original reports

Prevalence of (Epi)genetic Predisposing Factors in a 5-Year Unselected National Wilms Tumor Cohort: A Comprehensive Clinical and Genomic Characterization

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abstract

PURPOSE Wilms tumor (WT) is associated with (epi)genetic predisposing factors affecting a growing number of WT predisposing genes and loci, including those causing Beckwith-Wiedemann spectrum (BWSp) or *WT1*-related syndromes. To guide genetic counseling and testing, we need insight into the prevalence of WT predisposing (epi)genetic factors.

PATIENTS AND METHODS All children diagnosed with WT in the Netherlands between 2015 and 2020 were referred to a clinical geneticist. Phenotypic data, disease characteristics, and diagnostic test results were collected. If no genetic predisposition was identified by targeted diagnostic testing, germline (trio-)whole-exome sequencing and BWSp testing on normal kidney-derived DNA were offered.

RESULTS A total of 126 cases were analyzed of 128 identified patients. (Epi)genetic predisposing factors were present in 42 of 126 patients (33.3%) on the basis of a molecular diagnosis in blood-derived DNA (n = 26), normal kidney-derived DNA (n = 12), or solely a clinical diagnosis of BWSp (n = 4). Constitutional, heterozygous *DIS3L2* variants were identified as a recurrent predisposing factor in five patients (4%), with a second somatic hit in 4 of 5 tumors. Twenty patients (16%) were diagnosed with BWSp while four additional patients without BWSp features harbored chromosome 11p15 methylation defects in normal kidney tissue. Remaining findings included *WT1*-related syndromes (n = 10), Fanconi anemia (n = 1), neurofibromatosis type 1 (n = 1), and a pathogenic *REST* variant (n = 1). In addition, (likely) pathogenic variants in adult-onset cancer predisposition genes (*BRCA2*, *PMS2*, *CHEK2*, and *MUTYH*) were identified in 5 of 56 (8.9%) patients with available whole-exome sequencing data. Several candidate WT predisposition genes were identified, which require further validation.

CONCLUSION (Epi)genetic WT predisposing factors, including mosaic aberrations and recurrent heterozygous *DIS3L2* variants, were present in at least 33.3% of patients with WT. On the basis of these results, we encourage standard genetic testing after counseling by a clinical geneticist.

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ASSOCIATED CONTENT

See accompanying Oncology Grand Rounds on page 1853

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Wilms tumor (WT, nephroblastoma) arises from a developmental arrest in the embryonic kidney[1] and is frequently associated with (epi)genetic predisposing factors.[2,3] Our understanding of WT predisposition continues to evolve, as illustrated by the identification of novel WT predisposition genes (*TRIM28*, *REST*, and *CTR9*),[4-8] the role of mosaic aberrations,[9] and the range of phenotypic variability. With various study designs and definitions, previous reports identified WT predisposition syndromes in 5%-24% of children with WT.[10-13]

We hypothesized that the prevalence may be even higher when evaluating a cohort of patients with WT for all currently known predisposing factors. Therefore, we performed a phenotypic and genomic characterization of a 5-year nationwide WT cohort by a stepwise approach including targeted diagnostic testing and, after informed consent, whole-exome sequencing (WES) of germline and parental DNA (trio-analysis). We aimed to determine the prevalence of (epi)genetic predisposing factors, to correlate germline findings with patients' phenotypic and tumor characteristics, and to identify novel WT predisposition genes.

CONTEXT

Key Objective

To determine the prevalence and distribution of (epi)genetic predisposing factors in children with Wilms tumor (WT) and identify novel WT predisposition genes.

Knowledge Generated

A Dutch national cohort of children with WT (2015-2020) was referred for genetic evaluation. If no genetic predisposition was identified by targeted diagnostic testing, (trio-)whole-exome sequencing was offered. (Epi)genetic predisposing factors, including mosaic aberrations, were found to be present in 33.3% of the patients. We identified an important role for constitutional heterozygous *DIS3L2* variants.

Relevance

On the basis of these results, standard genetic testing after counseling by a clinical geneticist is encouraged for all children with WT.

PATIENTS AND METHODS

Patients and Data Collection

From 2015 onwards, Dutch hospitals referred all patients with (suspected) WT to the Princess Máxima Center for Pediatric Oncology. All patients diagnosed between January 1, 2015, and January 1, 2020, were retrospectively (2015-2018) or prospectively (2018-2020) invited for participation in this study. The study was referred to as the WES-KidTs study (whole-exome sequencing in children with kidney tumors). Parents, patients, and/or legal representatives were asked to give written informed consent for biomaterial and data collection (Medical Research Ethics Committee Utrecht: METC 18-033/M). This study conforms to the Declaration of Helsinki as revised in 2013.

In the definition of WT, we included all patients with WT and/or nephrogenic rests (WT precursor lesions[14]). Bilateral disease was defined as bilateral WT, bilateral nephrogenic rests, or WT with contralateral nephrogenic rests. Detailed data were collected, including patient characteristics (sex, birthweight, age at diagnosis, and medical and family history), tumor characteristics (stage, histology, and presence of nephrogenic rests as specified in the pathology report), and phenotypic findings during the clinical genetic consultation.

In the definition of (epi)genetic WT predisposition, we did not include (likely) pathogenic variants in adult-onset cancer predisposition genes nor genetic diagnoses, which are unrelated to WT development on the basis of current knowledge.

Diagnostic Procedures

Pediatric oncologists were instructed to refer all patients with WT to a clinical geneticist. Testing for Beckwith-Wiedemann spectrum (BWSp) was recommended for all patients, except for those with an alternative (suspected) diagnosis. BWSp testing was performed by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), primarily using blood-derived DNA. On a research basis, MS-MLPA was additionally performed using healthy kidney-derived DNA and tumor tissue, if this material was available after nephrectomy (Data Supplement, online only). Targeted *WT1* analysis was recommended for patients with a urogenital malformation, bilateral/multifocal disease, and/or age $<$ 2 years at diagnosis (Data Supplement). Other targeted genetic testing was performed according to the judgment of the clinical geneticist (Data Supplement).

Whole-Exome Sequencing

Patients in whom a clinical or molecular diagnosis of a WT predisposition syndrome was identified upon standard diagnostic testing were included for data collection only. In all remaining patients, informed consent for germline WES was requested. Patients were eligible if standard diagnostic testing had been completed by September 1, 2020.

