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Article

Germline Cancer Predisposition Variants in Pediatric Rhabdomyosarcoma: A Report From the Children's Oncology Group

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Abstract

Background: Several cancer-susceptibility syndromes are reported to underlie pediatric rhabdomyosarcoma (RMS); however, to our knowledge there have been no systematic efforts to characterize the heterogeneous genetic etiologies of this often-fatal malignancy. **Methods:** We performed exome-sequencing on germline DNA from 615 patients with newly diagnosed RMS consented through the Children's Oncology Group. We compared the prevalence of cancer predisposition variants in 63 autosomal-dominant cancer predisposition genes in these patients with population controls (n = 9963). All statistical tests were 2-sided. **Results:** We identified germline cancer predisposition variants in 45 RMS patients (7.3%; all FOXO1 fusion negative) across 15 autosomal dominant genes, which was statistically significantly enriched compared with controls (1.4%, $P = 1.3 \times 10^{-22}$). Specifically, 73.3% of the predisposition variants were found in predisposition syndrome genes previously associated with pediatric RMS risk, such as Li-Fraumeni syndrome (*TP53*) and neurofibromatosis type I (*NF1*). Notably, 5 patients had well-described oncogenic missense variants in *HRAS* (p.G12V and p.G12S) associated with Costello syndrome. Also, genetic etiology differed with histology, as germline variants were more frequent in embryonal vs alveolar RMS patients (10.0% vs 3.0%, $P = .02$). Although patients with a cancer predisposition variant tended to be younger at diagnosis ($P = 9.9 \times 10^{-4}$), 40.0% of germline variants were identified in those older than 3 years of age, which is in contrast to current genetic testing recommendations based on early age at diagnosis. **Conclusions:** These findings demonstrate that genetic risk of RMS results from germline predisposition variants associated with a wide spectrum of cancer susceptibility syndromes. Germline genetic testing for children with RMS should be informed by RMS subtypes and not be limited to only young patients.

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Rhabdomyosarcoma (RMS) is a highly malignant tumor believed to arise from developing skeletal muscle cells (myoblasts) and is the most common soft-tissue sarcoma in children and adolescents, with an overall incidence of 4.5 cases per million among those younger than 20 years of age (1). Relative to other pediatric cancers, the prognosis for many children with RMS

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remains poor. For example, treatment of children with intermediate-risk disease using maximal intensive therapy and application of new agents has only led to modest improvements in 5-year survival rates since the 1970s, which remain at only 43% to 67% by different estimates (2). Furthermore, for those RMS patients with metastatic disease, the 5-year survival rate is less than 30%, even with aggressive therapy (3).

The vast majority of RMS tumors have been classified by histology into embryonal RMS (ERMS), representing 70% of patients, and alveolar RMS (ARMS), representing approximately 20% (3,4). Further, nearly 80% of ARMS cases are driven by a characteristic balanced chromosomal translocation between either *PAX3* or *PAX7* and *FOXO1* (5-7). FOXO1 fusion-positive tumors have decreased tumor mutation burden compared with FOXO1 fusion-negative tumors (8), consistent with the driving role of FOXO1 fusions in disease pathogenicity. Also, FOXO1 fusion-negative ARMS tumors have gene expression profiles similar to those from ERMS (7,9,10). Notably, a wide range of somatic alterations have been identified in ERMS, including mutations in *TP53*, genes within the *RAS* family (*NRAS*, *KRAS*, *HRAS*), *PIK3CA*, *CTNNB1*, and *FGFR4* (8,11-14), as well as loss of heterozygosity at 11p15.5 (15).

Numerous reports and studies of individual genetic disease cohorts consistently highlight that children with certain genetic syndromes develop RMS more frequently than their unaffected peers (16). Cancer susceptibility syndromes that are most commonly reported among those with RMS include Li-Fraumeni (LFS) (17), neurofibromatosis type 1 (18,19), Costello (20,21), Noonan (20), and DICER1 (22). In an assessment by Zhang et al. (23) of germline mutations in cancer predisposition genes (CPGs) among 1120 pediatric cancer patients, only 43 patients in the cohort were diagnosed with RMS, of which 3 (7.0%) had a pathogenic variant in a known CPG. However, given the small sample size, it was difficult to evaluate the spectrum of cancer predisposition variants in CPGs among children with RMS. Additionally, there was no further stratification by RMS histology or fusion status. Thus, the burden of cancer susceptibility syndromes among children with RMS is still unclear. Delineating the germline risk for RMS could help to inform germline genetic testing strategies in this population. Therefore, we sought to determine the prevalence of predisposition variants in CPGs among a large, unselected cohort of children with newly diagnosed RMS. We also evaluated differences by histologic subtypes and compared the prevalence of these variants with a large population-based cohort.

