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ORIGINAL ARTICLE

Germline mutations in pediatric cancer cohort with mixed-ancestry Mexicans

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Abstract

Background: Childhood cancer is one of the primary causes of disease-related death in 5- to 14-year-old children and currently no prevention strategies exist to reduce the incidence of this disease. Childhood cancer has a larger hereditary component compared with cancer in adults. Few genetic studies have been conducted on children with cancer. Additionally, Latin American populations are underrepresented in genomic studies compared with other populations. Therefore, the aim of this study is to analyze germline mutations in a group of mixed-ancestry Mexican pediatric patients with solid and hematological cancers.
Methods: We analyzed genetic variants from 40 Mexican childhood cancer patients and their relatives. DNA from saliva or blood samples was used for whole-exome sequencing. All variants were identified following GATK best practices.
Results: We found that six patients (15%) were carriers of germline mutations in *CDKN2A*, *CHEK2*, *DICER1*, *FANCA*, *MSH6*, *MUTYH*, *NF1*, and *SBDS* cancer predisposition genes, and additional new variants predicted to be deleterious by in silico algorithms. A population genetics analysis detected five components consistent with the demographic models assumed for modern mixed-ancestry Mexicans.

Odontologia; Programa de Maestria y Doctorado en Ciencias Medicas, Odontologicas y de la Salud, UNAM; "Aqui nadie se rinde, ANSER (I.A.P); Max Planck Institute for Evolutionary Anthropology (MPI-EVA)

Conclusions: This report identifies potential genetic risk factors and provides a better understanding of the underlying mechanisms of childhood cancer in this population.

KEYWORDS

childhood cancer predisposition, exome sequencing, germline mutations, mixed-ancestry Mexicans

1 | INTRODUCTION

Childhood cancer is the leading cause of death by disease in 5- to 14-year-old children, and thus far, no prevention strategies have been identified. The global incidence of childhood cancer has increased by 1% per year (Steliarova-Foucher et al., 2017). However, considerable variations in incidence by age, sex, and geographic regions have been reported by The International Agency for Research on Cancer (IACR). Racial and ethnic disparities in survival rates have also been reported, mainly associated with socioeconomic status and access to medical care. That said, the impact of genetic differences between populations cannot be ruled out and has not been well addressed (Bhatia, 2011). High-income countries (HICs) have improved their survival rates in the last decades, reporting disease-free survival (DFS) in 80% of patients 5 years after diagnosis. Nevertheless, children with cancer that reside in low- and middle-income countries (LMICs), have lower estimates of overall survival (OS) (Bhakta et al., 2019). In Mexico, despite efforts to improve the diagnosis and treatment of childhood cancer, survival rates remain low (67%) (Rivera-Luna et al., 2017).

Previous authors have proposed that childhood cancer has a larger hereditary component than cancer in adults (Capasso et al., 2020; Sweet-Cordero & Biegel, 2019). It has been reported that the risk of childhood cancer in families with cases of pediatric cancer increases by 48% in first-degree relatives, compared with the general population of Sweden, which cannot be explained by currently known cancer predisposition germline variants (Stjernfelt et al., 2020). Additionally, previous studies have reported different cancer incidence rates between populations (Colton et al., 2018; Walsh et al., 2014). Few genetic studies have been performed on children with cancer. In 2015, Zhang et al. reported that 8.5% of patients under 20 years were positive for a germline mutation, in comparation to the control group (1%); interestingly, 60% of carriers did not report a history of family cancer (Zhang et al., 2016). Pan-cancer analysis reported a similar percentage (7.6%) of mutations in *MSH2*, *MSH6*, *PMS2*, *TP53*, *BRCA2*, *and CHEK2* (Gröbner et al., 2018). Germline sequencing in 1201 individuals performed by Staler and colleagues showed that 21% of patients with early onset cancer had an inherited genetic mutation, compared with 13% of young adults with tumors (Staler et al., 2020). The highest percentage of germline mutations in childhood cancer was reported by Oberg et al. (2016), where 14% of the children were carriers of a mutation in a cancer predisposition gene.

Despite this, the implications of genetic variants in childhood cancer remain unclear, and not all populations—such as mixed-ancestry Mexicans—have been included. It is crucial to elucidate the role of genetic variants in the development of childhood cancer, and their allele frequencies, to establish the basis of the causes of childhood cancer, cancer risk reduction strategies, surveillance, and treatment. The present study aims to assess the genes, alleles, and their frequencies, involved in childhood cancer predisposition in a group of pediatric cancer patients with mixed-ancestry Mexican.

2 | MATERIALS AND METHODS

2.1 | Patient recruitment

This work was performed in collaboration with the Hospital Infantil de Mexico "Federico Gomez." Patients were recruited from 2011 to 2016. The eligibility criteria for inclusion in the study were (a) confirmed diagnosis of a childhood malignancy and (b) age less than 18 years at diagnosis. A family history of cancer was not an inclusion or exclusion criterion. All samples were collected after obtaining informed consent. Clinical information was obtained through the review of medical records or phone calls to parents.

2.2 | Blood and saliva samples

Blood samples were drawn according to best practices in phlebotomy. Saliva samples were collected with Oragene DNA (*DNAgenotek*) following the kit instructions.

2.3 | DNA isolation and QC

Genomic DNA (DNAg) was purified of buffy coats of blood with Puregene Blood Kits (*Qiagen*). Whole saliva was purified with Gentra PureGene Blood kits (*Qiagen*). Tissue was purified with DNeasy Blood & Tissue Kits (*Qiagen*). All followed the manufacturer's protocols.

DNAg was quantified with Qubit dsDNA HS assay (*Life Technologies*) and its integrity was confirmed with agarose gels stained with GelRed (*Biotium*).

2.4 | Whole-exome sequencing and bioinformatic pipeline

Library preparation and subsequent whole-exome sequencing were performed at the Broad's Institute of Massachusetts Institute of Technology (MIT) and Harvard or at Instituto Nacional de Medicina Genomica, INMEGEN, following the previously described protocols (Melendez-Zajgla et al., 2018).

2.5 | Bioinformatics pipeline

The variant calling of germline genetic variants was done using a Genome Analysis Toolkit (GATK) HaplotypeCaller (HC) pipeline (version 4.1.7.0.) according to the GATK Best Practices (Poplin et al., 2018). A total of 572 most prevalent cancer predisposition genes in pediatric patients were selected for variant calling analysis. We merged the list of genes included in the Zhang et al. 2015 study (565 genes) with the genes analyzed in two commercial panels for cancer predisposition in children, Blueprint Genetics (71 genes) and USCF pediatrics panel (198), excluding all repeated genes. The merged list with 572 genes was included in a new BED file for variant calling analysis. After variant recalibration (Variant Quality Score Recalibration, VQSR), all VCF files were annotated with Variant Effect Predictor, VEP online tool (McLaren et al., 2016). All variants with clinical significance were filtered with a standardized tier approach following the recommendations of ACMG-CAP guidelines (Nykamp et al., 2017). In brief, variants were excluded from the analysis if they were: (1) Minor Allele Frequency (MAF) >1% using the genome aggregation (gnomAD) and 1000 genome (AMF population) databases, (2) classified as "low impact," (3) without HGVSc nomenclature, (4) classified as benign in ClinVar, (5) variants with conflicting interpretations of pathogenicity, and (6) all nonpathogenic variants found in more than one unrelated individual. All pathogenic or likely pathogenic variants were manually checked with the aid of the Integrative Genomics Viewer, IGV (v 2.16.0).

2.6 | Validation of germline variants

We confirmed pathogenic or likely pathogenic germline variants in patients with available DNA purified from the tumor tissue and compared both germline and matched tumor samples with variant allele frequencies.

2.7 | Estimation of relatedness and sex determination

We confirmed the sex and kinship status of all cases (mutation carriers) and controls (no mutation carriers) studied by pairing the genome-wide data with the clinical records data (Alexander & Lange, 2016; Schiffels, 2018). We used Sex.DetERRmine v. 1.1.2 to determine the genetic sex of our samples, calculating the relative coverage of X and Y chromosomes and their associated error bars (Lamnidis, 2020b). To assess the genetic kinship among the samples in both our data sets, we used the pairwise mismatch ratio statistics to assign twin/same sample (>87.5% of the median shared single nucleotide polymorphisms [SNPs]), first-degree (>75%), and second-degree (>50%) relationships (Lamnidis, 2020a). We used *samtools mpileup* (parameters –q 30 –Q 30 –B) to generate a pileup file from the merged sequence data of each individual, and used a custom script (pileupCaller ver. 8.6.5) to genotype the individuals, using a pseudo-haploid random draw approach (Schiffels, 2018). For each SNP position on a reference population genetics panel (the so-called 1240K panel), a random read was drawn for each individual and the allele of that read was assumed to be the homozygous genotype of the individual at that position (Mathieson et al., 2015).

