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Personalized Medicine and Imaging

Clinical Cancer Research

High Yield of Pathogenic Germline Mutations Causative or Likely Causative of the Cancer Phenotype in Selected Children with Cancer

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Abstract

Purpose: In many children with cancer and characteristics suggestive of a genetic predisposition syndrome, the genetic cause is still unknown. We studied the yield of pathogenic mutations by applying whole-exome sequencing on a selected cohort of children with cancer.

Experimental Design: To identify mutations in known and novel cancer-predisposing genes, we performed trio-based whole-exome sequencing on germline DNA of 40 selected children and their parents. These children were diagnosed with cancer and had at least one of the following features: (1) intellectual disability and/or congenital anomalies, (2) multiple malignancies, (3) family history of cancer, or (4) an adult type of cancer. We first analyzed the sequence data for germline mutations in 146 known cancer-predisposing genes. If no causative mutation was found, the analysis was extended to the whole exome.

Results: Four patients carried causative mutations in a known cancer-predisposing gene: *TP53* and *DICER1* ($n = 3$). In another 4 patients, exome sequencing revealed mutations causing syndromes that might have contributed to the malignancy (*EP300*-based Rubinstein–Taybi syndrome, *ARID1A*-based Coffin–Siris syndrome, *ACTB*-based Baraitser–Winter syndrome, and *EZH2*-based Weaver syndrome). In addition, we identified two genes, *KDM3B* and *TYK2*, which are possibly involved in genetic cancer predisposition.

Conclusions: In our selected cohort of patients, pathogenic germline mutations causative or likely causative of the cancer phenotype were found in 8 patients, and two possible novel cancer-predisposing genes were identified. Therewith, our study shows the added value of sequencing beyond a cancer gene panel in selected patients, to recognize childhood cancer predisposition.
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Introduction

A significant fraction of pediatric cancer is caused by a germline cancer-predisposing mutation, but the exact number is unknown. In literature on this topic, the relative contribution of genetic predisposition to pediatric cancer is considered to be approximately 10%. This number is based on a cancer registry and literature review already performed in 1991 (1).

Recognition of cancer susceptibility in children with cancer is of high clinical significance. Knowledge of a cancer-predisposing mutation may lead to modifications in treatment protocols and

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R.P. Kuiper, N. Hoogerbrugge, and M.C. Jongmans contributed equally to this article.

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Translational Relevance

Knowledge of a cancer-predisposing mutation in a child can be beneficial in terms of treatment choices, surveillance protocols, and genetic counseling of the family. This study sought to determine the proportion of germline genetic mutations in selected children with cancer, by applying whole-exome sequencing on patient–parent trios, with subsequent cancer gene panel-restricted analysis as well as exome-wide analysis. With this strategy, germline pathogenic mutations were identified in 20% of the children, showing the value of whole-exome sequencing in this selected cohort.

the application of cancer surveillance for early detection of second primary malignancies. In addition, it will enable genetic counseling and cancer surveillance for family members.

The presence of germline cancer-predisposing mutations in children can be suspected based on specific hallmarks such as a positive family history of cancer, the development of multiple malignancies, or the presence of congenital anomalies (2–4). Based on these hallmarks, we have developed a practical tool to improve the selection of childhood cancer patients eligible for referral to a clinical geneticist for genetic counseling and genetic testing (2). However, even if hallmarks suggesting a cancer-predisposing mutation are present in a child, the diagnosis of a specific syndrome relies on the physicians to recognize this syndrome and to ask for the right targeted genetic test. In daily practice, underlying syndromes are often missed due to their heterogeneity and the lack of a recognizable clinical phenotype (3, 5).

In recent years, next-generation sequencing (NGS) techniques have become available and are gradually being implemented in routine clinical care (6). For many syndromes, the application of these techniques has revealed that the phenotypic spectrum is much broader than the clinical diagnostic criteria set by physicians (7). In addition, NGS techniques have proven their value in the identification of novel disease genes (8, 9). In recent studies, the role of cancer-predisposing mutations was investigated by analyzing germline whole-genome or whole-exome sequencing (WES) data of 1,120 children with various types of cancer (10), 150 children with solid tumors (11), and 91 children with relapsed, refractory, or rare cancer (12). Germline mutations were found in 8.5%, 10%, and 10% of the patients, respectively. In these studies, the patients included were not selected based on features suggestive for a cancer-predisposing syndrome. Furthermore, the analysis was restricted to known cancer-predisposing genes, which excludes the possibility of finding novel cancer-predisposing genes. Therefore, the actual contribution of germline mutations may be higher.

