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Article

Heterozygous *BRCA1* and *BRCA2* and Mismatch Repair Gene Pathogenic Variants in Children and Adolescents With Cancer

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Abstract

Background: Genetic predisposition is has been identified as a cause of cancer, yet little is known about the role of adult cancer predisposition syndromes in childhood cancer. We examined the extent to which heterozygous pathogenic germline variants in *BRCA1*, *BRCA2*, *PALB2*, *ATM*, *CHEK2*, *MSH2*, *MSH6*, *MLH1*, and *PMS2* contribute to cancer risk in children and adolescents. **Methods:** We conducted a meta-analysis of 11 studies that incorporated comprehensive germline testing for children and adolescents with cancer. ClinVar pathogenic or likely pathogenic variants (PVs) in genes of interest were compared with 2 control groups. Results were validated in a cohort of mainly European patients and controls. We employed the Proxy External Controls Association Test to account for different pipelines. **Results:** Among 3975 children and adolescents with cancer, statistically significant associations with cancer risk were observed for PVs in *BRCA1* and *2* (26 PVs vs 63 PVs among 27 501 controls, odds ratio = 2.78, 95% confidence interval = 1.69 to 4.45; $P < .001$) and mismatch repair genes (19 PVs vs 14 PVs among 27 501 controls, odds ratio = 7.33, 95% confidence interval = 3.64 to 14.82; $P < .001$). Associations were seen in brain and other solid tumors but not in hematologic neoplasms. We confirmed similar findings in 1664 pediatric cancer patients primarily of European descent. **Conclusion:** These data suggest that heterozygous PVs in *BRCA1* and *2* and mismatch repair genes contribute with reduced penetrance to cancer risk in children and adolescents. No changes to predictive genetic testing and surveillance recommendations are required.

Genetic predisposition is an important etiologic factor in the development of cancer. Approximately 10% of children with cancer have a cancer predisposition syndrome (1,2). Cancer predisposition genes (CPGs) that play a role in this age group include *ALK*, *DICER1*, *ELP1*, *GATA2*, *NF1*, *PAX5*, *RB1*, *RET*, *RUNX1*, *SDHx*, *SMARCB1*, *SUFU*, *TP53*, and *WT1* (1). *TP53* is one of the most commonly mutated high-penetrance CPGs and is associated with a wide cancer spectrum (1-3). However, there are insufficient data on the quantitative contribution of germline *TP53* pathogenic and likely pathogenic variants (PVs) to childhood cancer risk. In addition to the autosomal dominantly acting CPGs mentioned above, several predominantly recessive disorders with abnormal DNA damage response are associated with increased cancer risks, including Fanconi

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anemia and especially subgroups caused by bi-allelic PVs in *BRCA2* and *PALB2*; constitutional mismatch repair (MMR) deficiency due to bi-allelic PVs in MMR genes *PMS2*, *MSH6*, *MSH2*, or *MLH1*; and ataxia-telangiectasia caused by bi-allelic PVs in *ATM* (4,5).

Less is known about the role of the so-called adult cancer predisposition syndromes in childhood cancer. These conditions include *BRCA1* and 2 (*BRCA1/2*) or *PALB2*-associated hereditary breast and ovarian cancer (HBOC) (6); susceptibility to breast cancer caused by PVs in *ATM* or *CHEK2*, among others (6); and Lynch syndrome (LS), caused by heterozygous PVs in one of the MMR genes (7). *BRCA1/2* PV carriers are also predisposed to male breast, pancreatic, stomach, prostate (only *BRCA2*), biliary tract (only *BRCA1*), and esophageal cancer (only *BRCA2*); PVs in *PALB2* are also associated with pancreatic and male breast cancer (8-11).

There are conflicting data on whether there is a higher occurrence of childhood cancer in HBOC (12,13) and LS families (13). Heterozygous PVs in *BRCA2* were associated with childhood and adolescent non-Hodgkin lymphoma (14) and rhabdomyosarcoma (15), and PVs in *BRCA2* and *PALB2* were associated with medulloblastoma (16). PVs in *BRCA2* among others have been associated with an increased risk of subsequent neoplasms (SNs) among long-term survivors of childhood cancer (17).

In HBOC and LS families and patients with heterozygous PVs in *ATM* and *CHEK2*, it is current practice to not offer predictive genetic testing of relatives at risk before adulthood because this would have no medical consequences. With the increasing use of next-generation sequencing (NGS), germline PVs in *BRCA1/2*, *PALB2*, *ATM*, *CHEK2*, *MSH2*, *MSH6*, *MLH1*, and *PMS2* are recurrently observed in children and adolescents with cancer (15,16,18-32). These findings suggest that heterozygous PVs in adult CPGs may contribute to childhood cancer risk. However, such PVs are also being observed in the general population (33-35), and their presence in a childhood cancer patient may represent a random, noncausal co-occurrence. Thus, the biological significance of such heterozygous PVs in the context of childhood cancer remains unclear. Mutational signatures in cancer genomes suggest that underlying heterozygous germline PVs in these genes drive the development of neoplasms in a portion of children carrying these PVs (36). In contrast, in others, the underlying germline defect may play no role in cancer development. However, the somatic landscape of cancer in children associated with germline PVs in these genes requires further investigation.

Our study aimed to investigate the hypothesis that heterozygous PVs in *BRCA1/2*, *PALB2*, *ATM*, *CHEK2*, *MSH2*, *MSH6*, *MLH1*, and *PMS2* represent reduced-penetrance cancer risk alleles in children and adolescents. We also aimed to study the proportion of *TP53* PV carriers among children and adolescents with cancer. We conducted a meta-analysis of 11 cohorts of children and adolescents with cancer investigated in comprehensive germline testing studies in Australia, Europe, and North America. We analyzed the frequency of heterozygous germline PVs in *BRCA1/2*, *PALB2*, *ATM*, *CHEK2*, *MSH2*, *MSH6*, *MLH1*, *PMS2*, and *TP53* in 3975 primary and relapsed pediatric cancer patients and compared the results with the frequency of heterozygous PVs in the same genes identified in 2 large control populations. Results were confirmed in a second cohort including 1664 children and adolescents with cancer.

Methods

Study Design and Systematic Review

The study design is depicted in Figure 1. We first conducted a systematic review and meta-analysis of published studies that included cohorts of children and adolescents with cancer who were comprehensively tested for underlying germline PVs in a broad spectrum of CPGs. The first study applying an NGS-based approach to investigate the role of germline PVs in multiple CPGs in children and adolescents with a range of cancer types was published in 2015 (32). We searched PubMed using the terms "childhood cancer" and "predisposition" or "pathogenic mutations in pediatric cancer" for subsequent studies published until February 28, 2022. We also searched the references of each selected study. We identified 17 studies that analyzed cohorts of children and adolescents with various cancer types including high-risk, refractory, and relapsed cancers (Table 1). We excluded 6 studies from the main analysis because we could not rule out isolated patient overlaps with other studies (15,16,18,22-24); 11 studies remained in the discovery data set (19-21,25-32). The excluded 6 studies were included in a supplementary analysis.

