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Genetic Predisposition to Childhood Cancer in the Genomic Era

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Abstract

Developments over the past five years have significantly advanced our ability to use genome-scale analyses—including high-density genotyping, transcriptome sequencing, exome sequencing, and genome sequencing—to identify the genetic basis of childhood cancer. This article reviews several key results from an expanding number of genomic studies of pediatric cancer: (*a*) Histopathologic subtypes of cancers can be associated with a high incidence of germline predisposition, (*b*) neurodevelopmental disorders or highly penetrant cancer predisposition syndromes can result from specific patterns of variation in genes encoding the SMARC family of chromatin remodelers, (*c*) genome-wide association studies with relatively small pediatric cancer cohorts have successfully identified single-nucleotide polymorphisms with large effect sizes and provided insight into population differences in cancer risk, and (*d*) multiple exome or genome analyses of unselected childhood cancer cohorts have yielded a 7–10% incidence of pathogenic variants in cancer predisposition genes. This work supports the increasing use of genomic sequencing in the care of pediatric cancer patients and at-risk family members.

INTRODUCTION

The last decade has seen an enormous increase in research on genetic susceptibility to childhood cancer, which has led to many important insights. In parallel, many cancer genetics clinics have been created to evaluate children at risk of cancer, resulting in increased numbers of pediatric-age patients diagnosed with cancer predisposition syndromes and followed for management of cancer risk. Given these developments, the American Association for Cancer Research sponsored a workshop in 2016 to develop and publish a series of 18 articles (see 2), introduced in a paper by Brodeur et al. (11), that made recommendations for cancer surveillance in the pediatric age range for more than 50 genetic conditions. Similarly, the American Society of Pediatric Hematology/Oncology has established the Cancer Predisposition Syndrome Working Group, which first met in Heidelberg in 2016 and published a concise review of genetic predisposition to individual pediatric cancer types (78). In addition, several scholarly reviews have described the large number of individual syndromes associated with an increased risk of pediatric cancer (69, 72, 86). Thus, in this article we do not attempt to describe these conditions in detail. We focus instead on examples that highlight the discoveries made over the last five years, particularly those that resulted from the increased ability to perform genome-scale analyses, including high-density genotyping, transcriptome sequencing, exome sequencing, and genome sequencing.[1]

The genomic era has been characterized by decreasing costs for genotyping and sequencing, which have increased the number of genes evaluated per sample for rare disorders and the number of samples analyzed per project. In some cancer predisposition syndromes, such as familial neuroblastoma (79) and familial glioma (81), affected members of the same family may develop distinct tumor subtypes; the predisposition is to the general tumor type (or several different tumor types), and patients with cancer predisposition syndromes represent a minority of patients with that tumor. By contrast, exome and genome sequencing has revealed that, in many situations, a high proportion (40–80%) of patients with a specific tumor histopathologic subtype have germline pathogenic or likely pathogenic (P/LP) variants in specific cancer predisposition genes (CPGs). In some cases, this association was identified by exome sequencing of samples from only three to five probands or families with the specific subtype. These histopathologic subtypes often represent a very small subset (often less than 3%) of the overall tumor type. Given the rarity of the subtype, older genetic and epidemiologic studies of cancer predisposition for the tumor in general resulted in negative findings. **Table 1** highlights the rich set of genes now associated

Table 1 Examples of specific histopathology pediatric tumor subtypes associated with a high likelihood of a cancer predisposition syndrome

Tumor type	Gene(s)
Low-hypodiploid acute lymphocytic leukemia	*TP53*
Juvenile myelomonocytic leukemia	*NF1*
Botryoid rhabdomyosarcoma	*TP53*
Medullary thyroid cancer	*RET*
Atypical teratoid/malignant rhabdoid tumor	*SMARCB1*, *SMARCA4*
Clear cell meningioma	*SMARCE1*
Ovarian small-cell carcinoma, hypercalcemic type	*SMARCA4*
Ovarian sex cord tumors with annular tubules	*STK11*

[1]The American College of Medical Genetics and Genomics recently recommended the latter two terms in place of the more common whole-exome sequencing and whole-genome sequencing, given that no methodology identifies all types of variation.

with specific histopathologic subtypes, and indications for genetic testing are based increasingly on the pathology reports for individual patients. In addition, the majority of the new discoveries described in the first half of this article resulted from genomic analyses of increasingly larger tumor (not germline) sample sets. The studies were designed to identify somatic (or cancer-specific) mutations that might affect treatment. However, recurrent (at the level of variant or gene) tumor findings resulted in subsequent analysis of corresponding germline samples, and this latter analysis showed that there is a strong germline component to cancer predisposition for the tumor type with these consistent genetic changes.

ACUTE LYMPHOCYTIC LEUKEMIA

The genomics revolution has substantially increased our knowledge about genetic predisposition to acute lymphocytic leukemia (ALL) over the last 10 years. ALL is the most common pediatric malignancy, and extensive research over several decades has aimed to elucidate its environmental and genetic etiologies. In addition, with the development of next-generation sequencing methods, large programs have applied genomic approaches to understanding the pattern of somatic mutation within leukemic cells, particularly for children with high-risk disease. Several landmark studies from St. Jude Children's Research Hospital applied exome, transcriptome, and eventually genome sequencing approaches to the study of ALL samples from large cohorts of children (see, e.g., 54, 55). Although these and many other studies described in this review focused initially on identifying important somatic events, the findings led to definitions of both somatic and germline events.

An early finding was the important role of *IKZF1* variation in pediatric ALL with poor outcome (27). Genomic studies demonstrated that the small subtype of ALL associated with *BCR:ABL1* translocations has a variety of somatic inactivating mutations in *IKZF1*, which encodes the Ikaros protein. That initial analysis did not identify any germline variants, although there had been one prior report of a patient with a large deletion of 7p11–p14, including *IKZF1*, with a clinical phenotype of Greig cephalopolysyndactyly and B cell ALL (48). More recently, initial analysis of one individual with *BCR:ABL1* ALL and a family history of leukemia identified a germline *IKZF1* loss-of-function (LOF) variant (c.del556, p.D186fs) (14). Further analysis of more than 4,000 pediatric ALL patients revealed several rare variants that were further characterized for pathogenicity. Overall, 28 unique germline *IKZF1* variants are consistent with a P/LP interpretation according to the interpretation guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (77) in 45 individuals, a total of 0.9% of all children with B cell ALL. However, the majority of these missense alleles were found across different subtypes of ALL and were not specific to the *BCR:ALB1* subtype. Also, as described below, common variation in *IKZF1* also plays a role in ALL risk.

Exome sequencing revealed transmission of a rare single-nucleotide variant, c.547G>A, in *PAX5* that was predicted to generate the p.Gly183Ser missense change in two extended familial leukemia kindreds (88). Somatic alterations of *PAX5* have been previously described as a somatic finding in approximately 30% of ALL samples. The leukemia of affected individuals in these families consistently exhibited 9p loss (in some cases as a result of isochrome 9q), resulting in retention of the *PAX5* allele with the missense variant. As with *IKZF1*, germline *PAX5* variants appear to result in predisposition to this specific form of ALL. For example, in the *PAX5* study (88), many other collaborating laboratories looked at this gene in a variety of familial leukemia cohorts without 9q loss and did not identify any additional families. To our knowledge, only a few subsequent *PAX5* families have been identified, with no additional reports of germline pathogenic missense variants in ClinVar.

