RECENT ADVANCES IN PEDIATRIC CANCER PREDISPOSITION SYNDROMES

EDITED BY: Angela Mastronuzzi, Luigi Boccuto and Riccardo Masetti
PUBLISHED IN: Frontiers in Pediatrics and Frontiers in Oncology

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ISSN 1664-8714
ISBN 978-2-88966-714-7
DOI 10.3389/978-2-88966-714-7

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RECENT ADVANCES IN PEDIATRIC CANCER PREDISPOSITION SYNDROMES

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We would like to acknowledge Dr. Giada Del Baldo and Dr. Mariachiara Lodi from IRCCS Bambino Gesù Children's Hospital have acted as coordinator and have contributed to the preparation of the proposal for this Research Topic.

Citation: Mastronuzzi, A., Boccuto, L., Masetti, R., eds. (2021). Recent Advances in Pediatric Cancer Predisposition Syndromes. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88966-714-7

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frontiers in Pediatrics

EDITORIAL
published: 08 March 2021
doi: 10.3389/fped.2021.661894

Editorial: Recent Advances in Pediatric Cancer Predisposition Syndromes

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Keywords: cancer, predisposition, syndromes, pediatric, oncology, children

Editorial on the Research Topic

Recent Advances in Pediatric Cancer Predisposition Syndromes

Cancer predisposition syndromes (CPSs) are an important cause of tumors in pediatric patients. Although a significant number of cancer predisposition genes have already been described, there are many pediatric patients with cancer in whom inherited cancer predisposition syndromes have yet to be detected.

The prevalence of childhood cancer attributable to genetic predisposition is difficult to be estimated but recent reports suggest that at least 10% of pediatric cancer patients harbor a germline mutation in a cancer-predisposition gene.

The advent of large-scale genome sequencing studies has profoundly helped our understanding of the biology of cancer predisposition, leading to better and earlier identification of individuals at high risk of cancer, selection of new molecular targets, and, in some cases, development of tailored approaches.

The Research Topic on "Recent Advances in Pediatric Cancer Predisposition Syndromes" included original contributions and reviews on different aspects of pediatric cancer predisposition syndrome.

Central nervous system tumors are the first cause of solid malignancies in children, and the leading cause of morbidity and mortality in young adults. Cancer predisposition syndromes are seen in children with brain tumors in much higher frequency than other childhood cancers. These syndromes predispose the individual and family members to multiple cancers in different sites. Recent genetic discoveries and careful observation and surveillance resulted in improved survival, reduced morbidity, and targeted therapies for these children. In some contributions of the present topic, the authors discuss clinical manifestations, genetic overview, and management of these complex syndromes in brain cancer.

Ceglie et al. described cancer predisposition syndrome in Pediatric High-Grade Gliomas (pHGG). In the review, the authors summarize the main pHGG-associated cancer predisposing disorders, suggesting indicationsfor suspecting these syndromes and referring for genetic counseling. Better understanding of pHGG-associated syndromes can not only help identify them more quickly and thus provide families with informative genetic counseling but can also lead to a broader knowledge of the tumor-specific genetic landscape and thus of the possible target therapies.

Medulloblastoma is the most frequent malignant brain tumor observed in infancy. Carta et al. presented a detailed overview of CPSs related to medulloblastoma, describing their clinical, epidemiological, genetic, diagnostic, and therapeutic features. Understanding the

OPEN ACCESS

Edited and reviewed by:
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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Pediatrics

Received: *31 January 2021*
Accepted: *12 February 2021*
Published: *08 March 2021*

Citation:
Mastronuzzi A, Boccuto L and Masetti R (2021) Editorial: Recent Advances in Pediatric Cancer Predisposition Syndromes. Front. Pediatr. 9:661894. doi: 10.3389/fped.2021.661894

associations between cancer predisposition syndromes and the different molecular subgroups of medulloblastoma can guide the development of novel targeted therapies, helping to elucidate differences in prognosis and therapeutic vulnerability. This may also help to further improve surveillance measures, to ensure the best quality of care for these patients.

Rhabdoid tumor predisposition syndrome (RTPS) is a rare condition characterized by a high risk of developing rhabdoid tumors such as atypical teratoid rhabdoid tumors (AT/RT), mainly aggressive and multifocal cancers that arise mostly before 1 year of age. RTPS1 is characterized by pathogenic variants in the *SMARCB1* gene, while RTPS2 has variants in *SMARCA4*. Del Baldo et al. provided a wide clinical and genetic description of RTPS types 1 and 2. Moreover, the authors highlighted the importance of early diagnosis of RTPS with references to surveillance proposition, genetic tests, and counseling recommendations to family members. Further research is needed to increase our understanding of rhabdoid tumor biology and the role of *SMARCB1*/*SMARCA4* tumor development.

DICER 1 syndrome (DS) is a cancer-predisposing disorder caused by pathogenic variants in the *DICER1* gene that confer an increased risk to develop a neoplasm in childhood of about 5.3% before 10 years of age. Its pathognomonic feature is the pleuropulmonary blastoma (PPB), but cancer can arise in many other sites. Caroleo et al. provided a review on this interesting topic. According to the authors, screening for DS should always be performed in patients with PPB and should be considered in the presence of other specific benign and malignant lesions. Early identification of DS is essential for planning an adequate follow-up to manage the risk of cancer occurrence in carriers of pathogenic *DICER1* variants.

About rare conditions, Miele et al. examined clinical and genetic features of 13 children affected by pediatric adrenocortical tumors, very rare endocrine neoplasms. They described an excellent prognosis, with a 5-year overall survival of 100% and 5-year disease-free survival of 84.6%. In 75% of patients tested the *TP53* gene was mutated, supporting the indication for genetic testing and family counseling in this disease.

The contribution of Chiang et al. offers an overview of predictive testing for CPSs in pediatric relatives in Asian countries. They conducted a retrospective analysis including families with germline pathogenic/likely pathogenic variants identified in genes associated with pediatric cancer susceptibility and conclude that the rate of predictive testing in pediatric first-degree relatives (FDRs) is higher than that of adults in Asia, albeit below the global average. They hypothesize that factors that may influence the uptake of predictive testing in pediatric FDRs include a lack of information about genetics, preoccupations regarding health insurance, and genetic discrimination.

To note, any cancerous transformation can result from mutations inherited or acquired throughout life. In this scenario, DNA repair mechanisms are crucial to preserve genomic integrity. DNA repair syndromes with a biallelic disorder of essential DNA damage response pathways generally occur early in life by exposing to a high susceptibility to develop hematologic and solid tumors. Sharma et al. described classic biallelic DNA repair cancer syndromes arising from defective single- and double-strand DNA break repair, as well as dysfunctional DNA helicases, providing a historical overview and discussion about complex biology and heterogeneous clinical manifestations.

Concerning vascular tumors in pediatric patients, Hinen et al. described major vascular tumors in the pediatric population with reference to International Society for the Study of Vascular Anomalies (ISSVA) classification guidelines for vascular anomalies (2018). A detailed description of vascular tumors (benign, locally aggressive/borderline, and malignant) and vascular malformations highlighted the importance to recognize high-risk characteristics of each cancer, including anatomic risks, morphology, potential for the co-occurrence of congenital defects, coagulopathy, and malignant evolution.

Capasso et al. provided a very detailed description of genetic variants that predispose to pediatric solid tumors (neuroblastoma, Wilms tumor, retinoblastoma, ependymoma, medulloblastoma, astrocytoma, osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma). They underlined the interactions between germline and somatic alterations as a determinant of cancer development and proposed future research directions focused on this and the importance to develop new molecular diagnostic tests.

As already known, overgrowth syndromes have been linked to an enhanced risk of cancer development and share key molecular pathways involved in cell growth and proliferation with several pediatric cancers. Griff et al. summarized the present data on cancer burden among these conditions and their associated cancer screening guidelines.

Cancer predisposition syndromes remain a challenging issue in pediatric cancer. The rapidly evolving scenario raises numerous biological, clinical, and ethical questions. Continuous efforts should be put into these issues by pediatric oncologists and hematologists in the near future. We believe that advancing knowledge in clinical and research fields would be important to improve the clinical outcome of patients.

AUTHOR CONTRIBUTIONS

All authors wrote and revised the editorial.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Oncology

ORIGINAL RESEARCH
published: 15 October 2020
doi: 10.3389/fonc.2020.554388

Clinical, Genetic, and Prognostic Features of Adrenocortical Tumors in Children: A 10-Year Single-Center Experience

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OPEN ACCESS

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This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Oncology

Received: 21 April 2020
Accepted: 20 August 2020
Published: 15 October 2020

Citation:
Miele E, Di Giannatale A, Crocoli A, Cozza R, Serra A, Castellano A, Cacchione A, Cefalo MG, Alaggio R and De Pasquale MD (2020) Clinical, Genetic, and Prognostic Features of Adrenocortical Tumors in Children: A 10-Year Single-Center Experience. Front. Oncol. 10:554388. doi: 10.3389/fonc.2020.554388

Background and Aims: Pediatric adrenocortical tumors (ACTs) are very rare endocrine neoplasms in childhood. In this study, we performed a retrospective analysis of children with ACT treated at our institution by examining clinical and genetic disease features, treatment strategies, and outcomes.

Methods: We retrospectively analyzed a cohort of 13 children treated at the Bambino Gesù Children's Hospital from November 2010 to March 2020.

Results: The median age at diagnosis was 17 months (range = 0–82 months). The female: male ratio was 3.3/1. Mixed symptomatology (>1 hormone abnormality) was the most common presentation (46.1%). In three cases, the tumor was detected during prenatal or perinatal echographic screening. All patients presented with localized disease at diagnosis and underwent total adrenalectomy. Six patients were identified as having malignancies according to the Wieneke scoring system, five benign, and two undetermined. Seven patients underwent mitotane adjuvant therapy for 12 months. There was metastatic disease in three patients, with no correlation with age or Wieneke score. The most common sites of metastases were the liver and lungs. Metastatic patients were treated with surgery (n = 2), mitotane (n = 1), chemotherapy (n = 2) associated with anti-EGFR (n = 1), or immunotherapy with anti-PD1 (pembrolizumab) (n = 1); two patients achieved complete disease remission. Overall 2- and 5-year survival rates were 100%, with a median follow-up of 5 years (range = 2–9.5 years). Two- and 5-year disease free survival was 76.9 and 84.6%, respectively (95% confidence interval = −66.78–114.76 months). All patients are alive, 12 without disease, and one with stable disease. Genetic analyses showed TP53 germline mutations in six of eight patients analyzed (five inherited, one *de novo*). One patient had Beckwith–Wiedemann syndrome, with mosaic paternal uniparental disomy of chromosome 11, in both neoplastic and healthy adrenal tissue.

Conclusion: We report the cases of 13 patients treated for ACT, including 12 aged <4 years at diagnosis, with a relative short time from symptoms onset. Our cohort experienced an excellent prognosis. TP53 mutation was found in 75% of tested patients (6/8) confirming the need to perform genetic tests and familial counseling in this disease.

Keywords: adrenocortical tumors, children, Li-Fraumeni Syndrome, Beckwith–Wiedeman syndrome, mitotane, immunotherapy, targeted therapies, prognosis

INTRODUCTION

Pediatric adrenocortical tumors (ACTs) include both benign adrenocortical adenomas (ACA) and highly aggressive adrenocortical carcinomas (ACC). They are very rare neoplasms of childhood, with a reported incidence of just 0.2–0.3 new cases per 1 million children per year (1, 2) and accounting for 6% of all adrenal cancers in children (3). ACC incidence rises of 10–15 times the worldwide rate in Southern Brazil, which is likely associated with high prevalence of the founder p.R337H TP53 mutation (4). ACT can occur in the context of several cancer predisposition syndromes; in fact, most childhood ACC are linked to genetic susceptibility, although their pathogenesis is not completely understood (5). Prognosis of pediatric ACT patients is highly heterogeneous and hardly predictable in clinical practice. There is considerable variability in clinical presentation, from tumors with an indolent clinical course to highly malignant tumors with dismal prognosis. Risk factors for poor outcomes in patients with ACT include older age, higher mitotic rate, higher percent of necrosis, and larger tumor size (3). In some cases, a delayed diagnosis may contribute to advanced stages and poor prognosis in these patients (3).

Pediatric ACC patients generally have overall 5-year survival ranging from 30 to 70%, depending on disease presentation (6–8). Despite multimodal therapeutic approaches, outcomes remain poor in patients with metastatic disease, with an estimated 5-year survival <20% (1, 2, 7, 9–11). No effective therapy is currently available for advanced and metastatic ACC; the only treatment leading to cure and long-term survival remains complete surgical resection (6, 7). Adjuvant mitotane, chemotherapy, and/or radiotherapy may reduce recurrence. Arterial chemoembolization, radiotherapy, and radiofrequency ablation are treatment options reported in cases of advanced disease in adulthood (2, 12, 13). However, because many children with ACT carry germline TP53 mutations, radiation therapy in pediatric ACTs has not been studied and should be avoided (6, 14). On the other hand, ACA histology is associated with excellent prognosis, but only about 20% of pediatric ACTs are identified as ACA, and the correct distinction between adenoma and carcinoma is difficult (15). Indeed, there are no well-defined pathological malignancy criteria for pediatric ACT, whereas adult tumors can be classified based on Weiss or Van Slooten criteria (16, 17). The Wieneke criteria, considering tumor size, local invasion, and histological features, are reported useful in discriminating benign from malignant tumors and predicting the prognosis of pediatric ACT (11, 18).

In the present study, we performed a retrospective analysis examining clinical and genetic disease features, treatment strategies, and outcomes in children with ACT in a single institution.

METHODS

We retrospectively reviewed medical records of children affected by ACT and admitted to our hospital between November 2010 and March 2020. All patients included in this study were <18 years old with ACT confirmed by pathological review. The following data were collected: general clinical features (gender, age, clinical symptoms, and signs), imaging, pathological characteristics, and prognosis.

Given our interest in examining genetic factors in this disease, *TP53* mutations analysis was performed on peripheral blood DNA samples from the patients and their parents, by using BigDye direct Sanger sequencing of exons 2–11 and intron–exon boundaries of polymerase chain reaction products by an ABI automated sequencer (Applied Biosystems, Foster City, CA). Gene dosage was evaluated by multiplex ligation-dependent probe amplification (MLPA) using the MRC-Holland SALSA MLPA PO56 TP53 probe set (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's instructions. Chromosome microarray analysis was performed in patients 1 (blood sample) and 9 (blood, saliva, skin fibroblasts, healthy and neoplastic adrenal samples) by using SNP- array (single-nucleotide polymorphism array) on platform CytoSNP-850K BeadChip (Illumina, San Diego, CA) with an average resolution of 100 Kb. Outcomes were reported as alive with no evidence of disease, alive with evidence of disease, and dead of disease. The Wieneke index was applied for diagnosis and prognosis definition.

RESULTS

This retrospective cohort included 13 children. Median age at pathological diagnosis was 17 months (range = 0–82 months). Female-to-male ratio was 3.3/1 (**Table 1**). Mixed symptomatology (>1 hormone abnormality) was the most common presentation (46.1%, $n = 6$), (**Table 1**). In three cases (patients 5, 7, and 13), diagnosis was performed in asymptomatic patients via prenatal (patient 13) or perinatal echographic screening for congenital dysplasia of the hip (patients 5 and 7). All patients presented with localized disease at diagnosis and underwent total adrenalectomy by laparotomy ($n = 12$) or

laparoscopic surgery ($n = 1$, patient 13; **Table 2**). The Wieneke score system was applied for diagnosis and prognosis definition: six patients were assigned to the malignant category, five to the benign category, and two had a diagnosis of tumor with uncertain biological behavior (indeterminate), (**Table 3**).

Seven patients underwent mitotane-based adjuvant therapy for 12 months (**Table 2**). Metastatic disease appeared in three patients after 3, 18, and 42, months, respectively, in one case under treatment and in two during follow-up. No correlation with age or with Wieneke category was observed in metastatic/relapsed patients (the Fisher exact test was not significant). The most common sites of metastases were the liver and lungs. Relapsed and metastatic patients were treated with surgery (2 patients), mitotane (1 patient), chemotherapy (2 patients) associated or not with anti-EGFR (1 patient), or immunotherapy with anti-PD1 (pembrolizumab) (1 patient); two patients achieved complete disease remission (**Figure 1**). Overall 2- and 5-year survival rates were both 100%, with a median follow-up of 5 years (range = 2–9.5 years). Two- and 5-year disease-free survival was 76.9 and 84.6%, respectively (95% confidence interval = −66.78–114.76 months). At present, 12 patients are alive with no evidence of disease, and one is alive with evidence of metastatic disease.

Genetic analyses were conducted for eight patients showing *TP53* germline mutations in six (five inherited and one *de novo*) (**Table 4**). The most part of detected mutations were already recognized as pathogenic. All the carrier parents were asymptomatic, but family history was positive for cancer in four patients (**Table 4**). In two cases, it was strongly suggestive of Li-Fraumeni syndrome (LFS) for the tumor histotypes (e.g., alveolar rhabdomyosarcomas, choroid plexus carcinoma) and the very young age of the affected individuals (**Figure 2**). One patient (patient 9) had Beckwith–Wiedemann syndrome (BWS) with clinical features (macrosomia, hyperinsulinism, hyperglycemia, and tumor) and paternal uniparental disomy of chromosome 11 on neoplastic and healthy adrenal tissue. Another patient (patient 1) showed a copy number variation of uncertain significance, involving a region 1.7 Mb on 8q21.3q22.1, not involving OMIM genes.

TABLE 1 | Clinical features at presentation of 13 pediatric patients with adrenocortical tumors.

Clinical feature	All patients (n = 13)	Age <24 months (n = 8)	Age ≥24 months (n = 5)
Age at onset of symptoms, months			
Median	17	5.5	39
Range	0–82	0–22	24–82
Sex, *n*			
Male	3	2	1
Female	10	6	4
Female:male ratio	3.3:1	3:1	4:1
Type of presentation, *n*			
Virilization only[a]	3 (23.1%)	2	1
Cushing syndrome only	0	0	0
Hypertension only	0	0	0
Mixed tumor	6 (46.1%)	3	3
Asymptomatic	3 (23.1%)	3	0
Unknown[b]	1 (7.7%)	0	1
Duration of symptoms, months			
Median	1	0.5	1
Range	0–10	0–10	1–3

[a]Indicated by clinical and/or laboratory evidence of abnormal production of more than one hormone, [b]The patient was diagnosed at another institution, and the initial medical records were not available.

DISCUSSION

Pediatric ACTs are very rare endocrine tumors in childhood with a highly heterogeneous and challenging prognosis. Recognized independent prognostic factors are older age (3, 10) and metastasis at the time of diagnosis (3), which in some cases could be attributed to delayed diagnosis. Our cohort is characterized by an excellent prognosis on long-term follow-up (media $n = 5$ years). Indeed, at present 12 patients are alive with no evidence of disease, and one is alive with evidence of metastatic disease. The 93% of patients were aged <4 years at diagnosis and with relative short time from symptoms onset (**Table 1**). Three patients were diagnosed in the course of other care, one prenatally and the other two through echographic evaluation for neonatal screening or urinary tract infection.

TABLE 2 | Therapeutic approach.

Surgery/no. of patients	Adjuvant mitotane/no. of patients	Chemotherapy/no. of patients	Immunotherapy/no. of patients	Outcome/no. of patients
LTUA/8	No/6	No/11	No/11	CR/12
LTUA + linfoadenectomy/3	Yes/7	Yes/2*^	Yes/2°	SD/1•
LTUA + bioptic sampling/1				
LUA/1				

*LTUA, laparotomic unilateral adrenalectomy; LUA, laparoscopic unilateral adrenalectomy. *Patient 9 received cisplatin (40 mg/mq) days 1 and 9, doxorubicin (20 mg/mq) days 1 and 8, etoposide (100 mg/mq) days 5–7; ^patient 8 received after relapse vincristine/irinotecan/panitumumab and then gemcitabine/oxaliplatin/panitumumab; °patients 8 and 11 received pembrolizumab; •patient 8.*

TABLE 3 | Pathological features in childhood ACT.

#	Tumor size	Growth pattern	Ki67 (%)	Atypical mitosis	Nuclear pleomorphism	Necrosis	Capsular invasion	Vascular invasion	N+	M+	Other	Wieneke score
1	77 g	Diffuse	2–8	No	No	Yes	No	Yes	No	No	Reticolinic pattern anomalies	Benign
	6 × 5.5 × 4 cm				–							
2	20 g	Diffuse	5	Yes	Yes	No	Yes	No	No	No	p53 + + + nuclear	Intermediate
	2.5 × 2 × 1 cm				++							
3	100 g	Diffuse	30	Yes	Yes	Yes	No	Yes	No	No	p53 + 70%	Malignant
	8 × 6.5 × 5 cm				+ + +							
4	159 g	Solid	10–40	Yes	Yes	Yes	Yes	Yes	No	No	/	Malignant
	9 × 7 × 4.5 cm				+ + +							
5	40 g	Solid	5–10	No	+/–	No	No	Yes	No	No	p53 neg	Benign
	3.2 × 2.5 × 2 cm											
6	50 g	Solid	2	No	Yes	No	No	No	No	No	p53+ nuclear 10%	Benign
	2.5 × 2 × 3.5 cm				+							
7	10 g	Diffuse	20–30	Yes	Yes	Yes	Yes	No	No	No	p53 +	Intermediate
	3.5 × 3 × 1.5 cm				+ + +							
8	49.3 g	Solid	30–40	Yes	Yes	Yes	No	Yes	No	No	p53 +	Malignant
	5.5 × 4.5 × 4 cm				+ + +							
9	50 g	Diffuse	20–30	Yes	Yes	Yes	Yes	Yes	Yes	No	p53 +	Malignant
	6 × 5 × 4 cm				+ + +							
10	33 g	Solid	8	No	No	Yes, focal	No	No	No	No	p53 neg	Benign
	5 × 4 × 2.5 cm											
11*	NA	Solid	High	Yes	Yes	Yes	na	No	na	No	/	Malignant
					+ + +							
12	48 g	Solid	15–20	Yes	Yes	Yes	Yes	Yes	No	No	p53 –/+	Malignant
	5.5 × 4.5 × 4 cm				+ + +							
13	18 g	Diffuse	5–30	No	No	No	No	No	No	No	p53 +/–	Benign
	5 × 3.7 × 2.5 cm											

**Diagnosis formulated in a different center. NA, not available; N+, nodal metastasis; M+, distant metastasis; p53+/–, positivity in <50% but more than 25% of cells; p53–/+, positivity in <25% of cells.*

Routine prenatal ultrasound examinations have increased the detection of fetal tumors; some specific imaging features together with magnetic resonance imaging may help in the differential diagnosis as other common fetal abnormalities can sometimes mimic fetal tumors (26). This is very important for appropriate prenatal management of pregnancy and delivery in order to facilitate prompt postnatal treatment (26). Similarly, ultrasound screening in pediatric population can be used to reveal lesions like tumors or other pathologies of developmental age that are undetectable by clinical examination, before the onset of clinical symptoms (27, 28). This is particularly appropriate for patients with cancer predisposition, for example, in children with BWS (27, 29).

Although adult ACCs are classified following Weiss score, Ki67 > 10% and European Network for the Study of Adrenal Tumors for tumor stage (17), there are no clear pathological malignancy criteria for pediatric patients. Higher mitotic rate, higher percent of necrosis, and larger tumor size are usually associated with aggressive behavior (3). The Wieneke criteria, which include tumor size, local invasion, and histological features, have been reported useful in pediatric ACT malignancy definition and prognosis prediction (11, 18, 30). Recently, Picard and colleagues (31) proposed a pathological scoring system incorporating the Ki67 index ≥15% in a prognostication algorithm to guide adjuvant treatment in pediatric ACTs, mostly for those with incomplete resection. In our cohort, the Wieneke score could not predict clinical outcomes in patients who experienced metastatic disease.

Treatment of pediatric ACTs is often based on the results of adult studies, and the same guidelines are applied (6). When achievable, radical surgery remains the only successful treatment strategy. Capsule rupture with consequent tumor spreading, however, can be a frequent complication due to the tumor friability, mostly during laparoscopic resection. Thus, adrenalectomy in laparotomy is considered the standard of care (6, 32). We were able to perform surgery in all of our patients;

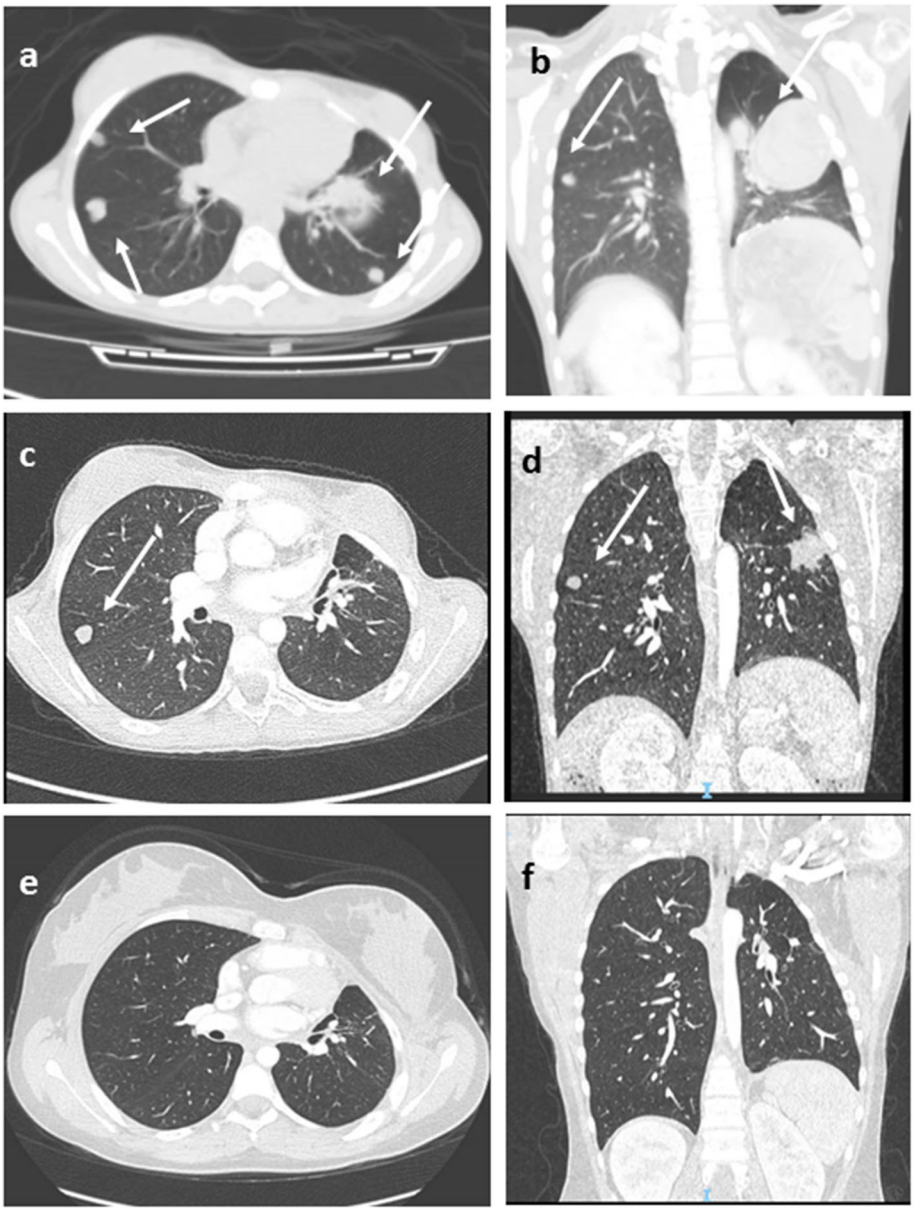

FIGURE 1 | Lung Computed tomography (CT) images (patient #11) showing multiple metastatic lesions (arrows), before **(a,b)**, after 3 months of pembrolizumab therapy **(c,d)**, and at last follow-up **(e,f)** in complete disease remission. Axial **(a,c,e)**, coronal **(b,d,f)**.

open laparotomy was the preferred choice. Laparoscopy was used in one case by the neonatal surgeon for the antenatal diagnosed lesion, given the suspicion of a benign adrenal tumor. Careful follow-up with clinical, radiographic, and endocrine evaluation is mandatory after surgery to detect recurrence and metastasis early.

Adjuvant therapies for ACC have not been successful (6). Both radiation and chemotherapy are poorly effective, and the role of mitotane is not completely clear. Mitotane is a derivative of the insecticide dichlorodiphenyltricholorethane and has been used for treating ACC for more than five decades, also in association with chemotherapy (18, 33). It is the only drug approved for ACC by the US Food and Drug Administration, characterized by low efficacy rate and a narrow therapeutic window, which often involves serious toxicity (34, 35). Current evidence highlighted by a comprehensive review indicates that adjuvant mitotane significantly reduced the recurrence rate and mortality after surgery in nonmetastatic ACC patients (13, 18, 32). In our cohort, mitotane-based adjuvant therapy was administered for 12 months in seven patients with an acceptable tolerability and quality of life.

Despite the known ACC radioresistance, adjuvant radiotherapy of the tumor bed has been proposed and recommended in adult patients with microscopically incomplete

TABLE 4 | Genetic finding: TP53 mutations features in tested patients.

#	Exon	Codon	Nucleotide mutation	Type of mutation	Amino acid change	Germline/Somatic	LFS association (according to ClinVar)	Family history
#2	6	607	G>A	Missense Heterozygosis	pVal203Met	Germline (maternal segregation)	Uncertain significance (19)	Negative
#3	5	538	G>A	Missense Heterozygosis	pGlu180Lys	Germline (maternal segregation)	Likely pathogenic (20)	Breast cancer in maternal grandmother
#4	4	358	A>T	Nonsense Heterozygosis	pLys120*	Germline *DE NOVO*	Pathogenic (21, 22)	Negative
#7	5	455	C>T	Missense Heterozygosis	pPro152Leu	Germline (maternal segregation)	Pathogenic (23)	Brain tumor (NOS) in maternal grandfather (50 years-old)
#8	7	742	C>T	Missense Heterozygosis	p.Arg248Trp	Germline (paternal segregation)	Pathogenic (23, 24)	Alveolar rhabdomyosarcoma in her brother (2 years old)
#13	5	472	G>A	Missense Heterozygosis	pVal143Met	Germline (maternal segregation)	Pathogenic (19, 25)	Choroid plexus carcinoma, neuroblastoma, soft tissue sarcoma and high-grade glioma in the maternal branch (see **Figure 2**)

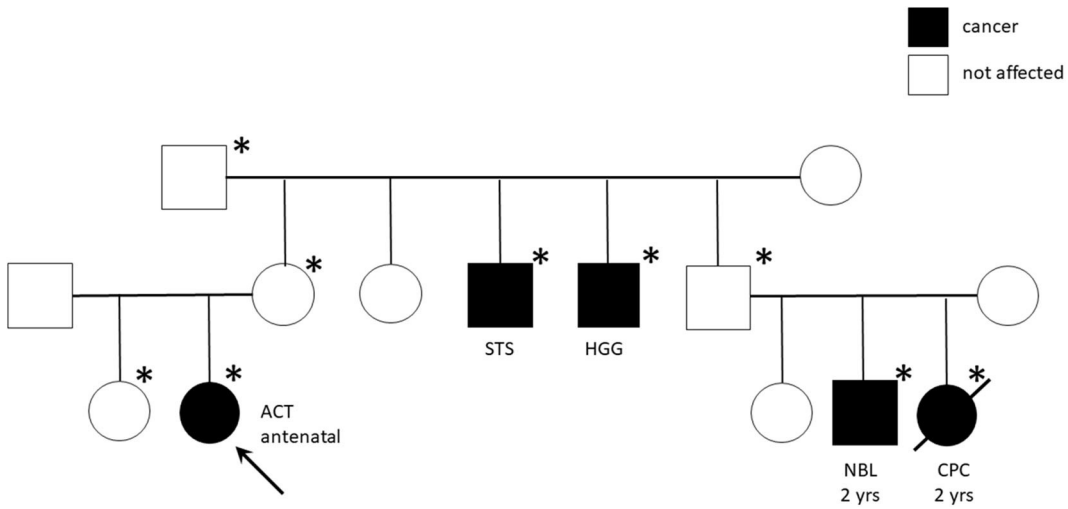

FIGURE 2 | Pedigree chart of Li-Fraumeni syndrome with TP53 mutation c427G>A (pVal143Met). The arrow indicates the proband (Case #13). Squares represent males, circles represent females, and black symbols indicate individuals, *indicates individuals carrying the mutation. ACT, adrenocortical tumor; STS, Soft Tissue Sarcoma; HGG, High Grade Glioma; NBL, Neuroblastoma; CPC, Choroid Plexus Carcinoma; yrs, years.

resection (17, 18, 36). In pediatric population, radiation therapy has not been investigated for the high probability for patients of carrying germline *TP53* mutations and thus should be avoided.

No effective therapy is currently available for advanced and metastatic ACC; the only treatment allowing cure and long-term survival remains complete surgical resection (12, 37). Systemic chemotherapy and mitotane therapy are considered valuable therapeutic options in the treatment of advanced pediatric ACC patients (6, 38–40). Duration of mitotane treatment longer than 6 months and mitotane levels >14 mg/L were found to be associated with significantly better survival (38). The FIRM-ACT trial was conducted to determine whether treatment with etoposide, doxorubicin, cisplatin, and mitotane (EDP/M) prolonged survival as compared to streptozotocin and mitotane (Sz/M) in patients with inoperable advanced ACC. Rates of response and progression-free survival were significantly better with EDP plus mitotane as first-line therapy, with similar rates of toxic events [58%), but no significant differences in OS were observed (12)]. In our experience, one of the three patients who experienced metastatic disease obtained complete remission with platinum-based chemotherapy and mitotane. Overexpression of the IGF2 and IGF1R genes was described in ACT also in the

pediatric setting (41), but trials testing the utility of insulin like growth factor receptor 1 inhibitors (e.g., linsitinib) have failed to provide advantage for adulthood ACC treatment (42).

Immunotherapy approaches have been recently investigated for this disease. In advanced ACC, pembrolizumab showed a significant and durable antitumor activity with a manageable safety profile (43–45). In the recent interim analysis of the phases 1–2 study, KEYNOTE-051 conducted in the pediatric setting, two of four patients with ACC showed partial responses to pembrolizumab therapy (46). In our cohort, two patients were treated by immunotherapy. Patient 8 showed early progressive disease. Patient 11 obtained durable complete remission after 24 months of pembrolizumab therapy (**Figure 1**). She is alive in not-evident disease after 3 years of follow-up.

Most childhood ACCs are reported in the context of cancer predisposition syndromes, in particular the Carney complex (CNC), the BWS, and the LFS. CNC, mostly due to germline inactivating mutations of PRKAR1A, is rarely associated with ACC but is the main cause of primary pigmented nodular adrenal diseases and usually linked to other tumors (somatotroph pituitary adenomas, thyroid, breast, and bone tumors, Sertoli tumors, melanocytic schwannoma, and cardiac and cutaneous myxomas) (5).

BWS is an overgrowth and tumor predisposition syndrome caused by genetic or epigenetic changes at the 11p15 locus. Childhood ACCs, together with embryonal tumors, represent the standard tumor spectrum of BWS (5). In our case studies, one patient was first clinically diagnosed with BWS, due to macrosomia, hyperinsulinism, hypoglycemia, and tumor at 1 month old. Then, the diagnosis of mosaic BWS was genetically confirmed by the evidence of chromosome 11 trisomy on healthy and neoplastic adrenal tissue but not on peripheral lymphocytes. Notably, this patient developed metastatic disease 3 months after surgery, treated by chemotherapy and mitotane, obtaining a complete remission with a 7-year follow-up.

LFS is a dramatic cancer predisposition syndrome, caused by germline inactivating mutations of *TP53* that highly expose to various and precocious cancer risk. Among the most common tumors in LFS are premenopausal breast cancer, soft-tissue sarcoma, osteosarcoma, central nervous system tumors, and ACC, the latter accounting for the 50–80% of pediatric cases. We found *TP53* mutation in 75% of tested patients (6/8) underlining the need to predict carrier and familial disease penetrance with potentially broad implications for clinical surveillance and counseling. Of note, the familial history was positive for cancer in four patients with *TP53* mutation and highly suggestive of LFS in two cases for the tumor histotypes and the very young age of the affected individuals. The most part of detected mutations were indeed already recognized as pathogenic (**Table 3**). In particular, the R248W missense *TP53* mutant that we found in patient 8 has been described to gain novel oncogenic activities (23, 26, 47). Interestingly, Pinto et al. (48) have investigated the clinicopathologic characteristics and outcomes of children with ACT without germline TP53 mutations. They found overlapping features with those reported for children with germline TP53 mutations, highlighting the central role of genetic or epigenetic alterations on chromosome 11p15 in pediatric ACT (48).

CONCLUSION

Our experience with an ACT patient cohort of very young patients (12/13 aged <4 years at diagnosis), with relative short time from symptoms onset and localized disease at diagnosis, suggests an excellent prognosis with appropriate and aggressive diagnosis, staging, and surgical treatment. Our experience confirms age and metastasis as independent prognostic factors and the importance of early diagnosis, supported by already recommended echographic screening in neonates. In our patients, use of the Wieneke index, which is reported to be most accurate in predicting clinical outcomes in younger children, could not predict clinical outcomes.

We were able to treat all patients with surgery. Adjuvant mitotane was offered to 7 of 13 patients for 12 months with acceptable tolerance and no disease recurrence during therapy. In patients who developed metastatic disease, both immunotherapy and chemotherapy led to disease remission or control.

TP53 mutation was found in 75% of tested patients confirming the need to perform genetic tests and familial counseling in this disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

INFORMED CONSENT

The authors declare that written informed consent was obtained from the patients' parents.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bambino Gesù Children's Hospital Ethical Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

EM: conception (ideation), design of the work, structuration, acquisition of the data, writing, revision, and final approval to be published. ADG: structuration and interpretation of the data ACro: surgery acquisition of

the data and revision. RC, AS, ACac, ACas, and MC: patients' management and revision. MDP: patients' management, structuration, and interpretation of the data. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank William Marc Silberg from the Department of Surgery, IRCCS Bambino Gesù Children's Hospital for providing writing support.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Pediatrics

REVIEW
published: 22 October 2020
doi: 10.3389/fped.2020.573023

Childhood Vascular Tumors

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Vascular tumors in pediatric patients are an important entity for the clinician to recognize and correctly diagnose. They may present at birth or develop at any point during infancy, childhood, or adolescence. Most are benign, but even benign lesions may have significant morbidity without proper intervention. Malignant vascular tumors are also rarely seen in the pediatric population, and may be associated with various syndromes.

Keywords: hemangioma, pyogenic granuloma, pediatric vascular tumor, PHACE, angioma

INTRODUCTION

Vascular tumors in pediatric patients are important for the clinician to be able diagnose, classify, and manage. They may present as a congenital lesion or develop at any point throughout infancy and childhood, and often follow a predictable clinical course depending on the type of tumor. The majority of vascular tumors occurring in children are benign, but even benign lesions may be associated with significant morbidity; it is important to be able to recognize the high-risk features associated with each type of tumor. The International Society for the Study of Vascular Anomalies (ISSVA) released updated classification guidelines for vascular anomalies in 2018; these guidelines divide vascular anomalies into vascular tumors (classified as benign, locally aggressive/borderline, and malignant) and vascular malformations (1). Vascular tumors will be further discussed in detail in this article.

DISCUSSION

Infantile Hemangioma

Infantile hemangiomas are the most common benign tumor of childhood with a reported incidence of 4–5% in children <1 year of age (2). A female preponderance has been observed, with a female-to-male ratio of 3:1 to 5:1. This ratio is even higher in PHACES syndrome, with a female-to-male ratio reported up to 7:1 (3). There is also a higher risk associated with prematurity, multiple gestation pregnancy, and in infants born to mothers who underwent chorionic-villus sampling (3, 4). These vascular tumors are comprised of a benign proliferation of endothelial cells. Pathogenesis is unknown and likely multifactorial. The tumor cells stain positive for GLUT-1 protein throughout all stages of growth, which is not found in other vascular tumors. GLUT-1 is expressed in many tissues that serve as blood-tissue barriers including the placenta, brain, and retina (5). The tumors are considered benign, but they may exist in critical locations, and therefore be threatening to form or function. While mostly isolated in occurrence, association with other findings will be discussed.

Infantile hemangiomas follow a predictable clinical course comprised of a proliferative phase, a period of plateau or stability, followed by spontaneous regression. Up to 50% of patient's have a skin lesion present at birth, though this may be subtle clinically (5). They may present as telangiectases or erythematous macules and patches, often with a surrounding zone of pallor. The growth phase

OPEN ACCESS

Edited by:
Angela Mastronuzzi, Bambino Gesù Children Hospital (IRCCS), Italy

Reviewed by:
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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Pediatrics

Received: *15 June 2020*
Accepted: *31 August 2020*
Published: *22 October 2020*

Citation:
Hinen HB, Boccuto L, Trenor CC III and Wine Lee L (2020) Childhood Vascular Tumors. Front. Pediatr. 8:573023. doi: 10.3389/fped.2020.573023

typically starts within the first month of life, with 80% of growth occurring within the first 5 months (6). Growth usually stops around 9–12 months of age. Most of these late-growth hemangiomas were classified as deep or mixed type hemangiomas (6). Growth after 36 months of age is rarely reported and more common in segmental hemangiomas of the head and neck and those involving deep and/or subcutaneous structures (7). Involution usually starts around 12–18 months of age, and can last for several years. Complete involution is predicted to occur at a rate of 10 percent per year, with the majority having completed involution by 5 years of age (5). It is important to note that complete involution does not imply normal skin left at the previous tumor site. Residual scarring, fibrofatty tissue, and telangiectases may persist (5). For this reason, it is important to determine the need for treatment early in the proliferative phase to prevent these sequelae in high risk lesions.

Classification of an infantile hemangioma is based on the pattern (anatomic configuration) or type of lesion (depth) (1). Patterns include focal, multifocal, segmental, and indeterminant. Segmental lesions are determined in embryonic development and may be associated with various syndromes. Focal hemangiomas may be an isolated and innocuous finding, or could be threatening to function or life depending on the location. For example, the nasal tip/bridge, ear, periorbital, and lip are all concerning anatomic locations due to risk of impaired function or disfigurement. Additionally, intertriginous sites and lips are high risk for ulceration (2, 5). Infantile hemangiomas may also be classified by depth. Superficial lesions classically appear as bright red papules or plaques, while deep lesions are blue to violaceous nodules or tumors, sometimes with overlying telangiectases. Mixed lesions also exist, which have both superficial and deep components (**Figure 1**) (1). Infantile hemangiomas, most often segmental IH, may fail to fully proliferate and therefore retain the course telangiectatic appearance of a precursor lesion. These are termed minimal growth hemangioma or IH-MAG (**Figure 2**) (8).

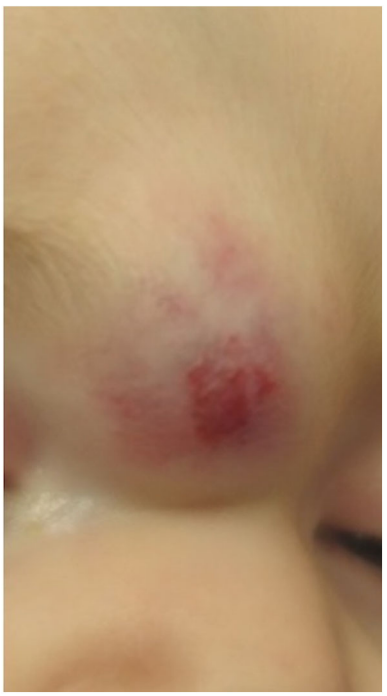

FIGURE 1 | Compound infantile hemangioma of the glabella in a 5 month old infant.

Infantile hemangiomas with a segmental morphology must be given special consideration. Large segmental hemangiomas of the face and neck may be seen in PHACE syndrome, most commonly >22 cm^2 (**Figure 3**) (9). Concomitant congenital anomalies in PHACE syndrome may include posterior fossa malformations, hemangiomas, arterial anomalies, coarctation of the aorta and cardiac defects, eye abnormalities, and sternal clefting or supraumbilical raphe (1, 10). Further evaluation is needed in patients suspected of having PHACE syndrome, including imaging and evaluations by neurology and ophthalmology. Vascular anomalies are the most common of the PHACE associations, and all patients with suspected PHACE should undergo MRI imaging of the cerebral vasculature. Based on these findings, patients should be risked stratified for risk of acute ischemic stroke and appropriate surveillance and intervention considered (11). Endocrinologic dysfunction has also been reported in PHACE syndrome including hypothyroidism, growth hormone deficiency, and pituitary dysfunction (12).

Another anatomic area of concern is the cervicofacial or "beard" distribution. Large or multifocal infantile hemangiomas in this area may be associated with airway involvement that can compromise respiratory function. One retrospective review found that 63 percent of patients with extensive infantile hemangiomas in this distribution had associated symptomatic airway involvement (13). Consideration of further imaging and referral to specialists should be given to these patients. Airway hemangiomas may occur in the absence of cutaneous hemangiomas.

Lumbosacral or extensive lower body segmental infantile hemangiomas are also lesions that may be associated with congenital anomalies (14). Multiple acronyms have been proposed to encompass findings associated with these hemangiomas including LUMBAR, SACRAL, and PELVIC syndromes (3). Extensive lower body hemangiomas with a minimal growth morphology were most commonly associated with LUMBAR syndrome. Associated findings include lower body hemangiomas, urogenital anomalies, ulceration, myelopathy, bony deformities, anorectal malformations, arterial anomalies, renal anomalies (1). Work-up including imaging should be guided by location of the hemangioma (14).

The majority of infantile hemangiomas occur in isolation, however approximately 20% of patients will have more than 1 lesion (4). If a patient has five or more cutaneous infantile hemangiomas involving any site, screening abdominal ultrasound should be performed to rule out the presence of hepatic hemangiomas. Hepatic hemangiomas can occur in three patterns; focal, diffuse, or multifocal. Focal lesions usually represent congenital hemangiomas, while diffuse and multifocal patterns are more classic for infantile hemangiomas (4, 15).

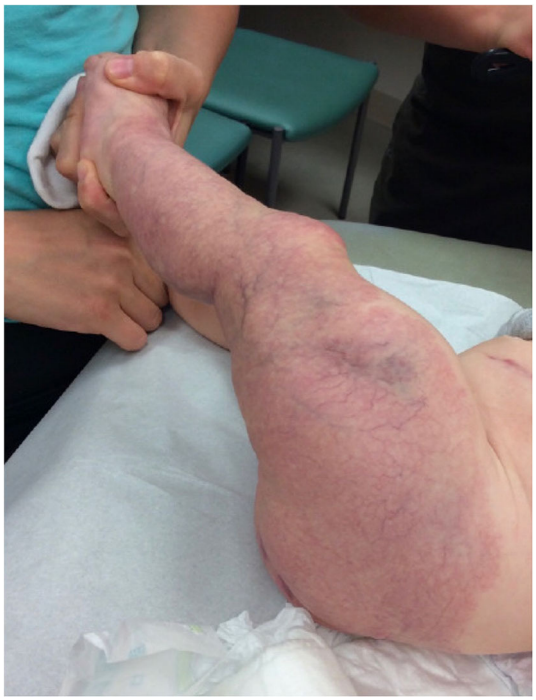

FIGURE 2 | Extensive minimal growth infantile hemangioma (IH-Mag) of the lower extremity in an infant with LUMBAR syndrome.

FIGURE 3 | Facial infantile hemangioma in a 6 week old infant with PHACE syndrome.

