertheless, all *RECQL4* and *RECQL* LOF variants are extremely rare with respect to both global and ethnicity-specific allele frequencies (Table 2; Supplemental Table 2). Therefore, it is unlikely that the differences in the composition of the two populations significantly impacts our results. To further examine this possibility, we evaluated for enrichment of germline heterozygous LOF variants in *RECQL4* using only non-Finnish Europeans who comprise the majority of individuals in the cancer and noncancer cohorts (Supplemental Table 3). Through this analysis, significant enrichment in pediatric OS was once again observed for variants affecting *RECQL4* ($P = 0.0012$, OR = 6.77 [2.79, 16.45]) and *RECQL* ($P = 0.024$, OR = 5.04 [1.60, 15.86]).

DISCUSSION

As genetic testing is increasingly applied to patients with cancer, our knowledge of the germline contributions to cancer risk is dramatically expanding. Through such efforts, it has become clear that heterozygous pathogenic variants in DNA damage repair genes, such as *ATM*, *BRCA2*, and *PALB2*, are associated with a significant increase in risk to develop certain cancers. Here, we examined whether heterozygous LOF variants in *RECQL4*, the

Table 3. Comparison of heterozygous *RECQL4* LOF variants in pediatric cancer patients and the gnomAD noncancer cohort

	Pediatric cancer patients		gnomAD noncancer cohort		Cancer risk	
Cancer diagnosis	Carriers	Noncarriers	Carriers	Noncarriers	Odds ratio (95% CI)	*P*-value
RECQL						
Pan-cancer (all diagnoses)	13	5549	388	133,799	0.81 (0.46, 1.4)	0.52
Osteosarcoma	3	246	388	133,799	4.2 (1.3, 13.1)	0.037*
BLM						
Pan-cancer (all diagnoses)	13	5549	279	133,908	1.1 (0.64, 2.0)	0.65
Osteosarcoma	0	249	279	133,908	0	1
WRN						
Pan-cancer (all diagnoses)	15	5547	273	133,914	1.3 (0.79, 2.2)	0.29
Osteosarcoma	0	249	273	133,914	0	1
RECQL4						
Pan-cancer (all diagnoses[a])	23	5539	385	133,802	1.4 (0.95, 2.2)	0.098
Osteosarcoma	5	244	385	133,802	7.1 (2.9, 17.0)	0.00087**
RECQL5						
Pan-cancer (all diagnoses)	10	5552	284	133,903	0.85 (0.45, 1.6)	0.76
Osteosarcoma	0	249	284	133,903	0	1

*$P < 0.05$.
**$P < 0.005$.
[a]Patient with pathogenic *RB1* variant was excluded from statistical analysis.

gene encoding an important DNA helicase that is mutated in individuals with RTS, are more prevalent in children with cancer.

After screening 5562 pediatric cases, we identified a significant enrichment of heterozygous germline *RECQL4* LOF variants in patients with OS, the most common cancer in RTS, which is caused by homozygous or compound heterozygous variants in *RECQL4*. Although not significantly overrepresented, such variants were also identified in children with other tumor types, such as ALL, AML, craniopharyngioma, GCT, HL, and NHL. In 12 cases for whom tumor data were available, no deletions or mutations within the remaining *RECQL4* allele were detected. Although other mechanisms might account for a second hit (e.g., changes in methylation) (Mazor et al. 2015; Di Ruscio et al. 2016), it remains possible that RECQL4 haploinsufficiency, perhaps in combination with other oncogenic events, is enough to promote malignant transformation.