Patients' germline DNA was assessed using a WES-based 30-gene WT gene panel (Data Supplement), including single-nucleotide variant, small indel, and copy number analyses (Data Supplement). If no causative variant was identified after panel analysis, exome-wide (trio-)analysis was performed using the patients' and (if available) parents' DNA. Participants could choose to limit the analysis to the WES-based WT gene panel only.

Variants were filtered on the basis of population frequency (gnomAD v3.1.1), quality metrics, protein effect, and in silico conservation and prediction scores. For genes included in the WT gene panel (Data Supplement), only (likely) pathogenic variants were communicated with the families. When variants of unknown significance were identified in the gene panel, tumor tissue (if available) was assessed by WES and/or single-nucleotide polymorphism array analysis (Data Supplement) for loss of heterozygosity (LOH) or somatic variants in this gene.

In the exome-wide trio-analysis, variants were additionally filtered on the basis of inheritance mode, prioritizing de

novo, homozygous, and compound heterozygous variants. Genes that were considered strong candidates were submitted to GeneMatcher[15], and if available, tumor tissue was assessed for LOH or somatic variants. A subset of genes was selected for meta-analysis on the basis of criteria specified in the Data Supplement. Germline sequencing data of all WT patients with informed consent for exome-wide analysis were combined, and variants in selected genes were extracted. In the resulting variant list, genes with multiple rare truncating and/or missense variants were prioritized.

Unsolicited findings were communicated with the families only after approval by a local multidisciplinary committee installed for this purpose at the Department of Genetics of the University Medical Center Utrecht.

RESULTS

A total of 128 patients with WT were identified. Two patients did not give informed consent for data collection (including one patient who died before 2018), leaving 126 patients (71 females and 55 males) available for analysis (Data Supplement). The median age at WT diagnosis was 3.0 years (range, 0-18.9 years). Five patients (4.0%) had a molecularly confirmed diagnosis of a WT predisposition syndrome at the time of WT diagnosis, including BWSp (n = 3), Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays syndrome (n = 1), and neurofibromatosis type 1 (NF1; n = 1; Table 1). One patient had a family history of WT.

Genetic Examination and Diagnostic Testing

Of the 121 patients without a prior diagnosis of a WT predisposition syndrome, 111 (91.7%) were examined by a clinical geneticist (Data Supplement). Seven patients were not referred, and three families refused referral for a clinical genetic consultation. For these patients, phenotypic data were extracted from the medical records. Targeted *WT1* testing was performed in 56 of 126 (44.4%) patients and diagnostic BWSp testing on blood-derived DNA in 97 of 126 (77.0%) patients. Additional MS-MLPA on normal kidney tissue was performed in 53 of 97 (54.6%) patients. Other targeted genetic testing was performed with various indications in six patients (Data Supplement).

Consent for Germline WES

Forty-three patients were not eligible for WES because a genetic predisposition had already been identified by germline-targeted testing and/or clinical criteria (n = 27) by MS-MLPA on kidney tissue (n = 3) or because diagnostic genetic testing had not been performed (n = 13). Of the 83 patients who were eligible for germline WES after diagnostic testing, we were able to approach 80 patients for WES, of whom (parents of) 57 patients (71.3%) gave informed consent. The consent was limited to the WT gene panel in four patients. DNA collection failed in one patient, and WES data were ultimately available for 56 patients. WES-based copy number variant analysis was informative in 52 of 56 patients (93%).

(Epi)genetic Predisposing Factors

When combining the results of standard diagnostic testing, BWSp testing on normal kidney tissue and WES panel analysis, an (epi)genetic WT predisposition was identified in 42 of 126 patients (33.3%; Fig 1 and Table 1). This included 26 patients with a molecular diagnosis in blood-derived DNA, 12 patients with a diagnosis in normal kidney-derived DNA, and four patients with solely a clinical diagnosis of BWSp. In seven patients (16.7%), the diagnosis was established by WES analysis (WT gene panel). Additionally, several variants of unknown significance were identified in the WT gene panel (Data Supplement) which were not considered to be causative on the basis of inheritance mode and lack of LOH/somatic variants in the tumor.

BWSp/11p15 aberrations. Twenty patients (15.9%) were diagnosed with BWSp (Table 1), including eight patients with a molecular diagnosis in blood-derived DNA. In eight more patients who had at least one additional feature of BWSp, a molecular diagnosis could not be confirmed in blood but was established in normal kidney-derived DNA (Data Supplement). Finally, in four patients, for whom no resected kidney tissue was available for analysis, a clinical diagnosis of BWSp was established according to the criteria of the 2018 consensus statement by Brioude et al.[16] Four patients were not diagnosed with BWSp because they lacked additional BWSp features, but they did display a gain of methylation of imprinting control region 1 in normal kidney-derived DNA (Data Supplement).

The 20 patients with BWSp had a median age of 3.6 years at WT diagnosis (range, 0.5-7.2 years), and 14 of 20 patients (70%) displayed lateralized overgrowth (hemihypertrophy), which was frequently subtle. WTs in patients with BWSp were not characterized by any specific histological subtype but frequently accompanied by perilobar nephrogenic rests (12 of 20, 60%). Among the eight patients with a confirmed molecular diagnosis in blood-derived DNA, one patient lacked BWSp features other than her WT diagnosis.

WT1 *aberrations.* Germline *WT1* aberrations were identified in 10 patients (7.9%), including one patient with Wilms tumor, anirida, genitourinary anomalies, and range of developmental delays syndrome (Table 1). These 10 patients were characterized by a young age at diagnosis (median 1.3 years, range, 0.6-3.0), stromal type WT (8 of 10 patients, 80%), and intralobar nephrogenic rests (7 of 10 patients, 70%). Seven patients (70%) had bilateral disease (n = 6) or unilateral WT with nephrogenic rests (n = 1), and 3 of 10 (30%) patients (all XY males) had urogenital malformations, including hypospadias, bifid scrotum, micropenis, and/or cryptorchidism.