Most centers for pediatric oncology currently may not be able to analyze all children with cancer using NGS techniques to search for germline-predisposing mutations. Selecting the right patients for germline testing will then be important. Therefore, we studied the yield of germline WES if applied on a selected cohort of childhood cancer patients with a high *a priori* chance of having a genetic cancer-predisposing mutation. Because we also aimed to identify novel cancer-predisposing genes, we used both a cancer gene panel analysis and whole-exome analysis. We included parental DNA to facilitate the interpretation of *de novo* and biallelic mutations. With this strategy, we found a high yield of pathogenic mutations in our selected childhood cancer cohort.

Materials and Methods

Enrollment of the patients

Patients with childhood cancer who fulfilled one or more of the following criteria were included: (1) The presence of congenital anomalies without a clear infectious or environmental cause and/or intellectual disability (ID), defined by an Intelligence Quotient score below 70, with an origin before the diagnosis and treatment of cancer; (2) the presence of a second primary malignancy, excluding patients in whom no clear distinction can be made between a second primary malignancy and a therapy-related secondary cancer; (3) the presence of a family history of cancer, defined as having one or more first- or second-degree relative(s) with childhood cancer; (4) the diagnosis of an adult type of cancer in a child, defined as a malignancy frequent in the adult population, but with an incidence of less than 1 in a million children (age 0–18) per year (Table 1). Patients who fulfilled our inclusion criteria were referred by pediatric oncologists and geneticists from different centers in the Netherlands ($n = 32$) and from abroad ($n = 8$). Prior to inclusion, most patients had undergone an extensive diagnostic workup for germline genetic aberrations, including 250K SNP array analysis, which did not lead to a diagnosis. When the clinician thought of a specific (cancer predisposing) syndrome, targeted gene tests were performed before inclusion in the study. Details about these tests can be found in Supplementary Appendix S1 for each patient. Written informed consent was obtained for all study participants and their parents (Radboudumc Medical Ethics Committee, study 2012/271).

WES

During the course of this study (2012–2016), WES was performed using the latest available SureSelect Human All Exon enrichment kits (Agilent Technologies) and Illumina HiSeq sequencing platforms (2 × 100 bp paired end; BGI). Reads were mapped to the hg19 reference genome, and variants were called using the Burrows–Wheeler aligner and annotated with an in-house annotation pipeline (13). WES was performed on germline

Table 1. Inclusion categories

	Method of analysis used				Total number of
Category	Trio	Patient only	Affected family members	Combination family + trio	patients (%)
1. Intellectual disability and/or congenital anomalies	17	2	N/A	N/A	19 (47.5)
2. Multiple malignancies	3	3	N/A	N/A	6 (15.0)
3. Positive family history	—	1	7	3	11 (27.5)
4. Adult type of cancer	1	—	—	—	1 (2.5)
5. Multiple reasons for inclusion	2	—	1	—	3 (7.5)
Total	23	6	8	3	40 (100)

Abbreviation: N/A, not applicable.

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DNA extracted from peripheral blood of the patients and both parents if available or, in case of inclusion because of a family history, their affected relatives (11 families with 24 affected patients; Table 1; Fig. 1). To determine the origin of variants in the index cases, a *de novo* analysis was performed for cases with available WES data for both parents ($n = 26$; ref. 6). For patients of whom affected family members were sequenced, the analysis focused on rare variants shared between related patients. Table 1 shows the different methods of analysis used in each inclusion category.

WES data were analyzed by a two-step approach (Fig. 1). First, we filtered for mutations in a panel of 146 well-known cancer-predisposing genes (Supplementary Table S1). This cancer gene panel includes genes that cause autosomal-dominant or autosomal-recessive diseases. All mutations found are shown in Supplementary Appendix S2, but mutations in genes known to cause autosomal-recessive cancer predisposition syndromes were only reported in the main article or patient descriptions if either homozygous or compound heterozygous mutations were found. If a causative mutation was found in this panel, whole-exome