Our findings are consistent with recent studies documenting heterozygous germline *RECQL4* variants in adults with a variety of malignancies (Schrader et al. 2016; Jalkh et al. 2017; Mandelker et al. 2017; Tedaldi et al. 2017; AlDubayan et al. 2018; Bonache et al. 2018; Lowery et al. 2018; Na et al. 2018; Paulo et al. 2018; Penkert et al. 2018; Quezada Urban et al. 2018; Slavin et al. 2018). In addition to these studies are reports of an increased prevalence of *RECQL4* variants in patients with bladder (Na et al. 2018) or colorectal cancer (AlDubayan et al. 2018) compared to the general population. Moreover, a heterozygous germline truncating variant in *RECQL4* has been implicated as a possible cancer risk factor in an individual with prostate cancer (Paulo et al. 2018). Curiously, a previous investigation did not find enrichment of *RECQL4* mutations in sporadic cases of OS compared to the general population (Nishijo et al. 2004); however, this prior study examined a much smaller cohort including only 71 patients with OS. Thus, this association could potentially have been missed.

Among the 24 patients identified as harboring a single germline *RECQL4* LOF variant, 17 (71%) of these variants reside in the highly conserved helicase domain, with nine of these carrying the same c.1573delT (p.Cys525Alafs) alteration. Most RTS patients also harbor variants that result in a truncated protein lacking some or all of the helicase domain. Correspondingly, it has been reported that many RTS patients who developed OS harbor at least one truncating variant in *RECQL4*, whereas individuals with a clinical diagnosis of RTS and either a missense variant or no identified variant in *RECQL4* were not reported to develop OS (Wang et al. 2003). In contrast to these deleterious truncating variants, the hallmark variant in RAPADILINO syndrome, a splice site variant that causes in-frame skipping of exon 7 (Van Maldergem et al. 2006), leaves the helicase domain largely intact (Kitao et al. 1999; Siitonen et al. 2003), which may correspond with the lower incidence of cancer.

In addition to the new association of heterozygous germline *RECQL4* LOF variants with pediatric OS, our data also showed evidence of an association linking *RECQL* variants with OS, which deserves further investigation. Germline *RECQL* variants have been reported in *BRCA1/2*-negative breast cancers (Cybulski et al. 2015; Sun et al. 2015; Kwong et al. 2016); however, this association is disputed (Kwong et al. 2016; Li et al. 2018). To date, *RECQL* variants have not been reported in pediatric cancer. No strong associations were found between pediatric cancer and germline heterozygous LOF variants in the other RecQ helicase genes (i.e., *BLM*, *WRN*, *RECQL5*). Although all RecQ helicase genes contain the highly conserved helicase domain (Hickson 2003), they each play unique roles in genome maintenance and stability (Croteau et al. 2014). Toward this end, previous work has demonstrated that only two of the five human RecQ helicases, *RECQL* and *RECQL4*, bind specifically to three well-defined DNA replication origins under native conditions (Thangavel et al. 2010), whereas the other helicases (i.e., *BLM*, *WRN*, *RECQL5)* can only be found at replication origins following treatment with replication inhibitors (Thangavel et al. 2010). The similar localization and putative roles of *RECQL* and *RECQL4* may underlie how disruption of these helicases promotes the development of OS and possibly other cancers.

This study has several limitations. First, the prevalence of germline *RECQL4* variants identified in this study might not reflect the prevalence in newly diagnosed pediatric cancer patients because 50% of the patients studied were long-term survivors of childhood cancer. If the presence of heterozygous germline LOF variants is associated with poorer (or better) outcomes, it is possible that their prevalence in our cohort will be lower (or higher) than in newly diagnosed patients. A second limitation is that the population admixture of the pediatric cancer cohort examined in this study is not equivalent to that found in the gnomAD noncancer cohort (Supplemental Data). We suspect that this is due to differences in outcomes or referral patterns among different populations. Regardless, all of the LOF alleles reported in this study are extremely rare with respect to both global and ethnicity-specific allele frequencies. Last, this study is limited by the small number of individuals who harbor germline LOF variants. This factor makes it difficult to define the true spectrum of cancers associated with these germline variants. Continued evaluation of patients with cancer for the presence of heterozygous germline *RECQL4* LOF variants, and LOF variants in the other RecQ helicase genes, is warranted to validate and refine this new association.

METHODS

Study Participants

Study participants were from the Pediatric Cancer Genome Project ($n = 1120$), Genomes for Kids protocol ($n = 309$), St. Jude Lifetime Cohort (SJLIFE) Study ($n = 3006$), and Therapeutically Applicable Research to Generate Effective Treatment (TARGET) program ($n = 1127$).