TABLE 1. Patients With (Epi)genetic Predisposing Factors Related to WT Development (n = 42)

ID	M/F, Age at WT	(Epi)genetic Predisposing Factors	Identification of Predisposing Factors	Disease Type	Additional Clinical Features
WESK054	M, 2 years 0 month	BWSp: pUPD chr.11p15[a]	MS-MLPA on blood and clinical criteria	Unilateral, blastemal WT and PLNR	Hemihypertrophy, helical ear pits, and horseshoe kidney
WESK058	F, 6 months	BWSp: pUPD chr.11p15[a]	MS-MLPA on blood and clinical criteria	Unilateral ILNR	Hemihypertrophy, diastasis recti, and macroglossia
WESK145	F, 3 years 2 months	BWSp: pUPD chr.11p15	MS-MLPA on blood	Unilateral, stromal WT	None[b]
WESK056	F, 4 years 8 months	BWSp: pUPD chr.11p15	MS-MLPA on blood and clinical criteria	Bilateral, WT with diffuse anaplasia and PLNR	Hemihypertrophy and pyloric hypertrophy
WESK129	M, 4 years 1 months	BWSp: pUPD chr.11p15[a]	MS-MLPA on blood and clinical criteria	Unilateral, WT with focal anaplasia and PLNR	Hemihypertrophy, macroglossia, and ear creases
WESK130	F, 4 years 11 months	BWSp: pUPD chr.11p15	MS-MLPA on blood and clinical criteria	Bilateral, WT with diffuse anaplasia, PLNR and NB	Facial nevus flammeus and umbilical hernia
WESK117	M, 4 years 9 months	BWSp: pUPD chr.11p15	MS-MLPA on blood and clinical criteria	Bilateral, regressive WT and PLNR	Macroglossia, birthweight > 2 SDS above mean, and mild developmental delay
WESK039	F, 7 years 1 months	BWSp: IC1 GOM	MS-MLPA on blood	Unilateral, mixed WT	Hemihypertrophy, mild developmental delay, and brother had leukemia (2 years)
WESK003	M, 2 years 11 months	BWSp: clinical diagnosis	Clinical criteria	Unilateral, mixed WT with PLNR	Hemihypertrophy
WESK009	M, 9 months	BWSp: clinical diagnosis	Clinical criteria	Unilateral, epithelial WT with PLNR	Hemihypertrophy and umbilical hernia
WESK022	F, 1 year 0 month	BWSp: clinical diagnosis	Clinical criteria	Unilateral, diffuse NB	Hemihypertrophy, epicanthal folds, facial nevus flammeus, hemangioma, and father had testicular seminoma (30 years)
WESK025	M, 4 years 4 months	BWSp: clinical diagnosis	Clinical criteria	Bilateral, blastemal WT and PLNR	Hemihypertrophy
WESK062	F, 4 years 6 months	BWSp: pUPD chr.11p15	MS-MLPA on kidney tissue and clinical criteria	Bilateral, regressive WT and PLNR	Hemihypertrophy
WESK055	F, 6 months	BWSp: IC1 GOM	MS-MLPA on kidney tissue and clinical criteria	Unilateral, mixed WT	Hemihypertrophy, facial nevus flammeus, helical ear pits, and sacral dimple
WESK096	F, 4 years 3 months	BWSp: IC1 GOM	MS-MLPA on kidney tissue and clinical criteria	Bilateral, regressive WT and diffuse NB	Hemihypertrophy and mother had *MITF*-related melanoma (39 years)
WESK128	M, 1 year 7 months	BWSp: IC1 GOM	MS-MLPA on kidney tissue and clinical criteria	Bilateral, mixed WT and PLNR	Hemihypertrophy
WESK014	F, 7 years 2 months	BWSp: IC1 GOM	MS-MLPA on kidney tissue and ≥ 1 BWSp feature	Unilateral, regressive WT and PLNR	Facial nevus flammeus and hemangioma
WESK088	F, 5 years 8 months	BWSp: IC1 GOM	MS-MLPA on kidney tissue and ≥ 1 BWSp feature	Unilateral, blastemal WT and PLNR	Birth weight > 2 SDS above mean

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TABLE 1. Patients With (Epi)genetic Predisposing Factors Related to WT Development (n = 42) (continued)

ID	M/F, Age at WT	(Epi)genetic Predisposing Factors	Identification of Predisposing Factors	Disease Type	Additional Clinical Features
WESK124	F, 2 years 0 month	BWSp: IC1 GOM	MS-MLPA on kidney tissue and ≥ 1 BWSp feature	Unilateral, mixed WT	Hemihypertrophy
WESK135	M, 2 years 0 month	BWSp: IC1 GOM	MS-MLPA on kidney tissue and ≥ 1 BWSp feature	Bilateral diffuse NB	Nephromegaly and syndactyly 3rd and 4th toe
WESK002	F, 3 years 8 months	IC1 GOM, and no BWSp features	MS-MLPA on kidney tissue	Bilateral, regressive WT and PLNR	None
WESK046	F, 5 years 0 month	IC1 GOM, and no BWSp features	MS-MLPA on kidney tissue	Unilateral, regressive WT and PLNR	None
WESK073	M, 4 years 9 months	IC1 GOM, and no BWSp features	MS-MLPA on kidney tissue	Unilateral, regressive WT and PLNR	Father had metastatic cancer (primary diagnosis unknown; 32 years)
WESK121	F, 3 years 3 months	IC1 GOM, and no BWSp features	MS-MLPA on kidney tissue	Unilateral, regressive WT and PLNR	None
WESK033	F, 2 years 11 months	*WT1*: c.1216_1223del and p.Ser406fs	Targeted *WT1* testing	Unilateral, mixed WT	None[b]
WESK049	F, 11 months	*WT1*: c.1-?_646+?del and start loss	Targeted *WT1* testing	Bilateral, stromal WT and ILNR	None
WESK060	F, 2 years 2 months	*WT1*: del exon 6	WES panel analysis	Unilateral, stromal WT and ILNR	None
WESK105	M, 9 months	*WT1*: c.1223_1225delinsAAAG and p.Leu408*	Targeted *WT1* testing	Bilateral, stromal WT and ILNR	Hypospadia and bifid scrotum
WESK108	F, 7 months	*WT1*: c.1213_1214del and p.Lys405fs	Targeted *WT1* testing	Unilateral, stromal WT	None
WESK113	M, 1 years 5 months	*WT1*: c.457G>T and p.Glu153*	Targeted *WT1* testing	Bilateral, stromal WT with ILNR and diffuse NB	Bilateral cryptorchidism
WESK120	F, 1 years 0 month	*WT1*: c.1387C>T and p.Arg463*	Targeted *WT1* testing	Bilateral, stromal WT and ILNR	None
WESK122	F, 1 years 9 months	*WT1*: c.514C>T and p.Gln172*	Targeted *WT1* testing	Unilateral, mixed WT	None
WESK144	F, 2 years 0 month	WAGR syndrome: del 11p15.1-p13[a]	SNP array	Bilateral, stromal WT and ILNR	Aniridia and nystagmus
WESK147	M, 7 months	*WT1*: c.1120C>T and p.Arg374*	Targeted *WT1* testing	Bilateral, stromal WT and ILNR	Micropenis and cryptorchidism
WESK006	F, 1 year 4 months	Fanconi anemia: *BRCA2* c.2548C>T, p.Gln850*, c.7875A>T, and p.Arg2625Ser	Targeted *BRCA2* testing	Unilateral, mixed WT	Café-au-lait spots, facial dysmorphisms, and polydactyly
WESK045	M, 5 years 7 months	NF1: *NF1* c.4169T>C and p.Leu1390Pro[a]	Targeted *NF1* testing	Unilateral, regressive WT and ILNR	Café-au-lait spots, axillar freckling, facial dysmorphisms, tibial bowing, and father had pancreatic carcinoma (38 years)
WESK102	F, 1 years 3 months	*REST*: c.843delC and p.Cys281*	WES panel analysis	Bilateral, blastemal WT and PLNR	Brother and aunt had WT (2 years, 3 years)
WESK018	F, 2 years 1 months	*DIS3L2*: del exon 9	WES panel analysis	Unilateral, mixed WT	Abnormal meatus