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Methods

Study Cohorts

We obtained germline DNA from diagnostic blood samples of 616 pediatric patients with RMS (age <25 years; 1 sample was removed due to quality, see below) from the Children's Oncology Group (COG) Biopathology Center. This study was approved by the institutional review board of Baylor College of Medicine. RMS patients were consented as part of the COG Soft Tissue Sarcoma Biology and Banking Protocol (COG Protocol D9902). There were no selection criteria in place for inclusion in D9902; therefore, these patients represent the demographic and clinical characteristics seen in previous population-based assessments of RMS (24,25). Additionally, all risk groups (low, intermediate, and high) were included in this analysis. The histologic diagnosis of RMS and histologic subtype status were confirmed by COG central review. Demographic and clinical characteristics of these patients were provided by COG.

For the population control cohort, we used exome-sequencing data from 10 853 European American and African American individuals included in the Atherosclerosis Risk in Communities (ARIC) cohort (dbGaP: phs000280.v4.p1) and 1030 Hispanic individuals from the Viva la Familia (VIVA, dbGaP: phs000616.v2.p2) study (26).

Exome-sequencing of the RMS cases and controls was performed at the Human Genome Sequencing Center at Baylor College of Medicine (see Supplementary Methods, available online). After quality control measurements and filtering, we included 615 RMS patients, 9663 controls from ARIC (73.7% of European Americans and 26.3% of African Americans), and 300 controls from VIVA (all of whom were Hispanic) in the final analyses.

Identification of Candidate Genes and Predisposition Variants

We precurated a set of autosomal-dominant CPGs (n = 24) from cancer susceptibility syndromes that have been specifically implicated in RMS predisposition as well as a set of additional autosomal dominant CPGs (n = 39) from the report by Zhang et al. (23). We also evaluated 7 genes underlying autosomal recessive disorders that are known to be associated with sarcomas (see Supplementary Methods for gene selection criteria). We annotated all the filtered variants using ANNOVAR (v2018.04.16) (27) in candidate CPGs for further analyses.

To identify germline predisposition variants in RMS, we focused on rare variants with minor allele frequency less than 0.01 in all of the following population databases: 1000 Genome Phase 3 (28), gnomAD (v2.1.1) (29), and ExAC (v0.3) (30). We defined germline predisposition variants as either 1) known pathogenic or likely pathogenic variants [reported in ClinVar (31) with 1 or more "stars" describing the level of support of clinical significance (version 20190305)]; or 2) novel, rare, potential loss-of-function variants (splicing variants, frameshift insertions or deletions, nonsense single-nucleotide variants, or stoploss variants).

Statistical Analyses

Logistic regression models were used to test associations between the occurrence of pathogenic variants and the status of the individuals while adjusting for the following covariates: ERMS vs ARMS: sex, age at diagnosis, tumor stage, and the first 10 principle components (PCs); case vs control: sex and the first 10 PCs. We used P less than .05/3 (Bonferroni correction) as a statistical significance threshold for both cohort-level (3 groups) and gene-level (3 genes) analyses. A linear regression model was used to determine the association between age at diagnosis and the pathogenic variant status within the ERMS subtype while using sex, tumor stage, and the first 10 PCs as covariates. All statistical tests were 2-sided and were performed using the *statsmodels* package (32). Odds ratios were calculated as the exponential of the estimated coefficient of the tested independent variable.

Results

Study Participants

To estimate the prevalence of germline predisposition variants among pediatric RMS, we analyzed samples from a total of 615

Table 1. Epidemiological features of the RMS cohort[a]

Characteristics	Embryonal RMS (n = 347)	Alveolar RMS (n = 167)	Other subtypes or not otherwise specified (n = 101)	Total (n = 615)
Sex, No. (%)				
Female	124 (35.7)	86 (51.5)	33 (32.7)	243 (39.5)
Male	223 (64.3)	81 (48.5)	68 (67.3)	372 (60.5)
Population, No. (%)				
European American	235 (67.7)	105 (62.9)	65 (64.4)	405 (65.9)
Hispanic	45 (13.0)	34 (20.4)	17 (16.8)	96 (15.6)
African American	45 (13.0)	18 (10.8)	15 (14.9)	78 (12.7)
East Asian	17 (4.9)	8 (4.8)	3 (3.0)	28 (4.6)
South Asian	5 (1.4)	2 (1.2)	1 (1.0)	8 (1.3)
Age at diagnosis, No. (%), y				
0-5	171 (49.3)	62 (37.1)	31 (30.7)	264 (42.9)
5-10	89 (25.6)	28 (16.8)	23 (22.8)	140 (22.8)
10-15	51 (14.7)	44 (26.3)	17 (16.8)	112 (18.2)
15-20	33 (9.5)	25 (15.0)	28 (27.7)	86 (14.0)
20-25	3 (0.9)	8 (4.8)	2 (2.0)	13 (2.1)
Median, (interquartile range)	5 (7)	9 (11)	9 (12)	6 (10)
Cancer stage, No. (%)				
1	135 (38.9)	21 (12.6)	24 (35.8)	180 (31.0)
2	47 (13.5)	30 (18.0)	4 (6.0)	81 (13.9)
3	117 (33.7)	53 (31.7)	16 (23.9)	186 (32.0)
4	48 (13.8)	63 (37.7)	23 (34.3)	134 (23.1)
NA	0	0	34	34

[a]NA = not available; RMS = rhabdomyosarcoma.