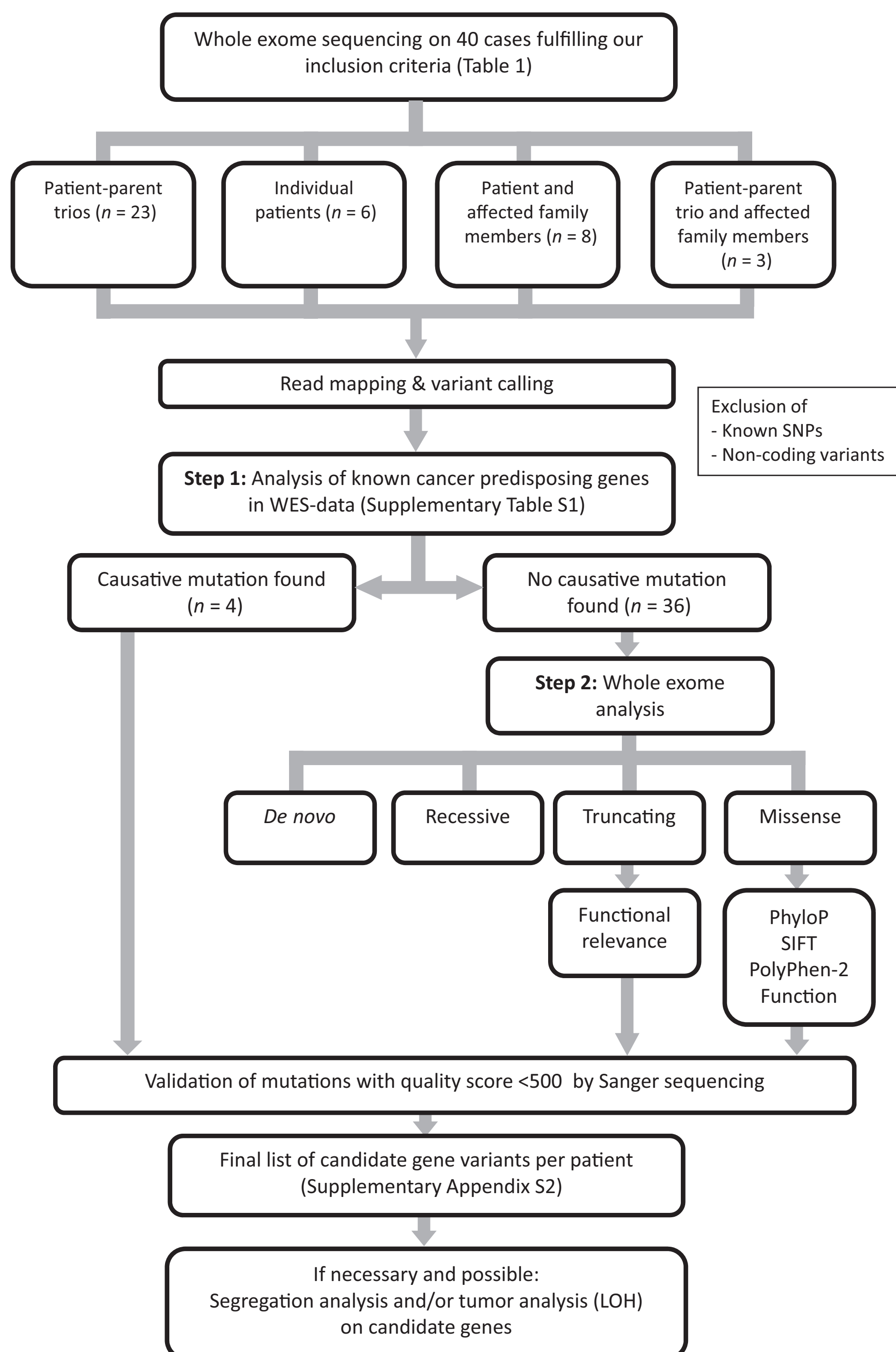

Figure 1.
Workflow of whole-exome sequencing in selected individuals with childhood cancer. As a first step, a cancer gene panel analysis was performed, by which 4 causative mutations were identified. In the remaining 36 patients, whole-exome analysis was performed, focusing on *de novo*, recessive, truncating, and missense variants. Abbreviations: LOH, loss of heterozygosity; SNP, single nucleotide polymorphism.

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analysis was not performed to prevent unsolicited findings. When no causative mutation was found, or in case of doubt about the association of the mutation found with the type of childhood cancer, an exome-wide analysis was performed. Variants were filtered according to a preset filtering template. Normal variation was excluded, which was defined as variants present in >10 cases of our in-house mutation database containing 15,576 exomes, and variants present with a frequency of >1% in dbSNP (build144), or the ExAC database (14). For recessive variants (homozygous or compound heterozygous variants inherited paternally and maternally), a slightly less stringent filtering for normal variation was used (2% frequency cut off).

All *de novo* and recessive variants were selected for Sanger sequencing validation if they had a GATK quality score below 500 (15). Missense variants were filtered based on Call quality ($\geq$10 total reads with $\geq$20% variant reads), conservation score (PhyloP $\geq$ 3.0), and pathogenic prediction in two out of three *in silico* prediction scores CADD PHREDD (16) (>20), SIFT (17) (damaging), and PolyPhen2 (ref. 18; possibly/probably damaging) using Alamut Visual software (v2.7.1., Interactive Biosoftware). Subsequently, missense or truncating variants (i.e., nonsense, frameshift, and canonical splice site variants) with a described role in cancer/tumorigenesis or involved in pathways that could be related to the type of tumor or the comorbidities present in the patient based on Genecards, PubMed, and OMIM were selected for Sanger sequencing validation if they had a GATK quality score below 500 (15). All variants that remained after filtering can be found in Supplementary Appendix S2.