2.8 | Inheritance of cancer susceptibility and risk assessment

Information on familial cancer diagnoses and age of onset in first- and second-degree relatives were obtained through a review of medical records or patient interviews for subsequent family tree construction with Progeny's Free Online Pedigree Tool Application (*Progeny's Free Online Pedigree Tool Application*). The genetic predisposition risk assessment for patients was performed using the questionnaire developed by Marjolijn C.J. Jongmans (Jongmans et al., 2016).

2.9 | Population genetics analysis

To validate our results in the context of the genetic demography of present-day mixed-ancestry Mexicans, quality control (QC) and population genetics analyses were carried out. In order to compare our genotypes with available data from the assumed parental populations, our SNPs were merged into approximately 600,000 SNPs of the Human Origins dataset (Patterson et al., 2012). We then used a set of ancestral populations from five continental regions to estimate admixture proportions in our mixed ancestry sample groups. We included Huichol (Raghavan et al., 2015), Mayan, Karitiana (Bergström et al., 2020), and Mixtec (Lazaridis et al., 2014) as part of a genetic pool to assess the Native American contribution to our samples. The African component was estimated by using the genetic data of individuals from Yoruba (Bergström et al., 2020; Patterson et al., 2012), Esan, Mende, and Luo (Lazaridis et al., 2014) groups. Spanish (Biagini et al., 2019; Lazaridis et al., 2014) and French (Bergström et al., 2020; Lazaridis et al., 2014) were used to model the European genetic contribution. The East Asian genetic contribution consisted of Han and Cambodian (Patterson et al., 2012); and the near Oceania was estimated with genotypes from Papuan (Patterson et al., 2012). We then used ADMIXTURE (Alexander & Lange, 2016) and Admixture Plotter (Lamnidis, 2019) to calculate the best *K* for our model (i.e., the one with the lowest cross-validation error) and to calculate the admixture proportions of the components that are maximized in each of the parental populations modeled.

3 | RESULTS

3.1 | Patients

From 2011 to 2016, a cohort of 40 cases with pediatric cancer were included for sequencing analysis. The index cases were grouped in trios (mother and father), duos (mother, father, or two siblings), or only the index case (Figure 1). The genetic relationships of index cases grouped in duos or trios were validated through the pairwise mismatch rates which confirmed the genetic kinship among the participants of the study (Data S1).

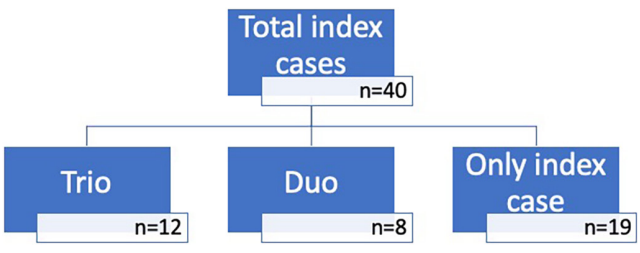


FIGURE 1 Schematic grouping of a childhood cancer patient's cohort that met all inclusion criteria and quality controls.

The mean age of childhood cancer patients at the time of diagnosis was 6.4 ± 4.91 years (mean ± SD).

Our cohort included more patients with solid tumors (75% solid tumors vs. 25% leukemia or lymphoma), with hepatoblastoma ($n = 12$) being the most common tumor type (Table 1), followed by lymphoid leukemias ($n = 9$) and ependymomas ($n = 5$). Only two patients presented with recurrent disease. Additionally, 82.5% of patients received chemotherapy and 37.5% received radiotherapy. The review of medical records confirmed a 77% overall 5-year survival rate.

3.2 | Frequency of germline mutations in childhood cancer patients and their relatives

After preprocessing the data, the QC metrics of the recalibrated BAM files were obtained with the CollectHsMetrics tool (*Picard-tools v 1.110*). A total of 72 BAM files passed the QC metrics. The average of "on target bases" was 174,662,570 bases, of which, the mean fraction of bases achieving ≥10× of depth sequencing was 0.86 and was sufficient for genotyping accuracy (Roy et al., 2018). Three BAM files were excluded from variant calling due to very low-aligned bases (Data S2).

Within the 572 genes used for variant filtering, we identified a total of 91,961 processed and annotated genetic variants in all index cases (a mean of 2312 variants per index, Figure 2a), 56.42% of them were synonymous variants, followed by 39% of missense, 1.41% of frameshift, 1.3% of stop-gained variants and 1.19% of inframe deletions or insertions (Figure 2b). The percentage of retained variants per filter level is shown in Figure 2c.

We identified 9 pathogenic/likely pathogenic germline mutations in cancer predisposition genes in 6/40 index cases (Table 2 and Data S3). Two coexisting mutations were detected in three patients: one of them (Family 2, III-1) carrying both a homozygous autosomal-dominant (*CDKN2A*) and heterozygous autosomal-recessive (*MUTYH*) mutation. Only two patients had a heterozygous mutation in genes associated with autosomal-recessive cancer syndromes, *FANCA* (Family 5, III-1), and *SBDS* (Family 4, III-1).

Additionally, we found that two patients were carriers of a different pathogenic mutation in *ABCA4*, which has been associated with eye diseases such as cone-rod dystrophy, Stargardt macular degeneration, and retinitis pigmentosa. We also identified two candidate nonsense variants in *HRAS* and *FANCL* that have not been reported

TABLE 1 Demographic and clinical characteristics of childhood cancer patients.

n = 40	Frequency	%
Gender		
Female	24	60.0
Male	16	40.0
Age at diagnosis (years)		
Mean (range) ± SD	6.5 (0–17) ±4.91	
Tumor type (International Classification of Childhood Cancer, Third edition)		
***n* = 40**	**Frequency**	**%**
Hepatic tumors	13	32.5
Hepatoblastoma	12	
Hepatic carcinoma	1	
Leukemias, myeloproliferative diseases, and myelodysplastic diseases	9	22.5
Lymphoid leukemias	9	
CNS and miscellaneous intracranial and intraspinal neoplasms	8	20.0
Ependymomas and choroid plexus tumor	5	
Intracranial and intraspinal embryonal tumors	3	
Malignant bone tumors	5	12.5
Osteosarcomas	4	
Other specified intracranial and intraspinal neoplasms (Giant cell tumor of the spine)	1	
Lymphomas and reticuloendothelial neoplasms	1	2.5
Hodgkin lymphomas	1	
Renal tumors	2	5.0
Nephroblastoma and other nonepithelial renal tumors	2	
Soft tissue and other extraosseous sarcomas	1	2.5
Fibrosarcoma, peripheral nerve sheath tumors, and other fibrous neoplasms	1	
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	1	2.5
Pathological background		
***n* = 40**	**Frequency**	**%**
None	26	65.0
Pox	8	20.0
Measles	1	2.5
No data available	4	12.5
Clinical data		
***n* = 40**	**Frequency**	**%**
Status at diagnosis		
First-time diagnosis	38	95.0
Relapse	2	5.0
Previous treatment		
Yes	27	67.5
No	10	25.0
Not confirmed	3	7.5
Familial history of cancer (<45 years)		
Yes	14	35
No	26	65
Metastasis (only solid tumors, *n* = 30)		
Yes	14	46.7
No	12	40.0
Not confirmed	4	10.3
Chemotherapy		
Yes	33	82.5
No	4	10.0
No data	3	7.5
Radiotherapy		
No	21	52.5
Yes	15	37.5
No data	4	10.0
Status		
Surveillance	19	47.5
Death	10	25.0
Data not available	11	27.5

in clinical or population databases, the last one, coexisting with an *ABCA4* pathogenic mutation (Tables S1 and S2).