The inclusion of leukemia and lymphoma as a component of Li–Fraumeni syndrome (39) generated studies in the 1990s to determine whether mutations in *TP53* might be one of the causes of familial ALL or lymphoma (20, 104). The studies limited the sequencing to the exons encoding the *TP53* somatic mutation hot spots in a small number of kindreds, with negative results. Hypodiploid leukemia is considered a high-risk subtype of ALL and is characterized by an absolute number of chromosomes less than 44. Although this subtype is rare (<2%), the use of single-nucleotide polymorphism (SNP) arrays at the time of ALL diagnosis has facilitated its diagnosis. Analysis of 124 leukemic bone marrow samples from patients with hypodiploid ALL at St. Jude Children's Research Hospital revealed a remarkably high proportion of either missense or LOF *TP53* pathogenic variants (30). This proportion was greater than 90% in the specific low-hypodiploid subtype of ALL (defined as 32–39 chromosomes). Further analysis of nontumor hematopoietic cells from these patients revealed that approximately 50% of the germline samples were positive for the *TP53* variant. The investigators did not have extensive family history data or fibroblast samples from these individuals to prove that the variants were inherited and not derived from bone marrow events; however, the results suggested that low-hypodiploid ALL patients have a high likelihood of carrying germline *TP53* variants. At the same time, a study found that one extended pedigree with five cases of ALL also transmitted a *TP53* nonsense variant, p.ARG306Ter (70), that was also described in the larger hypodiploid study. Based on these studies, multiple pediatric cancer genetics clinics now routinely refer any patients with hypodiploid ALL for consideration of *TP53* analysis.

These discoveries allowed a 2018 retrospective analysis of germline and tumor samples from 3,801 patients treated in the Children's Oncology Group ALL clinical trials (AALL0232 and P9900) for *TP53* status (71). Consistent with prior results, the investigators found that the group of patients with hypodiploid ALL had substantially more P/LP *TP53* variants than other ALL patients (65.4% versus 1.2%; $p < 0.001$) and were older at diagnosis. Unfortunately, patients with *TP53* P/LP variants also demonstrated much poorer prognosis. The poor prognosis in hypodiploid ALL was comparable for those with and without germline *TP53* variants, likely because more than 90% of leukemias of this type have *TP53* variants of either germline or somatic origin. However, nonhypodiploid patients with germline *TP53* variants had a poorer prognosis than those with wild-type germline *TP53*. This difference in overall survival was influenced by the strikingly higher development of a second neoplasm in the *TP53* germline group, consistent with a Li–Fraumeni phenotype. The five-year cumulative incidence of a second neoplasm was 25.1% [95% confidence interval (CI) = 1.5–48%] for the *TP53* germline variant versus 0.7% (CI = 0.4–1.1%) for the *TP53* wild-type groups ($p = 5.3 \times 10^{-11}$).

In summary, many of the more recently described leukemia CPGs (*IKZF1*, *PAX5*, and *TP53*) undergo either somatic mutation or rearrangement or are associated with germline P/LP variants resulting in genetic predisposition to leukemia. These germline variants result in an increased risk to specific subtypes of pediatric ALL (**Figure 1**). Acute myelogenous leukemia and ALL are distinct diseases with some overlap in oncogenic drivers, e.g., *BCR:ABL1* translocation in chronic myelogenous leukemia and rare children with high-risk ALL. In contrast to these ALL CPGs, there are rare families with individuals diagnosed with both myeloid and lymphoid malignancies. In three concurrent publications, exome sequencing of several pedigrees with thrombocytopenia and at least three members with hematopoietic malignancy (ALL plus or minus other myeloid malignancies) revealed specific missense mutations (e.g., p.Pro214Leu) in *ETV6*, a gene that normally undergoes translocation in sporadic hematopoietic cancers (57, 95, 113). The investigators performed subsequent targeted sequencing of *ETV6* in other probands and kindreds and found a variety of different rare missense alleles and at least one LOF frameshift variant, p.N385fs. *ETV6* is one of the few autosomal dominant genes identified that has been associated with both myeloid

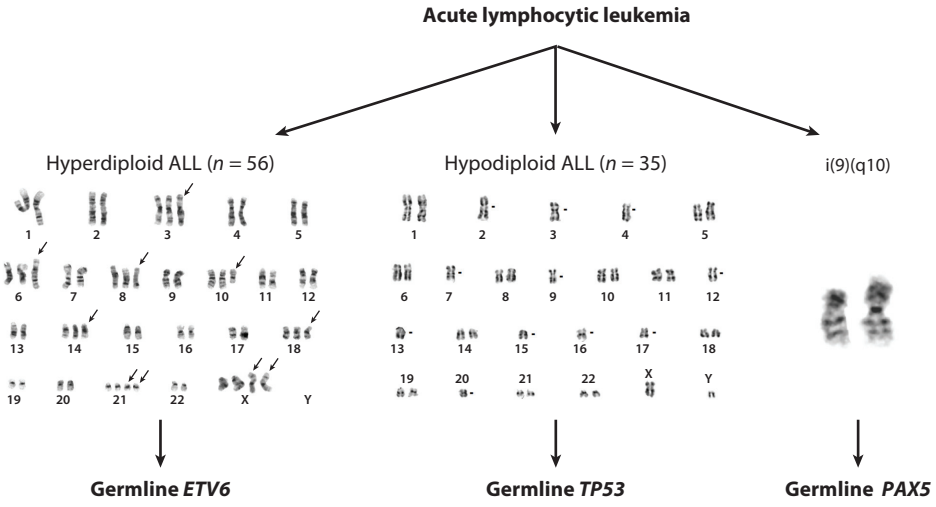

Figure 1

Schematic diagram with examples of key cytogenetic abnormalities in subtypes of pediatric acute lymphocytic leukemia (ALL) associated with specific cancer predisposition genes. Photographs courtesy of the Cytogenetics Laboratory of the Texas Children's Cancer Center, Texas Children's Hospital.

and lymphoid malignancy in the same kindred. Although this gene is associated with both myeloid and lymphoid malignancies, a large analysis of *ETV6* germline variants across the St. Jude and COG ALL patient cohorts demonstrated that patients with *ETV6* germline variants were highly overrepresented for hyperdiploidy (defined as more than 60 chromosomes in leukemic blasts or DNA index greater than 1.16) and older age of diagnosis (53). There is also a growing literature on disorders associated with thrombocytopenia and familial leukemia, predominantly acute myelogenous leukemia, including *RUNX1*, *DDX41*, *CEBPA*, and *GATA2*. This group of disorders has been recently reviewed (21).

SMARC GENES AND PREDISPOSITION TO PEDIATRIC TUMORS

An increasing number of studies have shown that genes that encode protein products that function in the same developmental pathway may result in predisposition to tumors (both the same and histopathologically distinct). The name SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily (SMARC) reflects the homology of these genes with the switch/sucrose nonfermentable (SWI/SNF) genes, which were first identified in budding yeast (*Saccharomyces cerevisiae*) for their role in gene regulation during mating-type switching and then subsequently by the biochemical evidence that they function together in a complex (e.g., the BAF complex) to regulate chromatin structure (10). Multiple members of the SMARC family have been identified as CPGs for specific histologic subtypes of tumors. This class of genes also plays an important role in development, as demonstrated by the frequent finding of de novo variants in these genes in children with neurodevelopmental disorders (34). However, there is little phenotypic and genotypic overlap in the developmental and cancer phenotypes, as best demonstrated by *SMARCB1*, where specific genotypes result in quite strikingly different phenotypes.