Multifocal hepatic infantile hemangiomas are usually asymptomatic, but may be associated with high-output cardiac failure due to vascular shunting. Diffuse hepatic infantile hemangiomas are higher risk, and may cause hepatomegaly resulting in abdominal compartment syndrome (4, 15). Both multifocal and diffuse patterns may also be associated with consumptive hypothyroidism due to the presence of intralesional type 3 iodothyronine deiodinase (16). Patients with symptomatic hepatic hemangiomatosis require prompt treatment with oral propranolol 2–3 mg/kg per day to avoid these life-threatening complications.

Several treatment options exist for infantile hemangiomas, and determination of therapy depends on multiple factors. A prospective study by Haggstrom et al. found that the most important predictors of poor outcomes associated with infantile hemangiomas are large size, segmental morphology, and facial location (17). Presence of ulceration is the most common complication, which may lead to scarring and pain.

The most common treatment for infantile hemangiomas is active observation, given the propensity for these lesions to completely regress. If a lesion is ulcerated or has high-risk features, other therapies should be considered. The first-line treatment for infantile hemangiomas requiring systemic therapy is oral propranolol 2–3 mg/kg/day, which has replaced systemic corticosteroids as the gold standard. If indicated, oral propranolol should be used for lesions throughout the entire proliferative stage. The medication may be initiated in the outpatient setting in infants older than 5–8 weeks corrected gestational age, without comorbid conditions. Heart rate and blood pressure should be monitored for upon initiation and with dose titrations (3). Extensive counseling with parents is required regarding potential side effects of propranolol, and they should be made aware of when to hold doses of the medication if needed. Additionally, doses should be given after a meal to prevent hypoglycemia (3). Hemangiomas falling in a high-risk category should have early referral to a hemangioma specialist for treatment initiation according to the AAP consensus guidelines (2).

Topical timolol and topical or intralesional corticosteroids may also be used as treatment for smaller, focal infantile hemangiomas. For ulcerated lesions, Pulse Dye Laser is a treatment option, though caution must be taken as this may induce ulceration of hemangiomas in the proliferative phase (3). Other therapies that have historically been used to treat infantile hemangiomas include interferon, vincristine, systemic corticosteroids, and cyclophosphamide, however these are now typically only used in rare circumstances for lesions resistant to treatment with propranolol. Systemic sirolimus has recently been successfully used to treat refractory hemangiomas, and is a promising emerging therapy for several vascular tumors (5, 18, 19). Embolization and surgical removal may also be an option, especially for larger, pedunculated lesions that are likely to heal with disfigurement. Finally, lasers are useful therapies both for lesions in the proliferative phase, as well as for treating sequalae in regressed lesions including telangiectases and scarring (5).

Congenital Hemangiomas

Congenital hemangiomas, unlike infantile hemangiomas, present fully formed at birth and may be diagnosed *in utero*. They are much more rare than infantile hemangiomas. There are

three defined types; rapidly involuting congenital hemangiomas (RICH), partially-involuting congenital hemangiomas (PICH), and non-involuting congenital hemangiomas (NICH) (1). RICHs often present as exophytic masses that start to involute shortly after birth, and completely regress by 6–14 months of age (**Figure 4**). They may be associated with a localized consumptive coagulopathy and thrombocytopenia, though less severe than in Kasabach-Merritt Phenomenon, an entity discussed later in this article (3, 20). Residual atrophy and scarring is often found following regression. NICHs are often broad plaques, and less exophytic. They do not involute, and grow proportionately with the patient.

These lesions can be differentiated from infantile hemangiomas by the natural history, histology, and immunophenotype (21). Congenital hemangiomas are GLUT-1 negative, unlike infantile hemangiomas. Histologically they are comprised of lobules of proliferating capillaries that are separated by dense, abnormal fibrotic stroma. The overlying epidermis is atrophic and there is loss of dermal adnexal structures. This is unlike infantile hemangiomas, in which the proliferating lobules of capillaries are separated by normal connective tissue and overlying epidermis is not atrophic in non-regressed lesions (21). Somatic activating mutations in GNAQ and GNA11 have been identified in a subset of congenital hemangiomas (22).

Treatment of congenital hemangiomas depends upon multiple factors including the type, size, and location. Observation is often recommended for initial management, and the clinician may consider imaging or biopsy to confirm diagnosis if it is in question. For large exophytic RICHs, redundant atrophic tissue may persist after involution that may require surgical excision. Congenital hemangiomas may be associated with ulceration and life-threatening hemorrhage and thrombocytopenia. In addition, large congenital hemangiomas, particularly in the liver, may induce a high-output cardiac state. Resection is the only known treatment for complicated congenital hemangiomas. Surgery may also be required for large NICHs. Pulse dye laser can be used to treat superficial telangiectases (3).

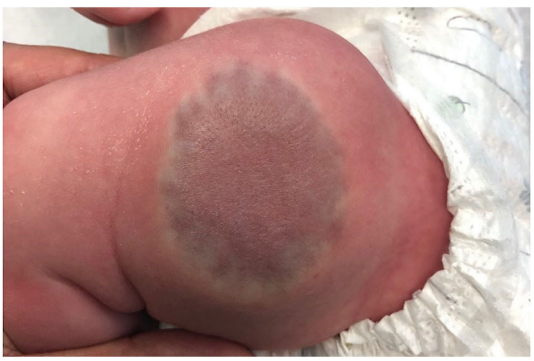

FIGURE 4 | Rapidly involuting congenital hemangioma on the thigh of a 2 month old infant.

Pyogenic Granuloma

Pyogenic granulomas (PGs), also known as lobular capillary hemangiomas, are a common acquired benign vascular tumor. Clinically, these lesions present as red to brown papules that may have a collarette of scale and bleed easily when traumatized (**Figure 5**). They can occur anywhere, but most commonly occur on exposed areas of skin in sites of trauma including the hands, face, and mucous membranes. They are usually solitary, but may be multiple and agminated, as seen in association with pre-existing capillary malformations (**Figure 6**) (23). PGs may occur more frequently during pregnancy or in association with certain medications (24).

The treatment of choice for pyogenic granulomas is most often surgical excision followed by electrodessication or curettage of the base of the lesion to help prevent recurrence. Small lesions can also be treated with the Pulse Dye Laser or combined continuous-wave/pulsed CO_2 laser to help minimize scarring and other adverse effects (25, 26). Topical imiquimod and topical or oral beta-blockers have also been successfully used as a non-invasive treatment option (27).

Tufted Angioma

Tufted angiomas are classified as benign vascular tumors by the ISSVA, but it is pertinent to distinguish them from infantile and congenital hemangiomas, as they may be complicated by Kasabach-Merritt Phenomenon. Tufted angiomas typically appear within the first 5 years of life and may be present at birth, though sporadic cases of acquired tufted angiomas in adult

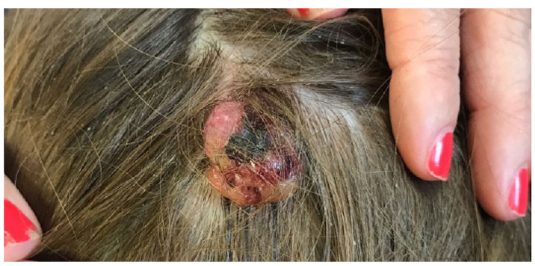

FIGURE 5 | Large pyogenic granuloma on the scalp.

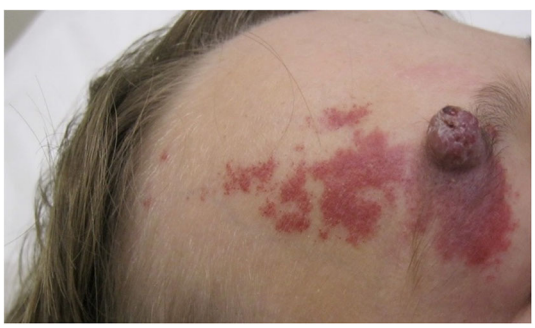

FIGURE 6 | Pyogenic granuloma-like growth arising within a capillary malformation.

patients have been reported (28, 29). They are slow-growing, erythematous to violaceous indurated plaques on the neck or upper trunk, often poorly-demarcated. Some lesions have been reported to have overlying hypertrichosis and hyperhidrosis. Histologically, they demonstrate tufts and lobules of capillaries in a cannonball pattern (28). Some consider tufted angiomas to be on a spectrum with kaposiform hemangioendotheliomas, as they often share similar histologic features and both can be associated with Kasabach-Merritt Phenomenon.

Kaposiform Hemangioendothelioma

Kaposiform hemangioendotheliomas (KHE) are rare tumors that present in infancy or early childhood; they are classified as locally aggressive or borderline vascular tumors (1). KHEs may present as a rapidly expanding firm violaceous plaque in the skin, that often infiltrates deep soft tissue and bone (**Figure 7**). They may occur in the retroperitoneum as well as visceral locations, making diagnosis particularly challenging. Histologically, the lesions demonstrate some features similar to tufted angiomas, though they can be distinguished by the presence of lymphangiomatosis and a sheet-like pattern of growth that may resemble Kaposi's sarcoma (28). Kaposiform hemangioendotheliomas are also larger and less well-defined tumors than tufted angiomas. Prognosis depends on the extent and location of the tumor. Poor prognosis is associated with visceral disease and consumptive thrombocytopenia, known as Kasabach-Merritt Phenomenon (KMP). KMP is associated with ∼70% of KHEs, and has a propensity to occurs in large lesions (>8 cm) that are located in the retroperitoneum or intrathoracic region (30, 31). Treatment of both KHE and tufted angioma is difficult, but management is primarily medical. Successful interventions have included systemic corticosteroids, cyclophosphamide, vincristine, and oral sirolimus. Sirolimus in particular is a promising emerging therapy for the medical management of these tumors. The first reported successful case of refractory KHE treated with sirolimus was in 2010 (32). Several studies published since that time have also showed promising results. A recent retrospective study by Wang et al. showed reduction in tumor size and normalization of platelet counts in 19 of 20 patients with KHE who completed therapy with oral Sirolimus. This study showed no evidence of recurrence after a median follow-up time of 32 months, and average time to response to therapy was 1 week (33). Though medical management predominates in the treatment of tufted angiomas and kaposiform hemangioendotheliomas, if a lesion is localized and well-circumscribed, surgery may be an option. Embolization can also be used to stabilize very large lesions until medical therapy can be initiated (34–36).

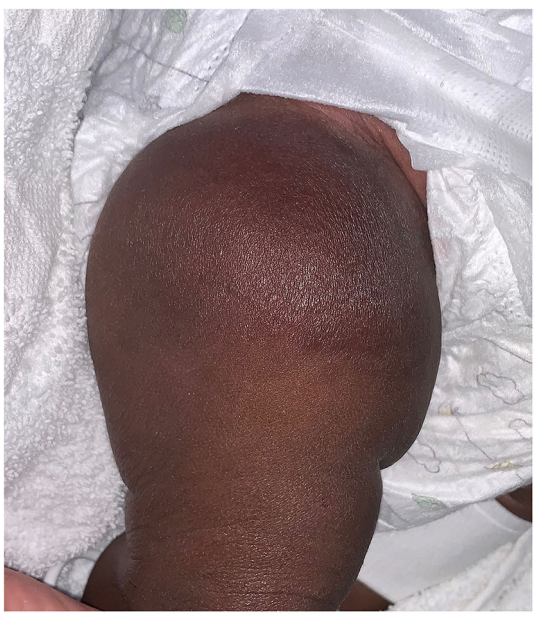

FIGURE 7 | Kaposiform hemangioendothelioma on the thigh of an infant.

Kasabach-Merritt Phenomenon is characterized clinically by a consumptive coagulopathy resulting in thrombocytopenia and hypofibrinogenemia, that can be seen in patients with tufted angiomas and kaposiform hemangioendotheliomas (37). Clinically, it is characterized by rapid enlargement of the vascular tumor with ecchymosis. KMP shows a variable response to treatment; both IV vincristine (combined with antiplatelet therapies) and oral sirolimus have shown promising results, though they have not been compared in a study. The conventional standard treatment was previously systemic corticosteroids which may be used initially, but should not delay the initiation of sirolimus or vincristine if indicated (35, 37). Other therapies for these lesions have included embolization, surgical excision, pulse dye laser, low-dose aspirin, and radiation therapy. Each of these therapies has limitations, and have shown mixed results regarding safety and efficacy (28, 38, 39).

Dabska Tumor

Papillary intralymphatic angioendotheliomas (PILA), also known as Dabska tumors, are rare vascular tumors that are most commonly found in children. They are categorized by the ISSVA as locally aggressive or borderline vascular tumors (1). The Dabska tumor was first described as a low-grade angiosarcoma in 1969 by Maria Dabska, who published a case series of six patients. Three of the six patients had lymph node involvement, and one patient had distant metastasis of the tumor resulting in death (40). Since it was first described, other cases have been reported that have seemingly behaved in a more benign manner (41). Histologically, the tumors are characterized by an intravascular proliferation of hobnail endothelial cells that form characteristic intraluminal papillary projections. They also have evidence of lymphatic vessels either histologically or immunophenotypically. Given the presence of these features histologically, the name Papillary Intralymphatic Angioendothelioma (PILA) was proposed by Fanburg-Smith et al. (41). Clinically, the tumor appears as a slow-growing violaceous to erythematous nodule or plaque, that over time may become more poorly-defined with palpable projections or satellite lesions. There is no predilection for gender or anatomic site (42). Treatment for these lesions is surgical excision with clear margins and close follow-up, given the potential for lymph node involvement and distant metastasis.

Hemangioendothelioma

Epithelioid Hemangioendothelioma

Epithelioid hemangioma (EHE) is a rare malignant vascular tumor that has overlapping of features of both angiosarcoma and epithelioid hemangioma. The tumor most commonly results from a translocation between chromosomes 1 and 3 that creates a pathopneumonic WWTR1-CAMTA1 fusion protein. Less often, it may result from a YAP1-TFE3 fusion (43–45). EHE most often occurs in middle age, however pediatric cases have been reported. Clinically, EHE has a variable presentation and has been reported to affect many different organs. Liver involvement is the most common presenting body site to be involved (21%), followed by both liver and lung involvement (18%), then bone alone (14%), and then lung involvement alone (12%) (43, 44). EHE may also involve the subcutaneous fat, presenting as subcutaneous nodules. Affected patients may have systemic symptoms, such as weight loss, fatigue, and fever, however the malignancy is commonly asymptomatic and often diagnosed by incidental findings on chest imaging (43, 44, 46). Prognosis is variable; poor outcomes are associated with systemic symptoms, metastases at time of diagnosis, and increased mitoses on pathology (43, 46, 47). Management is variable and data is limited given the rarity of the malignancy. Treatment options may include chemotherapeutic agents, immunotherapy, and targeted therapies (43). Surgical resection is an option for localized disease, and watchful waiting may be considered for asymptomatic disease, as spontaneous regression has been reported (46). Liver transplant for patients with hepatic EHE has also been reported as a successful treatment option. Interestingly, the presence of lymph node or extra-heptic involvement did not impact disease free survival in a series of 59 patients with hepatic EHE treated with liver transplant (43, 48).

Pseudomyogenic Hemangioendothelioma

Pseudomyogenic hemangioendothelioma (PHE) is a recently recognized, locally aggressive or borderline vascular tumor (1). The tumor expresses a fusion gene between FOSB and either SERPINE1, ACTB, or WWTR1, which results in an overexpression of FOSB (49). PHE most frequently occurs in young adult males, and often presents as grouped nodules on the lower limb. Histologically, the tumor is composed of sheets and cords of spindled cells with eosinophilic cytoplasm. Despite the resemblance of myoid cells, the tumor cells stain negative for desmin and positive for endothelial markers (45, 49, 50). Treatment of PHE is often determined by the size and location of the tumor; surgical excision is usually the treatment of choice, but given the propensity for PHEs to be multifocal, surgery may not be an option. Additionally, one third of patients have recurrence after surgical excision (51). In such cases, medical management with gemcitabine, sirolimus, and everolimus have been used successfully (50–52). Given the rarity and recent discovery of PHE, clinical trials have not yet been conducted, so further research is needed in medical management treatment options.

Other Hemangioendothelioma

There are several other borderline or locally aggressive vascular tumors that are classified as hemangioendotheliomas. These include the retiform hemangioendothelioma, composite hemangioendothelioma, and polymorphous hemangioendothelioma (1). Each of these tumors has unique histopathologic findings that aids in diagnosis. Most are low-grade neoplasms that have the potential to metastasize, though they rarely do. They vary in aggressiveness, and often recur after excision. Treatment of hemangioendotheliomas is typically handled on a case-by-case basis, and depends on histologic features and clinical aggressiveness (53).

Angiosarcoma

Angiosarcomas are uncommon, highly aggressive vascular tumors that usually present in the skin or soft tissue on the head and neck of elderly patients, but can affect any visceral organ. They are very rarely reported in children, and account for only 0.3% of pediatric sarcomas (54). The diagnosis portends a poor prognosis; angiosarcomas are often aggressive and have a tendency to metastasize (55). Known risk factors for developing angiosarcoma include long-standing lymphedema, prior radiation, and inherited familial syndromes including Neurofibromatosis Type I and Klippel-Trenaunay syndrome (54). Clinically, the tumor can present as an expanding bruise-like lesion, or as an erythematous to violaceous nodule or plaque. Visceral lesions often present as an expanding mass. Treatment of angiosarcoma is challenging, and recurrences are common. Successful therapies have included multi-agent cytotoxic chemotherapy, immunotherapy, tyrosine kinase inhibitors and propranolol, combined with surgical resection and radiation (56).

Vascular Syndromes With Malignancy Risk

Beckwith-Wiedemann syndrome (BWS) is a vascular syndrome associated with other characteristic congenital anomalies, and affected patients have an increased risk of developing various malignancies. BWS results from mutations on chromosome 11p15.5 and may present with hemihyperplasia, centrofacial capillary malformation, macrocephaly, macroglossia, hypoglycemia, and organomegaly (57, 58). Patients with BWS are at increased risk of developing several embryonal malignancies including Wilms tumor, hepatoblastoma, rhabdomyosarcoma, and neuroblastoma. Risk of tumor development in affected patients is ∼5–10 percent, with Wilms tumor being the most frequent tumor observed (58). Nephromegaly is considered to be a strong risk factor for developing Wilms tumor in these patients (59). The vast majority of the tumors in BWS occur intra-abdominally, therefore screening with abdominal ultrasound three to four times a year can be very useful in early detection and treatment of malignancies in these patients (58, 60, 61). Lapunzina et al. also suggests serial screening with physical examination, urinalysis, various serological tests, chest x-ray, and urine VMA, HVA, and catecholamines at varying intervals depending on age. As the majority of tumors are embryonal in origin, most malignancies occur in infancy or early childhood, so screening should be more frequent in younger patients.

CLOVES (congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and skeletal anomalies) syndrome is another vascular syndrome that has an increased

risk of malignancy. Affected patients have an increased risk of Wilms tumor that is reported to be similar to that seen in Beckwith-Wiedemann syndrome and other isolated hemihypertrophy disorders (57). CLOVES syndrome is caused by a postzygotic activating PIK3CA mutation, and is considered by many to be on a spectrum with other disorders characterized by PIK3CA somatic mutations. The PIK3CA-related overgrowth spectrum (PROS) disorders also include macrocephaly-capillary malformation, Klippel-Trenaunay syndrome (KTS), macrodactyly, isolated lymphatic malformation and others (57, 62). Outside of CLOVES syndrome, Wilms tumor has only been reported in 4 other patients with PIK3CA-related overgrowth spectrum (PROS) disorders including two cases seen in macrocephaly-capillary malformation (M-CM) (57, 62–64). Other PROS disorders, including Klippel-Trenaunay syndrome (KTS), have not been shown to be associated with an increased risk of Wilms tumor or other malignancy compared to the general population (57, 65, 66). Given the increased risk in patients with CLOVES syndrome and the benefit of early detection of Wilms tumor, these patients may benefit from screening ultrasounds. Peterman et al. proposes abdominal ultrasounds on a screening schedule similar to that for BWS; every 3 months until 7 years of age, with most tumors expected to be detected before 3 years of age (57).

Other syndromes with increased risk of malignancy include those with mutations in the PTEN tumor suppressor gene. Also known as PTEN hamartoma tumor syndromes (PHTS), these disorders include Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. These disorders are allelic to one another, but have clinically distinct phenotypes (67). Cowden syndrome is usually diagnosed in adolescence or adulthood, and is characterized by pathopneumonic dermatologic findings including trichilimmomas and numerous papillomatous lesions of the skin and mucosa. Patients with Cowden syndrome have a significantly increased risk of several malignancies including breast, endometrial, and thyroid carcinomas (67). Patient's with Bannayan-Riley-Ruvalcaba syndrome are usually diagnosed in childhood. They also have an increased risk of tumors, but unlike Cowden syndrome, most of these tumors are benign and include lipomas, angiolipomas, and hamartomatous GI polyps. Other common clinical findings include penile lentigines and macrocephaly (58, 67). Vascular tumors can be seen in both Cowden Syndrome and Bannayan-Riley-Ruvalcaba syndrome; hemangiomas and arteriovenous malformations have both been reported (67).

CONCLUSIONS

Infantile hemangioma is a common vascular tumor in infants, but not all benign vascular tumors are hemangiomas. Other vascular tumors in children are relatively rare and important to recognize, given difference in natural history, clinical prognosis, and treatment options. Though the vast majority of pediatric vascular tumors are benign and diagnosed clinically, it may be difficult to determine the diagnosis and predict risk of a particular lesion, so further imaging or biopsy for tissue diagnosis may be warranted. Once diagnosed, it is important for the clinician to recognize high risk features of each tumor, including anatomic risks, morphology, potential for co-existing congenital anomalies, coagulopathy, and malignant potential. Treatment of pediatric vascular tumors is often multi-disciplinary and is influenced heavily by individual risks and benefits. The options for medical therapies are actively evolving through genetic discoveries and compassionate use in selected patients.

AUTHOR CONTRIBUTIONS

HH, CT, and LW contributed to the writing of the manuscript. LW and CT supervised the project and provided clinical images. LB contributed to the conceptualization, design, and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Pediatrics

CORRECTION
published: 24 February 2021
doi: 10.3389/fped.2021.649610

Corrigendum: Childhood Vascular Tumors

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Keywords: hemangioma, pyogenic granuloma, pediatric vascular tumor, PHACE, angioma

OPEN ACCESS

Approved by:
Frontiers Editorial Office, Frontiers Media SA, Switzerland

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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Pediatrics

Received: *05 January 2021*
Accepted: *02 February 2021*
Published: *24 February 2021*

Citation:
Hinen HB, Boccuto L, Trenor CC III and Wine Lee L (2021) Corrigendum: Childhood Vascular Tumors. Front. Pediatr. 9:649610. doi: 10.3389/fped.2021.649610

A Corrigendum on

Childhood Vascular Tumors
by Hinen, H. B., Boccuto, L., Trenor, C. C. III., and Wine Lee, L. (2020). Front. Pediatr. 8:573023. doi: 10.3389/fped.2020.573023

Dr. Luigi Boccuto was not included as an author in the published article. The corrected Author Contributions Statement appears below.

HH, CT, and LW contributed to the writing of the manuscript. LW and CT supervised the project and provided clinical images. LB contributed to the conceptualization, design, and editing of the manuscript. All authors contributed to the article and approved the submitted version.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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frontiers in Pediatrics

REVIEW
published: 23 October 2020
doi: 10.3389/fped.2020.570084

DNA Repair Syndromes and Cancer: Insights Into Genetics and Phenotype Patterns

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DNA damage response is essential to human physiology. A broad spectrum of pathologies are displayed by individuals carrying monoallelic or biallelic loss-of-function mutations in DNA damage repair genes. DNA repair syndromes with biallelic disturbance of essential DNA damage response pathways manifest early in life with multi-systemic involvement and a high propensity for hematologic and solid cancers, as well as bone marrow failure. In this review, we describe classic biallelic DNA repair cancer syndromes arising from faulty single- and double-strand DNA break repair, as well as dysfunctional DNA helicases. These clinical entities include xeroderma pigmentosum, constitutional mismatch repair deficiency, ataxia telangiectasia, Nijmegen breakage syndrome, deficiencies of DNA ligase IV, NHEJ/Cernunnos, and ERCC6L2, as well as Bloom, Werner, and Rothmund-Thompson syndromes. To give an in-depth understanding of these disorders, we provide historical overview and discuss the interplay between complex biology and heterogeneous clinical manifestations.

Keywords: DNA repair, cancer predisposition, hematological malignances, hereditary cancer, pediatric cancer

OPEN ACCESS

Edited by:
Riccardo Masetti,
University of Bologna, Italy

Reviewed by:
Jean Soulier,
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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Pediatrics

Received: *06 June 2020*
Accepted: *18 September 2020*
Published: *23 October 2020*

Citation:
Sharma R, Lewis S and Wlodarski MW (2020) DNA Repair Syndromes and Cancer: Insights Into Genetics and Phenotype Patterns. Front. Pediatr. 8:570084. doi: 10.3389/fped.2020.570084

INTRODUCTION

Preservation of genomic DNA is fundamental to maintenance of life. Mammalian DNA can withstand at least 10^5 lesions in a single cell per day caused by intrinsic biological processes and extrinsic genotoxic agents (1). DNA repair mechanisms are highly complex and conserved pathways that have evolved over time. Their role is to restore genomic damage so that naturally occurring DNA lesions are rapidly neutralized and transmission of accurate genetic code across generations can occur (2). In this review, we discuss biological and clinical features of classic DNA repair disorders that predispose to hematologic and solid cancers early in life. Due to intricate genetic underpinnings and heterogeneous clinical manifestations, the diagnosis of these underappreciated syndromes is challenging and typically requires a high index of suspicion. Insight into specific phenotype spectrum and associated cancers can increase awareness of these rare syndromes. As a result, a timely diagnosis and multidisciplinary management with focus on structured surveillance can improve life expectancy in this pediatric population.

Sources of DNA damage are constant, innumerable, and divided into endogenous and exogenous culprits. Endogenous damage is caused by replication errors, as well as reactive intermediates secondary to essential cellular chemical reactions (reactive oxygen species, aldehydes). Exogenous damaging agents include ultraviolet (UV) and ionizing

radiation, environmental chemicals (polycyclic aromatic hydrocarbons, benzo[a]pyrene, aromatic compounds), and chemotherapeutic agents including DNA-alkylators (temozolomide), DNA crosslinkers (mitomycin C or cisplatin), topoisomerase inhibitors (etoposide), and radiomimetics (bleomycin) (2–4). These often unavoidable insults cause toxic DNA intermediates such as single-nucleotide lesions, helical distorting adducts and dimers, single-strand breaks (SSBs), and double-stranded breaks (DSBs), all of which activate the DNA damage response (**Figure 1**) (5).

The DNA damage response is a molecular surveillance system that regulates cell cycle progression at G1-S, intra-S, and G2-M checkpoints to maintain genomic stability (6). Heritable genetic mutations in this safeguard infrastructure results in cancer predisposition syndromes (5). Li-Fraumeni syndrome (LFS) is the prototypical cancer susceptibility disorder characterized by early onset of solid and hematological cancers due to germline monoallelic mutations in p53, a tumor suppressor gene (7) [excellent reviews can be found elsewhere (8)]. LFS highlights the central role of p53 as a bona fide genome guardian, which modulates G1-S and G2-M checkpoints in response to DNA damage pathways (9, 10). At least eight DNA repair mechanisms have been described to orchestrate the repair of mammalian DNA in a cooperative and redundant fashion (2). Importantly, nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), non-homologous end joining (NHEJ), and inter-strand DNA crosslink repair have been associated with Mendelian syndromes with cancer predisposition in children (**Figure 1**, **Table 1**).

Although classic DNA repair syndromes affect pediatric population, their rarity, complex genetics, and heterogeneous phenotypic features make them underrecognized. In the following, we highlight other (non-FA) DNA repair pathway deficiencies and the resulting clinical manifestations in hopes of minimizing missed opportunities for early diagnosis and risk-adapted treatment of aggressive cancers that increase morbidity and mortality in this biologically distinct patient population.

SYNDROMES CAUSED BY FAULTY SINGLE STRAND BREAK REPAIR

SSBs are the most common type of DNA lesion that represent discontinuity in one of the two strands of the DNA helix (11). Single-strand lesions induce replication block and can progress to lethal DSBs if unrepaired in active replicating cells (12) while causing cell death in post-mitotic cells (13, 14). Three repair mechanisms, BER, MMR, and NER, have evolved to mitigate single-strand breaks. BER ameliorates single base damage [detailed review available (15)], which when abrogated can lead to colorectal cancers in adults (16, 17) without evidence to cause childhood cancers. In contrast, both MMR, which resolves base mismatch and insertions–deletions (indels), and NER, which resolves bulky helix distorting lesions, are associated with pediatric cancer predisposition syndromes (**Figure 1**).

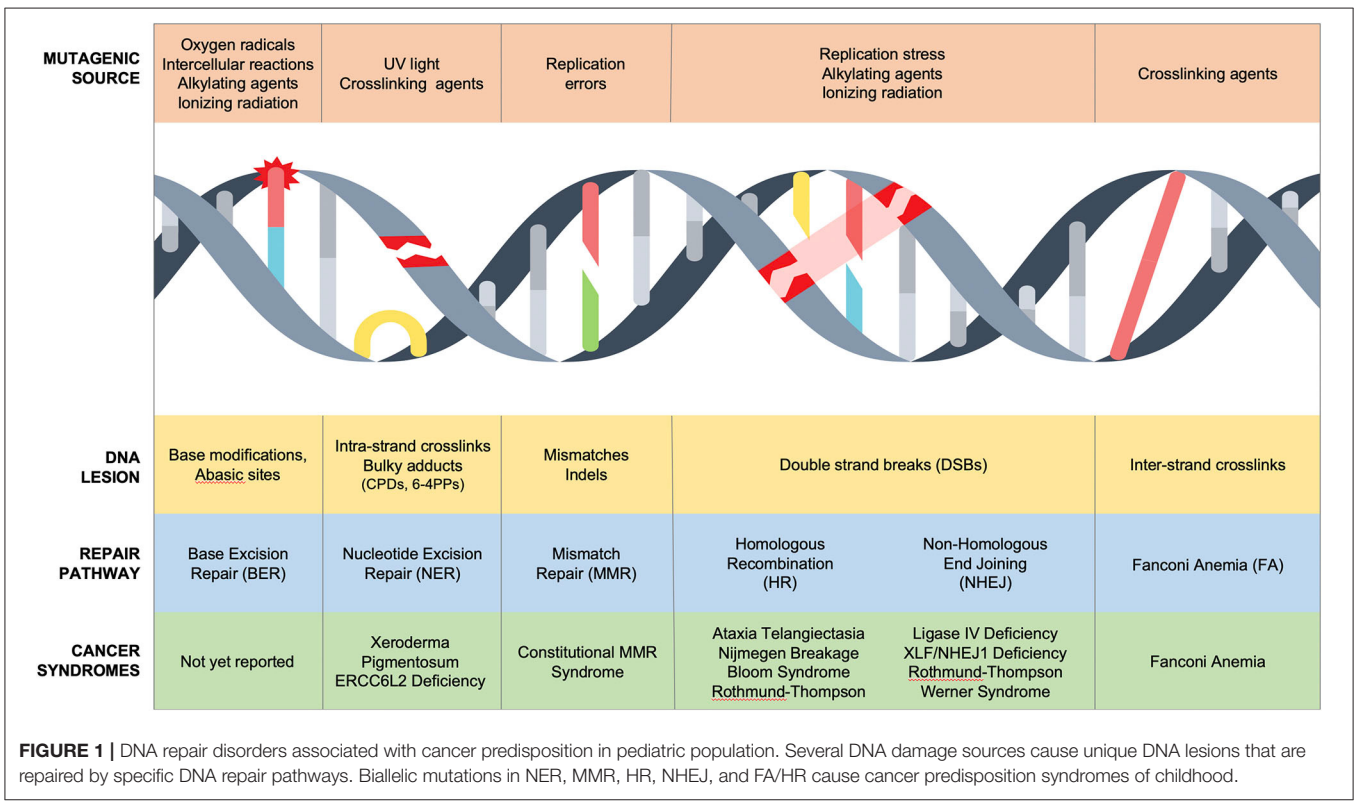

FIGURE 1 | DNA repair disorders associated with cancer predisposition in pediatric population. Several DNA damage sources cause unique DNA lesions that are repaired by specific DNA repair pathways. Biallelic mutations in NER, MMR, HR, NHEJ, and FA/HR cause cancer predisposition syndromes of childhood.

TABLE 1 | DNA repair deficiencies in single strand and double strand DNA repair and RECQ helicases result in classic DNA repair syndromes with multisystemic manifestations and oncogenic predisposition.

DNA repair pathway	Associated syndrome	Expected biallelic mutations	Clinical testing	Clinical features	Malignancy spectrum
SINGLE STRAND BREAK REPAIR DISORDERS					
NER[#]	Xeroderma Pigmentosum	*XPA, XPB, XPC, XPD, XPE, XPF, XPG, XPV*	Screening: UV hypersensitivity Confirmation: genetic testing	Skin Ocular Neurologic	Major: SCC, BCC, melanoma Minor: AML/MDS, brain/spinal cord
	ERCC6L2 deficiency	*ERCC6L2*	Genetic testing	Neurologic Bone marrow failure	MDS, erythroleukemia
MMR	Constitutional mismatch repair disorder	*MLH1*, *MSH2*, *MSH6*, *PMS2*	Screening: IHC, MSI, hypermutation (>100/MB) Confirmation: genetic testing	Skin	Major: brain, GI, T-NHL, ALL, AML Minor: sarcomas, GU
DOUBLE STRAND BREAK REPAIR DISORDERS					
HR	Ataxia telangiectasia	*ATM*	Screening: TREC, AFP, telomere length, t(7;14) Confirmation: Genetic testing	Neurologic Immunologic Endocrine	Major: B-NHL, HL, ALL, breast Minor: gastric, brain
	Nijmegen breakage syndrome	*NBN*	Screening: TREC, AFP, telomere length, t(7;14) Confirmation: Genetic testing	Neurologic Endocrine Immunologic	Major: B-NHL, T-LBL Minor: HL, ALL, AML, brain tumors, sarcoma
NHEJ	DNA Ligase IV Deficiency syndrome	*LIG4*	Screening: TREC Confirmation: Genetic testing	Endocrine Immunologic Bone marrow failure	Major: ALL, B-NHL Minor: AML, MDS
FA	Fanconi anemia	22 FA genes*	Screening: Chromosomal breakage, AFP, telomere length Confirmation: Genetic testing	Congenital anomalies Bone marrow failure Endocrine	Major: SCC (head/neck), AML, MDS Minor: anogenital
RECQ HELICASE DEFICIENT REPAIR DISORDERS					
HR	Bloom syndrome	*BLM*	Screening: SCEs, telomere length Confirmation: Genetic testing	Endocrine Skin Immunologic	Major: AML, ALL, B-NHL, colorectal Minor: breast, SCC, BCC, Wilm's
HR, NHEJ	Werner syndrome	*WRN*	Screening: telomere length Confirmation: Genetic testing	Aging, premature Heart Endocrine	Major: thyroid follicular carcinoma Minor: melanoma, sarcomas, MDS, AML
	Rothmund-thompson syndrome	*RECQL4*	Confirmation: Genetic testing	Skin Ocular	Major: Osteosarcoma, BCC, SCC, melanoma Minor: AML, MDS, lymphoma**
	Rapadilino			Endocrine Skeletal anomalies	Major: lymphoma**, osteosarcoma
	Baller-gerold syndrome			Skeletal anomalies	NK/T cell lymphoma

[#]*Cockayne syndrome and Trichothiodystrophy are important NER deficient syndromes that do not exhibit cancer predisposition risk.*

**Includes following 22 genes: FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ (BRIP1), FANCL, FANCM, FANCN (PALB2), FANCO (RAD51C), FANCP (SLX4), FANCQ (ERCC4), FANCR (RAD51), FANCS (BRCA1), FANCT (UBE2T), FANCU (XRCC2), FANCV (REV7).*

*** = types of lymphomas not reported in literature.*

*NER, nucleotide excision repair; MMR, mismatch repair; HR, homologous recombination; NHEJ, non-homologous end joining; FA, Fanconi anemia; RAPADILINO, (**RA**dial **RA**y defect; **PA**tellae hypoplasia or aplasia and cleft or highly arched **PA**late; **DI**arrhea and **DI**slocated joints; **LI**ttle size and **LI**mb malformation; **NO**se slender and **NO**rmal intelligence) syndrome; XPA-G, xeroderma pigmentosum A-G; XPV, xeroderma pigmentosum V; MLH1, MutL homolog 1; MSH2, MutS homolog. 2; MSH6, MutS homolog 6; PMS2, PMS1 homolog 2; ATM, Ataxia telangiectasia mutated; NBN, Nibrin; LIG4, DNA ligase 4; BLM, Bloom syndrome RecQ like helicase; WRN, Werner syndrome RecQ like helicase; RECQL4, REQ like helicase 4; UV, ultra-violet; IHC, immunohistochemistry; MSI, microsatellite instability; TREC, T cell receptor excision circles; AFP, Alpha-fetoprotein; SCEs, sister chromatid exchanges; SCC, squamous cell carcinoma; BCC, basal cell carcinoma; GI, gastrointestinal; GU, genitourinary; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; LBL, lymphoblastic lymphoma.*

Xeroderma Pigmentosum (XP)

XP, the first DNA repair disorder described in 1874 by Hebra and Kaposi (18), is an autosomal recessive syndrome with dermatological, ocular, and neurological manifestations with skin cancer predisposition (**Table 1**). XP is estimated to affect 1 per million in the United States and 2.3 cases per million in Western Europe (19, 20) with higher prevalence in Japan (21) and North Africa (22). XP patients are unable to repair UV radiation-induced DNA damage due to mutations in the NER pathway. Biallelic mutations in one of the eight XP genes [*XPA-G* and *XP-variant(V)*] of the NER pathway cause classic XP (23). Mutations in *XPA* through *XPG* account for about 80% of XP cases with the remaining attributed to *XPV* (24). Patients commonly present by 2 years of age with increased number of lentigines (freckle-like pigmentation) in sun-exposed areas, a diagnostic skin finding in XP. Extreme sensitivity to sunlight resulting in acute severe sunburns is the presenting feature in 50% of patients. Increased sun exposure and lack of sun protection correlates with development of telangiectasias, pigmented seborrheic warty lesions, and atrophic skin (20, 25). Patients with mutations in *XPA*, *XPB*, *XPD*, *XPF*, and *XPG* have severe photosensitivity at a young age (26). Photophobia is often present with ocular abnormalities limited to UV-exposed areas including eyelids, cornea, and conjunctiva (27). *XPC* patients are specifically hypersensitive to ocular damage with severe keratitis, corneal opacification, and vascularization (24). Approximately one third of patients exhibit progressive neuronal degeneration with *XPA*, *D*, and *G* groups considered to be the most severely affected (28). Clinical presentations can be as subtle as loss of deep tendon reflexes and high-frequency sensorineural hearing to intellectual disability, motor dysfunction (spasticity, ataxia, difficulties swallowing), and frank quadriparesis (25, 26, 29, 30).

XP patients have an estimated 10,000-fold greater risk of developing basal cell and invasive squamous cell carcinomas compared to the general population, with median onset age of <10 years (29). The risk of melanoma has been estimated to be 2,000-fold higher, with median age of onset of 20 years (29). Interestingly, *XPC*, *XPE*, and *XPV* mutations, which are classified as mild XP group due to only minor photosensitivity without neurological abnormalities, show the highest penetrance for cancers (24, 28). This is thought to be due to rapid accumulation of UV damage without sun protection in this patient population who lack overt skin findings resulting in late diagnosis (24). Mucosal cancers of the tongue, myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and tumors of the brain and spinal cord have also been described in XP patients (20, 24, 29, 31–33). Importantly, *TP53* somatic alterations are exceptionally common in XP-associated skin tumors and MDS/AML with high rate of del5q and del7q karyotype alterations in XP-C patients (33, 34). The broad phenotype spectrum seen in XP is a direct consequence of NER deficits at the molecular level. The NER pathway is orchestrated by 30 proteins, and two subbranches, namely, global genomic repair and transcription-coupled repair, recognize and remove UV-induced cyclobutene pyrimidine dimers (CPD) and 6-4 pyrimidine-primidone (6-4PPs) dimers. Global genomic repair relies on XPC and XPE to sense DNA adducts while transcription-coupled repair recognizes damage on the transcribed strand using NER proteins: Cockayne syndrome A and B (CSA, CSB). Both sub-pathways converge to recruit XPD and XPB helicase-containing transcription complex to unwind damaged DNA. This allows XPA to secure single-strand DNA followed by incision of damaged DNA portion by endonucleases XPF/ERCC1 and XPG and gap filling by replication polymerases (35, 36). XPV/POLH is involved in replicating past unrepaired UV-induced thymine dimers or AP sites during translesion synthesis (37, 38). Of note, Cockayne syndrome (39) and Trichothiodystrophy (40) are important NER-deficient syndromes that do not exhibit cancer predisposition risk.

ERCC Excision Repair 6 Like 2 (ERCC6L2) Deficiency

Biallelic loss-of-function mutations in ERCC6 like 2 (*ERCC6L2*) have been associated with BMF, MDS, and acute erythroid leukemia (AML M6). ERCC6L2 is a Snf2 helicase that belongs to SWI/SNF protein family, which makes chromatin accessible to transcription machinery (41). Along with its role in RNA processing, ERCC6L2 plays a role in DNA repair by facilitating cross talk between transcription-coupled NER and NHEJ DNA repair pathways. Specifically, ERCC6L2 repairs transcription-affiliated DNA lesions through its interaction with DNA-PK (42), a central component of the NHEJ DNA repair complex (43). The first report linked homozygous truncating *ERCC6L2* mutations to a bone marrow failure (BMF) syndrome manifesting with neurological and developmental findings in three index cases (9, 12, and 19 years of age) from consanguineous families (44). In another study, 7 patients, with median age of 13 years, were described to have hypocellular marrow in the setting of biallelic *ERCC6L2* mutations, 2 of which displayed dysplastic marrow features with monosomy 7 (45). Of note, only 1 patient from a consanguineous family had neurological and developmental delays. Most recently, biallelic germline mutations were identified in five patients with the unique phenotype of acute erythroleukemia with median age of onset at 49 years. Additionally, all *ERCC6L2*-mutated acute erythroleukemia cases harbored somatic *TP53* mutations at diagnosis (46). It remains to be answered if ERCC6L2 also plays a role in solid tumor predisposition and other types of hematologic malignancies.

Constitutional Mismatch Repair Deficiency (CMMRD)

CMMRD is a recessively inherited, cancer predisposition syndrome, which was described initially in 1999 (47, 48) and affects 1 in 1 million children (49). CMMRD is characterized by childhood onset of broad-spectrum malignancies secondary to biallelic (homozygous or compound heterozygous) germline mutations in the MMR pathway genes, mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH6*), and PMS1 homology 2 (*PMS2*) (50, 51). Parental consanguinity enriching for a founder mutation is observed in over 50% of CMMRD cancers (52, 53). However, in Western countries,

genotypes with compound heterozygous mutations among non-consanguineous families are more common (54). In adults, monoallelic (heterozygous) mutations in these MMR genes are known to cause Lynch syndrome (LS), with predisposition primarily to colorectal, and endometrial cancers (55, 56).

The biological relevance of the MMR pathway is underscored in CMMRD patient tumors, which have a hypermutator phenotype (defined as >10 mutations/Mb), as a result of the inability for MMR machinery to identify and excise DNA damage. Specifically, MSH2–MSH6 heterodimer recognizes base–base mismatch and MSH2–MSH3 heterodimer detects large indel mismatch followed by mismatch excision by MLH1–PMS2 (50). Abrogation of the essential MMR genes leaves behind a trail of incorrect base incorporation and indels, especially in microsatellite regions resulting in increased mutational burden and microsatellite instability, diagnostic hallmarks of CMMRD tumors. Finally, gap filling is accomplished by DNA polymerases *epsilon* (POLE) and *delta* (POLD1), which can acquire somatic mutations during tumorigenesis resulting in "ultra-hypermutated" (>100 mutations/Mb) CMMRD tumors (57, 58). POLE/POLD1 deficiency has been considered as a cancer susceptibility syndrome since mutation carriers with colonic and extra-colonic tumors have been reported (59–62). Importantly, childhood colorectal carcinoma and medulloblastoma in the setting of biallelic POLE mutations have been described (63, 64). Of note, heterozygous germline deletion of *EPCAM*, which causes epigenetic silencing of MSH2, thereby conferring an increased risk of colorectal cancer (65), in addition to biallelic mutation of MSH3, resulting in colorectal cancer (66), has expanded the spectrum of MMR deficient malignancies in humans.

CMMRD patients develop devastating malignancies at an early age with a median onset of 7.5 years (53). The cancer spectrum includes CNS tumors (estimated prevalence of 50%), digestive tract tumors (40%), hematological malignancies (33%), and other solid cancers (67). In a cohort study with 31 patients, the median age at diagnosis of hematologic malignancies, brain tumors, and gastrointestinal cancers was 6.6, 10.3, and 16 years, respectively (54). Commonly encountered brain tumors are high-grade gliomas with few reports of low-grade gliomas, CNS embryonal tumors, and medulloblastoma (49, 68, 69). Prevalent hematological malignancies are non-Hodgkin lymphoma (NHL), particularly T-lymphoblastic NHL followed by T cell-acute lymphoblastic leukemia (T-ALL) and AML (49, 53, 70). The affected MMR gene correlates with the cancer spectrum. MSH6 and/or PMS2 biallelic mutations "favor" brain tumors while MLH1 or MSH2 mutations are biased for development of aggressive hematological malignancies (53, 68). Greater than 40% of PMS2-mutated patients develop secondary neoplasms. However, MLH1/MSH2 patients have a secondary malignancy risk of 22% due to poor survival from the first malignancy (53, 68). Expectedly, colorectal carcinoma, the most prevalent Lynch syndrome associated cancer, has higher prevalence in CMMRD patients with biallelic MSH6 or PMS2 mutations (49, 53). Other solid tumors include osteosarcoma, rhabdomyosarcoma, neuroblastoma, and Wilms tumor (53).

Outside of cancers, certain features are recurrently found in patients with CMMRD. Many patients present with dermatological manifestations such as café-au-lait macules (CALMs), hyper- and hypopigmented skin alterations, venous anomalies, and pilomatricomas (benign hair follicle tumor). At least one CALM or hyperpigmented skin area is found in more than 60% of patients (53). Agenesis of the corpus callosum and mild immunodeficiency with decreased levels of immunoglobulins IgG and IgA were previously described (53). Collectively, oncologic and non-oncologic clinical criteria are used in a three-point scoring system established by the European consortium "Care for CMMRD" (C4CMMRD) for diagnosis of CMMRD (53).

SYNDROMES CAUSED BY FAULTY DOUBLE-STRANDED BREAK REPAIR

DSBs are the most destructive DNA lesions, which, when left unattended, result in cell death. HR and NHEJ are the two main DSB DNA repair pathways that differ in key aspects. HR is a high-fidelity repair pathway that dominates during S and G2 phase to repair DSB damage and relies on the presence of sister chromatids (71). In addition, it regulates essential cellular processes like meiotic recombination (72). On the other hand, an error-prone NHEJ pathway is active throughout the cell cycle (dominating in G1) and directly ligates two broken ends of a DSB. Outside of DNA repair, it is involved in T-cell receptor and immunoglobulin repertoire generation (73). The ability to resolve high-stake DSBs in a time-sensitive manner makes NHEJ a ubiquitous DSB repair pathway (74).

Since its first description by the Swiss pediatrician Guido Fanconi (75), Fanconi Anemia (FA) has been used as the prototypical example of a DSB repair syndrome associated with cancer. FA pathway recognizes and repairs toxic DNA inter-strand crosslinks that induce a replication block followed by formation and repair of DSBs. The inability to resolve these crosslinks results in FA, a cancer predisposition syndrome caused by biallelic mutations in 1 of 22 FA genes (76–81). FA usually manifests early in life with congenital anomalies involving many organ systems, progressive BMF and a very high risk for the development of MDS, AML, head and neck carcinomas, as well as multiple other cancer types. A number of comprehensive studies and reviews on FA and FA-associated cancers have been published elsewhere (82–85).