3.3 | Validation of germline mutations

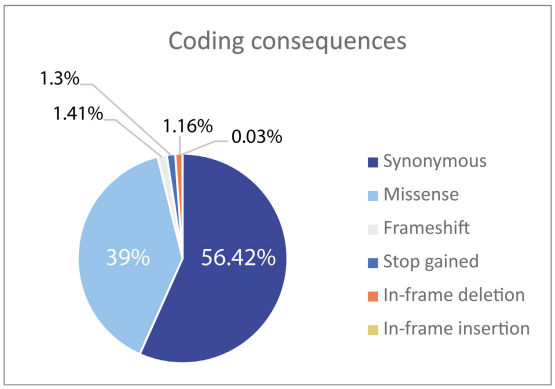
We validated five pathogenic or likely pathogenic germline variants in three patients (Data S3). In individual III-1 of family 2 with osteosarcoma, the c.928C>T (p.Gln310Ter) mutation in *MUTYH* was found to be heterozygous in both tumoral and germline DNA, while the c.146T>C (p.Ile49Thr) in *CDKN2A* was found to be homozygous in the germline DNA.

In individual III-2 of family 3, the double hit of c.5017dup (p.Ile1673fs) mutation in *DICER1* was confirmed through the analysis of the tumor sample. On the

(a)

Annotated variants	Count Mean (Range)±SD
Processed	2312 (2052 - 2608)±111
Novel	15(6-64)±9
Existing	2297 (2037 - 2571)±107

(b)



(c)

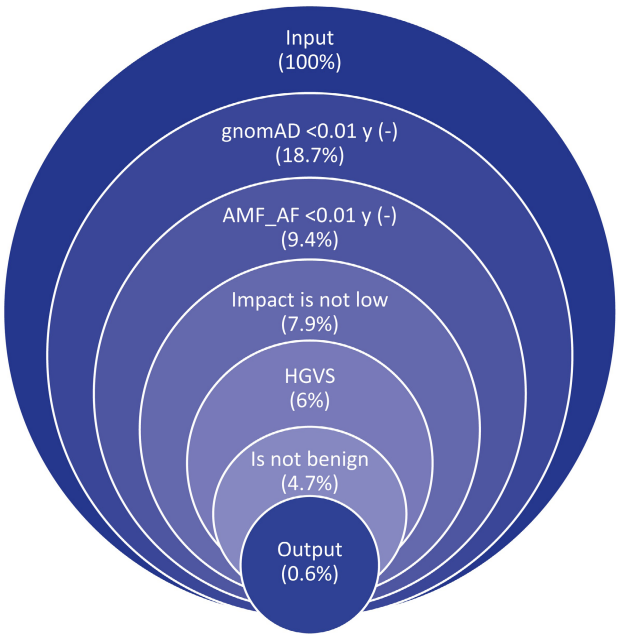


FIGURE 2 Statistics of variants annotated with VEP. (a) Mean of processed, novel, and existing output variants; (b) Types of mutations in coding regions and percentage. (c) Percentage of retained variants in each filter level.

other hand, the additional coexisting c.707T>C (p.Leu-236Pro) mutation in *CHEK2* was confirmed to be heterozygous in the tumor.

Finally, in individual III-1 of family 5, the double hit of the c.2639G>A (p.Arg880Gln) in *FANCA* was not confirmed through the matched tumor sample.

3.4 | Family history of cancer and suspicion criteria for cancer predisposition syndromes

A family history of cancer and the age at diagnosis are two of the main criteria to suspect a cancer predisposition syndrome in adults, in children, the young age of relatives, incomplete information on family history, de novo mutations, and incomplete penetrance may complicate the identification of individuals at high-risk for cancer. In our cohort, 25/40 patients (62.5%) reported a family history of cancer, and 14/25 (56%) had at least one relative with cancer diagnosed before the age of 45 (Table 3). Only one patient with leukemia had congenital anomalies associated with Down syndrome and two patients developed a secondary neoplasm (Family 3, III-2 with ovarian fibrosarcoma/contralateral ovarian granulose cell tumor, and Family 4, III-1 with Hodgkin lymphoma/Ewing sarcoma).

The Marjolijn C.J. Jongmans selection tool was used to assess cancer predisposition risk in the patients included in the study. We confirmed that 30% of all index cases (12/40) met at least one criterion and 7.5% (3/40) met two criteria (Table S3). Six patients carrying a pathogenic or likely pathogenic mutation in cancer predisposition genes

TABLE 2 Childhood cancer patients carrying of germline mutations.

Family	Member	Gender	Diagnosis/age at diagnosis	Family history of cancer	Overall survival	Current state	Gen	Variant	Zygosity	Classification	Inheritance pattern
1	III-1	Female	Spine giant cell tumor, 16-year-old	Osteosarcoma (brother, 14-year-old)	54.5 months	Alive	*NF1* *MSH6*	c.2953C>T (p.Gln985Ter) c.114del (p.Ala40fs)	Heterozygous Heterozygous	Pathogenic Pathogenic	Inherited (*MSH6*)
	III-2	Male	Osteosarcoma, 14-year-old	Spine giant cell tumor (sister, 16-year-old)	1.7 months	Dead	*MSH6*	c.114del (p.Ala40fs)	Heterozygous	Pathogenic	Inherited
2	III-1	Female	Osteosarcoma, 14-year-old	Cervix cancer (maternal aunt and maternal grandmother, unknown)	5.7 months	Dead	*MUTYH* *CDKN2A*	c.928C>T (p.Gln310Ter) c.146T>C (p.Ile49Thr)	Heterozygous Homozygous	Pathogenic Likely-pathogenic	Unknown
3	III-2	Female	Ovarian fibrosarcoma (7-year-old)/ contralateral ovarian granulose cell (11-year-old)	Wilms (brother, 13-year-old) Ovary tumor (Paternal cousin, unknown)	99 months	Alive	*CHEK2* *DICER1*	c.707T>C (p.Leu236Pro) c.5017dup (p.Ile1673fs)	Heterozygous Heterozygous	Likely-pathogenic Pathogenic	Unknown
4	III-1	Male	Ewing sarcoma (5-year-old)/ Non-Hodgkin lymphoma (7-year-old)	Negative	81.6 months	Alive	*SBDS*	c.184A>T (p.Lys62Ter)	Heterozygous	Pathogenic	De novo
5	III-1	Male	Hepatoblastoma, unknown	Cervix cancer (paternal grandmother, unknown)	6.5 months	Unknown	*FANCA*	c.2639G>A (p.Arg880Gln)	Heterozygous	Likely-pathogenic	Unknown

TABLE 3 Family history of cancer.

	Frequency	%
Index case with family history of cancer $n = 25$		
Relatives with cancer before 18 years of age	4	16.0
Relatives with cancer between 18 and 45 years of age	10	40.0
Relatives with cancer after 45 years	7	28.0
>2 first or second-degree relatives of the same family side with cancer before <45 years of age	1	4.0
Any relative with >2 tumors	1	4.0
Diagnosis and age unable to be confirmed	2	8.0

were identified by the tool (Data S4). However, no mutations were identified in the remaining nine patients who met at least one criterion. These results show a promising use of the Marjolijn tool as a screening tool for children with cancer who may benefit from genetic counseling and subsequent genetic testing.

3.5 | Estimation of ancestry admixture proportions

We were able to detect five components in both the cases and the paired controls (Data S5) that were consistent with the demographic models assumed for present-day mixed-ancestry Mexicans (Barquera et al., 2020; Price et al., 2007; Ruiz-Linares et al., 2014; Wang et al., 2007; Zúñiga et al., 2013). The best K for our admixture model was $K = 5$ (lowest CV error = 0.43606). For cases and controls, we detected a component maximized in Native Americans as the most prominent one (74.11% ± 15.72% vs. 68.54% ± 19.73%, respectively) followed by a component maximized in Europeans (22.29% ± 14.65% vs. 27.49% ± 18.50%, respectively). The component maximized in Africans (1.55% ± 2.90% vs. 1.23% ± 2.50%, respectively), East Asians (1.71% ± 2.07% vs. 1.95% ± 1.92%, respectively), and Near Oceanians (0.32% ± 0.69% vs. 0.78% ± 1.86%, respectively), although not that prominently, could also be detected, and especially that maximized in Africans showed a large variation, ranging from 0.00% to 12.85%. The cases and control groups did not differ significantly in any of the five ancestral components found for our model, but it is important to note that power was limited due to the sample size.

4 | DISCUSSION

The current study describes the frequency of germline mutations in cancer-predisposing genes in a cohort of mixed-ancestry Mexican pediatric cancer patients. We analyzed 40 pediatric cancer patients and identified at least one germline mutation in cancer predisposition genes in six patients (15%). Forty variants of uncertain significance in cancer predisposition genes were identified in 32 index cases and 35% of these variants were predicted to be deleterious by two in silico algorithms. Additional characterization and follow-up are needed to confirm the role of these variants in cancer predisposition or to make changes in their clinical classification (Moghadasi et al., 2016; Nykamp et al., 2017).