SMARCB1: Three Mendelian Disorders, Three Mechanisms of Action

Malignant rhabdoid tumors of the kidney frequently co-occur with tumors of the central nervous system (CNS) called atypical teratoid/rhabdoid tumors. As in predisposition to ALL,

the identification of the key rhabdoid CPGs was based not on family studies but on multiple genomic studies of rhabdoid tumor samples and subsequent study of matched germline samples. Cytogenetic analyses of the tumors through karyotypes, or subsequently by fluorescence in situ hybridization analysis, revealed abnormalities of chromosome 22q11.2, most commonly monosomy 22. Mapping of tumor samples with smaller deletions was used to identify *SMARCB1*[2] as the critical rhabdoid tumor suppressor gene, including some tumors with a LOF variant on one allele (then identified in the matched normal sample) and deletion or chromosome loss of the other allele (8, 99). Analysis of matched germline samples from larger cohorts of rhabdoid tumor patients demonstrated that 30% of patients with atypical teratoid/rhabdoid tumors, and a smaller percentage of patients with malignant rhabdoid tumors, have germline *SMARCB1* variants (17). The spectrum of variation in *SMARCB1* is typical for tumor suppressor genes, with nonsense, frameshift, and deletion variants reported but few missense or splice-site variants. This condition is now referred to as rhabdoid tumor predisposition syndrome. Given the poor mortality associated with these tumors, positive germline test results are often the result of de novo variants in *SMARCB1*. The few parents found to carry the variant often had not developed a rhabdoid tumor and demonstrate the incomplete penetrance of this disorder (13).

Coffin–Siris syndrome (CSS) is a distinct disorder characterized by severe intellectual disability, dysmorphic facies, CNS structural abnormalities, and variable congenital anomalies. In 2012, exome sequencing of only five individuals with CSS revealed two patients with de novo missense variants in *SMARCB1* (97). The investigators then performed targeted sequence analysis of six SMARC genes in 20 CSS patients, which revealed germline variants in 20 individuals, including in *SMARCA4* and *SMARCE1*, two genes subsequently identified as CPGs (described below). The results of exome and genome sequencing of many other patients with CSS in both clinical and research settings demonstrate *SMARCB1* variation in only a minority of patients with CSS, a type now referred to as CSS3 (OMIM 614608), with other chromatin-remodeling genes playing the majority role (10, 34). The spectrum of *SMARCB1* variation in CSS3 is quite distinct from rhabdoid tumor predisposition syndrome and includes a limited set of missense variants (primarily p.Arg374Gln and p.Arg377His) and in-frame deletions of single lysine residues (e.g., p.K364del) in exons 8 and 9 near the 3′ end of the SNF5 effector domain. The molecular pathogenesis of these variants has not been tested but is hypothesized to result from either gain-of-function or dominant negative actions (10). Another distinct neurodevelopmental phenotype has recently been reported to result from a missense variant in exon 2 (p.Arg37His) of *SMARCB1* (16).

Familial schwannomatosis is associated with patients developing multiple schwannomas (often in adult years) and is transmitted in an autosomal dominant fashion, without neurodevelopmental disorders or pediatric cancers. The link between familial schwannomatosis and *SMARCB1* was clarified through a combination of sequencing of samples from familial schwannomatosis kindreds and analysis of the tumors themselves, which demonstrated somatic mutations in *SMARCB1* (31). Schwannomas (both sporadic and inherited) demonstrate monosomy 22. This prompted earlier evaluation of the idea that a specific *NF2* gene variant might be responsible for familial schwannomatosis. However, multiple schwannomas from the same patient revealed distinct somatic variants in the *NF2* gene. Considering that both *SMARCB1* and *NF2* map to chromosome 22, detailed genomic analyses resulted in the four-hit mechanism (87) (**Figure 2**). A patient is born with a pathogenic variant in *SMARCB1*, which represents the first event. The order of the subsequent events is not clear. A larger event results in the loss of the second (wild-type) copy of the *SMARCB1* gene and the *NF2* gene, representing the second and third hits. Monosomy of chromosome 22 is

[2]Much of the older literature used *INI1* or *SNF5* as the gene name for *SMARCB1*, and the antigen that the antibody uses for tumor immunohistochemistry is referred to as BAF5.

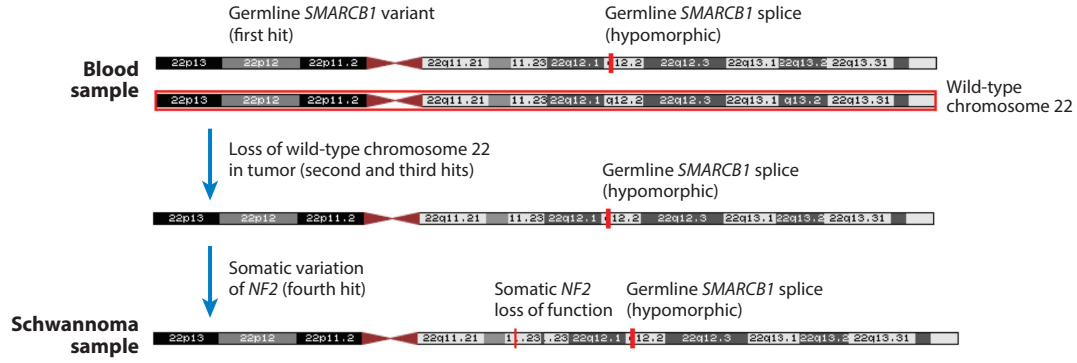

Figure 2

Diagram of the four-hit model of tumorigenesis in patients with schwannomatosis and germline hypomorphic *SMARCB1* variants, showing one potential order of events, as the loss of chromosome 22 and subsequent somatic *NF2* mutation may vary in sequence. The same mechanism applies to the schwannomatosis associated with variants in *LZTR1*, a gene that maps to 22q11.21.

one of the common events that simultaneously deletes both genes. Another LOF somatic variant in the *NF2* gene (the fourth hit) may be the final event, resulting in a tumor that has no *SMARCB1* or *NF2* gene function.

The germline variants in *SMARCB1* found in familial schwannomatosis are predicted to be hypomorphic, often disrupting splicing, in contrast to more typical LOF variants in rhabdoid tumor predisposition syndrome (33). A few families with overlap of rhabdoid tumors and schwannomas have been described, including one patient who survived an atypical teratoid/rhabdoid tumor as an infant and during adolescence developed a lesion on the tongue that a pathologist (agnostic to the *SMARCB1* finding) said resembled a schwannoma (66). One patient with the *SMARCB1* missense variant in exon 2, p.Arg374Gln, demonstrated both the CSS3 neurodevelopmental feature and multiple schwannomas (25). The concept of the four-hit model was further exploited in a study of the 50% of patients with schwannomatosis who do not have germline *SMARCB1* variants. Sequence analysis focused on other genes mapping to regions of 22q, which are lost in the schwannoma samples. This resulted in finding germline pathogenic LOF variants in *LZTR1* in the majority of *SMARCB1* wild-type schwannomatosis families analyzed (33, 65).

