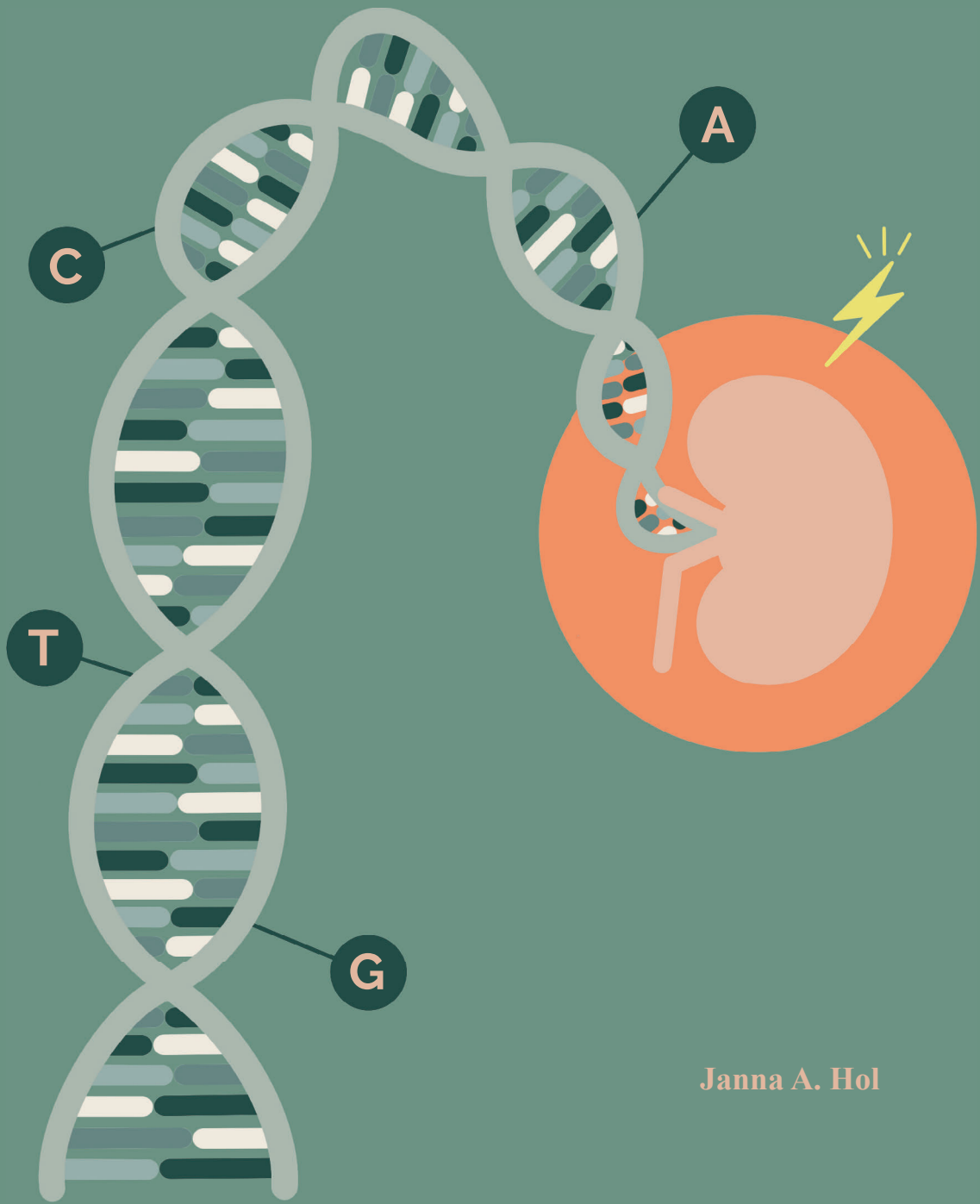
Renal tumors in children and the role of (epi)genetic predisposition



Janna A. Hol

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Renal tumors in children and the role of (epi)genetic predisposition

Niertumoren bij kinderen en de rol van (epi)genetische aanleg (met een samenvatting in het Nederlands)

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General introduction and outline of this thesis

RENAL TUMORS IN CHILDHOOD

Renal tumors account for approximately 5% of all childhood cancer diagnoses[1] and in the Netherlands, 30-35 children are diagnosed with a renal tumor each year. Wilms tumor (WT, nephroblastoma) is by far the most common renal tumor in childhood (**Figure 1**), with the majority of WTs occurring in preschool children.[2] WTs represent a relatively good prognostic cancer subtype as current treatment regimens, including a combination of chemotherapy, surgery and in some cases radiotherapy, lead to ~90% overall survival rates.[3] However, these treatment regimens are associated with short- and long-term side effects, and still, not all children with WT can be cured.

In teenagers and adolescents, the incidence of WT is overtaken by that of renal cell carcinoma (RCC).[2] RCC is the most common renal tumor in adults and, while localized disease can usually be treated with surgery alone, the development of novel targeted treatments is needed to improve survival for children and adults with metastatic RCC.[4] A variety of other renal tumor types can occur in childhood, all of which are characterized by their own unique clinical and genetic features.[3] Some pediatric renal tumors such as mesoblastic nephroma have an excellent outcome[5], while others such as malignant rhabdoid tumor of the kidney (MRTK) are hard to cure despite aggressive chemo- and radiotherapy.[6] In many of these malignancies, the underlying biological mechanisms are incompletely understood.

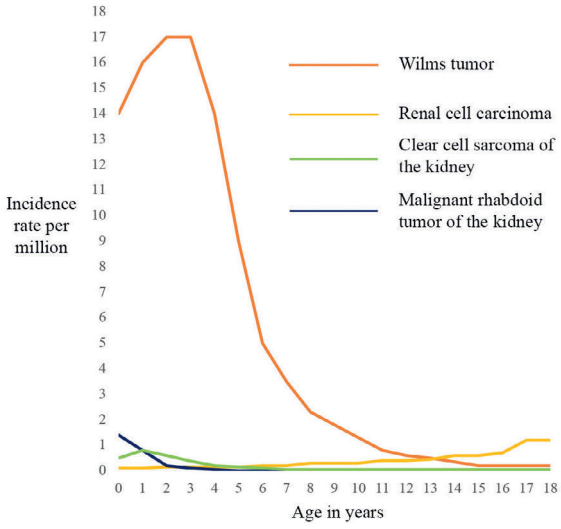

Figure 1. Most common renal tumors in childhood. Congenital mesoblastic nephroma (CMN), which is the most common renal tumor in children aged 0-3 months, is not shown here. Other renal tumors which are not displayed, have an estimated incidence rate <1 per million (e.g. metanephric tumors, cystic nephroma). Incidence rates are based on Nakata et al. 2020.[2]

In the pathogenesis of several pediatric renal tumor types, including WT, (epi)genetic predisposing factors play an important role.[7] Already in the 1960s, it was observed that phenotypic abnormalities such as hemihypertrophy and aniridia were common among children with WT.[8] We now know that many different germline (epi)genetic alterations can cause WT susceptibility and additional WT predisposition genes remain to be identified.[9-11]

GENETIC AND EPIGENETIC ALTERATIONS IN (PEDIATRIC) CANCER

Cancer is driven by genetic and/or epigenetic alterations. Whereas genetic alterations include DNA sequence and copy number variants, the term epigenetic refers to changes such as methylation defects, that affect gene transcription without altering the DNA sequence itself.

Genetic alterations are classified as germline or somatic (**Figure 2**). Germline alterations include alterations which are inherited from a parent or arise in gametes (sperm cells or oocytes), and which are therefore present in every cell of an individual's body. Somatic (acquired) alterations arise after fertilization (postzygotic) during embryonic development or during a person's lifespan. Within an individual, multiple cells may carry the same somatic alteration, which is known as somatic mosaicism. On top of this, all cells acquire their own, unique somatic alterations. Epigenetic alterations are somatic alterations which are generally not considered to be heritable, in the sense that they (usually) cannot be passed on to offspring. Yet, they can occur early during embryonic development and can have important consequences.

In adults, the development of cancer is usually a result of many (epi)genetic alterations, including those that were present in the germline (in some cases) and those that were acquired during life, ultimately causing single cells to undergo uncontrolled cell division. Compared to adult cancer, most types of childhood cancer are characterized by a relatively low number of mutations and a larger contribution of predisposing germline and/or early postzygotic alterations.

Detecting (epi)genetic alterations

Detecting genetic or epigenetic alterations requires specific molecular techniques. In the past, clinicians had to depend on targeted techniques which could usually analyze only a single gene or region of interest at a time. The detection of genetic alterations has become much more efficient over the last decade, due to the rise of next generation sequencing (NGS) technologies, by which many genes can be analyzed simultaneously. Whole exome and whole genome sequencing (WES/WGS) have provided insight into the landscape of genetic alterations across various adult[12] and childhood[13] cancer types, with important diagnostic, prognostic and therapeutic implications. Compared to somatic

alterations, finding a pathogenic (disease-causing) germline alteration has additional implications for the patient and his/her family members, who may be at risk for (second) malignancies and/or additional health problems. Therefore, before germline DNA is analyzed for any indication, patients need to be counseled about potential consequences of germline genetic findings.

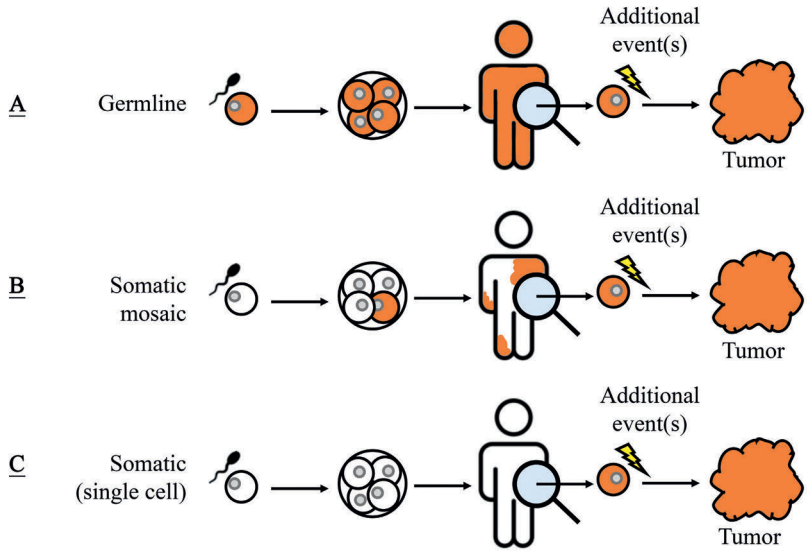

Figure 2. Germline versus somatic genetic alterations. Genetic alterations are classified as germline or somatic. Germline alterations (A) are inherited from a parent or arise in gametes (sperm cells or oocytes), causing them to be present in every cell of an individual's body. Somatic (acquired) alterations arise after fertilization (postzygotic) during embryonic development or during a person's lifespan. Within an individual, multiple cells may carry the same somatic alteration, which is known as somatic mosaicism (B). On top of this, all cells acquire their own, unique somatic alterations (C).

WILMS TUMORS

Wilms tumors (WTs) are the main focus of this thesis. They are named after the German surgeon Max Wilms (1867-1918).[14] Below, WT pathogenesis, treatment and histology are described, and (epi)genetic WT predisposition is introduced, summarizing what's known and highlighting current gaps of knowledge.

Wilms tumor pathogenesis

WTs result from a developmental arrest in the embryonic kidney.[15] Embryonic kidney development starts at around 4 weeks of gestation, and is completed at 34-37 weeks.[16] During a repetitive process which branches outwards, the structural units of the kidney (nephrons) are generated from the intermediate mesoderm. This starts with the formation of the ureteric bud from the Wolffian duct. As the ureteric bud protrudes into the adjacent

metanephric mesenchyme, mesenchymal cells condense into the cap mesenchyme at the tip of the ureteric bud (**Figure 3**). Through a process known as mesenchymal-to-epithelial transition (MET), the cap mesenchyme gives rise to the renal vesicle, comma-shaped body, S-shaped body and ultimately the mature nephron.[15] These developmental steps are orchestrated by (epi)genetic regulatory programs which sequentially activate or repress relevant signaling pathways. Disturbances in these regulatory programs due to (epi)genetic alterations, can lead to a developmental arrest and subsequent WT development. Manifestations of this developmental arrest include nephrogenic rests, which are embryonic remnants found in the surrounding kidney tissue of ~40% of WTs (~100% in bilateral cases) and are considered to be WT precursor lesions.[17] Additional events are necessary for nephrogenic rests to develop into WT.

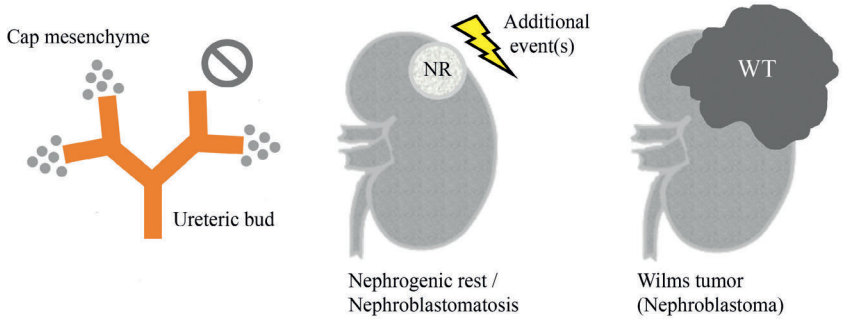

Figure 3. Wilms tumor (WT) development. In children with a WT predisposition, constitutional (epi) genetic alterations lead to a branching arrest in the embryonic kidney which may cause nephrogenic rests (NRs) to persist in the postnatal kidney. Additional events are necessary for NRs to develop into WT.

GENES DRIVING WT DEVELOPMENT

Currently, approximately 40 different genes have been identified as (possible) somatic drivers of WT development, with the most commonly mutated and established drivers being *WT1, H19/IGF2, AMER1 (*also known as *WTX*)*, CTNNB1, SIX1, SIX2, DROSHA, DICER1, DIS3L2*, *DCGR8* and *TP53*.[10,18,19] Notably, there is significant overlap between genes which are recognized as somatic drivers of WT development, and genes in which germline variants have been reported to cause WT predisposition (**Table 1**). However, given that a considerable proportion of WTs do not harbour alterations in any of these genes, the spectrum of WT driver and predisposition genes will likely be larger.[20,21]

Table 1. Currently identified Wilms tumor (WT) driver and predisposition genes.

Gene	Somatic driver variants?	Germline predisposing variants?	Associated syndromes		
			Syndrome(s)	**Inheritance**	**Estimated WT risk**
WT1	Yes [18,22,23]	Yes [24-32]	Denys-Drash / Frasier syndrome: now referred to as *WT1* disorders. WT may be the first or only manifestation in children with germline *WT1* variants. WAGR syndrome (11p13 deletion including *WT1* and PAX6).	AD	~50-80% [A]
H19/IGF2	Yes [18,22,33]	Yes [34-36]	Beckwith-Wiedemann spectrum	Postzygotic	<1%-21% [B]
DIS3L2	Yes [19,37]	Yes [38-40]	Perlman syndrome	AR	~64%
PIK3CA	Not reported	Yes [41,42]	*PIK3CA*-related overgrowth spectrum	Postzygotic	1-5%
GPC3	Not reported	Yes [43]	Simpson-Golabi Behmel syndrome	X-linked	~3%
TRIM28	Yes [44-46]	Yes [11,44-47]	*TRIM28*-related WT predisposition	AD	>50%
REST	Yes [13,19]	Yes [48,49, 50]	*REST*-related WT predisposition	AD	>50%
CTR9	Not reported	Yes [51,52]	*CTR9*-related WT predisposition	AD	Appears high
NYNRIN	Not reported	Yes [11]	*NYNRIN*-related WT predisposition	AR	Unknown
BRCA2	Not reported	Yes [53-57]	Fanconi anemia type D1	AR	~20%
PALB2	Not reported	Yes [53-57]	Fanconi anemia type N	AR	~40%
TRIM37	Not reported	Yes [58,59]	Mulibrey Nanism	AR	~6-8%
BUB1B	Not reported	Yes [60,61,62]	Mosaic variegated aneuploidy (MVA)	AR	~50%
TRIP13	Not reported		Mosaic variegated aneuploidy (MVA)	AR	~20%
MYCN	Yes [18,19]	Yes [63-65]	2p24.3 duplication syndrome	AD	Unknown
AMER1	Yes [18,66]	Yes [67,68,69]	Osteopathia striata with cranial sclerosis	X-linked	Appears >5%
BLM	Not reported	Yes [70]	Bloom syndrome	AR	~3%
DICER1	Yes [19,71]	Yes [9,72,73]	DICER1 syndrome	AD	<2%
TP53	Yes [18,74]	Yes [9,75]	Li Fraumeni syndrome	AD	Low

Table 1. Continued.

Gene	Somatic driver variants?	Germline predisposing variants?	Associated syndromes		
			Syndrome(s)	Inheritance	Estimated WT risk
NF1	Yes [37]	Yes [76]	Neurofibromatosis type 1	AD	<1%
CDC73	Not reported	Yes [77,78]	Hyperparathyroidism-jaw tumor syndrome	AD	<5%
ASXL1	Yes [13,18]	Yes [79,80]	Bohring-Opitz syndrome	AD	~7%
CTNNB1	Yes [18,23,81]	Not reported	None related to WT development	n.a.	n.a.
SIX1	Yes [18,19,82]	Not reported	None related to WT development	n.a.	n.a.
SIX2	Yes [18,19,82]	Not reported	None	n.a.	n.a.
DROSHA	Yes [18,19,82]	Not reported	None	n.a.	n.a.
DGCR8	Yes [18,19,82]	Not reported	None	n.a.	n.a.
MLLT1	Yes [18,83]	Not reported	None	n.a.	n.a.
BCOR	Yes [18]	Not reported	None related to WT development	n.a.	n.a.
MAX	Yes [18]	Not reported	None	n.a.	n.a.
NONO	Yes [18]	Not reported	None related to WT development	n.a.	n.a.
ACTB	Yes [18]	Not reported	None related to WT development	n.a.	n.a.
ARID1A	Yes [18,71]	Not reported	None related to WT development	n.a.	n.a.
CHD4	Yes [18,19]	Not reported	None related to WT development	n.a.	n.a.

Legend: WAGR: Wilms tumor, aniridia, genitourinary anomalies and range of developmental delays; AD: autosomal dominant, AR: autosomal recessive, n.a.: not applicable. [A] Depending on the location of the variant: *WT1* intron 9 variants are associated with low WT risk; [B] Depending on the molecular subtype. Note: genes with somatic WT driver variants are included in this table if such variants were reported in more than one publication, or at least three times in a single publication.

Wilms tumor treatment

In the 1950s, WT was the first human solid malignancy in which response to chemotherapy was reported.[84] The chemotherapeutic agent which was applied at the time (actinomycin-D), is still a crucial component of all WT treatment regimens.[85] In Europe and most countries outside North America and Canada, children with renal tumors are treated according to International Society of Pediatric Oncology Renal Tumor Study Group (SIOP-RTSG) protocols, which include preoperative chemotherapy for children aged ≥6 months at diagnosis.[85] After preoperative treatment, surgery is performed which usually consists of a resection of the tumor as well as the adjacent kidney (tumor-nephrectomy). Alternatively, nephron-sparing surgery can be safely performed in selected patients, depending on the size and location of the tumor. Following resection, postoperative treatment is planned according to tumor stage and histological subtype. In most cases, postoperative WT treatment includes an additional period of chemotherapy, and in selected cases, radiotherapy.

In contrast to SIOP-RTSG protocols, North American protocols developed by the Children's Oncology Group – Renal Tumor Group (COG-RTG) do not include preoperative chemotherapy unless a child has bilateral/metastatic disease or a known cancer predisposition syndrome.[86] Performing direct surgery as in COG-RTG protocols, eliminates the risk of misdiagnosis, which can have important benefits because some renal tumors require no or more aggressive chemotherapy. On the other hand, a randomized study has provided evidence that preoperative chemotherapy leads to easier surgery, downstaging of the tumor and a lower risk of tumor rupture. Moreover, it enables the identification of a treatment resistant subtype, the blastemal type, which is selected for intensive treatment in SIOP-RTSG protocols.[87] With either strategy, similar high survival rates are achieved. Challenges that remain, include the treatment of high-risk histological subtypes, metastatic and/or bilateral disease, as well as the reduction of treatment-related toxicity.[85] To overcome these challenges, tumors are ideally detected at an early stage, and treatment regimens need to be tailored to the patient's individual risk. In patients with a predisposition, this early detection can be pursued by surveillance.

Wilms tumor histology, staging and stratification

Treatment stratification is primarly based on the tumor's stage and histological subtype. The SIOP-RTSG and the COG-RTG use distinct classification systems for WT stage and histology. Both systems have evolved over time and will continue to be updated as novel prognostic factors emerge.[85]

WTs are morphologically composed of three components, which can be present in variable proportions and are used to define different WT subtypes. These three components include stroma, epithelium and blastema (**Figure 4**).[15] Additionally, WTs can display diffuse anaplasia, which is associated with somatic *TP53* mutations and more aggressive tumors.[74] Molecular aberrations identified in the tumor, including

somatic mutations, copy number changes (e.g. gain of chromosome arm 1q) and loss of heterozygosity, are associated with specific histological subtypes, and have been shown to be of prognostic value in COG-RTG settings. This is currently being validated in the SIOP-RTSG UMBRELLA protocol for pretreated patients.[88-94] Other prognostic factors which are used or considered for treatment stratification in SIOP-RTSG and/or COG-RTG protocols, include tumor weight/volume[95,96], volume of the blastemal component[97] and the patient's age at WT diagnosis.[95,96,98]

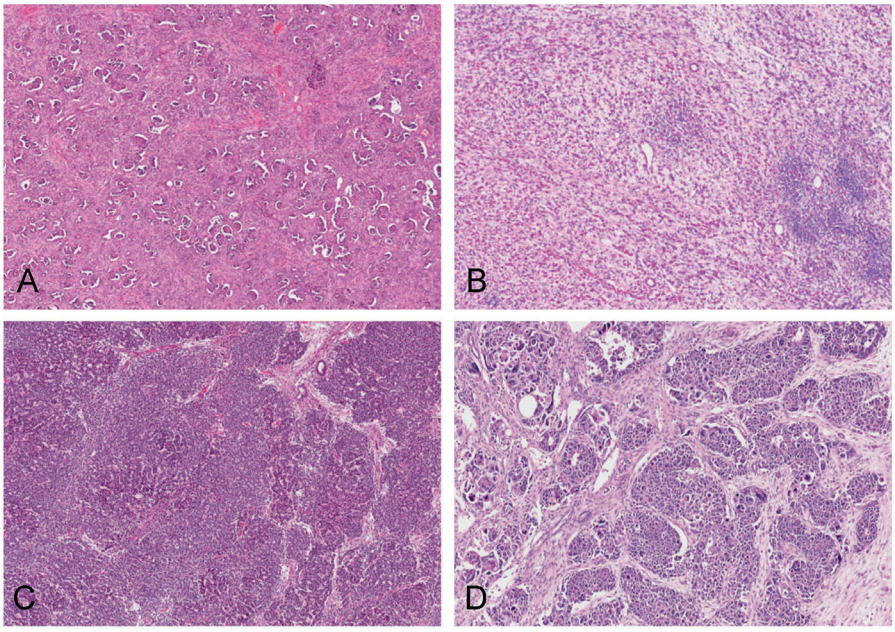

Figure 4. Histological components of Wilms tumor (WT). WTs typically consist of three histological components which can be present in variable proportions, including an epithelial (A), stromal (B) and blastemal (C) component. Additionally, WTs can display anaplasia, characterized by large hyperchromatic nuclei an atypical mitotic figures (D). *Adapted from: Vujanic, G.M. (2014). Pathology of Renal Tumors of Childhood. In K. Pritchard-Jones & J.S. Dome (Ed.) Renal Tumors of Childhood: Biology and Therapy (p. 56-63), Springer 2014.*

Wilms tumor predisposition

A subset of patients with WT has an underlying tumor predisposition syndrome. The fact that only 1-2% of all WT cases are familial, is due to the large contribution of *de novo* (epi)genetic alterations.[7,99] It has been estimated that (epi)genetic predisposing factors are present in 5-24% of children with WT[7,49,100-102], although until recently, reliable data from cohort studies were lacking and the high rate of phenotypic abnormalities suggested that this percentage may be higher.[100,101]

Relevance of identifying predisposition in children with WT

Identifying WT predisposition enables surveillance for children at-risk and can inform treatment decisions.[103] If the presence of a WT predisposition syndrome is known at the time of surgery, nephron-sparing surgery is recommended depending on the size and location of the tumor.[104,105] Moreover, stricter surveillance of the remaining kidney(s) may be warranted. Where necessary, family members can be counseled and tested. This is particularly relevant for parents with other young children and/or a desire to have additional children. In children with a WT predisposition syndrome, surveillance is recommended to detect smaller and lower-stage tumors[106-109], thereby reducing the need for toxic treatment and reducing direct and late side effects. Before this thesis, WT surveillance guidelines published by a British working group in 2006 were widely used in many European countries.[108] However, additional genes and syndromes associated with WT risk have been identified after 2006, and diagnostic molecular tests for previously known syndromes have improved. Updated WT surveillance recommendations were therefore urgently needed.