In our cohort, we observed two mutations that are convincingly associated with cancer predisposition in children. The first, a *DICER1* c.5017dup (p.Ile1673fs) truncation mutation with an accompanying mutation in *CHEK2* c.707T>C (p.Leu236Pro) in the individual III-2 of family 3 with ovarian fibrosarcoma diagnosis. *DICER1* pathogenic variants are associated with the autosomal dominant DICER1 syndrome. *CHEK2* c.707T>C (p.Leu236Pro) has been found to be prevalent in populations of Latin American backgrounds with a maximum subpopulation frequency of 0.25% in gnomAD (*Genome Aggregation Database*). However, an in vivo functional study in yeast suggests a deleterious effect (Delimitsou et al., 2019). Despite its high prevalence in populations of Latin American backgrounds, one study of women from Latin America found that this variant is associated with an increased risk of developing breast cancer in this population, suggesting that this may be a founder variant (Weitzel et al., 2019). In pediatric populations, mutations in *CHEK2* have been found in children affected with acute lymphoblastic leukemia (Douglas et al., 2022) and Wilms tumor (Gadd et al., 2017). This female patient was diagnosed at 9 years old and developed a secondary tumor at 11 years old (Granulosa cell tumor). Her 13-year-old brother was diagnosed with Wilms tumor and a parental cousin was diagnosed with an ovary tumor. The *DICER1* and *CHEK2* mutations were validated in the patient; however, the family did not accept the genetic testing for *DICER1* or *CHEK2*. Additional information on the *CHEK2* c.707T>C (p.Leu236Pro) variant in mixed-ancestry Mexican individuals is necessary to confirm its role in cancer predisposition in children from this genetic background.

The second mutation that is convincingly associated with cancer predisposition in children was found in *NF1* in a 16-year-old female patient (family 1, III-1) diagnosed with a spine giant cell tumor. The patient also harbored the *MSH6* mutation c.114del (p.Ala40fs). Her brother (family 1, III-2) was also diagnosed with osteosarcoma 1 year later, and interestingly, they shared the same mutation in *MSH6* c.114del (p.Ala40fs), but not *NF1* mutation c.2953C>T (p.Gln985Ter). *NF1* mutations have been reported in children with cancer, primarily those with brain and Central Nervous System (CNS) tumors and are less frequent in bone and soft sarcomas, peripheral nerve sheath tumors, leukemias, and myelodysplastic syndrome (Patil & Chamberlain, 2012; Peltonen et al., 2019). Three case reports showed individuals with spinal neurofibromatosis and *NF1* mutations (Carman et al., 2013; Ning et al., 2020), one of them without café-au-lait macules (Kaufmann et al., 2001). Unfortunately, clinical evaluations and tumor sample validation could not be performed because contact with the patient was lost.

Mutations in DNA repair genes have been recurrently observed in children and adolescents with cancer. However, some have also been observed in the general population and their presence in a childhood cancer patient may represent a random noncausal co-occurrence (Kratz et al., 2022). The pathogenic variant c.114del (p.Ala40fs) in *MSH6* identified by both siblings (family 1, III-1 and III-2) has not been observed in the general population so, the implications in cancer predisposition in these patients could be uncertain.

We observed osteosarcoma in a 14-year-old girl (family 2, III-1) with two coexisting mutations confirmed in the tumor: one homozygous in *CDKN2A* and one heterozygous mutation in *MUTYH*; two genes typically associated with adult-onset tumors. Variation in these genes has not been well studied in childhood cancer and these findings highlight the unexpected pleiotropy of even well-characterized cancer predisposition genes. To date, the mutation c.146T>C (p.Ile49Thr) in *CDKN2A* has been reported in 0.44% of Latino control individuals in the gnomAD database; that is approximately 15-fold of the estimated maximal expected allele frequency for a pathogenic variant in *CDKN2A* causing Cutaneous Malignant Melanoma phenotype. This suggests that the variant could be a benign polymorphism found primarily in populations of Latino origin (Puig et al., 2016). Interestingly, none of the carriers reported by gnomAD were homozygous and we cannot exclude a possible additive effect along with a heterozygous *MUTYH* variant.

We found a heterozygous *FANCA* mutation in the individual III-1 of family 5 who had a diagnosis of hepatoblastoma which was further confirmed through the tumor tissue analysis. Biallelic mutations in *FANCA* cause Fanconi anemia and increase cancer risk, mainly acute myeloid leukemia, but other phenotypes have also been observed (Del Valle et al., 2020; Tischkowitz & Hodgson, 2003). Despite the evidence supporting the hypothesis that monoallelic mutations confer an increased risk of cancer for adult carriers, more recent studies have shown that heterozygous variants affecting recessive genes can also increase the risk of predisposition to pediatric cancer, particularly in genes involved in DNA damage recognition and repair where modest reduction in the DNA repair efficiency could lead to tumor development (Savary et al., 2020). In fact, a recent case series study with 30 children with hepatoblastoma, found a 15-month-old girl with a germline heterozygous variant in *FANCA*. The patient presented craniofacial dysmorphisms, nail dysplasia, and developmental delays. The variant was classified as being of uncertain significance (Aguiar et al., 2022). Another study by St. Jude Children's Research Hospital also reported variants of *FANCA* in hepatoblastoma patients (Newman et al., 2021). The authors concluded that further validation in other cohorts can provide insights into the contribution of DNA repair genes in this hepatic tumor.

Another heterozygous variant in an autosomal recessive gene was found in a 5-year-old male patient with Ewing sarcoma, who developed Hodgkin Lymphoma 2 years later. We identified a mutation in *SDBS* (c.184A>T (p.Lys62Ter). Biallelic mutations in *SBDS* have been associated with Shwachman-Diamond syndrome. Patients with this disorder are at an increased risk of developing Acute Myeloblastic Leukemia and Myelodysplastic syndrome (MSD) (Dokal & Vulliamy, 2008). Mutations in *SDBS* have also been identified in refractory cytopenia, neutropenia, and aplastic anemia in a monoallelic context (Calado et al., 2007; Karow et al., 2010; Rother et al., 2021). *SDBS* mutations have not been associated with Ewing Sarcoma. That said, Sharma et al. reported two patients with Schwachman-Diamond syndrome who developed adult-onset lymphoma (Sharma et al., 2014; Verbrugge & Tulchinsky, 2012). The available clinical information for this patient did not report any hematological disease. However, tumor sequencing would be helpful to confirm the double-hit mutation.

We found three additional germline frameshift and nonsense variants in cancer-associated genes in three patients. Although they were not fully characterized, *in silico* mutation prediction suggests that these variants could impact protein structure and function (Table S4). One of them (*FANCL*) coexists with a pathogenic mutation in *ABCD4*. Interestingly, the patient was diagnosed with Acute Lymphoblastic Leukemia at 9 years old and has two relatives also diagnosed with cancer: his great-aunt, diagnosed with leukemia (unknown age at diagnosis), and a 38-year-old cousin whose diagnosis could not be confirmed by the relative.

Two patients were carriers of *ABCD4* pathogenic mutations which are associated with eye diseases. There are two case reports of children with retinoblastoma who were diagnosed with Stargardt disease (one of them, bilateral), showing a probable contribution of this gene to the development of cancer. This requires further exploration (Margalit et al., 2003; Steinmetz et al., 1991). The variant c.2453G>A (p.Gly818Glu) in *ABCD4* is reported to have a higher allele frequency in a mixed-ancestry American population than in other populations (53 of 54 total alleles). One study performed in a cohort of 31 unrelated Mexican subjects with Stargardt disease found that six patients were carriers of the genotype c.2453G>A (p.Gly818Glu). Five of these patients were heterozygous and one was homozygous (Chacón-Camacho et al., 2013). After a medical records review, we confirmed that neither of the two carriers of *ABCD4* mutations presented ophthalmological conditions of Stargardt disease.

Forty variants of uncertain significance in cancer predisposition genes were identified in 32 index cases. Most of them were inherited from the father, mother, or both (Data S6) as opposed to a de novo origin. In silico algorithms predicted 35% of these variants to be deleterious. Additional characterization and follow-up are needed to confirm their role in cancer predisposition (Moghadasi et al., 2016; Nykamp et al., 2017).