The index case consented to participate in an IRB-approved study at St. Jude Children's Research Hospital that specifically allowed for germline interrogation and reporting.

Variant Detection and Classification

Single-nucleotide variants, small insertions and deletions, and copy-number variations were detected from whole-exome and/or genome sequencing (WGS) of the germline as previously described (Zhang et al. 2015; Rusch et al. 2018). Genetic variants were annotated, and LOF variants were further analyzed.

Curation of LOF Variants in gnomAD

Variants in RecQ helicase genes (*RECQL*, *BLM*, *WRN*, *RECQL4*, *RECQL5*) in the noncancer subset of the Genome Aggregation Database (gnomAD v2.1) were queried (Supplemental Tables 4–8). The putative LOF variants in gnomAD noncancer set v2.1 were downloaded first, and subsequently all variants of dubious quality via the tags "Flag" = "lc_lof" (low confidence-loss-of-function) were excluded. Variants with the "Flag" = "lcr" or "Flag" = "segdup" were reviewed and retained if they passed the quality control and manual curation steps. In the case of *RECQL4*, the founder variant, c.1390+2delT (p.Ala420_Ala463del), was removed as it is commonly encountered in the Finnish population at a minor allele frequency of 0.4% (Lek et al. 2016) but was absent from our cohort.

Statistical Analysis

Statistical analysis of population enrichment was calculated via a 2 × 2 Fisher's exact test, and estimates of the OR were performed using the RStudio R statistical computing environment with the epiR package (https://CRAN.R-project.org/package=epiR). Statistical significance was defined by a two-sided $P = 0.05$.

Computational Prediction of Ethnicity

Data from our pediatric cancer cohort (comprised of 4435 pediatric cancer patients at St. Jude Children's Research Hospital and 1127 from the National Cancer Institute TARGET database) was combined with data from 1000 Genomes, and the principal components (PCs) were extracted. Ethnicity data from 1000 Genomes were then used to train a random forest using the top 10 PCs which was used to predict the ethnicity of individuals in the pediatric cancer cohort. A visualization of the unsupervised ethnicity clustering is shown in Supplemental Figure 1.

ADDITIONAL INFORMATION

Data Deposition and Access

Genomic sequence data from St. Jude Children's Research Hospital is available for request on the St. Jude Cloud Platform. The variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers SCV000288218.6 and SCV000890968.1.

Ethics Statement

Patients reported here provided written informed consent permitting genomic analysis. Family histories have been illustrated in a de-identified manner. Participants at St. Jude Children's Research Hospital were consented to at least one of the following protocols: institutional banking protocol for Collecting, Banking and Distributing Human Tissue Samples at St. Jude Children's Research Hospital (TBANK; NCT01354002), Genomes for

Kids (G4K; NCT02530658), and/or Establishment of a Lifetime Cohort of Adults Surviving Childhood Cancer (SJLIFE; NCT00760656).

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Author Contributions

J.L.M., K.V.H., K.E.N., and C.A.K. conceived the study and wrote the initial draft of the manuscript. K.V.H., R.B.M., R.N., R.M., and C.A.K. provided genetic counseling. A.S.P. and V.S. were and are involved with the clinical management of the index case. K.V.H., R.B.M., R.N., R.M., S.H.D., L.H., L.T., E.L.G., and A.O. provided participant recruitment, consent, and support. N.O., W.C., M.N.E., A.P., S.N., J.Z., Z.W., and G.W. developed software and pipelines for sequence data analysis and variant interpretation. N.O., W.C., Z.W., J.N., E.M.A., S.A.S., S.N., and G.W. analyzed sequence data and interpreted variants. J.L.M., N.O., G.W., and C.A.K. reviewed population databases and performed statistical analyses. D.W.E., J.R.D., M.M.H., L.L.R., S.N., J.Z., G.W., K.E.N., and C.A.K. provided project oversight. All coauthors reviewed the manuscript.

Competing Interest Statement

The authors have declared no competing interest.

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