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TABLE 1. Patients With (Epi)genetic Predisposing Factors Related to WT Development (n = 42) (continued)

ID	M/F, Age at WT	(Epi)genetic Predisposing Factors	Identification of Predisposing Factors	Disease Type	Additional Clinical Features
WESK019	F, 3 years 1 months	*DIS3L2*: c.2510_2513delinsGA and p.Phe837*	WES panel analysis	Unilateral, mixed WT and NR (type not specified)	Father had dermato-fibrosarcoma protuberans (38 years)
WESK036	F, 5 years 4 months	*DIS3L2*: c.1096G>T and p.Glu366*	WES panel analysis	Unilateral, mixed WT	None[b]
WESK057	M, 2 years 0 month	*DIS3L2*: del exon 9	WES panel analysis	Unilateral, blastemal WT and PLNR	None
WESK115	F, 3 years 9 months	*DIS3L2*: del exon 9	WES panel analysis	Unilateral, regressive WT	Ear creases

NOTE. Variants are described on the following transcripts: *WT1*: NM_024426.5, *BRCA2*: NM_000059.3, *NF1*: NM_000267.3, *REST*: NM_005612.5, *TRIM28*: NM_005762.3, and *DIS3L2*: NM_152383.5.

Abbreviations: BWSp, Beckwith-Wiedemann spectrum; F, female; GOM, gain of methylation; IC1, imprinting control region 1; ILNR, intralobar nephrogenic rests; M, male; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; NB, nephroblastomatosis; NF1, neurofibromatosis type 1; NR, nephrogenic rests; PLNR, perilobar nephrogenic rests; SDS, standard deviation score; SNP, single-nucleotide polymorphism; WAGR, Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays; WES, whole-exome sequencing; WT, Wilms tumor.

[a]Diagnosed before WT development.

[b]Patient would not have been selected for genetic referral using the MIPOGG decision-support algorithm.

Heterozygous* DIS3L2 *variants. Constitutional heterozygous variants in *DIS3L2*, which was in our WT gene panel because of the associated autosomal recessive Perlman syndrome, were identified in 5 of 126 patients (4%; Table 2). Among patients with available WES data, *DIS3L2* variants were identified in 4 of 56 (7.1%), including two truncating (stopgain) variants and two deletions of exon 9. The fifth constitutional variant, again a deletion of exon 9, was identified by single-nucleotide polymorphism array analysis performed for clarifying an ambiguous MS-MLPA result. A second somatic hit was identified in 4 of 5 tumors, including a deletion of exon 9, deletion of exons 1-10, and a somatic truncating *DIS3L2* variant.

All five patients with constitutional *DIS3L2* variants had inherited the variant from an unaffected parent. The median age at diagnosis was 3.1 years (range, 2.1-5.4). Two patients presented with metastatic WT while a third patient developed a metastatic relapse. None of the patients had bilateral disease, but one patient had multifocal WT with perilobar nephrogenic rests. Histological WT subtypes included mixed type WT (n = 3), regressive type WT (n = 1), and blastemal and regressive type WTs in the patient with multifocal disease. Minor phenotypic abnormalities were observed in two patients, including an abnormal meatus (n = 1) and ear creases (n = 1).

Other aberrations in known WT predisposition genes. Other (likely) pathogenic, germline variants in known WT predisposition genes were diagnosed in three patients. In these patients, the presence of a germline variant was suspected on the basis of the patient's phenotype or family history, and the findings included a familial *REST* variant, Fanconi anemia, and NF1 (Table 1).

Findings in adult cancer predisposition genes. (Likely) pathogenic variants in adult-onset cancer predisposition genes were identified in 5 of 56 (8.9%) patients with available WES data (Table 3). Two patients had heterozygous variants in *BRCA2* or *PMS2* (WESK132), the genes included in the WT gene panel because of the associated recessive conditions that predispose to WT. No somatic variant in the wildtype *BRCA2* allele was identified, and the mutational burden was too low to perform a mutational signature analysis. The tumor of WESK132 showed retained protein expression of PMS2 (immunohistochemical staining), and there were no signs of microsatellite instability (Idylla MSI v.1.4, seven MSI markers).

In one patient, exome-wide analysis revealed (likely) pathogenic heterozygous variants in three genes (*CHEK2*, *MUTYH*, and *RNASEL*), all inherited from her father who had a history of testicular cancer and osteoblastoma. Heterozygous *MUTYH* variants were identified in two additional patients. A single WT sample was available to assess the presence of a second-hit or *MUTYH*-related mutational signature (COSMIC signature SBS36)[17,18] which was not identified, suggesting that the *MUTYH* variant did not drive WT development in this patient.