Despite our results confirming a strong family history of cancer, only one patient with hepatoblastoma was referred for genetic counseling with clinical suspicion of Beckwith-Wiedemann syndrome. This information is vital for early detection of children with probable predisposition to cancer syndromes. However, in daily clinical practice, underlying syndromes and positive family histories are easily missed (Lu et al., 2014; Oncology, 2003; Wood et al., 2014). The Marjolijn C.J. Jongmans tool can be a very valuable option to help pediatric oncologists identify children with cancer with a high risk of carrying cancer predisposition genetic mutations (Data S4).

A critical aspect of quality control in trio or duo sequencing approaches is to ensure that each sequenced DNA sample originated from the expected individual, to avoid genetic misdiagnosis due to contamination, mislabeling, or sample swapping (Pedersen & Quinlan, 2017). Interestingly, after sex determination and verifying the family relationship, we confirmed second-grade consanguinity in the parents of one index case with *SBDS* mutation. This finding supports the importance of asking and/or confirming family relationships among parents of childhood cancer patients (Bittles, 2001; Jastaniah et al., 2018; Kakaje et al., 2020).

Populations of Latin American genetic backgrounds are poorly characterized since most of the genetic variant characterizations or exploratory studies have been conducted predominantly in populations of European descent (Popejoy & Fullerton, 2016). Our results show that the most prominent component of ancestry admixture proportions is maximized in Native Americans, which can be backed up by the patient's place of birth (mainly Mexico City, State of Mexico, and Puebla). An important notion emerging from this study is that additional efforts are needed to overcome the underrepresentation of Latin American and Indigenous American patients in genetic studies, which further strengthens the disparities of high incidence, recurrence, and lower survival rates reported in Latin American countries such as Mexico.

This study has some limitations. First, not all cancer subtypes were included and our cohort included a greater proportion of hepatoblastoma than other types of pediatric tumors. Second, not all index cases were grouped and the origin of the variants could not be confirmed in all patients. Third, although up to 85% of relevant clinical variants are found in coding regions, increasing evidence and characterization of intronic variants support the importance of performing more comprehensive sequencing. Last, the number of samples was limited, and therefore a clear correlation with ancestry could not be properly assessed.

Although this cohort is not representative of all Mexican pediatric cancer patients, this study represents the first effort to describe the frequencies of predisposing cancer genes in Mexican patients. The expected frequencies were higher than in previous reports (Gröbner et al., 2018; Oberg et al., 2016; Zhang et al., 2016). That said, a sampling bias cannot be disregarded given that most carriers were diagnosed with sarcomas and these tumors have a strong association with hereditary cancer syndromes (Chan et al., 2017; Farid & Ngeow, 2016).

5 | CONCLUSIONS

Germline predisposition gene mutations were found in 15% of mixed-ancestry Mexican children with cancer, but the confirmation of pathogenicity of additional VUS or candidate variants could increase the percentage of individuals with germline mutations.

Population genetic analysis confirmed a population of modern mixed-ancestry Mexicans that have not been included in other reports; its exploration could explain the disparities reported in our country. The Jongmans Marjolijn selection tool could be a good option for the identification of childhood cancer patients with underlying cancer syndromes.

The identification of mutation carriers could lead to an earlier diagnosis, since in Mexico, most childhood cancer diagnoses are made at advanced stages.

Further efforts are needed to assess the impact of genetic variants in populations of ancestries that are currently underresearched in the medical literature and unreported in population databases, including more mixed-ancestry Mexican children with cancer in trio aggrupation to confirm whether variants are new or segregated with a cancer phenotype among family members; additional cancer subtypes adjusting for the distribution reported in Mexico; the recruitment of patients should include individuals from the majority states of the country since Mexico has a large genetic diversity (Silva-Zolezzi et al., 2009); and finally, the evaluation of environmental factors and their interaction with genetic variants.

AUTHOR CONTRIBUTIONS

Oscar Alonso-Luna: Methodology, Software, Validation, Formal Analysis, Investigation, Data curation, Writing—Original draft, Visualization; **Gabriela E. Mercado-Celis**: Conceptualization, Methodology, Validation, Formal Analysis, Data curation, Writing—Reviewing and Editing, Visualization, Supervision, Project administration; **Jorge Melendez-Zajgla**: Conceptualization, Methodology, Validation, Formal Analysis, Software, Data curation, Writing—Reviewing and Editing, Visualization, Supervision; **Rodrigo Barquera**: Methodology, Software, Validation, Formal Analysis, Data curation, Writing—Reviewing and Editing; **Marta Zapata-Tarres**: Investigation, Resources, Data curation; **Luis Enrique Juárez-Villegas:** Investigation, Resources, Data curation; **Elvia Cristina Mendoza-Caamal:** Investigation, Data curation; **Elianeth Rey-Helo:** Investigation, Resources; **Socorro Aida Borges-Yañez**: Validation, Formal Analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information of this article.

ETHICAL STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Division de Estudios de Posgrado e Investigación, School of Dentistry, UNAM (CIE/0103/08/2018).

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REFERENCES

Aguiar, T., Teixeira, A., Scliar, M. O., Sobral de Barros, J., Lemes, R. B., Souza, S., Tolezano, G., Santos, F., Tojal, I., Cypriano, M., Caminada de Toledo, S. R., Valadares, E., Borges Pinto, R., Pinto Artigalas, O. A., Caetano de Aguirre Neto, J., Novak, E., Cristofani, L. M., Miura Sugayama, S. M., Odone, V., … Krepischi, A. (2022). Unraveling the genetic architecture of hepatoblastoma risk: Birth defects and increased burden of germline damaging variants in gastrointestinal/renal cancer predisposition and DNA repair genes. *Frontiers in Genetics*, *13*, 858396. https://doi.org/10.3389/fgene.2022.858396

Alexander, D. H., & Lange, K. (2016). *ADMIXTURE*.

Barquera, R., Hernández-Zaragoza, D. I., Bravo-Acevedo, A., Arrieta-Bolaños, E., Clayton, S., Acuña-Alonzo, V., Martínez-Álvarez, J. C., López-Gil, C., Adalid-Sáinz, C., Vega-Martínez, M. D. R., Escobedo-Ruíz, A., Juárez-Cortés, E. D., Immel, A., Pacheco-Ubaldo, H., González-Medina, L., Lona-Sánchez, A., Lara-Riegos, J., Sánchez-Fernández, M. G. J., Díaz-López, R., … Granados, J. (2020). The immunogenetic diversity of the HLA system in Mexico correlates with underlying population genetic structure. *Human Immunology*, *81*(9), 461–474. https://doi.org/10.1016/j.humimm.2020.06.008

Bergström, A., McCarthy, S. A., Hui, R., Almarri, M. A., Ayub, Q., Danecek, P., Chen, Y., Felkel, S., Hallast, P., Kamm, J., Blanché, H., Deleuze, J. F., Cann, H., Mallick, S., Reich, D., Sandhu, M. S., Skoglund, P., Scally, A., Xue, Y., … Tyler-Smith, C. (2020). Insights into human genetic variation and population history from 929 diverse genomes. *Science*, *367*(6484), eaay5012. https://doi.org/10.1126/science.aay5012

Bhakta, N., Force, L. M., Allemani, C., Atun, R., Bray, F., Coleman, M. P., Steliarova-Foucher, E., Frazier, A. L., Robison, L. L., Rodriguez-Galindo, C., & Fitzmaurice, C. (2019). Childhood cancer burden: A review of global estimates. *The Lancet Oncology*, *20*(1), e42–e53. https://doi.org/10.1016/S1470-2045(18)30761-7

Bhatia, S. (2011). Disparities in cancer outcomes: Lessons learned from children with cancer. *Pediatric Blood & Cancer*, *56*(6), 994–1002. https://doi.org/10.1002/pbc.23078

Biagini, S. A., Solé-Morata, N., Matisoo-Smith, E., Zalloua, P., Comas, D., & Calafell, F. (2019). People from Ibiza: An unexpected isolate in the Western Mediterranean. *European Journal*

of Human Genetics, *27*(6), 941–951. https://doi.org/10.1038/s41431-019-0361-1

Bittles, A. (2001). Consanguinity and its relevance to clinical genetics. *Clinical Genetics*, *60*(2), 89–98. https://doi.org/10.1034/j.1399-0004.2001.600201.x

Calado, R. T., Graf, S. A., Wilkerson, K. L., Kajigaya, S., Ancliff, P. J., Dror, Y., Chanock, S. J., Lansdorp, P. M., & Young, N. S. (2007). Mutations in the SBDS gene in acquired aplastic anemia. *Blood*, *110*(4), 1141–1146. https://doi.org/10.1182/blood-2007-03-080044