Types of Wilms tumor predisposition

A list of currently acknowledged WT predisposition genes is provided in **Table 1**. Classic examples are *WT1*, which can be affected by various genetic alterations, and *H19/IGF2*, which is affected by epigenetic alterations in children with Beckwith-Wiedemann spectrum (BWSp).[7,109,110] In 1990, *WT1* was the first WT predisposition gene to be identified.[111-113] The contiguous deletion of *WT1* and *PAX6* genes at 11p13 causes WAGR syndrome, which is the acronym for Wilms tumor, Aniridia, Genitourinary anomalies and Range of developmental delays.[29-32,114] Moreover, germline *WT1* pathogenic variants can cause various phenotypes which were previously referred to as Denys-Drash or Frasier syndrome, but which are now considered to be part of the same phenotypic spectrum.[24-28,115,116] In addition to an increased risk of WT, *WT1* pathogenic variants are associated with renal disease (glomerulosclerosis) and disorders of sexual development. In contrast to WAGR syndrome, which is usually recognized early due to aniridia, WT can be the first manifestation in children with *WT1* pathogenic variants.

BWSp is the most frequently diagnosed WT predisposition syndrome, affecting 1 in 10,500 children in Western populations.[117] It is an overgrowth syndrome which can be clinically diagnosed based on the presence of multiple phenotypic criteria including (lateralized) overgrowth, macroglossia, abdominal wall defects and neonatal hypoglycemia.[35] BWSp is caused by genetic and/or epigenetic changes at the 11p15.5 imprinted region, including *H19* and *IGF2* genes, which are frequently mosaic and may be missed by currently available diagnostic techniques.[35]

In addition to *WT1*-related syndromes and BWSp, many other syndromes have been associated with an increased risk of WT development[7] and genomic sequencing studies have led to the identification of additional WT predisposition genes such as *TRIM28,*

CTR9 and *REST*, that each in itself account for ≤1% of WT cases.[11,45,46,48,51] Still, (epi) genetic predisposing factors are not always diagnosed in children who are clinically suspected of having a WT predisposition syndrome, suggesting that additional genes remain to be identified.

GENETIC PREDISPOSITION AND NON-WILMS RENAL TUMORS

(Epi)genetic predisposing factors have not been well characterized for most non-Wilms pediatric renal tumors. An exception is MRTK, which is strongly associated with pathogenic germline variants in the *SMARCB1* gene, and to a lesser extent the *SMARCA4* gene.[118] Additionally, cystic nephromas are associated with pathogenic germline variants in the *DICER1* gene, which predispose to various benign and malignant tumors.[119] Moreover, the childhood onset of RCC, which is an adult-type tumor, warrants genetic evaluation to exclude RCC susceptibility.[120] Although most RCCs in children are MiT-family translocation-type RCCs which are typically sporadic[121], the diagnosis of specific RCC subtypes such as FH-deficient RCC should trigger awareness for an underlying syndrome.[122] So far, mesoblastic nephroma, clear cell sarcoma of the kidney (CCSK) and other rare renal tumor types have not been clearly associated with predisposing factors.

OUTLINE OF THIS THESIS

In children with WT, carefully designed protocols which include treatment stratification according to the patient's individual risk, can further improve survival and reduce treatment toxicity. Part I of this thesis deals with Wilms tumor treatment and outcome, describing the rationale behind the WT treatment recommendations in the current SIOP-RTSG 2016 UMBRELLA protocol (**Chapter 2**). Additionally, we describe the exploration of the prognostic significance of age in a large cohort of patients who were registered in SIOP 93-01 and SIOP 2001 protocols (**Chapter 3**). Furthermore, we describe the clinical characteristics and outcome of children with WT and WAGR syndrome (**Chapter 4**).

In Part II of this this thesis, (Epi)genetic predisposition to pediatric renal tumors, we present the phenotypic and genomic characterization of a nationwide WT cohort with the aim to determine the prevalence of (epi)genetic predisposing factors, correlate germline findings to clinical and tumor characteristics, and identify novel WT predisposition genes (**Chapter 5**). Moreover, we review histopathological and clinical features as well as potential underlying mechanisms of *TRIM28*-associated WT (**Chapter 6**). With a consensus group of pediatric oncologists, geneticists, a radiologist and an epidemiologist, we developed international guidelines for WT surveillance in children at-risk (**Chapter 7**). Finally, in **Chapter 8**, we report on young patients with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) caused by mutations in the *FH* gene.

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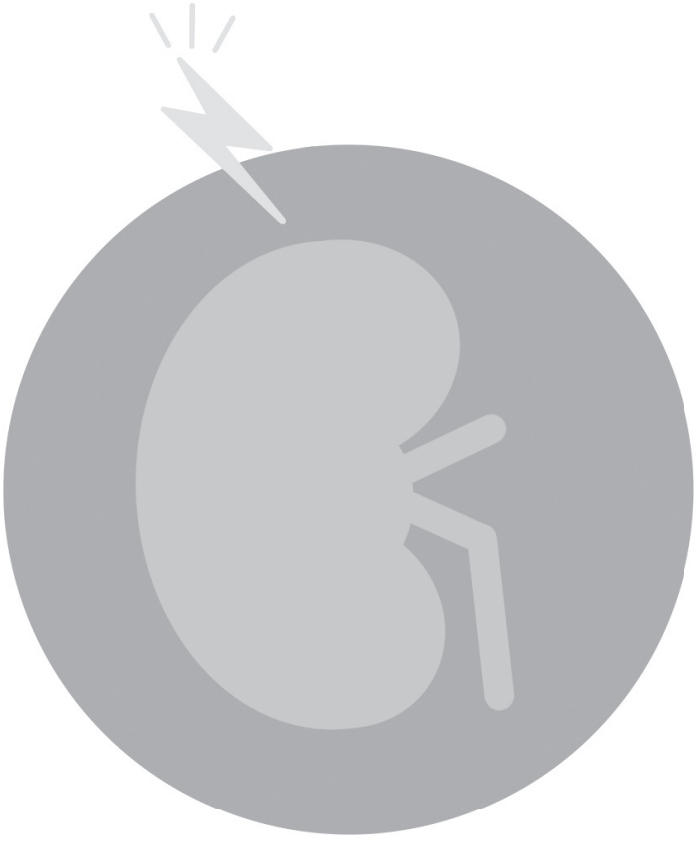

PART I

Wilms tumor treatment and outcome

2

Rationale for the treatment of Wilms tumor in the UMBRELLA SIOP-RTSG 2016 protocol

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ABSTRACT

The Renal Tumor Study Group of the International Society of Pediatric Oncology (SIOP-RTSG) has developed a new protocol for the diagnosis and treatment of childhood renal tumors, the UMBRELLA SIOP-RTSG 2016 (the UMBRELLA protocol), to continue international collaboration in the treatment of childhood renal tumors. This protocol will support integrated biomarker and imaging research, focussing on assessing the independent prognostic value of genomic changes within the tumor and the volume of the blastemal component that survives preoperative chemotherapy. Treatment guidelines for Wilms tumors in the UMBRELLA protocol include recommendations for localized, metastatic, and bilateral disease, for all age groups, and for relapsed disease. These recommendations have been established by a multidisciplinary panel of leading experts on renal tumors within the SIOP-RTSG. The UMBRELLA protocol should promote international collaboration and research and serve as the SIOP-RTSG best available treatment standard.

The Renal Tumor Study Group of the International Society of Pediatric Oncology (SIOP-RTSG) has developed a new protocol for diagnosis and treatment of childhood renal tumors, UMBRELLA SIOP-RTSG 2016 (referred to as the UMBRELLA protocol), to continue international collaboration in the treatment of childhood renal tumors.[1] The UMBRELLA protocol succeeds the SIOP-2001 protocol.[2] The name UMBRELLA signifies the ambitious aim to collect information concerning all pediatric primary renal tumors in a comprehensive multidimensional data registry, which includes embedded review of diagnostics, standardized biobanking, and treatment recommendations (**Figure 1**).[1] The UMBRELLA protocol will support integrated biomarker and imaging research, with a particular focus on assessing the independent prognostic value of genomic changes within the tumor (chromosomal gain of 1q and the extent of its intratumoral heterogeneity) and the volume of the blastemal component that survives preoperative chemotherapy.[3, 4]

Childhood renal tumors are relatively uncommon, accounting for ~5% of all pediatric malignancies. Of these tumors, around 80-90% are thought to be Wilms tumors, whereas other renal tumors (non-Wilms tumors), including clear cell sarcoma of the kidney, renal cell carcinoma, malignant rhabdoid tumor of the kidney, and congenital mesoblastic nephroma are even less common.[5] The exact incidence of non-Wilms tumors is unclear, owing to the probable under-registration of patients with these tumors in renal tumor protocols.

The UMBRELLA protocol addresses both Wilms tumors and non-Wilms tumors, and will be available on the SIOP-RTSG website (www.siop-rtsg.eu) after launch in 2017. All countries that are interested in joining the UMBRELLA protocol will be given full access to the treatment schedules, based on their commitment to register renal tumor patients. This Consensus Statement focuses on the rationale for treatment of Wilms tumors in the UMBRELLA protocol.

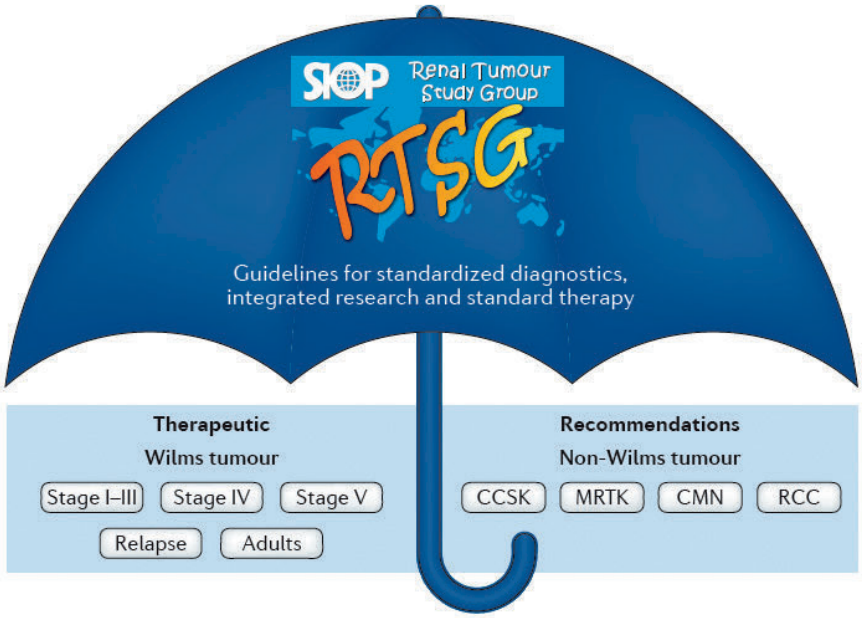

Figure 1. The UMBRELLA SIOP-RTSG 2016 protocol logo. The UMBRELLA signifies the ambitious aim to collect information concerning all pediatric primary renal tumors in a comprehensive multidimensional data registry, which includes embedded review of diagnostics, standardized biobanking, and treatment recommendations. CCSK, clear cell sarcoma of the kidney, MRTK, malignant rhabdoid tumor of the kidney, CMN, congenital mesoblastic nephroma, RCC, renal cell carcinoma.

Treatment guidelines for Wilms tumors in the UMBRELLA protocol include recommendations for localized, metastatic (stage IV), and bilateral disease, for all age groups, and for relapsed disease. These recommendations were established by a multidisciplinary panel of leading experts on renal tumors within the SIOP-RTSG, including pediatric oncologists, radiologists, pathologists, surgeons, radiation oncologists, statisticians, and scientists involved in basic research. Thorough communications were undertaken with colleagues with similar expertise involved in the Children's Oncology Group (COG), to ensure all relevant evidence was applied when deciding how to implement the results of the SIOP-2001 randomized trial, which investigated the safety of omitting doxorubicin in treating stage II–III intermediate-risk Wilms tumors, and to refine recommendations for patients with Wilms tumor. Over the past 15 years, wide-ranging discussions on global strategies for children with renal tumors have evolved between SIOP-RTSG and COG during meetings and workshops. These conversations have resulted in sharing of data and knowledge, which has been used in the design of the current UMBRELLA guideline for diagnostics and treatment.

TREATMENT RECOMMENDATIONS

In general, treatment of Wilms tumors is tailored to the patient based on tumor stage and histology, and involves a combination of chemotherapy, surgery, and, sometimes, radiotherapy. Since the first SIOP protocol started in 1971, treatment intensity has been successfully reduced for the majority of patients with Wilms tumors, and survival has risen to 90%.[2, 6-10] Consequently, the identification of additional predictive and prognostic factors is increasingly important to improve the stratification of patients according to their individual risk. Approximately two-thirds of patients with Wilms tumor now receive chemotherapy consisting of only two drugs, actinomycin D and vincristine.[11] Other patients, including those with metastatic disease and high-risk histological subtypes, are believed to benefit from doxorubicin.[12-16] Moreover, as innovative techniques emerge, surgical and radiotherapeutic procedures are improving.

Localized disease. Similar to the SIOP-2001 protocol, the UMBRELLA protocol continues to recommend preoperative actinomycin D and vincristine for patients newly diagnosed with Wilms tumor aged ≥6 months, based on results of previous SIOP trials that showed tumor downstaging using this regimen.[2, 6, 8, 9, 14, 17] This benefit was also independently observed in the randomized, controlled UKW3 trial conducted by the UK Children's Cancer and Leukaemia Group (UKCCLG, previously known as the UK Children's Cancer Study Group).[18] In patients receiving preoperative chemotherapy, the use of radiotherapy or doxorubicin could be reduced by 20% compared with those treated with direct nephrectomy, with no significant difference in survival.[18] The SIOP-RTSG accounts for the risk of misdiagnosis of Wilms tumor by recommending direct surgery instead of preoperative chemotherapy for children <6 months old, and the consideration of fine-needle biopsy for patients who have unusual clinical presentations or unusual findings on imaging. To avoid treatment delay, routine histological assessment before treatment is not advocated. This approach has been shown to be safe and identifies the vast majority of patients with non-Wilms tumors who are at risk of being unnecessarily treated with preoperative chemotherapy.

Furthermore, preoperative chemotherapy enables personalized assessment of tumor chemosensitivity, including identification of the high-risk, blastemal-type Wilms tumors. The centralized review process of histology, undertaken in the SIOP-RTSG, has shown that identification of the blastemal subtype is feasible and clinically relevant. Yet, the definition of blastemal-type histology might be improved by considering the absolute residual volume of blastema rather than the relative percentage, as will be investigated in the UMBRELLA protocol.[19]

Patients registered in the UMBRELLA protocol will continue to be stratified for postoperative treatment according to tumor stage and histological risk group, as was the protocol in SIOP-2001 (**Table 1**). Prospective data from patients who are stratified and treated based on standardized recommendations will be collected and analysed. This

data collection, in combination with the results of planned integrated biomarker and imaging studies (which will assess the relative contribution of gain of 1q and assessment of residual blastemal volume), might be used to guide stratification in future protocols.

Table 1. Overview of postoperative treatment for localized Wilms tumor in UMBRELLA SIOP-RTSG 2016. A, actinomycin D; D, doxorubicin; HR-1;etoposide, carboplatin, cyclophosphamide and doxorubicin (34 weeks); V, vincristine

Disease	Tumor volume after preoperative chemotherapy	Treatment		
		Stage I	Stage II	Stage III
Low-risk	All	None	AV (27 weeks)	AV (27 weeks)
Intermediate-risk, all subtypes	<500 ml	AV (4 weeks)	AV (27 weeks)	AV (27 weeks) + flank radiotherapy
Intermediate-risk, stromal or epithelial-type	≥500 ml	AV (4 weeks)	AV (27 weeks)	AV (27 weeks) + flank radiotherapy
Intermediate-risk, nonstromal, nonepithelial	≥500 ml	AV (4 weeks)	AVD (27 weeks)	AVD (27 weeks) + flank radiotherapy
High-risk blastemal type Wilms tumor	All	AVD (27 weeks)	HR-1 (34 weeks)	HR-1 (34 weeks) + flank radiotherapy
High-risk diffuse anaplasia	All	AVD (27 weeks)	HR-1 (34 weeks) + flank radiotherapy	HR-1 (34 weeks) + flank radiotherapy

The therapeutic regimen of the experimental arm of the SIOP-2001 trial has been adopted as the new standard management regimen for most patients in the UMBRELLA protocol with stage II–III intermediate-risk Wilms tumors. This regimen consists of 27 weeks of vincristine and actinomycin D without doxorubicin. This schedule resulted in a nonsignificant small decrease in event-free survival (EFS) and had no effect on overall survival compared with 27 weeks of vincristine and actinomycin D plus five doses of doxorubicin at 50 mg/m2 (the standard arm) in the SIOP-2001 trial.[2]

Post hoc analysis of data from SIOP-2001 was carried out to examine the association between omitting doxorubicin and the outcomes of patients with large-volume tumors (defined as tumors with a volume >500 ml after preoperative chemotherapy). Stromal and epithelial tumor types, which have excellent prognosis[20], were excluded from this analysis, leaving only stage II–III regressive, mixed, and focal anaplasia-type tumors (n = 429). In Kaplan Meier analysis, patients with large-volume tumors had an estimated 5-year EFS of 80% versus 90% for patients with small-volume tumors (log rank P = 0.01) (**Figure 2**).

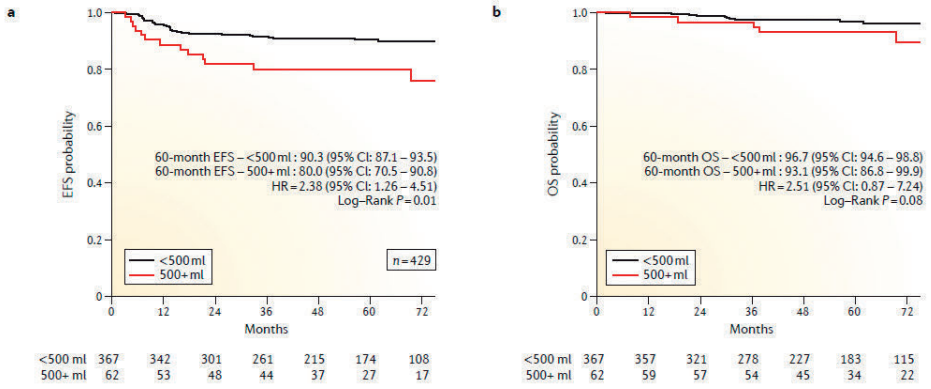

Figure 2. *Post hoc* analysis of data from patients with stage II or III intermediate-risk, nonstromal, nonepithelial Wilms tumor in the SIOP-2001 randomized controlled trial. Association of tumor volume with a) event-free survival (EFS) and b) overall survival (OS). Kaplan Meier curves.

Most importantly, EFS was significantly improved (93% versus 67%, log rank $P = 0.0005$) when doxorubicin was added to the treatment regimen for large-volume (≥500 ml) tumors (**Figure 3**). Thus, the inclusion of doxorubicin in postoperative treatment of patients with large-volume (≥500 ml) stage II–III nonstromal, nonepithelial tumors is recommended in the UMBRELLA protocol.

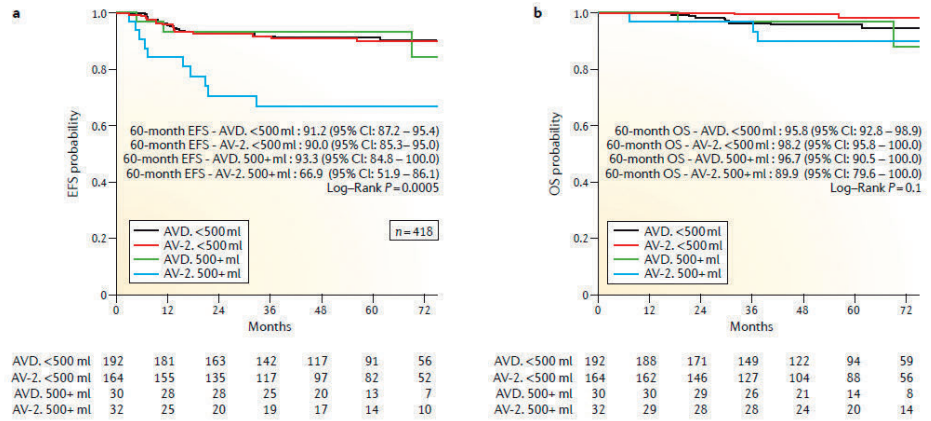

Figure 3. *Post hoc* analysis of data from patients with stage II or III, intermediate-risk, nonstromal, nonepithelial Wilms tumor in the SIOP-2001 randomized controlled trial. Association of tumor volume and treatment with a) event-free survival (EFS) and b) overall survival (OS). Kaplan Meier curves. AV-2, actinomycin D, vincristine (27 weeks); AVD, actinomycin D, vincristine and doxorubicin.

Furthermore, the UMBRELLA protocol will continue treatment for blastemal-type tumors according to the regimen used in SIOP-2001. A comparison of the results of the SIOP-2001 and SIOP-93-01 trials showed that in SIOP-2001, in which treatment was intensified by changing to the high-risk tumor treatment schedule for patients with blastemal-type Wilms tumor, EFS increased from 67% to 80% (log rank $P = 0.006$) avoiding intensive treatment for relapse in a considerable number of patients.[12]

Metastatic disease (stage IV). Overall, ~17% of patients with Wilms tumors present with stage IV disease at diagnosis, which is defined as hematogenous metastases to the lungs, liver, or other sites, or extra-abdominal lymph node metastases. Pulmonary metastases are by far the most frequently observed.[21-23] The increasing use of chest CT as routine imaging for staging has resulted in the detection of small pulmonary nodules not visible on chest radiography (CT-only nodules). Similar to the COG protocol, CT-only nodules are included in the definition of lung nodules and treated as metastases in the UMBRELLA protocol if they have a transverse diameter of at least 3 mm.[22, 24, 25] Presence of these CT-only nodules was associated with increased relapse risk and reduced survival in a SIOP-RTSG analysis comparing the outcomes of patients with CT-only lung lesions with those with true localized disease24. Results from the COG National Wilms Tumor Study Group (NWTS)-4 and NWTS-5 trials showed that patients with CT-only nodules who were treated with vincristine and actinomycin D plus doxorubicin had superior EFS to those who received vincristine and actinomycin D only, but overall survival was similar in both groups.[25] Including CT-only nodules in the definition of metastatic disease will benefit patients with intermediate-risk or low-risk histology who achieve a rapid complete response of their CT-only nodules. These patients do not need pulmonary radiotherapy and have, therefore, a reduced risk of severe long-term sequelae such as lung disease, cardiac complications or secondary malignancies.

Similar to SIOP-2001, preoperative treatment for metastatic (stage IV) disease in the UMBRELLA protocol includes a combined vincristine, actinomycin D, and doxorubicin regimen for 6 weeks, followed by reassessment imaging of local tumor (using MRI) and metastatic sites (using CT and/or MRI) before surgery. With this preoperative regimen, 61-67% of patients have complete metastatic response before surgery.[21, 23] Detailed guidelines are provided for the stratification of postoperative chemotherapy, in which the cumulative dose of doxorubicin has been lowered in order to reduce cardiac toxicity. The cumulative doxorubicin dose for patients with metastatic disease was 300 mg/m^2 in SIOP-2001, preliminary data from the COG AREN0533 trial suggest that using a cumulative doxorubicin dose of 150 mg/m^2 for patients with favourable histology does not considerably affect survival.[25, 26] For this reason, the UMBRELLA protocol recommends stratifying patients to either vincristine and actinomycin D plus doxorubicin with a cumulative doxorubicin dose of 150 mg/m^2, vincristine, and actinomycin D plus doxorubicin with cumulative doxorubicin of 250 mg/m^2, or a four-drug regimen including etoposide (150 mg/m^2/day), carboplatin (200 mg/m^2/day), cyclophosphamide

(450 mg/m^2/day), and doxorubicin (cumulative dose 300 mg/m^2). Stratification is based on local stage of the primary tumor, histology of the primary tumor and the metastatic tumor (if resected), the size of metastatic lesions, and their response to preoperative treatment and surgery (**Table 2**).

Notably, patients with metastatic disease and high-risk characteristics on histological examination are a rare subgroup, with recognized unfavourable prognosis. Only a few patients per year will be stratified into this category. Thus, UMBRELLA protocol advises that local centres discuss the best current treatment approach with the principle investigator for stage IV disease. Currently, the SIOP-RTSG board suggests a regimen based on unpublished but presented data from the COG[27], including combinations of vincristine, irinotecan, cyclophosphamide, carboplatin, etoposide, and doxorubicin, followed by high-dose chemotherapy and autologous stem cell transplantation at the discretion of the treating physician. The role of upfront high-dose chemotherapy for this subgroup is under debate, but a trend towards favourable outcomes has been reported by several groups in the primary and relapsed settings.[28-30] Details of this suggested regimen were added as an appendix to the UMBRELLA protocol. Data on the use of this regimen and outcomes will be prospectively captured in the SIOP database and can, therefore, be evaluated in a descriptive study.

Table 2. Treatment overview for stage IV WT based on response to treatment and histology.

Metastasis surgery	Wilms tumor histology	Treatment
Complete remission or very good partial remission		
Surgical complete resection if needed	Low-risk or intermediate-risk disease & lung nodules 3-5mm	AVD150, no pulmonary radiotherapy unless complete resection of viable metastasis, then pulmonary radiotherapy
	Low-risk or intermediate-risk disease & lung nodules >5mm or other site	AVD250, no pulmonary radiotherapy unless complete resection of viable metastasis, then pulmonary radiotherapy
	Low-risk or intermediate-risk disease No evidence of metastasis	Treatment as localized
Partial response or stable disease		
Representative nodule resection feasible	Low-risk disease Viable metastasis confirmed	AVD250, lung or metastasis radiotherapy, CT at week 10: if remaining nodules then surgery recommended to achieve complete response if feasible
	Low-risk disease Completely necrotic metastasis	AVD150, CT at week 10: if remaining nodules then surgery recommended to achieve complete response if feasible

Table 2. Continued.