We will review defects in the DNA repair machinery proteins of the HR system (ATM, NBN) and the NHEJ pathway (LIG4, NHEJ1, Artemis) that result in rare cancer predisposition disorders that exhibit radiosensitivity with overlapping clinical features including neurological deficits, cellular immunodeficiency with reduction or loss of T- and B-cells, hypogammaglobulinemia, and lymphoid cancers.

Ataxia-Telangiectasia (AT)

AT is an autosomal recessive disorder with an incidence of 1 per 40,000–100,000 births worldwide, initially described in 1941 by Louis-Bar but coined by Boer and Sedgwick in 1957 (86, 87).

AT is a multisystemic disease characterized by ataxia secondary to cerebellar degeneration, telangiectasias, immunodeficiency with recurrent pulmonary infections, premature aging, ionizing radiation sensitivity, and a high risk of developing cancers of lymphoid origin (88). AT is a result of biallelic mutations of *Ataxia Telangiectasia Mutated* (*ATM*) (89), a PI3K-related serine/threonine protein kinase located on chromosome 11q22.3 (90), with a chief function to maintain genomic integrity. Following damage by ionizing radiation, chemotherapy, and oxidative stress (91), DSBs are recognized by MRN complex (MRE11-RAD50-NBS1), which activates ATM (92). Activated ATM amplifies DNA damage signaling by phosphorylating several downstream effectors including cell cycle proteins (Chk1, Chk2) (93), DNA repair proteins (BRCA1) (94), apoptosis (TP53) pathway, and other collaborative DNA damage nodes, including DNA-dependent protein kinase and ATM-related (ATR) (95, 96). Most ATM mutations are truncating and associated with severe or classic phenotype of AT due to a lack of functional kinase. Missense and in-frame mutations allow for some residual ATM activity and are associated with milder clinical course and slow progression (97, 98).

AT classically presents in early childhood, between 1 and 4 years of age, with ataxia manifesting as abnormal gait pattern in a child with otherwise previously normal development. Common neurological symptoms include dysarthria, impaired oculomotor coordination, loss of fine motor skills, and development of sensory and motor neuropathy along with extrapyramidal symptoms. Most patients become wheelchair-bound by the second decade of life (99–102). Telangiectasias are the second most common feature with average onset at 5–8 years of life and occur generally within the bulbar conjunctiva but can also appear on sun-exposed areas such as face and ears (103). Ocular telangiectasias should be differentiated from physiologic ocular vessels due to their constant presence without changing with environment or time. Immunodeficiency is another pronounced feature in two thirds of AT patients, which is demonstrated by a lack of antibody response to vaccines, reduced B and T cell numbers, and decreased production of at least one immunoglobulin subclass (IgG, IgA, and IgM) (104–106). Of note, a minority of AT patients have elevated IgM concurrently with IgA or IgG deficiency, so care must be taken to not misdiagnose these patients as hyper-IgM syndrome (107). Sinopulmonary infections and increased risk of autoimmune or inflammatory diseases, such as ITP, cutaneous granulomatous disease, and vitiligo, is a direct result of immunodeficiency and immune dysregulation (106, 108, 109). Endocrine abnormalities including poor growth, gonadal atrophy, delayed pubertal development, and insulin-resistant diabetes are also common (110–112).

AT patients have a 25% lifetime risk of developing a malignancy, which is the main cause of death in the second or third decade of life along with respiratory insufficiency (113–115). The vast majority of these cancers are of lymphoid origin with B-cell NHL, Hodgkin lymphoma (HL), and ALL occurring at a higher rate in AT patients <20 years of age (113, 114). Strikingly, EBV infection was found to be associated with all HL and half of NHL cases. Other carcinomas including brain, gastric, and liver cancers have been reported (113, 114). Although previously debated, breast cancer is now considered as part of the cancer spectrum with a 30-fold increased risk in AT patients (113). It has been postulated that cancer risk correlates with gene dosage, where patients with classic AT and lack of ATM kinase function are at higher risk of developing lymphoid tumors than patients with some residual AT activity (113).

Nijmegen Breakage Syndrome (NBS)

NBS is an autosomal recessive disease caused by biallelic mutations in *NBN* located at 8q21.3. *NBN* gene codes for nibrin, which is one of three proteins that make up the MRN complex to activate and recruit ATM to DSBs (116). NBS was named after the Dutch city, Nijmegen, where it was first described in 1981 by Wermaes et al. (117). The prevalence is estimated to be 1 in 100,000 worldwide except in Central and Eastern European Slavic populations where it is more common due to founder mutation with a large cohort in Poland (118, 119).

Microcephaly at birth with distinct, "bird-like" craniofacial features as well as growth retardation and intellectual disability are early features of NBS (120, 121). Immunodeficiency is characterized by severe hypogammaglobulinemia in 20%, IgA deficiency in 50%, and reduced B and T cells in >80% of NBS patients, resulting in a spectrum from silent phenotype to recurrent, chronic respiratory tract infections requiring immunoglobulin replacement (122–124). Malignancy is a significant cause of mortality in NBS patients. More than 40% of patients develop cancer, predominantly of lymphoid origin, by 20 years of age (125). Diffuse large B-cell lymphoma and T cell lymphoblastic lymphoma predominate (126). Other hematological malignancies including HL, B- and T-cell ALL, and AML have also been described (125). Solid malignancies such as medulloblastoma, rhabdomyosarcoma, papillary thyroid carcinoma, glioma, meningioma, neuroblastoma, and Ewing sarcoma occur rarely (125, 127, 128).

DNA Ligase IV Deficiency (LIGIV)

LIGIV was clinically described in 1990 by Dr. Plowman et al., and in 1999, it was attributed to pathogenic mutations in DNA ligase IV (*LIG4*), located on 13q33.3 (129, 130). LIG4 mediates the final ligation step in the NHEJ pathway, a process utilized not only for NHEJ-mediated DSB repair but also for V(D)J recombination (131, 132). Approximately 40 cases have been reported with hypomorphic LIG4 mutations that correlate with clinical severity (133, 134). Patients present at variable ages with common features including microcephaly, facial dysmorphism, growth failure, infections, and severe immunodeficiency as well as hematological manifestations such as BMF and leukemia/lymphoma (134, 135). The immunologic phenotype can range from a radiosensitive T-B-NK+ severe combined immunodeficiency (SCID) to mild hypogammaglobulinemia and lymphopenia with restricted receptor repertoire (136). Hematological manifestations are largely due to accumulation of ionizing radiation and other genotoxic insults, resulting in BMF in 44% (134, 137, 138) and cancers in 24% of the patient population (134). Cancers of the hematopoietic system are most common and include lymphoid leukemia and lymphomas (EBV positive and negative) and AML

(130, 134, 135, 139, 140). Recently, in a cohort of patients with BMF/MDS, a novel homozygous mutation in LIG4 (c.2440C>T, p.R814X) was found in a 10-year-old boy presenting with MDS and monosomy 7 (141).

Genomic efforts have recently uncovered additional mutations in NHEJ repair genes, *Artemis* (*DNA Cross-Link Repair 1C*) and *Cernunnos* (*XLF/NHEJ1*), to cause hematological malignancies in anectodal reports. Compound heterozygous mutations in Artemis (*EX1_3del* and 1384_1390del), a key player in V(D)J recombinase machinery, was shown to cause EBV-associated B-cell lymphoma in a 9-month-old and a 5-year-old patient (142). In a targeted mutation screen in children with hematological cytopenias, a novel homozygous *NHEJ1* mutation (c.236T>C, p.L79P), involved in the final stage of DSB NHEJ repair, was identified as the causative genetic defect in a 21-year-old with MDS and monosomy 7 (143).

SYNDROMES CAUSED BY RecQ HELICASE FAMILY DEFICIENCIES

Helicases allow access to the genome during replication, recombination, transcription, and repair by unraveling the double helix and other complex DNA and RNA structures in an ATP-dependent manner. RecQ helicases all possess three highly conserved domains: N-terminal ATPase-dependent helicase domain, RecQ-C middle domain with ability to bind various DNA structures, and a C-terminal helicase-and-ribonuclease-D-like (HRDC) domain, which promotes DNA binding stability. *BLM*, *WRN*, *RECQL1*, *RECQL4*, and *RECQL5* are five human RecQ helicases that are essential in maintaining genomic stability during DNA damage repair (144). So far, disease-causing mutations have been described in BLM, WRN, and RECQL4 to cause cancer predisposition syndromes: Bloom, Werner, and Rothmund-Thompson syndrome, respectively.

Bloom Syndrome (BS)

BS, initially described by Dr. David Bloom in 1954 (145), is an autosomal recessive disorder caused by biallelic mutations in *BLM* located at 15q26.1 (146). As of 2018, almost 300 cases were known to the Bloom Syndrome Registry (147) with predominance of individuals of Eastern European descent, particularly within the Ashkenazi Jewish population who have an estimated carrier rate of 1 in 100 (148). BLM prevents erroneous HR during replication and resolves intermediate DNA structures such as displacement loops and double Holliday junctions (149). In the absence of BLM, dysfunctional HR results in a 10-fold increase in the rate of sister chromatid exchanges (SCEs) compared to healthy individuals (146).

Clinical features of BS include growth failure, sun-sensitive skin rash, endocrine disturbances, and immunodeficiency (150). BS neonates are small for gestational age with normal appearance with some exhibiting feeding difficulties resulting in failure to thrive (148). Photosensitive cutaneous rashes are among the most common manifestations that appear in infancy or early childhood and include telangiectasia erythema of the face (butterfly rash), hands, and forearms, as well as café-au-lait spots and hypopigmented macules (147). Immunodeficiency clinically manifests as frequent upper respiratory and gastrointestinal infections due to dysregulated T cells and hypogammaglobulinemia (particularly IgA and IgM deficiency) (150). Severe chronic lung disease is a common complication of BS thought to be secondary to repeated respiratory infections as a consequence of immunodeficiency (148). In addition to short stature, insulin resistance, type 2 diabetes, dyslipidemia, hypothyroidism, and impaired fertility are well-known endocrine sequalae that develop with age in BS patients (151, 152). Neurologically, BS patients have normal intelligence with very few cases reported with mild intellectual disability (152).

The distribution of cancers in BS patients is similar to that of the general population but with a younger age onset with at least one third of BS patients developing a malignancy by the age of 25 and 80% by the age of 40 years (147). Among 144 BS patients, 223 cancers were reported (147). Hematological cancers were most prevalent, with AML and ALL occurring most frequently with a median age of 18 years followed closely by lymphomas, (predominantly B-cell NHL) with a median age of diagnosis of 20 years (147). Colorectal carcinomas were the next most common solid tumors found in 28 of 223 cancers, with a median onset age of 37 years. Other common neoplasms include breast cancer, non-melanomatous basal and squamous cell skin carcinomas, and Wilms tumor (147).

Werner Syndrome (WS)

WS, previously known as adult onset progeria with cancer predisposition, is an autosomal recessive disorder initially reported by German medical student Otto Werner in 1904. He described a family of four siblings in their third decade of life that exhibited signs of premature aging, with graying of the hair, bilateral cataracts, scleroderma, and short stature (153), which was later attributed to biallelic mutations in the Werner (WRN) helicase (154). The prevalence is estimated at 1:380,000–1:1,000,000 (155) and is higher in the Japanese (156) and Sardinian (157) population with an estimated frequency of 1:20,000–1:40,000 and 1:50,000, respectively. More than 70 different pathogenic mutations were found in the helicase and exonuclease domains of *WRN* located on locus 8p12 (158, 159). WRN has well-established functions in several DNA repair pathways, including NHEJ (158), HR (160), BER (161), and telomere maintenance (162).

The first presenting sign of WS is often short stature in a pre-adolescent individual failing to undergo a growth spurt. By the early third decade, ectodermal changes will become prominent featuring skin atrophy, graying or loss of hair, and bilateral cataracts (154) with readily discernable bird-like facies. Skin atrophy and calluses, which can progress to intractable ulcers, are common along with Achilles tendon calcification, a highly characteristic of WS in older patients (163). Common older age-associated endocrine abnormalities appear in the late 30s, including type II diabetes, osteoporosis, and hypogonadism causing infertility (154, 163). Furthermore, WS patients suffer from premature and severe forms of atherosclerosis and medial artery calcification (154, 164). Surprisingly, there is a paucity of

neurodegenerative changes in these patients in addition to lack of skeletal anomalies or intellectual disability (154, 165). Heart attacks and malignancies are the leading cause of morbidity in WS patients resulting in a low median life expectancy of 54 years (164). WS patients have a 2–60-fold increased risk for neoplasms, with thyroid follicular carcinomas as the most common cancer followed by melanoma, meningioma, sarcomas, leukemia/MDS, and primary bone tumors (166, 167).

The International Registry of Werner Syndrome has provided five cardinal signs for WS diagnosis in individuals >10 years of age: bilateral cataracts, characteristic skin changes, short stature, parental consanguinity or affected siblings, and premature hair graying (154). More than 90% of affected individuals had four cardinal features (154, 164). There is a subgroup of patients classified as atypical Werner syndrome (AWS), which is used to describe individuals with a clinical diagnosis of WS but a lack an identifiable *WRN* mutation. Of the 71 patients with AWS, a subset was shown to carry mutations in *LMNA*, a gene known to be mutated in the Hutchinson-Gilford Progeria syndrome (HGPS) (168), or in *POLD1*, a DNA polymerase involved in several DNA repair pathways (169). Thus, far, malignancies have not been reported among these AWS patients (154).

Rothmund Thompson Syndrome (RTS)

RTS was initially described by the German ophthalmologist Dr. August von Rothmund in 1868 with unique ectodermal features followed by a similar description by Dr. Sydney Thomson, British dermatologist, in 1921. It was not until 1957 when Dr. Taylor coined the syndrome, which now has almost 500 patients described in all ethnicity groups (170). RTS results from autosomal recessive germline mutations in *RECQL4*, which organizes the DNA replication machinery, promotes DNA end resection with MRN and CtIP complex during HR and promotes NHEJ in G1 phase of the cell cycle (171, 172).

Cutaneous rash is the hallmark clinical sign in RTS, which commonly presents in infancy with an erythematous facial rash that spreads to buttocks and extremities while sparing the trunk. The rash progresses to poikiloderma (reticulated hypo- and hyperpigmentation, telangiectasias, and punctate atrophy) over months to years and persists throughout life. Hyperkeratotic lesions and café-au-lait spots can manifest later (170, 173). Skeletal abnormalities and long bone defects were found in 75% of RTS patients (174). Ocular abnormalities occur with varying prevalence of 10–50% with rapid-onset bilateral cataracts being most frequent (175). Other common features include short stature, sparse or absent hair, dental anomalies, and feeding difficulties (176, 177). Immunodeficiency is uncommon, although IgG and IgA deficiencies along with T-B+NK-combined immunodeficiency have been described (178–180). The most common malignancy among RTS patients is osteosarcoma with a prevalence of 30%, occurring at a younger median age of 11 years compared to the general population (177). Skin cancers, including melanoma and basal cell and squamous cell carcinoma, constitute the second most common cancer affecting 5% of patients (177, 181, 182). Rare hematological malignancies include MDS, lymphomas (NHL, HL), and AML (173).

Notably, germline mutations in *RECQL4* gene had also been associated with two other constitutional disorders with lymphoma risk. First, RAPADILINO (**RA**dial **RA**y defect; **PA**tellae hypoplasia or aplasia and cleft or highly arched **PA**late; **DI**arrhea and **DI**slocated joints; **LI**ttle size and **LI**mb malformation; **NO**se slender and **NO**rmal intelligence) syndrome. It has been initially described in Finland in 1989 (183) to affect an estimated 1 in 75,000 individuals and manifest with pre- and post-natal growth failure, cervical spine defects, failure to thrive, and juvenile diarrhea of unknown cause (184). Lymphoma was reported in 4 patients and osteosarcoma in 1 patient with RAPADILINO syndrome (185). Second, Baller–Gerold syndrome (BGS), first reported by Cohen in 1975, was based on three patients described in 1950 by Baller and 1959 by Gerold in German literature (186). Fewer than 40 patients have been described with an unknown prevalence (187). BGS patients with RECQL4 mutations have craniosynostosis, upper-limb anomalies, short stature, and poikiloderma (188). Thus, far, only one case of malignancy (NK/T-cell lymphoma) has been reported in a 2.5-year-old individual with BGS (189).

CANCER RISK AMONG HETEROZYGOUS MUTATION CARRIERS

Individuals with germline heterozygous (monoallelic) mutations in some DNA repair genes have an increased lifetime risk of cancer, which is often facilitated by the acquisition of a somatic mutation affecting the remaining wild-type allele. The spectrum and onset age of cancers in individuals with heterozygous mutations differ compared to individuals with biallelic mutations in the same gene. Genetic counseling is recommended for all patients with, or at risk for having, monoallelic or biallelic DNA repair disorders due to the complex nature of these conditions and their associated health risks (190).

Cancer screening guidelines have been established by multiple organizations to address the need for increased surveillance and/or prophylactic management for these high-risk individuals (191–193). Gene-specific cancer screening guidelines have also been established internationally for individuals with monoallelic variant for a DNA repair disorder gene with high risk of cancer development (194–196). Many of these guidelines are region specific and may differ from recommendations, when available, in other parts of the world. Continued efforts to harmonize these recommendations are needed to ensure patients have access to appropriate management worldwide.

Monoallelic pathogenic mutations in the mismatch repair genes, *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*, are associated with Lynch syndrome, a cancer predisposition syndrome characterized by an increased risk of colon cancer, uterine cancer, ovarian cancer, genitourinary tract cancers, and other gastrointestinal cancers. Cancer risk varies among the different MMR genes. Heterozygous mutations in PMS2, for instance, are associated with a lower risk of colon and endometrial cancers and are often diagnosed at later ages than in individuals with heterozygous mutations in MLH1 or MSH2 (197, 198).

Heterozygous mutations in FA genes involved in DSB repair predispose to development of breast, ovarian, and other cancers. These include *BRCA1* and *BRCA2* mutations that confer a 50–80% lifetime breast cancer risk, 10–40% lifetime ovarian cancer risk, and increased risk of male breast, pancreatic, and prostate cancer, as well as melanoma (199, 200). Heterozygous loss of *PALB2* has also been demonstrated to confer a susceptibility to breast and pancreatic cancer, as *PALB2* interacts directly with both *BRCA1* and *BRCA2* during HR. An elevated risk of later onset serous ovarian cancer has been demonstrated in individuals with heterozygous loss-of-function *BRIP1* mutations (201). Biallelic mutations in *BRCA1*, *BRCA2*, *PALB2*, and *BRIP1* result in FA groups S, D1, N, and J, respectively. Recent meta-analyses have estimated that the lifetime risk of breast cancer in *ATM* heterozygotes is 33–38% (115), although the c.7271T>G mutation may be associated with a significantly higher breast cancer risk (202). Heterozygous *ATM* mutations may also confer a susceptibility to pancreatic cancer (203). Heterozygous carriers of the *NBN* c.657del5 mutation (which is found in homozygous state in more than 90% of patients with Nijmegen breakage syndrome) who also carry two copies of the NBN polymorphism p.E185Q (GG allele) were shown to be at increased risk for breast and prostate cancers (204, 205). These recent studies are the first clear example of genetic modifier effect in a germline cancer syndrome, where the penetrance of a heterozygous allele is "activated" by the presence of an additional modifying polymorphism in the same gene.

DIAGNOSTIC CONSIDERATIONS

History and Examination

A thorough patient history, family history, and physical examination gives the first suspicion or a "red flag" pointing to an underlying DNA repair disorder (**Table 2**). Multisystem history should be obtained along with birth and developmental history since manifestations can appear at any location during the lifetime. If the patient has been treated for prior malignancy, age of diagnosis, type and location of cancer, treatment history, and hypersensitivity to chemotherapeutic agents should also be addressed. Family history features suggestive of one of these conditions include the presence of early-onset cancers in family members, multiple family members with cancer, or multiple cancers in one individual. Other concerning features include the presence of immunodeficiency, neurologic abnormalities, or deaths in young children from medical or unknown causes. Familial consanguinity should be noted because many of the DNA repair disorders are inherited in an autosomal recessive manner. Consideration should be given to the family's ethnic background as some of these disorders are enriched in specific ethnic populations secondary to founder mutations. Physical exam findings concerning DNA repair disorder include facial dysmorphology (particularly microcephaly, which should be evaluated by measuring head circumference); absent, sparse, brittle, or prematurely gray hair; as well as numerous dermatologic findings such as café-au-lait macules, hypopigmentation, multiple lentigines, telangiectasias, or rashes, especially if occurring on the face. An accurate height should also be obtained, as many patients with a DNA repair disorder are of short stature. Suggestive neurologic findings include loss of deep tendon reflexes, spasticity, ataxia, or other gait changes. Referral to a clinical geneticist may also be of benefit to further assess for features of these conditions.

TABLE 2 | The presence of multiple red flags in the medical and/or family history increases concern for an underlying DNA repair disorder and should warrant further evaluation.

	"Red flags"
Constitutional features	Short stature Microcephaly Sparse or premature gray hair
Skin	Photosensitivity Pigmentation changes (hypo/hyperpigmentation) Poikiloderma Café-au-lait spots Teleangiectasias Pilomatricoma/pilomatrixoma (benign, hair follicle associated tumor) Butterfly shaped facial skin rash
Neurologic	Intellectual disabilities Hyporeflexia Loss of fine or gross motor skills Ataxia
Immunodeficiency	Recurrent sinopulmonary infections Hypogammaglobulinemia T and B lymphocytopenia
Hematologic	Bone marrow failure
Cancers	Pediatric cancers including head and neck, brain, squamous cell carcinoma, melanoma, adrenocortical carcinoma, NHL, MDS, AML Family member with cancer below age 50, especially if of breast, endometrial, or colorectal origin 2 or more cancers in one individual/family Multiple family members with similar or related cancers

Functional Assays

Functional testing aids in the diagnostic workup of DNA repair disorders (**Table 1**). Telomere length is an important diagnostic tool that is used to diagnose short telomere syndromes such as dyskeratosis congenita, a BMF syndrome with mucocutaneous fragility and symptoms of premature aging with an increased predisposition to malignancies secondary to genetic deficiencies in telomere-associated genes such as *TERT*, *TERC*, *DKC1*, *TINF1*, and *RTEL1* to name a few [excellent review provided by (206)]. Importantly, telomere length should be measured in DNA repair disorders such as FA (207, 208), AT (209, 210), NBS (211), BS (212), and WS (213) where patients exhibit short telomeres and chromosome end fusions secondary to dysfunctional DNA damage response at the telomere.

Chromosome breakage studies are necessary to establish a diagnosis of FA, as individuals with this condition are hypersensitive to crosslinking agents such as mitomycin C (MMC) or diepoxybutane (DEB). When exposed to these agents, patient cells will have an increased rate of chromosome breaks

and aberrations such as radial figures and rearrangements. Rarely, mosaicism can occur in lymphocytes where two distinct lymphocyte populations are present with one subset having undergone spontaneous reversion resulting in normal sensitivity to clastogenic agents while the second population remains with the underlying genetic defect and retaining hypersensitivity features to damaging agents. Therefore, if breakage studies on lymphocytes are normal but there is still clinical suspicion for a DNA repair disorder, skin fibroblasts should be investigated to complete the diagnostic evaluation (76).

DNA repair disorders that present with profound immunodeficiency [AT, NBS, NHEJ deficiencies (Ligase IV, Artemis, Cernunnos)] can lead to absence or very low T-lymphocyte receptor excision circles (TRECs), which are detected on newborn screen (214, 215).

Spontaneous excess of immunoglobulin (Ig)/T-cell receptor (TCR) abnormal rearrangements of chromosomes 7 and 14 are common in patients with NBS (10–35%) (216) and AT (5–10%) (217). Alpha fetoprotein (AFP) is elevated in 95% of AT patients (218), but interestingly, it can also be increased in FA patients (219). Sister chromatid exchange (SCE) assay, which assess for increased SCE in metaphase cells with bromodeoxyuridine (BrdU) exposure, aids in the diagnosis of BS (148). UV hypersensitivity assay, where skin fibroblasts are exposed to UV light, is used for diagnosing NER defect in XP patients, but this testing is typically completed in a research setting and may not be available clinically (220). There is a lack of consensus and uniform availability for a routine radiosensitivity assay available for patients with HR and NHEJ biallelic genetic disorders. Radiation-induced lymphocyte apoptosis (RILA) assay and phospho-ATM assay have some predictive potential (221). Analysis of radiation-induced γH2AX foci accumulation in T and NK lymphocytes of LIG4-SCID individuals was recently implemented as a flow cytometry assay (222).

Genetic Testing

It has become a standard approach to perform genetic studies as part of the initial diagnostic workup in a patient with a suspected DNA repair disorder based on clinical features and/or history of related malignancies. The patient's clinical phenotype and results of functional testing can be used to guide the differential diagnosis and, in turn, the genes requiring further investigation. Genetic testing of individuals presenting with a related malignancy but lacking other clinical manifestations of a DNA repair disorder is unlikely to have a high yield, as these conditions are thought to be rare. However, the diagnostic pickup of a DNA repair disorder in individuals with a related malignancy in an unbiased manner requires further study.

When ordering genetic testing, issues to consider include sample source, optimal genetic testing type, and technical challenges limiting mutation identification. First, peripheral blood or saliva samples are the easiest and most preferred sample source to obtain. In patients with active hematologic malignancy, however, skin fibroblasts or hair follicles are the preferred germline specimen (223). Single gene analysis may be an appropriate rapid approach in scenarios where a specific gene is expected based on phenotype. A disease-specific multigene panel is a cost-effective approach for patients with clinical features consistent with multiple DNA repair disorders. Currently, clinical whole exome or genome sequencing represent the most comprehensive approach, generally used after obtaining negative results from targeted gene testing. Some genes may present technical challenges, such as the *PMS2* gene, which has multiple pseudogenes. One of these pseudogenes, *PMS2CL*, is part of a 100-kb inverted duplication and has close sequence homology to the regions of exons 9 and 11–15 in *PMS2*, making it difficult to differentiate whether the mutation is located within *PMS2* or the pseudogene (224).

When interpreting variants obtained in genetic studies, it is widely accepted to use consensus criteria established by the American College of Medical Genetics and Genomics to classify variants as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign (225). Pathogenic and likely pathogenic variants will confirm a clinical diagnosis and thus impact medical management decisions. If a patient with a suspected autosomal recessive DNA repair disorder is found to have a heterozygous pathogenic mutation in a gene consistent with the phenotype, one has to consider that a second mutation within the same gene was missed. A discussion with the reporting lab may be helpful to clarify limitations of their testing strategy and whether additional testing may be warranted to evaluate for a second gene alteration, which might include not only a mutation but also an intragenic deletion or intronic variant. An increasingly growing challenge in the clinical setting is the finding of a VUS, for which the available genetic and functional data are either lacking or conflicting and, therefore, at a given time, they generally should not influence clinical decision making. However, periodic communication with the testing lab is encouraged to learn of any changes in variant interpretation that may occur over time.

TREATMENT STRATEGIES

A unifying feature among most DNA repair disorders is hypersensitivity to DNA-damaging agents such as radiation and chemotherapy used to treat malignancies. However, the underlying genetic deficit of repair pathway genes in patients with DNA repair syndromes places them at high risk for therapy-related toxicities. For this reason, unique cancer treatment regimens are tailored that often employ reduced intensity doses to balance chemo- or radiotherapy-mediated toxicities while achieving clinical outcomes comparable to the standard of care. The high rate of treatment failures and secondary malignancies is problematic, especially in patients with CMMRD, NBS, and AT. Common strategies to avoid overt toxicities include avoiding radiomimetic drugs such as bleomycin and dactinomycin and being aware of cyclophosphamide- and/or ifosfamide-related hemorrhagic cystitis developing outside the normal range in patients with predisposition to telangiectasias. DSB DNA repair syndromes (AT, NBS, and LIGIV), due to their shared manifestations of immunodeficiency and increased risk for malignancies, benefit from reduced intensity conditioning-based hematopoietic stem cell transplantation (HSCT). However,

the role of HSCT in improving overall outcome of patients with AT remains debatable (215). Several clinical trials are aimed at innovative drugs that target DNA repair genes to provide effective therapy while minimizing toxicities for patients with DNA repair disorder-associated cancers (226).

CONCLUSIONS

Cancer can result from mutations that are inherited or acquired during lifetime. DNA repair mechanisms are essential to maintenance of genomic integrity and are abrogated in cancer. Defects in DNA repair pathways result in a chaotic and unstable genomic environment, which is a hot bed for oncogenic transformation. This biological phenomenon is well-recapitulated in classic DNA repair disorders that result from heritable mutations in genes essential for DNA damage response and result in early-onset cancers and premature aging. Because these syndromes are rare, a heightened awareness must be practiced to provide multidisciplinary care and surveillance and unique therapeutic considerations for patients with DNA repair disorders.

AUTHOR CONTRIBUTIONS

RS and SL wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by ALSAC/St. Jude Children's Research Hospital, and grants to MW: Fritz-Thyssen Foundation (10.17.1.026MN), ERAPERMED GATA2-HuMo (2018-123, 01KU1904), Deutsche Krebshilfe (109005), Deutsche Kinderkrebsstifung (DKS 2017.03), and BMBF MyPred (01GM1911A).

ACKNOWLEDGMENTS

The authors thank Mitch Weiss, Michelle Boals, and Nathan Gray and other members of the St. Jude Bone Marrow Failure/MDS program, as well as Dr. Kim Nichols and the St. Jude Division of Cancer Predisposition for patient management and helpful discussions.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Oncology

REVIEW
published: 28 October 2020
doi: 10.3389/fonc.2020.590033

Genetic Predisposition to Solid Pediatric Cancers

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Progresses over the past years have extensively improved our capacity to use genome-scale analyses—including high-density genotyping and exome and genome sequencing—to identify the genetic basis of pediatric tumors. In particular, exome sequencing has contributed to the evidence that about 10% of children and adolescents with tumors have germline genetic variants associated with cancer predisposition. In this review, we provide an overview of genetic variations predisposing to solid pediatric tumors (medulloblastoma, ependymoma, astrocytoma, neuroblastoma, retinoblastoma, Wilms tumor, osteosarcoma, rhabdomyosarcoma, and Ewing sarcoma) and outline the biological processes affected by the involved mutated genes. A careful description of the genetic basis underlying a large number of syndromes associated with an increased risk of pediatric cancer is also reported. We place particular emphasis on the emerging view that interactions between germline and somatic alterations are a key determinant of cancer development. We propose future research directions, which focus on the biological function of pediatric risk alleles and on the potential links between the germline genome and somatic changes. Finally, the importance of developing new molecular diagnostic tests including all the identified risk germline mutations and of considering the genetic predisposition in screening tests and novel therapies is emphasized.

Keywords: genetic predisposition, germline variants, cancer predisposition genes, pediatric tumors, cancer susceptibility, germline-somatic interaction, SNP, next generation sequencing

OPEN ACCESS

Edited by:
Angela Mastronuzzi, Bambino Gesù Children Hospital (IRCCS), Italy

Reviewed by:
Luigi Boccuto, Clemson University, United States
Jun Wei, University of Texas MD Anderson Cancer Center, United States
Xiaotu Ma, St. Jude Children's Research Hospital, United States

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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Oncology

Received: *31 July 2020*
Accepted: *08 September 2020*
Published: *28 October 2020*

Citation:
Capasso M, Montella A, Tirelli M, Maiorino T, Cantalupo S and Iolascon A (2020) Genetic Predisposition to Solid Pediatric Cancers. Front. Oncol. 10:590033. doi: 10.3389/fonc.2020.590033

INTRODUCTION

Genomic sequencing studies have highlighted that pediatric cancers typically have few somatic mutations but a higher prevalence of germline alterations in cancer predisposition genes (1). The contribution of germline variants in pediatric tumors has been estimated between 8 and 12% (2, 3). Genetic variants are generally classified on the basis of their clinical effect: pathogenic variant means any sequence change that, differing from the consensus wild-type sequence, directly contributes to the development of the disease; likely pathogenic variants, instead, are genetic changes with a high likelihood of being disease-causing, but additional evidence is expected to confirm their clinical significance. Variant classification can arise from different methodologies and algorithms, which can assign different weights to collected data. However, studies cited in the present review generally refer to the American College of Medical Genetics and Genomics (ACMG) guidelines for variants interpretation (4). In this process, multiple categories of data (such as frequency in affected and unaffected populations, computational prediction tools, functional studies, and

gene- or disease-specific information) are taken into account and combined to determine a variant pathogenicity classification.

It is also important to note that genetic variants can be detected through different genomic approaches and the type of identified alteration depends on the nature of the assay used. Large-scale genomic analyses such as whole-exome sequencing (WES) or whole-genome sequencing (WGS) can identify uncommon, moderate penetrant variants. Since WES investigates only the coding regions of the genome, it has proved very useful in detecting most of the causative variants of Mendelian diseases (5, 6). Furthermore, it has recently been used also to identify rare and uncommon causative mutations of complex diseases (7). On the other hand, WGS can capture nearly all known genetic variations, including those falling in regulatory elements, with much more uniform coverage of the genome, but it does not allow to detect mosaic variants with low clonality or variations causing DNA repetitions (8). Common, low-penetrance genetic variants, instead, are mostly identified by genome association study (GWAS), which assesses genotype–phenotype associations through testing of variants across genomes of many individuals, based on data obtained using numerous technologies, mostly WGS or genome-wide single-nucleotide polymorphism (SNP) arrays. Consequently, GWAS limitations are linked to the technology on which it is based: e.g., SNP array-based GWAS rely on pre-existing genetic variant reference panels (9). Finally, besides SNP array, copy-number variations (CNV) can be identified also through CGH array. Anyway, array methods cannot be used to detect single base pair changes, indels, balanced chromosome rearrangements, and low-percent mosaicism (10).

Recently, in addition to germline pathogenic and/or likely pathogenic variants in known cancer-predisposing genes, it has been estimated that a high percentage (61%) of children, adolescent and young adult patients with solid tumors carry germline pathogenic and likely pathogenic variants in new candidate genes, including *PRKN*, *SMACAL1*, *SMAD7*, and *TMPRSS3* (3). The detection of cancer predisposition can lead to clinical benefits for patients, both for the molecular diagnosis and for the presence of specific biological features, as well as to eventually refine therapeutic choices. We provide an overview of the most significant knowledge of germline predisposition for the main pediatric solid tumors, which are central nervous system tumors (medulloblastoma, ependymoma and astrocytoma), neuroblastoma, retinoblastoma, Wilms tumor, osteosarcoma, rhabdomyosarcoma, and Ewing sarcoma, altogether accounting for 34.8% of all childhood cancers (**Figure 1**). Each tumor description is organized into two subsections: "familial cancer" and "sporadic tumor." Familial cancer means a form of cancer that has higher incidence in families than in the general population due to rare, high-penetrance genetic variants. In this group, we also included rare genetic syndromes that are not usually considered as cancer syndromes but that predispose to the development of solid pediatric tumors. The second group, sporadic tumor, is referred to cancers which do not run in families and are intended as multifactorial diseases whose onset

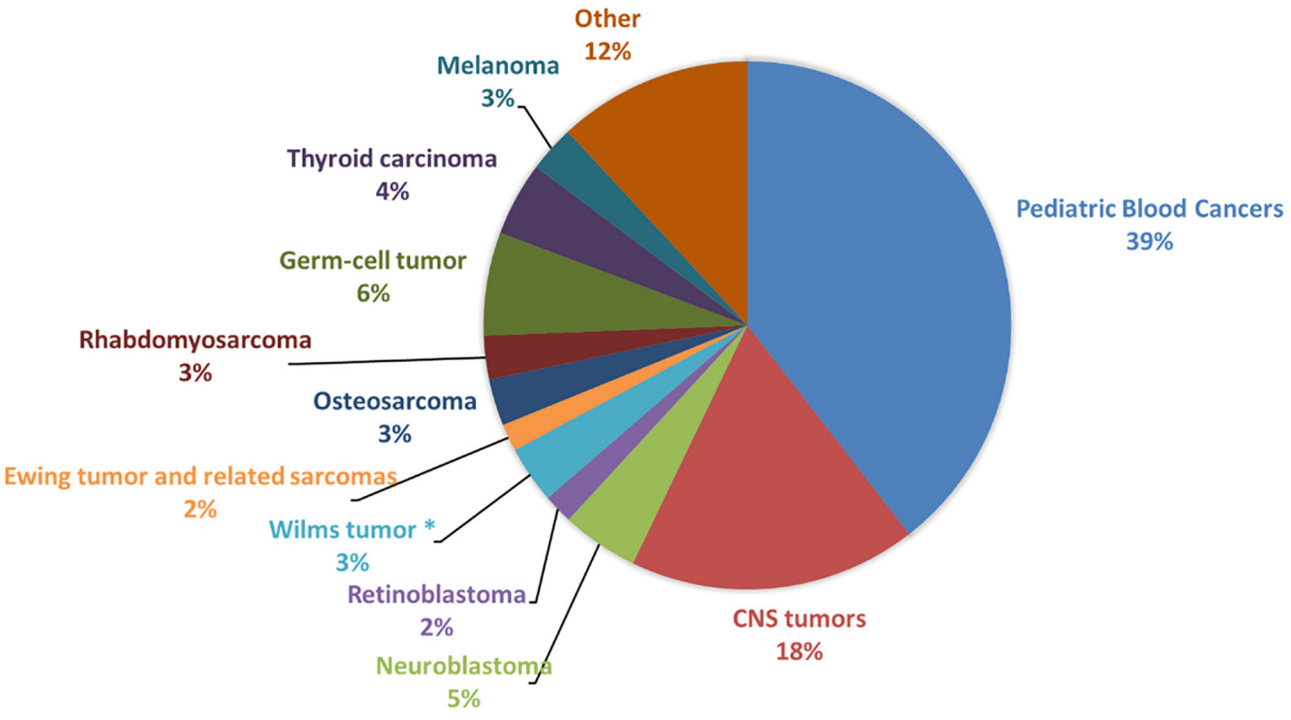

FIGURE 1 | Frequency of pediatric cancers in patients younger than 19 years. The figure shows the prevalence of the main pediatric cancer types among patients younger than 19 years of age, calculated from Centers for Disease Control and Prevention (CDC) data (United States Cancer Statistics Data, https://wonder.cdc.gov/cancer.html) and based on incidence in United States for the years 1999–2016. CNS, Central Nervous System. *This frequency is related to Wilms tumor and other non-epithelial renal tumors.

can be attributed to the combined effect of environmental and genetic factors. In sporadic cancers, genetic factors can be categorized into two types: uncommon, moderate-penetrance genetic variants, which for the studies considered in this review show a frequency lower than 1–0.001% in the general population and are not so rare as those associated with familial cancer, and common, low-penetrance genetic variants.

The knowledge of genetic mutations responsible for syndromic disorders associated with the risk of developing pediatric cancer has greatly increased over the past years (11). Indeed, several tumor predisposing syndromes are the underlying cause of at least 8.5% of cancers in pediatric patients (12). Thus, the role of general practitioners and pediatricians in recognizing the major cancer genetic-associated syndromes, in making appropriate referrals for genetic counseling and testing when indicated, is crucial for a specific monitoring and management of the patient.

Most cancer susceptibility genes are involved in fundamental biological pathways such as cell-cycle control, chromatin remodeling, or DNA repair. Therefore, alterations in these genes compromise the normal control of cell growth and lead to a substantial increase in the risk of developing cancer. Another element of great interest discussed here is the presence of cooperation between germline and somatic alterations, which can represent an early tool for evaluating the clinical outcome and for the stratification of patients in risk subgroups. We also discuss evidence that points to a need for more collaborative investigations in identifying driver events in pediatric cancers.

CENTRAL NERVOUS SYSTEM TUMORS

Central nervous system (CNS) tumors represent the most frequent types of cancer in children aged 0–14 years, with a mortality rate of 0.72 per 100,000 population (13). The three most frequent tumors are medulloblastoma (MB), ependymoma (EP), and astrocytoma (AS) (**Figure 2**).

Medulloblastoma

MB is an embryonal tumor of cerebellum (14) that affects children under the age of 14, with an average onset of about 6–8 years (**Figure 2**) and with a 5-year overall survival for standard-risk patients of 70–85% (14). It is classified into four genetic and molecular groups: the first two groups, WNT-activated (MB_{WNT}) and Sonic Hedgehog activated (MB_{SHH}), are named for the

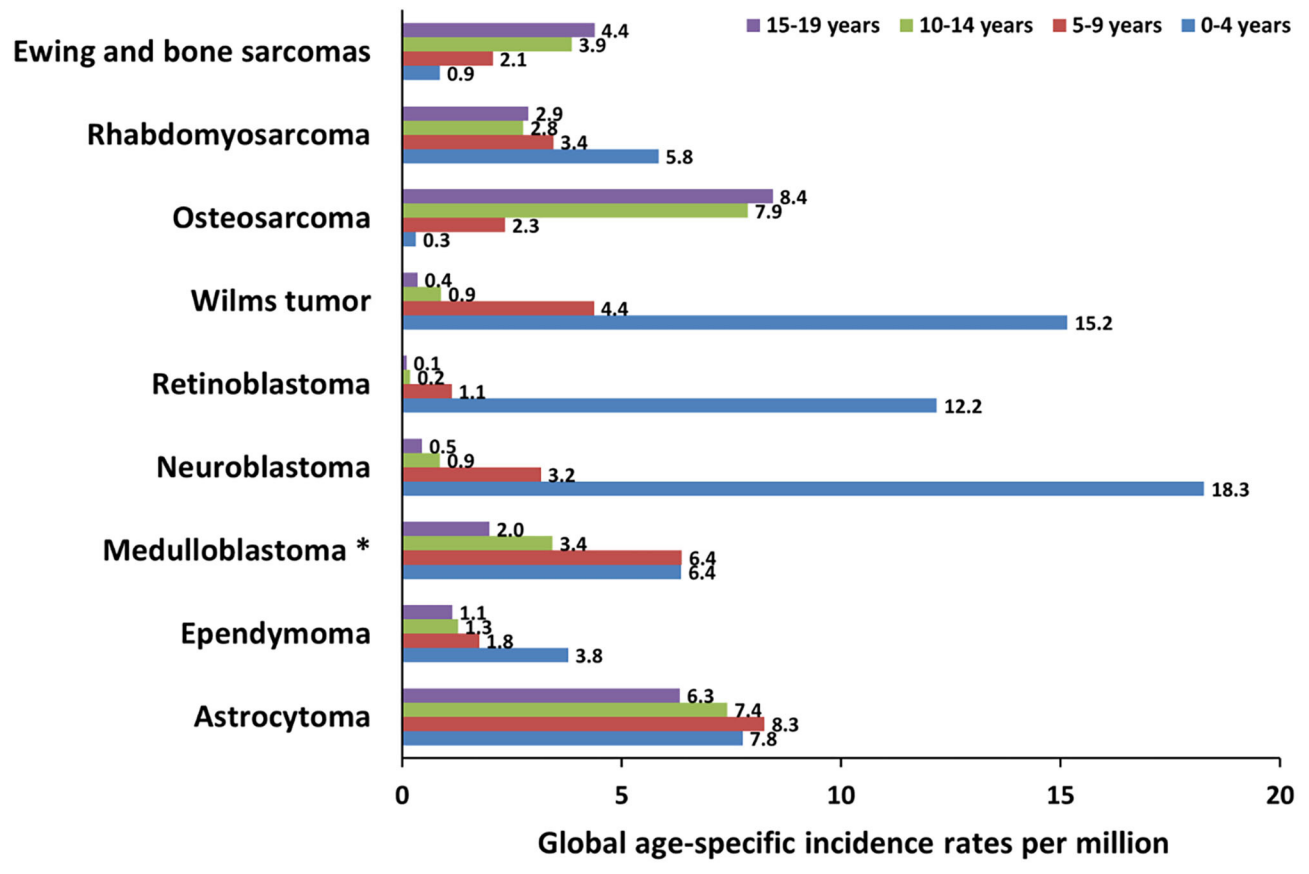

FIGURE 2 | Global incidence of pediatric cancers in patients younger than 19 years. The graph shows the global age-specific incidence rates (ASR) per million for individual age groups (0–4 years, 5–9 years, 10–14 years, and 15–19 years) of pediatric cancer types discussed in this review. ASR reported next to the bars are calculated from International Incidence of Childhood Cancer (IICC, https://iicc.iarc.fr/) data. *These ASR include also less frequent embryonal central nervous system tumors.

signaling pathways that play prominent roles in the pathogenesis of those subgroups, while, since less is known about the biology of the remaining two subgroups, they are numerically designated as "Group 3" and "Group 4" (14). Damaging germline mutations in known cancer-predisposing genes play an important role in two main subgroups, MB_{WNT} and MB_{SHH}, in which genetic testing is highly recommended (15). MB_{WNT} is characterized at somatic level by activating mutations in exon 3 of β-catenin (*CTNNB1*) and monosomy of chromosome 6, while MB_{SHH} by amplification of *GLI2* and *MYCN*, as well as loss of 17p (16).

Familial Medulloblastoma

To date, only germline mutations in *ELP1* have been found in two independent families with MB_{SHH} (17). Although inherited or familial MB is extremely rare, there are few rare inherited syndromes that are associated with increased risk of developing this tumor (**Table 2**). Germline mutations of *PTCH1* and *SUFU*, by causing activation of the SHH signaling pathway, predispose to MB_{SHH} in Gorlin syndrome, an autosomal dominant disease caused by mutations in *PTCH1* (67, 124). In Turcot syndrome, a rare disorder characterized by the association of colonic polyposis and primary brain tumors, germline mutations of *APC* predispose to the development of MB_{WNT} (114). In MB_{WNT}, activation of the WNT pathway is due to somatic mutations of *CTNNB1* in most of tumors but it is also observed in patients with only germline mutations of *APC*, stressing the importance of genetic predisposition in high-risk patients (15, 114). Germline mutations in *BRCA2* and *PALB2*, associated or not associated with Fanconi anemia, have been found in MB_{SHH} (58, 125) and are often observed in association with somatic homologous recombination repair defects (15). The role of germline mutations in *TP53* in MB is still widely debated today. *TP53* germline mutations affect MB prognosis differently according to the different subgroups: germline mutations in MB_{SHH} are associated with poor prognosis, while both germline and somatic mutations in MB_{WNT} are associated with better prognosis. This may be due to a different origin of the MB itself (14). Patients with germline *TP53* mutations can have tumors characterized by catastrophic DNA chromothripsis and are often associated with Li–Fraumeni syndrome (LFS), a cancer predisposition disorder caused by germline mutations of the tumor-suppressor p53 (71). Other MB-associated syndromes are Bloom's syndrome (31), ataxia telangiectasia (18), and Greig's cephalopolysyndactyly syndrome (14, 40, 45, 85, 122) (**Table 2**).

Sporadic Medulloblastoma

The association between MB and genetic syndromes explains most of the genetic predisposition to MB. However, sporadic forms are known in literature and are partially explained through uncommon, moderate penetrant mutations identified by whole-exome sequencing (WES) or whole-genome sequencing (WGS), or common, low-penetrance genetic variants identified by genome wide association study (GWAS) (**Table 1** and **Table 3**).

Uncommon, Moderate-Penetrance Variants

In a study on 1,022 MB patients, novel partial or total *APC* deletions were found (15). These mutations were not associated with any familial syndrome and predisposed to MB_{WNT}. In

TABLE 1 | Rare, high-penetrance, and uncommon, moderate-penetrance variants in genes predisposing to pediatric tumors and main biological pathways.