RMS patients who were consented to the COG D9902 protocol. These patients were newly diagnosed and were not specifically selected for the existence of known germline pathogenic variants or family history of cancer or any other diagnostic characteristics. Within this cohort, the patients were mostly of European American, Hispanic, and African American populations (Table 1). A majority of the patients were diagnosed with ERMS (56.4%, n = 347; including 38 botryoid and 30 spindle cell subtypes), followed by ARMS (27.2%, n = 167) (Table 1). Notably, among the ARMS patients whose FOXO1 fusion status was known, 82.5% (66 of 80) were FOXO1 fusion-positive. Both ERMS and ARMS subtypes showed bimodal peaks regarding age at diagnosis (Supplementary Figure 1, available online). The male-to-female ratio among RMS patients was approximately 1.5 to 1, which is largely driven by ERMS (Table 1) and consistent with previous reports (25). Also, ERMS patients were younger (74.9% were <10 years of age) compared with ARMS patients (53.9% were <10 years of age; Table 1). In addition, a larger proportion of the ARMS patients were diagnosed at a later stage (stage 4) compared with ERMS patients (37.7% ARMS vs 13.8% ERMS; Table 1). In summary, this unselected childhood RMS cohort had epidemiological features (such as sex ratio, subtype composition, and age distribution) that were representative of previous population-based studies (4,24,25).

Evaluation of Germline Predisposition Variants Among RMS Patients

We performed exome sequencing of DNA from blood samples of the 615 RMS patients. We curated 2 sets of autosomal-dominant genes for investigation: 1) RMS-associated CPGs (n = 24) that have been specifically associated in the medical literature with RMS as part of cancer susceptibility syndromes, such as LFS (*TP53*), neurofibromatosis type 1 (*NF1*), and Costello Syndrome (*HRAS*), as well as the 4 mismatch repair genes associated with both recessive constitutional mismatch repair deficiency syndrome and dominant Lynch Syndrome (Table 2); and 2) other autosomal dominant CPGs (n = 39) curated from previous studies (23).

Altogether, we identified germline predisposition variants in 45 out of 615 RMS patients (7.3%), 6 of which have not been reported in ClinVar previously (Table 3). Among them, 33 patients carried predisposition variants in RMS-associated CPGs, and the most common findings were in *TP53* (n = 11), *NF1* (n = 9), and *HRAS* (n = 5) (Table 3; Figure 1, A-C). Only 12 patients had a predisposition variant identified within the other general CPGs, among which *BRCA2* pathogenic variants were the most prevalent (n = 6; Table 3; Figure 1, D). Taken together, 7.3% of the childhood RMS patients have a molecular finding consistent with cancer predisposition, and the majority of those (33 of 45, 73.3%) are due to known RMS-associated cancer susceptibility syndrome genes.

Five ERMS patients from this cohort had germline *HRAS* codon 12 missense variants that correspond to well-described oncogenic variants: 4 patients had p.G12S and 1 had p.G12V variant (Table 3). Germline pathogenic variants of this codon, especially p.G12S, have been associated with Costello syndrome, a very rare multiple congenital anomaly and neurodevelopmental condition previously reported to be associated with increased risk of RMS and other cancers (20,21). We observed that 0.8% of patients with RMS, more specifically 1.4% of patients with ERMS, carried a germline pathogenic *HRAS* allele. Data collection on the COG D9902 protocol specifically asks about a list of syndrome diagnoses (including Costello syndrome). However, this was notated in only 1 of the 5 patients with the *HRAS* variant.

Prevalence of Cancer Predisposition Variants in RMS Subtypes

We compared the prevalence of germline predisposition variants between different RMS subtypes across the 63 CPGs.