Interpretation of variants

Within the cancer gene panel, variants were considered pathogenic if the mutation was found to have a deleterious effect on the function, and if the gene defect, based on available literature, correlates to the observed phenotype in the patient (positive genotype–phenotype correlation). Variants were considered deleterious if they were found to result in premature termination of the protein (i.e., truncating mutations), or if they involved a nonsynonymous substitution which had previously been described as pathogenic in literature. If a syndrome diagnosis was made by whole-exome analysis, the possible causality for the malignancy was judged by considering three different features: (1) a malignancy has been described before in patients with this syndrome, (2) the gene involved is affected by somatic mutations in cancer, and (3) the gene has a function clearly related to tumorigenesis. Mutations in genes not previously associated with disease were considered as candidate genes if mutations occurred in multiple patients, in addition to having a clear association with tumorigenesis. Findings related to each of these aspects are outlined in the clinical description of the patients (Supplementary Appendix S1, pages 15–43). Wherever possible, we have performed somatic analysis of tumor-derived DNA in patients with interesting candidate genes to analyze the tumor for second hit mutations or loss of heterozygosity (LOH). Furthermore, segregation analysis was performed in patients with variants of unknown significance.

Results

Cohort description

The number of patients per inclusion category is shown in Table 1. We included 29 sporadic patients and 11 patients with a positive family history of cancer (24 affected family members sequenced in total). In 23 of the 29 sporadic patients and in 3 of 11 patients with a positive family history, parents were included in the analysis to establish the mode of inheritance of variants identified. Detailed clinical descriptions of the patients and families are available in Supplementary Appendix S1. The tumor types observed were solid tumors (N = 14, 29.2%), leukemia (N = 13, 27.1%), brain tumors (N = 10, 20.8%), and lymphoma (N = 9, 18.8%; Fig. 2; Supplementary Table S3). Median age at diagnosis was 6.5 years (range, 0.5–18 years; mean, 7.25 years). In the first step of our analyses, we focused on a cancer gene panel, which revealed a total of 10 variants. Subsequently, we performed whole-exome analysis for patients that were unresolved after analysis of the cancer gene panel (n = 36). The mutational yield per inclusion category is shown in Figure 3.

Cancer panel: Causative pathogenic mutations

In 4 patients, mutations were identified that explained the development of cancer in these probands (Table 2). Three patients were found to carry germline *DICER1* mutations, but had very diverse phenotypes: a girl with neuroblastoma and a cervical embryonal rhabdomyosarcoma (Case #07), two siblings with thyroid carcinoma (Case #21), and a boy with pineoblastoma (Case #04; ref. 19). This boy additionally had a pulmonary lesion that was originally diagnosed as congenital bullous emphysema. After the identification of the *DICER1* mutation, this diagnosis was revised by the pathologist to pleuropulmonary blastoma. One patient (Case #18) carried a *de novo TP53* mutation (p.Gly245Ser; ref. 20). This patient was diagnosed with an anaplastic glioma (age 3) and a renal cell carcinoma (age 9), which is an atypical presentation of Li–Fraumeni syndrome. In addition, we included two half-siblings who were diagnosed with medulloblastoma. In one of the half-siblings, who also had polyposis and a pancreaticoblastoma, a truncating mutation in *APC* was identified before inclusion in the study. Because we hypothesized that there might have been an additional mutation present explaining why both half-siblings developed medulloblastoma, they were included in our study. No other possible causative mutations were identified.

Cancer panel: Pathogenic mutations of uncertain causality

In 3 patients, we identified pathogenic mutations in known cancer predisposing genes associated with a different type of cancer than was diagnosed in these children. A boy diagnosed with two primary leukemias (Case #05) carried a maternally inherited frameshift mutation in the breast and ovarian cancer-predisposing gene *BRCA2*. The mutation segregated in the family with multiple women diagnosed with breast cancer. Two patients with anaplastic ependymoma (Case #27) and acute myeloid leukemia (AML; Case #33), respectively, carried a *CHEK2* c.1100delC variant. In each of these patients, we proceeded with whole-exome analysis.

Cancer panel: *De novo* variants of uncertain significance

In 3 patients, we revealed *de novo* missense variants in known cancer-predisposing genes (*BUB1*, *BAP1*, and *ETV6*), but both the causal role and the pathogenicity of the identified variants need to be established (Supplementary Appendix S1). The *ETV6* variant p.Arg433Cys, for instance, was found in a girl with acute lymphoblastic leukemia (ALL; Case #36). *ETV6* has been associated with thrombocytopenia and leukemia predisposition (21). The

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Table 2. Germline mutations found in childhood cancer patients with cancer gene panel analysis