Metastasis surgery	Wilms tumor histology	Treatment
Representative nodule resection feasible	Low-risk or intermediate-risk disease No evidence of viable tumor	Contact principal investigator‡, potentially treatment as localized or AVD250, CT at week 10: if remaining nodules then surgery recommended to achieve complete response if feasible, no radiotherapy to metastases
	Intermediate-risk disease Viable metastasis confirmed	Four-drug regimen, radiotherapy to metastasis. CT at week 10: if remaining nodules then surgery recommended to achieve complete response if feasible
	Intermediate-risk disease Completely necrotic metastasis	AVD250 regimen, CT at week 10: if remaining nodules then surgery recommended to achieve complete response if feasible
Resection not feasible	Low-risk disease	AVD250, CT at week 10: reconsider resection and discuss radiotherapy to metastasis
	Intermediate-risk disease	Four-drug regimen, CT at week 10: if remaining nodules radiotherapy to metastasis is indicated
Progressive disease		
Representative nodule resection feasible	Intermediate-risk disease Metastasis confirmed	Four-drug regimen, radiotherapy to metastasis. CT at week 10: if remaining nodules then surgery is recommended to achieve complete response if feasible
	Intermediate-risk disease No evidence of viable or necrotic tumor	AVD250, CT at week 10: if remaining nodules then surgery:if viable metastasis then CDCV plus radiotherapy to metastases is indicated: contact principal investigator‡
All		
All	High-risk disease	Ask principal investigator‡ for advice, radiotherapy to metastases, CT week 10: if remaining nodules consider resection if feasible
Mixed		
Indicated	Confirm metastatic disease by histology	If metastases present then treat according to worst histology and worst response

Source: UMBRELLA-SIOP-RTSG-2016 protocol. AVD, actinomycin-D, vincristine and doxorubicin; CDCV, cyclophosphamide, doxorubicin, carboplatin and VP16. ‡arnauld.verschuur@ap-hm.fr

Bilateral disease (stage V). Synchronous bilateral Wilms tumor (stage V) accounts for ~5–8% of instances of Wilms tumor and long-term overall survival is currently ~80%.[31-35] End-stage renal disease (ESRD) is the most clinically significant morbidity for patients with bilateral Wilms tumors and can be caused by underlying germline genetic aberrations as well as treatment-related loss of functional renal tissue. Aronson *et al.*[35] observed that functional renal outcome was considerably better after bilateral nephron sparing surgery (NSS) than when other types of surgery were used.[35] Independently of the type of treatment, children with Wilms tumor, aniridia, genitourinary anomalies, and retardation (WAGR), Denys-Drash or other syndromes associated with *WT1* mutations, are at increased risk of ESRD.[36] Thus, avoiding total nephrectomy at initial

surgery is advised for bilateral tumors in the UMBRELLA protocol.[35] However, other important causes of ESRD exist, including tumor recurrence requiring bilateral nephrectomy or renal irradiation. Long-term monitoring of renal function is required after treatment of bilateral disease. In the SIOP-2001 study, patients with bilateral disease received preoperative chemotherapy including vincristine and actinomycin D until NSS was deemed feasible, with response evaluations performed every 4 weeks. However, several studies have shown that prolonged preoperative chemotherapy is often ineffective (especially as many bilateral tumors are the chemotherapy-insensitive stromal subtype) and could even result in an increased risk of the presence of anaplasia, disease progression, and development of metastases.[31, 32, 34] Thus, the UMBRELLA protocol limits preoperative chemotherapy to a maximum of 12 weeks, with time intervals for evaluation fixed to 6 weeks, to be comparable with the COG approach for future studies. The occurrence of misdiagnosis, in which synchronous bilateral renal tumors other than Wilms tumors are present, is, from experience, extremely rare. In instances of tumor nonresponsiveness or inoperability switching to treatment with etoposide and carboplatin is recommended, to avoid use of anthracyclines, and biopsy can be considered to determine histology.

Relapsed Wilms tumor. The UMBRELLA protocol provides structured guidelines for the treatment of patients with relapsed Wilms tumors. In retrospective studies, the best prognostic factors were initial histology and the first-line treatment used.[37-39] Thus, patients with relapsed tumors will be prospectively classified into three groups in the UMBRELLA protocol, group AA, group BB, and group CC, based on these factors.

Treatment of group AA relapsed Wilms tumors, defined as patients with initial stage I–II low-risk or intermediate-risk tumors, who received only vincristine and/or actinomycin D (no radiotherapy) in their first-line treatment, will include four drugs (combinations of doxorubicin and/or cyclophosphamide and carboplatin and/or etoposide). The combination of these drugs has already been tested in two comprehensive studies, the UKW-R protocol and the NWTS-5 relapse protocol, but drug combinations and doses varied.[40, 41]

Patients without initial diffuse anaplasia or blastemal-type histology, who have already received doxorubicin in their initial treatment, will be classified as group BB and receive an intensive reinduction drug regimen (including the combination of etoposide and carboplatin with either ifosfamide or cyclophosphamide), followed by either high-dose melphalan and autologous stem cell rescue (ASCR) or two further reinduction courses, at the discretion of the local physician.[28, 37] Acceptable response rates have been observed with both cyclophosphamide and etoposide and carboplatin and etoposide combinations, but ifosfamide showed an increased response rate in early-phase trials.[42] In an effort to reduce the risk of ifosfamide-related nephrotoxicity, cyclophosphamide will be alternated with ifosfamide in the group BB protocol recommendations. The heterogeneous settings

in which the role of high-dose chemotherapy and ASCR has been explored and the inconclusive results reported led us to propose a flexible approach to the consolidation phase, and high-dose chemotherapy is at the discretion of the treating physician, aiming to describe the results in a prospective observational fashion.[28]

Relapsed group CC includes patients with initial diffuse anaplasia or blastemal-type tumors. For patients in this category, and for the other relapsing patients showing no response to salvage treatment, the UMBRELLA protocol advises trying camptothecins (irinotecan or topotecan) or novel compounds, as these patients will have already received most conventional active agents in their first-line therapy and are likely to develop chemotherapy-resistant disease.[43] In the UMBRELLA protocol, the SIOP-RTSG commits to endorse initiatives dedicated to new drug development in children, such as those launched by the Innovative Therapies for Children with Cancer consortium.

Importantly, approaches to local treatment (including radiotherapy and surgical excision of relapsing tumor masses) have not been systematically explored, so the UMBRELLA protocol provides structured guidelines for administering radiotherapy and surgery at relapse. These guidelines include considering resection after proven reduction of relapsed disease after chemotherapy, independently of histological subtype or risk group, when radical surgery seems possible or when it is useful to evaluate histological tumor response. Applying radiotherapy to initially nonirradiated sites is uniformly accepted, but developing standard recommendations for the approach to previously irradiated sites is difficult, because of the many different situations encountered. For these instances, the UMBRELLA protocol recommends contacting radiotherapists on the SIOP-RTSG panel.

Infant Wilms tumors. Infants, defined as patients younger than 6 months (182 days), should be considered for primary surgery according to the UMBRELLA protocol, unless tumors are judged not amenable to immediate nephrectomy in a multidisciplinary team consensus. The reason for upfront nephrectomy is that, compared with older children, a higher proportion of renal tumors in infants are congenital mesoblastic nephroma or malignant rhabdoid tumors that either need surgery alone (congenital mesoblastic nephroma) or alternative chemotherapy at the outset (more intensive chemotherapy than actinomycin D and vincristine).[44, 45] Percutaneous cutting needle biopsy is recommended in instances of stage IV disease or when immediate surgery is deemed difficult. Postoperative chemotherapy for Wilms tumor is similar in infants to that in older children who underwent direct nephrectomy, with adjustment of drug doses according to age and body weight based on the experience from previous SIOP studies.[44]

Adult Wilms tumors. The UMBRELLA protocol registers and provides comprehensive guidelines for the management of adults with Wilms tumors, recognizing the long treatment delays and associated poor outcomes experienced by adult patients in the past.[46-49] The diagnosis of Wilms tumor in adults is exceptional and treatment recommendations are based on literature review and broad international and

multidisciplinary consensus, as published in 2011.[46] Adult Wilms tumor is often diagnosed unexpectedly after nephrectomy for a suspected renal cell carcinoma. In rare instances in which the diagnosis of Wilms tumor is histologically proven before surgery, preoperative chemotherapy is recommended, similar to treatment strategies for Wilms tumor diagnosed in childhood. In contrast to the histological classification of childhood Wilms tumors, both focal and diffuse anaplasia are considered high-risk subtypes in adults, as no evidence exists that patients with focal anaplasia have better outcomes.[50]

Adult patients often experience considerable delays before starting postoperative chemotherapy, owing to the time needed to verify histology.[46-49] For this reason, postoperative treatment recommendations for children cannot simply be applied to adult patients. For instance, the treatment regimen for pediatric stage I disease of actinomycin D and vincristine is only advised for a selected group of adult stage I patients without anaplasia. All other adult patients will receive more intensive treatment, either consisting of vincristine and actinomycin D plus doxorubicin for patients with nonanaplastic subtypes, or four drugs (carboplatin, cyclophosphamide, etoposide, and doxorubicin) for anaplastic tumors of any stage. Exceptions can be made in individual instances, in which diagnosis of a stage II Wilms tumor with favourable histology is timely and postoperative chemotherapy can start within 14 days after surgery. Notably, vincristine dose intensity is decreased in guidelines for treatment of adult Wilms tumors compared with standard guidelines for children, as adults more frequently develop severe neurological toxicities.[48]

SURGICAL RECOMMENDATIONS

After preoperative chemotherapy, radical tumor nephrectomy is the standard of care for children with Wilms tumor. The UMBRELLA protocol specifies surgical guidelines and emphasizes the importance of lymph node sampling, stating that the aim should be to sample seven locoregional lymph nodes, for the purpose of accurate staging.[51-53]

NSS is now acceptable for nonsyndromic unilateral Wilms tumors under certain conditions, specified in the UMBRELLA protocol, that include small tumor volume (<300 ml) and the expectation of a substantial remnant kidney function in patients with tumors <300 ml who never had lymph node involvement.[52] A new classification system for NSS, developed by a group of surgeons and pathologists from SIOP-RTSG, was adopted in the UMBRELLA protocol to optimize comparison of patient outcomes (**Table 3**).[54] For bilateral Wilms tumors, discussion with the SIOP-RTSG surgical panel is strongly recommended, in order to assess the feasibility of NSS and minimize the risk of upstaging by incomplete resection of the tumor. Where possible, surgery should be performed in identified reference centres. Issues related to minimally invasive or laparoscopic surgery are addressed in the UMBRELLA protocol, and although not advocated, owing to lack of evidence supporting its safety, these techniques will be

acceptable in selected circumstances, including small, central tumors with a rim of nonmalignant renal tissue, which still enable lymph node sampling. Minimally invasive or laparoscopic surgery should not be done in patients in whom NSS can be safely performed.

Table 3. Classification of nephron-sparing.

Aspect*	Description
Surgical technique	- NSS (A) = Partial Nephrectomy = resection of tumor with a rim of normal renal parenchyma - NSS (B) = Enucleation = resection of tumor without a rim of normal renal parenchyma
Surgical resection margin (SRM)	- Intact pseudo-capsule = (0) - Doubt = (1) - Tumor breach = (2)
Pathological resection margin (PRM)	- Safe rim of renal parenchyma on resection margin, except nephroblastomatosis = (0) - Intact pseudocapsule along the resection margin = (1) - Tumor breach = (2)
Remaining renal parenchyma (RRP)	- A subjective evaluation is done by the surgeon of the percentage of renal parenchyma remaining on the operated kidney = (*n* %) - For example, a polar nephrectomy usually corresponds to a RRP of 70%.

NSS, nephron-sparing surgery *A classification for each case would be reported as follows: NSS(X)-SRM(n)-PRM(n)-RRP(n%). Adapted from Godzinski, J. et al. Current concepts in surgery for Wilms tumor−the risk and function-adapted strategy. Eur. J. Pediatr. Surg. 24, 457–460 (2014). © Georg Thieme Verlag KG.

RADIOTHERAPY RECOMMENDATIONS

In SIOP-2001, around 25% of children with Wilms tumors underwent radiotherapy to the flank and/or metastatic sites. For the UMBRELLA protocol, the radiotherapy guidelines used in SIOP-2001 were refined based on the experience from a 2017 SIOP-2001 analysis and prior COG–NWTS trials (**Table 4-5**).[2, 8, 14, 55] The boost dose to the area of lymph node involvement for stage III intermediate-risk tumors is omitted in the UMBRELLA protocol, based on a 2017 analysis of SIOP-2001 data in which no locoregional control or survival benefit was observed (Davila Fajardo *et al.* unpublished data, manuscript submitted). Moreover, the dosage of whole-lung irradiation was decreased from 15 Gy to 12 Gy in the UMBRELLA protocol, to be in line with previous NWTS experience demonstrating high relapse-free and overall survival (72% and 78% respectively) for favourable-histology tumors after treatment with doxorubicin, actinomycin-D, vincristine, and 12 Gy to the lungs.[13]

Whole-abdominal radiotherapy is indicated for intermediate-risk or high-risk histology tumors with major (visible on imaging or during surgery) preoperative or intraoperative tumor rupture, or macroscopic peritoneal deposits.

Table 4. Radiotherapy guidelines in UMBRELLA SIOP-RTSG 2016 for locoregional disease.

	Stage I (total/ fraction dose)	Stage II (total/ fraction dose)	Stage III (total/ fraction dose)	Stage III (major rupture)‡ (total/fraction dose)
Low-risk	no	no	no	no
Intermediate-risk	no	no	14.41.8 Gy (± 10.8/1.8 Gy)*	15.0/1.5 Gy (± 10.8/1.8 Gy)§
High-risk blastemal-type Wilms tumor	no	no	25.2/1.8 Gy (± 10.8/1.8 Gy)*	19.5/1.5 Gy (± 10.8/1.8 Gy)*
High-risk diffuse anaplasia	no	25.2/1.8 Gy (± 10.8/1.8 Gy)*	25.2/1.8 Gy (± 10.8/1.8 Gy)*	19.5/1.5 Gy (± 10.8/1.8 Gy)*

*Boost dose indicated for localized residual tumor at the time of radiotherapy only. ‡Radiotherapy to the whole abdomen. §Boost only indicated for multiple residual peritoneal deposits (± 4.5/1.5 Gy).

Table 5. Radiotherapy guidelines in UMBRELLA SIOP-RTSG 2016 for metastatic disease.

	Lung (whole ± boost) (total/fraction dose)	Liver (whole ± boost) (total/fraction dose)	Brain (whole ± boost) (total/fraction dose)	Bone (total/fraction dose)
Low-risk	no	no	no	no
Intermediate-risk	12.0/1.5 Gy (± 10–13 Gy)*	14.4/1.8 Gy (± 10.8/1.8 Gy)*	15.0/1.5 Gy (± 10.8/1.8 Gy)*	30.6/1.8 Gy
High-risk	15.0/1.5 Gy (± 15–20 Gy)*	19.8/1.8 Gy (± 16.2/1.8 Gy)*	25.2/1.8 Gy (± 10.8/1.8 Gy)*	30.6/1.8 Gy

*Boost dose indicated for residual tumor at the time of radiotherapy only.

Pulmonary radiotherapy is administered for lung metastases lacking complete response by postoperative week 10. Evidence suggests that the majority of patients achieving a complete response after induction chemotherapy with or without surgery do not need radiotherapy to the lungs, as they have excellent survival even without radiotherapy (5-year EFS 84%, 5-year OS 92%).[21] Patients with viable metastases at surgery or high-risk histology, both of which are associated with poor survival of <40%, are the exception and receive radiotherapy to the lungs.[23] Given the inferior outcome with second-line treatment for patients with disease recurrence in the lung, whole-lung irradiation is recommended for patients who did not receive lung irradiation during first-line treatment, irrespective of histology.[56]

Radiotherapy recommendations are similar for adults and children with Wilms tumors, with the exception of stage II disease. In adult protocols, unlike pediatric protocols, radiotherapy is indicated for all stage II Wilms tumors, as lymph node sampling is often not performed.[46] Only for adult patients that are enrolled in UMBRELLA in time

to confirm negative lymph nodes and intermediate-risk histology can the avoidance of radiotherapy be discussed.

The UMBRELLA protocol also provides a detailed description of the radiotherapy target volumes so that advanced radiotherapy techniques can be applied if they are available. The potential role of proton therapy for flank irradiation in treating Wilms tumors has only been suggested in a dosimetric study, and needs further investigation before implementation in the UMBRELLA protocol.[57]

INTERNATIONAL COLLABORATION

The UMBRELLA protocol will guide treatment of Wilms tumor treatment in over 50 countries in Europe and beyond, making it the largest collaborative SIOP renal tumor protocol published to date, enabling international research to be conducted. In Europe alone, about 1,000 instances of pediatric renal tumors are diagnosed each year. In general, survival is excellent, but the SIOP-RTSG aims to address the current geographic inequalities in childhood cancer survival by providing a standardized approach to diagnosis, risk stratification, and treatment. Furthermore, estimates suggest that ~300 instances of complex renal tumors in Europe would benefit from multidisciplinary discussion of treatment with clinicians at centres of expertise.[5] Examples include most instances of bilateral Wilms tumors, patients with extensive intravascular tumor thrombus or complicated metastatic sites, and advanced diffuse anaplastic Wilms tumors. Patients with these diseases could benefit from international collaboration to access specialized surgical techniques, cardiothoracic expertise, innovative radiotherapy options, and guidance for phase I/II trials. European initiatives like the European Expert Pediatric Oncology Reference Network for Diagnostics and Treatment (ExPO-r-Net) pilot (http://www.expornet.eu/) aim to enhance such collaboration. The EXPO-R-Net is an online consultation platform for which national reference centres have been identified (**Figure 4**), and this platform should contribute to the establishment of international tumor boards, funding for coordinators, IT platforms and logistics, and future outreach to low-income countries.

CONCLUSIONS

As well as providing a useful guideline for routine clinical practice, the UMBRELLA protocol should stimulate international collaboration and research. By standardizing the treatment for all Wilms tumor types, prospectively collected data from a large, homogenous cohort of patients will be available for future validation of biomarkers, treatment stratification, and therapeutic targets. Moreover, the UMBRELLA protocol can serve as the SIOP-RTSG best-available treatment standard. It will be the backbone for new treatment approaches in future phase I/II and randomized trials, in which collaboration with the COG will continue to be sought. Global collaboration is still

necessary for finding effective treatments for the most unfavourable Wilms tumors, such as refractory metastatic, bilateral, and relapsed high-risk disease, and the UMBRELLA protocol will contribute to this aim.

Country	Institution and location
Preliminary European surgical expert referral sites	
Germany	University Hospital of Munich, Munich
Germany	University Hospital of Tuebingen, Tuebingen
Italy	Azienda Ospedaliera, Padova
Italy	Ospedale Pediatrico Bambino Gesù, Rome
Italy	Fondazione IRCCS Istituto Nazionale dei Tumori, Milano
Netherlands	Prinses Maxima Centrum, Utrecht
Poland	Marciniak Hospital, Wroclaw
Poland	Faculty of Medicine, Gdansk
Austria	St. Anna Kinderspital/CCRI, Vienna
France	Armand Trousseau Hospital, Paris
Spain	Hospital Universitario Virgin del Rocio, Seville
Spain	Hospital Vall d'Hebron, Barcelona
UK	Great Ormond Street Hospital, London
Overall subnetwork coordination	
Germany	University Hospital Homburg, Homburg
Netherlands	Princes Maxima Centrum, Utrecht
France	L'Institut d'Hématologie et d'Oncologie Pédiatrique de Lyon, Lyon
Italy	Fondazione IRCCS Istituto Nazionale dei Tumori, Milano
UK	Great Ormond Street Hospital, London

Figure 4. Centres involved in the European Expert Pediatric Oncology Reference Network for Diagnostics and Treatment.

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3

Prognostic significance of age in 5631 patients with Wilms tumor prospectively registered in International Society of Pediatric Oncology (SIOP) 93-01 and 2001

Hol JA, Lopez-Yurda MI, Van Tinteren H, Van Grotel M, Godzinski J, Vujanic GM, Oldenburger F, De Camargo B, Ramírez-Villar GL, Bergeron C, Pritchard-Jones K, Graf N, Van den Heuvel-Eibrink MM

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ABSTRACT

Background

To enhance risk stratification for Wilms tumor (WT) in a pre-operative chemotherapy setting, we explored the prognostic significance and optimal age cutoffs in patients treated according to International Society of Pediatric Oncology Renal Tumor Study Group (SIOP-RTSG) protocols.

Methods

Patients (6 months-18 years) with unilateral WT were selected from prospective SIOP 93–01 and 2001 studies (1993–2016). Martingale residual analysis was used to explore optimal age cutoffs. Outcome according to age was analyzed by uni- and multivariable analysis, adjusted for sex, biopsy (yes/no), stage, histology and tumor volume at surgery.

Results

5631 patients were included; median age was 3.4 years (IQR: 2–5.1). Estimated 5-year event-free survival (EFS) and overall survival (OS) were 85% (95%CI 83.5–85.5) and 93% (95%CI 92.0–93.4). Martingale residual plots detected no optimal age cutoffs. Multivariable analysis showed lower EFS with increasing age(linear trend $P<0.001$). Using previously described age categories, EFS was lower for patients aged 2-4 (HR 1.34, $P = 0.02$), 4-10 (HR 1.83, $P<0.0001$) and 10–18 years (HR 1.74, $P = 0.01$) as compared to patients aged 6 months-2 years. OS was lower for patients 4–10 years (HR 1.67, $P = 0.01$) and 10–18 years (HR 1.87, $P = 0.04$), but not for 2–4 years (HR 1.29, $P = 0.23$). Higher stage, histological risk group and tumor volume were independent adverse prognostic factors.

Conclusion

Although optimal age cutoffs could not be identified, we demonstrated the prognostic significance of age as well as previously described cutoffs for EFS (2 and 4 years) and OS (4 years) in children with WT treated with pre-operative chemotherapy. These findings encourage the consideration of age in the design of future SIOP-RTSG protocols.

INTRODUCTION

As treatment for Wilms tumor (WT) is evolving towards further risk adaptation, there is an increasing interest in additional factors that can help to stratify treatment intensity based on the patient's individual risk. One of these factors appears to be a patient's age at diagnosis. Older age has been suggested to be an adverse prognostic factor for recurrence and mortality [1–4] while younger patients may need less intensive treatment.[5–8]

Treatment stratification of WT has been primarily based on pathological stage and histology. More recently, potential molecular prognostic markers such as copy number changes and loss of heterozygosity (LOH) of specific chromosomal regions are emerging.[9–15] Currently, in addition to tumor weight, LOH 1p/16q, stage and histology, the Children's Oncology Group (COG) includes age in the risk stratification of its most recent protocols.[5,7] So far, the independent prognostic significance of age has not been sufficiently validated in a large cohort of patients treated with pre-operative chemotherapy, as recommended in International Society of Pediatric Oncology Renal Tumor Study Group (SIOP-RTSG) protocols (**S1 Table**).

Age as a prognostic factor was first described in 1976 when D'Angio et al.[1] reported that the addition of postoperative radiation therapy did not improve the already excellent outcomes of patients <2 years with stage I disease, treated with primary surgery in National Wilms tumor Study (NWTS)-1.1 After a pooled analysis of NWTS-1, -2 and -3, the 'very low risk' patients <2 years with stage I, non-anaplastic WT (lymph node sampling required), tumor weight <550 grams, without predisposition syndromes, were subsequently treated with nephrectomy only, in Children's Oncology Group (COG) protocols.[5–8]

Whether the age of 2 years is the optimal cutoff for risk stratification was debated in a later study by the UK Children's Cancer Study Group, suggesting that the age of 4 years may be a more relevant cutoff in the setting of minimal adjuvant chemotherapy.[3] The Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) study that used a cutoff at the age of 2 years, did not find older age to be an independent prognostic factor in stage I-IV WT.[16] By contrast, the relatively small subgroup of WT patients older than 10 years, revealed a particularly poor survival (63–70%) in reports from the Automated Childhood Cancer Information System (ACCIS) and UK Children's Cancer And Leukemia Group, compared to a survival of 80–90% in younger patients.[17, 18]

In the current study, we aimed to assess the prognostic significance of age in a large, prospectively registered cohort of pediatric patients with WT treated with pre-operative chemotherapy according to recent SIOP protocols. Moreover, we aimed to identify relevant age cutoffs for future stratification purposes.

PATIENTS AND METHODS

Patients

Patients with histologically proven stage I-IV WT, aged 6 months-18 years, treated according to SIOP 93–01[19] and SIOP 2001[20] protocols (including the SIOP WT 2001 trial with EudraCT number 2007-004591-39) from 1993–2016 and prospectively registered in the SIOP database, were included in this retrospective analysis. The SIOP-RTSG steering committee approved the research proposal for this specific study and anonymized data were made available to the researchers through statistical reports generated by data scientists of the SIOP-RTSG office. Patients <6 months were excluded as they received separate treatment regimen.[21] Moreover, patients with bilateral disease, non-Wilms tumors or extrarenal tumor sites were excluded. Subsets of patients from the SIOP database had been previously described in several reports.[9, 18–20, 22, 23] For both protocols ethical approval was obtained by ethical committees of all participating countries, and written informed consent for participation was obtained from the parents or legal representatives of the patients.