Table 2. Candidate CPGs[a]

Genes	Cancer susceptibility syndrome associated with RMS
RMS-associated CPGs	
TP53, *CHEK2*	Li-Fraumeni syndrome
NF1	Neurofibromatosis type 1
HRAS	Costello syndrome
DICER1	DICER1 syndrome
PTCH1, *SUFU*	Gorlin syndrome
SOS1, *PTPN11*, *RAF1*, *CBL*, *KRAS*, *NRAS*, *RIT1*, *SHOC2*	Noonan syndrome
BRAF, *MAP2K1*, *MAP2K2*	Cardiofaciocutaneous syndrome
MLH1, *MSH2*, *MSH6*, *PMS2*	Constitutional mismatch repair deficiency
CDKN1C	Beckwith-Wiedemann syndrome
CREBBP	Rubinstein-Taybi syndrome
Other CPGs (from non-RMS–associated cancer susceptibility syndromes)	
ALK, *APC*, *BAP1*, *BMPR1A*, *BRCA1*, *BRCA2*, *CDC73*, *CDH1*, *CDK4*, *CDKN2A*, *CEBPA*, *EPCAM*, *FH*, *GATA2*, *MAX*, *MEN1*, *NF2*, *PALB2*, *PAX5*, *PHOX2B*, *PRKAR1A*, *PTEN*, *RB1*, *RET*, *RUNX1*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *SMAD4*, *SMARCA4*, *SMARCB1*, *STK11*, *TMEM127*, *TSC1*, *TSC2*, *VHL*, *WT1*	NA

[a]CPG = cancer predisposition gene; NA = not applicable; RMS = rhabdomyosarcoma.

Notably, 10.0% (35 of 347) of children with ERMS (including the botryoid and spindle cell subtypes) harbored a predisposition variant compared with 3.0% (5 of 167) of those diagnosed with ARMS (odds ratio [OR] = 3.26, 95% confidence interval [CI] = 1.18 to 9.00, $P = .02$; logistic regression model). None of the FOXO1 fusion-positive ARMS patients (n = 66) had a predisposition variant in genes we investigated. Within the ERMS samples, patients who were positive for a predisposition variant were younger at diagnosis (median age = 3.0 years, all but 1 diagnosed before age 10 years) than those without a predisposition variant (median age = 5.5 years; $\beta = 3.07$, 95% CI = 1.26 to 4.87, $P = 9.9 \times 10^{-4}$; linear regression model; Figure 2, A). We did not observe the same trend in ARMS patients, which in part could be due to the small number of patients who carried a predisposition variant (Figure 2, B).

Prevalence of Cancer Predisposition Variants in Cases and Controls

To compare the prevalence of germline predisposition variants between RMS cases and the general population, we used a set of unrelated population controls consisting of 9963 samples with similar ethnicity characteristics (Supplementary Table 1, available online), sequenced at the same sequencing center and platform as the RMS cases and analyzed using the same bioinformatic pipelines. We evaluated the data for predisposition variants using the same approach in case and control cohorts.

We found that 143 samples (1.4%) from the population controls carried at least 1 predisposition variant from the 63 candidate genes, which is statistically significantly lower compared with RMS cases (OR = 6.34, 95% CI = 4.38 to 9.18, $P = 1.3 \times 10^{-22}$; logistic regression model, same below). This difference is largely driven by the RMS-associated CPGs (OR = 14.20, 95% CI = 8.72 to 23.11, $P = 1.3 \times 10^{-26}$) than by the other CPGs (OR = 2.32, 95% CI = 1.22 to 4.45, $P = .01$; Figure 3, A). In particular, we observed a 109-fold and 40-fold increase in the prevalence of predisposition variants among RMS cases compared with controls in *TP53* (OR = 109.42, 95% CI = 23.07 to 518.99, $P = 3.4 \times 10^{-9}$) and *NF1* (OR = 39.64, 95% CI = 11.49 to 136.81, $P = 5.8 \times 10^{-9}$), respectively (Figure 3, B). The most common germline findings in controls were from *BRCA2* (n = 28, 0.29%; Figure 3, B), yet we still observed an increase for *BRCA2* predisposition variants among RMS cases (n = 6, 0.98%) compared with controls (OR = 3.55, 95% CI = 1.34 to 9.40; $P = .01$). Notably, all the above-mentioned statistical results surpassed the Bonferroni correction threshold ($P < .0166$). Therefore, the prevalence of germline predisposition variants was statistically significantly more common in patients with RMS and also represented a different spectrum of germline variants in autosomal-dominant CPGs between childhood RMS patients and population controls.

In a separate analysis, we evaluated the prevalence of pathogenic variants among 7 genes underlying autosomal recessive disorders that are known to be associated with sarcomas. We did not observe any individuals with a biallelic pathogenic variant in these genes among our cases or controls. We identified carriers of pathogenic variants in 4 autosomal recessive genes in 1.14% (7 of 615) of RMS patients (*RECQL4* n = 3; *BLM* n = 2; *BUB1B* n = 1; *TRIP13* n = 1) and 0.75% (75 of 9963) of controls, which were not statistically different (OR = 1.37, 95% CI = 0.59 to 3.20, $P = .47$; logistic regression model).