Variant Calling and Pathogenicity Assessment

Details on pipelines for variant calling and pathogenicity assessment employed in the different studies included in this analysis are provided in Supplementary Table 1 (available online). Different alignment algorithms, variant callers, and indel callers were used across studies. Cancer types in individual patients and germline PVs in genes of interest were extracted from all articles (Supplementary Table 2, available online). All PVs were aligned to the human reference genome (UCSC build hg19) and annotated according to the Human Genome Variation Society standards. ClinVar annotations (https://www.ncbi.nlm.nih.gov/clinvar/) were systematically provided for all PVs using the Ensembl Variant Effect Predictor (37).

Control Sample Ascertainment

A cancer-free cohort from the Technical University of Munich served as the first control group. Of 27 501 controls, 95% were recruited in Western Europe. Variants were called with a pipeline described previously (38). The second control cohort was extracted from the Genome Aggregation Database (gnomAD) noncancer set version 3.1.1 (May 1 and 2, 2021) (39). This data set included 74 023 samples; 32 411 samples were from European individuals (gnomAD European).

Gene-Based Burden Testing

Gene-based burden testing was performed and odds ratios, 95% confidence intervals, and *P* values were calculated using the 2-sided Fisher exact test. *P* values were considered to be of relevance for a *P* value less than .05. To account for differences in variant curation across studies, only carriers of heterozygous PVs unequivocally classified as pathogenic/likely pathogenic according to ClinVar were included in the analysis. We excluded individual patients with bi-allelic PVs in the genes of interest. The frequency of occurrence of ClinVar PVs and corresponding

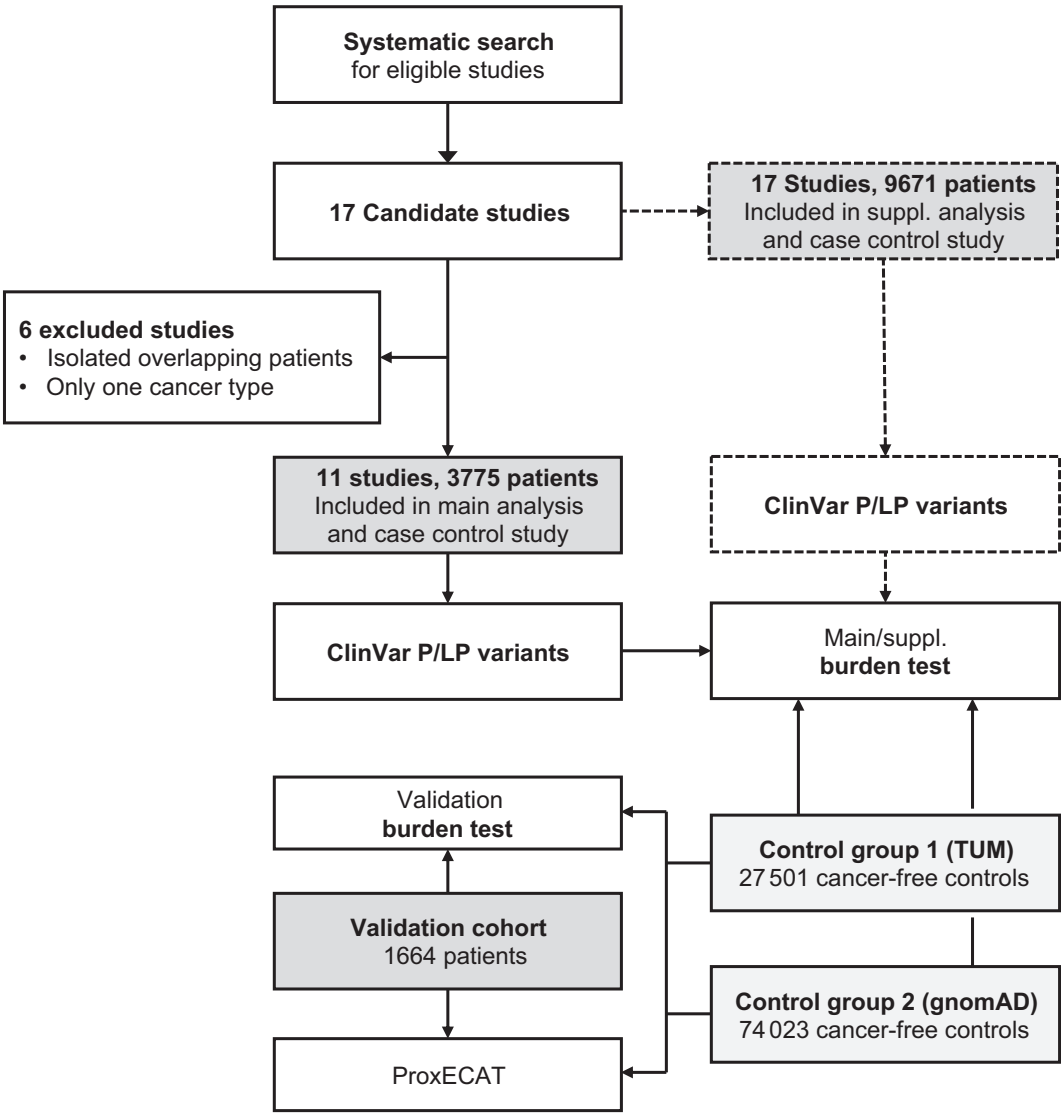

Figure 1. Study design of a combined meta-analysis and case-control study to analyze the cancer risk in children and adolescents associated with pathogenic variants in a range of adult cancer predisposition genes. See main text for explanations. gnomAD = Genome Aggregation Database; P/LP = pathogenic/likely pathogenic; ProxECAT = Proxy External Controls Association Test; suppl. = supplementary; TUM = Technical University of Munich.

95% Clopper-Pearson confidence intervals were calculated for individual studies. Estimating overall proportion was performed using fixed and random-effects models with generic invariance method (40). Between-study heterogeneity was measured using standard χ^2 tests and I^2 statistics (41). Meta-analysis results were summarized in forest plots.