SIOP 93–01 and SIOP 2001 protocols

Pre-operative chemotherapy consisted of 4 weeks of vincristine and actinomycin-D in case of localized disease, and 6 weeks of vincristine, actinomycin-D and doxorubicin in case of metastatic disease. Biopsy before start of treatment was not recommended as a standard procedure, but was allowed without upstaging if performed by a percutaneous fine needle or trucut procedure. This was a routine procedure in the Children's Cancer and Leukemia Group (CCLG), including the UK and Republic of Ireland, that participated in SIOP 2001 but not SIOP 93–01. Post-operative treatment stratification depended on SIOP stage and histological risk group[24, 25], and evolved over time. In SIOP 93–01, post-operative chemotherapy was randomized for stage I intermediate-risk and anaplastic WT, with the trial arm receiving a shorter treatment regimen, which was subsequently adopted for intermediate-risk WT in SIOP 2001.[19] Non-viable tumor tissue in the renal sinus and perirenal fat was no longer taken into account for upstaging histological risk group in SIOP 2001. Moreover, focal anaplasia (which was considered high-risk in SIOP 93–01) was considered intermediate risk and treated accordingly; while blastemal-type WT was considered high-risk and treated accordingly.[23, 24] For stage II-III intermediate risk tumors, the SIOP 2001 randomized trial provided evidence for omitting doxorubicin, and this was adjusted accordingly from 2011 onwards in the continuation of the SIOP 2001 protocol.[20]

In the current analysis, high-risk tumors included diffuse anaplastic and/or blastemal-type WT after pre-operative chemotherapy. Intermediate risk tumors were either stromal, epithelial, mixed or regressive type or focal anaplasia, while low risk was defined as completely necrotic tumors after pre-operative chemotherapy. Central pathology review was performed for 83.4% of patients in SIOP 2001, and for 94.4% of patients in SIOP

93–01. Tumor volume was not a factor for treatment stratification, with the exception of German Pediatric Oncology Haematology (GPOH) centers, where patients with non-stromal, non-epithelial intermediate risk WT and a tumor volume >500mL at surgery, received 'high-risk' post-operative chemotherapy (four drugs).[26]

Statistical methods

To search for potential relevant prognostic age cutoffs in relation to event-free survival (EFS) and overall survival (OS), martingale residual plots were evaluated.[27] Cox regression models were used to analyze the prognostic significance of age. Variables assessed in univariable analysis were age, sex, biopsy (yes/no), overall stage, histological classification and tumor volume at surgery (radiologically assessed, dichotomized as ≤500 ml and >500 ml). Variables that appeared to be associated with EFS/OS in univariable analysis ($P<0.10$) and/or were known confounders based on previous literature, were entered into the multivariable model, stratified by national/regional group and study protocol (SIOP 2001 and SIOP 93–01). Patient characteristics were compared using Pearson's Chi-Squared test for categorical variables, and Mann Whitney's U-test/ Kruskall-Wallis for continuous variables.

Tumor volume at surgery was missing in 18.3% of cases, and was imputed using multiple imputation techniques (fully conditional method) on 100 generated datasets, assuming it was missing at random. Missing volume was associated with center/consortium and not with patient characteristics, and there were no indications that it might be related to unobserved characteristics or the missing volume itself. Resulting model estimates were combined using SAS PROC MIANALYZE software (version 9.4).

RESULTS

Patient characteristics

Out of 7262 registered patients with histologically proven WT, 5631 met the inclusion criteria (78%) (**S1 Figure**). Baseline characteristics are summarized in **Table 1**.

Table 1. Baseline patient and disease characteristics (*N*=5631).

		TOTAL	
		N	**%**
Protocol	SIOP 93-01	**1980**	***35.2***
	SIOP 2001	**3651**	***64.8***
Sex	Female	3023	*53.7*
	Male	2608	*46.3*
	Missing	0	*0*
Age	6 mths – 2 yrs	1439	*25.6*
	2-4 yrs	1939	*34.4*
	4-10 yrs	2064	*36.7*
	10-18 yrs	189	*3.4*
	Missing	0	*0*
Histology	Low risk	315	*5.6*
	Intermediate risk	4566	*81.1*
	High risk – blastemal type	466	*8.3*
	High risk – diffuse anaplastic	278	*4.9*
	Missing	6	*0.1*
SIOP overall stage	I	2554	*45.5*
	II	1271	*22.6*
	III	949	*16.9*
	IV	857	*15.2*
	Missing	0	*0*
SIOP abdominal stage	I	2766	*49.3*
	II	1491	*26.6*
	III	1354	*24.1*
	Missing	4	*0.1*
Side	Left	2880	*51.2*
	Right	2749	*48.8*
	Missing	2	*0.03*
Biopsy	Yes	1367	*24.3*
	No	4264	*75.7*
	Missing	0	*0*

Table 1. Continued.

		TOTAL	
		N	%
	≤500 ml	3950	*70.1*
Volume at surgery	>500 ml	649	*11.5*
	Missing	1032	*18.3*

Median follow-up at time of data capture was 6.3 years (interquartile range, IQR: 3.0–8.7). Median age at diagnosis was 3.4 years (IQR: 2–5.1) with only 189 patients (3.4%) aged 10 years or older. Median age was 3.6 (IQR: 2–5.2) for females and 3.2 (IQR: 1.9–4.9) for males (P<0.001). The age distribution of females showed two peaks, at around 1 year and 4 years. For males there was an early peak in the age distribution but bimodality was less apparent than for females (**Figure 1**). Forty-five percent (N = 2554) presented with overall stage I disease, 23% (N = 1271) with stage II, 17% (N = 949) with stage III and 15% (N = 857) with stage IV. WT's were histologically classified as low-risk in 5.6% (N = 315), intermediate risk in 82% (N = 4566), high-risk blastemal type in 8.3% (N = 466) and high-risk diffuse anaplastic in 4.9% (N = 278). Biopsies were performed in 208 cases (10.5%) in SIOP 93–01 and 1159 cases (31.7%) in SIOP 2001. Tumor volume at surgery was available for 4599 patients (81.7%), of whom 14.1% (N = 649) had a tumor volume of >500ml at surgery.

Comparison of patient characteristics between age groups

The distribution of stage, histological risk and tumor volume differed between age groups, with the frequency of metastatic disease, high-risk histology types (most markedly for diffuse anaplastic WT) and high-volume tumors increasing with age (**Table 2**).

Optimal age cutoffs

Martingale residual plots (**Figure 2**) suggested that an increase in age was linearly associated with the risk of an event. However, since no specific change point (knot) could be clearly observed, hence, no optimal cutoffs for categorizing age could be identified. Therefore, in further analyses, age was included as a linear factor (per year), as well as categorized according to previous studies at the ages of 2, 4 and 10 years.

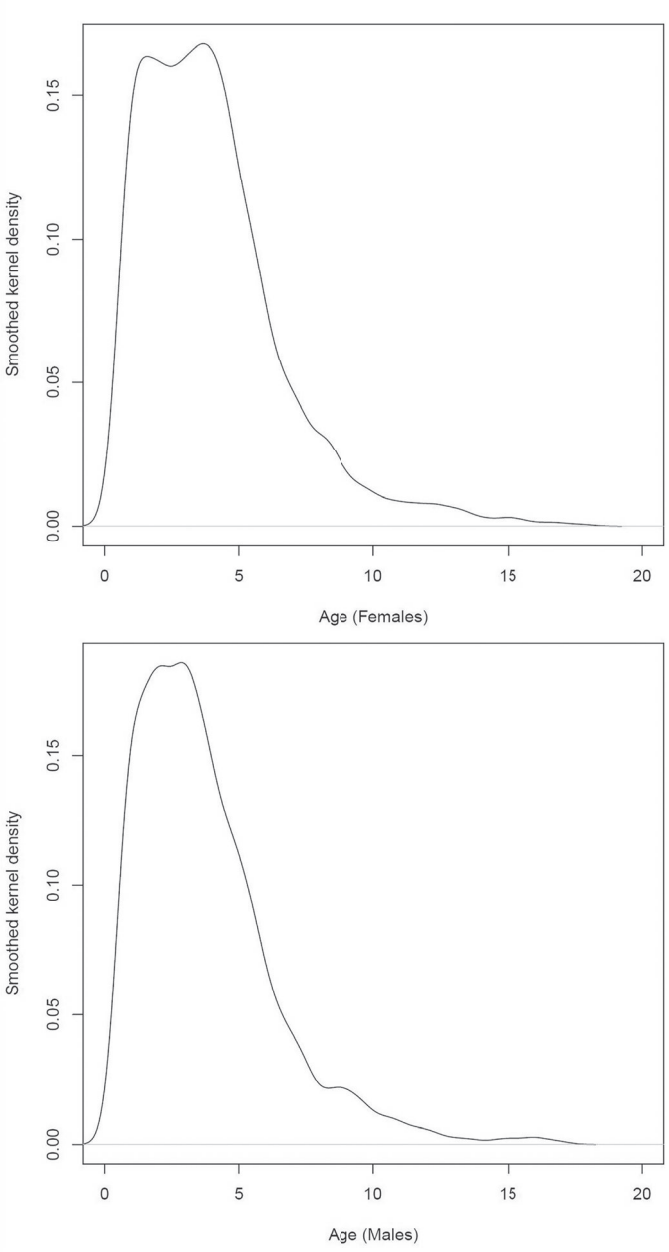

Figure 1. Distribution of age at diagnosis (smoothed kernel density), displayed for females (top) and males (bottom). Median age was 3.6 years (IQR: 2–5.2) for females and 3.2 years (IQR: 1.9–4.9) for males ($P<0.001$).

Table 2. Comparison of patient characteristics between age groups (SIOP 93-01 and SIOP 2001).

		0-2 years		2-4 years		4-10 years		10-18 years		Total		*p-value*
		N	%	N	%	N	%	N	%	N	%	
SIOP stage	Stage I	938	*65.2*	841	*43.3*	722	*35.0*	53	*28.0*	2554	*45.4*	<0.001
	Stage II	272	*18.9*	471	*24.3*	479	*23.2*	49	*25.9*	1271	*22.6*	
	Stage III	163	*11.3*	332	*17.1*	416	*20.2*	38	*20.1*	949	*16.9*	
	Stage IV	66	*4.6*	295	*15.2*	447	*21.7*	49	*25.9*	857	*15.2*	
Histology	Low risk	60	*4.2*	77	*4*	161	*7.8*	17	*9*	315	*5.6*	<0.001
	Intermediate risk	1261	*87.8*	1637	*84.5*	1533	*74.3*	135	*71.8*	4566	*81.2*	
	High risk: diffuse anaplastic	12	*0.8*	81	*4.2*	175	*8.5*	10	*5.3*	278	*4.9*	
	High risk: blastemal type	105	*7.3*	142	*7.3*	193	*9.4*	26	*13.8*	466	*8.3*	
Volume at surgery	≤500 ml	980	*83.2*	1411	*88.4*	1462	*86.9*	97	*68.3*	3950	*85.9*	<0.001
	>500 ml	198	*16.8*	185	*11.6*	221	*13.1*	45	*31.7*	649	*14.1*	

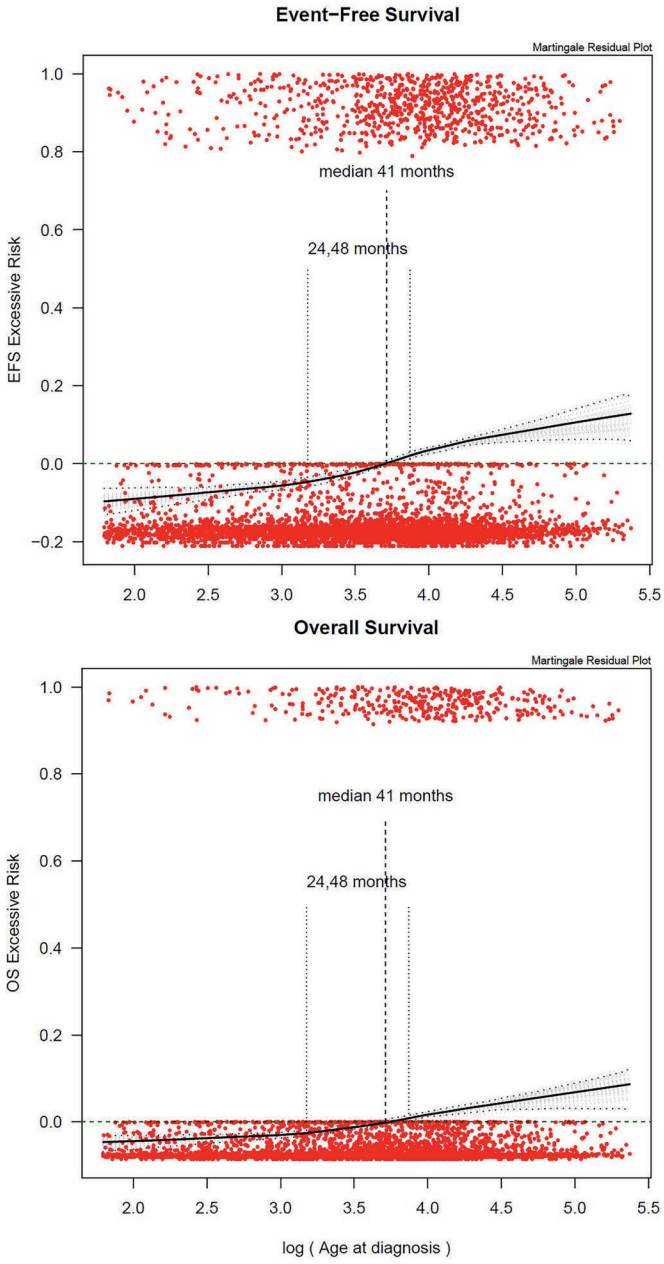

Figure 2. Martingale residual plots showing excessive risk for EFS (top) and OS (bottom) plotted versus log (age). The vertical axis in these plots can be interpreted as excess risk (increasing from bottom to top) and the horizontal axis is age (logarithmic scale). A smoothed curve (LOWESS: locally weighted scatterplot smoothing) is displayed forassessing the functional form for age. Median age (of the log or ratio) is indicated in the martingale residual plots witha dotted line. The grey lines in the plots correspond to the 95% bootstrapped confidence interval. The plots suggest thatan increase in age is linearly associated with the risk of an event. No specific change point (knot) can be clearly observed.

Survival and univariable analysis of prognostic factors

5585 patients were included in the survival analysis, after censoring 46 patients without available follow-up data. Estimated 5-year EFS and OS of the total cohort was 85% (95% CI 83.5–85.5) and 93% (95% CI 92.0–93.4) respectively. A total of 836 events occurred, of which 93.8% (N = 784) were relapses. In univariable analysis, significant differences in EFS and OS were found between age categories 6 months– 2 years, 2–4, 4–10 and ≥10 years (**Figure 3**). The 5-year EFS was 91.2% (95% CI 89.7–92.8) for ages 6 months-2 years, 86.3% (95% CI 84.7–87.9) for 2–4 years, 79.3% (95% CI 77.5–81.1) for 4–10 years and 73.5% (95% CI 66.8–80.9) for 10–18 years (log rank P<0.0001). OS was 96.8% (95% CI 95.9–97.8) for ages 6 months-2 years, 94.1% (95% CI 92.9–95.2) for 2–4 years, 89.5% 95% CI 88.1–90.9) for 4–10 years and 84.6% (95% CI 79.0–90.7) for 10–18 years (P<0.0001).

Multivariable analysis of prognostic factors for EFS

Age categorized as 6 months– 2 years, 2–4 years, 4–10 years and 10–18 years, was a significant prognostic factor for EFS in multivariable analysis (2–4 years: adjusted HR 1.34, P = 0.02, 4–10 years: adjusted HR 1.83, P<0.0001, 10–18 years: adjusted HR 1.74, P = 0.01), after stratifying for national/regional study group and study protocol, and including sex, overall stage, histological risk group, biopsy and tumor volume at surgery. Other independent prognostic factors for EFS were overall stage III and IV, histological subtype (low, intermediate or high-risk) and tumor volume at surgery (**Table 3**).When replacing the age categories by age per year in the multivariable model, the linear trend observed in the Martingale residual plot was confirmed for EFS (adjusted HR 1.06, P<0.0001). The same conclusions were obtained when imputing missing volume (**S2 Table**), and when limiting the analysis to SIOP 2001 patients only (N = 3132, **S3 Table**).

Multivariable analysis of prognostic factors for OS

For OS, the age category 2–4 years (adjusted HR 1.23, P = 0.29) did not retain significance in multivariable analysis. However, patients aged 4–10 (adjusted HR 1.67, P = 0.01) and 10–18 years (adjusted HR 1.87, P = 0.04) revealed lower OS compared to patients aged 6 months– 2 years. Other factors that were significantly associated with OS included overall stage III and IV, histological classification and tumor volume at surgery (**Table 4**). When including age as a continuous variable in the multivariable model, this did not reach statistical significance (adjusted HR 1.04, P = 0.06). These conclusions were maintained when imputing missing volume (**S4 Table**). When limiting the analysis to SIOP 2001 patients, age (categorized or continuous) did not retain significance (N = 3132, **S5 Table**).

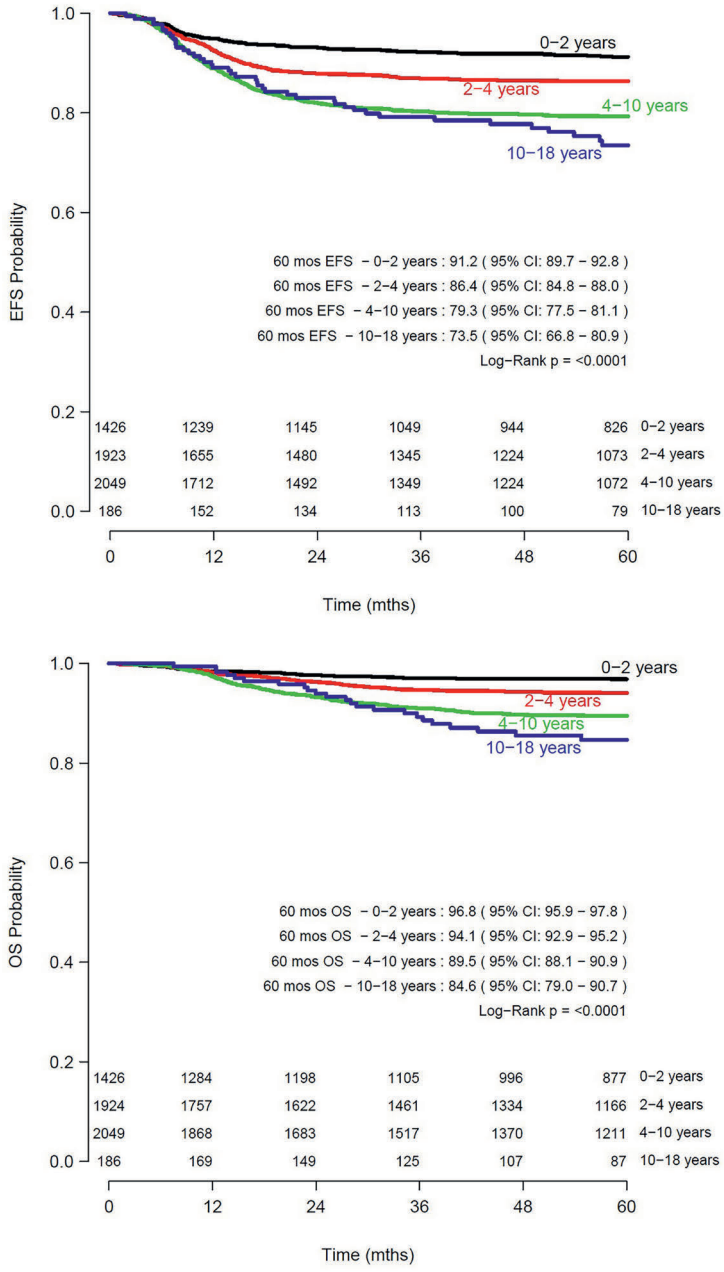

Figure 3. Survival of pediatric patients with Wilms tumor according to age. Kaplan Meier curves showing estimated 5-year event-free survival (EFS) (top) and estimated 5-year overall survival (OS) (bottom) per age category, N = 5585.

Table 3. Prognostic factors for event-free survival (EFS) in patients with Wilms tumor (N=4596).

Characteristic		Events	Univariable		Multivariable, age categorized		Multivariable, age linear	
			HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	Female	361	1		1		1	
	Male	305	0.98 (0.84-1.14)	0.78	1 (0.86-1.17)	1	0.99 (0.85-1.15)	0.88
Age at diagnosis, categorized (years)	0-2	97	1		1			
	2-4	203	1.56 (1.23-1.99)	0.0003	1.34 (1.05-1.72)	0.02		
	4-10	332	2.49 (1.99-3.12)	< 0.0001	1.83 (1.44-2.32)	< 0.0001		
	10-18	34	3.18 (2.15-4.70)	< 0.0001	1.74 (1.15-2.61)	0.01		
Age at diagnosis, linear (years)		666	1.12 (1.09-1.15)	< 0.0001			1.06 (1.03-1.09)	< 0.0001
Overall stage	I	204	1		1		1	
	II	134	1.30 (1.04-1.61)	0.019	1.13 (0.91-1.41)	0.28	1.17 (0.94-1.46)	0.17
	III	146	2.07 (1.68-2.56)	< 0.0001	1.6 (1.28-2)	< 0.0001	1.66 (1.33-2.07)	< 0.0001
	IV	182	3.08 (2.52-3.77)	< 0.0001	2.97 (2.4-3.67)	< 0.0001	3.13 (2.54-3.86)	< 0.0001
Histological risk group	Intermediate risk	471	1		1		1	
	High risk: diffuse Anaplastic	92	14.71 (12.44-17.38)	< 0.0001	2.9 (2.29-3.68)	< 0.0001	3.12 (2.48-3.94)	< 0.0001
	High risk: blastemal type	90	2.48 (1.94-3.17)	< 0.0001	2.16 (1.72-2.72)	< 0.0001	2.13 (1.69-2.69)	< 0.0001
	Low risk	13	0.59 (0.35-1.01)	0.052	0.27 (0.15-0.46)	< 0.0001	0.28 (0.16-0.48)	< 0.0001
Biopsy	No	463	1		1		1	
	Yes	203	1.44 (1.22-1.70)	< 0.0001	1.1 (0.89-1.37)	0.37	1.06 (0.85-1.31)	0.61
Volume at surgery	≤500 ml	502	1		1		1	
	>500 ml	164	2.24 (1.88-2.68)	< 0.0001	2.03 (1.69-2.44)	< 0.0001	1.93 (1.6-2.32)	< 0.0001

Univariable and Multivariable Cox regression models of event-free survival (EFS), stratified by national/regional study group and database (SIOP 93–01 and SIOP 2001), with age categorized and age linear.

Table 4. Prognostic factors for overall survival (OS) in patients with Wilms tumor (N=4596).

Characteristic		Events	Univariable		Multivariable, age categorized		Multivariable, age linear	
			HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	Female	168	1		1		1	
	Male	123	0.84 (0.67-1.06)	0.14	0.85 (0.67-1.07)	0.16	0.84 (0.66-1.06)	0.13
Age at diagnosis, categorized (years)	0-2	31	1		1			
	2-4	83	1.96 (1.30-2.96)	0.0014	1.29 (0.85-1.97)	0.23		
	4-10	159	3.63 (2.47-5.33)	< 0.0001	1.67 (1.11-2.51)	0.01		
	10-18	18	5.16 (2.89-9.23)	< 0.0001	1.87 (1.02-3.44)	0.04		
Age at diagnosis, linear (years)		291	1.15 (1.11-1.19)	< 0.0001			1.04 (1-1.08)	0.06
Overall stage	I	56	1		1		1	
	II	48	1.69 (1.15-2.49)	0.0076	1.43 (0.97-2.11)	0.07	1.47 (1-2.17)	0.05
	III	76	3.91 (2.77-5.52)	< 0.0001	2.76 (1.93-3.94)	< 0.0001	2.86 (2.01-4.09)	< 0.0001
	IV	111	6.65 (4.82-9.17)	< 0.0001	6.78 (4.82-9.53)	< 0.0001	7.14 (5.09-10.01)	< 0.0001
Histological risk group	Intermediate risk	152	1		1		1	
	High risk: diffuse Anaplastic	73	10.13 (7.66-13.40)	< 0.0001	6.91 (5.09-9.39)	< 0.0001	7.38 (5.47-9.95)	< 0.0001
	High risk: blastemal type	57	4.01 (2.96-5.44)	< 0.0001	4.55 (3.31-6.25)	< 0.0001	4.58 (3.33-6.3)	< 0.0001
	Low risk	9	0.86 (0.44-1.68)	0.65	0.53 (0.27-1.05)	0.07	0.55 (0.28-1.09)	0.09
Biopsy	No	192	1		1		1	
	Yes	99	1.66 (1.30-2.11)	< 0.0001	1.03 (0.75-1.4)	0.87	1.03 (0.75-1.41)	0.85
Volume at surgery	≤500 ml	201	1		1		1	
	>500 ml	90	2.99 (2.33-3.83)	< 0.0001	2.23 (1.7-2.91)	< 0.0001	2.19 (1.67-2.85)	< 0.0001

Univariable and Multivariable Cox regression models of overall survival (OS), stratified by national/regional study group and database (SIOP 93-01 and SIOP 2001), with age categorized and age linear.