Meta-analysis: novel candidate genes. On the basis of the exome-wide trio-analysis, 77 genes were selected for meta-analysis (Data Supplement). These included 31 genes with verified de novo variants and 46 genes with inherited variants (Data Supplement). For none of the genes, de novo variants were identified in more than one patient. Missense or truncating variants in the ubiquitin gene *USP45* were detected in four unrelated patients, including a de novo missense variant (WESK007) and three inherited variants (Data Supplement). For none of these patients, tumor

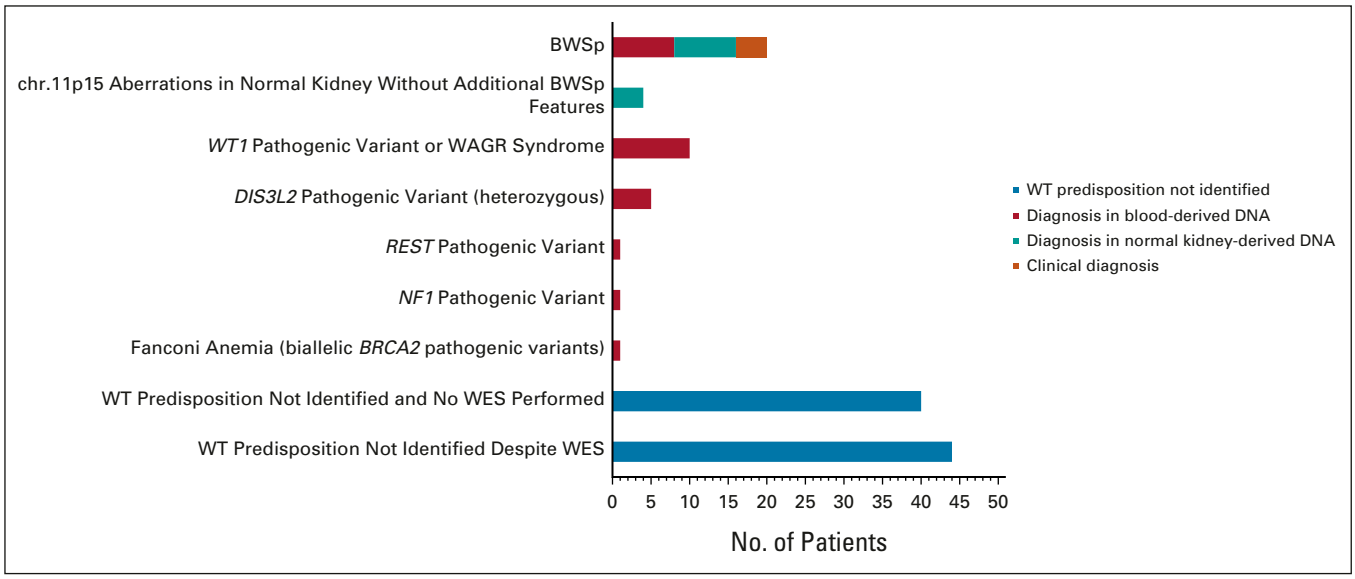

FIG 1. (Epi)genetic predisposing factors in patients with WT and/or nephroblastomatosis (N = 126). BWSp, Beckwith-Wiedemann spectrum; WAGR, Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays syndrome; WES, whole-exome sequencing; WT, Wilms tumor.

TABLE 2. Constitutional Heterozygous *DIS3L2* Variants: Inheritance and Second Somatic Events (n = 5)

ID	Germline *DIS3L2* Variant	Inheritance	Somatic *DIS3L2* Event in Tumor Sample
WESK018	Deletion of exon 9	Maternal	Deletion of exon 1-10
WESK019	c.2510_2513delinsGA p.Phe837* (exon 21)	Maternal	Deletion of exon 9
WESK036	c.1096G>T p.Glu366* (exon 9)	Paternal	Copy number neutral LOH of exon 9
WESK057	Deletion of exon 9	Paternal	c.1835dup (exon 15) and p.Pro613Alafs*8
WESK115	Deletion of exon 9	Paternal	Not identified

NOTE. Variants are described on transcript NM_152,383.5.
Abbreviation: LOH, loss of heterozygosity.

tissue was available to assess LOH or second-hit somatic variants. Notably, WESK007 had additional clinical characteristics including developmental delay, multiple dysmorphisms, and a urogenital malformation (shawl scrotum). In this patient, a second (mosaic) de novo variant affecting the *MTA1* gene was observed. Variants in other candidate genes were assessed in the meta-analysis but not considered to be convincing on the basis of the inheritance pattern, in silico conservation and prediction scores, and/or lack of LOH or second somatic variants in tumor tissue.

Unrelated genetic diagnoses. Four patients had a genetic diagnosis unrelated to WT development on the basis of current knowledge, including 47,XYY syndrome (n = 1), *KAT6A* syndrome (n = 1), and spondylodysplastic Ehlers-Danlos syndrome (biallelic *B3GALT6* variants, n = 1). The fourth patient was found to have *PHIP*-related developmental delay (de novo truncating variant in *PHIP*) and 16p12.2 deletion syndrome.

Family history of cancer. Apart from the patient with familial WT, 12 of 126 patients had a suspicious family history as defined in Jongmans' criteria.[19] Recurrent cancer types in affected relatives included childhood leukemia (4 relatives in three families), testicular cancer (three relatives in three families), melanoma (two relatives in two families), and neuroblastoma (three relatives in two families). In these families, we did not identify variants that could explain both the WT and the relative's cancer diagnosis.