Discussion

In one of the largest unselected cohorts of RMS patients assembled, we found that 7.3% of pediatric RMS patients carried a cancer predisposition variant in a known CPG. This is compared with 1.4% of the population-based controls included in our assessment. Notably, this is consistent with an independent assessment in a set of 394 RMS patients, which concluded that 6.6%-7.7% of patients carried a dominant-acting cancer predisposition variant in a known CPG (33). While there were no statistically significant differences in the overall frequency of pathogenic variants by ethnicity in RMS cases, relative differences compared with controls were consistent and statistically significant across groups (Supplementary Table 2, available online). As this study focused on newly diagnosed RMS patients rather than individuals from a survivor cohort, we limited the potential for survival bias. Previous genomic studies focusing on heterogeneous pediatric cancer populations report that 8%-10% of patients carry a germline predisposition variant (23,34,35). It is unclear if this is true for pediatric sarcomas, where there have been few large-scale assessments of CPGs. However, Mirabello et al. (36) recently reported that

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Table 3. Cancer predisposition variants identified from RMS patients

Variant information		ClinVar P or LP variant			
and genomic change (GRCh37)	Variant type	ClinVar status	Variation ID	Review status	Patient histology
RMS-associated CPGs					
TP53 (n = 11)					
17:7577046 C > A	Stopgain	P	93323	Criteria provided, multiple submitters, no conflicts	ERMS
17:7577120 C > T	Missense SNV	P/LP	12366	Criteria provided, multiple submitters, no conflicts	Spindle cell
17:7577144 A > G	Missense SNV	P/LP	245777	Criteria provided, multiple submitters, no conflicts	ARMS
17:7577538 C > T	Missense SNV	P/LP	12356	Criteria provided, multiple submitters, no conflicts	ERMS
17:7577551 C > T	Missense SNV	P/LP	376600	Criteria provided, multiple submitters, no conflicts	BRMS
17:7578290 C > G	Splicing	—	—	—	Spindle cell
17:7578457 C > T	Missense SNV	P/LP	141963	Criteria provided, multiple submitters, no conflicts	Mixed RMS
17:7578479 G > A	Missense SNV	P/LP	12370	Criteria provided, multiple submitters, no conflicts	ERMS
17:7578479 G > T	Missense SNV	LP	12369	Criteria provided, multiple submitters, no conflicts	ERMS
17:7579312 C > T	Synonymous SNV	P	177825	Criteria provided, multiple submitters, no conflicts	ERMS
17:7579320 TCA > T	Frameshift deletion	P	127809	Criteria provided, multiple submitters, no conflicts	BRMS
NF1 (n = 9)					
17:29541542 A > G	Missense SNV	P	354	Criteria provided, multiple submitters, no conflicts	ERMS
17:29553492 C > T	Stopgain	P	188280	Criteria provided, multiple submitters, no conflicts	BRMS
17:29560043 C > T	Stopgain	—	—	—	ERMS
17:29562746 C > T	Stopgain	P	237556	Criteria provided, multiple submitters, no conflicts	ERMS
17:29652987 G > A	Stopgain	P	573015	Criteria provided, single submitter	ERMS
17:29654553 C > T	Stopgain	P	228381	Criteria provided, multiple submitters, no conflicts	ERMS
17:29654857 G > A	Missense SNV	P	185354	Criteria provided, multiple submitters, no conflicts	ERMS
17:29664899 G > T	Splicing	P	547680	Criteria provided, multiple submitters, no conflicts	ERMS
17:29670115 CTAACTT > C	Nonframeshift deletion	P/LP	220715	Criteria provided, multiple submitters, no conflicts	ERMS
HRAS (n = 5)					
11:534288 C > G	Missense SNV	P	12603	Criteria provided, multiple submitters, no conflicts	ERMS
11:534289 C > T	Missense SNV	P	12602	Reviewed by expert panel	ERMS (n = 4)
CBL (n = 2)					
11:119149251 G > A	Missense SNV	P	13810	Criteria provided, single submitter	ERMS
11:119155742 C > T	Stopgain	—	—	—	ERMS
DICER1 (n = 2)					
14:95579443 G > A	Stopgain	P	242054	Criteria provided, multiple submitters, no conflicts	ERMS
14:95590735 G > A	Stopgain	P	429141	Criteria provided, multiple submitters, no conflicts	RMS-NOS
MSH2					
2:47656951 C > T	Stopgain	P	90554	Reviewed by expert panel	ARMS
MSH6					
2:48027422 C > T	Missense SNV	P	141058	Reviewed by expert panel	RMS-NOS
PMS2					
7:6017260 G > A	Stopgain	P	9237	Reviewed by expert panel	ERMS
PTCH1					
9:98279010 G > C	Stopgain	—	—	—	RMS-NOS
Other CPGs					
BRCA2 (n = 6)					
13:32900272 CAA > C	Frameshift deletion	P	51684	Reviewed by expert panel	ERMS
13:32911595 G > T	Stopgain	P	51400	Reviewed by expert panel	Spindle cell
13:32912089 CTG > C	Frameshift deletion	P	51493	Reviewed by expert panel	Spindle cell
13:32912337 CTG > C	Frameshift deletion	P	37859	Reviewed by expert panel	ARMS
13:32929123 C > G	Stopgain	P	38085	Reviewed by expert panel	ERMS
13:32936711 G > A	Stopgain	P	38122	Reviewed by expert panel	ARMS
SDHA (n = 2)					
5:218472 T > G	Missense SNV	P/LP	422382	Criteria provided, multiple submitters, no conflicts	ERMS
5:223624 C > T	Stopgain	P	142601	Criteria provided, multiple submitters, no conflicts	ARMS
BRCA1					
17:41215362 TTTTC > T	Frameshift deletion	P	37644	Reviewed by expert panel	ERMS
PTEN					
10:89692904 C > T	Stopgain	P	7819	Criteria provided, multiple submitters, no conflicts	RMS-NOS
SDHC					
1:161326611 G > A	Stopgain	—	—	—	ERMS
ALK					
2:29416655 TC > T	Frameshift deletion	—	—	—	ERMS