Pathways	Gene(s)	Tumors	References
Collagen chain polymerization	*COL7A1*	NB, RMS, WT	(3)
Cytoskeletal and adhesion signaling	*GJB2*	AS, CNS tumors, EWS, OS, RMS	(3, 126)
	CDH1	WT	(3)
DNA base excision repair (BER)	*ERCC2*	AS, OS	(127–129)
DNA double-strand break repair (DSB)	*BRCA1*	AS, CNS tumors, EWS, OS, RB	(3, 126, 129, 130)
	BRCA2	AS, NB, MB, RMS	(2, 3, 15, 58, 125, 126)
	CHEK2	CNS tumors, EWS, NB, OS, RB, RMS, WT	(3, 129, 131, 132)
	BAP1	RB	(3)
	BLM	EWS, MB	(15, 130)
	BRIP1	EWS, MB, OS	(2, 3, 15, 129, 130)
	NBN	MB	(15)
	WRN	MB	(15)
	PALB2	MB, OS, WT	(3, 15, 129, 131, 132)
DNA mismatch repair system (MMR)	*MSH2*	WT, OS	(2, 3)
	MSH6	RB, RMS, WT	(3, 133)
	PMS2	AS, CNS tumors, EWS	(2, 3, 127, 130)
DNA repair	*FANCA*	AS, MB	(15, 126)
	FANCC	EWS, MB	(2, 15, 130)
	FANCI	RMS	(133)
	FANCL	OS	(2, 129)
	FANCM	OS	(2, 129)
	ATR	RMS	(3)
	MUTYH	AS, EWS	(2, 127)
	RAD51D	WT	(3)
	RECQL4	OS	(129)
Genome stability and regulation of cell cycle	*ALK*	Familial/sporadic NB	(2, 3, 134, 135)
	ATM	EWS, MB, OS, RB, RMS	(3, 15, 129, 133)
	RB1	OS, familial/sporadic RB	(2, 3, 129, 135, 136)
	TP53	AS, EWS, MB, NB, OS, RMS, WT	(2, 3, 15, 127, 129–131, 133, 135, 137–139)
Metabolic pathways	*HMBS*	CNS tumors	(3)
	FAH	OS	(129)
	SDHA	NB	(3)
Protein interaction at synapsis	*PTPRD*	Advanced/metastatic EWS	(140)
Protein translation and modification	*KIF1Bβ*	Familial NB	(141)
RET signaling and G-protein signaling, H-RAS regulation pathway	*ERBB4*	NB	(3)
	NF1	AS	(126)
	RET	EWS	(2, 130)

(Continued)

TABLE 1 | Continued

Pathways	Gene(s)	Tumors	References
miRNA processing genes	*DIS3L2*	WT	(131, 132, 137)
	DROSHA	WT	(131, 137)
	XPO5	WT	(131)
	DICER1	Familial/sporadic WT, RMS	(3, 52, 55, 131, 137, 142)
Sonic Hedgehog pathway (SHH)	*GPR161*	MB	(143)
	PTCH1	MB	(15, 67)
	SUFU	MB	(15, 67)
Spindle assembly checkpoint (SAC)	*TRIP13*	Familial WT	(83)
Transcriptional regulation and chromatin remodeling	*CTR9*	Familial WT	(144)
	ELP1	MB	(17)
	LZTR1	CNS tumors, EWS	(3)
	PHOX2B	Familial NB	(145)
	POLE	EWS, NB	(3, 130)
	SMARCA4	NB	(3, 146)
	REST	Familial/sporadic WT	(147, 148)
	TRIM28	Familial/sporadic WT	(147)
	WT1	Familial/sporadic WT	(147, 149, 150)
WNT signaling pathway	*APC*	MB	(15)
Other	11p15	Familial/sporadic WT	(150, 151)

Rare, high-penetrance variants are related to familial forms of tumors, while uncommon, moderate-penetrance variants refer to sporadic forms. When the tumor form is not specified we refer to uncommon, moderate-penetrance variants. AS, astrocytoma; CNS, central nervous system; EP, ependymoma; EWS, Ewing sarcoma; MB, medulloblastoma; NB, neuroblastoma; OS, osteosarcoma; RB, retinoblastoma; RMS, rhabdomyosarcoma; WT, Wilms tumor.

the same study, 1% of patients (classified as MB_{SHH}) had *TP53* mutations but only 5/11 patients showed family history of cancer, emphasizing the role of *TP53* germline mutations in predisposing to sporadic MB. Notably, germline missense, frameshift, or non-sense mutations in the DNA-binding domain of *TP53* were found to be associated with a series of events at the somatic level such as rearrangements, chromothripsis, and loss of heterozygosity in MB_{SHH} patients, whereas germline mutations in *SUFU* and *PTCH1* co-occurred with somatic loss of heterozygosity (15) (**Table 4**). These results further provide evidence that novel associations between germline variants and specific somatic events, beyond those reported by Knudson in 1971, can play a role in carcinogenesis. Indeed, recent body of literature supports the hypothesis that specific germline variants determine which somatic events and mutations are generated and selected in cancer cells during tumorigenesis (179).

MB can also arise in patients with germline mutations in other known cancer genes such as *ATM*, *FANCA*, *FANCC*, *NBN*, *WRN*, *BLM*, and *BRIP1* and in candidate genes like *CHEK2*, *CREBBP*, *RAD51*, *ERCC2*, and *ERCC4.* All of these genes are involved in cell-cycle regulation and DNA repair (15). Frameshift, protein-truncating, and missense mutations occurring in *GPR161*, a gene never previously associated with MB, were found in 6 MB_{SHH} cases (143) that, at the somatic level, showed loss of heterozygosity with retention of the mutated allele, confirming its role as driver gene in MB_{SHH}. *GPR161* functions are essential for embryonic development and for the proliferation of granular cells (143). Germline mutations in *ELP1* have been very recently found to predispose to MB_{SHH} and to be associated with two consecutive somatic events: loss of the 9q arm, with consequent loss of the wild-type copy of *PTCH1* and *ELP1*, and a second independent mutation event in *PTCH1* (17) (**Table 4**). This study, importantly, showed that 40% of MB_{SHH} patients carry disease-predisposing mutations and that genetic predisposition to proteome instability may be a determinant in the pathogenesis of pediatric brain cancers (17) (**Table 1**).

Common, Low-Penetrance Variants

To date, there are no relevant GWAS conducted to identify common variants associated with MB. Only one study has been performed in a small sample including 244 MB cases and 247 control subjects from Sweden and Denmark, but no locus reached the significance threshold (154). The most significant locus was 18p11.23 including *PTPRM* (154). A different approach that starts from the most frequently mutated genes in MB such as *CCND2*, *CTNNB1*, *DDX3X*, *GLI2*, *SMARCA4*, *MYC*, *MYCN*, *PTCH1*, *TP53*, and *KMT2D* was proposed to identify MB-associated common variants (162). Eight variants, located in *CCND2*, *PTCH1*, and *GLI2*, associated with the risk of developing MB (162) (**Table 3**). However, these findings need further validation in independent cohorts of cases and controls.

Microsatellites are tandem repeats of 1–6 base pairs, and their variability is associated with numerous tumors, including MB. In a recent work, starting from WES and WGS data, the authors developed an algorithm able to identify a signature of 43 microsatellites that distinguished with high-sensitivity and specificity MB subjects from controls in two independent sets of MB cases and controls (180). Interestingly, *in silico* analyses revealed that genes harboring these microsatellite loci had cellular functions important for tumorigenesis (180).

Other Brain Tumors

EP originates from the walls of the ventricular system (79), arises between 0 and 4 years (**Figure 2**) (79), and has a 5-year overall survival of about 60% (181). EP is diagnosed in ∼33–53% of patients with type 2 neurofibromatosis, with high occurrence of truncating mutations in *NF2* (97). EP has recently been associated with Kabuki syndrome, with mutations in *KMT2D* (70) and rarely occurs in Turcot and MEN1 syndromes with mutations in *MSH2* and *MEN1*, respectively (79) (**Table 2**). To date, large studies on common variants and sporadic forms are lacking (**Table 1**). AS is classified into several forms including pilocytic, anaplastic, diffuse, and glioblastoma (182). Pilocytic AS is the most common form in children and young adults, with an average age at onset between 0 and 9 years (13) (**Figure 2**) and a 5-year survival of 94.1% (13). Regarding the genetic predisposition, one large study reported germline

TABLE 2 | Syndromes associated with pediatric tumors. Frequencies reported refer to the occurrence rate of pediatric cancers in patients with genetic syndromes.

Syndrome/disease	Inheritance pattern	Gene/s associated	Tumor	Frequency	References
Ataxia telangiectasia	AR	*ATM*	MB	Extremely rare	(18)
ATR-X syndrome	AR	*ATR-X*	OS	Extremely rare	(19)
Baller–Gerold syndrome	AR	*RECQL4*	OS	Extremely rare	(20, 21)
Beckwith–Wiedemann syndrome	Imprinting, AD	*CDKN1C*	NB	4–21%	(22, 23)
		KCNQ1OT1	RMS	7.5%	(24–28)
		11p15 or H19 loci	WT	7–30%/20%	(29, 30)
Bloom syndrome	AR	*RECQL3 (BLM)*	MB	Extremely rare	(31)
			OS	2%	(32, 33)
			WT	<5%	(29, 34)
Bohring-Opitz syndrome	AD	*ASXL1*	WT	7%	(35, 36)
CCHS/hirschsprung syndrome	AD	*PHOX2B*	NB	10–20%	(37–39)
Constitutional mismatch repair deficiency	AR	*MSH2, MSH6, MLH1, PMS2*	MB	11.6%	(33, 40)
Costello syndrome	AD	*HRAS*	NB	17%	(41)
			RMS	17%	(42–44)
Curry–Jones syndrome	Unknown	*GLI3*	MB	Extremely rare	(45, 46)
Diamond–Blackfan anemia	AD	*Unknown*	OS	<1%	(33, 47–50)
Denys–Drash syndrome	AD	*WT1*	WT	90%	(51)
DICER1 syndromes	AD	*DICER1*	RMS	Rare	(52–54)
			WT	<5%	(29, 55)
Familial paraganglioma/pheochromocytoma syndrome	AD	*SDHB*	NB	Rare	(56)
Fanconi anemia	AR	*BRIP1*, *BRCA2*, *PALB2*	NB	rare	(57)
		BRCA2, PALB2	MB,	25%	(58, 59)
			WT	>20%	(60–62)
Frasier syndrome	AD	*WT1*	WT	5–10%	(63)
Gorlin syndrome	AD	*PTCH1*	RMS	Rare	(64, 65)
			WT	<5%	(36, 65, 66)
		PTCH1	MB	<2%	(67, 68)
		SUFU		30-40%	
Hyperparathyroidism-jaw tumor syndrome	AD	*CDC73 (HRPT2)*	WT	<5%	(60)
Isolated hemihypertrophy	AD	*11p15 locus*	WT	6%/<5%	(69)
Kabuki syndrome	AD	*KMT2D*	EP	Extremely rare	(70)
Li–Fraumeni syndrome	AD	*TP53*	MB	14%	(68, 71)
			NB	rare	(72)
			OS	12%	(73–76)
			RMS	80%	(75, 77)
			WT	<5%	(29, 78)
MEN1 syndrome	AD	*MEN1*	EP	Rare	(79)
Mosaic variegated aneuploidy syndrome	AR	*BUB1B*	RMS	High	(80, 81)
		BUB1B, TRIP13	WT	>20%	(60, 80, 82, 83)
Muliebry nanism syndrome	AR	*TRIM37*	WT	<5%	(29, 84)
Nijmegen breakage syndrome	AR	*NBN*	MB	Extremely rare	(85)
		NBS1	RMS	Rare	(86, 87)
Noonan syndrome	AD	*PTPN11, KRAS*	NB	17%	(88)
		SOS1	RMS	Rare	(89–93)
Noonan-like syndrome	AD	*CBL*	RMS	Extremely rare	(94)
Neurofibromatosis type I	AD	*NF1*	NB	Rare	(95, 96)
			RMS	0.5%	(44)
Neurofibromatosis type II	AD	*NF2*	EP	3–6%	(68, 97)
Paget's disease of bone	AD	*Unknown*	OS	<1%	(98, 99)

(Continued)

TABLE 2 | Continued

Syndrome/disease	Inheritance pattern	Gene/s associated	Tumor	Frequency	References
Perlman syndrome	AR	*DIS3L2*	WT	50–60%	(33, 100)
PIK3CA-related segmental overgrowth	Unknown	*PIK3CA*	WT	<5%	(29, 101)
ROHHAD	Unknown	*Unknown*	NB	Rare	(39)
Rothmund–Thomson and RAPADILINO syndrome	AR	*RECQL4*	OS	30–60%, 13.3%	(33, 102–108)
Rubinstein–Taybi syndrome	AD	*CREBBP, P300*	MB	Extremely rare	(14)
		CREBBP	NB	Extremely rare	(77, 109)
Simpson–Golabi–Behmel syndrome	X-linked	*GPC3*	NB	10%	(77)
			WT	10%	(60, 82, 110)
Sotos syndrome	AD	*NSD1*	NB	Rare	(111, 112)
			WT	<5%	(36, 113)
Turcot syndrome	AR	*APC*	MB	<1%	(68, 114)
		MSH2	EP	53%	(68, 79)
WAGR syndrome	AD	*WT1*	WT	50%	(60, 115)
Weaver syndrome	AD	*EZH2*	NB	Rare	(116, 117)
Werner syndrome	AR	*RECQL2 (WRN)*	OS	7%	(108, 118–120)
Wolf–Hirschhorn syndrome	Unknown	*MSX1*	NB	Extremely rare	(121)
Xeroderma pigmentosum	AR	*DDB2, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, POLH, XPA, XPC*	MB	Extremely rare	(122)
13q deletion syndrome	Unknown	*RB1*	RB	Variable	(123)

AD, autosomal dominant; AR, autosomal recessive; EP, ependymoma; MB, medulloblastoma; NB, neuroblastoma; OS, osteosarcoma; RB, retinoblastoma; RMS, rhabdomyosarcoma; WT, Wilms tumor.

splicing mutations in the tumor-suppressor genes *MUTYH* and *ERCC2* and point mutations in *TP53* and *PMS2* (127) (**Table 1**). Pathogenic mutations in *NF1*, *BRCA2*, *FANCA*, and *GJB2* have been also identified in a recent study involving 280 patients with different forms of AS (126).

NEUROBLASTOMA

Neuroblastoma (NB) originates from neural crest cells and affects the nervous sympathetic system (183). NB exhibits unique features, such as early age of onset, high frequency of metastatic disease at diagnosis in patients over 1 year of age (**Figure 2**), and the tendency for spontaneous regression of tumors in infants. In high-risk cases, the survival rate is only 50% (183). NB tumors, as well as other pediatric cancers, present few recurrent somatic mutations but frequent chromosomic aberrations such *MYCN* amplification, 17q gain, 1p deletion, and 11q deletion (184).

Familial Neuroblastoma

Familial NB represents 1–2% of cases, with *PHOX2B* and *ALK* as major susceptibility genes (184) (**Table 1**). The first identified familial gene is *PHOX2B* (37, 145), already associated with congenital central hypoventilation syndrome (CCHS) (185) and encoding a transcription factor driving neural crest differentiation (186). NB-exclusive mutations are mainly missense and frameshift (187). *PHOX2B* germline mutations account for ∼10% of familial NB (188), but this gene is also mutated in 2% of sporadic cases (189). Subsequently, the major susceptibility gene was identified in *ALK*. Its gain-of-function mutations, which account for 75% of familial cases (134, 188), are mainly located in the kinase domain of the encoded tyrosine kinase receptor and show incomplete penetrance (190). *ALK* somatic mutations are also reported in 10–12% of primary sporadic NB tumors (134, 191). Additional NB-predisposing genes have not yet been discovered. Mutations in *KIF1Bβ* (141) and *GALNT14* (192) and in 16p12–13, 4p16, and 1p loci (193–195) (**Table 1**) have been reported in related patients, but further validations are needed.

Children suffering from specific cancer predisposition syndromes such as LFS and others (**Table 2**) show an increased NB risk (22, 38, 39, 41, 56, 57, 72, 77, 88, 95, 111, 116, 121). Thus, protocols for NB surveillance need to be established.

Sporadic Neuroblastoma

Only a small subset of sporadic NB cases has an identifiable somatic oncogenic point mutation (196, 197), suggesting that predisposing genetic factors found in GWAS studies could cooperate to increase disease occurrence (198, 199).

Uncommon, Moderate-Penetrance Variants

Recent studies focused on uncommon germline variants, which presumably have a larger effect on predisposition compared to common ones. In different studies, pathogenic and likely pathogenic variants were identified in predisposition genes such as *ALK*, *CHEK2*, *BRCA2*, *SMARCA4*, and *TP53* (**Table 1**) but also in candidate genes like *AXIN2*, *PALB2*, *BARD1*, *PINK1*, *APC*, *BRCA1*, *SDHB*, and *LZTR1* (2, 135, 146, 196, 197, 200) Specifically, *TP53* variants are strongly associated with NB

susceptibility (201). All the mentioned genes are involved in DNA repair and maintenance of genomic integrity (**Table 1**).

Common, Low-Penetrance Variants

GWAS studies identified several NB susceptibility loci (**Table 3**) including *CASC15* (160), *BARD1* (157), *LMO1* (175), *HACE1*, and *LIN28B* (155) associated with high-risk NB, whereas *DUSP12*, *HSD17B12*, *DDX4*, and *IL31RA* associated with the low-risk NB group (161, 198). Functional studies of these loci have highlighted the key role of GWAS in elucidating NB carcinogenesis. A SNP in the long non-coding RNA (lcnRNA) *CASC15* produces a truncated isoform, whose lower expression correlates with advanced disease (202). Loss of another lncRNA, *NBAT-1*, at the same locus, contributes to aggressive NB by increasing proliferation and impairing differentiation of neuronal precursors (203). Diverse functional studies have elucidated the role of *BARD1* and its variants in NB development (204). Variants in the *BARD1* promoter decrease the expression of the tumor-suppressor form which protects NB cells from DNA damage (205, 206), whereas variants in introns increase the expression of an oncogenic isoform, *BARD1β*, which stabilizes the Aurora kinases (207, 208). *LMO1* decreased expression, caused by a variant in a super-enhancer which disrupts GATA binding (209), reduces NB cell proliferation. Finally, the activation of *LIN28B*, due to genetic variants, can enhance MYCN levels via let-7 microRNA suppression (155, 210, 211). The genetic landscape of sporadic NB has been amplified with the discovery of additional susceptibility genes including *RSRC1*/*MLF1* and *CPZ* (159), *SPAG16* (177), *NEFL* (156), and *CDKN1B* (170).

Reanalyses of GWAS data have discovered novel mechanisms and genetic factors that promote NB development (**Table 3**). Two studies clearly demonstrate a cooperation between predisposing variants and somatic aberrations in NB initiation (**Table 4**). Indeed, SNPs in *MMP20* (167) and *KIF15* (168) increase NB susceptibility in the presence of 11q deletion and *MYCN* amplification, respectively, whereas another study shows that specific mtDNA haplogroups can influence the risk of NB (212). We have provided evidence that SNPs in *PARP1* and *IL6* might be predictive biomarkers of response to chemotherapy and prognosis (213, 214). Finally, our recent works found that NB shares risk loci with other complex diseases and tumors. Indeed, SNPs in 2q35, 3q25.32, and 4p16.2 are cross-associated with congenital heart disease (CHD) and NB (215), while 1p13.2 showed cross-association with NB and melanoma (216). Very recently, a cross-match investigation between germline alterations in pediatric patients with different solid tumors and CHD-related genes has identified that NB is among the tumors with the highest enrichment of germline pathogenic and likely pathogenic variants in these genes (3).

Constitutional Chromosomal Abnormalities

Highly associated with NB are hemizygous deletion in 1q21.1, disruption in *NBPF23* (217), and microdeletion in 16p11.2, containing *SEZ6L2* and *PRRT2* (218). Deletion including *SLFN11*, duplication of *SOX4*, and partial deletion of *PARK2* have been identified in three different patients, respectively (219).

TABLE 3 | Common, low-penetrance variants in genes predisposing to pediatric tumors and main biological pathways.

Pathways	Gene(s)	Tumors	References
Centrosome stabilization	*KIZ*	EWS	(152)
Cytoskeletal and adhesion signaling	*NHS*	WT	(153)
	PTPRM	MB	(154)
Differentiation	*NKX2-2*	EWS	(152)
	NEFL, *LIN28B*	NB	(155, 156)
DNA double-strand break repair (DSB)	*BARD1*	NB, WT	(157, 158)
Extracellular matrix remodeling	*MMP20*	NB	(159)
Genome stability and regulation of cell cycle	*BMF*	EWS	(152)
	CASC15/NBAT-1, *DUSP12*	NB	(160, 161)
	CCND2	MB	(162)
	MDM2, *MDM4*	RB	(163, 164)
Immunity pathways	*HACE1*, *IL31RA*	NB	(155, 161)
Metabolic pathways	*ACYP2*	OS	(165, 166)
	HSD17B12	NB	(161)
	PCSK9, *TCN2*	WT	(153)
Protein translation and modification	*CPZ*, *DDX4*, *KIF1*,	NB	(159, 161, 167, 168)
	DDX3X	MB	(162)
Replication and telomere maintenance	*TERC*, *NAF1*, *TERT*, *OBFC1*, *CTC1*, *RTEL1*	OS	(165, 166)
RET, RAS, and G-proteins signaling	*CDKN1A*	RB	(169)
	CDKN1B	NB	(170)
	KRAS	WT	(171)
RNA biogenesis and processing	*DDX1*	WT	(153)
	TARDBP	EWS	(172)
Sonic Hedgehog pathway (SHH)	*GLI2*	MB	(162)
Synaptic proteins and neurotransmitters	*DLG2*,	WT	(153)
	GRM4	OS	(173)
Transcriptional regulation and chromatin remodeling	*EGR2*, *NR0B1*, *RREB1*	EWS	(152, 172, 174)
	KMT2D, *MYC*, *MYCN*, *SMARCA4*	MB	(162)
	LMO1, *RSRC1/MLF1*	NB	(159, 175)
	NFIB	Metastatic OS	(176)
WNT signaling pathway	*CTNNB1*	MB	(162)
Others	2p25.2	OS	(173)
	SPAG16	NB	(177)

EWS, Ewing sarcoma; MB, medulloblastoma; NB, neuroblastoma; OS, osteosarcoma; RB, retinoblastoma; WT, Wilms tumor.

RETINOBLASTOMA

Retinoblastoma (RB) is a pediatric malignancy of the neural retina, commonly initiated by biallelic inactivation of *RB1* (220) and affecting one (unilateral) or both eyes (bilateral). The median age at diagnosis is 12 months in bilateral tumors and 24 months

TABLE 4 | Germline–somatic interactions identified in genes predisposing to pediatric tumors.

Tumors	Gene	Frequency	Somatic interaction	References
MB	*TP53*	Rare	DNA chromothripsis	(71)
	ELP1	Rare	Loss of the 9q arm and a second independent mutation event in *PTCH1*	(17)
NB	*KIF15*	Common	Increased NB risk in presence of *MYCN* amplification	(168)
	MMP20	Common	Increased NB risk in presence of 11q deletion	(167)
EWS	*EGR2*	Common	EWSR1-FLI1 chimera	(178)
	NR0B1	Common		(174)

EWS, Ewing sarcoma; MB, medulloblastoma; NB, neuroblastoma.

in unilateral ones (220) (**Figure 2**). Patient survival is >95% in high-income countries but <30% globally (220). The first studies on RB unveiled the importance of genetics in cancer; indeed, the "two-hit hypothesis" formulated by Knudson (221) on *RB1* has been paradigmatic for the understanding of tumor-suppressor genes and the study of familial cancers.

Familial Retinoblastoma

Hereditary RB encompasses about 40% of all cases with most having bilateral tumors, 15% unilateral, and 5% trilateral (associated with midline brain tumor) (220). Familial RB is distinctly associated with the *RB1* tumor-suppressor gene, which encodes pRB, a crucial regulator of the cell cycle. Germline mutations in *RB1* are inherited in 25% of cases in an autosomal-dominant manner. A broad spectrum of inactivating *RB1* germline mutations have been described, mainly nonsense and frameshifts affecting the coding region, few large deletions, and <5% silencing gene promoter (136). Penetrance and expressivity can vary within families due to partially functional *RB1* alleles (222, 223) or parent-of-origin effect (224). Influence of genetic modifiers such as *MDM2*, *MDM4* (225, 226), or *MED4* (227) and polymorphisms in p53 (228), *CDKN1A* (169), and *CDKN2A* (229) could also influence RB development. Reduced *MDM2* and *MDM4* expression may increase the *RB1* haploinsufficiency, whereas variants affecting the activity of p53 pathway effectors impact cell-cycle arrest. However, studies on larger cohorts of patients are required to confirm these findings. A small subset of hereditary RB patients is not carrier of *RB1* mutations. Investigation through a clinical exome gene panel within 3 families proposed *FGFR4*, *NQO1*, *ACADS*, *CX3CR1*, *GBE1*, *KRT85*, and *TYR* as possible candidate genes involved in RB oncogenesis, given their association with the retinoic acid pathway (230).

RB is generally described as retinoblastoma predisposition syndrome since germline *RB1* mutations lead to a high risk of second primary malignancies (231). Interestingly, RB onset is reported in 13q deletion syndrome, caused by deletion of part of the long arm of chromosome 13, where *RB1* is located (123, 232) (**Table 2**). Patients with this syndrome show a very wide phenotypic spectrum depending on the size and the location of the deletion (123, 232, 233).

Sporadic Retinoblastoma

Sporadic RB is always unilateral. Biallelic loss of *RB1* is found in 98% of cases, whereas 2% show *MYCN* amplification (234, 235). A significant proportion of sporadic RB exhibits somatic mosaicism for *RB1* mutations (236, 237).

Uncommon, Moderate-Penetrance and Common, Low-Penetrance Variants

Susceptibility variants have been investigated mostly in patients with hereditary RB. However, given the role of the p53 pathway in RB development, polymorphisms in genes such as *MDM2* (163), *MDM4* (164), and *CDKN1A* (169) could also influence the development of the sporadic form (**Table 3**). Uncommon variants conferring RB risk may be present in asymptomatic individuals. Indeed, high-throughput analysis revealed that several low-frequency *RB1* variants are present in the human population, including rare alleles disrupting splicing (238).

Constitutional Chromosomal Abnormalities

Mosaic and non-mosaic chromosomal deletions of 13q14 region are causative of RB (123, 239). Additionally, duplication of 1q21.1, containing the oncogene *BCL9*, has been reported in a patient with bilateral RB (240).

WILMS TUMOR

Wilms tumor (WT), also known as nephroblastoma, is the most common renal malignancy of childhood, with a median age at diagnosis between 2 and 3 years (241) (**Figure 2**). It is considered an embryonal tumor as it arises from the aberrant kidney development, due to genetic anomalies in genes essential for fetal nephrogenesis (29). WT treatment is successful with a 5-year overall survival of about 90% and 75% for localized and metastatic disease, respectively (82). It is estimated that about 10% of WT cases are caused by genetic predisposition factors, mainly represented by germline pathogenic variants or epigenetic alterations occurring early during embryogenesis (147, 242). The number of known susceptibility loci has significantly increased over the past years, even if our knowledge is still incomplete and further predisposition factors remain to be discovered. The landscape of somatic genetic alterations in WT is quite broad, with classical genetic changes involving *WT1*, the *IGF2* locus, the WNT pathway, *MYCN* and *TP53* but also driver mutations in several additional cancer genes including epigenetic remodelers, miRNA processing genes and transcription factors essential for nephrogenesis (29).

Familial Wilms Tumor

Several congenital malformation and cancer predisposition syndromes are associated with the risk of developing WT (**Table 2**). Some of the most known and characterized syndromes are associated with constitutional alterations in *WT1* at 11p13

(60). *WT1* was the first gene identified in WT and encodes a zinc-finger transcription factor, essential for renal and gonadal development (243). A syndrome frequently associated with high risk of developing WT (around 50%) is the Wilms tumor–aniridia syndrome (WAGR), caused by microdeletions of 11p13 including *WT1* and *PAX6* (115, 244). The second *WT1*-related disorder is Denys–Drash syndrome (DDS), due to missense variants in *WT1* exons 8 or 9, which affect critical residues in the zinc finger domains (51). The risk of WT in children with DDS is about 90% (241). Another syndrome, phenotypically similar to DDS but with a lower risk of WT development, is Frasier syndrome (FS), caused by splicing variants that result in an imbalance of *WT1* isoforms (63). The second major WT locus, identified at 11p15 (245), is also characterized by multiple germline epigenetic and genetic changes causing the overgrowth disorder Beckwith–Wiedemann syndrome (BWS). High WT risk is specifically associated with uniparental paternal disomy at 11p15 and to isolated H19 hyper-methylation that results in biallelic expression of *IGF2* and over-activation of the IGF signaling pathway (30, 246). **Table 2** reports other constitutional genetic mutations underlying both congenital syndromes and WT predisposition (34, 35, 61, 66, 69, 78, 80, 84, 100, 101, 110, 113).

WT is primarily a non-familial condition, with only about 2% of affected individuals belonging to familial pedigrees (29) (**Table 1**). A small proportion of familial cases are due to germline *WT1* variants (149, 150) and mutations in the H19 region of 11p15 (151). Two further predisposition loci at 17q21 (*FWT1*) and 19q13 (*FWT2*) were identified by genetic linkage studies, but the causative genes still remain not fully characterized (247). Another cause of familial WT is the presence of inactivating mutations in the *DICER1* miRNA processing gene, also causative of cancer susceptibility in DICER1 syndrome (55). Other recognized familial WT predisposition genes are *CTR9* and *REST* (144, 148, 248). *CTR9* encodes a key component of the PAF1 complex, implicated in maintenance of stem cell pluripotency (144), while *REST* encodes the RE1-silencing transcription repressor, well-known for its role in repressing neural development and differentiation (249). Rare biallelic *TRIP13* mutations have been found in a WES study on familial WT pedigrees (83). *TRIP13* encodes a member of the spindle assembly checkpoint complex, whose inactivation leads to chromosome segregation dysfunction and aneuploidy (83). Pathogenic inactivating mutations of *TRIM28* have been found in about 8% of familial WT in a sequencing study on 890 patients (147). These mutations have been found to show a strong parent-of-origin effect and a robust association with the epithelial subtype of WT (147, 250, 251). The same study reports constitutional mutations in *FBXW7*, *NYNRIN*, and *CDC73* as contributors to a small number of familial cases, and pathogenic mutations in *TRIM28, FBXW7,* and *KDM3B* as *de novo* events in children with sporadic tumors (147).

It is important to note that, to date, germline pathogenic variants have been identified only in a small proportion of familial WT cases and so that the underlying causative genetic events remain still obscure for the majority of individuals.

Sporadic Wilms Tumor

Many genetic causes of familial and syndromic WT also contribute to sporadic cases, e.g., constitutional *WT1* mutations and germline 11p15 anomalies (150, 151). It is currently estimated that in sporadic cases the number of predisposition genes is more than 20 (147). Next-generation sequencing (NGS) and GWAS approaches have allowed researchers to discover an ever-growing number of uncommon (**Table 1**) and common (**Table 3**) genetic variants associated with WT susceptibility.

Uncommon, Moderate-Penetrance Variants

Two recent WGS and WES studies have identified new pathogenic germline variants in *CHEK2* and *PALB2* in children with sporadic WT (131, 132). Both *PALB2* and *CHEK2* are involved in DNA repair pathways and are associated with breast cancer predisposition (62, 252). Germline mutations in *REST* and *TRIM28*, in addition to their role of familial WT predisposition genes, are also responsible for uncommon sporadic cases (148, 251). Additional pathogenic and likely pathogenic variants were identified in predisposition genes such as *TP53*, *DIS3L2*, and *MLLT1*, but also in candidate genes like *EP300*, *HDAC4*, *HACE1*, *ARID1A*, *NF1*, *MYCN*, and *GLI3* (131, 132, 137), that need to be validated in independent cohorts. Finally, exome and transcriptome sequencing studies have revealed constitutional mutations in the miRNA processing genes *DROSHA*, *DGCR8*, *DICER1*, and *XPO5* (131, 137), some of which associated with the blastemal subtype of WT (137).

Common, Low-Penetrance Variants

The first WT related GWAS study was performed by Turnbull et al. (153), using a dataset of 757 affected and 1.879 controls from North America and subsequently validated in two independent replication series from UK and US populations. They identified two significant SNPs at 2p24 (rs807624 and rs3755132), in the promoter of *DDX1*, and one SNP at 11q14 (rs790356) located near *DLG2*. They also identified candidate predisposition loci at 5q14, 22q12, and Xp22, located near the genes *PCSK9*, *TCN2*, and *NHS*, which need further validation (153). More recently, the group of Fu and colleagues performed two candidate gene studies on Southern Chinese populations and found a significant association between WT risk and *BARD1* (158) and *KRAS* (171) polymorphisms, respectively. However, both associations need to be validated in larger cohorts.

Constitutional Chromosomal Abnormalities

Few chromosomal aberrations and copy-number variations (CNVs) are known to be WT predisposing genetic factors. In addition to karyotypic abnormalities affecting 11p13 and 11p15 (60), a very small number of WT patients with gain of entire chromosomes have been reported, specifically with trisomy 18 and trisomy 13 (60). Rare chromosomal aberrations have been identified at 2q (60, 253, 254) and 7q (255, 256) regions, with terminal deletions and balanced and unbalanced translocations. A constitutional *de novo* balanced translocation was also identified in a child with bilateral WT, affecting the tumor-suppressor gene *HACE1*, also reported as NB susceptibility gene. *HACE1* controls growth and apoptosis and is often somatically

mutated in WT (257). Moreover, gain of *MYCN* (2p24), which is predominantly a somatic event, has been reported as a rare germline aberration (258). Finally, in 2020, a germline duplication of *SUZ12* has been detected in a WT patient carrying other germline pathogenic variants in new candidate cancer predisposition genes (3).

OSTEOSARCOMA

Osteosarcoma (OS) is the most common primary bone cancer. This tumor has a bimodal distribution with a high peak during adolescence and a smaller peak in elderly individuals (259) (**Figure 2**). Survival rates for children and young adults with non-metastatic disease have remained at 60–70%; however, outcome is reduced in patients with metastases (259). Unlike other childhood sarcomas, which are characterized by specific chromosome rearrangements and low mutation rate, complex genomic rearrangements are involved in OS. Indeed, OS exhibits extensive intra-tumoral heterogeneity and has a higher mutation rate (259).

Familial Osteosarcoma

OS is a sentinel cancer in many heritable cancer predisposition syndromes, including autosomal dominant cancer predisposition syndromes such as LFS (73–75) and Diamond–Blackfan anemia (47–50) (**Table 2**). Furthermore, recessive cancer syndromes associated with OS are Rothmund–Thomson syndrome (102–105), Baller–Gerold syndrome (20, 21), RAPADILINO syndrome (106, 107), Werner syndrome (118–120), Bloom syndrome (32), and ATR-X syndrome (19). OS has also been seen to arise in Paget's disease of bone (98, 99).

Sporadic Osteosarcoma

Targeted gene sequencing and WGS and WES studies have identified uncommon variants in tumor-suppressor and cancer predisposition genes (**Table 1**), while candidate gene, pathway studies, and GWAS have discovered common variants in genes involved in several key pathways for OS development (259) (**Table 3**).

Uncommon, Moderate-Penetrance Variants

In 2015, a sequencing study on 765 germline DNA samples showed the presence of uncommon *TP53* germline variants that could contribute to OS development; 3.8% of these variants were associated with LFS, and 5.7% were uncommon exonic variants of uncertain clinical significance (138). Another sequencing study on 1120 cases found 7/39 OS patients carrying pathogenic and likely pathogenic variants in *TP53*, *RB1*, *APC*, *MSH2*, and *PALB2* (2). In 2016, a targeted exon sequencing on 1162 patients with sarcoma found that >50% of all patients carried pathogenic variants in *TP53*, *BRCA2*, *ATM*, *ATR*, and in *ERCC2* (128). Among 11% of patients with OS, one patient showed a probable pathogenic variant in *ERCC2*. In the same work, an excess of functionally pathogenic variants in *ERCC2* was found to enhance cell sensitivity to cisplatin, commonly used in the treatment of OS (128). Recently, a sequencing study of 1244 OS patients showed that 28% of patients carried pathogenic and likely pathogenic variants in OS susceptibility genes, identifying new candidates (*CDKN2A*, *MEN1*, *VHL*, *POT1*, and *ATRX*) that require further confirmation in independent cohorts (129).

Common, Low-Penetrance Variants

In 2013, the first GWAS study on 941 cases and 3291 controls of European ancestry, identified two risk loci, one at 6p21.3 (rs1906953) mapping in intron 7 of *GRM4*, and the other at 2p25.2 (rs7591996) in an intergenic region (173). Subsequently, a GWAS study on OS metastasis at diagnosis identified rs7034162 at 9p24.1 (in *NFIB*) associated with metastasis (176). Functional investigations showed that reduced *NFIB* expression, due to the risk allele of the rs7034162 SNP, promoted an increase of OS cell migration, proliferation, and colony formation (176). In 2016, a case–control study identified that, for SNPs in genes associated with inter-individual variation in leukocyte telomere length (LTL) (*ACYP2*, *TERC*, *NAF1*, *TERT*, *OBFC1*, *CTC1*, and *RTEL1*), the allele associated with longer LTL increased OS risk, mainly rs9420907 in *OBFC1* (165). These findings were confirmed in 537 OS cases belonging to California Cancer Registry (166).

Constitutional Chromosomal Abnormalities

Next to the heterogeneous somatic CNV scenario present in OS, in a study conducted on 54 patients with childhood tumor, two large germinal CNVs were identified in 2 OS patients: dup4q13.33 of 476 kb containing *STATH*, *CSN1S2B*, *CABS1*, *CSN1S1*, *CSN2*, *HTN3*, *HTN1*, *CSN1S2A*, *C4orf40*, *ODAM*, *FDCSP*, and *CSN3*; and dup18q21.33 of 600 kb containing *RNF152*, *CDH20*, and *PIGN* (240). In 2020, a duplication of *DDX10* in an OS patient with a germline variant in *GJB2* has been reported (3).

RHABDOMYOSARCOMA

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood and represents a high-grade neoplasm of skeletal myoblast-like cells. Currently, 5-year overall survival of pediatric RMS exceeds 70% (260). The two major histological subtypes are embryonal (ERMS, 67%) and alveolar (ARMS, 32%) (261). ARMS is uniformly distributed among the different age groups (**Figure 2**) and has a worse prognosis; ERMS has a bimodal distribution (the first peak in early childhood and the second one in early adolescence) and has a better outcome (260, 262) (**Figure 2**). At somatic level, ARMS is often associated with fusion of *FOXO* and *PAX3* or *PAX7*, while ERMS does not show such translocations, but it is characterized by loss of heterozygosity at 11p15.5 as well as mutations in *TP53*, *NRAS*, *KRAS*, *HRAS*, *PIK3CA*, *CTNNB1*, and *FGFR4* (263). Since a small but substantial fraction of ARMS patients do not harbor one of these translocations, and tumors from those patients are biologically and clinically similar to ERMS, the disease classification has been further refined dividing RMS into "fusion-positive" RMS (FPRMS) and "fusion-negative" RMS (FNRMS) subtypes.

Familial Rhabdomyosarcoma

Although RMS is primarily sporadic (264, 265), it arises in several syndromes. Cancer predisposition syndromes appear to be more frequent in patients with ERMS than in those with ARMS (260). Among syndromes commonly associated with RMS and reported in **Table 2** (24–27, 42, 43, 52–54, 64, 75, 80, 81, 86, 87, 89–92, 94, 96), a high RMS risk is associated with RASopathies-like type I neurofibromatosis (NF1) (deletions in *NF1*), Costello syndrome (*HRAS* mutations), and Noonan syndrome (germline variants activating RAS-MAPK pathway), highlighting the tight dependence of RMS on the RAS pathway, which results to be activated in 40% of sporadic ERMS (263, 266, 267). In particular, up to 25% of children affected by Costello syndrome shows high RMS risk (43, 268). In addition, children who have a first-degree relative with cancer, particularly if the cancer occurred at a young age (<30 years), show an increase in RMS risk, especially of ERMS (269).

Sporadic Rhabdomyosarcoma

Unlike OS and Ewing sarcoma, GWAS studies for RMS have not been published (260) and few studies identified uncommon germline variants associated with tumor susceptibility (2, 52, 133, 139, 142, 270) (**Table 1**).

Many studies have found the presence of *DICER1* germline mutations in sporadic RMS patients for whom DICER syndrome has been ruled out (52, 142). WES and WGS on 1,120 patients with pediatric cancers identified germline pathogenic variants in 3/43 RMS patients in *TP53* and *BRCA2* (2). In a cohort of 66 patients with sarcoma, one patient with ARMS showed a protein-truncating variant (in *ERCC4*) co-occurring with predicted pathogenic mutations (in *ATM*, *FANCI*, and *MSH6*), suggesting a possible collective impact of these genetic variants on DNA repair and genomic instability, therefore conferring susceptibility to tumorigenesis (133).

EWING SARCOMA

Ewing sarcoma (EWS) is the second most frequent primary skeletal tumor that mainly affects bone and can also arise in soft tissue. It occurs in children, adolescents, and young adult (**Figure 2**). It is highly aggressive, with a survival of 70–80% for patients with standard-risk and localized disease and 30% for those with metastasis at diagnosis (20–25% of those resistant to intensive therapy) (271). EWS is characterized by low somatic mutation rate (272–274), mainly including fusions between *EWSR1* and members of the *ETS* gene family, usually *EWSR1-FLI1*, that play a key role in its pathogenesis. The chimeric protein EWSR1-FLI1 leads to the production of an oncogenic transcription factor that binds GGAA motifs (174, 271, 275, 276).

Familial Ewing Sarcoma

To date, no susceptibility genes to familial forms of EWS have been reported, and only case reports about siblings and cousins affected by this tumor have been documented (277, 278). On the basis of these isolated clinical cases, the presence of other cancer types among familial members of EWS patients (279, 280) suggests an important contribution of genetic susceptibility factors in this tumor. Nowadays, EWS is not considered part of predisposition syndromes because of its rare occurrence among these (281).

Sporadic Ewing Sarcoma

WES, WGS, and GWAS studies have led to the identification of uncommon (**Table 1**) and common (**Table 3**) germline variants associated with the risk of developing EWS. Despite the rarity and the paucity of information about familial cases, most of the known genetic scenario on this tumor concerns the sporadic form.

Uncommon, Moderate-Penetrance Variants

Two WGS and WES studies on EWS revealed an over-representation of uncommon pathogenic and likely pathogenic variants in DNA repair and cancer-predisposing syndrome genes (2, 130). Studies on small cohorts of patients identified other uncommon germline variants in *BRCA2* (146) and in *PTPRD* (140).

Common, Low-Penetrance Variants

In 2012, the first GWAS on EWS found 3 susceptibility loci at 1p36.22, 10q21, and 15q15, identifying a strong association of EWS risk with rs9430161 (25 kb upstream of *TARDBP*) and rs224278 (5 kb upstream of *EGR2*), and a modest association with rs4924410 (at 15q15) (172). The second GWAS detected a tagging variant strongly associated with EWS at 15q.15.1 (rs2412476 near *BMF*) and new risk loci at 6p25.1, 20p11.22, and 20p11.23 (152). Expression quantitative locus (eQTL) analyses identified candidate genes at 6p25.1 (*RREB1*) and 20p11.23 (*KIZ*) (152). Independent studies showed that a different number of germline GGAA repeats in polymorphic enhancer-like GGAA microsatellites impacts the binding between these regulatory elements and EWS cancer driver mutations (*EWSR1-FLI1*), affecting downstream genes expression (174, 178, 282).

These studies further suggest that cooperation between regulatory germline variants and somatic mutations can drive oncogenesis and create a major source of inter-tumor heterogeneity, determining clinical outcome and drug response through modulation of a druggable key downstream player.

Constitutional Chromosomal Abnormalities

Only one study reports the presence of germline CNV associated with EWS, describing a 14-year-old male with EWS carrying an intragenic deletion in *PTPRD* (283). Notably, germline and somatic variants in *PTPRD* have been already identified in a limited number of EWS patients (140).

CONCLUSIONS

For a long time, the prevalence of childhood cancer attributed to genetic predisposition was generally considered very low. However, to date, WGS, WES, and GWAS studies performed on pediatric cancers have made it possible to highlight a strong contribution of germline variants to tumorigenesis, helping us to better understand the etiology underlying pediatric tumors. Indeed, an important body of work allows us to highlight that

the prevalence of hereditable risk variants in pediatric solid cancers ranges between 6% and 18% (**Figure 3**). These variants generally affect the functions of genes belonging to biological processes linked to tumorigenesis, such as cell-cycle control, apoptosis, DNA repair, and transcriptional regulatory programs. The enrichment of genetic alterations in these pathways is often due to a bias because, since germline variant analysis is a highly challenging task in general, the vast majority of studies are based on a "candidate-gene" approach, which means they focus on specific subsets of genes already known to play a key role in cancer predisposition and tumorigenesis. For this reason, it may be useful exploiting a genome-wide scale approach, e.g., exome-wide association studies, to investigate the presence of genetic alterations predisposing to cancer also in genes involved in pathways others than the ones above mentioned. This approach may contribute in a meaningful way to the current knowledge of the mechanisms underlying solid pediatric tumors onset.

A very recent study reports a high number of germline variants in new candidate susceptibility genes, highlighting that some of them carry druggable alterations (3). It should be emphasized that the presence of germline variants in target therapeutic genes could improve current approaches of personalized therapy, making them more efficient and less toxic to patients. Furthermore, a more in-depth investigation of the germline component underlying tumor development should also be performed on pediatric solid tumors for which there is not yet a broad knowledge of germline landscape (e.g., thyroid carcinoma, melanoma) (284–289).