DISCUSSION

With a comprehensive and stepwise approach of diagnostic genetic testing and research-based WES analysis in a unique national unselected cohort of children with WT, we determined the prevalence of (epi)genetic predisposing factors, including mosaic aberrations and clinical BWSp diagnoses, to be at least 33.3%. This level of (epi)genetic predisposition is higher than 5%-24% that has been reported in previous studies.[10-13]

BWSp was diagnosed in 16% of all patients, compared with only 1%-8% in earlier reports.[10-12] This higher frequency was due to the fact that we applied clinical criteria[16] and performed MS-MLPA on resected healthy kidney tissue in addition to blood-derived DNA. It can be argued that chromosome 11p15 aberrations detected in resected kidney tissue, represent tissue-specific, somatic events.[9,20] However, for patients who had at least one additional feature of BWSp, we consider it likely that these aberrations were also present in other tissues. In these patients, methylation changes in blood-derived DNA may have been present below the detection threshold of our MS-MLPA (approximately 10%). In the future, the development of more sensitive molecular techniques may increase the yield of BWSp testing in blood-derived DNA.[21]

Constitutional, heterozygous *DIS3L2* variants were identified in 4% of all patients with WT (7% of patients with WES data), indicating that this gene is a bonafide WT predisposition gene. These children lacked a clearly recognizable phenotype. Biallelic *DIS3L2* pathogenic variants cause Perlman syndrome,[22] a congenital overgrowth syndrome with a high risk of WT development.[23] Somatic *DIS3L2* variants have been demonstrated in 1%-5% of WTs[22,24,25] and deletions or LOH in 4%-30%.[22,25,26] On the basis of incidental reports, heterozygous germline variants in *DIS3L2* were previously suggested to cause an increased WT risk.[24-27] Additionally, patients with rare constitutional deletions of 2q37.1/*DIS3L2* have been reported to develop WT.[28] In our cohort, three of five constitutional *DIS3L2* aberrations were exon 9 deletions, which are predicted to cause an in-frame deletion of 58 amino acids, resulting in reduced ribonuclease activity as demonstrated in transfected HEK293 cells.[22] Exon 9 is flanked by two approximately 5 Kb LINE-1 repeats causing genomic instability.[29] Homozygous exon 9 deletions have been reported in Perlman syndrome,[22] whereas heterozygous exon 9 deletions are present in 0.05% of healthy individuals (11 of 21,364 alleles in gnomAD SVs v.2.1). The identified second somatic hits strongly suggest that constitutional heterozygous *DIS3L2* variants contribute to WT development. However, their presence in unaffected parents and population databases implies a reduced penetrance.

Similar to previous childhood cancer studies,[27,30-33] we identified heterozygous, pathogenic germline variants in adult-onset cancer predisposition genes (*BRCA2*, *PMS2*, *CHEK2*, and *MUTYH*) in 8.9% of patients with available

TABLE 3. Patients With (likely) Pathogenic Variants in Genes Associated With Adult-Onset Cancer (n = 5)

ID	M/F, Age at WT	Germline Variant(s): *All Variants Are Heterozygous*	Inheritance	Family History of Cancer?	Disease Type (index)	Mutational Signature Analysis
WESK042	M, 4 years 6 months	*MUTYH*: c.536A>G and p.Tyr179Cys	Maternal	No	Unilateral, multifocal: blastemal and mixed WT	No relevant signatures identified
WESK051	F, 3 years 4 months	*MUTYH*: c.1187G>A and p.Gly396Asp *CHEK2*: c.1100delC and p.Thr367fs *RNASEL*: c.793G>T and p.Glu265*	Paternal (all three variants)	Testicular cancer and osteoblastoma (father, age 21 and 35 years, respectively)	Unilateral, mixed WT	NA
WESK072	F, 2 years 11 months	*BRCA2*: c.9672dup and p.Tyr3225fs	Paternal	Breast cancer (paternal grandmother, age 46 years) and childhood leukemia (mother, age 5 years and maternal grandfather's sister, age 5 years)	Unilateral, mixed WT and PLNR	Low mutational burden and signature analysis not possible
WESK110	F, 2 months	*MUTYH*: c.536A>G and p.Tyr179Cys	Maternal	No	Unilateral, mixed WT	NA
WESK132	M, 3 years 10 months	*PMS2*: c.137G>A and p.Ser46Asn	Maternal	No	Unilateral, regressive WT	NA

NOTE. Variants are described on the following transcripts: *MUTYH*: NM_001128425.1, *CHEK2*: NM_007194.3, *RNASEL*: NM_021133.4, *BRCA2*: NM_000059.3, and *PMS2*: NM_000535.7.

Abbreviations: F, female; M, male; NA, not applicable; PLNR, perilobar nephrogenic rests; WT, Wilms tumor.

WES data. It remains unclear whether these variants contributed to WT development. For comparison, in WES data of 1,640 healthy Dutch individuals, pathogenic variants in dominant cancer predisposition genes were identified in 0.7% and heterozygous pathogenic *MUTYH* variants in 1.9%.[34] Analysis of the mutational profile extracted from a single available WT sample did not reveal a contribution of *MUTYH* to tumor development.