Subgroup Analyses

To determine whether results were driven by specific cancer types, we conducted subgroup analyses focusing on patients with specific cancer types in addition to a combined analysis that included all patients. Across cohorts, data were presented in a heterogeneous manner. In some studies, details on tumor types were provided. In other studies, fewer details were available. We, therefore, categorized neoplasms into 3 groups: 1) brain tumors, 2) nonbrain solid tumors, and 3) hematological neoplasms, acknowledging that this crude categorization does not reflect the heterogeneous biology of different cancer types.

Validation Analysis

To validate the results of the main analysis, we used NGS data from 1664 patients analyzed at the Hopp Children's Cancer Center (Heidelberg, Germany). The variant calling pipeline used for these patients has been described previously (42). Whole genome and whole exome sequencing data for the entire set of 1664 patients (22,42) were available from the International Cancer Genome Consortium—Pedbrain Tumor and molecular mechanisms of malignant lymphoma (MMML)-seq (http://www.icgc.org), the German Cancer Consortium (https://dktk.dkfz.de/en), the Pediatric Cancer Genome Project (http://explore.pediatriccancergenomeproject.org/), the Heidelberg Institute for Personalized Oncology (http://www.dkfz.de/en/hipo), the Medulloblastoma Advanced Genomics International Consortium (43), the international case–control study on mobile phone use and brain tumour risk in children and adolescents (CEFALO) series (44), a series from France (45), and from 4 prospective clinical studies (SJMB03, SJMB12, SJYC07, and I-HIT-MED). All studies and acquisition of patient material

Table 1. Cohorts included in the main meta-analysis and the supplementary analysis

Reference	Included in the main analysis?	Case characteristics	No. of participants	DNA sequencing method
Byrjalsen et al. (19)	Yes	Children with all cancer types	198	WES
Chang et al. (20)	Yes	CAYA with relapsed and refractory cancer	59	WGS
Fiala et al. (21)	Yes	Children with solid tumors	751	NGS-based panel
Mody et al. (25)	Yes	CAYA with refractory or relapsed cancer	102	WES
Newman et al. (26)	Yes	Children with newly diagnosed or relapsed and/or refractory cancers	299	WGS
Oberg et al. (27)	Yes	Children with all cancer types, high risk	101	WES
Parsons et al. (28)	Yes	Children with solid tumors	150	WES
Stedingk et al. (29)	Yes	Children with all cancer types	790	Targeted sequencing
Wagener et al. (30)	Yes	Children with all cancer types	160	WES
Wong et al. (31)	Yes	Children with high-risk cancers	247	WGS
Zhang et al. (32)	Yes	CAYA with all cancer types	1120	WES, WGS
Akhavanfard et al. (18)	No[a]	CAYA with solid tumors	1507	WES
Grobner et al. (22)	No[a]	CAYA with all cancer types	914	WGS, WES
Kim et al. (23)	No[a]	CAYA with rhabdomyosarcoma	394	WGS, WES
Li et al. (15)	No[a]	Children with rhabdomyosarcoma	615	WES
Mirabello et al. (24)	No[a]	CAYA with osteosarcoma	1244	WES or targeted sequencing
Waszak et al. (16)	No[a]	Mainly children with medulloblastoma	1022	WES, WGS

[a]Reason for exclusion: We could not rule out isolated patient overlaps with studies included in the main analysis. These excluded studies were included in a supplementary analysis. CAYA = children, adolescents, and young adults; NGS = next-generation sequencing; WES = whole exome sequencing; WGS = whole genome sequencing.

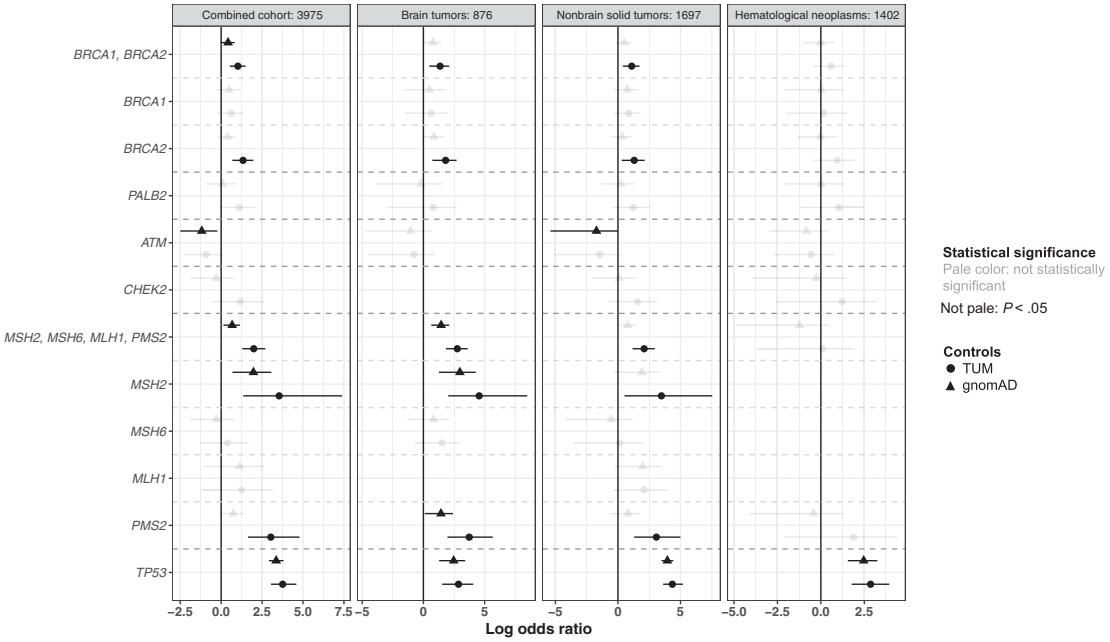

Figure 2. Burden test results of the main analysis including 11 studies. Results are shown for all patients combined, brain tumors, nonbrain solid tumors, and hematologic malignancies. Both control groups are included. Statistically significant associations with childhood and adolescent cancer were identified for PVs in *BRCA1* and 2, PVs in mismatch repair genes, and PVs in *TP53*. gnomAD = Genome Aggregation Database; PVs = pathogenic variants; TUM = Technical University of Munich.

were performed with written informed consent and in accordance with institutional review board guidelines. Of the 1664 patients, 83.7% were of European descent. To account for the heterogeneous variant calling pipelines used across studies, a Proxy External Controls Association Test (ProxECAT) (46) was performed using synonymous variants to produce weighted ClinVar PV counts as suggested by the method and detailed allele frequency information from this validation cohort.