[a]— = new pathogenic variants found in our cohort; ARMS = alveolar rhabdomyosarcoma; BRMS = botryoid rhabdomyosarcoma; CPG = cancer predisposition gene; ERMS = embryonal rhabdomyosarcoma; LP = likely pathogenic; NOS = not otherwise specified; P = pathogenic; RMS = rhabdomyosarcoma; SNV = single-nucleotide variant.

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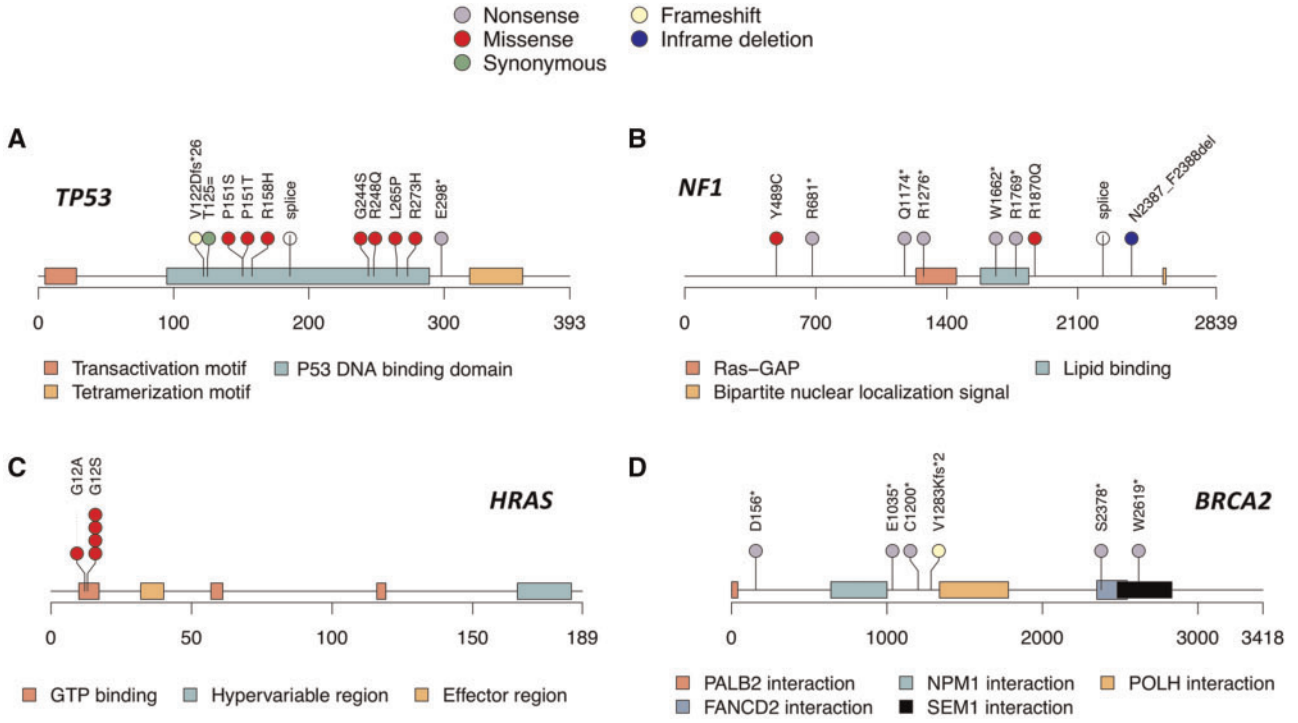

Figure 1. Predisposition variants identified in the rhabdomyosarcoma cohort. The location and type of the predisposition variants identified in (**A**) *TP53*, (**B**) *NF1*, (**C**) *HRAS*, and (**D**) *BRCA2*. Each **colored box** represents a protein domain in the gene. Each **colored circle** represents a variant of a specific type.