Gene and mutation		Patient ID[a]	Inheritance	Tumor type (age)	Additional medical history and family history	Syndrome diagnosis
Pathogenic mutations that explain the cancer phenotype						
***APC*[b]**	c.3180_3184del (p.Ile1060fs)	34	*De novo*	Medulloblastoma (8), polyposis, and pancreaticoblastoma	Half-sister (maternal) medulloblastoma (8), does not carry the mutation.	Familial adenomatous polyposis
DICER1	c.4407_4410del (p.Ser1470fs)	4[c]	Maternal	Pineoblastoma (1.5)	Pulmonal hypertension with lung cysts (revision after *DICER1* diagnosis showed pleuropulmonary blastoma)	*DICER* syndrome (42)
DICER1	c.2414T>C (p.Leu805Pro)	7[c]	Paternal	Neuroblastoma (3) Embryonal rhabdomyosarcoma cervix (4)	—	*DICER* syndrome
DICER1	c.1363del (p.Val455fs)	21[c]	Unknown; not maternal	Papillary thyroid carcinoma (17)	Sister follicular thyroid carcinoma (13); both carry the *DICER1*-mutation.	*DICER* syndrome
TP53	c.733C>T (p.Gly245Ser)	18[c]	*De novo*	Anaplastic glioma (3) Renal cell carcinoma (9)	—	Li–Fraumeni syndrome
Pathogenic mutations without a direct link to the phenotype						
***BRCA2*[b]**	c.4532del (p.Glu1511fs)	5	Maternal	Acute lymphoblastic leukemia twice (cytogenetically different) (3, 8)	Family history of breast- and ovarian cancer. Additional germline *TYK2* mutation (see Table 3)	
CHEK2	c.1100delC (p.Thr367fs)	27	Maternal	Anaplastic ependymoma (6)	Maternal aunt astrocytoma at age 3 (not tested), multiple family members with breast cancer.	
CHEK2	c.1100delC (p.Thr367fs)	33[d]	Paternal	Acute myeloid leukemia (13)	Congenital hypothyroidism, congenital hip dysplasia, learning difficulties. No family history of breast cancer.	
***De novo* variants of unknown significance**						
BAP1	c.2116G>A (p.Ile706Val)	33[d]	*De novo*	Acute myeloid leukemia (13)	Congenital hypothyroidism, congenital hip dysplasia, learning difficulties.	
BUB1	c.2971G>A (p.Val991Ile)	35[d]	*De novo*	Hepatocellular carcinoma (5)	No additional features	
ETV6	c.1297C>T (p.Arg433Cys)	36	*De novo*	Acute lymphoblastic leukemia (5)	Brother with acute lymphoblastic leukemia (3), does not carry *ETV6* mutation.	

[a]Additional clinical descriptions of all patients are available in Supplementary Appendix S1.
[b]These mutations were already known before inclusion in the study.
[c]In these patients, no whole-exome analysis was performed, because a causative mutation was found in the cancer gene panel.
[d]These patients have more than one (possible) causative mutation.

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brother of this patient, who also developed ALL, did not carry this mutation.

Whole-exome analysis: Diagnosis of known syndromes

In 4 of 36 patients, we revealed pathogenic mutations in genes that cause a specific syndrome, with a possible relation to the development of cancer in the affected child (Table 3). We found *ACTB*-based Baraitser–Winter syndrome in a boy with ALL, ID, and trigonocephaly (Case #01; p.Val209Leu; ref. 22). *ARID1A*-based Coffin–Siris syndrome was diagnosed in a boy with ALL, ID, slow dentition, and hypoplastic nails (Case #02; c.4993+1G>A). We found an *EP300* p.Pro1877fs frameshift mutation in a girl with AML, mild ID, and microcephaly (Case #19), indicating Rubinstein–Taybi syndrome. Furthermore, we found a pathogenic *EZH2* mutation (p.Ala682Thr) in a boy with Burkitt lymphoma, tall stature, and a horseshoe kidney (Case #37), resulting in the diagnosis Weaver syndrome (23). In hindsight, each of these patients presented with phenotypical features characteristic for the syndrome diagnosed, and for each of the syndromes, there are clues linking the syndrome to cancer predisposition (Table 3). More details about the relation of these mutations to cancer development can be found in Supplementary Appendix S1 in the patient description section.

Three additional patients carried deleterious mutations in genes associated with autosomal-dominant syndromes, but a causal relationship to the malignancy they developed is unlikely. In a girl with ALL and ID (Case #03), we found a *de novo* mutation in *SCN2A* (p.Arg856*). Heterozygous mutations in this gene cause epilepsy and/or ID (24). This mutation explains her delayed development, but most likely does not play a role in the onset of leukemia, based on the function of the gene in sodium channels. In a boy with multiple basal cell carcinomas (BCC; Case #32), a paternally inherited previously reported nonsense mutation in *SPRED1* (p.Arg16*) was found (25). Mutations in *SPRED1* cause Legius syndrome, a condition mainly characterized by café-au-lait macules, macrocephaly, lipomas, and learning disabilities (25). No association with BCCs has been described yet. The patient and his father showed no clinical features of Legius syndrome, and we did not find LOH of the *SPRED1* mutation in one of his BCCs. Finally, in a boy with hepatocellular carcinoma (Case #35), we found a paternally inherited heterozygous nonsense mutation in *APOB* (p.Tyr981*), causing hypobetalipoproteinemia (26, 27).