Results

Gene-Based Burden Testing

In this systematic review, combined meta-analysis, and case-control analysis, we included 3975 patients from 11 independent studies (19-21,25-32) (Figure 1 and Table 1). Data on variants and cancer types observed in individual patients are provided in Supplementary Table 2 (available online). Burden

Table 2. Frequencies of pathogenic/likely pathogenic variants in *BRCA1* and *2*, *PALB2*, *ATM*, *CHEK2*, MMR genes, and *TP53* identified among 3975 patients and 27 501 cancer-free controls

Cohort and gene(s)	Patients		Control group 1 (TUM)		Odds ratio (95% CI)	*P*
	Carrier No. (%)	Noncarrier No. (%)	Carrier No. (%)	Noncarrier No. (%)		
Combined cohort (n = 3975 patients[a])						
BRCA1, *BRCA2*	26 (0.7)	3949 (99.3)	63 (0.2)	27 438 (99.8)	2.78 (1.69 to 4.45)	<.001
BRCA1	9 (0.2)	3966 (99.8)	34 (0.1)	27 467 (99.9)	1.83 (0.77 to 3.91)	.11
BRCA2	17 (0.4)	3958 (99.6)	29 (0.1)	27 472 (99.9)	3.81 (1.97 to 7.10)	<.001
PALB2	6 (0.2)	3969 (99.8)	14 (0.1)	27 487 (99.9)	2.97 (0.93 to 8.23)	.03
ATM	4 (0.1)	3971 (99.9)	69 (0.3)	27 432 (99.8)	0.4 (0.11 to 1.07)	.08
CHEK2	3 (0.09)	3182 (99.91)	8 (0.0)	27 493 (100)	3.24 (0.55 to 13.51)	.09
MSH2, *MSH6*, *MLH1*, *PMS2*	19 (0.5)	3956 (99.5)	14 (0.1)	27 487 (99.9)	7.33 (3.64 to 14.82)	<.001
MSH2	5 (0.1)	3970 (99.9)	1 (0.0)	27 500 (100)	34.62 (3.87 to 1622.41)	<.001
MSH6	3 (0.1)	3972 (99.9)	10 (0.0)	27 491 (100)	1.48 (0.27 to 5.32)	.22
MLH1	2 (0.1)	3973 (99.9)	4 (0.0)	27 497 (100)	3.46 (0.31 to 24.15)	.17
PMS2	9 (0.2)	3966 (99.8)	3 (0.0)	27 498 (100)	20.80 (5.19 to 119.91)	<.001
TP53	55 (1.4)	3920 (98.6)	9 (0.0)	27 492 (100)	42.82 (20.98 to 98.95)	<.001
Brain tumors (n = 876 patients)						
BRCA1, *BRCA2*	8 (0.9)	868 (99.1)	63 (0.2)	27 438 (99.8)	3.89 (1.61 to 8.16)	.002
BRCA1	2 (0.2)	874 (99.8)	34 (0.1)	27 467 (99.9)	1.85 (0.21 to 7.23)	.31
BRCA2	6 (0.7)	870 (99.3)	29 (0.1)	27 472 (99.9)	6.11 (2.08 to 14.91)	<.001
PALB2	1 (0.1)	875 (99.9)	14 (0.1)	27 487 (99.9)	2.24 (0.05 to 14.77)	.38
ATM	1 (0.1)	875 (99.9)	69 (0.3)	27 432 (99.7)	0.45 (0.01 to 2.62)	.73
MSH2, *MSH6*, *MLH1*, *PMS2*	9 (1.0)	867 (99.0)	14 (0.1)	27 487 (99.9)	15.84 (6.25 to 37.24)	<.001
MSH2	3 (0.3)	873 (99.7)	1 (0.0)	27 500 (100)	94,68 (7.57 to 4776.60)	<.001
MSH6	2 (0.2)	874 (99.8)	10 (0.0)	27 491 (100)	4.49 (0.49 to 19.61)	.09
PMS2	4 (0.5)	872 (99.5)	3 (0.0)	27 498 (100)	42.02 (7.10 to 286.27)	<.001
TP53	5 (0.6)	871 (99.4)	9 (0.0)	27 492 (100)	17.53 (4.61 to 58.32)	<.001
Nonbrain solid tumors (n = 1697 patients)						
BRCA1, *BRCA2*	12 (0.7)	1685 (99.2)	63 (0.2)	27 438 (99.8)	3.01 (1.47 to 5.63)	.001
BRCA1	5 (0.3)	1692 (99.7)	34 (0.1)	27 467 (99.9)	2.39 (0.73 to 6.14)	.07
BRCA2	7 (0.4)	1690 (99.6)	29 (0.1)	27 472 (99.9)	3.67 (1.36 to 8.51)	.006
PALB2	3 (0.2)	1694 (99.8)	14 (0.1)	27 487 (99.9)	3.48 (0.64 to 12.47)	.07
ATM	1 (0.1)	1696 (99.9)	69 (0.3)	27 432 (99.7)	0.23 (0.01 to 1.35)	.19
CHEK2	2 (0.1)	1449 (99.8)	8 (0.0)	27 493 (100)	4.74 (0.49 to 23.79)	.09
MSH2, *MSH6*, *MLH1*, *PMS2*	9 (0.5)	1688 (99.5)	14 (0.1)	27 487 (99.9)	8.14 (3.22 to 19.12)	<.001
MSH2	2 (0.1)	1696 (99.9)	1 (0.0)	27 500 (100)	32.44 (1.69 to 1886.83)	.01
MSH6	1 (0.1)	1696 (99.9)	10 (0.0)	27 491 (100)	1.16 (0.03 to 7.62)	.59
MLH1	2 (0.1)	1695 (99.9)	4 (0.0)	27 497 (100)	8.11 (0.73 to 56.57)	.04
PMS2	4 (0.2)	1693 (99.8)	3 (0.0)	27 498 (100)	21.64 (3.66 to 148.15)	<.001
TP53	42 (2.5)	1655 (97.5)	9 (0.0)	27 492 (100)	77.56 (37.12 to 181.23)	<.001
Hematological neoplasms (n = 1402 patients)						
BRCA1, *BRCA2*	6 (0.4)	1396 (99.9)	63 (0.2)	27 438 (99.8)	1.81 (0.64 to 4.18)	.16
BRCA1	2 (0.1)	1400 (99.9)	34 (0.1)	27 467 (99.8)	1.15 (0.13 to 4.51)	.69
BRCA2	4 (0.3)	1398 (99.7)	29 (0.1)	27 472 (99.9)	2.54 (0.65 to 7.19)	.09
PALB2	2 (0.1)	1400 (99.9)	14 (0.1)	27 487 (99.9)	2.80 (0.31 to 12.23)	.18
ATM	2 (0.1)	1400 (99.9)	69 (0.3)	27 432 (99.7)	0.57 (0.07 to 2.13)	.59
CHEK2	1 (0.1)	1006 (99.9)	8 (0.0)	27 493 (100)	3.42 (0.08 to 25.51)	.28
MSH2, *MSH6*, *MLH1*, *PMS2*	1 (0.1)	1401 (99.9)	14 (0.1)	27 487 (99.9)	1.09 (0.03 to 6.91)	.61
PMS2	1 (0.1)	1401 (99.9)	3 (0.0)	27 498 (100)	6.54 (0.12 to 81.52)	.18
TP53	8 (0.6)	1394 (99.4)	9 (0.0)	27 492 (100)	17.52 (5.87 to 51.87)	<.001