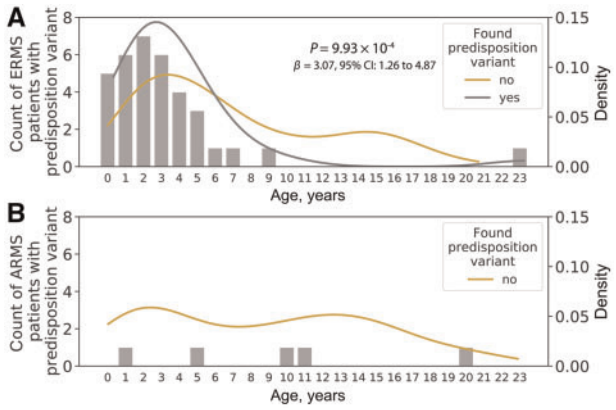

Figure 2. Age distribution of patients with and without a predisposition variant. **Bar plots** indicate the number of patients with a predisposition variant. (**A**) Age density among embryonal rhabdomyosarcoma (ERMS) patients with (**grey**) and without (**golden**) a predisposition variant. The *P* value was calculated from a linear regression model using age at diagnosis as the independent variable. (**B**) Age density among alveolar rhabdomyosarcoma (ARMS) patients without a pathogenic variant (**golden**). Density plot for ARMS patients who have predisposition variants (n = 5) was not generated due to the small patient count.

28.0% (281 of 1004) of osteosarcoma patients had a pathogenic or likely pathogenic variant in a CPG, although they analyzed a larger set of genes and variants, including several autosomal recessive disorders. We also evaluated the impact of autosomal recessive disorders in the context of RMS and showed that RMS is not strongly driven by autosomal recessive disorders, similar to other studies of pediatric cancer cohorts (23,35). Importantly, these results suggest distinct germline genetic susceptibility patterns underlying different types of sarcomas.

Of note, the majority of the molecular findings in RMS patients from the COG cohort (5.3% in total) are within CPGs previously reported in pediatric RMS patients. This prevalence is relatively consistent with previous clinic-based estimations for individual syndromes, where 2%-9% of RMS patients were thought to have a certain comorbid germline cancer susceptibility syndrome [eg, 2.0% with neurofibromatosis type I (19), 3.8% with DICER1 syndrome (22), and 9.1% with LFS (17)]. Additionally, individuals with a predisposition variant from RMS-associated CPG have higher odds (OR = 14.20) of developing RMS compared with individuals carrying a predisposition variant from CPGs not previously associated with RMS (OR = 2.32).

An important aspect of this study is the differences demonstrated in the frequency of cancer predisposition variants by demographic and clinical features of RMS. For example, predisposition variants were more common in ERMS patients compared with ARMS patients and were completely absent in the 66 FOXO1 fusion-positive patients in our cohort. This further supports the idea that there are etiologic differences based on histology and fusion status. Notably, we did not observe associations between predisposition variants and tumor stage (data not shown). Additionally, consistent with previous smaller reports (37), cancer predisposition variants were more frequent in younger patients. However, our findings also indicate that these variants are still present in older children diagnosed with RMS. For instance, 11 of the 35 (31.4%) ERMS patients (and 18 of the 45 [40.0%] RMS patients regardless of histology type) with predisposition variants were older than 3 years of age, suggesting that genetic testing should not be limited to only very young patients.

Within the genes where we discovered germline pathogenic variants in RMS, some of the same genes are frequently reported to also have somatic mutations in RMS tumors, such