Our literature review reveals that the presence of specific germline mutations is often associated with increased frequency of somatically acquired cancer-specific abnormalities (such as aberrations, rearrangements). The interplay between somatic and germline mutations may be at the basis of high interindividual tumor heterogeneity (290). For example, the cooperation between regulatory germline variants and somatic mutations underlines the importance of regulatory regions to stratify patients into risk groups to predict the clinical outcome and therapeutic approaches (290). In NB, inherited deleterious variants in genes that code for proteins involved in chromosomal segregation, centrosome segregation, DNA repair, and spindle apparatus machinery are thought to be the cause of chromosome instability at somatic levels (199). A similar germline–somatic interaction has been proposed for MB; indeed, germline *TP53* mutations are often found in combination with tumors characterized by catastrophic DNA chromothripsis. Determining if germline risk alleles predispose to genomic instability in

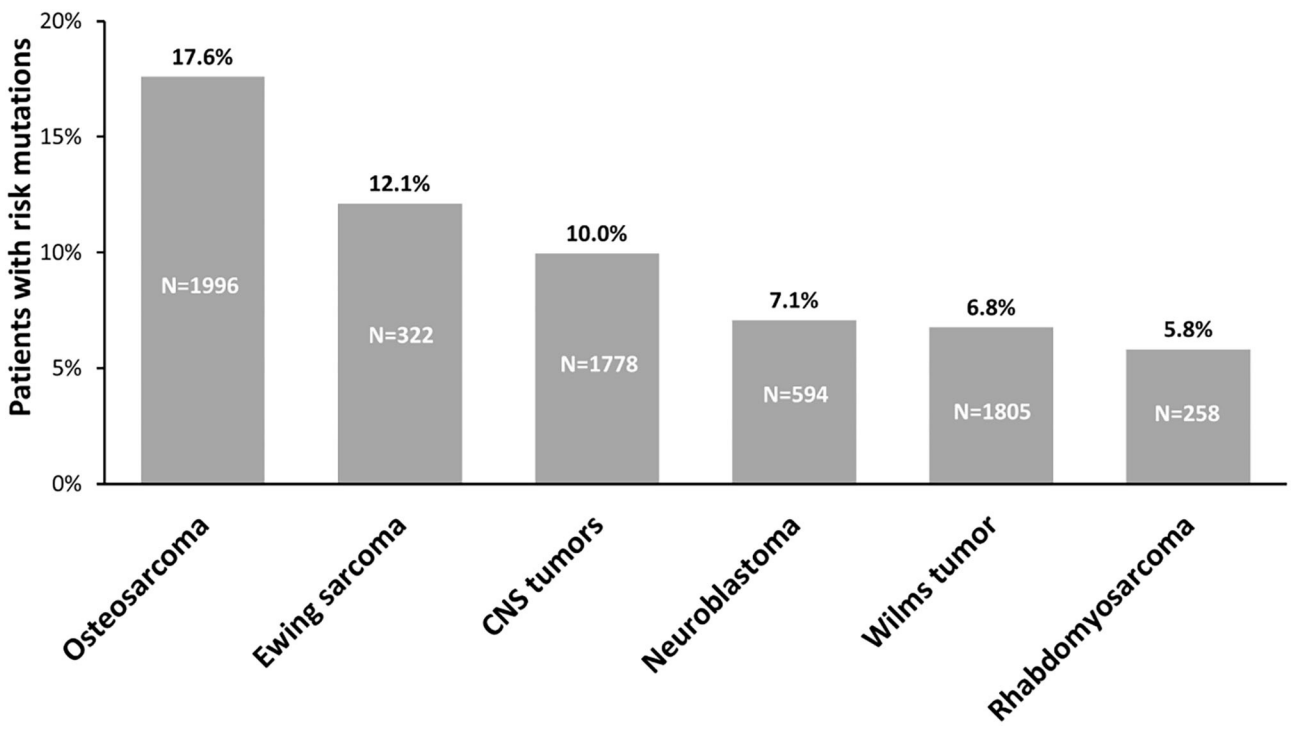

FIGURE 3 | Prevalence of germline predisposition in pediatric tumors. The percentage of germline predisposition due to uncommon, moderate-penetrance variants, reported above the bars, has been calculated evaluating the number of patients carrying pathogenic and likely pathogenic variants on the total number of patients from the cohorts analyzed for each tumor: CNS tumors: (3, 15, 17); neuroblastoma: (2, 3, 135, 146, 196, 197, 200); Wilms tumor: (3, 131, 132, 137, 148, 150, 251); osteosarcoma: (2, 3, 129, 138); rhabdomyosarcoma: (2, 3, 52, 139); Ewing sarcoma: (2, 3, 130, 146). N, number of patients analyzed in cohorts; CNS, central nervous system.

pediatric cancers is an important research objective for biologists and geneticists. Another interesting research field is related to the impact of risk alleles on genomic regions that regulate mutated cancer driver genes. The mechanisms underlying this type of interaction between germline–somatic variation have been elegantly elucidated in the EWS (174, 178, 282), and it is reasonable to think that it is common to other pediatric tumors as well. No relevant study has investigated the possible interplay between germline variations and epigenetic somatic events. For instance, there is an urgent need to find possible associations between germline risk alleles and DNA methylation of tumor. Studies integrating information on germline, somatic, and epigenomic variations using gene expression data as the intermediate phenotype may unravel the biological mechanisms underlying oncogenic interactions and cooperation of these different types of genomic variations.

The low number of recurrent somatic mutations in some pediatric cancers, compared to adult ones (135), does not explain the clinical heterogeneity and the resulting need for personalized therapies in tumors. Confirming a germline contribution to the clinical heterogeneity, some studies have highlighted that specific pathogenic variants are much more common in specific tumor histotypes (137, 147) and these associations could be used for the management and stratification of patients. Thereby, implementing screening tests with the introduction of germline detection would bring clinical benefits. In addition, screening for germline and somatic components of the tumor could lead to the identification of new prognostic markers to monitor cancer and predict clinical outcome. Finally, the use of these information in screening tests is important in the context of genetic counseling, to monitor and supervise family members of patients.

It is also important to note that many genetic syndromes such as Beckwith–Wiedemann, Costello, Fanconi anemia, Gorlin, Noonan syndrome, Li-Fraumeni, and others (**Table 2**) are both characterized by genetic and/or allelic heterogeneity and associated with the risk to develop different types of pediatric cancers. Therefore, NGS-based cancer gene panel tests should be performed in children with a genetic syndrome to ensure the patient a more precise diagnosis and to be able to assess the risk of developing a cancer disease. A clinical management that includes a cancer genetic test not only is useful to indicate a modification of the surveillance that also integrates periodic and cancer specific diagnostic tests, but over time it will increase our knowledge of genetic risk variants and thus will give a clearer picture of cancer risk in children affected by genetic syndrome. This surely can have a positive impact on improving patient care and survival.

AUTHOR CONTRIBUTIONS

MC and AI contributed to the design, reviewing, and editing of this manuscript. AM, SC, TM, and MT contributed to the design, writing, and editing of this manuscript. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by Associazione Italiana per la Ricerca sul Cancro (Grant no. 19255 to MC and Grant no. 20757 to AI), Fondazione Italiana per la Lotta al Neuroblastoma (to MC), Associazione Oncologia Pediatrica e Neuroblastoma (to MC), and Regione Campania SATIN grant 2018-2020 (to MC).

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Conflict of Interest: All authors were employed by the company CEINGE Biotecnologie Avanzate.

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frontiers in Oncology

REVIEW
published: 29 October 2020
doi: 10.3389/fonc.2020.566822

Cancer Predisposition Syndromes and Medulloblastoma in the Molecular Era

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OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Oncology

Received: 28 May 2020
Accepted: 30 September 2020
Published: 29 October 2020

Citation:
Carta R, Del Baldo G, Miele E, Po A, Besharat ZM, Nazio F, Colafati GS, Piccirilli E, Agolini E, Rinelli M, Lodi MC, Cacchione A, Carai A, Boccuto L, Ferretti E, Locatelli F and Mastronuzzi A (2020) Cancer Predisposition Syndromes and Medulloblastoma in the Molecular Era. Front. Oncol. 10:566822. doi: 10.3389/fonc.2020.566822

Medulloblastoma is the most common malignant brain tumor in children. In addition to sporadic cases, medulloblastoma may occur in association with cancer predisposition syndromes. This review aims to provide a complete description of inherited cancer syndromes associated with medulloblastoma. We examine their epidemiological, clinical, genetic, and diagnostic features and therapeutic approaches, including their correlation with medulloblastoma. Furthermore, according to the most recent molecular advances, we describe the association between the various molecular subgroups of medulloblastoma and each cancer predisposition syndrome. Knowledge of the aforementioned conditions can guide pediatric oncologists in performing adequate cancer surveillance. This will allow clinicians to promptly diagnose and treat medulloblastoma in syndromic children, forming a team with all specialists necessary for the correct management of the other various manifestations/symptoms related to the inherited cancer syndromes.

Keywords: pediatric brain tumors, cancer predisposition, hereditary neoplastic syndromes, cancer syndromes, medulloblastoma, cancer genes

INTRODUCTION

Medulloblastoma (MB) is the most frequent malignant tumor of the central nervous system (CNS) in childhood, representing 15–20% of all CNS neoplasms (1). It mainly affects the pediatric age with a 10-fold higher frequency than in adults (2). Children are diagnosed generally between 2 and 8 years old (median of 6 years old), with 50% of cases occurring in children under 5 years old and with a male/female ratio of 2:1 (3).

Clinical manifestations are initially related to intracranial hypertension and to the tumor's mass effect in the posterior fossa, including headaches, nausea, vomiting, ataxia, other motor deficits, and visual impairment. MB diagnosis is suspected based on neuroimaging of the brain and spine. Disease staging is established on magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) cytology (4), with about 35% of cases being metastatic at diagnosis (5).

Histological classification of MB distinguishes four variants: classic (68–80%); desmoplastic/nodular (7%), with a more favorable prognosis in children under 5 years old; MB with extensive nodularity (3%), generally found in young patients and sometimes associated with nevoid basal cell carcinoma syndrome; and large cell/anaplastic (10–22%), characterized by a more aggressive clinical behavior (6).

Treatment of MB is based on surgical resection, chemotherapy, and cranio-spinal irradiation (CSI). Due to the severe adverse effects of CSI, such as neurocognitive disability, endocrine dysfunction, impaired growth, infertility, and increased risk of secondary malignancies, great effort has been dedicated to reduce, differ, or omit radiation therapy, especially in children <3–5 years of age.

Among genetic defects, MYC amplification is the most recurrent and is associated with a worse prognosis (7–9).

A risk stratification based on histopathological subtype, age at diagnosis, staging, residual disease, MYC status, and molecular subgrouping allows a distinction of low-, average-, and high-risk patients (10). For low- and average-risk patients (characterized by age over three years old, absence of metastatic and/or residual disease, histotype other than anaplastic, absence of MYC amplification and/or TP53 mutations), 5-year overall survival (OS) is between 75% to over 90% (11–14), while high-risk patients show 5-year OS around 50–75% (11, 15–19).

More recently, four molecular MB subgroups have been identified and included in the 2016 WHO Classification of Tumors of the Central Nervous System (20): MB_{WNT}, MB_{SHH}, Group 3, and Group 4 (21). Molecular subgrouping reflects developmental aspects of the tumors' cell of origin and has been shown to have prognostic significance.

Cancer predisposition syndromes' importance has increasingly been recognized in pediatric neuro-oncology. According to Waszak et al. germline mutations in cancer predisposition genes account for about 5–6% of medulloblastoma diagnoses (22). Constitutional genetic defects are expected to result in deregulation of specific molecular pathways, leading to tumor development. Despite the significant amount of previous knowledge on inherited conditions predisposing to MB and the extensive molecular characterization of these tumors, limited attention has been given in the literature to their interconnection.

The main purpose of this review is to describe the association of cancer predisposition syndromes with MB molecular subgroups, including epidemiological, clinical, genetic, diagnostic, and therapeutic implications.

METHODS

The authors conducted a literature search describing the issue of CNS tumors and cancer predisposition syndromes. Research studies were selected based on research topics ("cancer predisposition syndrome," "brain tumor genetics," "brain tumor cancer predisposition syndrome," "medulloblastoma predisposition syndromes," "medulloblastoma in childhood") found in PubMed considering the last 10 years until April 2020. These studies were classified according to their relevance. In the selected studies the data were carefully evaluated, and they are described in detail and discussed in the following sections. The association between the different cancer predisposition syndromes described below and the related molecular subgroups of MB is summarized in **Figure 1**. The main cancer predisposition syndromes associated to pediatric MB and their related molecular, pathological, clinical, and prognostic features are summarized in **Table 1**.

Medulloblastoma Molecular Subgroups

Main features of MB subgroups are:

- Wingless (WNT) accounts for about 10% of diagnoses and is found mainly in girls with a peak between 10 and 12 years of age. The most common histological variant is classic. Approximately 85–90% of MB_{WNT} harbor somatic mutations in exon 3 of *Catenin beta 1 (CTNNB1)*, which causes stabilization and nuclear accumulation of β-catenin leading to uncontrolled activation of WNT signaling (23, 30). Patients with MB_{WNT} without *CTNNB1* mutations can harbor a mutant *APC* tumor suppressor gene, which is involved in the ubiquitination and consequently degradation of β-catenin (22). MB_{WNT} have a low tendency to metastasize and patients under 16 years of age have an excellent prognosis. Therefore, some ongoing clinical trials, PNET5 and SJMB12, are currently investigating de-escalation of therapy (19).
- Sonic hedgehog (SHH) accounts for about 30% of all MB diagnoses and has a bimodal distribution, with peaks in children <3 years of age and in young adults >16 years of age (21). This subgroup affects both sexes almost equally with a slight predominance in males among infants (31). The histological variant is frequently desmoplastic/nodular. MB_{SHH} harbor germline or somatic mutations in genes involved in SHH signaling pathway, leading to its constitutive activation, such as deletions or loss-of-function alterations in *Patched 1 (PTCH1)* (43% of patients) or *Suppressor of fused (SUFU)* (10%), activating mutations in *Smoothened (SMO)* (9%), amplification of *GLI1*/*GLI2* (9%) or *MYCN* (7%) (23, 32). More recently, four SHH subtypes have been identified (SHH_{α}, SHH_{β}, SHH_{γ}, SHH_{δ}) with distinct biological and clinical features (33). Older children with MB_{SHH} can harbor germline or somatic *Tumor Protein 53 (TP53)* mutations, associated with a poor prognosis (25, 32).
- Group 3 accounts for about 25–28% of all MB diagnoses and is exclusively found in childhood, with a male sex predominance. It is associated with metastatic disease at diagnosis and with large cell/anaplastic histological variant. About 17% of Group 3 MBs harbor *MYC* amplification. Among MB subgroups, Group 3 is characterized by the

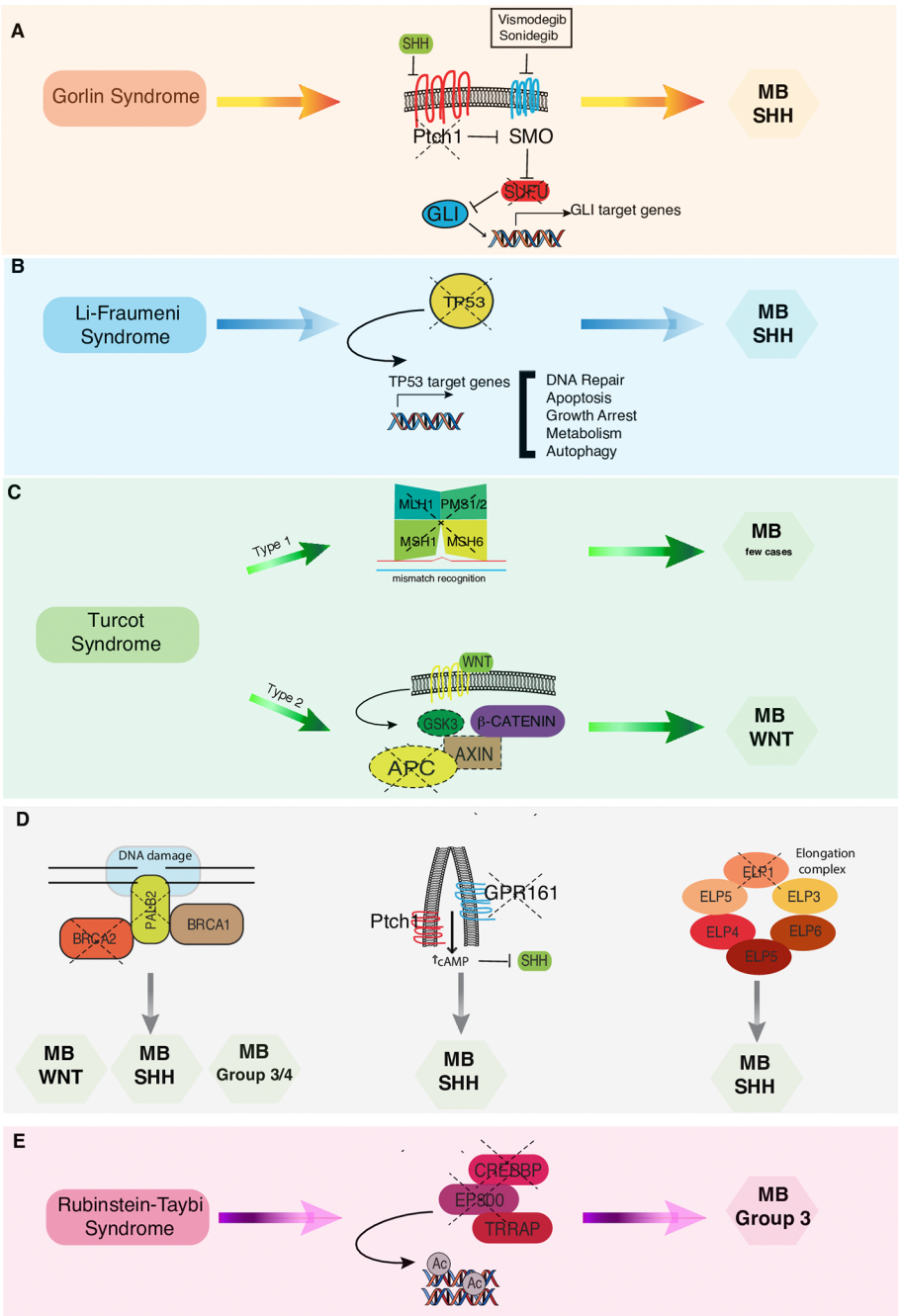

FIGURE 1 | Correlations between cancer predisposition syndromes and MB subtypes. **(A)** In Gorlin syndrome both PTCH1 and SUFU mutations have been associated to MB-SHH subgroup. Vismodegib and Sonidegib are selective antagonists of the transmembrane activator Smoothened (SMO). **(B)** In Li-Fraumeni syndrome los of TP53 finctions results in increased risk of developing MB-SHH subtype. **(C)** In Turcot syndrome, twotypes have been distinguished: Type 1 genetically related to the mutationof the mismatch repair genes and Type 2 related to APC mutation that are more commonly associated with MB-WNT subtype. **(D)** Pathogenic germline mutations in BRCA2, PALB2, GPR161, and ELP genes have been recently associated to an increased risk of developing different MB subtypes. **(E)** In Rubenstein-Taybi syndrome mutations in CREEBP and EP300 genes predispose to MB Group 3 onset.

poorest prognosis, especially in the presence of metastatic disease, isochromosome 17q, and *MYC* amplification (19).

- Group 4 is the most common MB molecular subgroup, accounting for about 35% of diagnoses. It is mostly found in males and more frequently associated to classic histological variant. It is characterized by an overall intermediate prognosis; however, a subset of patients with either chromosome 11 loss or 17 gain have an excellent prognosis (19).

TABLE 1 | Cancer predisposition syndromes associated to pediatric medulloblastoma and their related molecular, pathological, clinical, and prognostic features.

Predisposition genes	Cancer syndrome	MB prevalence (%)	MB median age at diagnosis (years)	Molecular subgroup	MB histologic subtype	Clinical features	5 year-OS (%)	References
PTCH1	Gorlin	<2–4.5	2	SHH	Desmoplastic/nodular with extensive nodularity	Palmar or plantar pits, odontogenic keratocysts, basal cell carcinomas	85*	Waszak et al. (23)
SUFU	Gorlin	2–33	2	SHH	Desmoplastic/nodular with extensive nodularity	Palmar or plantar pits, odontogenic keratocysts, basal cell carcinomas	85*	Waszak et al. (23); Smith et al (24)
TP53	Li Fraumeni	1	9.8	SHH WNT	LCA, Classic	Soft tissue sarcomas, osteosarcomas, glioblastomas/astrocytomas, choroid plexus carcinomas, breast cancers	27	Waszak et al. (23); Zhukova et al. (25)
MLH1, MSH2, MSH6, PMS1, PMS2	Turcot type1	unknown	unknown	unknown	unknown	Café-au-lait spots	unknown	
APC	Turcot type2	1	9.2	WNT SHH (rarely)	Classic	Gastrointestinal symptoms (diarrhea, constipation), neurological symptoms (headache, vomiting, visual and/or hearing and/or sensorimotor deficits)	80-100	Waszak et al. (23); Surun et al. (26)
BRCA2	unknown	1	5.7	SHH WNT SHH	Classic, desmoplastic/ nodular, LCA, with extensive nodularity Classic	unknown Fanconi Anemia phenotype (biallelic mutations)	25**;100***	Waszak et al. (23) Present report Present report
PALB2	unknown	<1		SHH Group3 Group 4	unknown	unknown	75	Waszak et al. (23)
GPR161	unknown	3.4****	unknown	SHH	unknown	unknown	unknown	Tischkowitz et al. (27)
ELP1	unknown	unknown	6.3	SHH	Desmoplastic/nodular	unknown	92	Hwang et al. (28)
CREBBP; EP300	Rubinstein-Taybi	0.05*****	unknown	Group3*****	unknown	Growth retardation, obesity, facial, skeletal and neurological anomalies, cognitive/psychiatric disorders, pilomatricomas	unknown	Carter et al. (29)

** cumulative PTCH1 and SUFU.*

*** compound heterozygous BRCA2.*

**** heterozygous germline BRCA2.*

***** referred to patients with MB_{SHH} subgroup.*

****** limited data.*

GORLIN SYNDROME

Gorlin syndrome (GS) (OMIM #109400), also known as Gorlin-Goltz syndrome, or nevoid basal cell carcinoma syndrome (NBCCS), or basal cell nevus syndrome (BCNS), was first described by Gorlin and Goltz in 1960 (34). The incidence of GS reported is about 1 in 15.000 births (35) and is equal between males and females (36). The prevalence varies from 1:30,000 to 1:256,000 based on different reports (37–40). Prevalence data could be even greater since milder cases of GS could remain undiagnosed (41, 42).

Clinical Phenotype

GS is characterized by the onset of multiple jaw keratocysts, most frequent in the second decade of life, and/or basal cell carcinomas (BCCs), generally starting from the third decade. Sixty percent of all patients have a recognizable phenotype. More than 100 features have been associated with GS, and the most representative are listed in **Table 2** (39, 40, 43).

Genetic Basis

Heterozygous germline mutations leading to the aberrant activation of SHH signaling are involved in GS, most frequently *PTCH1*, followed by *SUFU*. *PTCH1* and *SUFU* mutations work at different levels by disabling SHH pathway signaling, which is normally active during brain development, thus promoting proliferation and inhibiting apoptosis (24, 44–47).

Correlation With Medulloblastoma

In 1963 Herzberg and Wiskemann first described the association between GS and MB that has been also confirmed by various published studies (48).

In the first large population based study of GS, Evans et al. investigated the incidence of GS in 173 consecutive cases of MB in the North-West of England between 1954 and 1989; they observed a 5% incidence of GS in MB patients with less than 5 years of age, conversely, the incidence of MB in the GS population considered in this study was 3.6% (49). The mean age at MB diagnosis was 2 years in GS patients, earlier than that described in the general population with sporadic MB (38). The desmoplastic/nodular and the extensive nodularity subtypes of MB are the most frequently described (50, 51). The risk of MB in subjects with germline mutations of PTCH1 reported in a large series of 115 individuals with related GS-PTCH1 was <2%, while individuals with GS and SUFU germline mutations presented an approximately 20 times higher risk (33%) (24).

Diagnosis

Many individuals with GS are only recognized in adulthood. However, there are clinical signs that could appear early and guide the diagnosis, such as the presence of odontogenic keratocysts in children <20 years of age, basal cell carcinomas in persons <20 years of age, palmar or plantar pits, lamellar calcification of the falx cerebri, and MB with desmoplastic histology in combination with other major or minor criteria (52). Current diagnostic criteria for GS are summarized in **Table 3**. Diagnosis can be made if 2 major or 1 major and 2 minor criteria are fulfilled (36).

Cancer Surveillance

Surveillance protocols for individuals affected by GS have been proposed by several authors. As suggested in the consensus statement from the first international colloquium on GS, all individuals with GS should perform annually an assessment with a geneticist. A dermatological evaluation is also recommended annually until the first basal cell carcinoma is found, and then every 6 months. Baseline digital Panorex of jaw should be performed starting from the age of 3 years (or as soon as tolerated) and repeated annually before the detection of a first jaw cyst, and then every 6 months (until no jaw cyst for 2 years or until the age of 21).

A baseline echocardiographic evaluation is recommended to exclude cardiac fibromas; in females a pelvic ultrasound for fibromas is also recommended, starting from puberty.

A baseline spine film should be performed at age 1 or at time of diagnosis, and if a skeletal anomaly is found, it must be

TABLE 2 | Principal clinical features associated with Gorlin Syndrome.

Clinical features	Description
Macrocephaly	Head circumference increases above 97th percentile until age 10 to 18 months and then maintains its centile
Facies features	Frontal bossing, coarse facial features, and facial milia in about 60% of individuals with *PTCH1* mutation; more subtle in individuals with *SUFU* mutation
Jaw keratocysts	Can arise early as from five years of age, with a peak in the teenage years; usually present with painless swellings and if untreated can lead to tooth disruption and jaw fracture
Other congenital malformations	Cleft lip/palate; polydactyly; skeletal anomalies (bifid ribs, wedge-shaped vertebrae, short 4th metacarpal); various eye anomalies (strabismus, hypertelorism, cataract, orbital cyst, microphthalmia, retinal epithelium alterations)
Skin anomalies	Pits in the palm of the hand
Other anomalies	* Ectopic calcifications, frequently in the falx cerebri in more than 90% of patients by age 20 years

TABLE 3 | Current diagnostic criteria for Gorlin Syndrome.

Major Criteria	Multiple basal cell carcinomas (more than five in a lifetime) or basal cell carcinoma occurring at a young age (<30 years old)
	Jaw keratocysts
	Two or more palmar/plantar pits
	Lamellar calcifications of the falx cerebri or clear evidence of calcification in an individual younger than age of 20 years
	First degree relative with Gorlin Syndrome
Minor Criteria	Childhood medulloblastoma
	Lympho-mesenteric or pleural cysts
	Macrocephaly (>97th percentile)
	Cleft lip/palate
	Rib anomalies (bifid, splayed, extra ribs) or vertebral anomalies (bifid vertebrae)
	Ocular anomalies (cataract, developmental defects, pigmentary changes of the retinal epithelium)

repeated every 6 months, or sooner if necessary. A routine developmental screening, including an assessment of vision, hearing, and speech, is recommended annually.

Annual brain MRI with contrast has been recommended until the age of 8 (52).

However, Smith and colleagues recently described the risk stratification of MB development between *PTCH1* and *SUFU* mutation carriers, recommending the performance of brain MRI only for patients carrying *SUFU* mutation (24).

Expert consensus recommendations for tumor surveillance of gene carrier and family members were proposed in 2016 based on a literature review and discussion in the AACR Childhood Cancer Predisposition Workshop held in Boston, Massachusetts, in October 2016 (see **Table 4**) (53).

Therapeutic Approaches

Vismodegib and Sonidegib are selective antagonists of the SHH pathway that act by binding to the transmembrane activator SMO, inhibiting the activation of the downstream SHH pathway.

Vismodegib is the first SHH pathway inhibitor approved by U.S. Food and Drug Administration (FDA) in 2012 and by European Medicines Agency in 2013 for the treatment of advanced or metastatic basal cell carcinomas (54, 55).

Sonidegib is approved by the FDA in adult patients for the treatment of locally advanced recurrent basal-cell carcinomas after radiation or surgery or for patients that cannot undergo surgery or radiotherapy (56).

A systemic review and meta-analysis about phase I and phase II Sonidegib and Vismodegib clinical trials highlighted that they are both well tolerated and with anti-tumor activity in MB_{SHH}. The efficacy of Sonidegib was better than Vismodegib in pediatric MB_{SHH}; however, this has been observed in 3 pediatric patients and further studies are needed for a reliable result (57).

Since SHH signaling has a crucial role during development, along with reports of younger patients treated with SMO inhibitors that show various growth plate complications, their use is not recommended in skeletally immature patients (58).

TABLE 4 | Gorlin Syndrome surveillance recommendations.

***PTCH1* mutation carriers**	Basal cell carcinoma screening annually by age 10, with increased frequency after first basal cell carcinoma observed Baseline echocardiogram in infancy, dental exams with jaw X-ray every 12 to 18 months beginning at age 8, and an ovarian ultrasound by age 18 Low risk of medulloblastoma: no radiographic screening unless concerning neurologic exam, head circumference change, or other unusual signs or symptoms If medulloblastoma: radiation-sparing treatment given risk of radiation-induced skin cancers
***SUFU* mutation carriers**	Same as *PTCH1* mutation carriers, with the exception of no jaw X-rays, as keratocysts have not been described Additional medulloblastoma screening: consider every 4 month brain MRI through age 3 and then every 6 month brain MRI until the age of 5[a]. Radiation-sparing treatments are again recommended if a brain tumor should occur

[a]*Data to support optimal frequency and timing of imaging are not currently available.*

LI-FRAUMENI SYNDROME

Li-Fraumeni Syndrome (LFS) (OMIM #151623) is one of the most aggressive cancer predisposition syndromes, first described in 1969 by Frederick Li and Joseph Fraumeni Jr (59). LFS is a rare autosomal dominantly inherited disorder caused by germline mutation of *TP53*, the "guardian of the genome" (60–62). Loss of p53 function in affected individuals is responsible for an increased risk of developing various solid and hematologic cancers (63). LFS has an estimated prevalence of 1 in 5,000 to 1 in 20,000 (64, 65). However, according to Andrade et al., prevalence estimates of the LFS could be higher (1 in 3,555–5,476), reflecting the complexity linked to a wide phenotype and a variable penetrance (66).

Genetic Basis

TP53 gene is located at chromosome 17p13.1 and is composed by 14 coding exons: 10 encode TP53 protein, one a non-coding exon, and three alternative exons (67). *TP53* acts as a tumor suppressor gene: in unstressed cells TP53 is unstable and, after exposure to genotoxic stressors, it accumulates and induces the expression of various target genes involved in the regulation of critical cellular processes (growth suppression, apoptosis, DNA repair). Various mechanisms have been proposed to explain how the mutated TP53 protein contributes to tumor formation, including loss of TP53 tumor suppressor function and consequently the dysregulation of its target genes, the "dominant negative" effect in which the mutated TP53 protein inhibits wild-type TP53 protein and the "gain-of-function effect" in which the altered TP53 protein acquires new oncogenic properties.

Clinical Phenotype

Both children and adults affected by LFS have an increased risk of developing multiple primary tumors (68). The most frequent six "core" cancers, their relative prevalence estimates, and other less frequent types of tumor reported in LFS are summarized in **Table 5** (60, 69, 70). Considering all ages, the most frequent tumor reported in LFS families is breast cancer, with a median age at onset of 33 years in females (65, 70–73). Soft tissue sarcomas and osteosarcoma are the most common tumors in children and adolescents with LFS (65, 70, 74). The most common type of CNS tumors is glioblastoma/astrocytoma (65, 71). Choroid plexus carcinomas (CPC) are more tightly associated with LFS since 45–100% of children with CPC show a germline TP53 mutation (65, 75–78).

Correlation With Medulloblastoma

Although MB has been described in families with LFS, its prevalence in *TP53* carriers is not well known (79). About 5–10% of MBs present *TP53* mutations; however, most of these are somatic and only 1% of MBs have been associated with germline *TP53* mutations (22, 23, 80–82).

The correlation between TP53 mutation (both somatic and germline) and MB molecular subgroup has been investigated. In 2013, Zhukowa et al. analyzed a cohort of 397 individuals affected by MB (age 1.1 to 45 years) and reported a *TP53* mutation almost exclusively in WNT and SHH subgroups while it was virtually

TABLE 5 | Types of cancer associated with Li-Fraumeni Syndrome.

Cancer types in Li-Fraumeni Syndrome			Prevalence (%)
Most frequent six "core" cancers	Premenopausal Breast Cancer		27–31
	Soft Tissue Sarcomas		17–27
	Osteosarcoma		13.4–16
	CNS Tumors		9–14
	Adrenocortical Carcinoma		6–13
	Leukemia		2–4
Other less frequent cancer types	Myelodysplastic Syndrome	Thyroid	Prostate
	Lymphoma	Gastrointestinal tract	Ovarian
	Lung	Kidney	Skin
	Laryngeal	Testicular	Neuroblastoma

absent in subgroups 3 and 4. They described a high difference in age distribution between MB_{SHH}/*TP53* mutated, which are almost exclusively between ages 5 and 18 years, and MB_{SHH}/*TP53* wild-type, that showed a bimodal distribution with peaks before 9 and after 18 years of age. Another interesting fact was that all individuals with *TP53* germline mutation, therefore affected by LFS, had MB_{SHH}, and no germline mutations were observed in MB_{WNT}/*TP53* mutated. For individuals with *TP53* mutant tumors, a dramatic association between biologic subgroups and survival was observed. Patients with MB_{SHH}/*TP53* mutated showed a lower 5-year OS than those MB_{SHH} without *TP53* alteration (41% +/- 9% vs 81% +/- 5% respectively); on the contrary, individuals with MB_{WNT}/*TP53* mutated showed an almost similar 5-year OS than those MB_{WNT} without *TP53* alteration (90% +/- 9% vs 97% +/- 3% respectively), demonstrating that *TP53* mutation status is much more crucial in the SHH subgroup. Within the limitation of the small cohort, no significant difference was observed between LFS children with MB_{SHH} and MB_{SHH} with somatic mutations of *TP53* (25).

Diagnosis

The original definition of LFS requires one individual with a sarcoma diagnosed under the age of 45 that has at least one first-degree relative (parent, sibling, or child) with a cancer of any kind diagnosed under the age of 45 and a third family member who is either a first- or second-degree relative in the same parental lineage (grandparent, aunt, uncle, niece, nephew, or grandchild) with any cancer diagnosed under the age of 45, or a sarcoma at any age (83, 84). The finding of *TP53* mutations that did not fully respect classical criteria for LFS diagnosis led to the formulation of revised Chompret criteria. Individuals who meet classic and/or revised Chompret diagnostic criteria (**Appendix A**) should undergo TP53 genetic testing (65, 68, 71, 85).

Cancer Surveillance

Cancer screening in LFS individuals is challenging due to the wide range of associated tumors. Villani et al. in a prospective observational follow-up study of a comprehensive clinical surveillance protocol identified 89 carriers of *TP53* pathogenic variants in 39 unrelated families and divided them in two groups: carriers who accepted surveillance (45%) and carriers who did not accept (55%); 21% of patients crossed over from the non-surveillance to the surveillance group for a total of 66% patients undergoing surveillance for a median of 32 months (86). Over an 11-year period, they identified 40 asymptomatic tumors in 32% of individuals who underwent surveillance and 60 symptomatic neoplasms in 88% patients who initially declined surveillance. The authors highlighted a significant survival advantage in individuals who underwent surveillance reporting 5-year OS of 88.8% in patients with the surveillance group and 59.6% in patients in the non-surveillance group. The Villani et al. 2016 version of the surveillance protocol for children with germline *TP53* pathogenic variants is summarized in **Table 6** (86). According to Ballinger et al. baseline whole-body magnetic resonance imaging can be used to identify early tumors in a highly cancer-prone population such as LFS patients, although further studies are needed (87).

Therapeutic Approaches

Currently, there is no targetable therapy against tumors of LFS patients available. Generally, it is recommended to avoid use of DNA-damaging agents such as ionizing radiation in order to reduce the risk of secondary tumors with the exception of high grade CNS tumors. Notably, CNS tumor patients with LFS tend to show an overall worse outcome when compared to patients with the same CNS tumors but without TP53 alteration (78, 88, 89). Even though no guidelines exist, LFS patients should be subjected to physical examination annually with particular attention to neurologic functions. Radiologic approaches without ionizing radiation such as whole-body MRI are currently under investigation (81, 86).

TURCOT SYNDROME

Turcot syndrome (TS) is defined by the association of colorectal cancer (CRC) and primary brain tumors and is one of the clinical manifestations of the mismatch repair cancer syndrome (OMIM # 276300). The first clinical report of the association of primary brain tumor and colorectal polyposis dates back to 1949 by Crail et al.

TABLE 6 | Villani et al. 2016 version of the surveillance protocol for children (birth to age 18 years) with germline *TP53* pathogenic variants.

Adrenocortical Carcinoma	Ultrasound of abdomen and pelvis every 3–4 months Blood tests every 3–4 months: 17-OH-progesterone, total testosterone, dehydroepiandrosterone sulfate, androstenedione 24 h urine cortisol, if feasible
Brain tumor	Annual brain MRI
Soft tissue and bone sarcoma	Annual rapid whole-body MRI
Leukemia or lymphoma	Blood tests every 3–4 months*: complete blood count, erythrocyte sedimentation rate, lactate dehydrogenase
General assessment	Complete physical examination every 3–4 months, including anthropometric measurements plotted on a growth curve (with particular attention to rapid acceleration in weight or height), signs of virilization (pubic hair, axillary moisture, adult body odor, androgenic hair loss, clitoromegaly, or penile growth) and full neurological assessment Prompt assessment with primary care physician for any medical concerns

**Serial specimens obtained at the same time of day and processed in the same laboratory.*

(90). Ten years later Jacques Turcot described two siblings both affected by adenomatous colorectal polyposis and a malignant tumor of CNS, suggesting a common origin for this association (91). Two types of TS are known in literature. Type 1 (TS1) is characterized by the association between hereditary non-polyposis colorectal cancer (HNPCC), also called Lynch syndrome (LS), genetically related to the mutation of the mismatch repair (MMR) genes and CNS tumor (most frequently glioma). Type 2 (TS2) is characterized by the association of brain tumor and colorectal cancer due to familial adenomatous polyposis (FAP), caused by the mutation of the adenomatous polyposis coli (*APC*) gene, a suppressor gene in the long arm of chromosome 5 (92). Up to 10% of all CRC are inherited and among them a small number, commonly HNPCC or FAP, would be TS (93). Brain tumors in TS are mainly glioblastomas, associated with *MMR* genes mutations (TS1), and MB, associated with *APC* gene mutations (TS2).

Turcot Syndrome Type 1

Genetic Basis

There is a strong association between TS1 and LS. Lynch syndrome is caused by heterozygous germline mutations, inherited in an autosomal-dominant manner, in any of the MMR genes (*MLH1*; *MSH2*, *MSH6*, *PMS1*; *PMS2*), which are involved in DNA repair pathway. Unlike LS, TS1 is caused by homozygous mutations in the aforementioned genes (94, 95).

Clinical Phenotype

TS1 can clinically manifest with both gastrointestinal (diarrhea, constipation, and/or a positive fecal occult blood test) and neurological symptoms depending on which tumor arises first (95). Lynch syndrome is characterized by an average age of onset that is earlier than in sporadic cases (45 vs 63 years) and by CRC that develops most frequently proximal to splenic flexure and can often be synchronous and metachronous (94). Regarding the development of extracolonic cancers the most frequent are represented by carcinoma of the endometrium, ovary, stomach, small bowel, pancreas, hepatobiliary tract, brain, upper uroepithelial tract, sebaceous adenomas and carcinomas, and multiple keratoacanthomas (94). TS1 patients may have skin signs such as café-au-lait spots, resembling type 1 neurofibromatosis, which instead are not reported in TS2 patients (95).

Correlation With Medulloblastoma

MB cases within TS1 are less frequently described than those reported in the setting of TS2, while gliomas are the most frequently reported brain tumors in TS1 (96–99).

In 2007, Scott et al. described a 13-year-old girl with two colonic carcinomas and MB diagnosed at the age of 7 years caused by constitutional biallelic mutations in the mismatch repair gene *MSH6*, the first case of MB reported in literature that was caused by the aforementioned biallelic alteration (100). Another report by Lindsay et al. described a 12-year-old with colonic adenocarcinoma and classic MB due to biallelic deletion in *PMS2* gene (101). To our knowledge, a correlation between TS1 and various subgroups of MB has not yet been highlighted.

Diagnosis

Some aspects should be considered in TS1 diagnosis: individuals with TS1 are offspring of consanguineous in 20% of cases, with no family history of brain tumors or colon; in TS1 polyps are larger and less numerous than in TS2; in TS1 skin lesions are café-au-lait spots while in TS2 they resemble epidermal cysts (95).

According to the American College of Gastroenterology all newly diagnosed CRCs should be studied for MMR deficiency with immunohistochemical testing for the MLH1, MSH2, MSH6, PMS2 proteins and/or with testing for microsatellite instability. Individuals with a history of a tumor that is suspected to be determined by MMR deficiency, a known family mutation associated with LS, or a risk ≥5% of LS obtained with risk prediction models should undergo genetic testing: discovering LS may sometimes be the first step toward diagnosing TS1 (102).

Cancer Surveillance

Cancer surveillance guidelines for patients at risk of or affected by LS have been published while, to our knowledge, no specific guidelines regarding the brain tumor surveillance in patients with TS1 have been established (102).

Therapeutic Approaches

Immunotherapeutic agents such as checkpoint inhibitors have been used in children with biallelic MMR deficiency glioblastoma multiforme, with encouraging results in some studies (26, 103). Checkpoint inhibitors seems to be effective in patients whose tumors harbor a high mutation load, resulting in the expression of neoantigens that act as a target for immunotherapy. Checkpoint inhibitors, through different mechanisms, activate T cells that recognize cancer cells as foreign by destroying them. Nivolumab is an anti-programmed death-1 (PD-1) directed checkpoint inhibitor, approved for the treatment of non-small-cell lung cancer and melanoma, and is being tested in various adult and pediatric tumors (103). Ipilimumab is an anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) approved for the treatment of advanced melanoma and renal cell carcinoma and is also under clinical investigation in multiple adult and pediatric cancers (26). To our knowledge, there are no studies that have demonstrated the effectiveness of checkpoint inhibitors in children with MB, and therefore in those associated with MMR deficiency. Nivolumab and Ipilimumab are currently under investigation in a phase II trial of pediatric patients with high-grade CNS malignancies, including medulloblastoma (NCT03130959) (104).

Turcot Syndrome Type 2

Genetic Basis

APC mutation is generally inherited with an autosomal dominant manner for the development of FAP, while TS2 seems to require a biallelic loss of the *APC* gene (92, 105). Indeed, in patients with a germ-line alteration of *APC*, inactivation of the second copy of the gene seems to be crucial for brain tumor development.

Clinical Phenotype

Clinical findings are those typically associated with colorectal cancer and brain tumors, which can occur at different times. Patients with TS2 tend to develop a number of polyps, around thousands, and they frequently manifest gastrointestinal symptoms (similar to those mentioned for TS1). Either before or after the polyps are found, various neurological symptoms and signs can arise, depending on the location of the tumor: headache, vomiting, visual and/or hearing problems, and sensorimotor deficits. In TS2 patients brain tumors can occur without polyposis, and this could be explained by the hypothesis that affected individuals die before adenomatous polyps have time to develop. Skin lesions can also occur in patients with TS2 and are most commonly epidermal cysts.

Correlation With Medulloblastoma

About 40% of patients with TS develop MB (95). According to Hamilton et al., the relative risk of MB in patients with FAP was 92 times higher than in the general population (92). Surun et al. in their multicentric retrospective review of 12 patients, treated between 1988 and 2018 for MB with an identified or highly suspected *APC* germline pathogenic variant, described some recurrent features such as a constant classic histopathology, a frequent lateral location, and a predominant nonmetastatic status. They highlighted a strong correlation between *APC*-mutated MB and WNT subgroup, demonstrating their excellent outcome, as indeed have wild-type-MB_{WNT} (106). An international multicenter study by Waszak et al., which included 1022 patients with MB, highlighted a close association between *APC* germline mutations and WNT subgroup; in this study germline *APC* mutations were found in five (71%) of seven *CTNNB1*-wild type MB_{WNT} cases, representing 7.6% of all MB_{WNT}, which together with the counterpart constituted by somatic mutations of *CTNNB1* (89.4%), account for 97% of all MB_{WNT} (22).

Diagnosis

A key point in the diagnosis of TS2 patients is represented by family history. Individuals who have one or both parents with CRC diagnosed at an early age should be monitored for pre-cancerous colorectal polyps. According to the American College of Gastroenterology an individual with a history of ≥ 10 colorectal adenomatous polyps, or suggestive extracolonic manifestations, without a family history of an underlying pathogenic mutation, should be referred for genetic testing. In addition, the referral for genetic testing is also indicated for relatives of an individual with a known pathogenic mutation in order to establish the presence or absence of that specific mutation and to understand whether the relatives should be considered at-risk subjects (102).

Cancer Surveillance

The identification of family history of FAP and/or APC gene mutations may allow the clinician to perform surveillance in order to promptly identify the possible appearance of a brain tumor. An early diagnosis can allow an earlier treatment. However, it seems there is no advantage in terms of cost-effectiveness since not all individuals who present a CRC at an early age then develop a brain tumor and inversely (27, 95). Cancer surveillance guidelines for patients with FAP have been published, while, to our knowledge, no specific guidelines regarding brain tumor surveillance in patients with TS2 have been established (95, 102, 107).

Therapeutic Approaches

There is currently no targeted therapy available against tumors arising in the setting of TS2.

RECENTLY IDENTIFIED GENETIC SYNDROMES ASSOCIATED WITH MEDULLOBLASTOMA PREDISPOSITION

Pathogenic germline mutations in *BRCA2*, *PALB2*, *GPR161*, and *ELP* genes have been recently associated with an increased risk of developing MB.

Germline BRCA2 and PALB2 Mutations

The international multicenter study by Waszak et al. identified germline *BRCA2* mutations in 11 (1%) of 1022 patients with MB, 10 children and one adult, with a median age at diagnosis of 5.7 years (22). They observed compound heterozygosity at *BRCA2* in 4 (36%) of 11 patients, of which all developed MB_{SHH} and showed a worse Progression-Free Survival (PFS) and OS (25% at 5 years, respectively) compared to patients with heterozygous germline *BRCA2* mutations, which instead showed a 100% OS and PFS, without secondary neoplasms. Germline mutations in *BRCA2*, compared with 53105 controls, were associated with increased risk of MB_{SHH} and $MB_{Group3/4}$ (22).

BRCA2 biallelic mutations are known to be responsible for **Fanconi Anemia (FA)**. The association of FA with MB has been described in literature (108). FA is a syndrome characterized by a chromosomal instability associated with congenital anomalies, bone marrow failure, and an increased risk of developing acute myeloid leukemia, myelodysplastic syndrome, and a number of solid tumors. It is a genetically and phenotypically heterogeneous disorder, inherited with an autosomal recessive pattern (rarely X-linked). We reported a novel *BRCA2* mutation (c.2944_2944delA.) in a 35-month-old female with FA and diagnosis of two distinct MBs that had been previously treated for a nephroblastoma at the age of 15 months. Genetic testing on the patient's DNA extracted from both peripheral blood and MB cells revealed the presence of compound heterozygosis for BRCA2 frameshift mutations. Molecular analysis showed a MB_{SHH} for both the first- and the second-diagnosed MB. However differences in localization, more aggressive histology, and distinct gene expression pattern led to hypothesize a second distinct tumor rather than a distant relapse from the first one (109). The identification of SHH subgroup in FA patients may play a crucial role for their treatment with the use of targeted therapies, especially in these individuals extremely sensitive to conventional treatments.

In 2016 we described a case report of a 7-year-old girl with a classic histotype MB_{WNT} and whose family history was negative

for cancer (28). After six years of complete remission from MB the patient developed a secondary glioblastoma. Genetic testing for cancer predisposition syndromes was performed despite a negative family history for neoplasms, and we identified a maternal inherited heterozygous germline *BRCA2* mutation, an unusual finding, since cases described in literature were non-WNT subgroups and, to our knowledge, this was the first case of *BRCA2*-mutated MB_{WNT} reported so far.

Waszak et al. also reported pathogenic heterozygous germline *PALB2* mutations in five (<1%) of 1022 patients with MB, of which there were 3 with MB_{SHH}, 1 with MB_{Group3}, and 1 with MB_{Group4}. Five-year OS and PFS for patients with germline *PALB2* mutations was 75% (22).

Interestingly, a correlation was described between germline BRCA2 and PALB2 mutations and homologous recombination repair deficiency (HRD)-like mutation spectrum, specifically for pediatric MB_{SHH} (89% of cases), revealing HRD as potential biomarker for cancer predisposition in this subgroup (22). Furthermore, the association between germline *BRCA2* and *PALB2* with HRD-like mutation spectrum can be exploited to evaluate the susceptibility to combination therapies with PARP inhibitors.