Given the distinct clinical phenotypes of the *SMARCB1* disorders and the very different recommendations for tumor surveillance, it is critical to provide families with accurate genetic evaluation and counseling. A parent searching the web for the *SMARCB1* gene is likely to be very confused, given the different information provided by the three different disease support groups. Specifically, as described above, the phenotypes associated with rare germline *SMARCB1* variants that encode varying levels of SMARCB1 activity include a serious neurodevelopmental disorder, a cancer predisposition syndrome associated with infantile onset of highly lethal tumors of the brain and kidney, and an adolescent- or adult-onset disorder associated with multiple benign tumors along the nerves.

SMARCE1 and Meningiomas

Meningiomas are among the most common types of brain tumors. They are considered to have better prognosis than gliomas, although 20% are characterized as atypical or malignant and often recur after initial surgery (106). It has been well established that meningiomas occur in the setting of neurofibromatosis type 2, and this Mendelian disorder is found particularly in the rare

circumstance of meningioma diagnosis during childhood (28). Analysis of *SMARCB1* in patients with meningioma, particularly localized to the falx cerebri of the cranium, has also found germline LOF variants.

Spinal meningiomas are more likely to be associated with clear cell meningioma (CCM) histopathology and often occur in childhood or adolescence. Multiple CCMs have been reported to be transmitted as an autosomal dominant disorder. Exome sequencing of three unrelated probands from familial CCM kindreds revealed distinct LOF variants (one nonsense and one splice abnormality) in *SMARCE1* in two of the kindreds (91). Targeted sequencing of a small number of additional patients revealed additional LOF *SMARCE1* variants, but again only in patients with spinal tumors. Analysis of the tumor samples revealed loss of staining consistent with a typical tumor suppressor function. Many subsequent studies of CCM patients have consistently found *SMARCE1* mutation and protein loss (when detected via immunohistochemistry). For example, a recent prospective analysis of 27 CCM tumors, from 26 patients diagnosed as young as six years of age, demonstrated the absence of SMARCE1 staining in all samples and *SMARCE1* LOF variants (both hits identified) in 10 of 10 samples where both germline and tumor samples were available. The pattern of biallelic variants was similar to that of other tumor suppressor genes, with a variety of LOF alleles (93). Again highlighting the specificity for this subtype, the investigators found no loss of SMARCE1 staining or *SMARCE1* variants in patients with other forms of meningioma, including microcystic meningioma, which can resemble CCM pathologically.

SMARCA4 and Rhabdoid Tumors and Small-Cell Ovarian Cancer, Hypercalcemic Type

Two different experimental approaches have associated *SMARCA4* with two distinct tumor types. As described above, loss of *SMARCB1* (through mutations, deletions, and/or monosomy 22) was identified as the key driver in more than 95% of rhabdoid tumors (both atypical teratoid/rhabdoid tumors and malignant rhabdoid tumors). Further analysis revealed that some of the rare *SMARCB1* wild-type rhabdoid tumors were associated with LOF *SMARCA4* variants (84). Subsequently, exome sequencing of three individuals from kindreds with small-cell carcinoma of the ovary, hypercalcemic type (SCCOHT), showed that all three had LOF variants in *SMARCA4* (22). SCCOHT is a rare subtype of ovarian cancer that has now been shown to be almost uniformly associated with loss of SMARCA4 staining (BRG1 antigen) by immunohistochemistry and *SMARCA4* LOF variants (both germline and tumor) (68, 107). As with meningioma, patients with SCCOHT and *SMARCA4* variants are typically diagnosed in the second or third decade of life, with a mean age of 24 years—much earlier than other forms of ovarian cancer.

In sum, LOF germline variants in several different SMARC genes result in a high frequency of rare, highly malignant histopathologic tumor subtypes. These examples follow the tumor suppressor gene two-hit mechanism with complete loss of SMARC gene function in the tumor sample. Thus, immunohistochemical staining of the corresponding protein product is now in routine diagnostic practice. Schwannomatosis results from hypomorphic *SMARCB1* alleles, LOF *NF2* alleles, and loss of the remaining alleles. Even though the SMARC proteins function together in chromatin-remodeling complexes (BAF complexes), there is little overlap between the genes and their specific tumor predisposition other than rhabdoid tumors resulting from variants in both *SMARCB1* and *SMARCA4*. These genes play important roles in development, and specific missense variants (or in-frame deletions) result in severe neurodevelopmental disorders characterized by multiple congenital anomalies without significant cancer risk.

TUMOR MUTATIONAL CHARACTERISTICS

In addition to specific histologic subtypes, there is a growing appreciation that the pattern of somatic alteration (sometimes referred to as the mutation signature) can reflect inherited cancer predisposition syndromes. Research in this area has focused primarily on adult cancers, particularly given the smaller number of somatic variants identified in pediatric malignancies. However, several examples have emerged in which the pattern of mutation identified in the pediatric tumor specimen is highly suggestive of specific cancer predisposition syndromes.

Medulloblastoma

As with hypodiploid ALL, there has been extensive exome, genome, transcriptome, and array analysis of medulloblastoma, resulting in a new molecular-based classification of this cancer into four subtypes: WNT pathway alteration, SHH pathway alteration, group 3, and group 4 (60a). Before these analyses, studies had already reported that patients with familial adenomatous polyposis have an increased risk of medulloblastoma (although still less than 2% absolute risk). Similarly, LOF germline variants in the SHH pathway genes *PTCH1* and *SUFU* patients result in Gorlin syndrome and familial medulloblastoma, respectively, both of which include a hereditary risk of medulloblastoma (35). Patients with familial medulloblastoma often have the desmoplastic/nodular histopathology form of this cancer (90). Even more strikingly, analysis of SHH medulloblastoma with chromothripsis pattern of DNA breakage events specifically demonstrated an extremely high proportion of patients with *TP53* P/LP variants in both tumor and matched normal DNA (73).

An international team of investigators led by Stefan Pfister in Heidelberg recently reported results from the largest study to date (more than 1,000 patients from several cohorts) of the hereditary nature of medulloblastoma (9). Overall, 11% of medulloblastoma patients in the retrospective cohort carried P/LP variants in known CPGs. The study compared the germline findings with those reported in the Exome Aggregation Consortium (ExAC) cohort of adult exomes in a burden analysis that revealed that six genes were significantly overrepresented: *APC*, *BRCA2*, *PALB2*, *PTCH1*, *SUFU*, and *TP53*. Subsequent prospective analysis of additional subjects revealed that 6% of patients had germline variants in one of these six genes. This study confirmed the prior work, with *TP53* variants underlying the SHH tumors with chromothripsis (~50% germline), *PTCH1* and *SUFU* variants present in the SHH tumors, and *APC* germline variants responsible for most of the WNT medulloblastoma that lacked somatic *CTNNB1* variants. Four of 11 patients were compound heterozygous for *BRCA2* variants, suggesting the Fanconi D1 subtype (SHH medulloblastoma), although the remaining heterozygous *BRCA2* patients retained the wild-type allele in the tumor. The *PALB2* variants were heterozygous in all five patients. Interestingly, germline *PALB2* and heterozygous *BRCA2* variants were found across the medulloblastoma subtypes. However, these tumors demonstrated a homologous recombination deficiency tumor mutation profile (signature 3 and 8), which has been previously described in breast cancer specimens and other adult-onset cancers associated with germline *BRCA1* mutations.