[a]Two patients were excluded because of benign tumors. CI = confidence interval; MMR = mismatch repair; TUM = Technical University of Munich.

test results are shown in Figure 2, Table 2 (control group 1), and Table 3 (control group 2). Among 3975 children and adolescents with cancer, statistically significant associations with cancer risk were observed for PVs in *BRCA1/2* and MMR genes combined and *MSH2* alone using both control groups. Subgroup analysis revealed that enrichment of PVs in these genes was most pronounced for patients with brain tumors followed by patients with nonbrain solid tumors. The statistically significant *BRCA1/2* association (*BRCA1/2* combined and *BRCA2* alone) was reproduced with control group 1, when we analyzed patients with brain tumors or nonbrain solid tumors alone. Statistically significant associations between childhood cancer and PVs in MMR genes were reproduced in the brain tumor group (both control groups, statistically significant associations with MMR genes combined, *MSH2*, and *PMS2* alone) and in the nonbrain solid tumor group (control group 1, statistically significant associations with MMR genes combined, *MSH2*, and *PMS2* alone). We did not observe statistically

Table 3. Frequencies of pathogenic/likely pathogenic variants in *BRCA1* and 2, *PALB2*, *ATM*, *CHEK2*, MMR genes, and *TP53* identified among 3975 patients and 74 023 cancer-free controls

Cohorts and gene(s)	Patients		Controls group 2 (gnomAD)		Odds ratio (95% CI)	*P*
	Carrier No. (%)	Noncarrier No. (%)	Carrier No. (%)	Noncarrier No. (%)		
Combined cohort (n = 3975 patients[a])						
BRCA1, *BRCA2*	26 (0.7)	3949 (99.3)	318 (0.4)	73 705 (99.5)	1.53 (0.99 to 2.28)	.05
BRCA1	9 (0.2)	3966 (99.8)	103 (0.1)	73 920 (99.9)	1.63 (0.73 to 3.21)	.18
BRCA2	17 (0.4)	3958 (99.6)	215 (0.3)	73 808 (99.7)	1.47 (0.84 to 2.42)	.13
PALB2	6 (0.2)	3969 (99.8)	103 (0.1)	73 920 (99.9)	1.08 (0.39 to 2.45)	.83
ATM	4 (0.1)	3971 (99.9)	243 (0.3)	73 780 (99.7)	0.31 (0.08 to 0.79)	.008
CHEK2	3 (0.09)	3182 (99.91)	95 (0.1)	73 928 (99.9)	0.73 (0.15 to 2.21)	.80
MSH2, *MSH6*, *MLH1*, *PMS2*	19 (0.5)	3956 (99.5)	181 (0.2)	73 842 (99.8)	1.96 (1.15 to 3.15)	.009
MSH2	5 (0.1)	3970 (99.9)	13 (0.0)	74 010 (100)	7.17 (2.00 to 21.44)	.002
MSH6	3 (0.1)	3972 (99.9)	75 (0.1)	73 948 (99.9)	0.74 (0.15 to 2.26)	.80
MLH1	2 (0.1)	3973 (99.9)	12 (0.0)	74 011 (100)	3.10 (0.34 to 13.95)	.16
PMS2	9 (0.2)	3966 (99.8)	81 (0.1)	73 942 (99.9)	2.07 (0.91 to 4.13)	.05
TP53	55 (1.4)	3920 (98.6)	36 (0.1)	73 987 (99.9)	28.82 (18.57 to 45.29)	<.001
Brain tumors (n = 876 patients)						
BRCA1, *BRCA2*	8 (0.9)	868 (99.1)	318 (0.4)	73 705 (99.6)	2.14 (0.91 to 4.28)	.06
BRCA1	2 (0.2)	874 (99.8)	103 (0.1)	73 920 (99.9)	1.64 (0.20 to 6.10)	.35
BRCA2	6 (0.7)	870 (99.3)	215 (0.3)	73 808 (99.7)	2.37 (0.86 to 5.26)	.05
PALB2	1 (0.1)	875 (99.9)	103 (0.1)	73 920 (99.86)	0.82 (0.02 to 4.68)	>.99
ATM	1 (0.1)	875 (99.9)	243 (0.3)	73 780 (99.67)	0.35 (0.01 to 1.96)	.54
MSH2, *MSH6*, *MLH1*, *PMS2*	9 (1.0)	867 (99.0)	181 (0.2)	73 842 (99.76)	4.23 (1.90 to 8.26)	<.001
MSH2	3 (0.3)	873 (99.7)	13 (0.0)	74 010 (99.98)	19.56 (3.57 to 71.36)	.001
MSH6	2 (0.2)	874 (99.8)	75 (0.1)	73 948 (99.9)	2.26 (0.27 to 8.46)	.23
PMS2	4 (0.5)	872 (99.5)	81 (0.1)	73 942 (99.9)	4.19 (1.11 to 11.18)	.02
TP53	5 (0.6)	871 (99.4)	36 (0.1)	73 987 (99.9)	11.80 (3.60 to 30.25)	<.001
Nonbrain solid tumors (n = 1697 patients)						
BRCA1, *BRCA2*	12 (0.7)	1685 (99.2)	318 (0.4)	73 705 (99.6)	1.65 (0.84 to 2.93)	.09
BRCA1	5 (0.3)	1692 (99.7)	103 (0.1)	73 920 (99.9)	1.12 (0.67 to 5.12)	.09
BRCA2	7 (0.4)	1690 (99.6)	215 (0.3)	73 808 (99.7)	1.42 (0.56 to 2.99)	.36
PALB2	3 (0.2)	1694 (99.8)	103 (0.1)	73 920 (99.9)	1.27 (0.26 to 3.83)	.52
ATM	1 (0.1)	1696 (99.9)	243 (0.3)	73 780 (99.7)	0.18 (0.00 to 1.01)	.049
CHEK2	2 (0.1)	1449 (99.8)	95 (0.1)	73 928 (99.9)	1.07 (0.13 to 4.00)	.71
MSH2, *MSH6*, *MLH1*, *PMS2*	9 (0.5)	1688 (99.5)	181 (0.2)	73 842 (99.8)	2.18 (0.98 to 4.23)	.04
MSH2	2 (0.1)	1696 (99.9)	13 (0.0)	74 010 (100)	6.72 (0.74 to 29.72)	.04
MSH6	1 (0.1)	1696 (99.9)	75 (0.1)	73 948 (99.9)	0.58 (0.01 to 3.34)	>.99
MLH1	2 (0.1)	1695 (99.9)	12 (0.0)	74 011 (99.9)	7.28 (0.79 to 32.70)	.04
PMS2	4 (0.2)	1693 (99.8)	81 (0.1)	73 942 (99.9)	2.16 (0.57 to 5.75)	.12
TP53	42 (2.5)	1655 (97.5)	36 (0.1)	73 987 (99.9)	52.14 (32.51 to 83.81)	<.001
Hematological neoplasms (n = patients cases)						
BRCA1, *BRCA2*	6 (0.4)	1396 (99.9)	318 (0.4)	73 705 (99.6)	1.00 (0.36 to 2.20)	>.99
BRCA1	2 (0.1)	1400 (99.9)	103 (0.1)	73 920 (99.9)	1.03 (0.12 to 3.81)	.72
BRCA2	4 (0.3)	1398 (99.7)	215 (0.3)	73 808 (99.7)	0.98 (0.26 to 2.56)	>.99
PALB2	2 (0.1)	1400 (99.9)	103 (0.1)	73 920 (99.9)	1.03 (0.12 to 3.81)	.72
ATM	2 (0.1)	1400 (99.9)	243 (0.3)	73 780 (99.7)	0.43 (0.05 to 1.59)	.34
CHEK2	1 (0.1)	1006 (99.9)	95 (0.1)	73 928 (99.9)	0.77 (0.02 to 4.42)	>.99
MSH2, *MSH6*, *MLH1*, *PMS2*	1 (0.1)	1401 (99.9)	181 (0.2)	73 842 (99.8)	0.29 (0.01 to 1.65)	.27
PMS2	1 (0.1)	1401 (99.9)	81 (0.1)	73 942 (99.9)	0.65 (0.02 to 3.74)	>.99
TP53	8 (0.6)	1394 (99.4)	36 (0.1)	73 987 (99.9)	11.79 (4.73 to 25.86)	<.001