GPR161 Mutations

Germline G protein-coupled receptor 161 (*GPR161*) mutations have recently been described by Begemann et al. as variants predisposing to pediatric MB (110). *GPR161* is located on chromosome 1q24.2 and is involved in various aspects of embryonic development, including granule cell proliferation (111, 112). Proliferation of granule cells in cerebellum is regulated by SHH ligand and becomes abnormal when SHH-signaling pathway is constitutively activated. GPR161 acts as a SHH-pathway suppressor and its loss of function causes MB development (113). The frequency of germline *GPR161* mutations in the general population is about 6 in 10,000 individuals (110). *GPR161* biallelic inactivation, most frequently by copy-neutral loss of heterozygosity of chromosome 1q in individuals with heterozygous germline mutation, in the absence of other driver somatic events, has been associated with early TP53-wild-type-MB_{SHH} development (110). According to Begemann et al., overall prevalence of germline *GPR161* mutations among pediatric (age<18 years) and infant (age<4 years) patients with MB_{SHH} was 3.4% and 5.5%, respectively (110). Copy-neutral loss of heterozygosity of chromosome 1q was never reported in *GPR161* wild-type MB_{SHH}; therefore, it can be considered a molecular feature (110).

Germline ELP1 Mutations

Germline loss of function (LOF) variants in *ELP1* have recently been identified in strong association with MB in pediatric age (114). ELP1 is a molecule that is part of the Elongator Complex, involved in epitranscriptomic tRNA modifications, whose main function is to modify wobble base uridines in the anticodon loop of tRNAs in order to ensure a correct translational elongation (29, 115–117). The loss of even a single subunit causes the dysregulation of the Elongator Complex with consequent proteome instability. The cerebellum is described as the site of greatest ELP1 expression during brain development (118, 119). According to Waszak et al., three consecutive mutational events are probably required for the development of *ELP1*-associated $MB_{SHH:}$ a heterozygous germline *ELP1*LOF variant; somatic biallelic inactivation of *ELP1* with monoallelic inactivation of PTCH1 via loss of chromosome arm 9q and biallelic inactivation of the residual PTCH1 allele via a somatic mutation or focal deletion (114). Interestingly, Waszak et al. found a strong association between germline LOF variants in *ELP1* and MB_{SHH} subgroup, especially with SHHα subtype (114). Patients with *ELP1*-associated MB_{SHH} showed a median age at diagnosis of 6.3 years, older than patients with MB_{SHH} and germline SUFU or PTCH1 LOF variants and younger than those with MB_{SHH} and germline TP53 mutations. These patients most frequently presented a desmoplastic nodular histotype and showed a favorable clinical outcome with 92% 5-year OS (114).

Rubinstein-Taybi syndrome (RSTS) is an extremely rare genetic disease, with an incidence of 1 in 100,000 to 125,000 live births, characterized by intellectual disability, unusual behavior, postnatal growth retardation, and multiple congenital anomalies, most frequently of the face and distal limbs (120, 121). RSTS is caused by a heterozygous mutation in *cyclic-AMP regulated enhancer binding protein* (*CREBBP*) gene, a transcriptional co-activator gene on chromosome 16p13.3, in about 60% of affected individuals (122), a submicroscopic deletion on chromosome 16p13.3 in about 10% of individuals (RSTS1, OMIM #180849) (123), alteration of *E1A binding protein p300 (EP300)* on chromosome 22q13.2 in about 5–10% of individuals (RSTS2, OMIM #613684) (124, 125). *CREBBP* gene and *EP300* genes act as transcriptional co-activators and are involved in DNA repair, cellular growth, differentiation, apoptosis, and tumor suppression (126). According to Boot et al. that reviewed the literature from 1963 to 2017, a total of 132 tumors have been reported in 115 individuals with RSTS and MB was the second most frequent CNS neoplasm with 6 reported cases, after meningioma (121). However, an increased risk for malignant tumors in RSTS could not be confirmed given the small numbers of affected individuals reported in literature, and additional studies are warranted.

GENETIC TESTING OF CANCER PREDISPOSITION SYNDROMES

With the advent of next generation sequencing (NGS) and implementation of genetic testing for adult cancer predisposition syndromes into routine clinical practice, cancer genetics research has extended the use of molecular testing for tumor and germline analysis in pediatric cancer patients. Molecular diagnosis of cancer predisposition syndromes can influence cancer screening initiation or frequency, to either prevent or detect cancer at an earlier and more treatable stage, and directly impact treatment decisions. However, even if medulloblastoma can be associated with rare hereditary cancer predisposition syndromes, screening guidelines for genetic counseling and testing of pediatric patients are not available (23). For genetic testing of cancer predisposition syndromes, different approaches are being used, and, currently, most molecular diagnostics laboratories that offer NGS are

performing targeted gene panel testing or clinical whole exome sequencing (WES), more rarely whole genome sequencing (WGS). A multi-gene panel usually includes high and moderate penetrance genes and, sometimes, some low or of yet unknown risk genes, offering the advantage of identifying germline pathogenic variants in genes that would normally not be tested based on the patient's diagnosis. However, it is possible that variants in genes not included in the panels contribute to the cancer risk and WES or WGS can be used to explore other genetic basis of familial syndromes in a more extensive way, permitting to identify new high- and moderate-risk genes of cancer predisposition. Genome-wide approaches generate huge amounts of genetic data and it remains a challenge to interpret the identified variants. Such data interpretation needs close collaboration among molecular geneticists, bioinformaticians, and clinicians. However, as sequencing costs are decreasing and computer and technological resources are expanding, genome-wide analysis in clinical practice will become more common.

CONCLUSIONS

MB is the most frequent malignant CNS tumor in children, and additionally to the sporadic form, MB can occur in association with a cancer predisposition syndrome. Knowledge of the clinical findings, etiopathogenic basis, and diagnostic criteria of each syndrome described in this review allow the pediatrician to make a correct diagnosis, start cancer surveillance, and suspect precociously a MB on its onset, providing a prompt treatment. Conversely, when MB is diagnosed, the correct identification/detection of a cancer predisposition syndrome can allow the clinician to make a more appropriate and complete management of treatment involving several medical specialists in a multidisciplinary team. The molecular studies conducted in the last years have evidenced an association between the various cancer predisposition syndromes and the different MB subgroups. Knowing these relationships can help further clarify the difference not only from a biological point of view but also in prognostic terms. Notably, the extremely poor outcome of MB_{SHH} in children expressing germline *TP53* mutations has already been reported. Based on the findings described by Waszak et al., pediatric MB_{SHH} development could be explained by a high genetic predisposition (about 40%); therefore, the effort to carry out genetic testing and surveillance program for affected patients and families in this subgroup becomes even more crucial.

According to Waszak et al. we suggest that patients with MB_{SHH} should be tested for germline *TP53* (when older than 3 years), *SUFU* and *PTCH1* mutations (when younger than 3 years), and if negative, also for germline mutations in *BRCA2* and *PALB2*. Furthermore, we suggest that patients with MB_{SHH} should be tested for germline *ELP*1 mutations, especially those presenting outside of infancy, and for germline *GPR161* mutations, particularly those presenting in infancy. We suggest, also, genetic counselling for germline *APC* mutations in children with MB_{WNT}.

Considering that only 5–6% of MB are associated with cancer predisposition syndromes, our current knowledge is probably still limited. Given the importance that the recognition of a cancer predisposition syndrome can have in the management of a child with MB, we suggest to extend genetic testing also in patients with family history for cancer and/or finding of a dysmorphic phenotype. Knowledge of the associations between molecular subgroups and cancer predisposition syndromes can also be useful in clarifying the differences in terms of therapeutic vulnerability, guiding the development of new targeted therapies. Finally, the comprehension of these biological and molecular differences can help to further improve cancer surveillance measures, with the aim of guaranteeing the best quality of care for the patients.

AUTHOR CONTRIBUTIONS

RC and GB equally contributed to this manuscript. EM, AP, ZMB, FN, GC, EP, EA, MR, ML, and AC contributed to the finishing of the work. FN provided the figure. AM, ACar, EF, LB, and FL revised it critically for important intellectual content. All authors finally approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors thank Megan Eckley for helping in the English final version and the Association "Il Laboratorio di Chiara".

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.566822/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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APPENDIX A. LI-FRAUMENI SYNDROME CLASSIC DIAGNOSTIC CRITERIA AND REVISED CHOMPRET CRITERIA

Classic diagnostic criteria

A proband with Sarcoma diagnosed under the age of 45 years

AND

A first degree relative with any cancer under 45 years

AND

Another first or second degree relative with either cancer under 45 years or a sarcoma at any age

Chompret diagnostic criteria (revised)

A proband with an LFS spectrum tumor (soft tissue sarcoma, osteosarcoma, brain tumors, pre-menopausal breast cancer, adrenal cortical carcinoma, leukemia, lung bronchoalveolar cancer) before 46 years

AND one of the following criteria:

At least one first- or second-degree relative with an LFS tumor (except breast cancer, if the proband has breast cancer) before 56 years or with multiple primary tumors

OR

A proband with multiple primary tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum and the first of which occurred before 46 years

OR

A proband with adrenal cortical carcinoma or choroid plexus carcinoma or embryonal anaplastic subtype rhabdomyosarcoma independent of the family history

OR

Breast cancer before the age of 31 years

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 30 October 2020
doi: 10.3389/fped.2020.568528

Predictive Testing for Tumor Predisposition Syndromes in Pediatric Relatives: An Asian Experience

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Approximately 10% of pediatric cancer patients possess germline pathogenic/likely pathogenic variants (PV/LPV) in known tumor predisposition genes. Predictive testing is the optimal approach to identify asymptomatic at-risk relatives to guide gene-directed surveillance for early cancer detection and/or risk-reducing strategies. However, the uptake rate for predictive testing remains low in Asian countries. We aim to evaluate the uptake rate of predictive testing in a pediatric population (aged under 21-years-old) in a multi-ethnic Asian cancer center. Our retrospective analysis included families with PV/LPVs identified in genes associated with pediatric tumor predisposition. Of the 83 pediatric first-degree relatives (FDRs) from 49 unrelated families, 20 FDRs (24.1%) originating from 13 families (26.6%) underwent predictive testing. Genes tested in pediatric FDRs were *APC, RB1, SBDS, SDHA, SDHB, SDHD,* and *TP53*. All pediatric FDRs of probands with PV/LPVs in *RB1* and biallelic PVs in *SBDS* underwent predictive testing, while <45% of pediatric FDRs had predictive testing for familial PV/LPVs identified in the *APC, SDHA, SDHB, SDHD,* and *TP53* genes. Amongst the 13 families who underwent pre-test counseling, 80% of pediatric FDRs in these families proceeded with predictive testing. Malay pediatric FDRs and siblings of probands were more likely to undergo predictive testing. We conclude that the predictive testing rate in pediatric FDRs is higher than that of adult FDRs in Asia, but still below the global average. We postulate factors that may influence predictive testing uptake in pediatric FDRs includes a lack of genetics awareness, concerns regarding insurance, and genetic discrimination.

Keywords: predictive testing, cascade, hereditary cancer, pediatric, Asia

OPEN ACCESS

Edited by:
Luigi Boccuto,
Clemson University, United States

Reviewed by:
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HIMA San Pablo Oncologic, United States
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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Pediatrics

Received: *01 June 2020*
Accepted: *08 October 2020*
Published: *30 October 2020*

Citation:
Chiang J, Yuen J, Shaw T, Goh HX, Li S-T, Courtney E and Ngeow J (2020) Predictive Testing for Tumor Predisposition Syndromes in Pediatric Relatives: An Asian Experience. Front. Pediatr. 8:568528. doi: 10.3389/fped.2020.568528

INTRODUCTION

Approximately 10% of pediatric cancer patients have a hereditary monogenic cause (1–3), although the true prevalence is likely higher due to unknown syndromes or the limitations of current DNA sequencing methods (4). Tumor predisposition syndromes, such as familial adenomatous polyposis (FAP) and hereditary retinoblastoma (RB) can affect children, afflicting individuals as young as 10 years old with adenomatous polyposis (5) and new-born infants with retinoblastoma (6), respectively. The majority of pediatric tumor predisposition

syndromes follow an autosomal dominant inheritance pattern; first-degree relatives (FDRs) of a proband have a 50% chance of inheriting the familial pathogenic/likely pathogenic variant (PV/LPV). Genetic testing allows for the identification of a PV/LPV in probands, which then sets in motion predictive testing within the family. High rates of predictive testing are beneficial to both the proband's family and the healthcare system. Predictive testing can reduce public healthcare costs and increase efficiency compared to genetic testing of symptomatic probands (7, 8). The uptake rate of predictive testing has a direct impact on cost-effectiveness of genetic testing programs (7, 8) and overall health outcomes (9). On a larger scale, this likely translates to greater cost-savings for the healthcare system as such a model of preventive medicine aims to reduce the burden of cancer-related morbidity and mortality (8–10). Predictive testing is important for pediatric-onset conditions as it provides potentially actionable information for screening asymptomatic children. Correspondingly, family members who test negative can avoid unnecessary screening, medical interventions, and associated costs. Increased genetic awareness and accessibility has improved the uptake of germline genetic testing globally (11–14), providing probands and parents/guardians the opportunity to ascertain if the personal or family history of cancer is hereditary. Results from genetic testing can empower decisions that promote early cancer detection through options, such as intensified surveillance and/or risk-reducing strategies to mitigate cancer risk (15–20).

Rates of predictive testing vary globally, however uptake is consistently lower in Asian countries (7, 21–24). The uptake of predictive testing is dependent on several factors, such as the cost of testing with limited coverage by healthcare institutions, genetic discrimination and reliance on probands to disclose the identification of a hereditary condition among family members (25). Cost remains a significant barrier despite reduction over the past decade with the advent of next-generation sequencing (7, 25). The cost of genetic testing in most parts of Asia is paid out-of-pocket, with minimal government or insurance subsidy. Secondly, there is a lack of legislation to protect against genetic discrimination, including health insurance. This plays an even larger role in the pediatric population who may find that they are unable to obtain insurance coverage due to their underlying hereditary condition. Thirdly, the dissemination of genetic testing results relies solely on the proband (or parents/guardians in cases where the proband is a minor). This hampers predictive testing uptake as proband - initiated disclosure is often complicated by several factors on an individual, familial and cultural basis (21, 23, 26–28). In most parts of Asia, the diagnosis of cancer is stigmatized and rarely discussed among family members, creating another barrier to uptake of genetic testing (28). The proband or parents/guardians may choose not to share genetic results due to distant family relations, fear of discrimination, backlash from family members, as well as perceived burden knowing one has an increased risk of cancer (22, 23).

The Cancer Genetics Service (CGS) at the National Cancer Center Singapore (NCCS) follows the American Academy of Pediatrics (AAP) and the American College of Medical Genetics and Genomics (ACMG) guidelines (4, 29, 30) and recommends predictive testing for pediatric patients only in childhood-onset conditions. To our knowledge, there has been no published literature on predictive testing in pediatric FDRs to date. This study evaluated the uptake rate of predictive testing for pediatric tumor predisposition syndromes in minor FDRs in an Asian cancer center and explores potential factors that affect the uptake rate.

METHODS

Probands who were seen at the CGS at NCCS from March 2014 to December 2019 and had an identified PV/LPV following genetic testing were recruited. Probands were included up until December 2019 to allow for a follow-up period for any delay in predictive testing decisions. Demographic, clinical data, and pedigrees of probands and their pediatric FDRs were extracted from the CGS database (REDCap Software, version 6.10.3, 2017, Vanderbilt University). The database and pedigrees were reviewed by two independent study personnel. Pediatric FDRs of probands who did not attend the CGS clinic were assumed to have declined predictive testing, in tandem with their parents/guardians' decision. Demographic and clinical data for untested FDRs were obtained from pedigrees provided by probands. Financial status of untested pediatric FDRs were assumed to be similar to that of the proband as they are likely to reside in the same household.

Only probands with a PV/LPV in genes associated with pediatric-onset tumor predisposition syndromes were included in the study, in line with AAP and ACMG guidelines. These included *AIP, ALK, APC, ATM, AXIN2, BAP1, BLM, BMPR1A, CDC73, CDKN1C, CEBPA, DICER1, DIS3L2, EPCAM, EXT1, EXT2, FH, GATA2, GPC3, HRAS, LZTR1, MAX, MEN1, MLH1, MSH2, MSH6, NBN, NF1, NF2, PHOX2B, PMS2, PRKAR1A, PTCH1, PTEN, RB1, RECQL4, REST, RET, RUNX1, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TERC, TERT, TMEM127, TP53, TSC1, TSC2, VHL, WRN,* and *WT1*. The mismatch repair genes and the *SBDS* gene were tested only if FDRs were at risk of constitutional mismatch repair deficiency (CMMRD) and Shwachman-Diamond syndrome, respectively. Probands were excluded from the study if they were not Singapore residents as their family members were unlikely to be living in Singapore and would have been unable to attend the CGS for predictive testing. A minor, by Singapore law, is defined as an individual under age 21 years and hence the pediatric population is defined as individuals below 21 years old. Written informed consent and assent for medical record research was obtained from all probands and tested FDRs at the point of genetic testing. The study was approved by the Singhealth Centralized Institutional Review Board (CIRB number 2010/826/B).

Genetic counseling services at NCCS are provided by medical oncologists with specialization in genetics and/or Master's trained genetic counselors. A shared decision-making approach for pre-test genetic counseling is adopted in the CGS (31). Following the identification of a PV/LPV in a proband, family notification

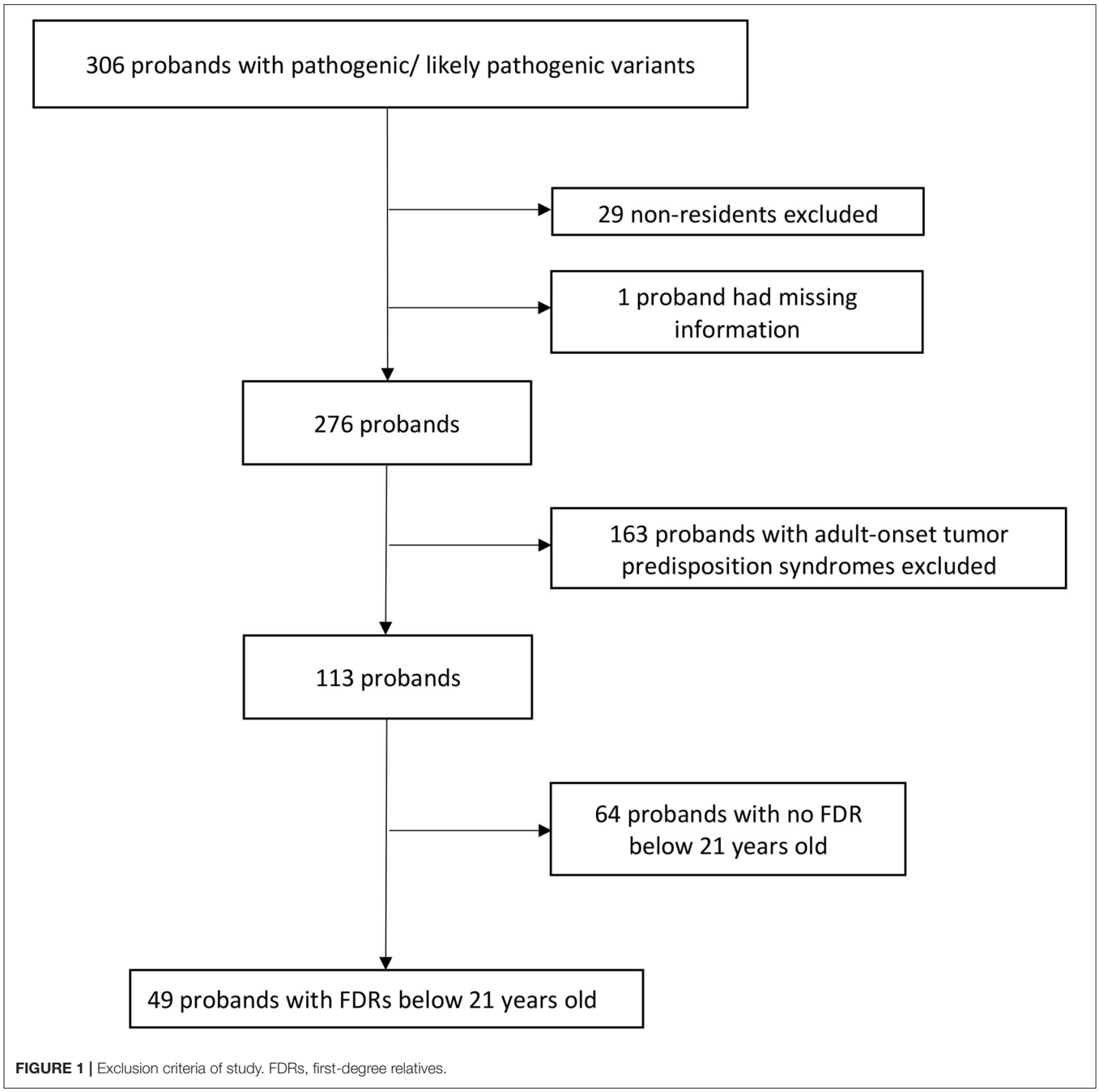

FIGURE 1 | Exclusion criteria of study. FDRs, first-degree relatives.

letters were provided to assist the proband/family members with dissemination of the result. Family members who were keen to undergo genetic testing were referred to the CGS where an appointment for pre-test genetic counseling was scheduled to facilitate predictive testing.

Tested and untested pediatric FDRs were compared for potential prognostic factors of predictive testing uptake. Two-tailed chi-square test and independent samples *t*-test were performed for categorical and normally distributed continuous variables, respectively. For categorical variables with a 2 × 2 distribution, a two-tailed Fisher's exact test was used when the expected count was below 5. Statistical significance was set at $P<0.05$. All statistical analyses were performed using IBM SPSS version 25.

RESULTS

Overall, 306 probands who underwent genetic testing between March 2014 and December 2019 were found to have PV/LPVs in known tumor predisposition genes. After excluding 29 non-residents, one proband with missing information, 163 probands with adult-onset tumor predisposition syndromes and 64 probands with no FDRs below 21 years old (**Figure 1**), there were 83 pediatric FDRs from 49 unrelated probands. A total of

TABLE 1 | Proportion of FDRs below 21 years old and families who had predictive testing.

FDRs below 21 years old			Source families		
Total	**Tested (%)**	**Not tested (%)**	**Total**	**Tested (%)**	**Not tested (%)**
83	20 (24.1)	63 (75.9)	49	13 (26.6)	36 (73.5)

TABLE 2 | Demographic and clinical factors of probands and tested pediatric FDR.

	Probands ($n = 49$)	**Pediatric FDRs ($n = 20$)**
AGE		
Mean (range)	35.0 (1–57)	11.3 (3–20)
SEX		
Male (%)	18 (36.7)	10 (50.0)
Female (%)	31 (63.3)	10 (50.0)
RACE		
Chinese (%)	38 (77.6)	14 (70.0)
Malay (%)	6 (12.2)	6 (30.0)
Indian (%)	2 (4.1)	0 (0.0)
Others (%)	3 (6.1)	0 (0.0)
PERSONAL HISTORY OF CANCER		
Yes (%)	45 (91.8)	2 (10.0)
No (%)	4 (8.2)	18 (90.0)
FINANCIAL ASSISTANCE		
Yes (%)	16 (32.7)	7 (35.0)
No (%)	33 (67.3)	13 (65.0)
GENETIC RESULT		
Positive (%)	49 (100.0)	11 (55.0)
Negative (%)	0 (0.0)	9 (45.0)

20 pediatric FDRs (24.1%), originating from 13 families (26.6%), underwent predictive testing (**Table 1**).

Demographic and clinical information of the 49 probands whom carried an identified PV/LPV in a pediatric-onset tumor predisposition gene and the 20 pediatric FDRs who had predictive testing are shown in **Table 2**. The mean age of the probands and pediatric FDRs were 35.0 and 11.3 years, respectively. The majority of probands were female (63.3%), Chinese (77.6%), and had a personal history of cancer (91.8%). In comparison, the pediatric FDRs who underwent testing were similar in terms of gender (female; 50.0%) and ethnicity (Chinese; 70.0%). The ethnic distribution in probands and pediatric FDRs is representative of the Singaporean population (32). Most of the pediatric FDRs did not have a personal history of cancer (90.0%). The need for financial assistance was similar between probands and pediatric FDRs, at 32.7 and 35.0%, respectively. Overall, the familial PV/LPV was detected in 11/20 (55.0%) of tested pediatric FDRs.

Pediatric FDRs underwent predictive testing for familial PV/LPVs identified in the following genes: *APC, RB1, SBDS, SDHA, SDHB, SDHD,* and *TP53* (**Table 3**). Among six unrelated probands with identified *APC* PV/LPVs, there were 18 pediatric FDRs. Eight pediatric FDRs (44.4%) from three families (50.0%) underwent predictive testing for the familial *APC* variant. Two pediatric FDRs from one family had genetic testing for familial PVs in *APC* and *MUTYH* as there were two PVs found in the proband. There were two unrelated probands with identified *RB1* PV/LPVs with three pediatric FDRs from both families. All three pediatric FDRs from both families (100.0%) had predictive testing. One family had a PV in both *RB1* and *TP53*. One proband with biallelic *SBDS* PVs had one pediatric FDR who underwent predictive testing (100.0%). Of 16 pediatric FDRs from 12 families with *SDHx* PV/LPVs, seven pediatric FDRs (43.8%) from six families (50.0%) underwent predictive testing. Out of nine pediatric FDRs from seven families with *TP53* PV/LPVs, three FDRs (33.3%) from two families (28.6%) had predictive testing for the familial variant. More than half of the eligible pediatric FDRs did not proceed with predictive testing for familial PV/LPVs identified in *APC, SDHx,* and *TP53*. Among the 13 families that presented for predictive testing, 20/25 (80.0%) pediatric FDRs underwent predictive testing.

We identified two factors that shows significant association with the uptake of predictive testing in pediatric FDRs—ethnicity and relationship to proband (**Table 4**). Malay pediatric FDRs were more likely to undergo predictive testing as compared to other ethnic groups (66.7 vs. 23.0%, $p = 0.005$). In addition, pediatric siblings of probands were more likely to undergo predictive testing compared to children of probands (53.3 vs. 17.6%, $p = 0.003$). We examined other potential factors that may affect the uptake of predictive testing, although we did not find any significant associations with gender, age of FDR, age of parents/guardians, or socioeconomic status.

DISCUSSION

This study reports the predictive testing uptake rate in pediatric FDRs of probands with PV/LPVs in pediatric tumor predisposition genes. Concurrently, it provides insight into the uptake of commonly tested genes among pediatric FDRs of Asian families.

We observed a 24% uptake rate of predictive testing for tumor predisposition syndromes in the Singaporean pediatric population, almost double the predictive testing rate of 13% in Singaporean adults (25). The lack of predictive testing data for pediatric-onset tumor predisposition syndromes meant that there were no available data for comparison. We postulate that the low predictive testing rate in our Asian pediatric population may be due to a combination of factors relating to poor genetics knowledge and awareness, concerns regarding insurance and genetic discrimination, and Asian familial culture.

There is a general lack of understanding of the clinical utility of genetic testing in Singapore (23). This could explain the poor uptake of predictive testing amongst potential pediatric *APC, SDHA, SDHB, SDHD, a*nd *TP53* PV/LPV carriers, who may be at increased risk for a range of different cancer types from a young age. Our data demonstrates that pediatric FDRs are significantly

TABLE 3 | Proportion of FDRs who underwent predictive testing by gene.

Gene	Genetic Condition	FDRs below 21 years old			Source families		
		Eligible for testing	Tested (%)	Not tested (%)	Eligible for testing	Tested (%)	Not tested (%)
APC	Familial adenomatous polyposis	18	8 (44.4)	10 (55.6)	6	3 (50.0)	3 (50.0)
*RB1**	Hereditary retinoblastoma	3	3 (100.0)	0 (0.0)	2	2 (100.0)	0 (0.0)
SBDS	Shwachman-Diamond syndrome	1	1 (100.0)	0 (0.0)	1	1 (100.0)	0 (0.0)
SDHx (SDHA, SDHB, SDHD)	Hereditary paraganglioma-pheochromocytoma syndrome	16	7 (43.8)	9 (56.2)	12	6 (50.0)	6 (50.0)
*TP53**	Li-Fraumeni Syndrome	9	3 (33.3)	6 (66.7)	7	2 (28.6)	5 (71.4)

**Two FDRs within one family underwent predictive testing for pathogenic variants in TP53 and RB1, both found in the proband.*

TABLE 4 | Factors associated with uptake of predictive testing in FDRs below 21 years old.

	Tested	Not tested	*P*-value
AGE OF FDR			
Mean (range)	11.3 (3–20)	9.1 (0–20)	0.141
SEX			
Male (%)	10 (25.0)	30 (75.0)	0.853
Female (%)	10 (23.3)	33 (76.7)	
RACE			
Chinese (%)	14 (23.0)	47 (77.0)	**0.006**[a]
Malay (%)	6 (66.7)	3 (33.3)	
Indian (%)	0 (0.0)	8 (100.0)	
Others (%)	0 (0.0)	5 (100.0)	
MEAN AGE OF PARENTS/GUARDIANS			
Mean (range)	40.3 (32–56)	42.4 (28–57)	0.251[b]
RELATIONSHIP TO PROBAND			
Child (%)	12 (17.6)	56 (82.4)	**0.003**
Sibling (%)	8 (53.3)	7 (46.6)	
FINANCIAL ASSISTANCE			
Yes (%)	7 (25.9)	20 (74.1)	0.787
No (%)	13 (23.2)	43 (76.8)	

[a] *Fisher's Exact test.*
[b] *Independent sample t-test.*
Chi-square test was used, unless otherwise specified.
Bold values indicate statistical significance p <0.05.

less likely to undergo predictive testing if the proband is the parent. We hypothesize that parents/guardians may want to minimize invasive procedures, such as blood tests, which cause the child unnecessary worry. They may also be concerned that knowledge of a hereditary condition may result in stigma from the family/community and have an impact on the child's psychological well-being, which in turn could impact schooling, social interaction, and self-esteem. Parents/guardians may also have difficulty broaching the subject of hereditary conditions and explaining the risk to their children, possibly stemming from guilt of passing it on to the next generation (33). Furthermore, parents/guardians may project assumptions onto the child, which may make for inaccurate assessments of the child's ability to understand and/or cope with the implications of undergoing predictive testing. Such assumptions may be overly paternalistic, as there are varying levels of cognitive maturity in the two decades spanning the pediatric age group, where adolescents are known to be capable of independent thoughts that may be distinct from their parents/guardians. Parents/guardians may worry that the child is not mature enough to understand the impact of genetic information (33, 34). Often in Asia, clinical consultations with pediatric FDRs comprises of an extended discussion with the parents/guardians, with minimal interaction with the child. The CGS at NCCS actively overcomes this by involving the child in an age-appropriate way throughout the pre-test counseling process with developmentally-appropriate explanations, child-friendly assent forms, and engaging them in the final decision-making, where appropriate. Unfortunately, we are aware of instances where information has been intentionally withheld by parents/guardians to protect their at-risk child(ren) from the knowledge of an increased risk of cancer, despite the provision of family communication strategies between parents/guardians and child.

From an ethical point of view, the subject of predictive testing in pediatric FDRs is keenly debated (35–38). Advocates highlight the actionability of identifying pediatric PV/LPV carriers to guide early screening to detect cancer at an earlier and more manageable stage or risk-reducing interventions, with the aim of decreasing mortality. This is especially observed in pediatric patients with familial adenomatous polyposis (FAP), where colorectal adenomatous polyposis and cancer can develop at a young age (39, 40). Genetic testing for the purpose of enhancing medical monitoring, prophylaxis or treatment in pediatric FDRs may be in the best interest of the child in such conditions (41). Detractors cite the right to autonomy and self-determination of the child as a reason to defer germline testing until they are able to comprehend the spectrum of benefits and limitations (42), especially as there are often reproductive and insurance implications following germline genetic testing. The best interests of the child must be respected at all times and healthcare providers need to balance the autonomy of the child and medical need for genetic testing carefully. The balance might come from testing children only when cancer risk begins in childhood and where there are evidence-based interventions to mitigate such risks.

Interestingly, our service reports a predictive testing rate in pediatric FDRs that is nearly double that of adult FDRs (25). Previous studies of adults in Singapore who underwent genetic testing found several barriers to disclosure of results by the proband, including cost, concerns regarding insurance, potential genetic discrimination, as well as perceived burden of genetic results (27). This barrier of proband-mediated disclosure is not unique to Asia, with literature demonstrating similar challenges in other countries (43, 44). In the case of a pediatric FDR, proband-mediated disclosure is not a relevant factor as parents/guardians are often involved in the entire genetic counseling process.

Medical decision making in Asia usually includes significant input from the family, especially in the Malay community (22, 28, 45). In Asian culture, the concept of illness is familial, rather than individual, and involvement of the family provides hope, support, and strength (46). This pattern of familial decision-making can be seen as entire families often come together for testing if they choose to do so and vice versa (25). In our dataset, Malay pediatric FDRs, whom traditionally apply a familial decision-making approach (28), are more likely to undergo predictive testing than other ethnic groups. We observed that predictive testing tends to happen in clusters within families which suggests the strong influence of the family in decision-making for genetic testing. Further research on family-based genetic counseling should be considered in Asia.

Based on our study, *RB1* was the most common gene tested when predictive testing was offered to pediatric FDRs. Even though all *SBDS* pediatric FDR had predictive testing, this should be interpreted with caution as it is based on a single proband with biallelic *SBDS* PVs with one pediatric FDR. Hereditary retinoblastoma is a disease of childhood and curative intervention can be performed if detected early. The *RB1* gene is highly penetrant with most carriers presenting with retinoblastoma before age five (6). Parents/guardians may thus be more likely to opt for early testing to improve detection and prospects of cure.

Complete data, with minimal missing information, is a strength of this study. Though numbers are small, our study addresses a gap in the literature by looking at the issue of predictive testing uptake in pediatric FDRs and sets a benchmark for comparison with future studies. Further studies with larger datasets would be beneficial for comparison. Our study did not explore the reasons for or against predictive testing in children, such as the breakdown of age, education, and socioeconomic status. Future qualitative studies are required to understand the concerns and needs of pediatric FDRs and their parents/guardians (47, 48). Additionally, pedigree and family information was dependent on proband's recall which may be subject to recall bias. Our study has limited access to FDRs who may have undergone predictive testing via other services, which may have led to an underestimation of predictive testing uptake rates. Nevertheless, this is unlikely to be a significant number as our center has funding assistance for testing and the majority of predictive testing is done at the same center as the proband.

CONCLUSION

This study addresses a question that has not been reviewed in literature, by demonstrating that a quarter of pediatric FDRs undergo predictive testing for childhood-onset tumor predisposition syndromes in Asia. While the rate is higher than that observed in adult FDRs in Singapore, it is still below global predictive testing rates. Factors, such as ethnicity and relationship-to-proband are positive predictors for the uptake of predictive testing amongst pediatric FDRs. Future directions for further exploration include facilitators and barriers to predictive testing unique to a pediatric population, addressing lack of protective legislature especially for health insurance, the effectiveness of family-based genetic counseling in improving pediatric predictive testing uptake, and/or the approach of directly contacting FDRs for predictive testing without proband-mediated dissemination.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Singhealth Centralized Institutional Review Board (CIRB number 2010/826/B). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JC and JN: conception and design and provision of study materials or patients. JC: administrative support, collection and assembly of data, and data analysis and interpretation. JC, JY, TS, HG, S-TL, EC, and JN: manuscript writing and final approval of manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Pediatrics

REVIEW
published: 12 November 2020
doi: 10.3389/fped.2020.561487

Cancer Predisposition Syndromes Associated With Pediatric High-Grade Gliomas

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Pediatric High-Grade Gliomas (pHGG) are among the deadliest childhood brain tumors and can be associated with an underlying cancer predisposing syndrome. The thorough understanding of these syndromes can aid the clinician in their prompt recognition, leading to an informed genetic counseling for families and to a wider understanding of a specific genetic landscape of the tumor for target therapies. In this review, we summarize the main pHGG-associated cancer predisposing conditions, providing a guide for suspecting these syndromes and referring for genetic counseling.

Keywords: brain tumors, cancer predisposition, genetics of cancer, pediatric neuro- oncology, high grade gliomas

OPEN ACCESS

Edited by:
Anat Erdreich-Epstein, Children's Hospital of Los Angeles, United States

Reviewed by:
Joshua John Breunig, Cedars Sinai Medical Center, United States
George Michaiel, University of Southern California, United States

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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Pediatrics

Received: *12 May 2020*
Accepted: *26 October 2020*
Published: *12 November 2020*

Citation:
Ceglie G, Del Baldo G, Agolini E, Rinelli M, Cacchione A, Del Bufalo F, Vinci M, Carta R, Boccuto L, Miele E, Mastronuzzi A, Locatelli F and Carai A (2020) Cancer Predisposition Syndromes Associated With Pediatric High-Grade Gliomas. Front. Pediatr. 8:561487. doi: 10.3389/fped.2020.561487

INTRODUCTION

Central Nervous System (CNS) tumors are the most common pediatric solid tumors and represent the second most frequent neoplasm in pediatric age, second only to leukemias. They count for 1.12–5.14 cases per 100,000 people in individuals aged 0–19 years, with variable incidence rates across different countries, the highest being in the USA (1). Management of pediatric CNS tumors is challenging and requires specific oncological training.

Among brain tumors in the pediatric age, gliomas are the most represented. Approximately 21% of all primary pediatric gliomas are high-grade tumors (2, 3). Even though from a histopathological point of view pediatric high-grade gliomas (pHGGs) are similar to their adult counterpart, their genetic and epigenetic features reflect intrinsic differences compared to adult HGGs. Despite an increased understanding of their biological basis, therapeutic options for these tumors are still very limited, and the long-term prognosis remains poor, with high levels of both morbidity and mortality (3, 4) and a 5-year survival rate of $< 20\%$ (4).

Risk factors for pHGG seem to be mostly genetic in nature, even though some predisposing environmental factors such as irradiation have been described (5). In contrast to adult population, where cancer associated mutations are mostly somatic and resulting from external causes, germline mutations are frequently encountered in children.

Several cancer predisposing syndromes (CPS) associated with an increased risk of developing to pHGG have been identified so far, including Neurofibromatosis type 1 (NF1), Turcot syndrome and Li-Fraumeni syndrome. In this review we will address the impact of these syndromes for the management of pHGG.

METHODS

The authors conducted a literature search describing CNS tumors and cancer predisposing syndromes. Selection of studies were based on research topics (such as cancer predisposition syndrome AND/OR brain tumor genetics, brain tumor cancer predisposition syndrome, HGG predisposition syndromes, HGG in childhood) found in the PubMed. Only papers written in the English language and those published from the year 2000 up to May 2020 were selected. We included reviews, case series and research studies that were classified according to their relevance. No abstracts were included. The information found in the selected studies was carefully evaluated, which is described and discussed in the following sections.

LI-FRAUMENI SYNDROME

Li-Fraumeni Syndrome (LFS) (OMIM #151623) was reported for the first time in 1969 by Frederick Li and Joseph Fraumeni (6). LFS is an autosomal dominant, highly penetrant cancer predisposition syndrome associated with germline mutations in the *TP53* gene. It lacks additional clinical features and is only characterized by the high frequency of malignancies in multiple organs, making it a difficult syndrome to diagnose in the absence of a significant family history of multiple cancers (7). The involved gene encodes the *TP53* transcription factor, tumor protein p53, also known as the "guardian of the genome" (8). *TP53* is involved in cellular growth control by regulating the expression of several genes causing cell-cycle arrest and apoptosis in response to DNA damage.

Epidemiology and Cancer Spectrum

LFS prevalence is estimated between 1/5.000 and 1/20.000 of the population (9, 10), even if the estimated prevalence of pathogenic and likely pathogenic germline *TP53* variants seems to be higher, as described by Andrade et al. (9).

LFS is characterized by a high lifetime cancer risk and, due to its extremely high penetrance, by a familiar clustering of tumors. Cancer types are variable and often present during childhood. Osteosarcoma, soft-tissue sarcomas, brain tumors, early-onset breast cancer, leukemia, and adrenocortical tumors are the most frequently observed tumors (10). It can also be associated with myelodysplastic syndromes, lymphoma and other benign and malignant tumors (11, 12). In children with LFS, brain tumors are the second most common malignancies following adrenocortical carcinoma. A quarter of childhood tumors involved CNS compared to only 13% of adult LFS related tumors (13). In LFS, the median age of onset of brain tumors is 16 years, compared to 57 years in the general population.

CNS tumors related to LFS have a prevalence ranging from 9 to 14% (14) and the most frequent types are glioblastoma and astrocytoma. Nonetheless, medulloblastoma, ependymoma, choroid plexus carcinomas, and other embryonal tumors are also described.

Etiopathology

The main gene disrupted in LSF is *TP53*, a tumor suppressor gene encoding the p53 protein, fundamental for the transcription of target genes involved in cell cycle arrest, DNA repair and response to DNA damage (15). *TP53* gene is located on chromosome 17p13.1 and more than 250 different germline alterations have been reported in medical literature to date. In brain tumors, most mutations reside within the DNA binding domain, even though all the genotypic-phenotypic correlations are not fully understood (16). Despite genetic lesions in LFS have been widely studied, not all the underlying genetic defects responsible for LFS have been found. In fact, several families fulfill the definition of classical LFS without the recognition of any known *TP53* defect being found (16). Although few LFS cases have been reported with germline mutations in the *CHK2* gene, no pediatric CNS tumors have been detected in these patients, suggesting a genotype-phenotype correlation between such malignancies and *TP53* mutations (17, 18). See **Figure 1** for details.

Clinical and Therapeutic Considerations

As already mentioned, there are no clinical characteristics associated with LFS other than an increased cancer risk. Considering this and the highly penetrance of LFS, clinical and familial diagnostic criteria are essential for the diagnosis. Classic diagnostic criteria and revised Chompret criteria for LFS are reported in **Supplementary Table 1** (19). It is essential to individuate families where LFS might be present as it has been demonstrated that intense tumor surveillance leads to increased survival (20).

It has been proven that *TP53* mutations are a negative prognostic factor in several tumor types, including pHGG (21). Despite the high risk of secondary malignancies after exposure to ionizing radiation, no specific treatment is available for LFS pHGG patients. Treatment strategies in these patients can be challenging, since mutations in the *TP53* gene have been associated with resistance to both chemotherapy and radiation (22). Also, LFS patients with CNS tumors show an overall worse outcome if compared to non-affected patients (22).

NEUROFIBROMATOSIS

Neurofibromatosis type 1 (NF1) (OMIM #162200), also known as von Recklinghausen disease, is a common autosomal dominant disorder with a prevalence of 1:4.000 individuals due to mutations of the *NF1* gene on chromosome 17q11.2 (23). The protein product of the NF1 gene, neurofibromin, regulates several intracellular processes, including the RAS/ERK/MAP kinase cascade and cytoskeletal assembly. Loss-of-function mutations of *NF1* gene lead to a high risk of tumor development due to decreased RAS signaling inhibition (24). Clinically, NF1 is characterized by *café au lait* macules, skin fold freckling, optic pathway gliomas, neurofibromas and plexiform neurofibromas, osseous lesions, and iris hamartomas (Lisch nodules) (23). The clinical diagnosis requires the fulfillment of at least two of the criteria as listed in **Supplementary Table 2**, however there are other possible manifestations that are not

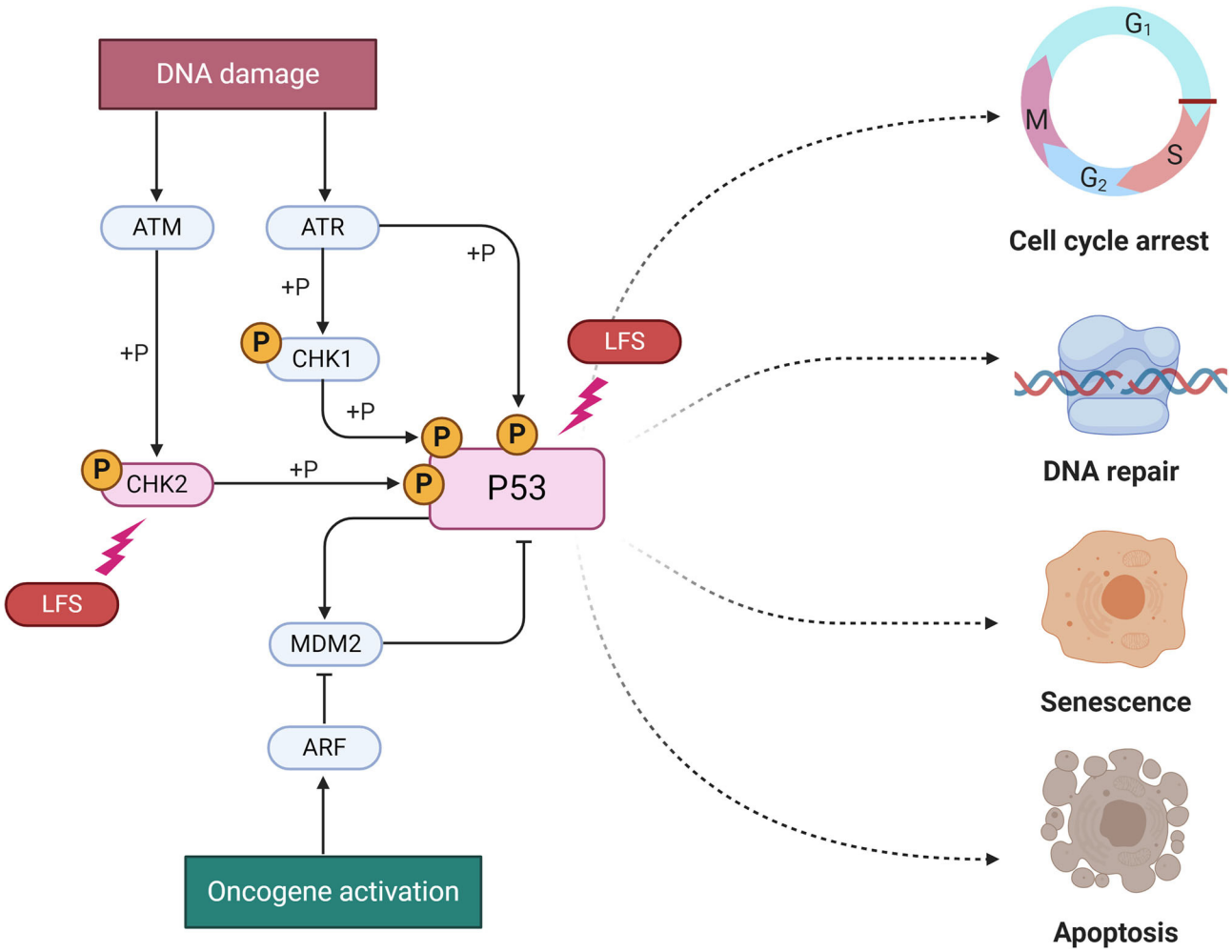

FIGURE 1 | Molecular pathways of Li-Fraumeni (LFS) Syndrome. The two known mutation for LFS are represented here (*P53* and *CHK2*) as lighting bolt. A group of protein kinases such as ATM, ATR, CHK1, CHK2 is implicated in the genome integrity checkpoint, a molecular cascade that detects and responds to several forms of DNA damage caused by genotoxic stress. Oncogenes also stimulate p53 activation, mediated by the protein ARF. In a normal cell, p53 is inactivated by its negative regulator, *MDM2*. Upon oncogene activation, various pathways will lead to the dissociation of the *P53* and *MDM2* complex. Once activated, p53 will induce a cell cycle arrest to allow either repair and survival of the cell or apoptosis to discard the damaged cell. Adapted from "P53 Regulation and Signaling," by BioRender.com (2020). Available online at: https://app.biorender.com/biorender-templates.

included in the diagnostic criteria but that can be present in patients harboring the mutation, such as macrocephaly, learning disabilities, vasculopathies and scoliosis. NF1 is associated with some CNS neoplasms in infancy, namely optic pathway gliomas and brainstem gliomas.