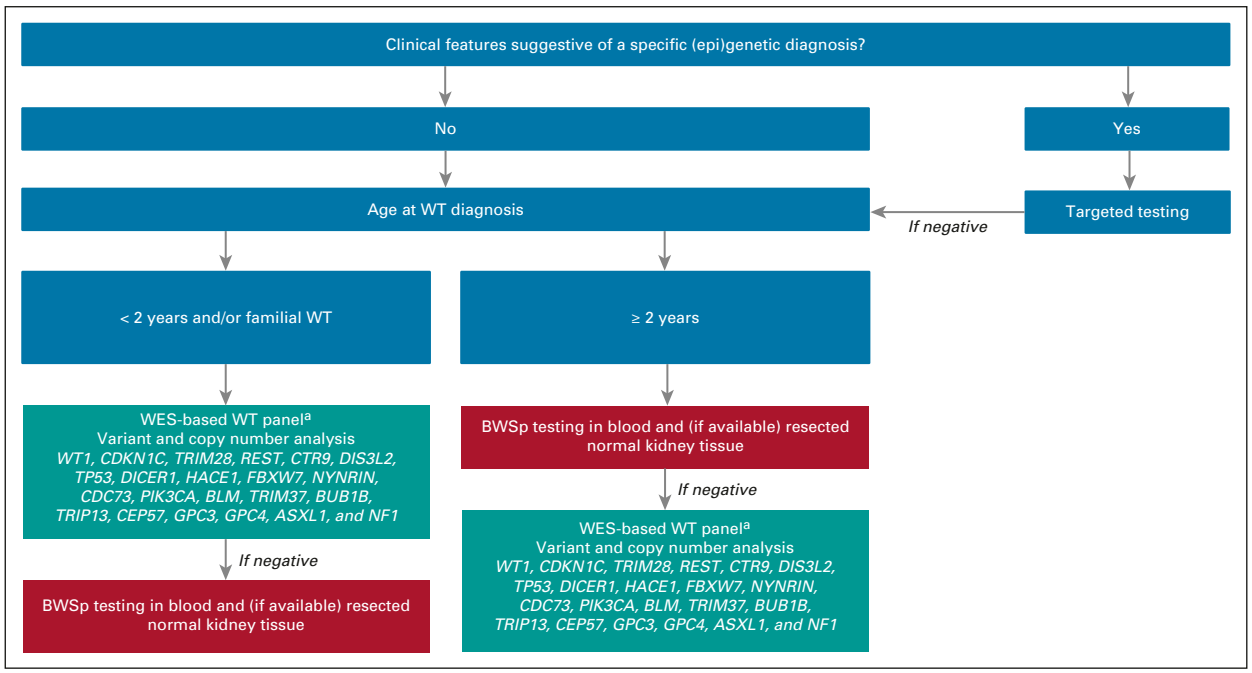

FIG 2. Suggested strategy for germline genetic testing in children with WT. [a]Adult-onset cancer predisposition genes were excluded for ethical reasons and may be assessed by targeted testing in children who are clinically suspected of Fanconi anemia (*BRCA2* and *PALB2*) or constitutional mismatch repair deficiency (*PMS2*, *MSH2*, *MSH6*, and *MLH1*). BWSp, Beckwith-Wiedemann spectrum; WES, whole-exome sequencing; WT, Wilms tumor.

Similarly, the contribution of NF1 to WT development is not entirely clear. An association has been suggested in a report in which 3 of 342 children with WT were found to have NF1.[35] Since then, several case reports have been published supporting this association.[36-39] However, the risk of WT development in patients with NF1 is considered too low (< 1%) to recommend WT surveillance.[40]

This study was limited by the fact that not all patients underwent (complete) genetic testing and/or WES analysis because of physicians' and families' personal choices. Moreover, future reanalysis of the WES data may provide novel insights, when, for instance, even better tools for splice effect prediction and copy number variant detection become available. This study reflects (epi)genetic aberrations in a Dutch population of children with WT and does not account for the differences in (epi)genetic predisposing factors which appear to exist between different geographical populations.[41-43]

Our exome-wide trio-analysis approach did not yield strong candidate WT predisposition genes outside the gene panel, which illustrates the complexity of searching for novel WT predisposition genes. In contrast to unsolved familial WT pedigrees, where a monogenic cause is suspected,[4] epigenetic factors and postzygotic mosaicism play an important role in isolated (nonfamilial) WT. Moreover, yet to be identified WT predisposition genes may exhibit reduced penetrance as demonstrated for *DIS3L2*.

On the basis of the results of this study, we encourage standard genetic testing after counseling by a clinical geneticist for all children with WT. In settings where this is not feasible, decision-support algorithms such as the MIPOGG tool[12] can be used to prioritize children for genetic testing. Using such a tool reduces the rate of genetic referrals, although our findings indicate that some diagnoses are missed with this approach. Among the 42 patients with identified WT predisposition in this study, three (7%) would not have been selected for genetic testing using MIPOGG, including a patient with a germline *WT1* variant, germline *DIS3L2* variant, and molecularly confirmed BWSp. Targeted testing is advised if a child has clinical features suggestive of a specific (epi)genetic diagnosis. For all other patients, we propose a diagnostic strategy (Fig 2) which includes (mosaic) BWSp testing and/or WES-based panel analysis. This is justified by the high prevalence of (epi)genetic predisposing factors, including mosaic aberrations and recurrent heterozygous *DIS3L2* variants, as demonstrated in this study.

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REFERENCES

1. Hohenstein P, Pritchard-Jones K, Charlton J: The yin and yang of kidney development and Wilms' tumors. Genes Dev 29:467-482, 2015
2. Scott RH, Stiller CA, Walker L, et al: Syndromes and constitutional chromosomal abnormalities associated with Wilms tumour. J Med Genet 43:705-715, 2006
3. Miller RW, Fraumeni JF Jr, Manning MD: Association of Wilms's tumor with aniridia, hemihypertrophy and other congenital malformations. N Engl J Med 270: 922-927, 1964

