SUPPLEMENTARY MATERIALS

Germline cancer-predisposition variants in pediatric rhabdomyosarcoma: a report from the Children's Oncology Group

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Supplementary Methods

Exome sequencing and variant filtering

Exome sequencing of the RMS samples was performed at the Human Genome Sequencing Center (HGSC) at Baylor College of Medicine. Specifically, genomic DNA samples underwent exome capture using HGSC VCRome2.1 (35Mb covering ~24K genes; Roche) and were then sequenced on the Illumina NovaSeq platform with ~100X coverage. Alignment was performed using BWA-MEM (version 0.7.15-r1140) (1) against the human hs37d5 assembly, followed by Base Score Recalibration and Indel Realignment using GATK (version 3.4-0) (2). Variant calling was performed using xAtlas (version 0.2.1) (3) to generate gvcf files for merging different sets of data. We did not call larger deletions in this study. Samples from the ARIC and VIVA cohorts have been sequenced at the HGSC on the same platform and described previously (4,5). Of note, both studies have used similar exome capture reagent (VCrome2.1) and were subjected to the same analytical pipelines as the RMS cohort.

Next, we performed joint genotyping using GLnexus (v1.1.3) (6) to combine samples from the three datasets (RMS patients, ARIC controls, and VIVA controls) to increase consistency of variant calling. A series of quality control (QC) measures were then employed. First, for each variant in each sample, we assigned a missing genotype if the variant fulfilled one of the following criteria: read depth less than 15; quality score less than 15; allelic ratio less than 0.25 or greater than 0.75; or non-PASS variants from xAtlas variant call results. We then removed the variants with a genotyping rate less than 85% within each dataset. These QC measures were performed independently for single nucleotide variants (SNVs) and insertion/deletions (INDELs). For all the pathogenic variants found in RMS patients, we manually reviewed each of them in IGV (V2.3) (7) to validate the variant. In addition, we performed sample-wise QC by removing those samples with a missing genotyping rate >10%. We also removed samples with a heterozygosity rate >3 standard deviations to

eliminate potential sample contamination. For each set of the cohorts, we calculated individual-wise identity by descent (IBD) using PLINK (v1.90b2t) (8) and kept unrelated samples with IBD <0.1 via PRIMUS (v1.9.0) (9). None of the RMS samples were related based on the above criteria. Genetic ancestry of each individual was determined using ADMIXTURE (v1.3.0) (10). After filtering, we included 615 RMS patients (99.7% of variants > 30X depth), 9,663 controls from ARIC (97.8% of variants > 30X depth), and 300 controls from VIVA (99.0% of variants > 30X depth) in the final analyses.

Curation of cancer-predisposition genes (CPGs)

We pre-curated a set of autosomal dominant CPGs (n = 24) from cancer-susceptibility syndromes that have specifically been implicated in RMS predisposition including Li-Fraumeni (11), neurofibromatosis type 1 (12,13), Costello (14,15), DICER1 (16), Gorlin (17), Noonan (14), Cardiofaciocutaneous (14), constitutional (biallelic) mismatch repair deficiency (18), Beckwith-Wiedemann (19), and Rubinstein-Taybi (20). Notably, 21 of these 24 RMS-associated CPGs were included in the assessment by Zhang *et al.* (21) for the prevalence of germline pathogenic variants in other cancers. We also included a set of additional autosomal dominant CPGs (n = 39) from the report by Zhang *et al.* (21).

We also conducted a separate analysis to evaluate the frequency of pathogenic variants in autosomal recessive genes associated with sarcomas. We searched the "Online Mendelian Inheritance in Man" catalog using the search terms "rhabdomyosarcoma" and "sarcoma" to identify genes implicated in autosomal recessive disorders associated with sarcomas and selected the seven following genes: *RECQL2*, *RECQL4*, *BUB1B*, *NBN*, *CEP57*, *TRIP13*, and *BLM*.

Supplementary References

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Supplementary Tables

Supplementary Table 1. Epidemiological features of cases and controls used in this study