Epidemiology and Cancer Spectrum

NF1 (von Recklinghausen disease) is one of the most common CPS (13). It is an autosomal dominant inherited condition and about 50% of cases are found *de novo* with no associated family history (25).

CNS neoplasms predominantly associated with NF1 are optic pathway gliomas (15–20%) and brainstem -gliomas (1–2%). Other malignant tumors can be observed in these patients such as malignant peripheral nerve sheath tumors (MPNST) and juvenile myelomonocytic leukemia (JMML) (26).

Etiopathology

The gene involved in the pathogenesis of this syndrome is *NF1*, an onco-suppressor located on chromosome 17q11.2. The protein encoded by this gene is called Neurofibromin and is a GTPase activating protein that inhibits the product of the *RAS* oncogene, mediating the passage from GTP-RAS to GDP-RAS. RAS, in turn, is an activator of cell-cycle signaling pathways such as MAPK (RAF-MEK-ERK) and PI3K/AKT/mTOR pathways (27). *NF1* loss-of-function mutations remove this inhibition on RAS and the downstream pathways, leading to abnormal cell proliferation and tumorigenesis.

Clinical and Therapeutic Considerations

NF1 brain tumors are considered more indolent than same histology counterparts observed in patients without NF1, and can even regress over time without treatment (28). Histologically, most of them are low-grade gliomas (LGG), with a smaller representation of pHGG (81). It is notable that NF1 associated pHGG exhibit the same genetic alterations found in sporadic pHGG (such as *P53* and *CDKN2A* alterations) (29). On the other hand, NF1 alterations are frequently found as somatic genetic lesions in sporadic HGGs of childhood (30).

Apart from LGG, differential diagnosis of pHGG in NF1 children has to include the frequent finding of Focal Areas of Signal Intensity (FASI) in these patients. These are benign lesions, usually multiple and radiologically characterized as non-enhancing, small areas without mass effect or edema. They can be found in around 70% of NF1 pediatric cases and must be differentiated from gliomas (31).

Being pHGGs very uncommon in NF1, surveillance neuroimaging is controversial and not universally recommended (24). Regardless, families should be instructed to recognize the warning signs of brain tumors.

Treatment of pHGG in NF1 is similar to sporadic cases, some reports suggest that prognosis might be better than sporadic pHGG (32, 33). As for target specific therapies, MEK inhibitors have shown promising results in NF1 patients with low grade gliomas, this result may pose the basis for future treatment strategies also in NF1-pHGG (34).

Radiotherapy is generally part of the treatment protocol, despite increased complications, namely secondary malignancies and stroke (35).

CONSTITUTIONAL MISMATCH-REPAIR DEFICIENCY SYNDROME

Constitutional mismatch repair deficiency (CMMRD) syndrome (OMIM #276300) is a childhood autosomal recessive cancer predisposition syndrome caused by a biallelic germline mutation in the DNA mismatch repair (MMR) genes, namely *mutL homolog1* (*MLH1*), *mutS homolog1* (*MSH2*), *pms2 c-terminal like pseudogene* (*PMS2),* or *mutS homolog6* (*MSH6)* (36). Patients with monoallelic mutations in the MMR genes develop hereditary non-polyposis colorectal carcinoma (HNPCC), also known as Lynch syndrome, an autosomal dominant genetic disorder associated with increased risk of colorectal cancer, endometrial carcinoma, and other gastrointestinal and genitourinary malignancies in the fourth and fifth decades of life (37).

Epidemiology and Cancer Spectrum

CMMRD is a rare disease with roughly 200 cases reported to date (38, 39). However, its prevalence might be underestimated and a consistent number of cases might go undiagnosed in South Asian and Middle Eastern countries where consanguinity is more prevalent (40). In CMMRD, the tumor spectrum is very broad including CNS (glioblastoma, oligodendroglioma, low-grade glioma, medulloblastoma, and other embryonal tumors), hematological, genitourinary and intestinal tract tumors (41). Among brain tumors, malignant gliomas are the most frequent CMMRD-associated tumors, typically presenting within the first 2 decades of life and accounting for 25–40% of CMMRD cancers (41). Overall, there is a high degree of consanguinity within the family, indicating that inbreeding is a major risk factor for this otherwise rare disorder.

Etiopathology

MSH2, *MSH6*, *MLH1*, and *PMS2* genes are involved in the mismatch repair mechanisms, one of the most important DNA repair machinery of the cell (36). Its main role is to correct errors arising during DNA replication, thus tumors arising in the context of CMMRD exhibit an extraordinary number of DNA mutations. The most common type of defects found in these "hypermutated cancers" are point mutations (single nucleotide variations) and microsatellite instability (MSI) where repetitive sequences (microsatellites) are not adequately repaired. Recently, new genetic alterations affecting this machinery have been described, such as *MSH3* variants (42), deletions of the *EPCAM* gene (43), and mutations in DNA polymerases epsilon and delta 1 (*POLE*, *POLD1*) (44). See **Figure 2** for details.

Clinical and Therapeutic Considerations

In addition to cancer, CMMRD patients frequently have other physical features such as cutaneous *café-au-lait* spots and hypo- or hyperpigmented spots that may mimic some of the skin features usually observed in NF1. Also neurofibromas, Lisch nodules and freckling have been reported, although less frequently than in NF1 (39, 45). Other findings have occasionally been described in these patients such as vascular anomalies, pilomatrixomas, agenesis of the corpus callosum (46), and decreased levels of immunoglobulins IgG2/4 and IgA (39). However, none of these features are mandatory to diagnose the syndrome. The penetrance of the disease is very high, reaching more than 90% by the first two decades of life. Most patients will have childhood cancer and more than one tumor, often presenting synchronously (13).

Initial screenings can be performed by immunohistochemistry showing loss of MMR protein both in normal and malignant cells. Diagnosis can be confirmed by genetic testing for the presence of biallelic mutations in one of the four MMR genes. Evidence of low grade glial lesions and premalignant, dysplastic polyps advocates for surveillance protocols to intercept asymptomatic tumors at early stages, when they are more amenable to complete resection (47). Current protocols suggest annual whole-body MRI (WBMRI) from the age of 6 years. In addition, it is recommended to start colon surveillance by colonoscopy from 6 years of age. Treatment of CMMRD tumors is complicated by resistance to standard therapies for pHGG such as temozolomide, since it requires adequate mismatch repair to perform its action.

Interestingly, immunotherapy has proved to be a promising strategy in these tumors. One of the main mechanisms through which tumors escape immune recognition and induce immunosuppression is PD-L1 overexpression of cancers that acts as a binding site for PD1. The binding of PD1 to PDL1 activates PD1 signaling that inhibits T cells allowing the tumor

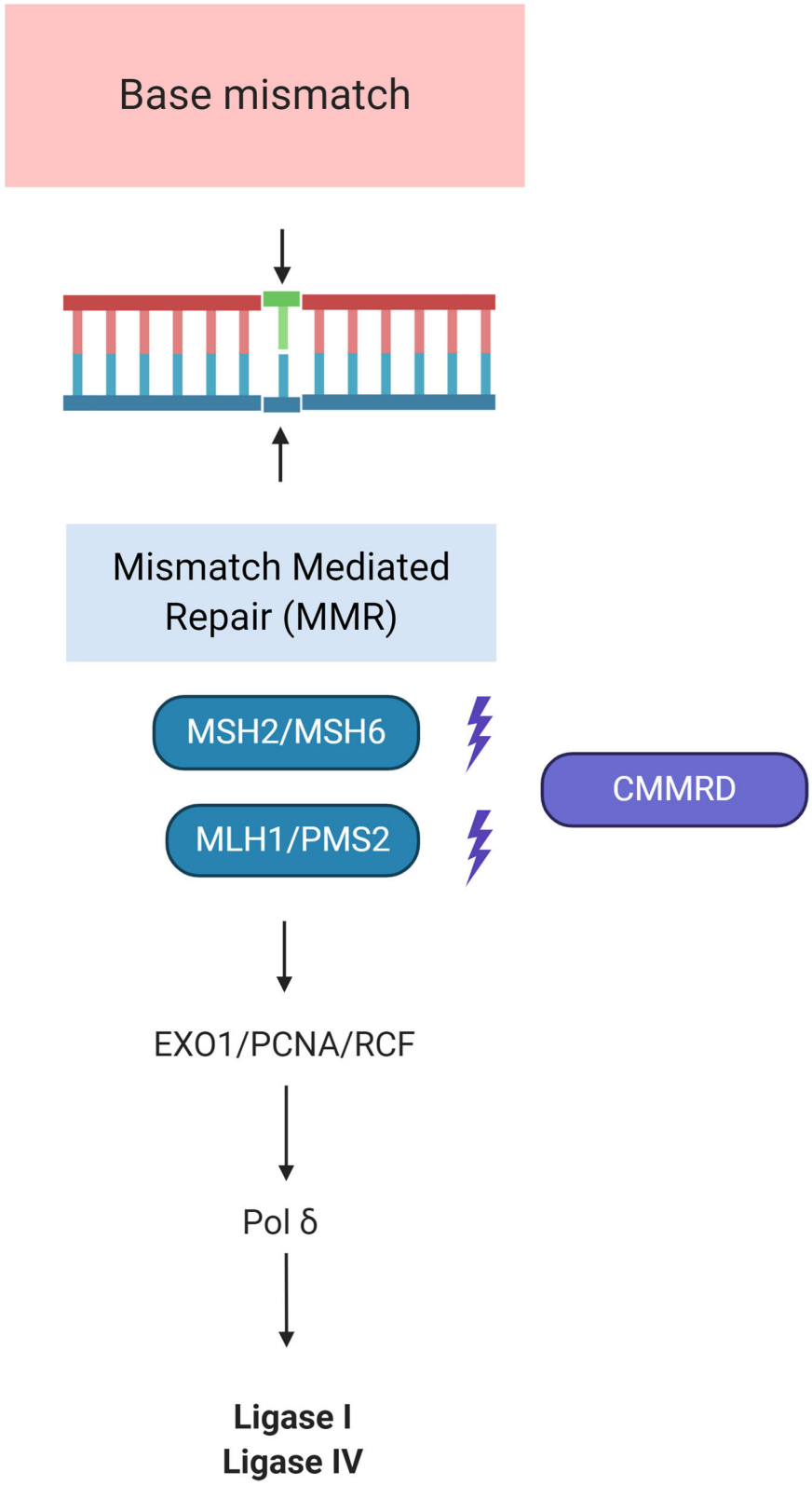

FIGURE 2 | Molecular pathways of Constitutional Mismatch Repair Deficiency Syndrome (CMMRD). MSH2 dimerizes with MSH6 to form the MutSα complex, which is involved in base mismatch repair and short insertion/deletion loops. The formation of the MSH2-MSH6 heterodimer accommodates a second heterodimer of MLH1 and PMS2. This protein complex formed between the 2 sets of heterodimers enables initiation of repair of the mismatch defect by recruiting PCN/EXO1/RCF. RFC is

(Continued)

FIGURE 2 | essential for PCNA loading and function in DNA replication. PCNA loads onto double-strand breaks and promotes Exo1 damage association through direct interaction with Exo1. By tethering Exo1 to the DNA substrate, PCNA confers processivity to Exo1 in resection. This role of PCNA in DNA resection is analogous to its function in DNA replication where PCNA serves as a processivity co-factor for DNA polymerases such as polymerases δ. DNA Pol δ is an enzyme used for both leading and lagging strand synthesis by engaging Ligase I and IV. Adapted from "DNA Repair Mechanisms by BioRender.com (2020). Available online at: https://app.biorender.com/biorender-templates.

to evade immune attack (48). These principles have been used to develop drugs named checkpoint inhibitors that counteract the interactions of the PD1 protein. It has been demonstrated that CMMRD tumors are more responsive to PD1 blockers than MMR proficient tumors. In particular, in children with CMMRD with recurrent glioblastoma, shrinking of tumors was observed on MRI, suggesting these tumors as ideal candidates for such therapies (49).

OLLIER DISEASE AND MAFFUCCI SYNDROME

Ollier disease (OD, OMIM 166000) and Maffucci syndrome (MS, OMIM 6145692) are related conditions characterized by multiple endochondromas and caused by somatic mutations in the *IDH1* and *IDH2* genes, respectively (50, 51). The main difference between the two conditions is the presence of hemangiomata in MS, moreover, while OD presents with multiple enchondromas, typically unilateral in distribution with a predilection for the appendicular skeleton, MS is often characterized by multiple enchondromas bilaterally distributed (51).

Epidemiology and Cancer Spectrum

Most cases of OD and MS have been reported as sporadic, with an estimated prevalence of 1 out of 100,000 individuals, although the description of few familial cases of OD suggests a possible autosomal dominant pattern of inheritance (51). About half of the individuals with OD or MS develop a malignancy, such as chondrosarcoma (with a prevalence of 30% in both conditions), glioma, and ovarian juvenile granulosa cell tumor, accompanied by other clinical features, such as multiple swellings on the extremity, deformity around the joints, limitations in joint mobility, scoliosis, bone shortening, leg-length discrepancy, gait disturbances, pain, loss of function, and pathological fractures (51).

Etiopathology

Mutations in the *IDH1* or *IDH2* genes have been detected in a large number of adult diffuse grade II and grade III gliomas; such high frequency has suggested a possible role for those variants as the earliest oncogenic event in these malignancies (52). It has been proven that pathogenic variants in these two genes cause an abnormal production of 2-hydroxyglutarate (2-HG), a structural analog of alpha-ketoglutarate, a key intermediate of the Krebs cycle. 2-HG competitively inhibits the active sites of multiple alpha-ketoglutarate enzymes, resulting in hypermethylation of histones and DNA, altered cell differentiation, and activation of a series of downstream enzymes (53, 54). Some of these enzymes are involved in the degradation of HIF-1 (hypoxia-induced factor 1), a key player in the cellular adaptation to low oxygen and nutrient-deprived environment and in the progression to malignancy in human solid cancers, and in the overexpression of platelet-derived growth factor receptor A (PDGFRA), implicated in the pathogenesis of leukemias, lymphomas, gastrointestinal stromal tumors, and various types of brain tumors (53–55).

Clinical and Therapeutic Considerations

The clinical management of individuals with OD and MS is mostly focused on treating via surgery the complications arising from the enchondromas, such as fractures, growth defects, and tumors. The prevailing strategy aims to treat and remove any extraneous bone tissue preserving the limb function (51). Although gliomas are not the most frequent types of malignancies reported in OD and MS, imaging surveillance is recommended. The gliomas described in these conditions are similar to the ones caused by sporadic variants in *IDH1* or *IDH2* for their frequent location in the frontal lobe and their prevalent histological type: more commonly diffuse low-grade or anaplastic gliomas than glioblastomas (53). However, they present some substantial differences as compared to the sporadic forms: they are diagnosed at an earlier age and involve more frequently the brainstem, hinting toward an earlier origin of gliomas associated with enchondromatosis.

OTHER SYNDROMES AND pHGG

Some less-known syndromes have been associated with pHGG with lower frequency than the afore-mentioned syndromes.

One of those is the Familial Melanoma Astrocytoma Syndrome (56, 57). It is caused by germline inactivating deletion of the *CDKN2A* tumor suppressor gene. Affected individuals have a predisposition to develop melanoma and CNS tumors, most commonly astrocytoma.

Since familial predisposition to glioma has been consistently observed within non-syndromic families, an international consortium named GLIOGENE was formed in order to collect such non-syndromic glioma families, and possibly identify new genes involved in the pathogenesis of these tumors. One of the genomic regions identified by the consortium lies in chromosome 17q. According to these linkage studies the *MYO19* and *KIF18B* genes and rare variants in *SPAG9* and *RUNDC1* have been identified as candidates worthy of further investigation (58). Also, whole exome sequencing allowed the identification of mutations in *POT1* (p.G95C, p.E450X), a member of the telomere shelterin complex

(59). These new findings may not only have a leading role in identifying new pathogenic pathways in gliomas but may also contribute to improve targeted treatment of this disease.

Mutations in *BRCA1* and *BRCA2*, tumor suppressor genes involved in DNA repair, have been traditionally associated with an increased risk of breast and ovarian cancer. More recently, they have been recognized to also play a role in CNS tumors (60). In particular, germline variants of *BRCA2* which is also essential for normal neurogenesis (61) have been described in individuals with brain tumors including glial tumors, meningioma and medulloblastoma (62–64).

There have been some anecdotal reports of pHGG in other syndromes (65), such as tuberous sclerosis (66), Beckwith-Wiedemann and Fanconi Anemia (67). However, these case reports do not prove a real increased risk for pHGG.

MOLECULAR DIAGNOSTICS OF CPSs

Genetic testing in pediatric oncology is of great interest for the investigation into potentially underlying CPSs. Molecular diagnosis of a CPS can influence cancer surveillance program initiation or frequency, and directly impact treatment decisions. Genetic diagnostic laboratories have introduced next-generation sequencing (NGS) technologies into their practices. NGS has specific advantages over traditional Sanger sequencing, considered the gold standard for mutation analysis for many years, as multiple genes in several patients can be tested simultaneously. Different approaches are being used, and currently, most laboratories that use these technologies are performing targeted gene panel testing or clinical whole exome sequencing (WES), more rarely whole genome sequencing (WGS). These revolutionary technological advances have drastically reduced sequencing costs and shortened the turnaround time, increasing the detection rate (68). Multi-gene panels usually include high and moderate penetrance genes, and sometimes, some low or unknown risk genes, that offer the advantage of identifying germline pathogenic variants in genes that would not normally be tested based on the patient's diagnosis (69). Unfortunately, depending on the disease, between 70 and 92% of the patients remain mutation-negative or undiagnosed after gene-panel testing (70). It is possible that variants in genes not included in these panels contribute to the cancer risk and WES or WGS can explore the genetic basis of familial syndromes in a more extensive way, permitting to identify new high- and moderate-risk cancer predisposition genes. WES of parent-child trios has become a widely used strategy to identify presumably pathogenetic genetic variants in children with rare diseases. However, it has not yet been routinely implemented in pediatric oncology, with few exceptions (71). Genome-wide approaches generate huge amounts of genetic data and it remains challenging to interpret the identified variants. Such data interpretation needs close collaboration among bioinformaticians, molecular geneticists and clinicians. However, as sequencing costs are decreasing and computer and technological resources are expanding, genome-wide analysis will become more common in the clinical practice and hopefully help to advance on the path of personalized medicine, by providing more precise genetic diagnoses and better molecular information for more effective treatments.

DNA METHYLATION PROFILING

Recently, a machine learning approach for classification of CNS tumors based on the analysis of global DNA methylation profiling has been developed and introduced to reach a histopathological-molecular integrated diagnosis, discriminating tumor classes and ameliorating diagnostic precision (72, 73). In detail, the developed "Classifer" provides a methylation-based classification assigning a subgroup score for an index tumor compared to 91 different brain tumor entities. Furthermore, it also provides a chromosomal copy-number variation (CNV) analysis.

Interestingly, Capper and colleagues found that a high proportion of unclassifiable CNS tumors were associated with various hereditary tumor syndromes, and/or diagnosed in childhood (73). Additional chromothripsis and unusual complex chromosomal changes should also be considered as a cue for Li–Fraumeni syndrome-associated tumors.

CONCLUSIONS

Pediatric HGG cancer predisposition syndromes are rare and diverse pathological conditions that may be present in children with CNS tumors and deserve consideration.

Knowing when to suspect one of these predisposing syndromes is essential for the pediatric oncologist, not only to make the correct diagnosis, but also to formulate a more accurate prognostic judgment and provide an adequate treatment. Moreover, it is mandatory to refer the family for genetic counseling when such conditions are suspected. This latter aspect is of particular relevance since it has been demonstrated that close surveillance can decrease the morbidity and mortality in these patients.

The ever-growing knowledge of the genetic mechanisms underlying cancer is a key tool in the understanding of this disease, opening new scenarios for the introduction of molecular target therapy.

Since these conditions are extremely rare, several patients' associations have been created to help families find the nearest structure for follow-up and to raise funds and consciousness for these diseases.

AUTHOR CONTRIBUTIONS

GC, GD, AM, and AC designed the study. GC, GD, MV, LB, RC, and EM cured the literature research and its organization. GC, EA, FD, and MR cured the literature research focusing on the genetics aspect. GC, GD, EM, and LB wrote the final version of the manuscript. AM, AC, FL, and LB critically revised the

manuscript for intellectual content. Finally, all authors approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

We thank the association Heal for the support.

ACKNOWLEDGMENTS

The author thanks Megan Eckley for the english language editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.561487/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Pediatrics

REVIEW
published: 17 December 2020
doi: 10.3389/fped.2020.613260

Characterization and Childhood Tumor Risk Assessment of Genetic and Epigenetic Syndromes Associated With Lateralized Overgrowth

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Lateralized overgrowth (LO), or segmental overgrowth, is defined as an increase in growth of tissue (bone, muscle, connective tissue, vasculature, etc.) in any region of the body. Some overgrowth syndromes, characterized by both generalized and lateralized overgrowth, have been associated with an increased risk of tumor development. This may be due to the underlying genetic and epigenetic defects that lead to disrupted cell growth and proliferation pathways resulting in the overgrowth and tumor phenotypes. This chapter focuses on the four most common syndromes characterized by LO: Beckwith-Wiedemann spectrum (BWSp), *PIK3CA*-related overgrowth spectrum (PROS), Proteus syndrome (PS), and *PTEN* hamartoma tumor syndrome (PHTS). These syndromes demonstrate variable risks for tumor development in patients affected by LO, and we provide a comprehensive literature review of all common tumors reported in patients diagnosed with an LO-related disorder. This review summarizes the current data on tumor risk among these disorders and their associated tumor screening guidelines. Furthermore, this chapter highlights the importance of an accurate diagnosis when a patient presents with LO as similar phenotypes are associated with different tumor risks, thereby altering preventative screening protocols.

Keywords: lateralized overgrowth, hemihypertrophy, hemihyperplasia, Beckwith-Wiedemann spectrum, Beckwith-Wiedemann syndrome (BWS), PIK3CA-related overgrowth spectrum (PROS), Proteus syndrome (PS), PTEN hamartoma tumor syndrome

OPEN ACCESS

Edited by:
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Reviewed by:
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Joseph Louis Lasky,
Cure 4 the Kids, United States

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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Pediatrics

Received: *01 October 2020*
Accepted: *03 November 2020*
Published: *17 December 2020*

Citation:
Griff JR, Duffy KA and Kalish JM (2020) Characterization and Childhood Tumor Risk Assessment of Genetic and Epigenetic Syndromes Associated With Lateralized Overgrowth. Front. Pediatr. 8:613260. doi: 10.3389/fped.2020.613260

INTRODUCTION

Lateralized overgrowth (LO) is defined as any type of segmental overgrowth (1) (**Figure 1**). The nomenclature was developed to classify patients who were previously described with overgrowth due to both hyperplasia (OMIM 235000), a proliferation of cells, and hypertrophy (OMIM 235000), an increase in cell size. The overgrowth defined by LO is not specific to the type of tissue affected and can include skeletal, muscular, adipose, and/or vascular tissues. Some patients present with isolated LO, in which patients are primarily affected by LO. Overgrowth of organs is not required for the designation of LO, but it can be present and typically occurs in patients with overgrowth syndromes associated with LO.

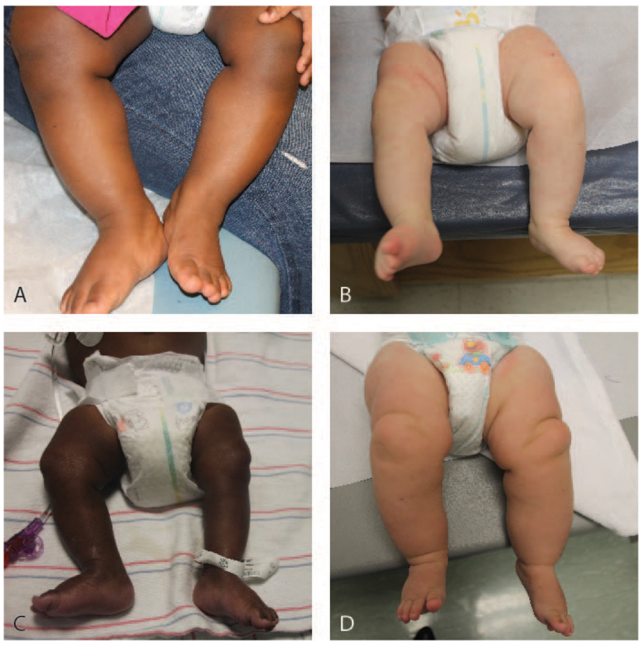

FIGURE 1 | Legs of patients with lateralized overgrowth. **(A)** 12-month old patient with Beckwith-Wiedemann syndrome. **(B)** 3-month old patient with Beckwith-Wiedemann syndrome. **(C)** 9-month old patient with Beckwith-Wiedemann syndrome. **(D)** 6-month old patient with PIK3CA-related overgrowth spectrum.

Patients with isolated lateralized overgrowth (ILO), those affected by LO but lacking other features and patterns of malformations, dysplasia, and morphologic variants, have been reported to have an increased development of tumors, primarily the embryonal tumors Wilms tumor (WT) and hepatoblastoma (HB) (2, 3), similar to the most common tumor types observed among patients affected by LO and overgrowth disorders (4). A prior study of patients with isolated hemihypertrophy, now referred to as ILO, reported 9 out of 168 developing a tumor (5) and two cases of HB in patients with isolated hemihyperplasia, now also termed ILO (6). Retrospectively, it is likely that many of these patients could be classified with an overgrowth or cancer predisposition syndrome.

There are several genetic and epigenetic syndromes associated with LO and ILO. These molecular changes may influence the tissue type, location of the observed overgrowth, and associated tumor risk in patients. In this chapter, we review the clinical characteristics of the most common genetic and epigenetic syndromes associated with LO. We focus on tumor development and risks associated within each syndrome and summarize current screening recommendations.

Common considerations for all suspected LO-related overgrowth disorder include the underlying molecular cause and appropriateness for tumor surveillance.

Molecular Considerations

The underlying mechanisms for the disorders described are complex and beyond the focus of this review. A brief description of the currently understood mechanisms for each disorder is summarized and includes both genetic and epigenetic mechanisms. One consideration for molecular investigation for these disorders is that some defects can present as mosaic, in which the proportion of normal cells to cells with the molecular change varies in any given tissue, leading to patients with somatic molecular defects. This means that positive molecular detection may only be found in affected tissue(s), whereas blood sample analyses may yield negative results. Other patients affected by LO and overgrowth have the molecular defect change(s) detectable in blood samples (constitutional defects), with some patients affected by changes that are inheritable or considered germline defects.

Tumor Risk and Screening

Specific recommendations and implementation of tumor surveillance protocols are determined by the risk of tumor development in a particular syndrome, the uniformity of the tumors that develop (i.e., can they be screened for in a non-invasive manner), and the health care environment in which the screening is occurring (i.e., the threshold of acceptable risk) (4). In some syndromes with an established tumor risk, tumor screening has been demonstrated to detect tumors at an early age. For example, in Beckwith-Wiedemann spectrum (BWSp), patients who underwent ultrasonographic screening had on average earlier tumor stages at diagnosis than those who did not undergo screening (7). Diagnosing tumors in their earlier stages may allow for less invasive treatment and the prevention of possible metastasis.

Here, we review the most common syndromes characterized by LO: BWSp (OMIM 130650), *PIK3CA*-related overgrowth spectrum (PROS), Proteus syndrome (PS) (OMIM 176920), and *PTEN* hamartoma tumor syndrome (PHTS). Tumor development in these four syndromes is variable and discussed below.

BECKWITH-WIEDEMANN SPECTRUM (OMIM 130650)

Overview

BWSp is the most common and well-characterized overgrowth and cancer predisposition disorder and is caused by a variety of molecular defects in the chromosome 11p15 region. The disorder is estimated to affect 1 in 10,340 live births and disproportionately affects patients conceived by assisted reproduction techniques, estimated to affect 1 in 1,100 live births (8, 9). The clinical manifestations and subsequent phenotype of patients with BWSp can be highly variable, leading to the reclassification of the disorder from a syndrome [Beckwith-Wiedemann syndrome (BWS)] to a spectrum [BW spectrum (BWSp)] by an international consensus group (10). The consensus group created a clinical scoring system to guide molecular and clinical diagnosis. They classified features as those classically associated with the disorder (cardinal features) and features associated with the disorder but that can also occur in the general population (suggestive features). This scoring system was implemented to determine if genetic testing is necessary (10). Cardinal features

include macroglossia, omphalocele, muscular LO, bilateral WT, hyperinsulinism, adrenal cytomegaly, pancreatic adenomatosis, and placental mesenchymal dysplasia, and suggestive features include macrosomia, facial nevus simplex, polyhydramnios or placentomegaly, ear creases or pits, transient hypoglycemia, embryonal tumors, nephromegaly and/or hepatomegaly, and umbilical hernia or diastasis recti. Each cardinal feature receives two points, and each suggestive feature receives one point. A total clinical score greater or equal to 2 indicates the need for genetic testing for BWSp. A clinical score greater or equal to 4 (typically including at least one cardinal feature) is sufficient for a clinical diagnosis of BWSp even if no molecular defect on chromosome 11p15 is identified. Genetic testing is also recommended for patients with a family history of BWSp caused by a heritable alteration.

Molecular Considerations

BWSp is caused by a variety of genetic and epigenetic alterations in the BWS critical region on chromosome 11p15.5 (10). The BWS critical region contains two imprinted regions, which control the normal regulation of fetal and postnatal growth genes through a process called methylation. The majority of patients are affected by abnormal methylation in the imprinting control 1 (IC1) and/or imprinting control 2 (IC2) regions, with the most common cause being loss of methylation at KCNQ1OT1:TSS DMR (IC2 LOM) (∼50% of patients) (10). Other causes of BWSp include paternal uniparental isodisomy of chromosome 11p15 (pUPD11), gain of methylation at H19/IGF2:IG DMR (IC1 GOM), mutations of *CDKN1C*, and other genetic aberrations including deletions, duplications, and translocations that affect chromosome 11p15 (10).

Tumor Risk in BWSp

The risk for WT, HB, and neuroblastoma in BWSp is well documented (11–18). A patient's tumor risk varies based on the molecular etiology of BWSp. According to the recent international consensus for BWSp, for patients with IC1 GOM, the overall risk of tumor development is 28%, and the risk for WT is 24%. For patients with IC2 LOM, the overall tumor risk is 2.5%. For patients with pUPD11, the overall tumor risk is 16%. The risk for developing a WT is 8%, and the risk for developing a HB is 3.5%. Screening guidelines are constantly evolving based on ongoing research on this topic and are dependent on geographical location and cultural context of clinical practice. The European guidelines include abdominal ultrasounds every 3 months until the age of 7 years for patients with BWSp due to IC1 GOM, pUPD11, *CDKN1C* mutations, and other chromosome aberrations of the BWS region (10). The United States guidelines developed by the American Association for Cancer Research (AACR) Childhood Predisposition Workshop include abdominal ultrasounds and alpha-fetoprotein (AFP) screening every 3 months until the 4th birthday and renal ultrasounds every 3 months from the 4th to the 7th birthday for all patients with BWSp (4). In addition, patients with *CDKN1C* mutations, those at the highest risk for developing a neuroblastoma among patients with BWSp, should receive urine vanillylmandelic acid (VMA), homovanillic acid (HVA), and chest X-rays screening every 3 months until the 6th birthday and every 6 months from the 6th to the 10th birthday (10). Patients with BWSp caused by genome-wide paternal isodisomy (GWpUPD) have been reported to have additional tumors and beyond these screening windows. Patients with this molecular subtype should be monitored closely (10, 19).

PIK3CA-RELATED OVERGROWTH SPECTRUM

Overview

The phenotypic variety and overlap of individual syndromes caused by *PIK3CA* mutations prompted the establishment of the term PROS (20). The specific overgrown tissue observed in patients with PROS is typically adipose or vascular; however, muscular and skeletal overgrowth has also been observed (20). Other common clinical characteristics include epidermal nevus, macrodactyly, hemimegalencephaly (HMEG), seborrheic keratoses, and benign lichenoid keratoses (20). To determine the eligibility for genetic testing, clinical characteristics are divided into two categories: category A, which includes a spectrum of overgrowth, vascular malformations, and epidermal nevus phenotypes, and category B, which includes isolated features, such as lymphatic malformations or macrodactyly. Genetic testing is warranted if a patient presents with two or more features from category A, or one feature from category B, that was/were congenital or developed during early childhood.

A diagnosis of PROS is confirmed with a pathogenic variant found in the *PIK3CA* gene; however, if a mutation is not detected, the patient retains a clinical diagnosis of PROS if the clinical criteria are met (20, 21). In patients affected by clinical diagnoses of PROS, it is likely that the negative genetic result(s) observed are due to the somatic and therefore mosaic nature of the *PIK3CA* mutation leading to the phenotype, which may be difficult to detect from a single sample (such as blood).

Molecular Considerations

PIK3CA is a protein coding gene for p110α that is the α subunit of a collection of catalytic subunits for phosphatidylinositol 3-kinase (PI3K) (22). This protein is important for regulating signals for cell proliferation and survival. Mutations in *PIK3CA* have been identified as the driver for many cancers in asymptomatic patients (those without phenotypes related to *PIK3CA* abnormalities), with common cancer types including breast (>30%), endometrial (>30%), bladder (>20%), colorectal carcinoma (>17%), and head and neck squamous cell carcinoma (>15%) (23).

PIK3CA mutations have also been identified in patients with the following syndromes: fibroadipose overgrowth (FAO) (24), congenital lipomatous overgrowth, vascular malformations, epidermal nevi, scoliosis/skeletal and spinal (CLOVES) syndrome (25, 26), megalencephaly-capillary malformation (MCAP) syndrome (27), Klippel-Trenaunay syndrome (KTS) (26, 28), and HMEG (29). Typically, *PIK3CA* mutations occur post-fertilization (somatic mutations), but there have been germline *PIK3CA* mutations reported (30, 31). Allelic heterogeneity in PROS (and other overgrowth disorders) and

the overlap of common variants in the genes responsible may influence cancer predisposition, but further study is required.

Tumor Risk in PROS

Tumor risk and surveillance for patients with PROS is currently debated. Gripp et al. suggested similar screening guidelines for patients affected by PROS to the guidelines for patients affected by ILO or BWS, which includes abdominal ultrasounds until the 7th birthday (32). Peterman et al. suggest sonographic screening for patients with CLOVES, MCAP, and diffuse capillary malformations only if LO is present (33, 34). To our knowledge, there have been 12 patients with PROS reported with malignant or potentially malignant renal findings [including WT, nephrogenic rests (NR), and indeterminate WT/NR findings]. NR and nephroblastomatosis (NBL) are capable of transforming into WT, but they are not tumors themselves (35, 36). Among the PROS patients with renal findings, eight patients with findings reported had a molecularly confirmed PROS diagnosis: four reported with WT development, two with reported indeterminate WT/NR findings, and two with NR (26, 28, 32, 37–40). Four additional patients with renal findings and without molecular PROS confirmation have also been reported (41–44). Postema et al. estimated the tumor risk between 1 and 2%, suggesting that under European standards, screening is not warranted (39); however, at that risk level by US guidelines, screening would be warranted (4).

As the focus of this review is to discuss common syndromes associated with LO and tumor screening guidelines, determining the true WT risk in PROS is beyond the scope of this chapter. A meta-analysis of PROS patients and WT development is currently being performed and will be reported separately in the literature once completed. Based on the current literature, the risk depends on how the reported cases are classified (for example, true WT vs. those with indeterminant malignant potential, such as NBL and NR). The tumor risk in the PROS population appears to be slightly less than what Postema et al. reported (∼1–2%), and therefore it is unclear whether screening is warranted. The AACR tumor screening guidelines suggest screening when the risk of developing cancer is 1% or greater (4). It is suspected that the total patients with *PIK3CA* mutations currently classified may be higher than reports suggest (due to difficult detection of low levels of mosaicism). If this is true, the number of patients affected by PROS with tumors and the associated tumor risk for this disorder are likely well below the 1% threshold to warrant screening. Additionally, through our experience and discussions with colleagues, we are aware of many unreported patients with molecularly confirmed PROS who have not developed a WT or NR. We suspect that it is likely that the overall risk falls below 1%, indicating that screening is not warranted. It is also possible there are more patients with PROS and NR that have not been reported, as the NR did not progress to NBL or WT requiring treatment. There is a clear need for further publication of known cases and collaboration among institutions, so the denominator of patients with PROS can be further adjusted to understand true WT risk in this population. In terms of current recommendations, tumor screening should be performed at the discretion of the provider based on the genetic change and clinical features of the PROS presentation, as well as the family perspective.

In addition to WT and NR, there are four case reports of patients with PROS who developed other cancers including leukemia, vestibular schwannoma, retinoblastoma, and a meningioma (45–47); however, these do not suggest a specific predisposition or warrant surveillance.

Additional Considerations

Studies on cell-free DNA of urine of patients with PROS found *PIK3CA* mutations in urine samples of patients who developed renal abnormalities, but not in patients with PROS who did not have a history of kidney irregularities (48, 49). As a result, it has been suggested that urine may be useful in detecting *PIK3CA* mutations, and those patients with positive results in urine may represent an increased risk for WT development (48). There may be other specific circumstances that could increase tumor risk, such as known *PIK3CA*-related changes in proximity to the kidneys or patients affected by specific germline or somatic mutations, but further study using larger cohorts is needed to better understand mechanisms and individual risk.

PROTEUS SYNDROME (OMIM 176920)

Overview

PS is caused by postzygotic *de novo* activating mutations in *AKT1* (50). Clinical features of the syndrome include asymmetric skeletal growth, connective tissue nevi, epidermal nevi, vascular malformations, and dysregulated adipose tissue (lipomas, lipohyperplasia, fatty overgrowth, and partial lipohyperplasia) (51). Overlapping disorders, such as CLOVES, under the umbrella of PROS prompted the creation of a new diagnostic scoring system for PS (52). Five points are attributed for cerebriform connective tissue nevus, disproportionate overgrowth, and organ/visceral overgrowth. Two points are attributed for bullae or cysts of the lungs, dysregulated adipose tissue, linear verrucous epidermal nevus, vascular malformations, deep vein thrombosis/pulmonary embolism, and certain facial features, such as dolichocephaly and a low nasal bridge. Single points are attributed for specific tumors including genital cystadenomas, parotid monomorphic adenoma, and meningiomas (52). Points are subtracted for features, such as substantial prenatal extracranial overgrowth and ballooning overgrowth (52).

A diagnosis is confirmed if a patient has a score of 15 or more regardless of the presence of an *AKT1* variant. A patient with 10 or more points with an identified mosaic *AKT1* variant is considered to have PS. Those with scores between 2 and 9 points with an AKT1 variant are considered to have *AKT1*-related overgrowth spectrum (AROS) (52).

Molecular Considerations

The *AKT1* gene located on chromosome 14q.32.33 is involved in the mTOR pathway that is responsible for regulating cell proliferation and survival (50). Patients with PS have a somatic, activating mutation in this gene that causes the observed

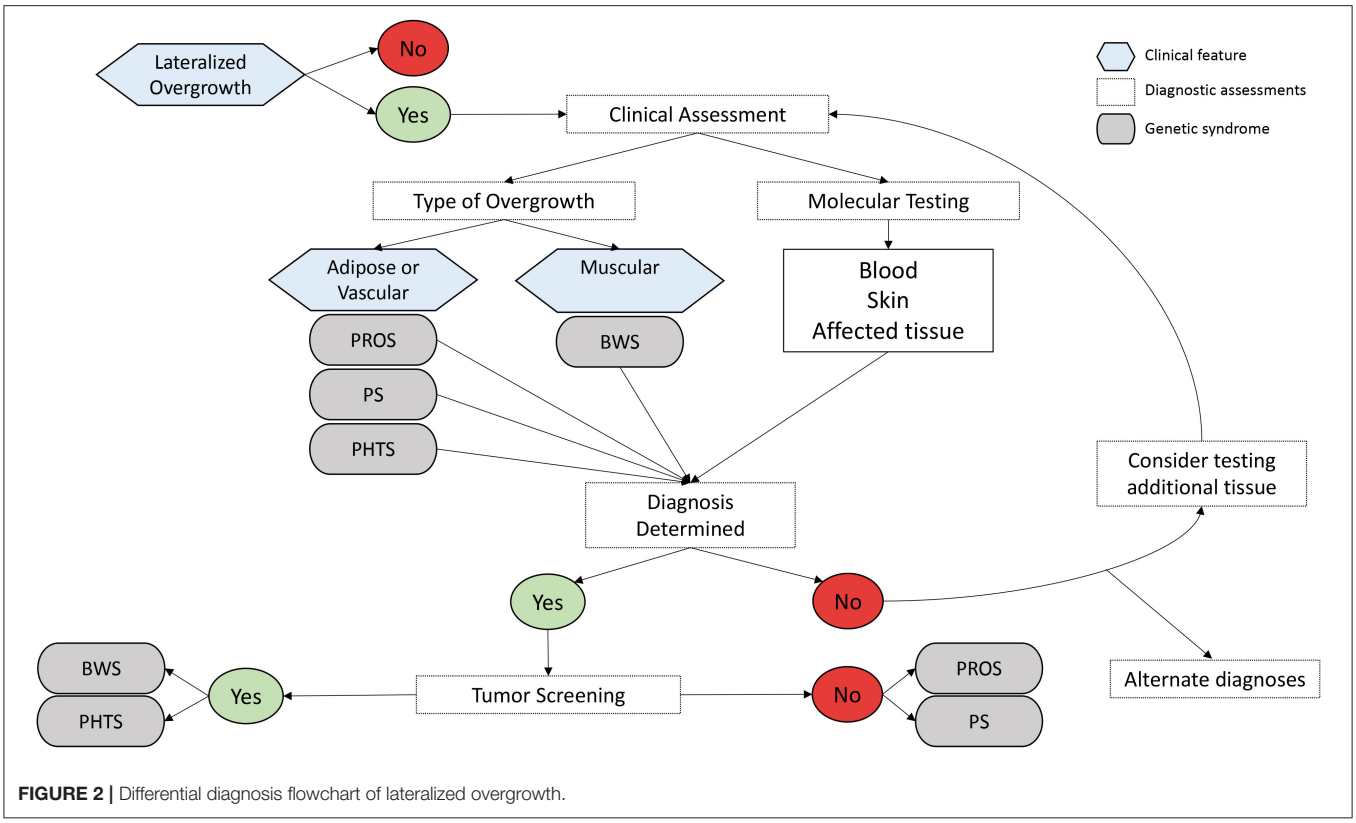

FIGURE 2 | Differential diagnosis flowchart of lateralized overgrowth.

abnormal growth. This mutation is not found in blood cells, and therefore a biopsy of the affected skin or tissue is required for a molecular diagnosis.

Tumor Risk in PS

There are currently no tumor screening guidelines for patients with PS. However, a variety of benign and malignant tumors have been reported. Common neoplasms in patients with PS include lipomas, hamartomas, and vascular malformations (53). There have been multiple reports of patients with PS who developed genital cysts as well as meningiomas (52–55). Other case reports of benign tumors include an optic nerve tumor, pinealoma, monomorphic parotid adenoma, intraductal papilloma, goiter, leiomyomas, papillary adenoma of appendix testis, papillary adenoma of kidney, and epibulbar tumor (53, 54, 56–58). Malignant tumors in patients with PS have also been reported. They include papillary thyroid carcinoma, mesothelioma of tunica vaginalis and peritoneal surface, intraductal carcinoma of the breast, endometrial cancer, ovarian carcinoma, and paratesticular ovarian-type papillary serous carcinoma (53, 54, 59–64).

Early mortality in patients with PS is high yet does not appear to be related to the development of cancer (65), as pulmonary embolisms, postoperative embolisms, and pneumonia are responsible for mortality in 20% of patients with PS (51). It is possible that tumor risk is higher in this population, especially benign tumors, but due to the high mortality, an increased tumor risk is not observed.

PTEN HAMARTOMA TUMOR SYNDROME

Overview

PHTS is the umbrella term for genetic syndromes caused by germline *PTEN* mutations. Common clinical features of pediatric patients with PHTS include macrocephaly, hamartomas, lipomas, cardiac defects, and autism (66). LO is due to adipose and vascular anomalies. Major and minor criteria were implemented to aid in diagnosis. Major criteria include the presence of macrocephaly, macular pigmentation of the glans penis, and multiple mucocutaneous lesions, and minor criteria include autism, lipomas, and vascular malformations (66, 67).

Molecular Considerations

PTEN is a tumor suppressor gene on chromosome 10q23 and is also involved in the mTOR signaling pathway (68). Germline mutations of *PTEN* cause PHTS and have been identified in patients with Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome (69). There have also been case reports of patients with an initial clinical diagnosis of PS, but a *PTEN* mutation was identified, leading to the term Proteus-like syndrome (70–72).

Tumor Risk in PHTS

The tumor risk in patients with PHTS is well-documented although the syndrome is not typically associated with early childhood cancer risks. Tumors tend to develop in females more

TABLE 1 | Summary of tumor risks in genetic and epigenetic syndromes with lateralized overgrowth.

	Genetic cause	Type of overgrowth	Malignant tumors	Tumor risk	Childhood surveillance recommendation(s)
PROS	*PIK3CA* mutations*	Adipose, vascular	Wilms tumor	∼1%	None (to be determined)
BWSp	Genetic and epigenetic alterations on chromosome 11p15.5	Muscular	Wilms tumor Hepatoblastoma (Neuroblastoma)	0.2–24% 0–3.5% 0.5–4.2%	Abdominal ultrasound and AFP screening every 3 months until the 4th birthday and renal ultrasounds from the 4th until the 7th birthday
PS	*AKT1* mutations	Skeletal, adipose, vascular	None	Unknown	None
PHTS	*PTEN* mutations	Adipose, vascular	Breast Thyroid Endometrium Melanoma Kidney Colorectal	25–50% 3–17% 9–27% 1–6% 4–16% 3–13%	Annual thyroid ultrasounds beginning at the time of diagnosis

**Majority are somatic mutations, but there have been case reports of patients with germline PIK3CA mutations.*

than males. The cumulative cancer risk by age 50 for females is 81% and for males is 48% (73).

Malignant tumors commonly observed in patients with PHTS include breast (25–50%), thyroid (3–17%), endometrium (9–27%), melanoma (1–6%), renal (4–16%), and colorectal cancers (3–13%) (73–82). Lhermitte-Duclos disease (LDD) also known as gangliocytoma of the cerebellum is common to develop late in life in patients with germline *PTEN* mutations (83, 84). Common benign tumors including hamartomas and lipomas can develop in patients at any age and require attention (evaluation and work-up) because of secondary complications that can arise.

There is no international consensus for tumor screening protocols in PHTS. In pediatric patients with PHTS, annual thyroid ultrasounds for thyroid cancer surveillance are recommended although the age to initiate surveillance is debated. The National Comprehensive Cancer Network (NCCN) guidelines for pediatric patients with PHTS include annual thyroid ultrasounds at the time of diagnosis, but Schultz et al. suggest starting ultrasounds at age 7 since the youngest reported case of thyroid cancer in a patient with PHTS was 7 years old (85, 86). In adult patients, colorectal screening beginning at age 40 is recommended (87), and the NCCN guidelines outline additional cancer surveillance recommendations in adults with PHTS.

DISCUSSION

Narrowing the differential diagnosis and attaining confirmatory molecular testing results are critical for patient care management related to LO (**Figure 2**). The most common disorders and syndromes leading to LO have many overlapping clinical characteristics, making genetic testing useful for determining the underlying mechanism for the observed phenotype. For instance, PHTS is caused by a germline mutation (i.e., the genetic defect is present in every cell of the body), whereas PROS is mostly due to somatic alterations of the *PIK3CA* gene, leading to a mosaic distribution of the genetic defect throughout the body (i.e., some positive and negative cells). It is suspected that certain regions of the body are more likely to develop tumors if that region contains the genetic defect. If the genetic defect is widespread as it is in germline mutations and constitutional defects, it is logical that the tumor risks may be higher; however, further research is needed to explore this hypothesis.