Capasso, M., Montella, A., Tirelli, M., Maiorino, T., Cantalupo, S., & Iolascon, A. (2020). Genetic predisposition to solid pediatric cancers. *Frontiers in Oncology*, *10*, 590033. https://doi.org/10.3389/fonc.2020.590033

Carman, K., Yakut, A., Anlar, B., & Ayter, S. (2013). Spinal neurofibromatosis associated with classical neurofibromatosis type 1: Genetic characterisation of an atypical case. *BML Case Reports*, *2013*, bcr2012008468. https://doi.org/10.1136/bcr-2012-008468

Chacón-Camacho, O. F., Granillo-Alvarez, M., Ayala-Ramírez, R., & Zenteno, J. C. (2013). ABCA4 mutational spectrum in Mexican patients with Stargardt disease: Identification of 12 novel mutations and evidence of a founder effect for the common p.A1773V mutation. *Experimental Eye Research*, *109*, 77–82. https://doi.org/10.1016/j.exer.2013.02.006

Chan, S. H., Lim, W. K., Ishak, N. D. B., Li, S. T., Goh, W. L., Tan, G. S., Lim, K. H., Teo, M., Young, C. N. C., Malik, S., Tan, M. H., Teh, J. Y. H., Chin, F. K. C., Kesavan, S., Selvarajan, S., Tan, P., Teh, B. T., Soo, K. C., Farid, M., … Ngeow, J. (2017). Germline mutations in cancer predisposition genes are frequent in sporadic sarcomas. *Scientific Reports*, *7*(1), 10660. https://doi.org/10.1038/s41598-017-10333-x

Colton, M. D., Hawkins, M., Goulding, D., Cockburn, M., & Green, A. L. (2018). Socioeconomics, race, and ethnicity in childhood cancer survival: Accessing and addressing root causes of disparities. *Cancer*, *124*(20), 3975–3978. https://doi.org/10.1002/cncr.31558

Del Valle, J., Rofes, P., Moreno-Cabrera, J. M., López-Dóriga, A., Belhadj, S., Vargas-Parra, G., Teulé, À., Cuesta, R., Muñoz, X., Campos, O., Salinas, M., de Cid, R., Brunet, J., González, S., Capellá, G., Pineda, M., Feliubadaló, L., & Lázaro, C. (2020). Exploring the role of mutations in Fanconi anemia genes in hereditary cancer patients. *Cancers (Basel)*, *12*(4). https://doi.org/10.3390/cancers12040829

Delimitsou, A., Fostira, F., Kalfakakou, D., Apostolou, P., Konstantopoulou, I., Kroupis, C., Papavassiliou, A. G., Kleibl, Z., Stratikos, E., Voutsinas, G. E., & Yannoukakos, D. (2019). Functional characterization of CHEK2 variants in a *Saccharomyces cerevisiae* system. *Human Mutation*, *40*(5), 631–648. https://doi.org/10.1002/humu.23728

Dokal, I., & Vulliamy, T. (2008). Inherited aplastic anaemias/bone marrow failure syndromes. *Blood Reviews*, *22*(3), 141–153. https://doi.org/10.1016/j.blre.2007.11.003

Douglas, S. P. M., Lahtinen, A. K., Koski, J. R., Leimi, L., Keränen, M. A. I., Koskenvuo, M., Heckman, C. A., Jahnukainen, K., Pitkänen, E., Wartiovaara-Kautto, U., & Kilpivaara, O. (2022). Enrichment of cancer-predisposing germline variants in adult and pediatric patients with acute lymphoblastic leukemia. *Scientific Reports*, *12*(1), 10670. https://doi.org/10.1038/s41598-022-14364-x

Farid, M., & Ngeow, J. (2016). Sarcomas associated with genetic cancer predisposition syndromes: A review. *The Oncologist*, *21*(8), 1002–1013. https://doi.org/10.1634/theoncologist.2016-0079

Gadd, S., Huff, V., Walz, A. L., Ooms, A. H. A. G., Armstrong, A. E., Gerhard, D. S., Smith, M. A., Auvil, J. M. G., Meerzaman, D., Chen, Q. R., Hsu, C. H., Yan, C., Nguyen, C., Hu, Y., Hermida, L. C., Davidsen, T., Gesuwan, P., Ma, Y., Zong, Z., … Perlman, E. J. (2017). A Children's Oncology group and TARGET initiative exploring the genetic landscape of Wilms tumor. *Nature Genetics*, *49*(10), 1487–1494. https://doi.org/10.1038/ng.3940

Gröbner, S. N., Worst, B. C., Weischenfeldt, J., Buchhalter, I., Kleinheinz, K., Rudneva, V. A., Johann, P. D., Balasubramanian, G. P., Segura-Wang, M., Brabetz, S., Bender, S., Hutter, B., Sturm, D., Pfaff, E., Hübschmann, D., Zipprich, G., Heinold, M., Eils, J., Lawerenz, C., … Pfister, S. M. (2018). The landscape of genomic alterations across childhood cancers. *Nature*, *555*(7696), 321–327. https://doi.org/10.1038/nature25480

Jastaniah, W., Aljefri, A., Ayas, M., Alharbi, M., Alkhayat, N., Al-Anzi, F., Yassin, F., Alkasim, F., Alharbi, Q., Abdullah, S., Abrar, M. B., & Alsultan, A. (2018). Prevalence of hereditary cancer susceptibility syndromes in children with cancer in a highly consanguineous population. *Cancer Epidemiology*, *55*, 88–95. https://doi.org/10.1016/j.canep.2018.05.006

Jongmans, M. C., Loeffen, J. L., Waanders, E., Hoogerbrugge, P. M., Ligtenberg, M. J., Kuiper, R. P., & Hoogerbrugge, N. (2016). Recognition of genetic predisposition in pediatric cancer patients: An easy-to-use selection tool. *European Journal of Medical Genetics*, *59*(3), 116–125. https://doi.org/10.1016/j.ejmg.2016.01.008

Kakaje, A., Alhalabi, M. M., Ghareeb, A., Karam, B., Mansour, B., Zahra, B., & Hamdan, O. (2020). Interactions of consanguinity and number of siblings with Childhood acute lymphoblastic leukemia. *BioMed Research International*, *2020*, 7919310. https://doi.org/10.1155/2020/7919310

Karow, A., Flotho, C., Schneider, M., Fliegauf, M., Niemeyer, C. M., & European Working Group of Myelodysplastic Syndromes in Childhood. (2010). Mutations of the Shwachman-Bodian-diamond syndrome gene in patients presenting with refractory cytopenia–do we have to screen? *Haematologica*, *95*(4), 689–690. https://doi.org/10.3324/haematol.2009.015008

Kaufmann, D., Müller, R., Bartelt, B., Wolf, M., Kunzi-Rapp, K., Hanemann, C. O., Fahsold, R., Hein, C., Vogel, W., & Assum, G. (2001). Spinal neurofibromatosis without café-au-lait macules in two families with null mutations of the NF1 gene. *American Journal of Human Genetics*, *69*(6), 1395–1400. https://doi.org/10.1086/324648

Kratz, C. P., Smirnov, D., Autry, R., Jäger, N., Waszak, S. M., Großhennig, A., Berutti, R., Wendorff, M., Hainaut, P., Pfister, S. M., Prokisch, H., Ripperger, T., & Malkin, D. (2022). Heterozygous BRCA1 and BRCA2 and mismatch repair gene pathogenic variants in children and adolescents with cancer. *Journal of the National Cancer Institute*, *114*(11), 1523–1532. https://doi.org/10.1093/jnci/djac151

Lamnidis, T. C. (2019). *AdmixturePlotter*.

Lamnidis, T. C. (2020a). *pMMRCalculator*.

Lamnidis, T. C. (2020b). *Sex.DetERRmine*.