DISCUSSION

This study, which included 5631 patients with unilateral WT registered over 23 years in the recent SIOP trials, demonstrated that age is an independent prognostic factor for EFS in patients treated with pre-operative chemotherapy. Although optimal age cutoffs for risk stratification could not be identified, the prognostic significance of previously described cutoffs was confirmed for EFS (2 and 4 years) and OS (4 years). Despite the observation that older patients more frequently have a higher stage at diagnosis, high-risk histology types and large-volume tumors, age retained independent prognostic significance. Interestingly, the strong prognostic value of tumor volume >500mL confirmed previous findings from the posthoc analysis of the SIOP 2001 randomized trial.[20, 26] While previous studies have reported conflicting results on the prognostic value of age, depending on sample size and whether age was included as a categorized or continuous variable, our findings are in line with the results described in other large cohorts (>1000 patients, **S1 Table**).

We observed that the presence of diffuse anaplasia increases with age, and is a strong adverse prognostic factor. We could not include molecular markers in the analysis, since copy number status was only available for a subset of the SIOP 2001 cohort (N = 586), as previously described.[9] Gain of 1q and loss/LOH of 1p/16q, which are thought to reflect genomic instability, have been associated with adverse outcome in various reports.[9–15] These and other copy number changes/LOH appear to be more prevalent in older patients.[11, 12, 15] Three recent studies that assessed age, 1q gain and 1p/16q loss/LOH in multivariable analysis[9, 13, 14] found 1q gain to be independently associated with relapse and/or survival, while age and 1p/16q loss/LOH did not retain significance (**S6 Table**). A large study on prognostic molecular markers (*N* = 1114) showed that 1p/16q loss/LOH was not independently associated with EFS when correcting for 1q gain, but suggested prognostic value in the group of patients lacking 1q gain.[11] Age and 1q gain have not been combined in multivariable models with >1000 patients, but will be prospectively validated in the UMBRELLA SIOP-RTSG protocol.[26, 28, 29] Noteworthy, different biomarkers may be important in patients aged <2 years, particularly in a nephrectomy-only setting, where 11p15 status was shown to be associated with relapse.[7, 30] Furthermore, Wilms tumor predisposition syndromes may be a relevant factor to consider in relation to age at diagnosis and survival, but could not be assessed in this study due to incomplete data. Wilms tumor predisposition syndromes have been associated with a younger age at diagnosis and depending on the genetic aberration, a more favorable tumor biology.

On the other hand, these syndromes carry a higher risk of bilateral/second tumors and subsequent renal failure. Wilms tumor predisposition was not always evaluated or recognized in the past, and incompletely registered, as this was beyond the objectives of SIOP 93–01 and 2001. Therefore, we were unable to reliably distinguish between patients with and without a Wilms tumor predisposition syndrome in the current study.

As genomic sequencing becomes more widely implemented in pediatric oncology, more data will become available to unravel these associations in the SIOP-RTSG UMBRELLA protocol.[26]

Other limitations of this study included the long period of time during which treatment evolved based on the results of two successive clinical trials, and missing data requiring imputation. When limiting the analysis to SIOP 2001 only, a more uniform but slightly smaller cohort, age retained significance in relation to EFS but not OS. The two most recent COG protocols have provided some insight into the outcomes after reduced treatment for young patients[5, 7], but this is difficult to compare to SIOP-RTSG protocols, in which response to pre-operative chemotherapy influences risk stratification.[24] Yet, since age seems to emerge as an even more important adverse prognostic factor in reduced therapy settings[3], it seems sensible to remove older patients from minimal treatment strategies. A decision analysis approach, simulating reduced treatment to model the clinical course in different age categories, could aid the design of future guidelines for treatment stratification.[31]

Overall, these results encourage the consideration of age in the design of future SIOP-RTSG protocols, albeit after validation of 1q gain, other molecular markers and age as independent prognostic factors in the UMBRELLA SIOP-RTSG protocol.

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S1 Table. Previously published literature including age as a variable for outcome in Wilms tumor (WT).

Studies with patients treated according to SIOP protocols (with pre-operative chemotherapy):

Reference (year)	N	Population	Outcomes studied	Age prognostic?	Age categories (years)	Univariable analysis (95% CI or *P*-value)	Multivariable analysis (95% CI or *P*-value)
Chagtai et al. (2016)[1]	585	Stage I-IV in SIOP 2001 registry	EFS	No	*Per year*	*Not described*	HR 1.01 (1.00-1.01)
			OS	No	*Per year*	*Not described*	HR 1.00 (0.99-1.01)
Van den Heuvel-Eibrink et al. (2015)[2]	238	Stage I-III blastemal-type histology in SIOP 2001 RCT	EFS	No	2-16 versus <2	*Not described*	HR 2.21 (1.00–5.93)
			OS	No	2-16 versus <2	*Not described*	HR 1.61 (0.55–4.78)
Weirich et al. (2004) [3]	334	Stage I-V in SIOP9 (registered by GPOH), treated with pre-operative chemotherapy	RFS	**Yes**	2-16 versus <2	RR 3.1 (*P*=0.0051)	RR 2.4 (*P*=0.038)
			OS	No	2-16 versus <2	RR 2.7 (*P*=0.0396)	*Eliminated by backward selection of the proportional hazards model*

Studies with patients treated with primary surgery:

Reference (year)	N	Population	Outcomes studied	Age prognostic?	Age categories (years)	Univariable analysis (95% CI)	Multivariable analysis (95% CI)
You et al. (2018) [4]	1924	Stage I-V in SEER registry	OS	**Yes**	*Per year*	HR 1.045 (1.03-1.06)	HR 1.049 (1.032-1.065)
Fernandez et al. (2018)[5]	535	Stage III FH WT in AREN0532	EFS	Univariable only	*Per year*	HR 1.16 (1.08-1.24)	*Age not included in multivariable analysis*
			OS	No	*Per year*	HR 0.94 (0.77-1.15)	
Saltzman et al. (2018)[6]	2340	Stage I-IV FH WT in NCDB	OS	**Yes**	*Per year*	HR 1.106 (1.077-1.136)	HR 1.092 (1.055-1.131)
Spreafico et al. (2017) [7]	453	Stage I-IV in AIEOP WT 2003	EFS	No	2-18 versus <2	HR 0.72 (0.38-1.35)	HR 1.36 (0.74-2.48)
			OS	No	2-18 versus <2	HR 2.76 (1.07-7.15)	HR 1.63 (0.61-4.34)
Jastaniah et al. (2017) [8]	71	Stage I-V in a single center in Saudi Arabia	EFS	No	2-14 versus <2	HR 0.917 (0.195-4.323)	*Age not included in multivariable analysis*
			OS	No	2-14 versus <2	HR 1.030 (0.293-3.618)	

S1 Table. Continued.

Reference (year)	N	Population	Outcomes studied	Age prognostic?	Age categories (years)	Univariable analysis (95% CI)	Multivariable analysis (95% CI)
Irtan et al. (2015) [9]	635	Stage I-IV in UKW3 trial	Distant relapse	**Yes, for ≥4 only**	<2 2-4 4-16	HR 1 HR 1.61 (0.76–3.45) HR 3.87 (1.95–7.67)	HR 1 HR 1.55 (0.66–3.64) HR 3.39 (1.56–7.33)
Wang et al. (2014) [10]	1832	Stage I-V in SEER registry	OS	**Yes**	*Per year*	HR 1.10 (1.05-1.15)	HR 1.09 (1.03-1.14)
Provenzi et al. (2014) [11]	45	Stage I-V in a single center in Brazil	OS	**Yes**	*Per year*	Not described	HR 1.05 (1.01–1.08)
Aronson et al. (2014) [12]	57	WT beyond stage I in a single center in South Africa	EFS	No	0-3 4-7 8-12	Log rank $P = 0.956$	*Not performed*
			OS	No	0-3 4-7 8-12	Log rank $P = 0.797$	*Not performed*
Segers et al. (2013) [13]	331	Stage I-V WT in the United Kingdom (stage V excluded from survival analysis)	EFS	No	<2 2-4 4-14	*Not described*	HR 1 HR 1.01 (0.48-2.13) HR 0.75 (0.34-1.66)
			OS	No	<2 2-4 4-14	*Not described*	HR 1 HR 1.15 (0.42–3.15) HR 0.85 (0.3-2.4)
Perotti et al. (2012) [14]	77	Stage I-IV WT from AIEOP protocols	RFS	No	2-18 versus ≤2	HR 3.776 (0.863-16.520)	HR 2.661 (0.557-12.705)
Gutierrez et al. (2010) [15]	790	Stage I-V in COG and non-COG centres	OS	No	<1 1-4 5-8 9-18	*Not described*	HR 1 HR 3.6 4 (0.50–26.67) HR 3.10 (0.40–23.79) HR 6.10 (0.74–49.95)
Pession et al. (2008) [16]	555	Stage I-V in AIEOP registry	OS	**Yes**	3-14 versus <3	*Not described*	HR 2.3 (1.4-4.1)

S1 Table. Continued.

Reference (year)	N	Population	Outcomes studied	Age prognostic?	Age categories (years)	Univariable analysis (95% CI)	Multivariable analysis (95% CI)
Pritchard-Jones et al. (2003) [17]	242	Stage I FH WT in UKW2 and UKW3 trial	EFS	**Yes, for ≥4 only**	<2 2-4 4-16	93.2% 87.2% 71.3% (Log rank *P* = 0.001)	HR 1 HR 1.53 (0.57-4.11) HR 3.42 (1.45-8.04)
			OS	Univariable only	<2 2-4 4-16	98.2% 95.0% 87.4% (Log rank *P* = 0.01)	*Not described*
Shamberger et al. (1999) [18]	282	Stage I-III from NWTS-4	Local relapse	No	<2 2-4 4-16	RR 1 RR 0.86 (0.39-1.9) RR 2.6 (1.4-5.1)	- - RR 2.0 (0.95-4)
Breslow et al. (1991) [19]	1466	Nonmetastatic FH WT in NWTS-3	Any relapse	**Yes**	<2 2-4 4-16	5.4% 9.5% 16.3% (Log rank *P*<0.001)	Regression coefficient 0.15 +/- SE 0.03
			Tumor death	**Yes**	<2 2-4 4-16	2.1% 4.6% 8.0% (Log rank *P*<0.001)	Regression coefficient 0.15 +/- SE 0.04
Breslow et al. (1985) [20]	632	Nonmetastatic WT in NWTS-2	Any relapse	No	<2 2-4 4-16	10.7% 15.8% 22.4% (Log rank *P*<0.001)	"Explained by correlation with other variables"
			Death	No	<2 2-4 4-16	10.2% 13.0% 16.1% (Log rank *P*=0.075)	
D'Angio et al. (1976) [21]	154	"Group 1" WT patients in NWTS-1	DFS at 2 years	**Yes**	<2 ≥2	0.89 +/- SE 0.04 0.67 +/- SE 0.06	*Not performed*

SIOP: International Society of Pediatric Oncology, NWTS: National Wilms Tumor Study, COG: Children's Oncology Group, AIEOP: Associazione Italiana di Ematologia e Oncologia Pediatrica, OS: overall survival, EFS: event-free survival, RFS: relapse-free survival, HR: hazard ratio, RR: relative risk, FH: favourable histology, NCDB: National Cancer Database.

References for S1 Table:

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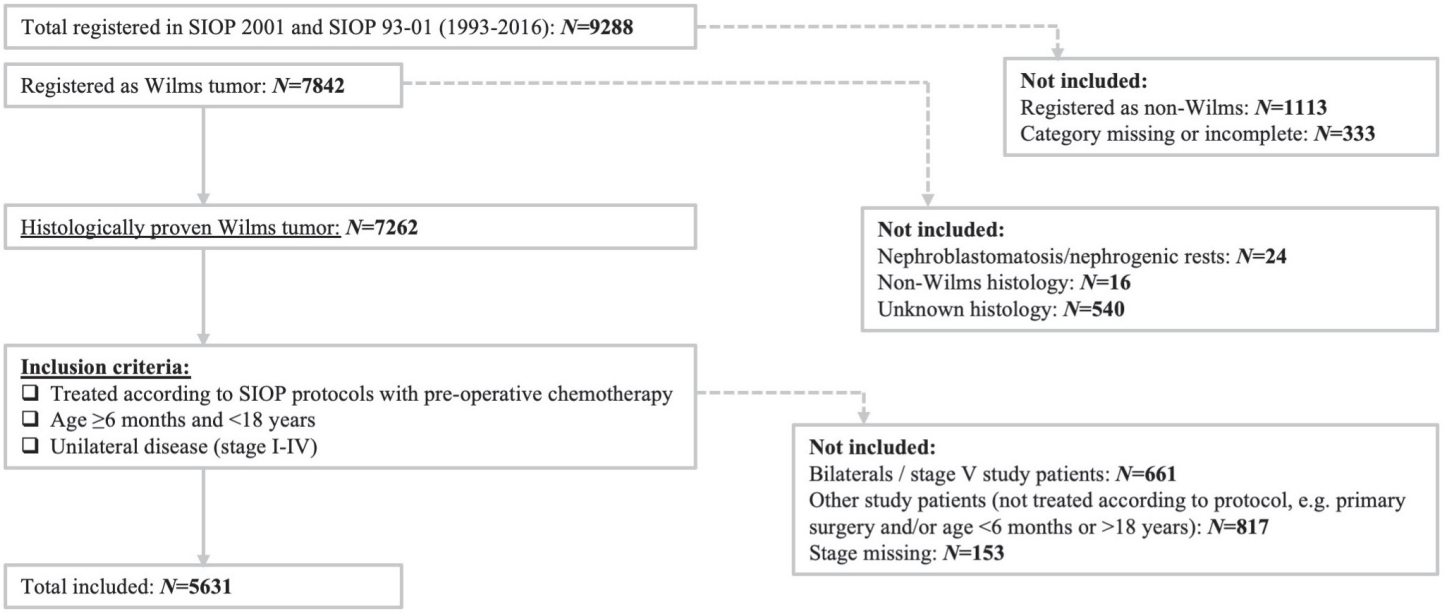

S1 Figure. Inclusion flowchart of patients with histologically proven Wilms tumor from the SIOP 93-01 and SIOP 2001 database.

S2 Table. Prognostic factors for event-free survival (EFS) in patients with Wilms tumor, missing volume imputed (*N*=5631).

Characteristic		Multivariable, age categorized		Multivariable, age linear	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	Female	1		1	
	Male	0.99 (0.86-1.13)	0.85	0.98 (0.85-1.12)	0.73
Age at diagnosis, categorized (years)	0-2	1			
	2-4	1.34 (1.07-1.67)	0.0092		
	4-10	1.80 (1.45-2.22)	<0.0001		
	10-18	1.74 (1.21-2.49)	0.0028		
Age at diagnosis, linear (years)				1.06 (1.03-1.08)	<.0001
Overall stage	I	1			
	II	1.10 (0.90-1.34)	0.37	1.13 (0.93-1.38)	0.22
	III	1.55 (1.27-1.89)	<0.0001	1.61 (1.32-1.95)	<0.0001
	IV	2.85 (2.37-3.44)	<0.0001	2.99 (2.48-3.59)	<0.0001
Histological risk group	Intermediate risk	1			
	High risk: diffuse anaplastic	2.98 (2.41-3.69)	<0.0001	3.18 (2.57-3.92)	<0.0001
	High risk: blastemal type	2.24 (1.83-2.74)	<0.0001	2.23 (1.82-2.73)	<0.0001
	Low risk	0.32 (0.20-0.51)	<0.0001	0.33 (0.20-0.53)	<0.0001
Biopsy	No	1			
	Yes	1.05 (0.87-1.28)	0.601	1.02 (0.84-1.24)	0.86
Volume at surgery	≤500 ml	1			
	>500 ml	1.99 (1.66-2.40)	<0.0001	1.92 (1.60-2.30)	<0.0001

S3 Table. Prognostic factors for event-free survival (EFS) in patients with Wilms tumor, SIOP 2001 only (*N=3132*).

Characteristic		Multivariable, age categorized		Multivariable, age linear	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	Female	1		1	
	Male	1.05	0.62	1.04	
Age at diagnosis, categorized (years)	0-2	1			
	2-4	1.32	0.07		
	4-10	1.82	<0.0001		
	10-18	1.85	0.01		
Age at diagnosis, linear (years)				1.07	<0.0001
Overall stage	I	1		1	
	II	1.23	0.13	1.26	0.09
	III	1.48	0.004	1.51	0.003
	IV	2.87	<0.0001	3.01	<0.0001
Histological risk group	Intermediate risk	1		1	
	High risk: diffuse anaplastic	3.24	<0.0001	3.42	<0.0001
	High risk: blastemal type	1.60	0.002	1.58	0.003
	Low risk	0.25	0.0001	0.26	0.0002
Biopsy	No	1		1	
	Yes	1.19	0.16	1.14	0.32
Volume at surgery	≤500 ml	1		1	
	>500 ml	1.79	<0.0001	1.73	<0.0001

S4 Table. Prognostic factors for overall survival (OS) in patients with Wilms tumor, missing volume imputed (*N*=5631).

Characteristic		Multivariable, age categorized		Multivariable, age linear	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	Female	1		1	
	Male	0.84 (0.68-1.04)	0.11	0.83 (0.68-1.03)	0.09
Age at diagnosis, categorized (years)	0-2	1			
	2-4	1.17 (0.81-1.69)	0.40		
	4-10	1.52 (1.07-2.17)	0.02		
	10-18	1.75 (1.04-2.96)	0.03		
Age at diagnosis, linear (years)				1.04 (1.00-1.07)	0.05
Overall stage	I	1		1	
	II	1.56 (1.09-2.23)	0.01	1.60 (1.12-2.28)	0.01
	III	3.13 (2.26-4.33)	<0.0001	3.22 (2.33-4.46)	<0.0001
	IV	6.98 (5.13-9.50)	<0.0001	7.28 (5.36-9.88)	<0.0001
Histological risk group	Intermediate risk	1		1	
	High risk: diffuse anaplastic	6.80 (5.16-8.96)	<0.0001	7.12 (5.43-9.32)	<0.0001
	High risk: blastemal type	4.44 (3.37-5.85)	<0.0001	4.51 (3.42-5.94)	<0.0001
	Low risk	0.47 (0.25-0.89)	0.02006	0.48 (0.25-0.92)	0.03
Biopsy	No	1		1	
	Yes	1.00 (0.75-1.34)	0.98	1.01 (0.76-1.35)	0.95
Volume at surgery	≤500 ml	1		1	
	>500 ml	2.19 (1.69-2.84)	<0.0001	2.17 (1.67-2.81)	<0.0001

S5 Table. Prognostic factors for overall survival (OS) in patients with Wilms tumor, SIOP 2001 only (*N=3132*).

Characteristic		Multivariable, age categorized		Multivariable, age linear	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	Female	1		1	
	Male	0.83 (0.60-1.11)	0.22	0.82 (0.61-1.11)	0.2
Age at diagnosis, categorized (years)	0-2	1			
	2-4	1.08 (0.63-1.87)	0.78		
	4-10	1.58 (0.94-2.66)	0.08		
	10-18	1.52 (0.7-3.3)	0.29		
Age at diagnosis, linear (years)				1.05 (0.99-1.1)	0.09
Overall stage	I	1		1	
	II	1.93 (1.13-3.32)	0.02	1.96 (1.14-3.35)	0.01
	III	3.49 (2.12-5.72)	<0.0001	3.52 (2.15-5.76)	<0.0001
	IV	9.87 (6.15-15.86)	<0.0001	10.27 (6.42-16.42)	<0.0001
Histological risk group	Intermediate risk	1		1	
	High risk: diffuse anaplastic	10.06 (6.9-14.67)	<0.0001	10.8 (7.48-15.6)	<0.0001
	High risk: blastemal type	3.56 (2.33-5.46)	<0.0001	3.57 (2.33-5.47)	<0.0001
	Low risk	0.51 (0.21-1.28)	0.15	0.53 (0.22-1.33)	0.18
Biopsy	No	1		1	
	Yes	1.13 (0.77-1.66)	0.53	1.11 (0.75-1.63)	0.6
Volume at surgery	≤500 ml	1		1	
	>500 ml	2.13 (1.51-3.0)	<0.0001	2.14 (1.52-3.0)	<0.0001

S6 Table. Previously published studies including 1q gain in multivariable analysis.

Reference (year)	N	Population	Outcomes studied	Factors included in multivariable analysis (95% CI or *P*-value) - *significant factors in bold*				
				Age	**1q gain**	**1p loss/LOH**	**16q loss/LOH**	**Other factors:**
Perotti et al. (2012)[1]	77	Stage I-IV WT in AIEOP registry	RFS	HR 2.66 (0.56-12.71)	HR 6.11 (1.70-21.96)	*Not included*	*Not included*	Stage, presence of diffuse anaplasia
Segers et al. (2013)[2]	331	Stage I-V WT in the United Kingdom (stage V excluded from survival analysis)	EFS	0-2: HR 1 2-4: HR 1.01 (0.48-2.13) >4: HR 0.75 (0.34-1.66)	HR 2.45 (1.17-5.15)	HR 0.37 (0.1-1.37)	HR 1.83 (0.8-4.16)	Stage, 11q-. 16q-, 22-, cx, 14q-, 4q-, 8+, 10+, 12+
			OS	0-2: HR 1 2-4: HR 1.15 (0.42-3.25) >4: HR 0.85 (0.3-2.4)	HR 4.28 (1.59-11.53)	HR 0.23 (0.05-1.1)	HR 1.55 (0.56-4.33)	
Chagtai et al. (2016)[3]	586	Stage I to IV WT in SIOP 2001	EFS	HR 1.01/year (1-1.01)	HR 1.98 (1.27-3.07)	HR 0.98 (0.5-1.91)	HR 1.14 (0.68-1.91)	Gender, stage, histology (high vs. intermediate risk)
			OS	HR 1/year (0.99-1.01)	HR 1.61 (0.83-3.15)	HR 0.67 (0.23-1.89)	HR 1.37 (0.67-2.83)	
Gratias et al. (2016)[4]	1114	Stage I-IV non-anaplastic WT, exluding patients with stage I very-low risk WT in NWTS	EFS	Not included in MVA, however: patients with 1q gain were found to be older (median age 51.5 months) than those without 1q gain (median age 36.5 months), *P*=0.001	RR 2.4 (*P*<0.001)	Not significant in MVA; however: in the group of patients lacking 1q gain; 1p and/or 16q loss seems to be associated with EFS (EFS 84% vs. 91%, *P*=0.03).		Stage
			OS		RR 3.08 (*P*=0.067)*			

WT: Wilms tumor, SIOP: International Society of Pediatric Oncology, NWTS: National Wilms Tumor Study, AIEOP: Associazione Italiana di Ematologia e Oncologia Pediatrica, OS: overall survival, EFS: event-free survival, RFS: relapse-free survival, HR: hazard ratio, RR: relative risk.

References for S6 Table:

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4

Clinical characteristics and outcome of children with WAGR syndrome and Wilms tumor and/or nephroblastomatosis: 30-year SIOP-RTSG experience

Hol JA, Jongmans MCJ, Sudour-Bonnange H, Ramírez-Villar GL, Chowdhury T, Rechnitzer C, Pal N, Schleiermacher G, Karow A, Kuiper RP, De Camargo B, Avcin S, Redzic D, Wachtel A, Segers H, Vujanic GM, Van Tinteren H, Bergeron C, Pritchard-Jones K, Graf N, Van den Heuvel-Eibrink MM, on behalf of the International Society of Pediatric Oncology-Renal Tumor Study Group (SIOP-RTSG)

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ABSTRACT

Background

WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays) is a rare contiguous gene deletion syndrome with a 45% to 60% risk of developing Wilms tumor (WT). Currently, surveillance and treatment recommendations are based on limited evidence.

Methods

Clinical characteristics, treatments, and outcomes were analyzed for patients with WAGR and WT/nephroblastomatosis who were identified through International Society of Pediatric Oncology Renal Tumor Study Group (SIOP-RTSG) registries and the SIOP-RTSG network (1989-2019). Events were defined as relapse, metachronous tumors, or death.

Results

Forty-three patients were identified. The median age at WT/nephroblastomatosis diagnosis was 22 months (range, 6-44 months). The overall stage was available for 40 patients, including 15 (37.5%) with bilateral disease and none with metastatic disease. Histology was available for 42 patients; six nephroblastomatosis without further WT and 36 WT, including 19 stromal WT (52.8%), 12 mixed WT (33.3%), one regressive WT (2.8%) and two other/indeterminable WT (5.6%). Blastemal type WT occurred in two patients (5.6%) after prolonged treatment for nephroblastomatosis; anaplasia was not reported. Nephrogenic rests were present in 78.9%. Among patients with WT, the five-year event-free survival rate was 84.3% (95% confidence interval, 72.4%-98.1%), and the overall survival rate was 91.2% (95% confidence interval, 82.1%-100%). Events (n = 6) did not include relapse, but contralateral tumor development (n = 3) occurred up to seven years after the initial diagnosis, and three deaths were related to hepatotoxicity (n = 2) and obstructive ileus (n = 1).

Conclusion

Patients with WAGR have a high rate of bilateral disease and no metastatic or anaplastic tumors. Although they can be treated according to existing WT protocols, intensive monitoring of toxicity and surveillance of the remaining kidney(s) are advised.