Constitutional Mismatch Repair Deficiency Syndrome

Constitutional mismatch repair deficiency syndrome (cMMRD) is an autosomal recessive disorder where an individual inherits a P/LP variant on both alleles in one of the four mismatch repair (Lynch syndrome) genes: *MSH2*, *MLH1*, *MSH6*, or *PMS2*. The International Constitutional Mismatch Repair Deficiency Consortium published several reports describing the diagnostic criteria, tumor spectrum, and surveillance recommendations (7). As in Li–Fraumeni syndrome,

children with cMMRD are at high risk for a wide variety of tumor types (median age of onset 7.5 years); brain tumors, leukemia and lymphoma, and colorectal tumors are the most frequent types, and demise from malignancy during childhood is the most common outcome. These individuals also have dermatologic features (café au lait spots and axillary freckling) that overlap with those of neurofibromatosis type 1.

Relevant to this review is the impact of mismatch repair deficiency and subsequent somatic variants in replication polymerase genes (*POLE/POLD1*) on the tumor mutational burden. Evaluation of 81,000 tumors analyzed by the same somatic mutation platform, including 2,885 pediatric samples, revealed 160 pediatric hypermutated cancers and a smaller subset described as an ultra-hypermutated tumor phenotype (more than 100 somatic variants per megabase sequenced), which were all associated with replication errors (38). Using the data from this large tumor set with the addition of tumor samples from other children with cMMRD, the investigators were able to establish mutational signatures that distinguish patients who have cMMRD and subsequently acquire somatic *POLE/POLD1* variants (notably, this group of tumors is microsatellite stable), those who have only cMMRD, and those who have germline *POLE/POLD1* pathogenic variants. Evaluation of tumors for evidence of hypermutation is increasingly being performed given the improved response of adult patients to treatment with immune checkpoint inhibitors, including early experience with children with cMMRD.

GENOME-WIDE ASSOCIATION STUDIES OF PEDIATRIC CANCER

Studies evaluating genetic predisposition to pediatric cancer have focused largely on identifying rare variants underlying rare Mendelian conditions, but there have also been efforts to characterize the role of common genetic variation in susceptibility. An important shift in this direction was the advent of the genome-wide association study (GWAS). GWASs test millions of SNPs for association with a disease in hundreds or thousands of individuals, and this approach has revolutionized the search for the genetic influences of complex traits (44). Complex traits (or multifactorial traits), in contrast to Mendelian conditions, are caused by many genetic and environmental factors acting together, each having a relatively small effect and few (if any) being either necessary or sufficient for disease to occur. As noted, GWASs have focused largely on the role of common genetic variation (e.g., SNPs with a minor allele frequency of >1%) in disease susceptibility (62).

Prior to the development of GWASs, genetic association studies relied primarily on the so-called candidate gene approach. In these studies, investigators would select candidate genes and SNPs based on hypothesized gene–disease associations (e.g., the role of DNA repair genes in specific pediatric cancers) or functions. Notably, candidate gene studies were often characterized by weak or imprecise estimates of association, as well as a lack of consistent replication across studies. Therefore, the candidate gene approach has been largely set aside in favor of the agnostic GWAS (45).

Also in response to the apparent failure of many candidate gene studies, an important feature of GWASs is the requirement for replication in an independent group of individuals (44, 45). Therefore, investigators rely on identifying signals in a discovery set, which must then be confirmed in a replication set. While no particular epidemiologic study design is required for a GWAS, the most common approach is a case–control study, in which genotype frequencies are compared between cases (affected individuals) and controls (unaffected individuals). This is especially true for genetic association studies of cancer (44, 45).

Also in contrast to candidate gene studies, where functional SNPs are selected for association analysis, the top hits in GWASs are often in intronic regions of genes or in gene deserts, which makes understanding the function underlying these associations difficult and also challenges

Table 2 Genes and chromosomal locations identified in genome-wide association studies of pediatric cancer

Tumor type	Genes or chromosome locations
Acute lymphocytic leukemia	*ARID5B*, *IKZF1*, *CEBPE*, *CDKN2A*, *GATA3*, *BMI1*, *PIP4K2A*
Neuroblastoma	*CASC15/NBAT-1*, *BARD1*, *LMO1*, *HACE1*, *LIN28B*, *DUSP12*, *DDX4*, *IL31RA*, *HSD17B12*
Ewing sarcoma	1p36.22, 10q21.3, 15q15.1, 6p25.1, 20p11.22, 20p11.23
Wilms tumor	2p24, 11q14
Osteosarcoma	*GRM4*, 2p25.2
Langerhans cell histiocytosis	*SMAD6*

assumptions about the role of genetic susceptibility to disease (41, 44, 45, 62). This has been true for GWASs of most pediatric cancers.

Over the past 10 years, GWASs have identified thousands of robustly replicated loci (i.e., specific genomic locations) for complex traits (41). However, compared with adult cancers, there have been relatively few GWASs of pediatric cancers, likely due to the large sample sizes required for GWASs. Specifically, it was assumed that more than 1,000 affected individuals (i.e., cases) and at least as many controls were required to detect small effects [e.g., odds ratios (ORs) < 1.5] when applying a genome-wide level of statistical significance (commonly $p < 5.0 \times 10^{-8}$) to account for the number of comparisons and number of independent chromosomal segments (41, 44, 45). However, several investigators hypothesized that, based on the limited number of GWASs for pediatric conditions, diseases of younger onset may demonstrate stronger effects (e.g., ORs > 1.5) than are seen in diseases of adult onset (1, 75). Therefore, in spite of smaller sample sizes, GWASs of pediatric cancers may inform our understanding of susceptibility to these conditions, and several successful studies have been reported in the last five years.

To date, pediatric cancers for which there have been multiple GWASs have been limited largely to ALL (19, 49, 59, 60, 64, 89, 96, 100, 101, 105, 109) and neuroblastoma (46, 94). Additionally, a few assessments have focused on Ewing sarcoma (42, 67), and there is currently one published GWAS each for Wilms tumor (98), osteosarcoma (82), and Langerhans cell histiocytosis (LCH) (63). Finally, there has been one published assessment of maternal genetic effects and ALL (4). No GWASs have been published for pediatric lymphomas, CNS tumors, or soft-tissue sarcomas, including rhabdomyosarcoma. Genes and genomic regions identified in GWASs of pediatric cancer are summarized in **Table 2** and described below.

Acute Lymphoblastic Leukemia

The first two GWASs of ALL were published in 2009, included fewer than 1,000 cases, and reported susceptibility loci in *ARID5B*, *IKZFI*, and *CEBPE* (60, 96). These susceptibility loci have been replicated in several larger GWASs (100, 101, 105). The top variants in these loci are relatively common (minor allele frequency > 0.30) and have relatively strong effects (ORs > 1.5) compared with GWASs of adult cancers (75). The *ARID5B* locus is typically one of the strongest hits in GWASs of ALL (96, 101, 105, 109). Notably, the frequency of risk variants in *ARID5B* varies by genetic ancestry, with the highest frequency in Latinos, an intermediate frequency in Caucasians, and the lowest frequency in African Americans, which corresponds with the incidence of pediatric ALL in these groups (i.e., highest in Latinos, followed by Caucasians and then African Americans) (5, 108). As described above, rare germline LOF variants in *IKZF1* are found in about 0.5% of apparently sporadic ALL patients, along with somatic deletions and mutations

in patients with *BCR:ABL1*-positive ALL. Thus, *IKZF1* plays a role in multiple different aspects of leukemogenesis.