Possible novel candidate genes

We identified mutations in two genes that we regard as possible novel cancer susceptibility genes (Table 3), because of the presence of mutations in multiple patients, in addition to having a function that could be linked to cancer development. In a boy with two primary leukemia occurrences (Case #05), a mutation in *TYK2* was identified (p.Pro760Leu), located in the DPG motif of the pseudokinase domain. Interestingly, as we published earlier (28), another mutation in *TYK2* (p.Gly761Val) was found in a second patient with two primary leukemia occurrences. Both mutations promote *TYK2* autophosphorylation and activate downstream STAT family members. Because activation of the JAK–STAT pathway is an important oncogenic event in the pathogenesis of leukemia, these mutations in *TYK2* may contribute to the development of leukemia (28).

Mutations in *KDM3B* were identified in 2 patients in our cohort, a girl with AML, mild ID, and congenital hip dysplasia (Case #33; p.Glu93*), and a boy with Hodgkin lymphoma and moderate ID (Case #25; p.Asp1032Val). The mutation in the boy was *de novo*, whereas the nonsense mutation in the girl was inherited from her mother, who also presented with mild ID, but did not develop a malignancy. *KDM3B* is involved in H3K9 demethylation, which is part of chromatin remodeling (29). Mutations in several components of chromatin remodeling pathways have been found to cause both syndromes characterized by ID and syndromes with cancer predisposition (30, 31).

Discussion

Our strategy of patient selection and data analysis yielded a high number of mutations, as we identified pathogenic mutations in eight children for which the link to the patients' cancer phenotype is confirmed or plausible (20%), therewith contributing to improved recognition of cancer predisposition in children. The approach used in our study is unique compared with previously published studies (10–12) for several reasons. First, the patients included were preselected for having a high *a priori* chance of carrying a germline cancer-predisposing mutation, showing that specifically this group of patients benefits highly from WES. Second, in previous studies the data analysis was limited to a cancer gene panel analysis only, whereas we added a whole-exome analysis, which enabled the detection of novel cancer-predisposing genes and increased the yield of mutations found. Third, we performed WES on patient–parent trios. Of the 21 (possible) causative mutations found as shown in Tables 2 and 3, 11 were *de novo* (52.4%), underscoring the effectiveness of child–parent trio sequencing for this group of patients.

Our study illustrates that WES can aid in diagnosing clinically heterogeneous syndromes, enabling physicians to diagnose children with nontypical presentations of known syndromes. An example is the mutation identified in *DICER1* in two siblings with thyroid cancer. In this family, the clinical phenotype had not led to a suspicion of this syndrome at the time of inclusion in this study. We conclude that the application of our strategy will improve the understanding of phenotypic heterogeneity within known cancer syndromes.

Most currently used cancer gene panels are not specifically designed for the analysis of childhood cancer predisposition. The panels include adult cancer-predisposing genes for which a link to childhood cancer is unknown, making it difficult to interpret mutations identified in children. Examples from this study include heterozygous mutations in *BRCA2* and *CHEK2*. Contradicting papers about the risk of childhood cancer in families with mutations in *BRCA1* and *BRCA2* have been published (32, 33). Furthermore, both Zhang and colleagues and Parsons and colleagues report the finding of multiple *BRCA1/2* and *CHEK2* mutations in their cohort (10, 11), but it is unclear whether the incidence of mutations they found exceeds that of the general population. It is possible that these mutations have contributed to cancer development in our patients, but it seems unlikely that they fully and singularly explain the phenotype.

Importantly, if the analysis had been restricted to the cancer gene panel, we would have missed relevant mutations in 10 children (Table 3), which underlines the added value of an exome-wide analysis. In two recent studies, genetic predisposition was found in 8.5% (10) and 10% (11) of the patients. In these studies, the analysis was restricted to genes known to have a role in cancer predisposition, which smoothens the interpretation of identified mutations, but prevents the discovery of new and less

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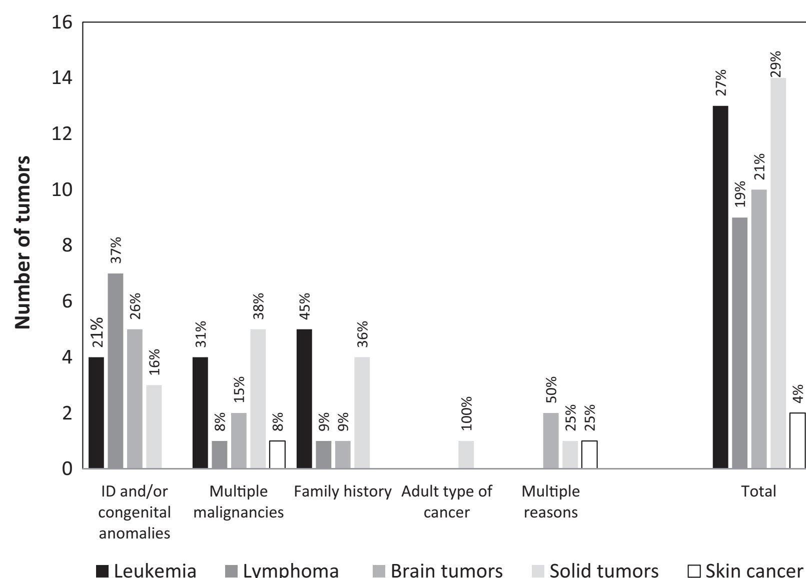