INTRODUCTION

WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays) is caused by a rare contiguous germline gene deletion involving chromosome band 11p13. Children with WAGR syndrome have a 45% to 60% risk of developing Wilms tumor (WT).[1-3] Currently, surveillance and treatment recommendations for children with WAGR syndrome are based on limited evidence.

Historically, WAGR syndrome has played an important role in our understanding of WT genetics; it contributed to the identification of *WT1*, the first WT predisposition gene to be identified, in 1990.[4-9] The genetic diagnosis of WAGR syndrome requires the involvement of both *WT1* and the aniridia gene *PAX6* in the deletion, whereas patients with isolated *PAX6* deletions are not at risk of developing WT.[3] The size of the deletion as well as the phenotype of patients with WAGR syndrome can vary widely, and they only partially depend on whether or not additional genes such as *BDNF* are involved.[10] One previous report, based on North American National Wilms Tumor Studies (NWTSs) 1 to 5, described the tumor characteristics, treatments, and outcomes of a cohort of patients with WAGR who developed WT and were treated according to consecutive NWTS protocols without preoperative chemotherapy.[2] However, the characteristics and outcomes of patients with WAGR and WT registered for the International Society of Pediatric Oncology Renal Tumor Study Group (SIOP-RTSG) protocols, according to which preoperative chemotherapy is administered, have never been described. Currently, also in North American treatment protocols (Children's Oncology Group), preoperative chemotherapy is recommended for children with a genetic predisposition.[11] Here, we report the clinical and tumor characteristics and outcomes of patients with WAGR syndrome who developed WT and/or nephroblastomatosis and were identified through the two last SIOP-RTSG protocol registries and the SIOP-RTSG network in order to support surveillance and treatment recommendations.

MATERIALS AND METHODS

Patients

In the SIOP-RTSG studies (SIOP 93-01[12] and SIOP 2001[13]), patients were prospectively registered from 1993 onward, in some countries up to and including 2019. These studies were not designed to register tumor predisposition syndromes, but the presence or absence of aniridia was recorded. The SIOP-RTSG steering committee approved the research proposal for the current analysis, and we retrospectively identified all patients with aniridia in the SIOP 93-01 and SIOP 2001 databases. Patients diagnosed before 2007 may have been previously reported by Van Heyningen et al,[3] but because this study did not describe WT characteristics or outcomes, we did not exclude patients with possible overlap.

By using SIOP study IDs, national and/or local principal investigators (PIs) were requested to confirm the diagnosis of WAGR and to complete missing data. Patients for whom the diagnosis of WAGR could not be confirmed were excluded from the analysis, whereas additional patients with WAGR and WT, identified by national and/or local PIs, were added to the series. They included ten patients who were not in the central SIOP databases but were registered locally and three patients who were registered on prior or subsequent SIOP protocols (SIOP 9[14] and SIOP-RTSG UMBRELLA[15]).

For SIOP protocols, ethical approval was obtained from ethics committees of all participating countries, and written informed consent for participation was obtained from the parents or legal representatives of the patients. For those patients not registered in the central databases, national and/or local PIs confirmed that informed consent was obtained.

Additional data collection

National and/or local PIs were requested to complete an additional data collection form for each patient (see the supporting information) to obtain clinical information on various items, including the type of genetic testing, age at diagnosis of WAGR syndrome, presentation and symptoms of WT (symptomatic vs asymptomatic), birth weight, congenital abnormalities, cognitive impairment, chronic kidney disease status, and other clinical findings. Chronic kidney disease was defined as a decreased estimated glomerular filtration rate (eGFR; not further specified) and/or proteinuria 2+, at least on dipstick testing. End-stage renal disease was defined as "requiring dialysis and/or kidney transplantation."

Stage and histology

Stage and histology were classified according to the SIOP-RTSG staging system and histological classifications.[16,17] Bilateral disease was defined as synchronous bilateral WT, bilateral nephroblastomatosis, or WT with contralateral nephroblastomatosis.

For the analysis of the overall stage at diagnosis, patients with metachronous tumors were considered unilateral if they had unilateral disease at their initial diagnosis. A local (abdominal) stage could be assigned only to patients with a diagnosis of WT. Nephroblastomatosis was defined as the presence of multiple or diffuse nephrogenic rests visible on imaging studies and, where possible, confirmed histologically. In the current SIOP-RTSG histological classification, histological subtypes are assigned after preoperative chemotherapy and include completely necrotic WT (low risk); stromal, epithelial, mixed, or regressive WT and WT with focal anaplasia (intermediate risk); and diffuse anaplastic or blastemaltype WT (high risk). For the majority of tumors registered in SIOP-RTSG studies, a central pathology review was performed by national and/or regional pathology panels as well as the international SIOP-RTSG pathology panel.[18] For the current analysis, the reviewed histological diagnosis was used if available.

Statistical methods

Frequency distributions for age at diagnosis and tumor volume were analyzed nonparametrically. Event-free survival and overall survival analysis was performed with the Kaplan-Meier method. Patients diagnosed with nephroblastomatosis without further WT were excluded from the survival analysis, whereas patients who were initially diagnosed with nephroblastomatosis but went on to develop WT were included. The survival time was defined as the time from the diagnosis of WT to an event or last follow-up. Events were defined as relapse, the development of metachronous tumors, or death.

RESULTS

In the SIOP 93-01 and SIOP 2001 databases, aniridia was recorded in 47 of 7842 patients with WT (0.6%). After further exploration, the diagnosis of WAGR could not be confirmed in 17 of the 47 patients, and they were, therefore, excluded: 12 did not have aniridia (they had been misclassified), and for the remaining five patients, we received no response from national/local

PIs. Thirteen additional cases with WAGR and WT that were identified by national/local PIs but apparently were not registered in the central SIOP 93-01 and SIOP 2001 databases were subsequently added to this study (**Figure 1**). Overall, a total of 43 patients (18 phenotypic males and 25 phenotypic females) were included (**Table 1**). Patients had been treated according to the

SIOP 93-01 protocol (n = 8, 18.6%), the SIOP 2001 protocol (n = 31, 72.1%), the SIOP 9 protocol (n = 2, 4.7%), or the SIOP-RTSG UMBRELLA protocol (n = 1, 2.3%). The treatment protocol was not specified for one patient (2.3%).

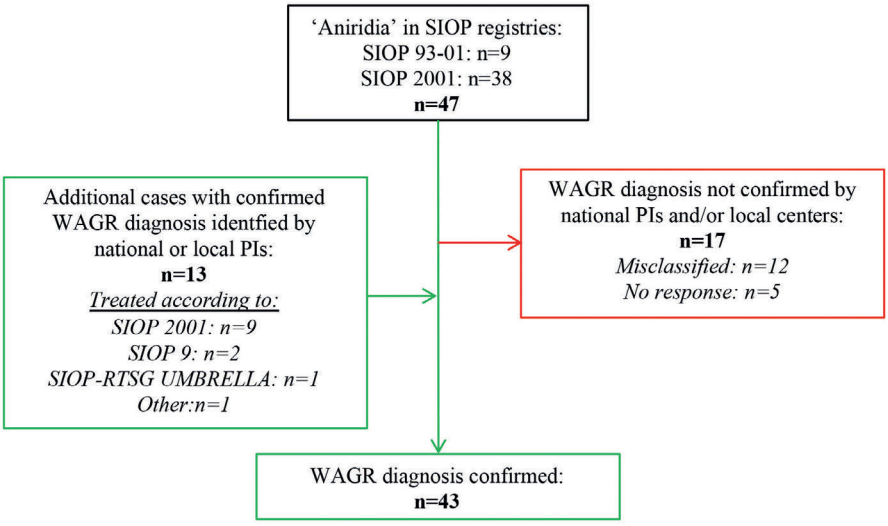

Figure 1. Inclusion of patients with WAGR syndrome and Wilms tumor/nephroblastomatosis based on aniridia registration in the SIOP 93-01 and SIOP 2001 registries. PI: principal investigator; SIOP: International Society of Pediatric Oncology; SIOP-RTSG: International Society of Pediatric Oncology Renal Tumor Study Group; WAGR: Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays.

Table 1. Identified Patients With WAGR Syndrome and WT Based on the SIOP 93-01 and SIOP 2001 Registries (n = 43).

#	Protocol	M/F	Age at WT (mths)	Unilateral/ Bilateral	Abd. stage	Pre-operative Tx (wks)	Surgery	Histology	Nephro-genic rests	Notes	FU (mths)
1	SIOP 2001	F	15	Unilateral	II	4	TN	Stromal-type WT	ILNR	-	96
2	SIOP 2001	F	21	Unilateral[a]	I	None	TN	Stromal-type WT	ILNR	Contralateral tumor at age 9yrs	119
3	SIOP 2001	F	38	NA	NA	4	TN & NSS	Nephroblastomatosis	ILNR	-	5 [b]
4	SIOP 2001	M	14	Bilateral	NA	26	TN & NSS	L: Nephroblastomatosis R: Nephroblastomatosis*	ILNR	*Progression to blastemal type WT 12mths after Dx	38
5	SIOP 2001	M	39	Unilateral	I	4	TN	Regressive-type WT	ILNR	-	152
6	SIOP 2001	M	18	Bilateral	NA	3	None	NA	NA	Death due to hepatic failure 3wks after Dx	0 †
7	SIOP 2001	F	25	Unilateral[a]	I	4	TN	Stromal-type WT	No	Contralateral tumor at age 3yrs	39
8	SIOP 2001	F	25	Bilateral	II	5	TN & NSS	L: Stromal-type WT R: Nephroblastomatosis	ILNR	-	114
9	SIOP 2001	M	36	Unilateral	III	6	TN	Mixed-type WT	ILNR	ESRD at age 16yrs	158
10	SIOP 2001	F	13	Unilateral	II	4	TN	Stromal-type WT	ILNR	-	104
11	SIOP 2001	M	20	Unilateral	I	6	NA	Stromal-type WT	ILNR	-	8
12	SIOP 2001	F	23	Unilateral	III	4	NA	Stromal-type WT	No	-	95
13	SIOP 2001	F	12	Bilateral	NA	22	NSS	L: Nephroblastomatosis (radiology only) R: Nephroblastomatosis	ILNR	-	79 [b]
14	SIOP 2001	F	14	Unilateral	I	4	NSS	Mixed-type WT	ILNR	-	0
15	SIOP 2001	F	37	Unilateral	III	4	TN	Stromal-type WT	No	-	132
16	SIOP 2001	F	35	Unilateral	NA	4	NSS	Nephroblastomatosis	ILNR	Death, cause not specified	9 † [b]
17	SIOP 2001	M	26	Bilateral	I	8	NSS	L: Mixed-type WT R: Stromal-type WT	ILNR	-	118

Table 1. Continued.

#	Protocol	M/F	Age at WT (mths)	Unilateral/ Bilateral	Abd. stage	Pre-operative Tx (wks)	Surgery	Histology	Nephro-genic rests	Notes	FU (mths)
18	SIOP 2001	F	9	Bilateral	I	5	TN & NSS	L: Stromal-type WT R: Stromal-type WT	No	-	189
19	SIOP 2001	F	23	Unilateral	I	4	TN	Stromal-type WT	No	-	183
20	SIOP 2001	M	22	Unilateral	I	4	TN	Stromal-type WT	ILNR	-	142
21	SIOP 2001	F	16	Unilateral	III	4	TN	Stromal-type WT	PLNR+ ILNR	-	0
22	SIOP 2001	M	7	Unilateral	I	4	NSS	Stromal-type WT	ILNR	-	95
23	SIOP 9	M	20	Bilateral	I	4	None	Mixed-type WT (L/R not specified)	NA	Death due to hepatic failure 10 days after Dx	0 †
24	SIOP 9	M	27	Unilateral	I	4	NA	Mixed-type WT	NA	-	26
25	SIOP 93-01	F	11	Bilateral	I	NA	NSS	L: Nephroblastomatosis R: Mixed-type WT	Yes	Obstructive ileus and death, 19mths after Dx	19 †
26	SIOP 93-01	F	27	Unilateral	I	4	TN	Stromal-type WT	Yes	-	166
27	SIOP 93-01	F	36	Bilateral	NA	NA	TN & NSS	L: Nephroblastomatosis R: Nephroblastomatosis	Yes	-	155 [b]
28	SIOP 93-01	F	19	Unilateral	I	NA	NSS	Mixed-type WT	Yes	-	91
29	SIOP 93-01	M	32	Unilateral	NA	4	NA	Nephroblastomatosis	Yes	-	34 [b]
30	SIOP 93-01	F	16	Unilateral	I	4	TN	Stromal-type WT	No	-	45
31	SIOP 93-01	F	31	Unilateral	II	4	TN	Stromal-type WT	No	-	107
32	SIOP 93-01	M	26	Bilateral	I	4	TN	L: No histology R: Indeterminable	NA	-	96
33	SIOP 2001	M	12	Unilateral	I	4	TN	Mixed-type WT	ILNR	-	45
34	SIOP 2001	M	22	NA	NA	NA	TN	Nephroblastomatosis	ILNR	SAE, no relapse	141 [b]
35	SIOP 2001	F	23	Unilateral	I	NA	TN	Stromal-type WT	ILNR	-	115
36	SIOP 2001	M	44	NA	NA	NA	NSS	Mixed-type WT	PLNR	-	50

Table 1. Continued.

#	Protocol	M/F	Age at WT (mths)	Unilateral/ Bilateral	Abd. stage	Pre-operative Tx (wks)	Surgery	Histology	Nephro-genic rests	Notes	FU (mths)
37	SIOP 2001	M	21	Bilateral	NA	NA	TN & NSS	L: Nephroblastomatosis* R: Nephroblastomatosis	ILNR	*Progression to blastemal type WT 11mths after Dx	16
38	SIOP 2001	M	16	Bilateral	NA	NA	NSS	L: Nephroblastomatosis (radiology only) R: Stromal-type WT	Yes	-	11
39	SIOP 2001	F	16	Bilateral	I	8	NSS	L: Nephroblastomatosis R: 'Non-anaplastic'	Yes	-	148
40	Other	M	28	Unilateral	II	None	TN	Mixed-type WT	No	-	22
41	SIOP 2001	F	6	Bilateral	NA	8	TN & NSS	L: Nephroblastomatosis* R: Nephroblastomatosis*	PLNR+ ILNR	*Progression to stromal/Mixed-type WT, 13 and 55 mths after intitial Dx	128
42	SIOP-RTSG UMBRELLA	F	24	Bilateral	II	12	TN	L: Stromal-type WT R: Nephroblastomatosis (radiology only)	ILNR	-	7
43	SIOP 2001	F	39	Unilateral	I	None	NSS	Mixed-type WT	NA	-	110

Legend for Table 1: ESRD: end-stage renal disease; F: female; ILNR: intralobar nephrogenic rests; L: left; M: male; NA: not available; NSS: nephron-sparing surgery; PLNR: perilobar nephrogenic rests; R: right; SAE: serious adverse event; SIOP: International Society of Pediatric Oncology; SIOP-RTSG: International Society of Pediatric Oncology Renal Tumor Study Group; TN: total nephrectomy; WAGR: Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays; WT: Wilms tumor.
[a]Unilateral at diagnosis but developed metachronous disease.
[b]Not included in the survival analysis (nephroblastomatosis only).
†The patient died

WAGR diagnosis

For 38 of the 43 patients, the diagnosis of WAGR had been established by genetic testing, whereas the other five patients had been diagnosed with WAGR on the basis of their clinical characteristics alone. Details on the type of genetic testing were available for 16 cases, and the tests included fluorescence in situ hybridization (n = 10), array comparative genomic hybridization (n = 2), karyotyping (n = 2), a single-nucleotide polymorphism array (n = 1), and quantitative polymerase chain reaction (n = 1). The exact span of the deletion was available for only 5 cases and ranged from 5 to 14 Mb in size. In one case, the deletion was mosaic (patient 33 in **Table 1**). The median age at the diagnosis of WAGR syndrome, available for 19 patients, was two months (range, 0-47 months).

Presentation, stage, and preoperative treatment

The median age at WT/nephroblastomatosis presentation was 22 months (range, 6-44 months). The majority of the tumors were asymptomatic and were detected by surveillance (27 of 39, 69.2%), whereas 12 patients (12 of 39, 30.8%) presented with a palpable/visible abdominal mass and/or other symptoms such as hematuria. Among these 12 patients, 3 had been previously diagnosed with WAGR syndrome, and 2 had not yet been diagnosed with WAGR syndrome; for seven patients, this information was not available. In four cases, the presence or absence of symptoms was not specified.

The overall stage was available for 40 patients. Fifteen patients (15 of 40, 37.5%) had bilateral disease at diagnosis. This included bilateral nephroblastomatosis (n = 5; 3 progressed to WT on 1 or both sides), unilateral WT with contralateral nephroblastomatosis (n = 5), and bilateral WT (n = 2); in 3 patients with bilateral disease, it was not known whether they had bilateral WT or (a combination of WT and) nephroblastomatosis. None of the patients had metastatic disease. The local (abdominal) stage was available for 31 patients and included stage I for 21 (67.7%), stage II for 6 (19.4%), and stage III for 4 (12.9%).

Information regarding preoperative treatment (yes/no) was available for 42 patients; 39 of these patients (92.9%) received preoperative chemotherapy, including actinomycin D and vincristine in 30 cases and doxorubicin in two cases. The type of preoperative treatment was not available for the other seven patients. The median duration of preoperative treatment was eight weeks for bilateral cases (range, 4-26 weeks; missing in five cases) and four weeks for unilateral cases (range, 4-6 weeks; missing in eight cases).

Tumor volume and response to preoperative treatment

The median tumor volume at diagnosis, available for 36 patients, was 46.5 mL (range, 1-659 mL). Three patients presented with tumors larger than 500 mL, and all of these patients had been symptomatic at diagnosis. Tumors that were symptomatic at diagnosis had a median volume of 375 mL (range, 4.2-659 mL), whereas tumors

detected by surveillance had a median volume of 18 mL (range, 1-396 mL; *P* = .001). For 28 patients, the response to preoperative chemotherapy was recorded; 14 patients showed a decrease in tumor volume (14 of 28, 50%), two patients revealed a stable tumor volume (2 of 28, 7.1%), and 12 patients revealed tumor growth (12 of 28, 42.9%) during preoperative treatment. The histological subtype of tumors that increased in volume during preoperative treatment was the stromal type (n = 6), the mixed type (n = 2), or nephroblastomatosis (n = 4).

Surgery

Two patients with bilateral disease died of hepatic failure before surgery (patients 6 and 23 in **Table 1**). Among the other 13 patients with bilateral disease, 11 (85%) underwent nephron-sparing surgery (NSS), including bilateral NSS (n = 4), unilateral NSS (no surgery on the other side; n = 2), and NSS preceded or followed by total nephrectomy on the contralateral side (n = 5). In two patients with bilateral disease, only a unilateral total nephrectomy was performed (patients 32 and 42 in **Table 1**). The type of surgery was specified for 21 patients with unilateral disease: NSS for five (23.8%) and total nephrectomy for 16 (76.2%).

Histological subtype and nephrogenic rests

The histological subtype was available for 42 patients (with central review available for 32 of 42, 76.2%). Six patients (6 of 42, 14.3%) were diagnosed with nephroblastomatosis only. This was histologically confirmed in five patients; one patient with bilateral disease underwent unilateral resection (showing nephroblastomatosis), whereas the other kidney was diagnosed with nephroblastomatosis on the basis of imaging (patient 13 in Table 1). Three patients who were initially diagnosed with bilateral nephroblastomatosis on imaging experienced disease progression and were diagnosed with WT after histological assessment (11-13 months after their first presentation; patients 4, 37, and 41 in **Table 1**).

Among patients with WT, the histological subtypes (n = 36) included stromal WT in 19 (52.8%), mixed WT in 12 (33.3%), regressive WT in one (2.8%) and other/ indeterminable WT in two (5.6%). Blastemal-type WT occurred in two patients (5.6%) after prolonged treatment for nephroblastomatosis, whereas (focal or diffuse) anaplasia was not reported.

Upon histological assessment, nephroblastomatosis or nephrogenic rests were present in 30 of 38 patients (78.9%), including patients with intralobar (n = 20), perilobar (n = 1), or both intralobar and perilobar rests (n = 2). For the remaining seven patients, the type of nephrogenic rests (intra- or perilobar) was not specified.

Survival and events

Six patients with nephroblastomatosis only, without further (progression to) WT, were excluded from the survival analysis. Among these patients, one death (cause not specified) occurred after nine months, and one serious adverse event (not further specified) occurred after 141 months of follow-up. Survival data were subsequently available for 37 patients with WT (including one patient without a histological diagnosis because the patient died before surgery) with a median follow-up of 95 months (range, 0-189 months). The estimated five-year event-free survival rate was 84.3% (95% confidence interval, 72.4%-98.1%; **Figure 2**), and the overall survival rate was 91.2% (95% confidence interval, 82.1%-100%; **Figure 3**). Events occurred in six patients, including three patients (patients 2, 7, and 41 in **Table 1**) who developed metachronous contralateral tumors. All three patients had been treated for stromal-type WT, and the contralateral tumors occurred one, seven and three years after the first tumor at the ages of three, nine and five years, respectively. These three patients were alive at last follow-up.

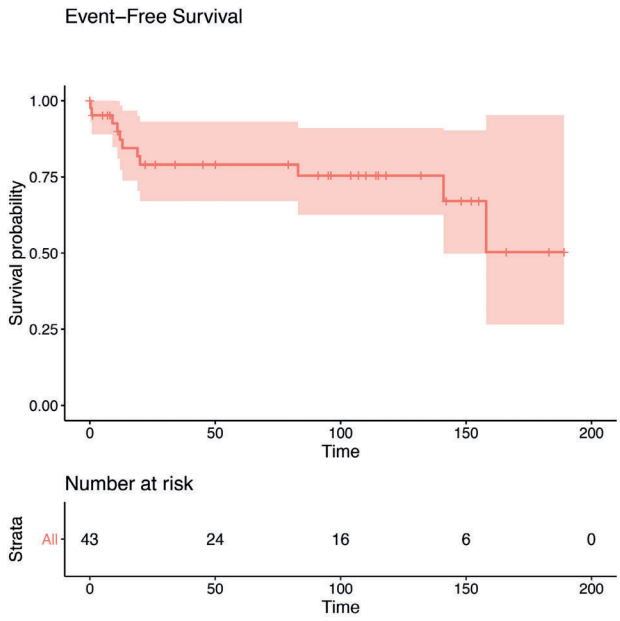

Figure 2. Kaplan-Meier curve showing estimated event-free survival and 95% confidence intervals for patients with WAGR syndrome and Wilms tumor (n = 37). WAGR: Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays.

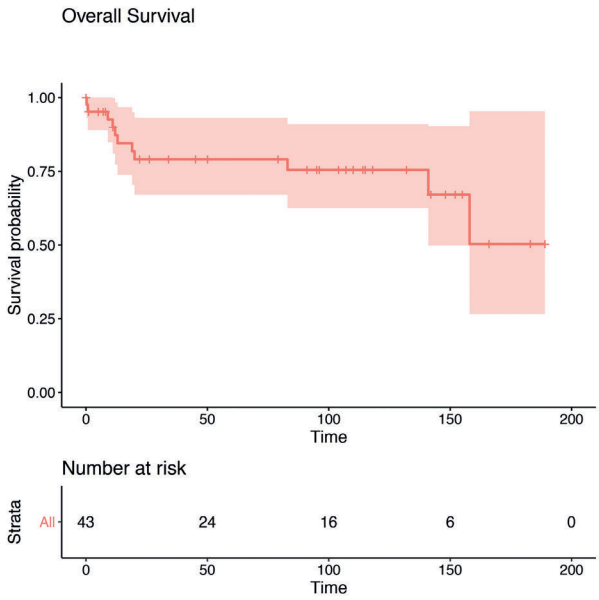

Figure 3. Kaplan-Meier curve showing estimated overall survival and 95% confidence intervals for patients with WAGR syndrome and Wilms tumor (n = 37). WAGR: Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays.

Among patients with WT, three deaths occurred, including the deaths of two patients who died of hepatoxicity as a result of sinusoidal obstruction syndrome during preoperative chemotherapy (patients 6 and 23 in **Table 1**). In one of these patients, an incorrect dose (overdose) of actinomycin D had been administered (patient 23). The third patient died 19 months after diagnosis, and at this same date, obstructive ileus was registered as an event (patient 25 in **Table 1**). Because this patient was treated more than 20 years ago, we were unable to confirm the exact cause of death.

Chronic kidney disease

Data on chronic kidney disease were collected on the additional data collection form (see the supporting information) and were subsequently available for 20 patients. In five of these 20 patients (25%), a decreased eGFR, proteinuria (2+), or both were reported, with the age of onset varying from three to 16 years (time to onset, 2-13 years after WT diagnosis). One of these patients had been treated for bilateral disease, whereas the other four patients had been treated for unilateral WT (unilateral nephrectomy in three cases and the surgery type not specified in the fourth case). One patient, treated for unilateral WT, was reported to have end-stage renal disease at the age of 16 years (patient 9 in **Table 1**).

Additional clinical conditions

The additional data collection form (see the supporting information) was completed for 30 of the 43 patients, and for many items, the requested data could not be retrieved. Birth weight was available for 15 patients, and three of these patients (20%) were reported to have a birth weight below the 10th percentile for gestational age; the remaining 12 patients had a birth weight within the normal range. Congenital abnormalities other than aniridia, including ocular and genitourinary abnormalities as well as polydactyly (n = 2), macrocephaly (n = 1), Pierre- Robin sequence (n = 1), and atrial septal defects (n = 2), were reported in 13 patients (**Table 2**). Cognitive impairment was reported in 18 of 22 patients (81.8%) with available data. The four patients who were reported to have normal cognitive function were two to 16 years old at the last follow-up. Additional clinical findings are summarized in **Table 2**. Other tumors such as gonadoblastoma were not reported.