Since the original GWAS of ALL, larger studies have identified other susceptibility loci in several genes, including *CDKN2A*, *GATA3*, *BMI1*, and *PIP4K2A*, which may demonstrate subtype specificity (19, 49, 59, 64, 89, 100, 101, 105, 109). For example, inherited *GATA3* variants are strongly associated with Philadelphia chromosome (Ph)–like ALL (OR = 3.9) (64). This association was identified among 75 Ph-like ALL cases, which demonstrates the importance of evaluating associations among well-defined subtypes. Other subtype-specific associations include variants in *ARID5B* and *CEBPE* with hyperdiploid B cell ALL (101).

While these associations have led to new insights into the etiology of pediatric ALL, work is ongoing to understand the mechanisms underlying these findings. Additionally, newer studies have focused on assessing genetic susceptibility in non-European populations (105) as well as alternative genetic mechanisms, including maternal genetic effects (e.g., the role that maternal genotypes play in the child's phenotype during development). To this end, Archer et al. (4) conducted the first GWAS of maternal genetic effects in ALL. While the findings have yet to be fully validated, this is an important first step in exploring the missing heritability of ALL.

Neuroblastoma

A small proportion of neuroblastoma cases are considered to be familial (79). However, for the 95% of neuroblastoma cases that occur sporadically, investigators have long hypothesized that common germline genetic variants could influence the probability of disease occurrence (24). Building from this hypothesis, Maris et al. (46) published the first GWAS of neuroblastoma in 2008, consisting of 720 neuroblastoma cases and 2,128 controls. They observed a significant association between neuroblastoma and common minor alleles of three SNPs on chromosome band 6p22. Homozygosity for the risk allele of the most significantly associated SNP, rs6939340, resulted in an increased likelihood of developing neuroblastoma (OR = 2.0, CI = 1.6–2.5). As with ALL, the effect sizes were larger than those reported for GWASs of adult cancers.

This original GWAS has been expanded as additional patient samples have been gathered, leading to the confirmation and identification of multiple susceptibility loci significantly associated with both high- and low-risk neuroblastoma, including *CASC15*, *BARD1*, *LMO1*, *LIN28B*, *HACE1*, *DUSP12*, *DDX4*, *IL31RA*, and *HSD17B12* (94). Notably, the main impact of neuroblastoma GWASs has been in identifying genes critical to neuroblastoma progression and maintenance, thus uncovering potential oncogenic vulnerabilities. Work is ongoing to characterize the functions of variants in these genes and leverage this information for improved treatment (94). Several of these loci, such as *BARD1*, have also been implicated in susceptibility to adult malignancies.

Ewing Sarcoma

Compared with other pediatric cancers, Ewing sarcoma is not classically considered to be part of known cancer predisposition syndromes. However, there is a notable disparity in the incidence of this pediatric cancer: Caucasians have the highest incidence, followed by Asians and then African Americans, with incidence rates of 0.155, 0.082, and 0.017 per 100,000 individuals, respectively (32), meaning that Caucasians are approximately nine times more likely than African Americans to develop Ewing sarcoma. In populations worldwide, individuals of European ancestry exhibit the highest incidence rates regardless of geography. Furthermore, individuals with African ancestry on different continents still exhibit the lowest incidence, suggesting that racial disparities in incidence could be due partly to differences in genetic susceptibility (23, 32).

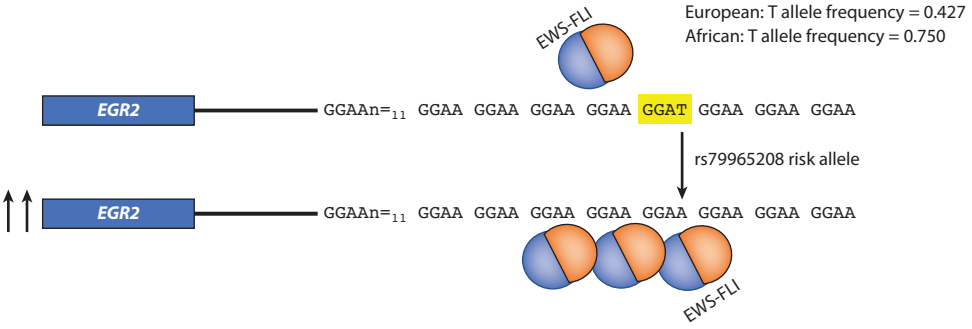

Figure 3

Schematic of the proposed mechanism for the Ewing sarcoma risk allele rs79965208. The T-to-A change increases the number of perfect GGAA microsatellites, which is proposed to improve binding of the EWS-FL1 fusion protein. This binding results in increased *EGR2* expression. The rs79965208 polymorphism is more common in Caucasian populations than it is in African American populations (data shown are from the 1000 Genomes Project) and may influence the difference in Ewing sarcoma frequency between the two populations.

An important finding in recent years came from the first GWAS of Ewing sarcoma, which included only 401 cases in the discovery cohort (67). Specifically, this study identified two notable risk loci: one located upstream of *TARDBP* ($p = 1.4 \times 10^{-20}$, OR = 2.2) and one located upstream of *EGR2* ($p = 4.0 \times 10^{-17}$, OR = 1.7). Interestingly, *EGR2* also contains a GGAA microsatellite with a Ewing sarcoma–associated SNP that appears to alter *EWS/FLI1* binding (29) (**Figure 3**). The authors also showed that *EGR2* knockdown induced regression of Ewing sarcoma xenografts, increasing its plausibility as a candidate for contributing to disease development. While it is unclear how these candidates would fit with Ewing sarcoma development, the risk haplotypes were less prevalent in African Americans. A more recent GWAS of Ewing sarcoma replicated the loci identified in the first assessment and identified three new susceptibility loci (42) (**Table 2**) that will hopefully shed more light on this important pediatric cancer.

Wilms Tumor

Wilms tumor is the most common childhood renal malignancy. To identify common variants that confer susceptibility to Wilms tumor, Turnbull et al. (98) conducted the first GWAS of this cancer in 757 individuals with Wilms tumor and 1,879 controls. Two regions were replicated in an independent set of cases and controls: 2p24 (rs3755132, $p = 1.03 \times 10^{-14}$; rs807624, $p = 1.32 \times 10^{-14}$) and 11q14 (rs790356, $p = 4.25 \times 10^{-15}$). The investigators also identified candidate association signals at 5q14, 22q12, and Xp22. They concluded that these loci could ultimately provide insights into biological pathways that may be important in the genesis of this embryonal kidney cancer. Additionally, these findings strongly suggest that multiple loci of equivalent or weaker effect are likely to exist and may be identified through follow-up analysis of additional SNPs that have shown evidence of association in this study and through further GWASs.

Osteosarcoma

Unlike other pediatric sarcomas, there have been several candidate gene studies of osteosarcoma (51, 56, 80, 83). However, as with previous candidate gene studies of other cancers, none of these associations were significant in the only GWAS of osteosarcoma published to date (82). The only

two variants that reached genome-wide significance were rs1906953 near the *GRM4* gene and rs7591996 in an intergenic region of 2p25.2 (a gene desert). The function of these variants in relation to osteosarcoma development has not been investigated.

A GWAS of osteosarcoma metastasis at diagnosis identified the rs7034162 variant in the *NFIB* gene, which more than doubled the likelihood of metastasis at diagnosis (OR = 2.4, CI = 1.8–3.2). This finding was supported by in vitro experiments that showed that cell lines with this variant behaved more aggressively (50). These findings point to the importance of evaluating not only susceptibility to pediatric cancers through GWASs but also differences in presentation and outcome.