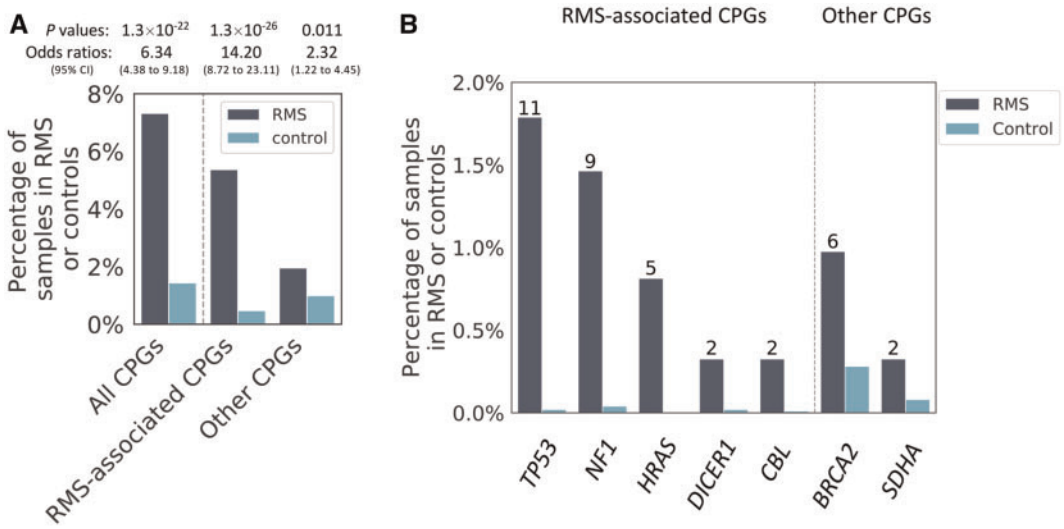

Figure 3. Prevalence of predisposition variants in cancer predisposition genes (CPGs) among rhabdomyosarcoma (RMS) patients and controls. (**A**) Comparison of the prevalence of germline predisposition variants between RMS patients and population controls stratified by gene types on the x-axis. (**B**) Percentage of samples in RMS cases and controls that carry a predisposition variant. This figure only shows genes containing predisposition variants in greater than 1 RMS cases. **Numbers on top of the RMS bars** indicate patient count.

as *TP53*, *NF1*, and *HRAS* (8,11-13). For example, Shern et al. (8) evaluated the frequency of somatic mutations across 147 RMS patients using paired germline and tumor samples, and somatic mutations in *TP53* (3.4%) and *NF1* (3.4%) were found in the RMS patients. In our study, 1.7% and 1.5% of our pediatric RMS patients had a germline pathogenic variant in *TP53* and *NF1*, respectively. Therefore, patients with a somatic pathogenic variant in these genes should have further germline analysis to determine the origin of the variant and implications for family members (38,39).

Five ERMS patients had a well-described oncogenic variant in codon 12 of *HRAS*. The p.G12S pathogenic variant seen in 4 patients represents the only recurrent germline pathogenic variant we found in this cohort. In addition, germline mutations in *HRAS* (more commonly p.G12S than p.G12V) are known to cause Costello syndrome, a rare multiple congenital anomaly and neurodevelopmental syndrome associated with an increased risk of developing childhood RMS and other cancers (21,40). Although 1 patient was noted to have Costello syndrome in the case report form, additional phenotypic information was not available for the other patients. Further study is required to determine if children with these pathogenic alleles develop RMS without the many other features of Costello syndrome.

We observed 6 RMS patients with a pathogenic variant in *BRCA2*, which was statistically significantly higher than our control population (OR = 3.55, 95% CI = 1.34 to 9.40, $P = .01$). Furthermore, 5 of the 6 were diagnosed before 6 years old. We did not expect such an enrichment in *BRCA2* as it has not traditionally been thought of as an RMS-associated gene and is usually implicated in adult-onset cancers. Interestingly, 1 of the 3 pathogenic variants identified in RMS patients by Zhang et al. (23) was also in *BRCA2*. Also, in a recent study of germline pathogenic variants across 1162 adult sarcoma patients, Ballinger and Goode et al. (41) reported an enrichment for pathogenic variants in *BRCA2*. In addition, an excess number of breast cancer cases have been reported in mothers of children with sarcomas (42). Similarly, first-degree relatives of women with breast cancer are found to have increased prevalence of soft tissue and bone sarcomas (43,44). These studies suggest that, while LFS with underlying *TP53* pathogenic variants have been known to play a role in the aggregation of breast cancer and sarcomas (45), breast and ovarian cancer genes, such as *BRCA2*, may also play a role in this aggregation.

Notably, our analysis did not include pathogenic variation that may result from intragenic or whole-gene deletions, gene imprinting, or epigenetic alterations. Therefore, the overall prevalence of germline pathogenic genetic variation among children diagnosed with RMS is likely to be higher when considering these other types of variants. Additionally, our assessment did not include an evaluation of outcomes for those with pathogenic variants in CPGs but rather focused on the prevalence of cancer predisposition syndromes associated with RMS in an unselected pediatric RMS cohort at diagnosis. Finally, while we did use population-based controls for our assessment, future assessment should leverage other noncancer populations to evaluate the impact of underlying characteristics, which could influence effect estimates.

In this study, we investigated the role of germline predisposition variants in one of the largest pediatric RMS cohorts. Notably, the prevalence of the predisposition variants differs by histology and age at diagnosis as well as by the type of cancer susceptibility syndromes with which the genes are associated. These results shed light on the spectrum of CPGs that may cause RMS. These findings warrant further discussion about the clinical management of children newly diagnosed with RMS and the modification of guidelines for when to consider germline genetic testing. Additionally, there is a growing awareness that leveraging germline information of cancer predisposition could provide innovative therapeutic strategies, thereby improving patient care (46). Finally, future studies should evaluate novel genes, through the assessment of both common and rare variants on RMS susceptibility, to yield new insights into the mechanisms underlying pediatric RMS.

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Data Availability

All of the pathogenic and likely pathogenic variants observed in RMS patients from this manuscript have been submitted to and are searchable in ClinVar. The individual sequencing and phenotypic data for the 615 RMS cases are available through dbGaP requests (accession ID phs002304.v1.p1).

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