Table 2. Additional findings in patients with WAGR syndrome and Wilms tumor and/or nephroblastomatosis, as reported in the additional data collection form (n=30).

Category	Finding	Occurrences (N)
Ocular findings other than aniridia	Cataracts	10
	Peter's anomaly	2
	Nystagmus	5
	Ocular hypotonia	1
	Retinal detachment	2
Genitourinary findings	Cryptorchidism	5
	Hypospadias	2
	Renal cysts	1
	Ovarian cysts	1
	'Renal tissue disorganization'	1
	Ureteric reflux	1
	Horseshoe kidney	1
Neurologic findings	Hypotonia	1
	Hypertonia	1
	Epilepsy	1
Metabolic findings	Obesity	4
	Hypothyroidism	1
	Insulin resistance	1
Pulmonary findings	Lung hypoplasia	1
Behavioral findings	Attention deficit (hyperactivity) disorder	4
	Aggression	1
	Autism (spectrum)	3
	Sleep disturbances	1

Table 2. Continued.

Category	Finding	Occurrences (N)
Musculoskeletal findings	Osteochondrome	1
Other	Hypertrophic pyloric stenosis	1
	Mild pulmonary artery stenosis	1
	Polydactyly	2
	Macrocephaly	1
	Pierre-Robin sequence	1
	Atrial septal defects	2

WAGR: Wilms tumor, aniridia, genitourinary anomalies, and range of developmental delays.Note that each item was only completed for a subset of these patients, and for other patients it is unclear whether the condition was absent or whether the requested data could not be retrieved.

DISCUSSION

We identified 43 patients with confirmed WAGR syndrome and WT/nephroblastomatosis in the SIOP-RTSG database and through their identification by national and/or local PIs within the SIOP-RTSG network. Overall, we demonstrated a high rate of bilateral disease (37.5%, including patients with bilateral or contralateral nephroblastomatosis, versus 6%-7% in general cohorts[19,20]) and no anaplastic tumors. Blastemal-type WT, which is considered high-risk after preoperative chemotherapy, was observed in only two patients after prolonged treatment for nephroblastomatosis. Metastatic disease was not observed in the current study; this was similar to the findings described by Breslow et al in 2003.[2]

Event-free and overall survival rates at five years after diagnosis appear to be similar to those described for nonsyndromic WT[19,20] except that relapses did not occur and mortality was exclusively due to non–tumor- related causes. Because of the long-term morbidity and mortality associated with the underlying syndrome, Breslow et al.[2] reported that 20-year overall survival was only 47.8% for patients with WAGR versus 85.5% for non-WAGR patients, but our follow-up data were insufficient to confirm this. Longer follow-up data are also needed to reliably establish the risk of chronic kidney disease, which was reported in only five of 20 patients with available data in the current study but has previously been estimated to occur in 50% to 60% of patients with WAGR.[1,2] Our study was further limited by the fact that our data collection form did not specify the level of creatinine/eGFR changes required for the definition of chronic kidney disease.

Although two deaths were related to hepatic failure in our cohort of 42 patients, we are not aware of other studies reporting hepatotoxicity in patients with WAGR. Considering the fact that in one of the patients hepatic failure was related to an overdose of actinomycin D, we are uncertain about any potential association with the underlying *WT1* defect. In addition to kidneys and other organs, WT1 protein is expressed in the

developing liver.[21] If future studies report additional patients with *WT1* aberrations and hepatotoxicity, this may warrant further investigation.

The difference in volume between tumors detected by surveillance and those that were symptomatic illustrates the benefit of surveillance, which enables a high rate of NSS even for unilateral cases (23.8%). Currently, different groups offer different WT surveillance recommendations in which surveillance is continued until the age of five[22], six[1], seven[23] or eight[24] years. On the basis of the current study, we would recommend that surveillance for WT be continued until the age of five years, at which 100% of the initial tumors were diagnosed. Notably, only approximately 90% were diagnosed before this age in the NWTS cohort.[2,25] Breslow et al.[2] did not specify whether patients diagnosed at older ages had previously been under surveillance, and we hypothesize that they may have had nephroblastomatosis before their WT diagnosis. For patients in whom WT or nephroblastomatosis has been previously diagnosed, an extended surveillance of the (remaining) kidney(s) may be warranted. We observed the occurrence of contralateral tumors up to seven years after the initial diagnosis, with the latest occurrence at the age of nine years; this suggests that nephrogenic rests in patients with WAGR carry a long-lasting risk of progression to WT. It would be very useful to develop treatment modalities that can prevent this malignant transformation. A drug that has been suggested to induce differentiation of nephrogenic rests is retinoic acid (a metabolite of vitamin A), but clinical studies are limited to case reports.[26,27] In addition, metformin has been speculated to induce cell differentiation by inhibiting the mTOR pathway.[28] Although its potential role in cancer prevention is being studied in several adult populations, it has not been assessed in the context of nephrogenic rests and/or WT predisposition.

In patients with a genetic predisposition such as WAGR syndrome, preoperative chemotherapy is relevant to facilitate NSS.[11] However, we observed a high rate of progressive or nonresponsive tumors, which were frequently of the stromal subtype, as has been previously reported.[29] For patients suspected of bilateral nephroblastomatosis, it is challenging to decide whether or not, and at which time point, surgery should be performed, with the risks of disease progression being balanced against a loss of renal function. It has been suggested that a long period of pretreatment for bilateral nephroblastomatosis increases the risk of anaplastic WT and mortality[30], but this was not observed in patients with WAGR syndrome.

Although the risk of progression of (bilateral) nephroblastomatosis to WT appears to be high (three of five patients), all three patients who experienced progression were alive and disease-free at last follow-up (1-10 years after progression had occurred). Gonadoblastoma, which has been occasionally reported in patients with WAGR syndrome[1], was not reported in the current study, and the risk of developing gonadoblastoma appears to be lower with WAGR syndrome versus germline *WT1*

mutations, particularly in comparison with Frasier syndrome (intron 9 mutations), in which complete sex reversal (XY females) and gonadoblastoma are common.[31]

Our study was limited by its retrospective design. The inclusion of 13 additional patients who were not registered in the central SIOP database may have introduced a bias; the exclusion of five patients for whom we were unable to get in contact with national/local PIs may have as well (notably, none of these five patients had metastatic or anaplastic disease, and all were alive and disease-free at last follow-up). For the collection of additional data, physicians had to rely on medical chart notes of patients who had been treated many years ago. A more complete picture of the phenotypic spectrum of WAGR syndrome can be achieved by involving parents, as has been previously shown[1], and by recording both genetic and clinical features in prospective WT registries such as the SIOP-RTSG UMBRELLA study, which is currently ongoing.

In conclusion, we confirm a lack of metastatic and anaplastic tumors and observe that patients with WAGR syndrome who develop WT and/or nephroblastomatosis can be successfully treated with current WT protocols. Our results illustrate the value of surveillance for enabling NSS and support the recommendation to continue surveillance until the age of five years, which can be further extended for patients with a prior diagnosis of WT/ nephroblastomatosis. Because of the high rate of bilateral disease and the risk of contralateral tumor development and comorbidity, patients with WAGR syndrome need to be treated by multidisciplinary, expert teams.

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Supplementary file 1. Additional data collection form sent to national and/or local PIs.

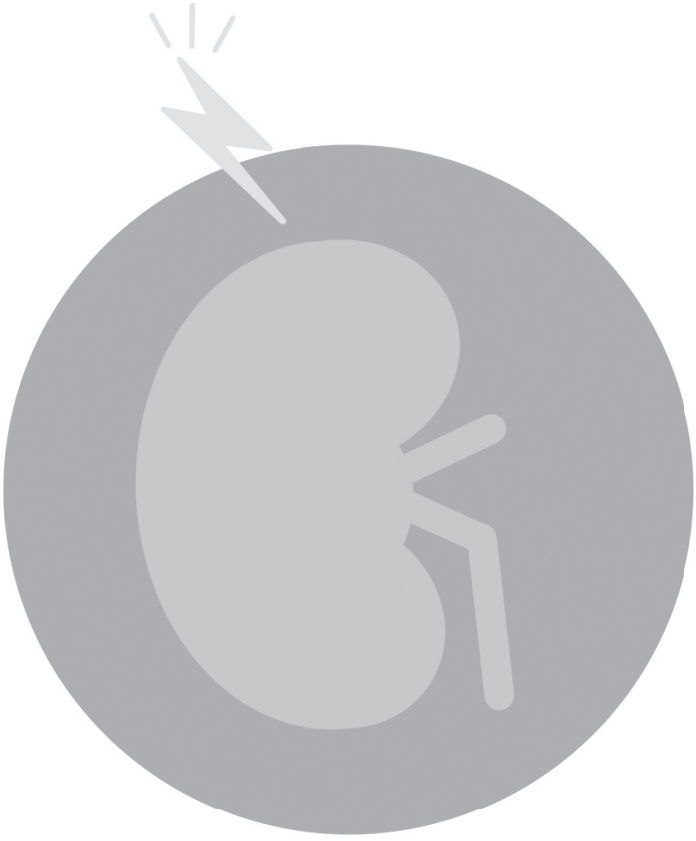
Center: …….. SIOP sequence number: ……..

SIOP-RTSG WAGR Study

GENETIC DIAGNOSIS	
Date of diagnosing WAGR syndrome:	_____ / _____ (month, year)
Genetic test used for diagnosis:	☐ SNP array ☐ FISH ☐ Array CGII ☐ MLPA ☐ Unknown ☐ Other, please specify: ……..……..
Test result:	☐ Deletion ☐ Translocation ☐ Mosaic deletion
Specify result (including breakpoints):	……..……..…… ……..……..
WILMS TUMOUR DIAGNOSIS	
Clinical presentation:	☐ Detected on surveillance imaging ☐ Visible/palpable abdominal mass without other symptoms ☐ Symptomatic ☐ Other, please specify: ……..……..
PREGNANCY & BIRTH	
Birth weight:	☐ Within normal range (p10-p90) ☐ Low birth weight (<p10) ☐ High birth weight (>p90) ☐ *Not available*
Congenital abnormalities:	X Aniridia ☐ Other, specify: ……..……..…… ……..……..…… ……..……..
MEDICAL HISTORY & CLINICAL FINDINGS	
Other malignancies / tumours diagnosed in this patient:	☐ No ☐ Yes ☐ Unknown **If yes:** Specify type: ……..……..…… Date of diagnosis other malignancy/tumour: _____ / _____ (month, year)

Center: …….. SIOP sequence number: ……..

Chronic kidney disease / renal failure? *= decreased eGFR and/or proteinuria 2+*	☐ No ☐ Yes ☐ Unknown **If yes:** Onset of chronic kidney disease: _____ / _____ (month, year) Dialysis or renal transplant indicated? ☐ No ☐ Yes ☐ Unknown	
Did neurotoxicity occur?	☐ No ☐ Yes ☐ Unknown **If yes:** Specify type: …… Onset: _____ / _____ (month, year)	
Other findings:	**Ocular:** ☐ Cataracts ☐ Glaucoma ☐ Nystagmus ☐ Other ocular findings, specify: ……..……... **Genitourinary:** ☐ Cryptorchidism ☐ Ambiguous genitalia ☐ Other genitourinary findings, specify: ……..…… **Neurologic:** ☐ Hypertonia/hypotonia ☐ Epilepsy ☐ Other neurologic findings, specify: ……..…… **Renal:** ☐ Focal segmental glomerulosclerosis (FSGS) ☐ Other renal findings, specify: ……..…… **Metabolic:** ☐ Obesity ☐ Diabetes ☐ Hyperlipidemia ☐ Other metabolic, specify: ……..……	**Pulmonary:** ☐ Asthma ☐ Recurrent pneumonia ☐ Other pulmonary findings, specify: …… **Behavioral:** ☐ Attention-deficit / hyperactivity disorder ☐ Autism / autism spectrum disorder ☐ Obsessive compulsive disorder ☐ Other behavioral findings, specify: …… **Musculoskeletal:** ☐ Scoliosis / kyphosis ☐ Hemihypertrophy ☐ Other musculoskeletal, specify: …… **Cognitive impairment:** ☐ Yes ☐ No **OTHER CLINICAL FINDINGS:** …… …… …… ……
Room for remarks:	……	



Part II

(Epi)genetic predisposition to pediatric renal tumors

5

Prevalence of (epi)genetic predisposing factors in a 5-year unselected national Wilms tumor cohort: a comprehensive clinical and genomic characterization

Hol JA, Kuiper RP, van Dijk F, Waanders E, Van Peer SE, Koudijs MJ, Bladergroen R, Van Reijmersdal SV, Morgado LM, Bliek J, Lombardi MP, Hopman S, Drost J, De Krijger RR, Van den Heuvel-Eibrink MM, Jongmans MCJ

Submitted

ABSTRACT

Background
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 better insight into the prevalence of WT predisposing (epi)genetic factors.

Methods
All children diagnosed with WT and/or nephroblastomatosis in The Netherlands between 2015-2020 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 (WES) and BWSp testing on normal kidney-derived DNA was offered.

Results
126 cases were analyzed out of 128 identified patients. (Epi)genetic predisposing factors were present in 42/126 patients (33.3%) based on 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 4/5 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, MUTYH)* were identified in 5/56 (8.9%) patients with available WES 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 in this national cohort. Based on these results, we encourage standard genetic testing after counseling by a clinical geneticist.

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, 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 WT patients 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 to patients' phenotypic and tumor characteristics, and to identify novel WT predisposition genes.

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 1st, 2015 – January 1st, 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).

In the definition of WT, we included all patients with WT and/or nephrogenic rests (WT precursor lesions[14]). Detailed data were collected, including patient characteristics (sex, birthweight, age at diagnosis, medical and family history), tumor characteristics (stage, histology, 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 based on 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 as well as tumor tissue, if this material was available after nephrectomy (**Supplementary Methods**). Targeted *WT1* analysis was recommended for patients with a urogenital malformation, bilateral/multifocal disease and/or age <2 years at diagnosis (**Supplementary Methods**). Other targeted genetic testing was performed according to the judgement of the clinical geneticist (**Supplementary Figure 1**).

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 1st, 2020.

Patients' germline DNA was assessed using a WES-based 30-gene WT gene panel (**Supplementary Table 1**), including single nucleotide variant, small indel and copy

number analyses (**Supplementary Methods**). 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.

Variant filtering and interpretation

Variants were filtered based on 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 (**Supplementary Table 1**), only (likely) pathogenic variants were communicated with the families. When variants of unknown significance (VUS) were identified in the gene panel, tumor tissue (if available) was assessed by WES and/or SNP array analysis (**Supplementary Methods**) for loss of heterozygosity (LOH) or somatic variants in this gene.

In the exome-wide trio-analysis, variants were additionally filtered based on inheritance mode, prioritizing *de novo*, homozygous and compound heterozygous variants. Genes that were considered strong candidates were submitted to GeneMatcher (https://genematcher.org/), and if available, tumor tissue was assessed for LOH or somatic variants. A subset of genes was selected for meta-analysis based on criteria specified in **Supplementary Table 2**. 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 (UMCU).

RESULTS

A total of 128 patients with WT were identified. Two patients did not give informed consent for data collection, leaving 126 patients (71 females, 55 males) available for analysis (**Supplementary Table 3**). 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), WAGR syndrome (N=1) and neurofibromatosis type I (N=1) (**Table 1**). One patient had a family history of WT.

Genetic examination and diagnostic testing

Out of the 121 patients without a prior diagnosis of a WT predisposition syndrome, 111 (91.7%) were examined by a clinical geneticist (**Supplementary Figure 1**). 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 56/126 (44.4%) patients and diagnostic BWSp testing on blood-derived DNA in 97/126 (77.0%) patients. Additional MS-MLPA on normal kidney tissue was performed in 53/97 (54.6%) patients. Other targeted genetic testing was performed with various indications in 6 patients (**Supplementary Figure 1**).

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). Out 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 CNV analysis was informative in 52/56 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 42/126 patients (33.3%) (**Figure 1** & **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 4 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 VUS were identified in the WT gene panel (**Supplementary Table 4**) which were not considered to be causative based on inheritance mode and lack of LOH/somatic variants in the tumor.

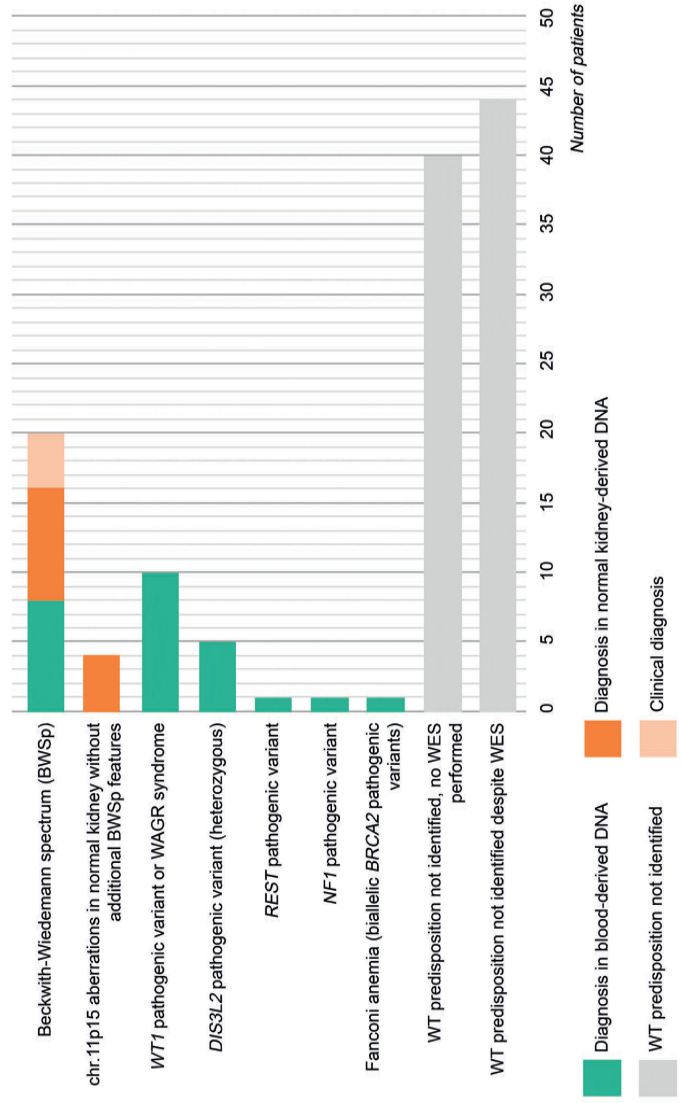


Figure 1. (Epi)genetic predisposing factors in patients with Wilms tumor (WT) and/or nephroblastomatosis (N=126).

WAGR: Wilms tumor, Aniridia, Genitourinary anomalies and Range of developmental delays (WAGR) syndrome, WT: Wilms tumor, WES: whole exome sequencing.

Table 1. Patients with (epi)genetic predisposing factors related to Wilms tumor (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, 2y0m	**BWSp**: pUPD chr.11p15 [A]	MS-MLPA on blood & clinical criteria	Unilateral, blastemal WT & PLNR	Hemihypertrophy, helical ear pits, horseshoe kidney
WESK058	F, 6m	**BWSp**: pUPD chr.11p15 [A]	MS-MLPA on blood & clinical criteria	Unilateral ILNR	Hemihypertrophy, diastasis recti, macroglossia
WESK145	F, 3y2m	**BWSp**: pUPD chr.11p15	MS-MLPA on blood	Unilateral, stromal WT	None
WESK056	F, 4y8m	**BWSp**: pUPD chr.11p15	MS-MLPA on blood & clinical criteria	Bilateral, WT with diffuse anaplasia & PLNR	Hemihypertrophy, pyloric hypertrophy
WESK129	M, 4y1m	**BWSp**: pUPD chr.11p15 [A]	MS-MLPA on blood & clinical criteria	Unilateral, WT with focal anaplasia & PLNR	Hemihypertrophy, macroglossia, ear creases
WESK130	F, 4y11m	**BWSp**: pUPD chr.11p15	MS-MLPA on blood & clinical criteria	Bilateral, WT with diffuse anaplasia, PLNR & NB	Facial nevus flammeus, umbilical hernia
WESK117	M, 4y9m	**BWSp**: pUPD chr.11p15	MS-MLPA on blood & clinical criteria	Bilateral, regressive WT & PLNR	Macroglossia, birth weight >2SDS above mean, mild developmental delay
WESK039	F, 7y1m	**BWSp**: IC1 GOM	MS-MLPA on blood	Unilateral, mixed WT	Hemihypertrophy, mild developmental delay, brother had leukemia (2y)
WESK003	M, 2y11m	**BWSp**: clinical diagnosis	Clinical criteria	Unilateral, mixed WT with PLNR	Hemihypertrophy
WESK009	M, 9m	**BWSp**: clinical diagnosis	Clinical criteria	Unilateral, epithelial WT with PLNR	Hemihypertrophy, umbilical hernia
WESK022	F, 1y0m	**BWSp**: clinical diagnosis	Clinical criteria	Unilateral, diffuse NB	Hemihypertrophy, epicanthal folds, facial nevus flammeus, hemangioma, father had testicular seminoma (30y)
WESK025	M, 4y4m	**BWSp**: clinical diagnosis	Clinical criteria	Bilateral, blastemal WT & PLNR	Hemihypertrophy
WESK062	F, 4y6m	**BWSp**: pUPD chr.11p15	MS-MLPA on kidney tissue & clinical criteria	Bilateral, regressive WT & PLNR	Hemihypertrophy

Table 1. Patients with (epi)genetic predisposing factors related to Wilms tumor (WT) development (N=42) (*continued*)

ID	M/F, age at WT	(Epi)genetic predisposing factors	Identification of predisposing factors	Disease type	Additional clinical features
WESK055	F, 6m	**BWSp**: IC1 GOM	MS-MLPA on kidney tissue & clinical criteria	Unilateral, mixed WT	Hemihypertrophy, facial nevus flammeus, helical ear pits, sacral dimple
WESK096	F, 4y3m	**BWSp**: IC1 GOM	MS-MLPA on kidney tissue & clinical criteria	Bilateral, regressive WT & diffuse NB	Hemihypertrophy, mother had *MITF*-related melanoma (39y)
WESK128	M, 1y7m	**BWSp**: IC1 GOM	MS-MLPA on kidney tissue & clinical criteria	Bilateral, mixed WT & PLNR	Hemihypertrophy
WESK014	F, 7y2m	**BWSp**: IC1 GOM	MS-MLPA on kidney tissue &≥1 BWS feature	Unilateral regressive WT & PLNR	Facial nevus flammeus, hemangioma
WESK088	F, 5y8m	**BWSp**: IC1 GOM	MS-MLPA on kidney tissue &≥1 BWS feature	Unilateral blastemal WT & PLNR	Birth weight >2SDS above mean
WESK124	F, 2y0m	**BWSp**: IC1 GOM	MS-MLPA on kidney tissue &≥1 BWS feature	Unilateral, mixed WT	Hemihypertrophy
WESK135	M, 2y0m	**BWSp**: IC1 GOM	MS-MLPA on kidney tissue &≥1 BWS feature	Bilateral diffuse NB	Nephromegaly, syndactyly 3rd and 4th toe
WESK002	F, 3y8m	IC1 GOM, no BWSp features	MS-MLPA on kidney tissue	Bilateral, regressive WT & PLNR	None
WESK046	F, 5y0m	IC1 GOM, no BWSp features	MS-MLPA on kidney tissue	Unilateral, regressive WT & PLNR	None
WESK073	M, 4y9m	IC1 GOM, no BWSp features	MS-MLPA on kidney tissue	Unilateral, regressive WT & PLNR	Father had metastatic cancer (primary diagnosis unknown) (32y)
WESK121	F, 3y3m	IC1 GOM, no BWSp features	MS-MLPA on kidney tissue	Unilateral, regressive WT & PLNR	None
WESK033	F, 2y11m	***WT1***: c.1216_1223del, p.Ser406fs	Targeted *WT1* testing	Unilateral, mixed WT	None
WESK049	F, 11m	***WT1***: c.1-?_646+?del, start loss	Targeted *WT1* testing	Bilateral, stromal WT & ILNR	None

Table 1. Patients with (epi)genetic predisposing factors related to Wilms tumor (WT) development (N=42) (*continued*)

ID	M/F, age at WT	(Epi)genetic predisposing factors	Identification of predisposing factors	Disease type	Additional clinical features
WESK060	F, 2y2m	***WT1*: del exon 6**	WES panel analysis	Unilateral stromal WT & ILNR	None
WESK105	M, 9m	***WT1***: c.1223_1225delinsAAAG, p.Leu408*	Targeted *WT1* testing	Bilateral, stromal WT & ILNR	Hypospadia, bifid scrotum
WESK108	F, 7m	***WT1***: c.1213_1214del, p.Lys405fs	Targeted *WT1* testing	Unilateral, stromal WT	None
WESK113	M, 1y5m	***WT1***: c.457G>T, p.Glu153*	Targeted *WT1* testing	Bilateral, stromal WT with ILNR & diffuse NB	Bilateral cryptorchidism
WESK120	F, 1y0m	***WT1***: c.1387C>T, p.Arg463*	Targeted *WT1* testing	Bilateral, stromal WT & ILNR	None
WESK122	F, 1y9m	***WT1***: c.514C>T, p.Gln172*	Targeted *WT1* testing	Unilateral, mixed WT	None
WESK144	F, 2y0m	**WAGR syndrome**: del 11p15.1-p13 [A]	SNP array	Bilateral, stromal WT & ILNR	Aniridia, nystagmus
WESK147	M, 7m	***WT1***: c.1120C>T, p.Arg374*	Targeted *WT1* testing	Bilateral, stromal WT & ILNR	Micropenis, cryptorchidism
WESK006	F, 1y4m	**Fanconi anemia**:*BRCA2* c.2548C>T, p.Gln850* & c.7875A>T, p.Arg2625Ser	Targeted *BRCA2* testing	Unilateral, mixed WT	Café-au-lait spots, facial dysmorphisms, polydactyly
WESK045	M, 5y7m	**Neurofibromatosis type 1:** *NF1* c.4169T>C, p.Leu1390Pro [A]	Targeted *NF1* testing	Unilateral, regressive WT & ILNR	Café-au-lait spots, axillar freckling, facial dysmorphisms, tibial bowing, father had pancreas carcinoma (38y)
WESK102	F, 1y3m	***REST***: c.843delC, p.Cys281*	WES panel analysis	Bilateral, blastemal WT & PLNR	Brother and aunt had WT (2y, 3y)
WESK018	F, 2y1m	***DIS3L2***: del exon 9	WES panel analysis	Unilateral, mixed WT	Abnormal meatus
WESK019	F, 3y1m	***DIS3L2***: c.2510_2513delinsGA, p.Phe837*	WES panel analysis	Unilateral, mixed WT & NR (type not specified)	Father had dermato-fibrosarcoma protuberans (38y)

Table 1. Patients with (epi)genetic predisposing factors related to Wilms tumor (WT) development (N=42) (*continued*)

ID	M/F, age at WT	(Epi)genetic predisposing factors	Identification of predisposing factors	Disease type	Additional clinical features
WESK036	F, 5y4m	***DIS3L2**:* c.1096G>T, p.Glu366*	WES panel analysis	Unilateral, mixed WT	None
WESK057	M, 2y0m	***DIS3L2***: del exon 9	WES panel analysis	Unilateral, blastemal WT & PLNR	None
WESK115	F, 3y9m	***DIS3L2**:* del exon 9	WES panel analysis	Unilateral, regressive WT	Ear creases

WT: Wilms tumor, M: male, F: female, PLNR: perilobar nephrogenic rests, ILNR: intralobar nephrogenic rests, NR: nephrogenic rests, BWSp: Beckwith-Wiedemann spectrum, VAF: variant allele frequency. 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, *DIS3L2:* NM_152383.5. ^ Diagnosed prior to WT development.