[a]Two patients were excluded because of benign tumors. CI = confidence interval; gnomAD = Genome Aggregation Database; MMR = mismatch repair.

significant associations between childhood and adolescent cancer and heterozygous germline PVs in *ATM*, *CHEK2*, and *PALB2*. None of the statistically significant associations with PVs in *BRCA1/2* or MMR genes were observed in the hematologic neoplasm group. The strongest association was observed for *TP53*, mutated in the germline of 1.4% of studied patients diagnosed with cancer. The effects were consistent for patients with brain, nonbrain solid tumors, and hematologic neoplasms. The meta-analysis results for the occurrence rates are depicted in the forest plots in Supplementary Figure 1 (available online; control group 1) and Supplementary Figure 2 (available online; control group 2). When adding the initially excluded 6 additional studies (15,16,18,22-24) to the primary analysis (Figure 1 and Table 1), results were reproduced with both control groups (Supplementary Figure 3, available online).

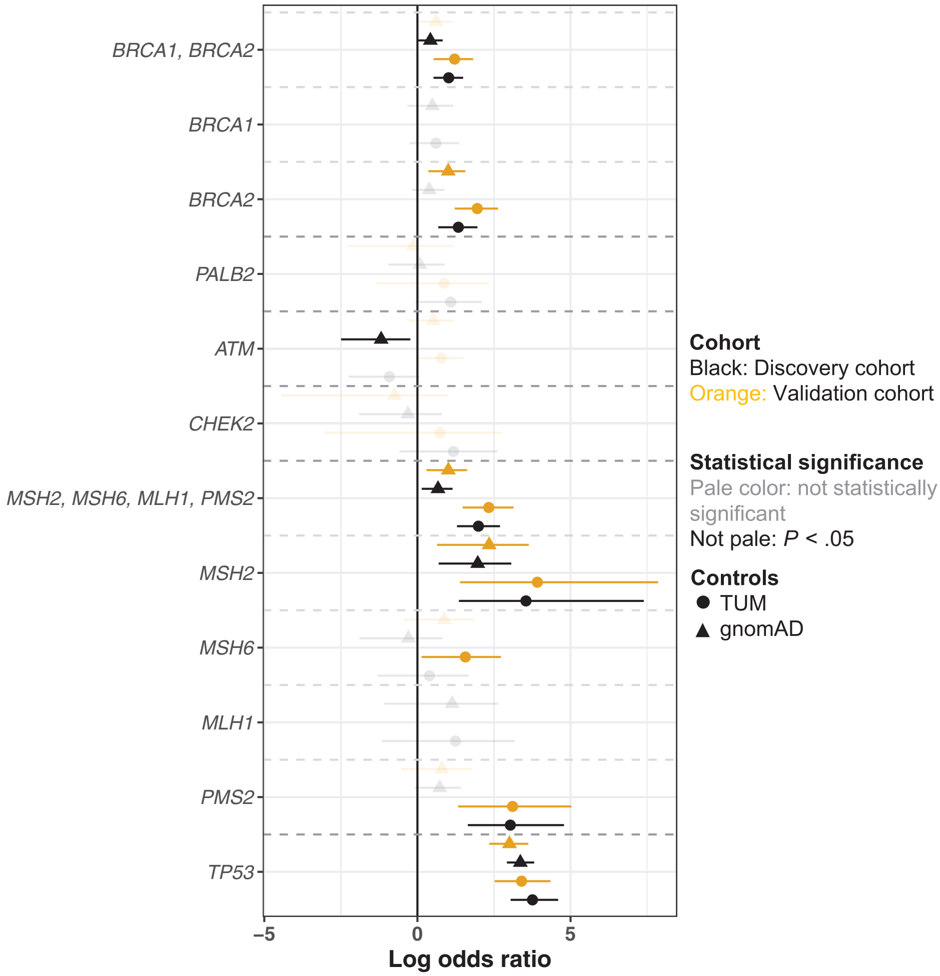

Figure 3. Results of the validation case-control study with 1664 patients. A total of 1664 mainly European childhood and adolescent cancer patients served as a validation cohort. Statistically significant associations with childhood and adolescent cancer were identified for PVs in *BRCA2*, PVs in mismatch repair genes, PVs in *MSH2*, and PVs in *TP53*. Results of the main analysis are also shown. gnomAD = Genome Aggregation Database; PVs = pathogenic variants; TUM = Technical University of Munich.