Figure 2.
Tumor types of index patients per inclusion category. The total number of tumors ($N = 48$) exceeds the total number of included index patients ($N = 40$), because both primary and secondary malignancies of the index patients are shown.

well-characterized cancer predisposition genes. For example, by analysis beyond the cancer panel, we found syndrome diagnoses in 7 patients. These patients showed a clinical phenotype compatible with the syndrome diagnosed. In five of these syndromes (Baraitser–Winter syndrome, Coffin–Siris syndrome, Rubinstein–Taybi syndrome, Weaver syndrome and *APOB*-based hypobetalipoproteinemia), malignancies have been described before (22, 23, 34–37), and the genes involved are also affected by somatic mutations in several cancer types (38–40). This suggests a role of these mutations in cancer development in these patients, but more studies are needed to firmly establish this relationship.

These findings illustrate that cancer gene panels are likely incomplete, especially for syndromes with reduced cancer penetrance. For instance, the causative gene for Weaver syndrome, *EZH2*, was not present in our panel, nor in the panel used by Parsons and colleagues, whereas enough data are available to regard this gene as a low-to-moderate penetrant childhood cancer-predisposition gene (23, 41). Obviously, we cannot exclude that our inclusion criteria may have caused an ascertainment bias, and the association between these specific syndromes and childhood cancer needs to be studied in more detail. This could be achieved by registration of syndrome diagnoses in the national pediatric cancer registries, which will facilitate insight in syndrome/cancer associations. Nevertheless, if WES for children with cancer will be implemented in diagnostic settings on a large scale, cancer gene panels need to be optimized.

In general, a positive family history is the most well-known indicator of genetic predisposition. However, in only 2 of 11 patients with a positive family history, mutations were identified that explain the phenotype of the family, in both cases affecting *DICER1* (family 4 and 21; Fig. 3). Two families were included that consisted of two siblings with leukemia (family 36) and two half-siblings with medulloblastoma (family 34), respectively. Strikingly, in each of these families, a likely causative *de novo* mutation was found in only 1 of the 2 affected patients: a mutation in *ETV6* (p.Arg433Cys) in one sibling with leukemia and a mutation in *APC* (p.Ile1060fs) in one sibling affected by medulloblastoma. It is possible that the cancer in the siblings without the mutation is

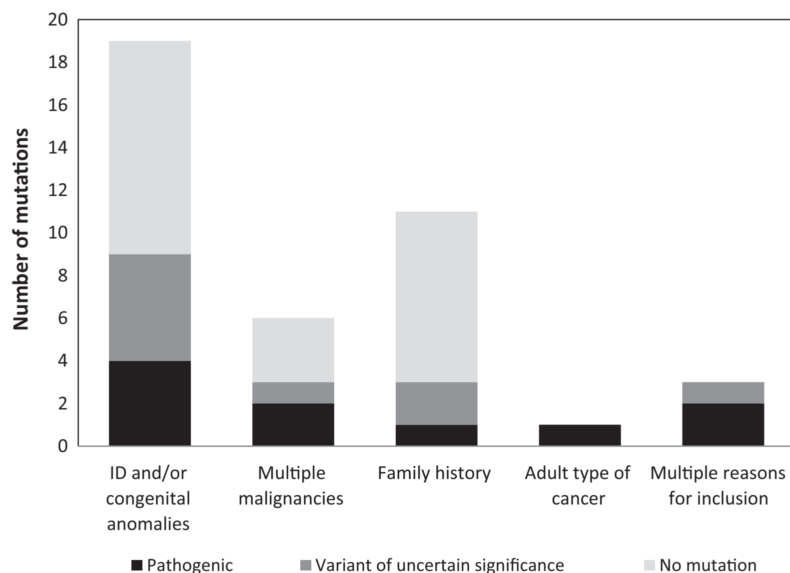

Figure 3.
Mutation yield per inclusion category. The mutations found are subdivided in pathogenic mutations, variants of uncertain significance, and no plausible causative mutations found.