4. Mahamdallie S, Yost S, Poyastro-Pearson E, et al: Identification of new Wilms tumour predisposition genes: An exome sequencing study. Lancet Child Adolescent Health 3:322-331, 2019
5. Halliday BJ, Fukuzawa R, Markie DM, et al: Germline mutations and somatic inactivation of TRIM28 in Wilms tumour. PLoS Genet 14:e1007399, 2018
6. Diets IJ, Hoyer J, Ekici AB, et al: TRIM28 haploinsufficiency predisposes to Wilms tumor. Int J Cancer 145:941-951, 2019
7. Mahamdallie SS, Hanks S, Karlin KL, et al: Mutations in the transcriptional repressor REST predispose to Wilms tumor. Nat Genet 47:1471-1474, 2015
8. Hanks S, Perdeaux ER, Seal S, et al: Germline mutations in the PAF1 complex gene CTR9 predispose to Wilms tumour. Nat Commun 5:4398, 2014
9. Coorens THH, Treger TD, Al-Saadi R, et al: Embryonal precursors of Wilms tumor. Science 366:1247-1251, 2019
10. Dumoucel S, Gauthier-Villars M, Stoppa-Lyonnet D, et al: Malformations, genetic abnormalities, and Wilms tumor. Pediatr Blood Cancer 61:140-144, 2014
11. Segers H, Kersseboom R, Alders M, et al: Frequency of WT1 and 11p15 constitutional aberrations and phenotypic correlation in childhood Wilms tumour patients. Eur J Cancer 48:3249-3256, 2012
12. Cullinan N, Villani A, Mourad S, et al: An eHealth decision-support tool to prioritize referral practices for genetic evaluation of patients with Wilms tumor. Int J Cancer 146:1010-1017, 2020
13. Merks JH, Caron HN, Hennekam RC: High incidence of malformation syndromes in a series of 1,073 children with cancer. Am J Med Genet A 134A:132-143, 2005
14. Beckwith JB: Nephrogenic rests and the pathogenesis of Wilms tumor: Developmental and clinical considerations. Am J Med Genet 79:268-273, 1998
15. Gene Matcher. https://genematcher.org/
16. Brioude F, Kalish JM, Mussa A, et al: Expert consensus document: Clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: An international consensus statement. Nat Rev Endocrinol 14:229-249, 2018
17. Tate JG, Bamford S, Jubb HC, et al: COSMIC: The catalogue of somatic mutations in cancer. Nucleic Acids Res 47:D941-D947, 2019
18. COSMIC, Catalogue Of Somatic Mutations In Cancer; signature SBS36. cancer.sanger.ac.uk
19. Jongmans MC, Loeffen JL, Waanders E, et al: Recognition of genetic predisposition in pediatric cancer patients: An easy-to-use selection tool. Eur J Med Genet 59:116-125, 2016
20. Foulkes WD, Polak P: Bilateral tumors—Inherited or acquired? N Engl J Med 383:280-282, 2020
21. Fiala EM, Ortiz MV, Kennedy JA, et al: 11p15.5 epimutations in children with Wilms tumor and hepatoblastoma detected in peripheral blood. Cancer 126: 3114-3121, 2020
22. Astuti D, Morris MR, Cooper WN, et al: Germline mutations in DIS3L2 cause the Perlman syndrome of overgrowth and Wilms tumor susceptibility. Nat Genet 44: 277-284, 2012
23. Alessandri JL, Cuillier F, Ramful D, et al: Perlman syndrome: Report, prenatal findings and review. Am J Med Genet A 146A:2532-2537, 2018
24. Gadd S, Huff V, Walz AL, et al: A Children's Oncology Group and TARGET initiative exploring the genetic landscape of Wilms tumor. Nat Genet 49:1487-1494, 2017
25. Ciceri S, Gamba B, Corbetta P, et al: Genetic and epigenetic analyses guided by high resolution whole-genome SNP array reveals a possible role of CHEK2 in Wilms tumour susceptibility. Oncotarget 9:34079-34089, 2018
26. Wegert J, Ishaque N, Vardapour R, et al: Mutations in the SIX1/2 pathway and the DROSHA/DGCR8 miRNA microprocessor complex underlie high-risk blastemal type Wilms tumors. Cancer Cell 27:298-311, 2015
27. Parsons DW, Roy A, Yang Y, et al: Diagnostic yield of clinical tumor and germline whole-exome sequencing for children with solid tumors. JAMA Oncol 2: 616-624, 2016
28. Falk RE, Casas KA: Chromosome 2q37 deletion: Clinical and molecular aspects. Am J Med Genet C Semin Med Genet145C:357-371, 2007
29. Higashimoto K, Maeda T, Okada J, et al: Homozygous deletion of DIS3L2 exon 9 due to non-allelic homologous recombination between LINE-1s in a Japanese patient with Perlman syndrome. Eur J Hum Genet 21:1316-1319, 2013
30. Zhang J, Walsh MF, Wu G, et al: Germline mutations in predisposition genes in pediatric cancer. N Engl J Med 373:2336-2346, 2015
31. Grobner SN, Worst BC, Weischenfeldt J, et al: The landscape of genomic alterations across childhood cancers. Nature 555:321-327, 2018
32. Diets IJ, Waanders E, Ligtenberg MJ, et al: High yield of pathogenic germline mutations causative or likely causative of the cancer phenotype in selected children with cancer. Clin Cancer Res 24:1594-1603, 2018
33. Byrjalsen A, Hansen TVO, Stoltze UK, et al: Nationwide germline whole genome sequencing of 198 consecutive pediatric cancer patients reveals a high incidence of cancer prone syndromes. PLoS Genet 16:e1009231, 2020
34. Haer-Wigman L, van der Schoot V, Feenstra I, et al: 1 in 38 individuals at risk of a dominant medically actionable disease. Eur J Hum Genet 27:325-330, 2019
35. Stay EJ, Vawter G: The relationship between nephroblastoma and neurofibromatosis (Von Recklinghausen's disease). Cancer 39:2550-2555, 1977
36. Shvartsbeyn M, Bassani L, Mikolaenko I, et al: Brain metastasis of Wilms tumor with diffuse anaplasia and complex cytogenetic phenotype in a child with neurofibromatosis type 1. J Neurosurg Pediatr 8:353-356, 2011
37. Ito F, Watanabe Y, Ito T: Synchronous occurrence of Wilms tumor and ganglioneuroblastoma in a child with neurofibromatosis. Eur J Pediatr Surg 7:308-310, 1997
38. Perilongo G, Felix CA, Meadows AT, et al: Sequential development of Wilms tumor, T-cell acute lymphoblastic leukemia, medulloblastoma and myeloid leukemia in a child with type 1 neurofibromatosis: A clinical and cytogenetic case report. Leukemia 7:912-915, 1993
39. Chu JY, O'Connor DM, Danis RK: Neurofibrosarcoma at irradiation site in a patient with neurofibromatosis and Wilms' tumor. CA Cancer J Clin 31:333-335, 1981
40. Hol JA, Jewell R, Chowdhury T, et al: Wilms tumour surveillance in at-risk children: Literature review and recommendations from the SIOP-Europe Host Genome Working Group and SIOP Renal Tumour Study Group. Eur J Cancer 153:51-63, 2021
41. Haruta M, Arai Y, Watanabe N, et al: Different incidences of epigenetic but not genetic abnormalities between Wilms tumors in Japanese and Caucasian children. Cancer Sci 103:1129-1135, 2012
42. Fukuzawa R, Breslow NE, Morison IM, et al: Epigenetic differences between Wilms' tumours in white and east-Asian children. Lancet 363:446-451, 2004
43. Kaneko Y, Okita H, Haruta M, et al: A high incidence of WT1 abnormality in bilateral Wilms tumours in Japan, and the penetrance rates in children with WT1 germline mutation. Br J Cancer 112:1121-1133, 2015

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Prevalence of (Epi)genetic Predisposing Factors in a 5-Year Unselected National Wilms Tumor Cohort: A Comprehensive Clinical and Genomic Characterization

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