Lazaridis, I., Patterson, N., Mittnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P. H., Schraiber, J. G., Castellano, S., Lipson, M., Berger, B., Economou, C., Bollongino, R., Fu, Q.,

Bos, K. I., Nordenfelt, S., Li, H., de Filippo, C., Prüfer, K., ... Krause, J. (2014). Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature*, *513*(7518), 409–413. https://doi.org/10.1038/nature13673

Lu, K. H., Wood, M. E., Daniels, M., Burke, C., Ford, J., Kauff, N. D., Kohlmann, W., Lindor, N. M., Mulvey, T. M., Robinson, L., Rubinstein, W. S., Stoffel, E. M., Snyder, C., Syngal, S., Merrill, J. K., Wollins, D. S., Hughes, K. S., & American Society of Clinical Oncology. (2014). American Society of Clinical Oncology expert statement: Collection and use of a cancer family history for oncology providers. *Journal of Clinical Oncology*, *32*(8), 833–840. https://doi.org/10.1200/JCO.2013.50.9257

Margalit, E., Sunness, J. S., Green, W. R., Kelman, S. E., Schachat, A. P., Fiergang, D., & Allikmets, R. (2003). Stargardt disease in a patient with retinoblastoma. *Archives of Ophthalmology*, *121*(11), 1643–1646. https://doi.org/10.1001/archopht.121.11.1643

Mathieson, I., Lazaridis, I., Rohland, N., Mallick, S., Patterson, N., Roodenberg, S. A., Harney, E., Stewardson, K., Fernandes, D., Novak, M., Sirak, K., Gamba, C., Jones, E. R., Llamas, B., Dryomov, S., Pickrell, J., Arsuaga, J. L., de Castro, J. M., Carbonell, E., ... Reich, D. (2015). Genome-wide patterns of selection in 230 ancient Eurasians. *Nature*, *528*(7583), 499–503. https://doi.org/10.1038/nature16152

McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R. S., Thormann, A., Flicek, P., & Cunningham, F. (2016). The Ensembl variant effect predictor. *Genome Biology*, *17*(1). https://doi.org/10.1186/s13059-016-0974-4

Melendez-Zajgla, J., Mercado-Celis, G. E., Gaytan-Cervantes, J., Torres, A., Gabiño, N. B., Zapata-Tarres, M., Juarez-Villegas, L. E., Lezama, P., Maldonado, V., Ruiz-Monroy, K., & Mendoza-Caamal, E. (2018). Genomics of a pediatric ovarian fibrosarcoma. Association with the DICER1 syndrome. *Scientific Reports*, *8*(1), 3252. https://doi.org/10.1038/s41598-018-21663-9

Moghadasi, S., Eccles, D. M., Devilee, P., Vreeswijk, M. P., & van Asperen, C. J. (2016). Classification and clinical management of variants of uncertain significance in high penetrance cancer predisposition genes. *Human Mutation*, *37*(4), 331–336. https://doi.org/10.1002/humu.22956

Newman, S., Nakitandwe, J., Kesserwan, C. A., Azzato, E. M., Wheeler, D. A., Rusch, M., Shurtleff, S., Hedges, D. J., Hamilton, K. V., Foy, S. G., Edmonson, M. N., Thrasher, A., Bahrami, A., Orr, B. A., Klco, J. M., Gu, J., Harrison, L. W., Wang, L., Clay, M. R., ... Nichols, K. E. (2021). Genomes for kids: The scope of pathogenic mutations in pediatric cancer revealed by comprehensive DNA and RNA sequencing. *Cancer Discovery*, *11*(12), 3008–3027. https://doi.org/10.1158/2159-8290.CD-20-1631

Ning, Z., Yang, Z., Chen, G., Wu, W., He, L., Sun, Y., Cai, D., & Zhang, W. (2020). Spinal neurofibromatosis with NF1 mutation in a classic neurofibromatosis type 1 family: A case report and literature review. *Molecular Genetics & Genomic Medicine*, *8*(1), e1035. https://doi.org/10.1002/mgg3.1035

Nykamp, K., Anderson, M., Powers, M., Garcia, J., Herrera, B., Ho, Y. Y., Kobayashi, Y., Patil, N., Thusberg, J., Westbrook, M., Invitae Clinical Genomics Group, & Topper, S. (2017). Sherloc: A comprehensive refinement of the ACMG-AMP variant classification criteria. *Genetics in Medicine*, *19*(10), 1105–1117. https://doi.org/10.1038/gim.2017.37

Oberg, J. A., Glade Bender, J. L., Sulis, M. L., Pendrick, D., Sireci, A. N., Hsiao, S. J., Turk, A. T., Dela Cruz, F. S., Hibshoosh, H., Remotti, H., Zylber, R. J., Pang, J., Diolaiti, D., Koval, C., Andrews, S. J., Garvin, J. H., Yamashiro, D. J., Chung, W. K., Emerson, S. G., ... Kung, A. L. (2016). Implementation of next generation sequencing into pediatric hematology-oncology practice: Moving beyond actionable alterations. *Genome Medicine*, *8*(1), 133. https://doi.org/10.1186/s13073-016-0389-6

Oncology, A. S. o. C. (2003). American Society of Clinical Oncology policy statement update: Genetic testing for cancer susceptibility. *Journal of Clinical Oncology*, *21*(12), 2397–2406. https://doi.org/10.1200/JCO.2003.03.189

Patil, S., & Chamberlain, R. S. (2012). Neoplasms associated with germline and somatic NF1 gene mutations. *The Oncologist*, *17*(1), 101–116. https://doi.org/10.1634/theoncologist.2010-0181

Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., & Reich, D. (2012). Ancient admixture in human history. *Genetics*, *192*(3), 1065–1093. https://doi.org/10.1534/genetics.112.145037

Pedersen, B. S., & Quinlan, A. R. (2017). Who's who? Detecting and resolving sample anomalies in human DNA sequencing studies with Peddy. *American Journal of Human Genetics*, *100*(3), 406–413. https://doi.org/10.1016/j.ajhg.2017.01.017

Peltonen, S., Kallionpää, R. A., Rantanen, M., Uusitalo, E., Lähteenmäki, P. M., Pöyhönen, M., Pitkäniemi, J., & Peltonen, J. (2019). Pediatric malignancies in neurofibromatosis type 1: A population-based cohort study. *International Journal of Cancer*, *145*(11), 2926–2932. https://doi.org/10.1002/ijc.32187

Popejoy, A. B., & Fullerton, S. M. (2016). Genomics is failing on diversity. *Nature*, *538*(7624), 161–164. https://doi.org/10.1038/538161a

Poplin, R., Ruano-Rubio, V., DePristo, M. A., Fennell, T. J., Carneiro, M. O., Van der Auwera, G. A., Kling, D. E., Gauthier, L. D., Levy-Moonshine, A., Roazen, D., Shakir, K., Thibault, J., Chandran, S., Whelan, C., Lek, M., Gabriel, S., Daly, M. J., Neale, B., MacArthur, D. G., & Banks, E. (2018). Scaling accurate genetic variant discovery to tens of thousands of samples. *bioRxiv*, 201178. https://doi.org/10.1101/201178

Price, A. L., Patterson, N., Yu, F., Cox, D. R., Waliszewska, A., McDonald, G. J., Tandon, A., Schirmer, C., Neubauer, J., Bedoya, G., Duque, C., Villegas, A., Bortolini, M. C., Salzano, F. M., Gallo, C., Mazzotti, G., Tello-Ruiz, M., Riba, L., Aguilar-Salinas, C. A., ... Reich, D. (2007). A genomewide admixture map for Latino populations. *American Journal of Human Genetics*, *80*(6), 1024–1036. https://doi.org/10.1086/518313

Progeny's Free Online Pedigree Tool Application. https://pedigree.progenygenetics.com

Puig, S., Potrony, M., Cuellar, F., Puig-Butille, J. A., Carrera, C., Aguilera, P., Nagore, E., Garcia-Casado, Z., Requena, C., Kumar, R., Landman, G., Soares, C., de Sá, B., Gargantini Rezze, G., Facure, L., de Avila, A. L. R., Achatz, M. I., Carraro, D. M., Duprat Neto, J. P., ... Badenas, C. (2016). Characterization of individuals at high risk of developing melanoma in Latin America: Bases for genetic counseling in melanoma. *Genetics in Medicine*, *18*(7), 727–736. https://doi.org/10.1038/gim.2015.160

Raghavan, M., Steinrücken, M., Harris, K., Schiffels, S., Rasmussen, S., DeGiorgio, M., Albrechtsen, A., Valdiosera, C., Ávila-Arcos, M. C., Malaspinas, A. S., Eriksson, A., Moltke, I., Metspalu, M., Homburger, J. R., Wall, J., Cornejo, O. E., Moreno-Mayar, J. V., Korneliussen, T. S., Pierre, T., ... Willerslev, E. (2015). POPULATION GENETICS. Genomic evidence for the Pleistocene and recent population history of native Americans.