Langerhans Cell Histiocytosis

Through a better understanding of the biology of LCH and its associated somatic features (e.g., *BRAF* mutations), this condition has emerged as an important malignancy affecting children, with an incidence similar to that of non-Hodgkin lymphoma. Despite advances to elucidate the somatic mutational landscape underlying LCH pathogenesis, the germline risk factors remain largely unknown. Therefore, Peckham-Gregory et al. (63) conducted the first GWAS of LCH, which identified and replicated a risk variant in *SMAD6* (rs12438941) associated with increased LCH risk (OR = 3.7, CI = 2.5–5.4). There are functional data to support this association. Specifically, SMAD6 inhibits bone morphogenetic protein (BMP) and transforming growth factor-beta (TGF-β)/activin signaling, which are determinants of Langerhans cell differentiation (111). Additionally, this variant appears to suppress SMAD6 protein expression without a decrease in SMAD6 messenger RNA expression in patients carrying the risk allele. Notably, this risk allele is more common in Hispanics, who have the highest risk of developing LCH, and is absent in individuals of African ancestry, who have the lowest risk of LCH (76). This particular GWAS leveraged a case–parent trio design rather than a case–control approach, which is an important alternative for genetic studies of pediatric cancer, as appropriate controls may be difficult to obtain.

Future Directions for Genome-Wide Association Studies

GWASs of pediatric cancer reveal several themes: (*a*) Larger sample sizes (>1,000 cases) are not always required, as inherited genetic effects are much stronger than those observed in GWASs of adult cancers (i.e., larger effect sizes overcome smaller sample sizes); (*b*) common SNPs could explain some differences in the incidence of pediatric cancer by ancestry (e.g., ALL, Ewing sarcoma, and LCH); (*c*) for some tumors, the same gene (e.g., *IKZF1*) is implicated in both Mendelian syndromes and GWASs; and (*d*) GWAS analysis is similar to rare-variant analysis in that certain SNPs have a strong tumor subtype specificity. Ongoing and future GWASs of pediatric cancer are focusing on multiple issues, including characterizing less frequent variants for pediatric cancer risk and common variants for less common cancers and discovering genetic variants that can be leveraged for predicting outcomes in those with pediatric cancer.

Most GWASs have focused on inherited genetic effects; however, other genetic mechanisms may also play a role in susceptibility. In particular, little is known about whether and (if so) to what extent the maternal genotype might influence the risk of pediatric cancer in offspring. It has been hypothesized that the maternal genotype could influence a child's phenotype by affecting the intrauterine environment independent of the inherited genotype (4). This is particularly important because the evaluation of maternal genetic effects may serve as a proxy for maternal environmental exposures. Therefore, understanding the role of maternal genetic variation in pediatric cancer risk may inform studies evaluating the role of environmental exposures in susceptibility (4). Finally,

future studies must also determine the mechanisms underlying genetic associations, which may inform prevention and treatment strategies.

EXOME AND GENOME SEQUENCING OF UNSELECTED PEDIATRIC CANCER COHORTS

By 2013, the rapid advances in next-generation sequencing technology and bioinformatics over the previous five years had resulted in effective methods for interrogating genomic sequences for diverse mutation types. In particular, remarkable progress has been made in the field of medical genomics and its application to analysis of pediatric patients, including (*a*) paired tumor/normal sequencing in order to identify somatic variants in specific pediatric tumor types (e.g., 18, 40) and (*b*) clinical sequencing using clinical exome and genome sequencing of germline samples from heterogenous patient cohorts, often with developmental disorders or multiple congenital anomalies (e.g., 110). In this section, we describe several studies published over the last five years that report quite consistent results from germline exome and/or genome cancer predisposition analyses for heterogeneous cohorts of pediatric cancer patients.

St. Jude Children's Research Hospital and the Genome Center at Washington University in St. Louis embarked on extensive exome and genome sequencing of paired tumor and blood samples for cancer patients being treated at the hospital. As described above, initial studies reported the novel discovery of somatic mutations resulting from tumor/normal sequencing, particularly in high-risk leukemia samples. Subsequently, investigators retrospectively reviewed the results of the paired normal samples from 1,100 patients across multiple tumor types (50% leukemia patients and the remainder split between CNS and non-CNS solid-tumor patients), unselected for family history (112). Overall, 9% of the subjects had a P/LP variant (using an assessment similar to the interpretation guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology) in the matched blood sample. This cohort had substantial representation of *TP53* variants (50 patients), with the remainder including a long tail of genes, each with only a few patients with a P/LP variant. It is important to note that St. Jude Children's Research Hospital is a tertiary care facility, and the distribution of tumor types for the patients in the study is not typical of the childhood cancer population as a whole. In particular, this cohort was overrepresented for several tumor types associated with a high prevalence of germline *TP53* findings, including adrenal cortical carcinoma. The authors adjusted for this distribution and estimated that the overall germline frequency would be 7.3–9.8% for a tumor diagnosis distribution comparable to that of the general population. The CPGs represented a mixture of genes previously associated with a hereditary risk of the tumor found in the patient and a substantial minority of genes not previously associated with the tumor type, including those associated with adult malignancies, such as *BRCA1* and *BRCA2*.

In parallel with the St. Jude retrospective analysis of germline variation (112), two National Institutes of Health Clinical Sequencing Exploratory Research (CSER) genomic projects (3) prospectively explored the likelihood of finding variants associated with cancer predisposition in smaller childhood cancer cohorts. The University of Michigan CSER project (MiOncoSeq) reported on 100 patients with difficult-to-treat or recurrent tumors, including both leukemia and CNS and non-CNS solid tumors (52). They performed genome sequencing and reported that 9 of 102 subjects (8.8%) had a germline cancer predisposition finding that would affect either their own medical management or that of relatives. The Baylor College of Medicine Advancing Sequencing in Childhood Cancer Care (BASIC3) CSER trial included Clinical Laboratory Improvement Amendments (CLIA)–certified germline and tumor exome sequencing of an unselected cohort of 278 ethnically and racially diverse childhood cancer patients with malignant solid tumors and

brain tumors (61). Like the two other studies, patients were unselected for family history or other features suggestive of hereditary status (such as bilateral tumors) but included only solid-tumor patients. P/LP variants in CPGs were found in 9.8% of patients. Unlike the St. Jude cohort, *TP53* variants were not predominant, and this difference might represent the wider diversity of tumor types in this cohort, which included both low-grade and high-grade malignancies. The Precision in Pediatric Sequencing (PIPseq) program at Columbia University Medical Center performed tumor and germline exome sequencing for 101 high-risk oncology patients (along with transcriptome sequencing of their tumors) and found that 14% of the patients had reportable variants in CPGs (58).

In all four of these studies, pathogenic variants were identified in genes known to increase risk of the tumor type in question, and a number of patients had P/LP variants in CPGs previously associated with adult cancers. A variety of germline DNA repair gene mutations have also been reported from cohorts of patients with neuroblastoma (26) and Ewing sarcoma (12).