		Controls			RMS cases
		ARIC	VIVA	Total	
Number of samples		9,663	300	9,963	615
Sex	Female	5,417 (56.1%)	163 (54.3%)	5,580 (56.0%)	243 (39.5%)
	Male	4,246 (43.9%)	137 (45.7%)	4,383 (44.0%)	372 (60.5%)
Population	European American	7,122 (73.7%)	0	7,122 (71.5%)	405 (65.9%)
	African American	2,541 (26.3%)	0	2,541 (25.5%)	78 (12.7%)
	Hispanic	0	300 (100%)	300 (3.0%)	96 (15.6%)
	East Asian	0	0	0	28 (4.6%)
	South Asian	0	0	0	8 (1.3%)
Age* (year)	0 - 10	0	127 (42.3%)	127 (1.3%)	404 (65.7%)
	10- 20	0	146 (48.7%)	146 (1.5%)	198 (32.2%)
	20 - 30	0	0	0	13 (2.1%)
	30 - 40	0	0	0	0
	40 - 50	1,979 (20.5%)	0	1,979 (19.9%)	0
	50 - 60	2,986 (30.9%)	0	2,986 (30.0%)	0
	> 60	1,114 (11.5%)	0	1,114 (11.1%)	0
	NA	3,584 (37.1%)	27 (9.0%)	3,611 (36.2%)	0

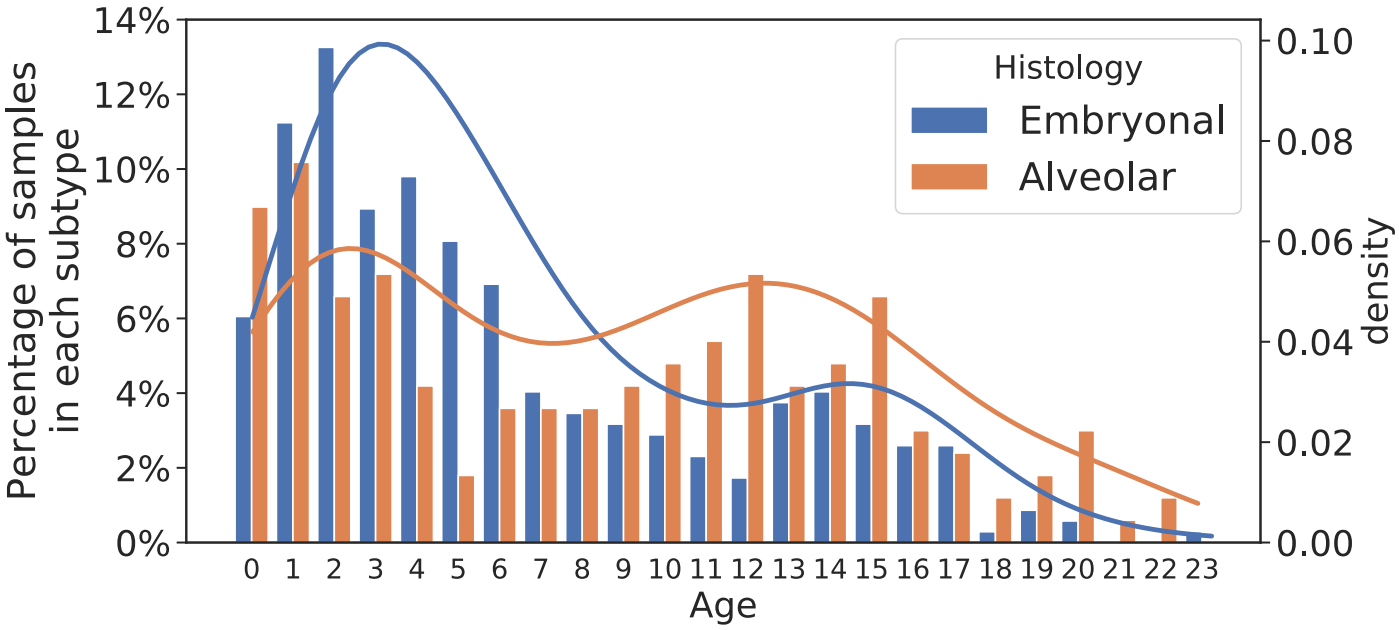
* For controls, Age represents the time when the sequencing experiment was performed. For RMS cases, Age represents the time when a diagnosis was made.

Supplementary Table 2. Associations between pathogenic or likely pathogenic (P/LP) variant frequencies and case / control status in different populations

	RMS cases		Controls		p value
	Total	with P/LP	Total	with P/LP	OR (95% CI)*
European American	405	33 (8.2%)	7122	96 (1.4%)	2.20E-19 7.27 (4.72-11.20)
African American	78	5 (6.4%)	2541	47 (1.9%)	5.96E-3 3.97 (1.49-10.60)
Hispanic	96	5 (5.2%)	300	3 (1.0%)	3.27E-3 13.2 (2.37-73.72)
Chi-square tests across populations	$p = 0.57$; $\chi^2 = 1.11$		$p = 0.16$; $\chi^2 = 3.73$		

*The control groups were served as the reference group for calculating odds ratio

Supplementary Figures



Supplementary Figure 1. Age distributions for embryonal and alveolar rhabdomyosarcoma patients. Bar plots indicate the percentage of patients of a given age from each histology group.