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Table 3. Germline mutations found in childhood cancer patients with whole-exome analysis

Gene	Mutation	Patient ID[a]	Inheritance	Tumor type(s) (age)	Additional medical history and family history	Syndrome diagnosis	Evidence[b]
Pathogenic mutations							
ACTB	c.625C>A (p.Val209Leu)	1	*De novo*	Acute lymphoblastic leukemia (8)	Intellectual disability, trigonocephaly	Baraitser–Winter syndrome (22)	1, 2
ARID1A	c.4993+1G>A	2	*De novo*	Acute lymphoblastic leukemia (16)	Intellectual disability, slow dentition, hypoplastic nails	Coffin–Siris syndrome	1, 2
EP300	c.5630insGGTA (p.Pro1877fs)	19	*De novo*	Acute myeloid leukemia (8)	Mild intellectual disability, microcephaly, mild learning problems	Rubinstein–Taybi syndrome	1, 2
EZH2	c.2044G>A (p.Ala682Thr)	37	*De novo*	Burkitt-type non-Hodgkin lymphoma (3)	Tall stature (>+2.5SD), horseshoe kidney	Weaver syndrome (23)	1, 2, 3
APOB	c.2943C>G (p.Tyr981*)	35[c]	Paternal	Hepatocellular carcinoma (5)	Low cholesterol levels in boy and paternal relatives that carry the mutation[d]	Hypobetalipoproteinemia (34)	1, 2, 3
SCN2A	c.2566C>T (p.Arg856*)	3	*De novo*	Acute lymphoblastic leukemia (2)	Intellectual disability, congenital hip dysplasia	Early infantile epileptic encephalopathy (24)	—
SPRED1	c.46C>T (p.Arg16*)	32	Paternal	Multiple BCCs (>10) (18)	No additional features	Legius syndrome (25)	1, 2
Potential novel cancer-predisposing genes based on occurrence in multiple patients							
KDM3B	c.3095A>T (p.Asp1032Val)	25	*De novo*	Hodgkin lymphoma (14)	Intellectual disability, feeding difficulties, umbilical and inguinal hernia		
KDM3B	c.277G>T (p.Glu93*)	33[c]	Maternal	Acute myeloid leukemia (13)	Congenital hypothyroidism, congenital hip dysplasia, mild intellectual disability. Mother ID.		
TYK2	c.2279C>T (p.Pro760Leu)	5[c]	Paternal	Acute lymphoblastic leukemia twice (cytogenetically different) (3, 8)	Maternal family history of breast- and ovarian cancer due to BRCA2 mutation which this boy carries as well	Published in (28)	

[a]Additional clinical descriptions of all patients are available in Supplementary Appendix S1.
[b]In this column, the evidence for a relationship with cancer development is depicted. The numbers represent: 1 = Somatic mutations in this gene are described in cancer based on the COSMIC database; 2 = Additional cases with this syndrome and a malignancy are described; 3 = Exactly this type of malignancy is described in a patient with this syndrome.
[c]These patients have more than one (possible) causative mutation.
[d]This was ascertained after the identification of the *APOB* mutation.

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attributable to the population risk of developing childhood cancer. These cases illustrate that the possibility of a causative *de novo* mutation cannot be excluded in patients with a positive family history.

We did not find a genetic explanation for the cancer in 23 probands, even though based on the phenotype of these children, a genetic cause seems likely. There are several hypotheses that can explain this. First, WES has limitations, for example in the detection of causative intronic mutations, structural variation, or epigenetic defects. Second, it is possible that mosaic mutations have been missed. This happened in Case #02, in whom the *ARID1A* mutation was initially filtered out based on the low number of mutant reads (Supplementary Fig. S2). Third, it is possible that we have identified causative mutations in novel cancer-predisposing genes that we currently do not recognize as such. And finally, it is to be expected that at least a subset of the patients that we have included in our study do not carry a cancer-predisposing germline mutation.

Based on our study, several recommendations with respect to the identification of genetic predisposition in a diagnostic- and research setting can be made. First, cancer gene panels should be optimized for childhood cancer predisposition. Second, performing an exome-wide analysis in addition to the cancer gene panel is the preferred method. Third, patient–parent sequencing will aid in interpreting the mutations found. And finally, collaborating studies and data sharing will improve the recognition of genetic predisposition in children with cancer.

Conclusion

The selection of patients combined with a trio-based whole-exome analysis approach, and the subsequent two-step analysis of a cancer gene panel and the whole exome, led to a high yield of established and possible childhood cancer-predisposing mutations. Our study shows that WES is a useful diagnostic tool for selected patients with childhood cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: P.M. Hoogerbrugge, J. Loeffen, R.P. Kuiper, N. Hoogerbrugge, M.C. Jongmans
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Study supervision: P.M. Hoogerbrugge, R.P. Kuiper, N. Hoogerbrugge, M.C. Jongmans

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High Yield of Pathogenic Germline Mutations Causative or Likely Causative of the Cancer Phenotype in Selected Children with Cancer

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