Validation Analysis

To investigate the reproducibility of our findings, we conducted a validation analysis using a cohort of 1664 patients. With both control groups, gene burden analysis confirmed statistically significant associations between childhood and adolescent cancer and *BRCA2*, MMR genes combined, *MSH2*, and *TP53* (Figure 3). Additionally, to obtain more control over technical confounders, we employed the ProxECAT (46), which confirmed the results of the validation burden test (Figure 4). To account for the fact that the validation cohort consisted of mainly European cases, we conducted a separate burden analysis using gnomAD European controls only. This analysis showed similar results (Figure 5).

Tumor Spectra Associated With PVs in *BRCA1/2* and MMR Genes

The cancer types and specific heterozygous PVs in *BRCA1/2* and MMR genes are depicted in Supplementary Table 2. The cancer patterns were broad and included brain tumors, nonbrain solid tumors, and, more rarely, hematologic neoplasms. Among individuals included in the main and validation analyses, PVs in *BRCA1/2* were found in patients with acute lymphoblastic leukemia, ependymoma, Ewing sarcoma, glioma, Langerhans cell histiocytosis, medulloblastoma, neuroblastoma, rhabdoid tumor, or rhabdomyosarcoma. In the *BRCA1/2* group, medulloblastoma was the predominant condition (Supplementary Table 2, available online). PVs in MMR genes were found in children and adolescents with acute myeloid leukemia, Ewing sarcoma, high- and low-grade glioma, malignant germ cell tumor, malignant peripheral nerve sheath tumor, medulloblastoma, neuroblastoma, osteosarcoma, or rhabdomyosarcoma. In the MMR group, high-grade glioma was the predominant condition (Supplementary Table 2, available online).

Discussion

We conducted a combined meta-analysis and subsequent case-control study of 11 studies that included 3975 children and adolescents diagnosed with cancer. We compared the frequency of germline ClinVar PVs in 9 adult-onset cancer predisposition genes with the frequency of ClinVar PVs in the same genes in 2 independent control groups. PVs in *BRCA1/2* and MMR genes were enriched among cases compared with 2 large external

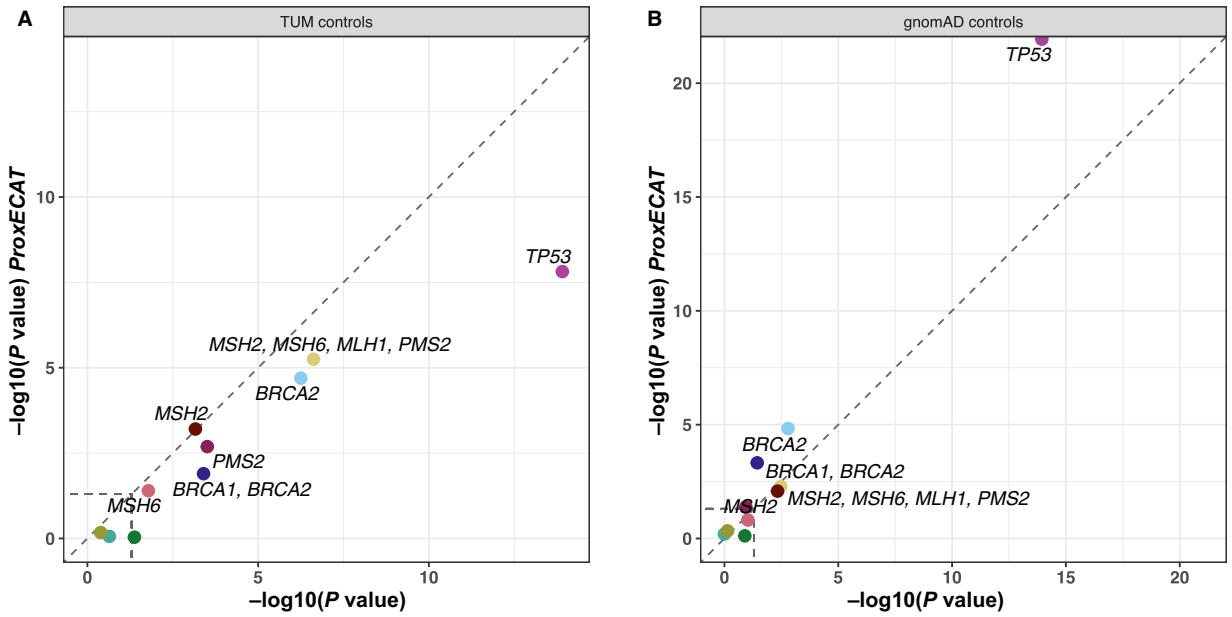

Figure 4. Correlation of *P* values, results in burden test, and ProxECAT. The ProxECAT showed similar results indicating that differences in variant calling pipelines did not heavily bias the results of the validation analysis. **A**) Control group 1. **B**) Control group 2. gnomAD = Genome Aggregation Database; ProxECAT = Proxy External Controls Association Test; TUM = Technical University of Munich.

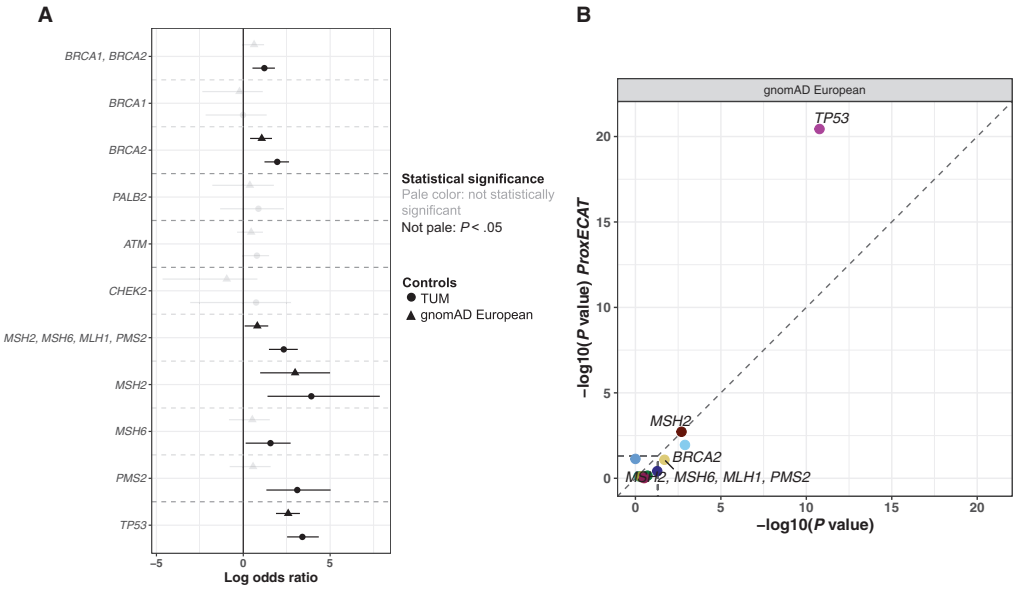

Figure 5. Results of the validation case-control study with 1664 patients. **A**) Results of the validation case-control study with 1664 patients using gnomAD European controls. Results with TUM controls are also shown. Statistically significant associations with childhood and adolescent cancer were identified for PVs in *BRCA2*, PVs in mismatch repair genes combined, PVs in *MSH2*, and PVs in *TP53*. **B**) Proxy external controls association test results with gnomAD European controls. gnomAD = Genome Aggregation Database; PVs = pathogenic variants; TUM, Technical University of Munich.

control cohorts. Results were further reproduced in a cohort of 1664, mainly European cases.