As described above, *PALB2* and *BRCA2* are statistically associated with medulloblastoma predisposition (9). However, the increasing number of reports of P/LP variants in adult-onset CPGs in a few pediatric cancer patients have lacked an appropriate case–control analysis to determine whether these variants are clearly enriched compared with a noncancer pediatric cohort. In addition, where tumors are available, there has been no evidence of loss of heterozygosity, which is typically seen in adult cancers. However, heterozygous mutations (or haploinsufficiency) in these DNA repair genes could result in some increase in pediatric cancer risk, and this needs to be systematically studied.

In 2018, a European consortium published an analysis of both somatic and germline findings from comprehensive genomic analyses of 961 pediatric and adolescent patients across 24 types of cancer, including both hematopoietic and solid tumors, with CNS tumors overrepresented (27). Focusing their germline sequence analysis on 162 CPGs, the authors found germline variants likely to be pathogenic in 7.6% of patients. These findings included several of the known associations described above, such as *TP53* with hypodiploid ALL. However, there was also an excess of children with germline variants in some genes not previously associated with specific malignancies, including associations of *TSC1*, *CHEK2*, and *SDHA* with medulloblastoma. Germline variants in *SDHA* were also overrepresented in an adult pan-cancer germline analysis (30a). Despite the dramatically different patient population compared with the previously described projects, this study also had an overall frequency of 8.6% of P/LP variants in CPGs.

In contrast to studies performed on pediatric cancer patient populations at the time of diagnosis or relapse, a recent study reported the results of genome sequencing of 3,006 childhood cancer survivors (at least five years after diagnosis) enrolled in the longitudinal St. Jude Lifetime Cohort Study (103). This cohort provides detailed information on the treatment for the initial malignancy as well as the subsequent development of a second neoplasm. Among this cohort, 5.8% of survivors carried P/LP variants in CPGs. This modest decrease in germline variant prevalence may represent a different spectrum of tumor diagnoses compared with those of prior studies; in particular, 19% of survivors had lymphoma as their primary diagnosis, in comparison with less than 5% of all pediatric cancer patients. The genes with germline variants are similar to those in the prior studies, including genes strongly associated with pediatric cancer (*RB1* and *NF1*) and adult malignancies (*BRCA2*). Patients with germline findings had a substantial increase in the likelihood of a second neoplasm (relative risk = 1.8, CI = 1.2–2.6), particularly for patients where breast cancer was the second neoplasm (relative risk = 9.4, CI = 4.8–18.2). The relationship between a second neoplasm and prior radiation treatment and germline status was more complex, with the germline status having a smaller effect for patients who underwent radiation therapy for their first cancer.

Mendelian Mechanism of Disease

An interesting observation from these cohorts of patients undergoing exome or genome sequencing is the distribution of Mendelian mechanisms. X-linked cancer predisposition disorders (e.g., dyskeratosis congenita and Fanconi anemia type B) are rare and were only reported in a few patients. There are hundreds of autosomal recessive cancer predisposition disorders, including some diagnoses with substantial genetic heterogeneity (e.g., at least 16 different Fanconi anemia genes). Even so, the St. Jude cohort reported that only 1 of 1,100 patients had a molecular recessive diagnosis (biallelic variants in *PMS2*-associated cMMRD) (112). Exome analysis of the BASIC3 cohort also revealed only one recessive diagnosis (*TJP2*-associated liver disease) compared with 27 dominant molecular diagnoses (61; S. Plon, unpublished data). Despite the large number of recessive cancer predisposition syndromes, they appear to account for less than 1% of children diagnosed with solid tumors in the United States. The initial study from Columbia University and a follow-up analysis that included only hematopoietic malignancies and other hematologic disorders, such as hemophagocytic lymphohistiocytosis, reported additional patients with biallelic variants (two hemophagocytic lymphohistiocytosis patients, one Kabuki syndrome patient, and one cMMRD patient), suggesting a potentially higher prevalence of recessive disorders (47, 58). The proportion in solid-tumor patients may be higher in countries with higher rates of consanguinity. Although we are unaware of any similar study reporting on genome-scale sequencing in an unselected cohort of pediatric cancer patients, there was an evaluation of mismatch repair genes in children diagnosed with high-grade gliomas or supratentorial primitive neuroectodermal tumors in distinct populations (92). The investigators found that the prevalence of cMMRD was significantly higher in a population of children in Jordan with frequent consanguinity than in a population of children with the same diagnoses evaluated in Toronto.

In contrast to the rare recessive diagnoses, both the St. Jude and BASIC3 studies noted that approximately 6% of pediatric cancer cohorts carry single P/LP variants in genes associated with rare recessive cancer predisposition syndromes, such as *BUB1B* and *FANCC* (61, 112). The patients with single recessive variants do not appear to have features of the syndromic diagnosis, and the cancer diagnosed is not necessarily the type associated with the syndrome. Thus, without appropriate comparison with genomic data from control populations, it is unclear whether single variants in any of the hundreds of recessive CPGs are enriched in these patients compared with the general population.

Future Directions in Genetic Analysis of Pediatric Cancer Patients

The results from the studies of large-scale unselected pediatric cancer cohorts provide a consistent picture that 6–10% of childhood cancer patients carry a germline P/LP variant in an autosomal dominant CPG. Although specific tumor types may be associated with a much higher likelihood of identifying a cancer predisposition syndrome (e.g., rhabdoid tumors), there have been no reports of genome-scale sequencing of a pediatric patient cancer population that resulted in a germline predisposition finding substantially less than 6%. Similarly, studies have consistently shown that other features previously associated with an indication for genetic evaluation (e.g., family history) are not always present due to the possibility of either de novo or somatic mosaicism for the CPG variant, incomplete penetrance in family members who carry the variant, or a lack of family history information (102). In addition, exome or genome sequencing tests have consistently reported P/LP variants in both genes already associated with the specific cancer type and genes associated with a high risk of other malignancies but not previously established to be associated with the patient's tumor type. This latter situation may result from a combination of both truly incidental findings and tumor–gene associations that have not yet been thoroughly established. The

prevalence of germline findings and the crossover of gene and tumor type results are very similar to the results from large series of adult cancer patients (85). Finally, although not reviewed in detail here, an increasing number of pediatric cancer patients are undergoing tumor-only somatic mutation analysis, which can result in findings that are highly associated with cancer predisposition, such as chromothripsis in medulloblastoma. Preparation for these types of somatic findings is needed for both tumor-only testing laboratories and the physicians ordering these tests (74).

There will be many considerations involved in deciding whether to expand germline analysis to all pediatric cancer patients. For example, the Netherlands recently determined that a 5% risk of germline predisposition for a given cancer warranted testing (37), and one could argue that a childhood cancer diagnosis as a whole meets that threshold. Many diverse approaches to systematic germline analysis of pediatric cancer patients have been proposed. For example, several studies are implementing germline exome or genome sequencing for all patients (instead of panel testing focused on pediatric cancer genes), and the National Cancer Institute/Children's Oncology Group Pediatric Molecular Analysis for Therapy Choice (MATCH) precision oncology trial includes targeted germline panel analysis and reporting for every patient undergoing somatic panel screening. Other approaches to deciding whether to perform germline analysis of pediatric cancer patients include systematic checklists of germline features to evaluate in each patient (78) and a richly annotated decision support algorithm for health-care workers to use for each newly diagnosed pediatric cancer patient (26). The sensitivity and specificity of unbiased sequencing versus these selective approaches, availability of genetics professionals, and costs of genomic diagnostics will all inform how to best implement germline genomics for pediatric cancer patients and their at-risk relatives.

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