From this review, it is evident that there are drastic differences in tumor risks for patients with syndromic LO, some of which warrant childhood tumor surveillance programs and others that do not seem to contribute an increased tumor risk as part of the phenotype (**Table 1**). It is therefore of utmost importance to correctly diagnose these patients, so they can receive proper screenings and care. Patients with ILO due to increased muscle bulk but without an identifiable genetic cause are now included under the BWSp umbrella and should undergo routine screening like other patients with BWS (17). Given that the guidelines are still being developed for PROS, a discussion with the family about the risk is recommended. In LO disorders with increased tumor risks, the effectiveness of tumor screening goes beyond diagnosing tumors at earlier stages. One study found that parents of patients with elevated tumor risks prefer screening because when educated about their child's risk, it reduced their worry and psychological stress (88).

Overall, syndromes involving LO are heterogenous both within a given syndrome and between syndromes. As a result, tumor risk across the spectrum of LO disorders varies greatly due to the underlying cause of the syndrome, as well as personal tumor risk due to specific abnormalities present. Therefore, following diagnostic criteria to diagnosis, each patient will aid in assessing his/her individualized tumor risk and screening program.

AUTHOR CONTRIBUTIONS

JG performed the literature search and drafted the manuscript and figures. KD helped conceptualize the project, assisted with literature search, and edited the manuscript. JK conceptualized,

organized, and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Alex's Lemonade Stand Foundation for Childhood Cancer provided support for this work.

ACKNOWLEDGMENTS

We would like to thank patients and their families for participating in the many studies compiled to present this review. We thank Christina Gonzalez-Gandolfi for helping put together **Figure 1**. We thank our funding foundation for supporting this work.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Oncology

MINI REVIEW
published: 21 January 2021
doi: 10.3389/fonc.2020.614541

DICER1 Syndrome and Cancer Predisposition: From a Rare Pediatric Tumor to Lifetime Risk

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OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Oncology

Received: 06 October 2020
Accepted: 24 November 2020
Published: 21 January 2021

Citation:
Caroleo AM, De Ioris MA, Boccuto L, Alessi I, Del Baldo G, Cacchione A, Agolini E, Rinelli M, Serra A, Carai A and Mastronuzzi A (2021) DICER1 Syndrome and Cancer Predisposition: From a Rare Pediatric Tumor to Lifetime Risk. Front. Oncol. 10:614541. doi: 10.3389/fonc.2020.614541

DICER1 syndrome is a rare genetic condition predisposing to hereditary cancer and caused by variants in the *DICER1* gene. The risk to present a neoplasm before the age of 10 years is 5.3 and 31.5% before the age of 60. *DICER1* variants have been associated with a syndrome involving familial pleuropulmonary blastoma (PPB), a rare malignant tumor of the lung, which occurs primarily in children under the age of 6 years and represents the most common life-threatening manifestation of DICER1 syndrome. Type I, II, III, and Ir (type I regressed) PPB are reported with a 5-year overall survival ranging from 53 to 100% (for type Ir). *DICER1* gene should be screened in all patients with PPB and considered in other tumors mainly in thyroid neoplasms (multinodular goiter, thyroid cancer, adenomas), ovarian tumors (Sertoli-Leydig cell tumor, sarcoma, and gynandroblastoma), and cystic nephroma. A prompt identification of this syndrome is necessary to plan a correct follow-up and screening during lifetime.

Keywords: DICER1, cancer predisposition, pediatric, PPB, cystic nephroma

INTRODUCTION

DICER1 syndrome is a cancer-predisposing disorder caused by pathogenic variants in the *DICER1* gene (OMIM 606241), which are known to confer a lifetime risks for a variety of neoplastic and dysplastic lesions (1).

Germline *DICER1* variants have been detected in individuals affected with familial pleuropulmonary blastoma (PPB) (2–5), a rare malignant tumor of the lung, which occurs primarily in children under the age of 6 years (6). The International PPB Registry collected data from PPB patients and their families, reporting a variety of tumors in individuals with PPB and/or their relatives (6). A study on 207 carriers of DICER1 pathogenic variants reported that the risk to develop a neoplasm is 5.3% before the age of 10 years and of 31.5% before the age of 60, while in the American general population is estimated to be respectively 0.17 and 6.57% (1, 7). DICER1 syndrome occurs in children and young adults and its clinical presentation may include, beyond PPB, cystic nephroma, ovarian Sertoli-Leydig cell tumor (SLCT),

multinodular goiter, cervix embryonal rhabdomyosarcoma, Wilms' tumor, nasal chondromesenchymal hamartoma, ciliary body medulloepithelioma, differentiated thyroid carcinoma, pituitary blastoma, pineoblastoma, and sarcomas of different sites including, amongst others, the uterine cervix, kidney, and brain (8).

This syndrome shows an autosomal dominant inheritance pattern with reduced penetrance, which likely decreases the rate of familial cases. In cases with PPB, about 80% of the *DICER1* germline pathogenic variants are inherited by a parent and nearly 20% are *de novo* (9).

This paper aims to review the clinical and genetic features of DICER1 syndrome, with particular focus on the description of the different types of cancer reported in this syndrome, grouped by systems.

DICER1 SYNDROME GENETICS

The *DICER1* gene, located on chromosome 14q32.13, encodes an RNA endonuclease (Dicer) that is involved in the post-transcriptional gene expression of over 30% of protein-coding genes by modulating microRNAs (miRNAs) (10, 11).

miRNAs are transcribed as pri-miRNAs, that are longer precursor, which are elaborated into pre-miRNAs in the nucleus. The pre-miRNAs, transported to the cytoplasm, are processed by Dicer to give a ∼21-bp RNA duplex intermediate. One strand of this RNA is incorporated into the RNA-induced silencing complex (RISC), and matched to complementary mRNA targets to regulate gene expression, inhibiting mRNA degradation (12).

In most syndrome's neoplasms a biallelic pathogenic variant in DICER1 has been detected: usually a germline loss-of-function pathogenic variant in one allele and a tumor-specific somatic hotspot variant in the second allele. Several studies have shown that "monoallelic *DICER1* inactivation promotes tumorigenesis, whereas biallelic loss is inhibitory, and although inactivation of one *DICER1* allele is the initiating event in DICER1 syndrome", leading "to dysregulation of miRNA levels, other events must be required for cancer to occur " (13, 14). Only one third of DICER1 carriers present a neoplasm during the life, hinting that multiple additional events are required (13, 14).

This process suggests a predominant haploinsufficient tumor-suppressor function, where one copy of Dicer, albeit mutated, is functioning, rather than a more classical "two-hit" tumor suppressor model, which has been described in association with earlier diagnosis of *DICER1*-related conditions, where no function of the oncosuppressor gene is preserved (5, 15, 16).

Complete loss of Dicer is incompatible with life (4, 17, 18), while somatic mosaic mutations in the RNase IIIb domain have been associated with a more serious form of DICER1 syndrome, named GLOW syndrome from Global developmental delay, Lung cysts, Overgrowth, and Wilms tumor (19). Functional evidence links the hotspot mutations in the RNAse IIIb domain to specific dysregulation of certain miRNAs leading to activation of the PI3K/AKT/mTOR pathway (20). This mechanistic link to the PI3K/AKT/mTOR pathway may explain the fact that GLOW syndrome shares some clinical features with other conditions characterized by somatic gain-of-function mutations of genes of this pathway, such as lung cysts, reported in Proteus syndrome, and segmental overgrowth, a prominent feature of PROS (21).

The recurrent involvement of specific organs (lungs, thyroid, kidneys, ovaries) in presence of *DICER1* alterations may lead to infer that the effects of miRNAs on gene expression are tissue-specific (19). Nonetheless, the penetrance of each of the *DICER1*-associated neoplasms in inherited conditions is not fully understood. Individuals carrying germline loss-of-function mutations may present clinical features in few sites (0–2) of their body, while patients with mosaic "hotspot" mutations are more prone to manifestations in multiple site (6).

CLINICAL FEATURES OF TUMORS COMMONLY ASSOCIATED WITH *DICER1* VARIANTS

Different tumors are related to *DICER1* syndrome as reported by Foulkes et al. and by Stewart et al. In **Figure 1** we resumed the principal neoplasms according to the age of onset.

Foulkes et al. in 2014 described the *DICER1*-associated features and their characteristics, as reported in **Table 1** (5).

Stewart et al. recently published the first quantitative analysis of site-specific neoplasm risk, analyzing the standardized incidence ratios of 207 individuals carrying *DICER1* variants, selected combining data from three large cohorts of patients. The most remarkable rates were noted in PPB, in gynecologic tumors, especially SLCTs and rhabdomyosarcoma, and in cystic nephroma (1).

Lung

Pleuropulmonary Blastoma

PPB is a rare tumor that develops during fetal life/infancy and constitutes the most common life-threatening manifestation of DICER1 syndrome (22). Type I PPB is typically a purely cystic mass occurring before age of 2 years, with a 5-year overall survival (OS) of 89% if it does not progress to type II or III PPB. Type II is a solid-cystic tumor while type III is purely solid; both types present from approximately 2 to 6 years of age and are malignant, although type III is generally more aggressive. If treated with chemotherapy and radiotherapy, OS rates may reach up to 74% in type II and 53% in type III. The fourth type, named type Ir, as "type I regressed", is a cystic tumor lacking malignant cells and is supposed to represent regressed/non-progressed type I PPB. OS for this type of PPB is 100%. Cystic PPB is reported to be common in carriers of *DICER1* variants, and only a limited number of cases had a type II II or III PPB progression (1).

The PPB begins as a cystic lung lesion, also defined as a Type I PPB, a well-defined pathology entity with a potential evolution in a more aggressive tumor. We need to underline that the imaging findings of Type I PPB is overlapped with congenital lung cyst; congenital lung cyst with congenital pulmonary airway malformation (CPAM) are almost

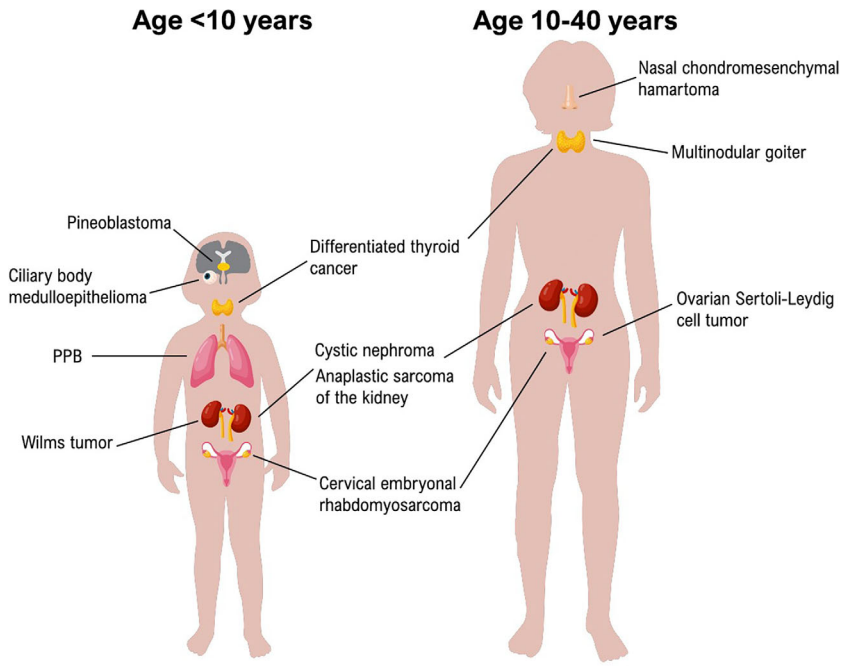

FIGURE 1 | Principal DICER1-Syndrome neoplasms according to the age of onset.

TABLE 1 | Key clinical phenotypes (ordered by relative frequency) associated with germline *DICER1* mutations.

Phenotype	Age (peak)
PBB	
Type I (cystic) PPB	0–24 m (8 m)
Type II (cystic/solid) PPB	12–60 m (31 m)
Type III (solid) PPB	18–72 m (44 m)
Type Ir (cystic) PPB	Any age
Multinodular goiter	5–40 y (10–20 y)
Cystic nephroma	0–48 m (undetermined)
Ovarian Sertoli-Leydig cell tumor	2–45 y (10–25 y)
Cervical embryonal rhabdomyosarcomas	4–45 y (10–20 y)
Differentiated thyroid cancer	5–40 y (10–20 y)
Wilms tumor*	3–13 y (undetermined)
Juvenile hamartomatous intestinal polyps*	0–4 y (undetermined
Ciliary body medulloepithelioma	3–10 y (undetermined)
Nasal chondromesenchymal hamartoma	6–18 y (undetermined)
Pituitary blastoma	0–24 m (undetermined)
Pineoblastoma	2–25 y (undetermined)
Anaplastic sarcoma of the kidney	Estimated 2–20 y
Medulloblastoma*	Undetermined
ERMS bladder*	Estimated <5 y
ERMS ovary	Undetermined
Neuroblastoma*	Estimated <5 y
Congenital phthisis bulbi*	Birth
Juvenile granulosa cell tumor*	Undetermined
Gynandroblastoma	Undetermined
Cervix primitive neuroectodermal tumor	Undetermined

**The association of these conditions with DICER1 variants may not be so strong to warrant testing in the absence of other features suggestive of DICER1 syndrome.*
PBB, Pleuropulmonary blastoma; ERMS, embryonal rhabdomyosarcoma; m, months; y, years.

diagnosed in prenatal period or over the first year and a surgical approach—with pathology study—was mandatory only in symptomatic cases. Indeed, in more than 70% of CPAM, a wait and see strategy is addressed (23); in this cases a DICER1 variants should always be considered in order to identify promptly with a strict follow-up and genetic screening patients at risk of more aggressive PBB. The pathology should always consider PPB evaluating a CPAM.

Shortness of breath and pneumothorax due to cyst rupture may be the presenting symptoms of PPB.

Thyroid

Multinodular Goiter and Epithelial Differentiated Thyroid Cancer

Multinodular goiter (MNG) is characterized by the development of thyroid nodular lesions. MNG is common in individuals with *DICER1* pathogenic variants, as reported by Khan et al. (24). Germline *DICER1* mutations have been reported in children with both MNG or familial MNG (25). The risk of DTC in carriers of *DICER1* variants is elevated as compared to the general population and its occurrence is typically related to an indolent course (26).

Kidney

Cystic Nephroma, Wilms' Tumor, and Anaplastic Sarcoma of the Kidney

Cystic nephroma is a benign multicystic kidney tumor that constitutes the most common neoplasm associated with PPB

(3). It has a bimodal incidence: 65% of cases occur in the pediatric band, before the age of 4, while 35% of cases appear in adulthood and are usually seen between the fourth and the sixth decade (27, 28).

DICER1 syndrome also includes an elevated risk of Wilms' tumor, an embryonal cancer of the kidney that affects children before the age of 6, without evidence to be a consequence of a prior cystic nephroma (29, 30).

Recent reports enumerate anaplastic sarcoma of the kidney in DICER1 syndrome, correlating the germline DICER1 mutations with the development of these tumors, and postulate that they may arise from pre-existing pediatric cystic nephromas (31–33).

Gynecologic Manifestations

The gynecologic tumors most frequently associated to DICER1 syndrome are ovarian SLCTs and embryonal rhabdomyosarcoma of the cervix. These neoplasms, as well as PPB and MNG, constitute key features leading to consider an underlying cancer predisposition syndrome, especially if found in children or adolescents (34).

Ovarian Sertoli-Leydig Cell Tumor

Unlike PPB, the age range of increased risk for genital tract tumors is wide (2 to 40 years), even if some data suggest that ovarian SLCTs arising in patients carrying *DICER1* variants occur mostly in the second decade (18, 35). Moderately differentiated SLCTs are most common, but juvenile granulosa cell tumor (JGCT), gynandroblastoma, and unclassified sex cord-stromal tumors have also been described. Most tumors are stage I, presenting with androgenic symptoms and a pelvic mass, that rarely may be bilateral (34). The prognosis of ovarian SLCT is generally favorable, but a recent report indicates that somatic *DICER1* variants SLCTs may be linked to a higher relapse risk than others (36).

Cervical Embryonal Rhabdomyosarcomas

Even though rhabdomyosarcoma is the most common cervical sarcoma, it is still very rare (37). Most *DICER1* cases are confined to the cervix at diagnosis, presenting with polypoid appearances (botryoides) and with vaginal bleeding. Even if ERMS is one of the more common sarcomas in childhood, approximately a third of DICER1-related ERMS arises in patients older than 20 years (38, 39). Studies report a quite favorable prognosis with an EFS over 50%, and an OS around 90% (34, 38–41).

Other Ovarian Neoplasms

Poorly differentiated ovarian sarcoma (42), retiform SLCT, and primitive neuroectodermal tumor (PNET) of the cervix (43) have also been reported in individuals with possible germline *DICER1* variants.

Central Nervous System

Pituitary Blastoma

Pituitary blastoma is an extremely rare tumor of the anterior pituitary. Genetic tests performed on 14 cases, on a total of 16 described to date, showed that all have at least one pathogenic variant in *DICER1* (44–46). For such reason, pituitary blastoma may be considered pathognomonic for DICER1 syndrome (46).

Pineoblastoma

Pineoblastoma is a rare primitive neuroectodermal grade IV tumor originating in the pineal gland (47). Only a few genes have been implicated in the pathogenesis of pineoblastomas, for instance, *RB1* in the setting of "trilateral retinoblastoma" (48).

To date, in *DICER1*-related pineoblastomas loss of heterozygosity of the wild-type *DICER1* allele seems to be the somatic event, in contrast from the typical missense hotspot mutations that usually lead to a factual germline heterozygosity (49–53). Moreover, somatic *DROSHA* and *DGCR8* mutations, both related to the Dicer miRNA-regulating pathway, have been recently documented in pineoblastomas, in addition to germline and somatic *DICER1* mutations (50), indicating that pineoblastoma development is influenced by disturbances of miRNA processes (46).

Others

Other brain tumors associated to DICER1 alterations have also been reported but their genetic association has not been clearly demonstrated. These include medulloblastoma (6, 54), intracranial medulloepithelioma (55), anaplastic meningeal sarcoma (53), glioblastoma multiforme (56, 57), and embryonal tumor with multilayered rosettes (ETMR) (58).

Head and Neck

Ciliary Body Medulloepithelioma

Ciliary body medulloepithelioma is a rare embryonal ocular tumor, that arises from the eye's ciliary body, which generally occurs during infancy and constitutes the second most common eye tumor of childhood, after retinoblastoma (59–61).

Some cases suspected to be *DICER1*-related have been documented but further studies are required to support their association with the syndrome (6, 15, 62–71).

Nasal Chondromesenchymal Hamartoma

Nasal chondromesenchymal hamartoma is a rare benign tumor of the sinus and nasal cavities that have been described in children with PPB. This peculiar association has led to the assumption that this hamartoma is also a manifestation of DICER1 syndrome (5, 72).

MOLECULAR DIAGNOSTICS

Molecular genetic testing methods, including single-gene or multigene panel testing, may be considered when clinical, imaging, and/or histopathological features evoke a DICER1 syndrome's diagnosis. Heterozygosis is the most common condition through DICER1 syndrome's patients, where commonly a germline loss-of-function gene variant (nonsense, frameshift, or splice-affected) generates a truncated protein. These variants can be identified by Sanger sequencing or next-generation sequencing (NGS). NGS has specific advantages over traditional Sanger sequencing, considered the gold standard for mutation analysis for many years, as multiple genes in several patients can be tested simultaneously. Indeed, when the phenotype is hard to distinguish from many other cancer

predisposition syndromes, extensive genetic testing, based on multigene panels or exome analysis can be useful to identify the molecular defects underlying the condition.

Besides point mutations, other predisposing *DICER1* alterations have also been documented, including deletion of the entire *DICER1* locus (62), or intragenic deletions involving one or more exons (73). Methods used to detect these kinds of alterations may include quantitative polymerase chain reaction (PCR), multiplex ligation-dependent probe amplification (MLPA) and gene-targeted microarray. Finally, molecular genetic testing of tumor DNA may be necessary to identify somatic mosaicism, which is observed in 10% of individuals with DICER1 syndrome.

SURVEILLANCE

Although risks of malignancy are elevated, most patients with pathogenic germline *DICER1* variants live healthy lives. Indeed, a tumor occurs in 19,3% of the patients who carry germline pathogenic variation by the age of 50 years old and the neoplastic risk rises with age, especially in females, that are exposed to the risk to present with gynecologic neoplasms (1).

Schultz et al. have defined the indications for *DICER1* genetic counseling and testing, and they also provided specific screening strategies to manage risk in carriers of *DICER1* pathogenic variants (2). Germline DICER1 genetic testing is to consider in individuals with one major or two minor criteria. "Major criteria are: PPB, lung cysts in childhood, thoracic embryonal rhabdomyosarcoma, cystic nephroma, genitourinary sarcomas including undifferentiated sarcoma, ovarian Sertoli–Leydig cell tumor, gynandroblastoma, uterine cervical or ovarian embryonal rhabdomyosarcoma, genitourinary/gynecologic neuroendocrine tumors, multinodular goiter or thyroid cancer in two or more first-degree relatives or in an index patient with a family history consistent with DICER1 syndrome, childhood-onset multinodular goiter or differentiated thyroid cancer, ciliary body medulloepithelioma, nasal chondromesenchymal hamartoma, pineoblastoma, pituitary blastoma. Minor criteria are: Lung cysts in adults, renal cysts, Wilms tumor, multinodular goiter or differentiated thyroid cancer, embryonal rhabdomyosarcoma other than thoracic or gynecologic, poorly differentiated neuroendocrine tumor, undifferentiated sarcoma, macrocephaly" (2).

Surveillance guidelines for individuals with a germline *DICER1* pathogenic variant have been established. The current guidelines include "chest radiograph every 4–6 months until age 8 years, and every 12 months until 12 years; a chest computed tomography scan should be considered. Baseline chest radiograph or chest CT should be considered when the diagnosis is performed after age 12 years. Thyroid ultrasound is recommended by the age of eight years with subsequent ultrasounds every three to five years. Individuals with a history of chemotherapy exposure should begin thyroid ultrasound within three to five years from treatment. Pelvic ultrasounds for surveillance for gynecologic tumors in females are recommended every 6 to 12 months by the age of eight years and extending until at least age 40 years. Screening for cystic nephroma and other renal tumors includes abdominal ultrasounds every six months until age eight years and then annually until age 12 years. Visual acuity measurement and dilated ophthalmology examination for ciliary body medulloepithelioma is recommended annually from age three years until at least age ten years. Annual physical examination should be considered by an expert clinician" (2).

THERAPEUTIC PERSPECTIVES

Some studies explored the use of metformin to upregulate DICER1 and linked proteins in mice, to counter the DICER1 syndrome's effects (74–77). Despite patients affected by biallelic DICER1 mutations may not benefit from this treatment, metformin will be may proposed to patients with a single allele alteration, to try to augment DICER1 protein production and compensate the deficit, preventing the oncogenetic cascade.

CONCLUSIONS

DICER1 syndrome is a rare condition caused by germline variants of *DICER1*; the occurrence of a second somatic tissue-specific mutation leads to different phenotypes ranging from benign lesions to malignant tumors. Screening for *DICER1* variants should be performed in all patients with PPB and considered in few benign lesions and malignant tumors. A prompt identification of this syndrome is necessary to plan a correct follow-up and screening for tumor occurrence during the patient's lifetime.

AUTHOR CONTRIBUTIONS

AC reviewed the literature and was a major contributor in writing the manuscript. MI reviewed the literature and wrote the manuscript. LB contributed to the concept and reviewed critically the manuscript. AM contributed to the concept, reviewed the literature, and reviewed critically the manuscript. All the authors state that no honorarium, grant, or other form of payment was given to anyone to produce the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Oncology

MINI REVIEW
published: 22 February 2021
doi: 10.3389/fonc.2021.586288

Rhabdoid Tumor Predisposition Syndrome: From Clinical Suspicion to General Management

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pediatric Oncology, a section of the journal Frontiers in Oncology

Received: *22 July 2020*
Accepted: *05 January 2021*
Published: *22 February 2021*

Citation:
Del Baldo G, Carta R, Alessi I, Merli P, Agolini E, Rinelli M, Boccuto L, Milano GM, Serra A, Carai A, Locatelli F and Mastronuzzi A (2021) Rhabdoid Tumor Predisposition Syndrome: From Clinical Suspicion to General Management. Front. Oncol. 11:586288. doi: 10.3389/fonc.2021.586288

Rhabdoid tumors are rare aggressive malignancies in infants and young children with a poor prognosis. The most common anatomic localizations are the central nervous system, the kidneys, and other soft tissues. Rhabdoid tumors share germline and somatic mutations in *SMARCB1* or, more rarely, *SMARCA4*, members of the SWI/SNF chromatin-remodeling complex. Rhabdoid tumor predisposition syndrome (RTPS) is a condition characterized by a high risk of developing rhabdoid tumors, among other features. RTPS1 is characterized by pathogenic variants in the *SMARCB1* gene, while RTPS2 has variants in *SMARCA4*. Interestingly, germline variants of *SMARCB1* and *SMARCA4* have been identified also in patients with Coffin-Siris syndrome. Children with RTPS typically present with tumors before 1 year of age and in a high percentage of cases develop synchronous or multifocal tumors with aggressive clinical features. The diagnosis of RTPS should be considered in patients with rhabdoid tumors, especially if they have multiple primary tumors and/or in individuals with a family history. Because germline mutations result in an increased risk of carriers developing rhabdoid tumors, genetic counseling, and surveillance for all family members with this condition is recommended.

Keywords: rhabdoid tumors, atypical teratoid/rhabdoid tumors, cancer surveillance, genetic test, cancer risk, cancer predisposition syndromes

INTRODUCTION

Rhabdoid tumor predisposition syndrome (RTPS) is characterized by an elevated risk of developing malignancies called rhabdoid tumors (RTs). RTs are rare, aggressive tumors, typically diagnosed in infants (1).

Primary rhabdoid tumor sites can include the central nervous system (65%), kidney (9%) and in the remaining 26% of cases: head and neck soft tissues, paravertebral muscles, liver, bladder, mediastinum, retroperitoneum, and pelvis (2).

Immunohistochemical characteristics of these tumors include loss of the BAF47/BRG1 protein (3). Among newly diagnosed cases, 25%–35% will harbor a germline variant of the *SMARCB1* gene

(OMIM*601607) (4, 5). Recently, pathogenic variants in the *SMARCA4* gene (OMIM*603254) have also been associated with RT (6); while the involvement of other genes appears to be exceedingly rare in RTs (7, 8).

The most frequent pediatric tumor associated with RTPS is atypical teratoid/rhabdoid tumor (AT/RT). AT/RTs are rare, accounting for 1%–2% of all brain cancers, 90% of cases being diagnosed in children of less than 3 years of age (9–12), with a slight male predominance (13). At the time of presentation, 65.4% are in the posterior fossa, 31% supratentorial and 3.6% multifocal (14).

Histologically, AT/RT shows areas of rhabdoid phenotype containing rhabdoid cells with eccentric nuclei, prominent nucleoli, abundant eosinophilic cytoplasm, and a mesenchymal component with spindle cells. In the last years, molecular characterization of RT has become increasingly relevant. *SMARCB1* and *SMARCA4* are tumor suppressor genes playing a critical etiologic role in all rhabdoid tumors including AT/RT, which is linked to somatic and germline mutations of *SMARCB1* or, more rarely, *SMARCA4*.

AT/RTs are biologically heterogeneous. In the last few years, different authors described transcriptional features of AT/RTs that can be summarized in three molecular subgroups (12, 15–17) with different genetic profile, age at onset, prognosis, and brain localization:

1) AT/RT-TYR tumors are characterized by infratentorial location, younger age at diagnosis (<1 year) and overexpression of the melanosomal markers such as DCT, TYR, and MITF and many genes involved in ciliogenesis (*DNAH11* and *SPEF1*). Other pathways described include bone morphogenetic protein (BMP) and orthodenticle homeobox 2 (OTX2). Chromosome 22q loss is the most common cytogenetic anomaly.
2) AT/RT-MYC tumors are generally supratentorial, affected individuals are older (age 4–5 years), and the cluster genes *MYC*, *HOTAIR*, and *HOX* are overexpressed. Focal deletions of *SMARCB1* are the most common molecular anomaly. Supratentorial location is the more frequent site. Spinal tumors are included in this subgroup.
3) AT/RT-SHH tumors location may be infratentorial or supratentorial with similar frequency, diagnosis is in the age interval 2 to 5 years. Genes of the sonic hedgehog pathway (*GLI2*, *BOC*, *PTCHD2*) and NOTCH signaling (*ASLC1*, *CBL*, *HES1*) are overexpressed.

Patients outcome for each group is not homogeneous among the different data published to date and prognosis is still unclear (12, 15–17).

The most common extra-cerebral site for the primary onset of an RT is the kidney (48% of cases), followed by head and neck (14%), liver (13%), and other sites such as trunk and arms (25%) (18, 19).

RTs of the kidney account for about 2% of all pediatric renal cancers (20). Renal RT is highly aggressive and has a poor prognosis, with a 12-month survival rate of only 30% (18). Patients presenting with renal RT in the first year of life tend to develop brain tumors in 10%–15% of cases (21). These patients often harbor a germline mutation of *SMARCB1* and have a worse prognosis, as compared to those with sporadic RTs (22).

RHABDOID TUMOR PREDISPOSITION SYNDROME

RTPS is an autosomal dominant cancer predisposition syndrome. When the mutation pathogenic variants occur in the *SMARCB1* gene, the syndrome is called RTPS1, and RTPS2 has variants in the *SMARCA4* gene.

BAF47/BRG1 proteins encoded by *SMARCB1/SMARCA4* genes are key components of the ATP-dependent chromatin-remodeling SWI/SNF complex, which is essential for lineage specification, gene regulation, and maintenance of stem cell pluripotency (23).

RTs are the most frequent malignancies associated with these syndromes, but not the only ones. In most cases these arise *de novo* but there is a small percentage of familial cases having RTPS. RTs can present in a familial setting, with up to 35% of cases due to germline mutations in *SMARCB1* (4) or, in 2%–3% of cases, in *SMARCA4* (24, 25).

Children with RTPS typically present with tumors before 12 months of age and in 35% of cases develop synchronous or multifocal tumors with aggressive clinical features (20, 22, 26). RTs can be detected in the prenatal period or during childhood with a median age at onset of 4–7 months (range prenatally – 60 months) (1, 27, 28) versus sporadic RTs that are detected at a median age of 13–30 months (range: age 1 day–228 months). Often RTs in RTPS are synchronous, with advanced stage at diagnosis and clinically aggressive. Progression occurs during chemotherapy in 58% of individuals with RTPS and RTs (24). In the EU-RHAB Registry 28% of cases had synchronous RT: eight individuals AT/RT and extracranial malignant rhabdoid tumors (eMRT), four had AT/RT and rhabdoid tumor of the kidney (RTK), and two AT/RT, multiple eMRT and RTK (28).

Furthermore, other conditions are known to be related to RTPS. Family history of RT or cribriform neuroepithelial tumor (CRINET) and/or combination of RT with one of the following: schwannoma, malignant peripheral nerve sheath tumor, meningioma are highly suggestive for RTPS (29).

The diagnosis of RTPS is established in a proband with a rhabdoid tumor and/or a family history of RT and/or multiple SMARCB1/SMARCA4 deficient tumors (synchronous or metachronous) and identification of a germline pathogenic variant in *SMARCB1* or *SMARCA4* by genetic testing (30). In **Figure 1** are summarized the main clinical and genetics features of RTPS.

Rhabdoid Tumor Predisposition Syndrome 1

Rhabdoid Tumor Predisposition Syndrome 1 (RTPS1, OMIM #609322) is caused by heterozygous germline mutations in the *SMARCB1* gene, which maps to chromosome 22q11.2 (31). The protein involved is an SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (30).

Clinical Features

As described above, the syndrome predisposes to the development of RTs, including brain tumors, renal and extrarenal cancers. AT/RT is the most frequent brain cancer in

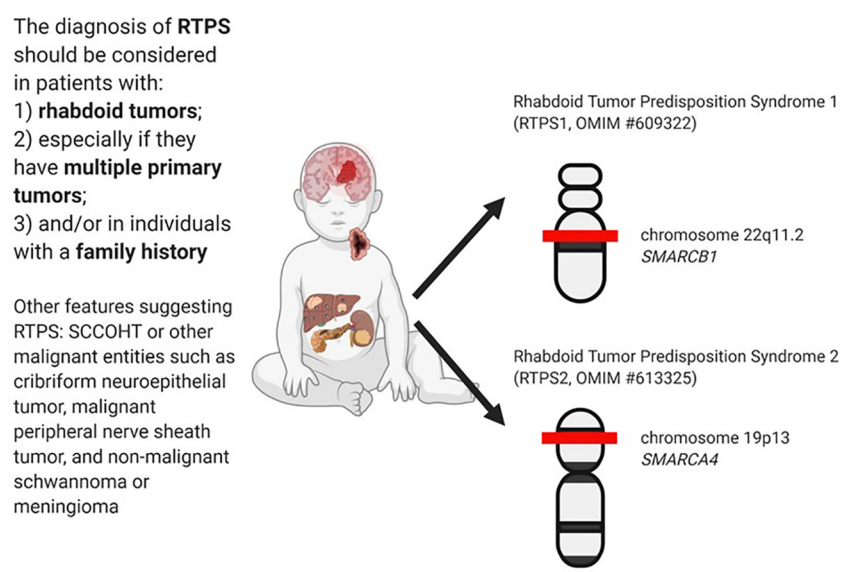

FIGURE 1 | RTPS tumors spectrum and related genes involved.

patients with *SMARCB1* mutations, but other CNS tumors are described (32).

Interestingly, Thomas et al. (33) described a case of RTPS1 in an infant with AT/RT in which supratentorial and infratentorial parts of the tumor demonstrated different DNA methylation profiles suggesting synchronous or metachronous AT/RT with different molecular subgroup and cell of origin.

Recently, the *SMARCB1* gene has been found also in familial and sporadic schwannomatosis. Hulsebos et al. (34) described two family members with schwannomatosis and a germline mutation of *SMARCB1*, suggesting it as a candidate predisposing gene. Swensen et al. reported a family with hereditary schwannomatosis associated with a germline mutation of *SMARCB1*. Three members of the family developed RTs and died before 2 years of age (35). About 40%–50% of familial schwannomatosis and 8%–10% of sporadic cases harbor a constitutional mutation in *SMARCB1* (25). Interestingly, *SMARCB1* and *NF2* loci map very close to each other on the long arm of chromosome 22 (25).

Furthermore, Schmitz et al. found the same somatic mutation of *SMARCB1* in four of 126 meningiomas. The data suggest that *SMARCB1* is a tumor suppressor gene that may be important also for the oncogenesis in a subset of meningiomas (36).

Moreover, *SMARCB1* mutation carriers may be at risk for developing other tumors such as malignant peripheral nerve sheath tumors and cribriform neuroepithelial tumors (37).

Genetics

SMARCB1 inactivation can be caused by different mechanisms like gross chromosomal aberration or loss of heterozygosity of 22q11.2 or loss-of-function mutations including nonsense, frameshift, splicing and missense mutations (6).

Concerning cytogenetics, the most frequent alteration described in AT/RT is the monosomy of chromosome 22 (14, 38, 39). Biegel et al. described also a rhabdoid tumor with an unbalanced 9;22 translocation (40).

Penetrance. Penetrance may vary according to the mutation type. Incomplete penetrance has been observed in three of nine published families with RTPS due to *SMARCB1* mutations (6). Rarely a SMARCB1 pathogenic variant is inherited from an unaffected parent or a parent with late-onset or undiagnosed RTPS (41). Germline mosaicism must be taken into account for at least half of the families with sibs affected by RTPS (30).

Rhabdoid Tumor Predisposition Syndrome 2

Rhabdoid Tumor Predisposition Syndrome 2 (RTPS2, OMIM #613325) is caused by heterozygous germline mutations in the *SMARCA4* gene, which maps to chromosome 19p13 (6) and encodes a protein involved in the transcription activator BRG1, a catalytic component of the ATP-dependent SWI/SNF chromatin remodeling complex (30).

Clinical Features

The main tumor resulting from germline pathogenic variants in *SMARCA4* is small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) (37, 42). It seems that up to 40% of females with SCCOHT may harbor a germline variant in *SMARCA4* (43), therefore the detection of SCCOHT in young women is high evocative for RTPS2 (44–46).

Although more rarely than *SMARCB1* mutations, pathogenic germline *SMARCA4* variants are found in children with AT/RT and it seems that *SMARCA4*-mutated AT/RT may be associated with a worse prognosis (24, 47). The risk of other RTs in *SMARCA4* germline heterozygotes is unknown, but probably very low.

Other epithelial cancers, such as lung cancer, have been reported in some adults with pathogenic germline variants in *SMARCA4*, but again, the risks remain unquantified (46).

Recently, a novel entity designated "*SMARCA4*-deficient thoracic sarcoma" (SDTS) was described by Le Loarer et al. in 19 adult individuals, supporting the carcinogenic effect of *SMARCA4* inactivation, with consequences beyond the pediatric age range (48).

Genetics

Among the different *SMARCA4* pathogenic variants reported to date, nonsense, and intragenic deletions are the prevalent types, while only a single missense variant has been detected (24).

Penetrance. It appears that *SMARCA4* mutations are less penetrant for AT/RT than *SMARCB1* ones (37). In contrast to *SMARCB1*, most reported patients with RTs and a *SMARCA4* mutation inherited it from an unaffected parent (30). In *SMARCA4*-related RTPS, the penetrance for RT in the preceding generation of seven informative families was zero. However, in one family, two sibs with a SMARCA4 pathogenic variant were both affected (6, 24, 30).

Other Rare Manifestations Related to SMARCB1 and SMARCA4 Mutations

Interestingly, germline variants of *SMARCB1* and *SMARCA4* have been identified also in patients with Coffin-Siris syndrome three (CSS3, OMIM #614608) and four (CSS4, OMIM #614609). CSS is a congenital malformation syndrome characterized by developmental delay, intellectual disability, coarse facial features, feeding difficulties, and hypoplastic or absent fifth fingernails and fifth distal phalanges (49). Individuals with CSS carrying *SMARCB1* or *SMARCA4* mutations seem to show no predisposition to develop RTs or other forms of tumor. This can be explained by the fact that mutations resulting in CSS3 are non-truncating, implying that they exert gain-of-function or dominant-negative effects (excluding haploinsufficiency as a cause) (50). Very rare exceptions have been described. To date, a single CSS individual with schwannomatosis and a *SMARCB1* variant has been reported (51): the *SMARCB1* c.1121G>A (p.Arg374Gln) germline transition in exon 9 lead to the inactivation of the second allele in the tumor tissue. More recently, a pediatric patient with mild CSS who concomitantly developed small-cell carcinoma of the ovary hypercalcaemic type has been found to harbor a germline heterozygous nonsense mutation and a somatic frameshift mutation in *SMARCA4* (52).

TABLE 1 | Surveillance recommendations for rhabdoid tumor predisposition syndrome (RTPS) carriers.

Foulkes et al. (37)	Teplick et al. (55)
Germline truncating mutations:	- From 0–1 year: is recommended abdominal US every 2 to 3 months and head US monthly - From 1–4 years: abdominal US every 6 months. Brain and spine MRI every 6 months
SMARCB1	
- Brain: MRI every 3 months to age 5 years	
- Abdomen: Ultrasound every 3 months through 5 years. Consider WB-MRI, undetermined frequency	
SMARCA4	
- Brain: No data available, risks likely very low	
- Abdomen: No data available, risk likely low to very low	
- Ovary: No data available, abdominal ultrasound every 6 months may be justified, role, if any, of MRI unknown. Preventive oophorectomy may be justified outside of the pediatric age range	
Germline missense mutations:	
No screening, generally no/very low risk	

MRI, magnetic resonance imaging; WB-MRI, whole body magnetic resonance imaging; US, ultrasound.

GENOTYPE-PHENOTYPE CORRELATION

According to Smith et al. and Holsten et al. a clear genotype-phenotype correlation could be identified (53, 54). Germline *SMARCB1* mutations located in the central portion of the gene, involving multiple exon deletions or duplications and truncating mutations, likely responsible for a loss of SMARCB1 protein product, are most frequently associated with rhabdoid tumors. Instead, *SMARCB1* mutations located at the ends of the gene, particularly non-truncating alterations, including missense variants, are most frequently associated with non-oncologic diseases and low-grade tumors such as the ones reported in CSS, meningiomas, and schwannomas. Unlike the germline *SMARCB1* mutations detected in RT cases, schwannomatosis-associated alterations determine reduced expression levels or a partial loss of function of the SMARCB1 protein (53). Moreover, a correlation was identified between the type of *SMARCB1* variant and the time of onset of the disease: truncating variants are associated with early-onset disease, non-truncating variants with late-onset disease.

SURVEILLANCE

To date, no universally accepted surveillance recommendations for RTPS carriers have been established. In **Table 1** are summarized two surveillance propositions suggested by Foulkes et al. (37) and Teplick et al. (55). Nemes et al. (30) proposed a protocol of surveillance not only in pre-symptomatic RTPS carriers but also in individuals affected by RTs.

Foulks et al. (37) give more detailed indications about monitoring of *SMARCB1* or *SMARCA4* carriers as opposed to Teplick et al. (55), even if they failed to stratify cancer monitoring for age range. They recommended brain MRI in *SMARCB1* carriers every 3 months for the first 5 years of life. As known, AT/RTs in RTPS1 arise generally within the first year of life and MRI is an expensive examen, and sedation is needed in young children. After the first year of life, a brain MRI should be performed every 6 months. About abdominal monitoring, they recommended ultrasound every 3 months through 5 years and consider whole-body MRI, with undetermined frequency. Whole-body MRI will guarantee high diagnostic accuracy as opposed to ultrasound, but it is an expensive procedure and requires sedation in little patients.

Regarding *SMARCA4* carriers they suggest an abdominal ultrasound every 6 months with no mention of the beginning or end of the follow-up. Considering the rarity of the condition and the very low risk, unfortunately, there is no data available for monitoring of brain and abdominal RTs in *SMARCA4* carriers.

Interestingly, Folkes et al. (37) proposed a separated surveillance protocol for germline truncating mutations versus germline missense mutations, underlining that germline missense mutations need no screening for their very low risk of RTs. On the other hand, they proposed MRI surveillance for patients with a germline missense mutation of *SMARCB1* to allow the early detection of schwannomas.

Teplick et al. (55) did not take into account the due separated conditions RTPS1 and 2 and different germline kinds of mutations. They suggested the use of ultrasound in the first year of life to monitor the brain and abdomen every 2–3 months. Between 1 and 4 years of age, they suggest extending abdominal ultrasound monitoring every 6 months and using brain and spine MRI to exclude the onset of brain tumors every 6 months. In their proposal, there is no mention of whole-body MRI.

GENETIC TEST

Molecular genetic testing for RTPS is appropriate in any patients with:

- RTs, familial RTs, multifocal or synchronous tumor, congenital or early-onset disease, other conditions known to be related to RTPS
- *SMARCB1*- or *SMARCA4*-deficient tumors with a positive family history.

Point variants of *SMARCB1* and *SMARCA4* can be identified by Sanger sequencing or next-generation sequencing (NGS). Besides point mutations, other alterations of *SMARCB1* and *SMARCA4* have also been documented, including deletion of the entire *SMARCB1* locus or intragenic deletions involving one or more exons (5). Methods used to detect this kind of alteration may include quantitative PCR, multiplex ligation-dependent probe amplification (MLPA), and a gene-targeted microarray designed to detect single-exon deletions or duplications.

GENETIC COUNSELING AND RISK TO FAMILY MEMBERS

Siblings and Parents

When a pathogenic variant of *SMARCB1* or *SMARCA4* is detected in a proband, molecular genetic evaluation of parents and siblings is required.

As mentioned above, carriers of *SMARCA4* mutation inherited a pathogenic variant from an unaffected parent (24), while the vast majority of individuals with RTPS1 have a *de novo* germline *SMARCB1* mutation, and only in extremely rare cases, they inherited a *SMARCB1* pathogenic variant from an unaffected parent.

A healthy parent with a pathogenic germline variant has to start surveillance as for siblings, but at longer intervals, as the risk of malignancies is very low.

If the *SMARCA4* or *SMARCB1* pathogenic variant found in the proband cannot be detected in either parent, it raises the possibility of a *de novo* pathogenic variant in the proband or germline mosaicism in a parent. Parental germline mosaicism in *SMARCB1* has been rarely described (5, 27, 32, 56, 57), while the overall incidence of germline mosaicism in RTPS is unknown.

The cancer risk for the siblings of a proband depends on the genetic status of the proband's parents:

- 50% risk of inheriting the variant if the proband harbors a *SMARCA4* or *SMARCB1* pathogenic variant, although penetrance can be incomplete.
- 1% risk of inheriting the variant if the parent is negative for *SMARCA4* or *SMARCB1* mutations, considering the possibility of parental germline mosaicism (5, 27, 56, 57).

Offspring of a Proband

As mentioned above, patients with RTPS1 die at a young age. Despite it occurs very rarely, it should be considered the cancer risk in offsprings. If children are affected by a *de novo* germline *SMARCB1* mutation and survive to adulthood, they can potentially transmit the mutation to their offspring (25).

The family history of most individuals with RTPS may appear to be negative for many reasons: failure of detection of the disorder in family members, reduced penetrance (more evident in *SMARCA4*-related RTPS), late onset in the affected parent.

PREVENTION AND PRENATAL DIAGNOSIS

There is no possibility of preventing cancer development in patients with RTPS, but in case of detected *SMARCB1*/*SMARCA4* mutations, the advice of surveillance and follow-up must be followed. Prophylactic oophorectomy may be discussed in women with *SMARCA4*-related RTPS for the high risk to develop SCCOHT (58).

It would also be important to prevent secondary complications related to aggressive treatments.

Once *SMARCB1* and *SMARCA4* pathogenic variants are detected, prenatal testing for a pregnancy at increased risk and preimplantation genetic diagnosis are possible. The preferred tests used to assess if a product of conception carries a known *SMARCB1*/*SMARCA4* mutation are chorionic villus sampling and amniocentesis.

CONCLUSION

Germline variants play a role in 8.5%–10% of all pediatric cancer with the prevalence of certain genes such as *TP53*, *APC*, *NF1*, *PMS2*, *RB1*, and *RUNX1*. The increasing implementation and availability of genetic testing lead to the opportunity to identify the risk of cancer and early detection of tumors with the aim of reducing mortality and morbidity (21).

RTPS is characterized by a high risk of developing RTs and other unfrequent conditions. RTs are a rare, aggressive form

of malignancies typically diagnosed in young infants that can arise in multiple anatomical sites. About 25%–35% of RTs carry a germline variant of *SMARCB1* (4, 5), or more rarely *SMARCA4*. The diagnosis of RTPS should be taken into account in patients with RTs, especially if early and multiple primary tumors and/or if a positive family history of RTs is present (25).

The ongoing new characterization of AT/RTs and RTs (12) will likely lead to further biological insights that can delineate molecular subtypes and may lead to novel therapeutic options. Despite these promising advancements, surveillance for cancer risk and prevention remains the focus of current management. Further research is needed to increase our understanding of RTs biology and gather further knowledge of the role of SMARCB1/SMARCA4 in RTs development and other rare manifestations.

AUTHOR CONTRIBUTIONS

GB and RC wrote the manuscript. PM provided the figure. AS, IA, and GM contributed to the finishing of the work. EA and MR contributed to the genetic details of the manuscript. AM, AC, LB, and FL revised it critically for important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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