The main burden analysis and the validation analysis indicate that *BRCA1/2* and the MMR genes play a role in the pathogenesis of childhood cancer with reduced penetrance. The subgroup analysis revealed the most statistically significant associations with PVs in *BRCA1/2* or MMR genes in the groups of patients with brain and nonbrain solid tumors. Brain tumors belong to the established LS tumor spectrum (7) and are a hallmark of constitutional MMR deficiency (5). Therefore, an association between PVs in MMR genes and childhood brain tumors is plausible. Likewise, PVs in *BRCA2* have been implicated in the pathogenesis of sarcoma in adults (47), and patients with Fanconi anemia due to bi-allelic PVs in *BRCA2* have a high risk of developing myeloid neoplasms as well as a range of childhood brain and nonbrain tumors (4), rendering the observed statistically significant association between PVs in *BRCA1/2* and childhood tumors biologically highly plausible. No

statistically significant associations were identified between childhood cancer and PVs in *ATM*, *CHEK2*, and *PALB2*. Statistically significant associations between cancer and PVs in *BRCA1/2*, *PALB2*, *ATM*, *CHEK2*, or MMR genes were absent in patients with hematologic neoplasms, which is consistent with results of genome-wide association studies (48,49). Larger, better informed studies may be necessary to identify weaker associations.

Our results do not implicate changes in the current predictive testing practice, which is to defer predictive testing for heterozygous *BRCA1/2* and the MMR gene variants until adulthood. Cancer surveillance is not indicated in healthy children carrying PVs in *BRCA1/2* or MMR genes. PVs in *BRCA1/2* or MMR genes may increase the risk of SNs among childhood cancer survivors, and this risk may be further increased by genotoxic treatment elements such as radiation and alkylating agents (17). The finding of PVs in DNA repair genes such as *BRCA2* in a child with cancer may inform treatment decisions in the future, and an increased awareness for SNs may be indicated in survivors of childhood cancer with a PV in *BRCA1/2* or an MMR gene. In addition, the detection of a heterozygous germline PV in *BRCA1/2* or one of the MMR genes allows genetic counseling and cascade testing in the family.

We found that approximately 1.2% of children and adolescents with cancer carried a heterozygous PV in *BRCA1/2* or one of the MMR genes. This combined percentage is similar to the percentage of patients with a germline PV in *TP53* and more common than PVs in most other highly penetrant CPGs that play a role in childhood cancer, suggesting that *BRCA1/2* and MMR genes are among the most commonly mutated CPGs in children and adolescents with cancer. The neoplasm spectrum is broad (Supplementary Table 2, available online) and, in this regard, similar to the cancer spectrum observed in patients with Li-Fraumeni syndrome (3,50). A detailed investigation of the somatic landscape of patients with *BRCA1/2*- or MMR-related childhood cancer will be required to distinguish tumors caused by an underlying PV in *BRCA1/2* or an MMR gene from tumors that occurred coincidentally or without evidence of causality. We recommend enrolling children and adolescents carrying a PV in an adult tumor gene into registries to further study the biological significance, natural history, and clinical implications of this association in minors.

Approximately 1.4% of children and adolescents with cancer carried a PV in *TP53*. This percentage may have been influenced by an overrepresentation of tumors that are known to be associated with *TP53*; however, despite this possible bias, these data confirm that *TP53* is among the most commonly mutated CPGs in children and adolescents with cancer and that PVs in *TP53* are present in 1%-2% of patients in this age group. The associated cancer risks are high and confirm the need for intensive cancer surveillance in *TP53* PV carriers.

Our study has limitations:

1) Different variant calling pipelines were used across the included studies and controls. To address this issue, we first performed a validation analysis using regular burden testing. Second, to account for the potential resulting bias, we used the ProxECAT (46), which confirmed the results of the validation analysis.
2) Variant classification did not occur identically across all studies and controls. Differences in PV classification can substantially impact enrichment testing results leading to uncertainty regarding results and conclusions. To account for this potential bias, all PVs in cases and controls were reclassified, and only PVs with a ClinVar designation as pathogenic or likely pathogenic were included in the final data set.
3) Selection bias may have influenced our results because included cancers tended to be high risk. This possible bias does not compromise the general conclusions of our study.
4) The population frequencies of heterozygous PVs vary between populations and are influenced by factors like founder PVs (33-35). We reproduced the results in a cohort that included mainly European cases and controls. Therefore, results are unlikely to be driven by occult population stratification
5) We did not analyze valuable information from pediatric cancer genomes because this information was not consistently available across the 11 studies.
6) We did not analyze whether variants may contribute to polygenic effects to cancer risk as has been recently demonstrated in adult patients with sarcoma (47).
7) Precise histology information was not available in all studies. Therefore, we grouped patients into the 3 disease categories: brain tumors, nonbrain solid tumors, and hematologic neoplasm.
8) We noted a patient overlap between 1 of the 11 cohorts of the main analysis (32) and the validation cohort, however, this overlap does not compromise the ProxECAT results.
9) We used 2 separate controls of convenience resulting in lingering uncertainties of the results. For these reasons, large, better-informed epidemiologic studies are essential to independently confirm these results and to further study the spectrum of associated cancers as well as specific cancer risks.

Despite these limitations, the results of this combined meta-analysis and case-control study and the ProxECAT validation suggest that heterozygous PVs in *BRCA1/2* and MMR genes contribute to cancer risk in children and adolescents. In children and adolescents, the penetrance of these PVs is reduced, not requiring changes to current genetic testing or surveillance practices. Further studies are needed to study the precise childhood and adolescent tumor spectrum and the somatic mutation landscape associated with PVs in these and other adult-onset CPGs.

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Notes

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Data Availability

All data used for the analysis are available online in Supplementary Table 2 (available online).

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