Science, *349*(6250), aab3884. https://doi.org/10.1126/science.aab3884

Rivera-Luna, R., Zapata-Tarres, M., Shalkow-Klincovstein, J., Velasco-Hidalgo, L., Olaya-Vargas, A., Finkelstein-Mizrahi, N., Cárdenas-Cardós, R., & Aguilar-Ortiz, M. R. (2017). The burden of childhood cancer in Mexico: Implications for low- and middle-income countries. *Pediatric Blood & Cancer*, *64*(6). https://doi.org/10.1002/pbc.26366

Rother, C., Gebauer, N., Schneider, J., Bauer, A., Holzhausen, F., Mayer, T., Riecke, A., Müller, M., Merz, H., Steinestel, K., & Witte, H. M. (2021). Autoimmune neutropenia associated with heterozygous variant of *SBDS* gene mimicking Shwachman-Bodian-Diamond syndrome. *Leukemia & Lymphoma*, *62*(12), 3047–3050. https://doi.org/10.1080/10428194.2021.1941941

Roy, S., Coldren, C., Karunamurthy, A., Kip, N. S., Klee, E. W., Lincoln, S. E., Leon, A., Pullambhatla, M., Temple-Smolkin, R. L., Voelkerding, K. V., Wang, C., & Carter, A. B. (2018). Standards and guidelines for validating next-generation sequencing bioinformatics pipelines: A joint recommendation of the Association for Molecular Pathology and the College of American Pathologists. *The Journal of Molecular Diagnostics*, *20*(1), 4–27. https://doi.org/10.1016/j.jmoldx.2017.11.003

Ruiz-Linares, A., Adhikari, K., Acuña-Alonzo, V., Quinto-Sanchez, M., Jaramillo, C., Arias, W., Fuentes, M., Pizarro, M., Everardo, P., de Avila, F., Gómez-Valdés, J., León-Mimila, P., Hunemeier, T., Ramallo, V., Silva de Cerqueira, C. C., Burley, M. W., Konca, E., de Oliveira, M. Z., Veronez, M. R., … Gonzalez-José, R. (2014). Admixture in Latin America: Geographic structure, phenotypic diversity and self-perception of ancestry based on 7,342 individuals. *PLoS Genetics*, *10*(9), e1004572. https://doi.org/10.1371/journal.pgen.1004572

Savary, C., Kim, A., Lespagnol, A., Gandemer, V., Pellier, I., Andrieu, C., Pagès, G., Galibert, M.-D., Blum, Y., & de Tayrac, M. (2020). Depicting the genetic architecture of pediatric cancers through an integrative gene network approach. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-58179-0

Schiffels, S. (2018). *SequenceTools*.

Sharma, A., Sadimin, E., Drachtman, R., & Glod, J. (2014). CNS lymphoma in a patient with Shwachman diamond syndrome. *Pediatric Blood & Cancer*, *61*(3), 564–566. https://doi.org/10.1002/pbc.24743

Silva-Zolezzi, I., Hidalgo-Miranda, A., Estrada-Gil, J., Fernandez-Lopez, J. C., Uribe-Figueroa, L., Contreras, A., Balam-Ortiz, E., del Bosque-Plata, L., Velazquez-Fernandez, D., Lara, C., Goya, R., Hernandez-Lemus, E., Davila, C., Barrientos, E., March, S., & Jimenez-Sanchez, G. (2009). Analysis of genomic diversity in Mexican mestizo populations to develop genomic medicine in Mexico. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(21), 8611–8616. https://doi.org/10.1073/pnas.0903045106

Staler, Z., Maio, A., & Padunan, A. (2020, June 22). Germline mutations prevalence in young adults with cancer. AACR Annual Meeting 2020, Virtual Annual Meeting.

Steinmetz, R. L., Garner, A., Maguire, J. I., & Bird, A. C. (1991). Histopathology of incipient fundus flavimaculatus. *Ophthalmology*, *98*(6), 953–956. https://doi.org/10.1016/s0161-6420(91)32197-3

Steliarova-Foucher, E., Colombet, M., Ries, L. A. G., Moreno, F., Dolya, A., Bray, F., Hesseling, P., Shin, H. Y., Stiller, C. A., & IICC-3 contributors. (2017). International incidence of childhood cancer, 2001–10: A population-based registry study. *The Lancet Oncology*, *18*(6), 719–731. https://doi.org/10.1016/S1470-2045(17)30186-9

Stjernfelt, K. J., von Stedingk, K., Wiebe, T., Hjorth, L., Kristoffersson, U., Stenmark-Askmalm, M., Olsson, H., & Øra, I. (2020). Increased cancer risk in families with pediatric cancer is associated with gender, age, diagnosis, and degree of relation to the child. *Cancer Epidemiology, Biomarkers & Prevention*, *29*(11), 2171–2179. https://doi.org/10.1158/1055-9965.EPI-20-0322

Sweet-Cordero, E. A., & Biegel, J. A. (2019). The genomic landscape of pediatric cancers: Implications for diagnosis and treatment. *Science*, *363*(6432), 1170–1175. https://doi.org/10.1126/science.aaw3535

Tischkowitz, M. D., & Hodgson, S. V. (2003). Fanconi anaemia. *Journal of Medical Genetics*, *40*(1), 1–10. https://doi.org/10.1136/jmg.40.1.1

Verbrugge, J., & Tulchinsky, M. (2012). Lymphoma in a case of Shwachman-diamond syndrome: PET/CT findings. *Clinical Nuclear Medicine*, *37*(1), 74–76. https://doi.org/10.1097/RLU.0b013e3182335f1f

Walsh, M., Wu, G., Edmonson, M., Gruber, T. A., Easton, J., Yergeau, D., Vadodaria, B., Ma, X. T., Chen, X., Mcgee, R., Odom, C., Shurtleff, S. A., Parker, M., Rusch, M., Hedlund, E., Huether, R., Lemmon, G., Nakitandwe, J., Becksfort, J., … Downing, J. R. (2014). Incidence of germline mutations in cancer-predisposition genes in children with hematologic malignancies: A report from the pediatric cancer genome Project. *Blood*, *124*(21), 127. https://doi.org/10.1182/blood.V124.21.127.127

Wang, S., Lewis, C. M., Jakobsson, M., Ramachandran, S., Ray, N., Bedoya, G., Rojas, W., Parra, M. V., Molina, J. A., Gallo, C., Mazzotti, G., Poletti, G., Hill, K., Hurtado, A. M., Labuda, D., Klitz, W., Barrantes, R., Bortolini, M. C., Salzano, F. M., … Ruiz-Linares, A. (2007). Genetic variation and population structure in native Americans. *PLoS Genetics*, *3*(11), e185. https://doi.org/10.1371/journal.pgen.0030185

Weitzel, J. N., Neuhausen, S. L., Adamson, A., Tao, S., Ricker, C., Maoz, A., Rosenblatt, M., Nehoray, B., Sand, S., Steele, L., Unzeitig, G., Feldman, N., Blanco, A. M., Hu, D., Huntsman, S., Castillo, D., Haiman, C., Slavin, T., & Ziv, E. (2019). Pathogenic and likely pathogenic variants in PALB2, CHEK2, and other known breast cancer susceptibility genes among 1054 BRCA-negative Hispanics with breast cancer. *Cancer*, *125*(16), 2829–2836. https://doi.org/10.1002/cncr.32083

Wood, M. E., Kadlubek, P., Pham, T. H., Wollins, D. S., Lu, K. H., Weitzel, J. N., Neuss, M. N., & Hughes, K. S. (2014). Quality of cancer family history and referral for genetic counseling and testing among oncology practices: A pilot test of quality measures as part of the American Society of Clinical Oncology quality Oncology practice initiative. *Journal of Clinical Oncology*, *32*(8), 824–829. https://doi.org/10.1200/JCO.2013.51.4661

Zhang, J., Nichols, K. E., & Downing, J. R. (2016). Germline mutations in predisposition genes in pediatric cancer. *The New England Journal of Medicine*, *374*(14), 1391. https://doi.org/10.1056/NEJMc1600338

Zúñiga, J., Yu, N., Barquera, R., Alosco, S., Ohashi, M., Lebedeva, T., Acuña-Alonzo, V., Yunis, M., Granados-Montiel, J., Cruz-Lagunas, A., Vargas-Alarcón, G., Rodríguez-Reyna, T. S.,

Fernandez-Viña, M., Granados, J., & Yunis, E. J. (2013). HLA class I and class II conserved extended haplotypes and their fragments or blocks in Mexicans: Implications for the study of genetic diversity in admixed populations. *PLoS One*, *8*(9), e74442. https://doi.org/10.1371/journal.pone.0074442

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