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 (**Supplementary Table 5**). 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.[15] Four patients were not diagnosed with BWSp because they lacked additional BWSp features, but they did display a gain of methylation (GOM) of imprinting control region 1 (IC1) in normal kidney-derived DNA (**Supplementary Table 5**).

The 20 patients with BWSp had a median age of 3.6 years at WT diagnosis (range 0.5-7.2 years) and 14/20 (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 (12/20, 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 ten patients (7.9%), including one patient with Wilms tumor, Anirida, Genitourinary anomalies and Range of developmental delays (WAGR) syndrome (**Table 1**). These ten patients were characterized by a young age at diagnosis (median 1.3 years, range 0.6-3.0), stromal type WT (8/10 patients, 80%) and intralobar nephrogenic rests (7/10 patients, 70%). Seven patients (70%) had bilateral (N=6) or multifocal (N=1) disease and 3/10 (30%) patients (all XY males) had urogenital malformations, including hypospadias, bifid scrotum, micropenis and/or cryptorchidism.

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 5/126 patients (4%) (**Table 2**). Among patients with available WES data, *DIS3L2* variants were identified in 4/56 (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 SNP array analysis performed for clarifying an ambiguous MS-MLPA result. A second somatic hit was identified in 4/5 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. 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 based on the patient's phenotype or family history, and findings included a familial *REST* variant, Fanconi anemia and neurofibromatosis type 1 (NF1) (**Table 1**).

Findings in adult cancer predisposition genes

(Likely) pathogenic variants in adult-onset cancer predisposition genes were identified in 5/56 (8.9%) patients with available WES data (**Table 3**). Two patients had heterozygous variants in *BRCA2* or *PMS2* (WESK132), 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, 7 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; cancer.sanger.ac.uk)[16] which was not identified, suggesting that the *MUTYH* variant did not drive WT development in this patient.

Meta-analysis: novel candidate genes

Based on the exome-wide trio-analysis, 77 genes were selected for meta-analysis (**Supplementary Methods**). These included 31 genes with verified *de novo* variants and 46 genes with inherited variants (**Supplementary Table 6-7**). 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 (**Supplementary Table 8**). For none of these patients, tumor 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 based on inheritance pattern, *in silico* conservation and prediction scores and/or lack of LOH or second somatic variants in tumor tissue.

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 loss of heterozygosity of exon 9
WESK057	Deletion of exon 9	Paternal	c.1835dup (exon 15), p.Pro613Alafs*8
WESK115	Deletion of exon 9	Paternal	Not identified

Variants are described on transcript NM_152383.5.

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, 4y6m	*MUTYH:* c.536A>G, p.Tyr179Cys	Maternal	No	Unilateral, multifocal: blastemal & mixed WT	No relevant signatures identified
WESK051	F, 3y4m	*MUTYH:* c.1187G>A, p.Gly396Asp *CHEK2:* c.1100delC, p.Thr367fs *RNASEL:* c.793G>T, p.Glu265*	Paternal (all three variants)	Testicular cancer and osteoblastoma (father, age 21 and 35)	Unilateral, mixed WT	n.a.
WESK072	F, 2y11m	*BRCA2:* c.9672dup, p.Tyr3225fs	Paternal	Breast cancer (paternal grandmother, age 46) & childhood leukemia (mother, age 5, and maternal grandfather's sister, age 5)	Unilateral, mixed WT & PLNR	Low mutational burden, signature analysis not possible
WESK110	F, 2m	*MUTYH:* c.536A>G, p.Tyr179Cys	Maternal	No	Unilateral, mixed WT	n.a.
WESK132	M, 3y10m	*PMS2:* c.137G>A, p.Ser46Asn	Maternal	No	Unilateral, regressive WT	n.a.

WT: Wilms tumor, M: male, F: female, PLNR: perilobar nephrogenic rests. Variants are described on the following transcripts: *MUTYH*: NM_001128425.1, *CHEK2:* NM_007194.3, *RNASEL*: NM_021133.4, *BRCA2:* NM_000059.3, *PMS2:* NM_000535.7.

Unrelated genetic diagnoses

Four patients had a genetic diagnosis unrelated to WT development based on 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*), as well as 16p12.2 deletion syndrome.

Family history of cancer

Apart from the patient with familial WT, 12/126 patients had a suspicious family history as defined in the Jongmans' criteria.[17] Recurrent cancer types in affected relatives included childhood leukemia (4 relatives in 3 families), testicular cancer (3 relatives in 3 families), melanoma (2 relatives in 2 families) and neuroblastoma (3 relatives in 2 families). In these families, we did not identify variants which could explain both the WT as well as 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 the 5-24% that has been reported in previous studies.[10-13]

BWSp was diagnosed in 16% of all patients, compared to only 1-8% in earlier reports.[10-12] This higher frequency was due to the fact that we applied clinical criteria[15] 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.[9,18] 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 (~10%). In the future, the development of more sensitive molecular techniques may increase the yield of BWSp testing in blood-derived DNA.[19]

Constitutional, heterozygous *DIS3L2* variants were identified in 4% of all WT patients (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[20], a congenital overgrowth syndrome with a high risk of WT development.[21] Somatic *DIS3L2* variants have been demonstrated in 1-5% of WTs[20,22,23], and deletions or LOH in 4-30%.[20,23,24] Based on incidental reports, heterozygous germline variants in *DIS3L2* were previously suggested to cause an increased WT risk.[22-25] Additionally, patients with rare constitutional deletions of

2q37.1/*DIS3L2* have been reported to develop WT.[26] In our cohort, three out 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.[20] Exon 9 is flanked by two ~5Kb LINE-1 repeats causing genomic instability.[27] Homozygous exon 9 deletions have been reported in Perlman syndrome[20], whereas heterozygous exon 9 deletions are present in 0.05% of healthy individuals (11/21364 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[25,28-31], we identified heterozygous, pathogenic germline variants in adult-onset cancer predisposition genes (*BRCA2, PMS2, CHEK2, MUTYH*). It remains unclear whether the prevalence of these variants exceeds that of the general population and/or whether they contributed to WT development. Analysis of the mutational profile extracted from a single available WT sample did not reveal a contribution of these DNA repair genes in tumor development, as was demonstrated in a recent study.[32]

This study was limited by the fact that not all patients underwent (complete) genetic testing and/or WES analysis, due to 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 CNV 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.[33-35]

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 (non-familial) WT. Moreover, yet to be identified WT predisposition genes may exhibit reduced penetrance, as demonstrated for *DIS3L2*.

Based on 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 (**Figure 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.

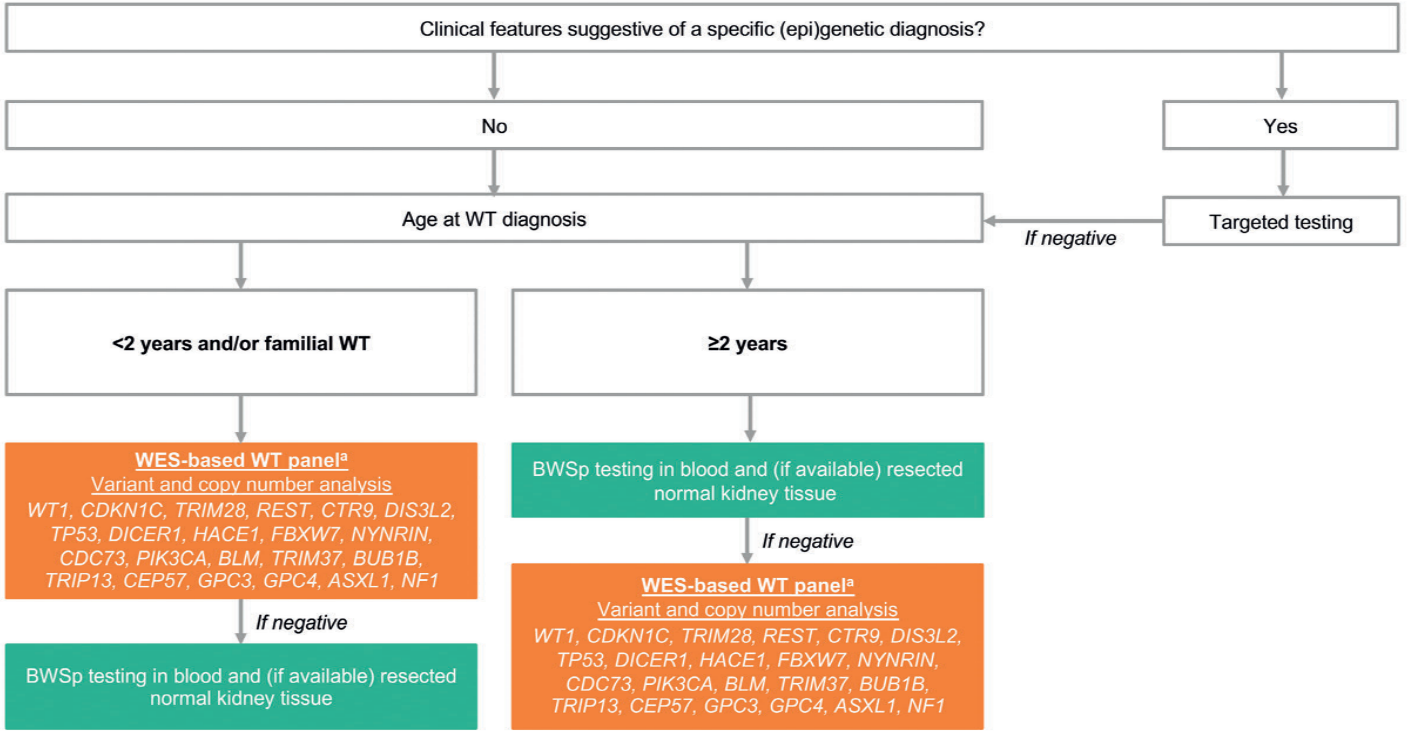


Figure 2. Suggested strategy for germline genetic testing in children with Wilms tumor (WT).

[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, PALB2*) or constitutional mismatch repair deficiency (*PMS2, MSH2, MSH6, MLHI*). WT: Wilms tumor, BWSp: Beckwith-Wiedemann spectrum, WES: whole exome sequencing.

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SUPPLEMENTARY MATERIAL – CHAPTER 5

Includes:
- Supplementary Methods
- Supplementary Table 1: Wilms tumor (WT) gene panel used in this study.
- Supplementary Table 2: Gene selection for meta-analysis.
- Supplementary Table 3: Baseline characteristics of the cohort.
- Supplementary Table 4 (Excel): Variants of unknown significance identified in the WT gene panel.
- Supplementary Figure 1: Flowchart of genetic evaluation, testing and research.
- Supplementary Table 5: Patients with a molecular and/or clinical diagnosis of BWSp (N=20) or epigenetic 11p15 aberrations limited to kidney tissue (N=4).
- Supplementary Table 6 (Excel): Index variants in genes selected for meta-analysis.
- Supplementary Table 7 (Excel): Meta-analysis: all variants in selected genes.
- Supplementary Table 8 (Excel): Exonic variants in *USP45*.

Supplementary tables 4, 6, 7 and 8 are provided in an Excel workbook and are available online.

SUPPLEMENTARY METHODS

DNA isolation

Germline DNA was extracted from peripheral blood lymphocytes using magnetic bead based DNA isolation on a Chemagic™ MSM-I Instrument (PerkinElmer Chemagen Technologie GmbH, Baesweiler, Germany) and in some cases from saliva collected using Oragene®-DNA self-collection kits (DNA Genotek Inc., Ottawa, Canada). DNA was extracted from fresh frozen healthy kidney and tumor tissue using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).

Methylation specific multiplex ligation-dependent probe amplification (MS-MLPA)

Methylation specific multiplex ligation-dependent probe amplification (MS-MLPA) on blood-derived DNA was performed in an ISO15189 accredited diagnostic laboratory (Amsterdam University Medical Center) using MRC Holland MS-MLPA probe mix ME030-C3 BWS/RSS and the MRC Holland MS-MLPA General Protocol version MSP-v006 (MRC Holland, Amsterdam, The Netherlands).

MS-MLPA on DNA derived from healthy kidney and tumor tissue was performed in a research laboratory (research laboratory of the Princess Máxima Center for Pediatric Oncology) using the same probe mix and protocol. Reference DNA was extracted from healthy resected kidney tissue from children with renal cell carcinoma, or from children

with Wilms tumors that did not display chromosome 11p15 aberrations. Data were analyzed using Coffalyser.Net Software (MRC Holland, Amsterdam, The Netherlands).

Targeted *WT1* testing

Targeted *WT1* testing to detect single nucleotide variants (SNVs), small indels and copy number variants (CNVs) in the *WT1* gene, was performed in an ISO15189 accredited diagnostic laboratory (Amsterdam University Medical Center) by Sanger sequencing and MLPA (MRC Holland MLPA probe mix P118 WT1).

Whole exome sequencing

Whole exome sequencing (WES) was performed using Illumina NovaSeq sequencing platforms (Illumina Inc., San Diego, United States). Library preparation and target enrichment was performed using either the latest available SureSelectXT Human or SureSelectXT Clinical Reseach Exome V2 enrichment kits (Agilent Technologies Inc., Santa Clara, United States), or KAPA HyperExome probes (Roche Molecular Systems, Inc., Pleasanton, United States).

Sequencing data were processed with either one or both of the following in-house developed pipelines:

1. IAP pipeline[1] developed by the diagnostic laboratory of the University Medical Center Utrecht (UMCU). In this pipeline, reads were mapped to genome build GRCh37 using BWA-MEM[2], duplicates were marked, lanes were merged using Sambamba[3] and indels were realigned using GATK IndelRealigner. Subsequent mapping and variant calling was performed using GATK[4] implemented according to best practices.[5] Variants were annotated, filtered and prioritized using the Alissa Interpret Clinical Informatics Platform (v5.3, Agilent) and evaluated according to the American College of Medical Genetics (ACMG) criteria[6] by a trained clinical genetic labspecialist reviewer. CNV detection was performed using a modified version of ExomeDepth[7,8] and in-house developed scripts[9]. The Exomedepth tool uses an algorithm to determine CNVs based on the read depth information of a patient compared to a matched reference set. Only CNVs spanning the regions of the genes in our gene panel were assessed.

2. Research pipeline developed by the research department of the Princess Máxima Center. In this pipeline, reads were aligned to genome build GRCh38 using the Burrows-Wheeler Alignment tool (BWA).[10] Subsequent mapping and variant calling was performed using GATK[4] implemented according to best practices.[5] Variants were annotated using ANNOVAR[11] (version 20180416) software and converted to tabular format using a custom script. Variants were filtered based on population frequency (gnomAD V3.1.1), quality metrics, protein effect and in silico conservation and prediction scores. They were evaluated by a clinical researcher (JAH). (Likely)

pathogenetic variants and selected variants of unknown significance in genes associated with disease (OMIM) were reassessed by a trained clinical genetic labspecialist reviewer. CNV detection was performed following GATK best practices using an in-house compiled panel of normals. To aid interpretation the segments were afterwards annotated using a custom R script. Only CNVs spanning the regions of the genes in our gene panel were assessed.

SNP array

SNP array copy number profiling and analysis of regions of homozygosity were performed on DNA isolated from blood, fresh frozen kidney and tumor tissue according to standard procedures using the Infinium Human CytoSNP-850K v1.2 or Infinium Omni5Exome-4 v1.3 BeadChips (Illumina, San Diego, CA, USA). Subsequently, visualizations of SNP-array results and data analysis were carried out using NxClinical software v5.1 (BioDiscovery, Los Angeles, CA, USA). Human genome build Feb. 2009 GRCh37/hg19 was used. Results were classified with BENCH Lab CNV software v5.1 (Agilent, Santa Clara, CA, USA).

References – Supplementary Methods, Chapter 5

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Supplementary Table 1. Wilms tumor (WT) gene panel used in this study.

WT1, CDKN1C, TRIM28, REST, CTR9, DIS3L2, TP53, DICER1, HACE1, FBXW7[a], *NYNRIN*[a], *CDC73, KDM3B*[a,b], *PIK3CA, NSD1*[b], *BLM, TRIM37, BUB1B, TRIP13, GPC3, GPC4, ASXL1, NF1, PMS2*[b], *MSH2*[b], *MSH6*[b], *MLH1*[b], *EPCAM*[b], *BRCA2*[b], *PALB2*[b]

[a] Added after update in February 2019. Data analyzed before February 2019 were reassessed using the updated panel.
[b] These genes were included in the gene panel used for this study, but later removed from the recommended diagnostic WT gene panel due to lack of a clear association with WT development (*KDM3B, NSD1*) or for ethical reasons in the case of adult-onset onset cancer predisposition genes (*PMS2, MSH2, MSH6, MLH1, EPCAM, BRCA2* and *PALB2*).

Supplementary Table 2. Gene selection for meta-analysis

Selected #1 : all genes with verified *de novo* variants (no filters applied)
☐ Either visually confirmed in the IGV browser or by Sanger sequencing

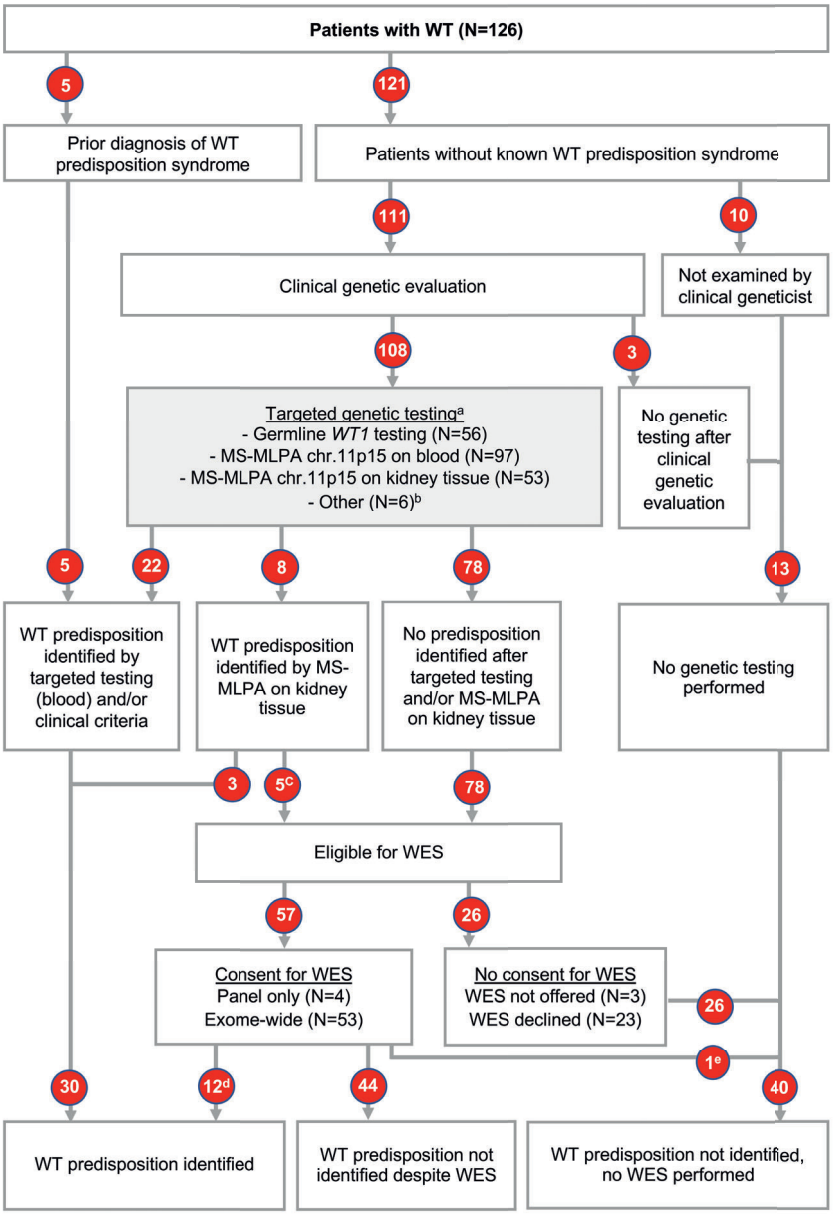
Filters applied: quality and variant allele frequency in the population
☐ Mapping quality (MQ) ≥ 30 ☐ Read depth (DP) ≥ 10 ☐ Number of alternative allele reads (AD_ALT) ≥ 5 ☐ Population frequency (gnomAD AF) ≤ 1%

Selected #2 : all genes with inherited variants meeting one of the following criteria:
☐ gnomAD pLi score ≥0.5 and truncating variants (stop-, frameshift or splicing) with gnomAD AF<0.0001; ☐ Homozygous variants with phyloP≥2 and CADD15_phred≥25 ☐ Compound heterozygous variants with phyloP≥2 and CADD15_phred≥30 ☐ Somatic WT driver genes with truncating variants or missense variants with CADD15_phred≥15

Supplementary Table 3. Baseline characteristics of included patients (N=126)

Clinical characteristics	No. (%)
Gender	
Female	71 (56.3)
Male	55 (43.7)
Disease type	
Unilateral – single lesion	76 (60.3)
Unilateral – multifocal[a]	28 (22.2)
Bilateral	22 (17.5)
Metastatic disease at diagnosis	
No	100 (79.4)
Yes	26 (20.6)
SIOP histological classification	
Mixed type	37 (29.4)
Regressive type	37 (29.4)
Stromal type	15 (11.9)
Epithelial type	5 (4.0)
Focal anaplasia	3 (2.4)
Non-anaplastic (direct nephrectomy)	3 (2.4)
Blastemal type	11 (8.7)
Diffuse anaplastic	6 (4.8)
Completely necrotic	2 (1.6)
Cystic partially differentiated nephroblastoma	2 (1.6)
Nephrogenic rests/nephroblastomatosis only	5 (4.0)
Presence of nephrogenic rests	
Intralobar rests	11 (8.7)
Perilobar rests	27 (21.4)
Diffuse nephroblastomatosis or intralobar & perilobar rests	8 (6.3)
Not specified in pathology report	3 (2.4)
No rests	77 (61.1)

[a] Includes patients with Wilms tumor and nephrogenic rests/nephroblastomatosis.



Supplementary Figure 1. Flowchart of genetic evaluation, testing and research.

[a]Multiple tests were performed in single patients. [b]Targeted analysis of *BRCA2* (Fanconi anemia) (N=1), *TRIM28* (N=2), *DICER1* (N=1), *NF1* (N=1) and *GPC3* (N=1). [c]In five cases, MS-MLPA results were pending while patients were selected for WES analysis. [d]This includes 7 cases where a predisposition was identified by WES, and 5 cases where a predisposition was identified by MS-MLPA on kidney tissue. [e]In one patient, DNA isolation failed and WES could not be performed.

Supplementary Table 5. Patients with a clinical diagnosis of Beckwith-Wiedemann Spectrum (BWSp) and/or chr.11p15 aberrations.

ID	MS-MLPA blood	MS-MLPA kidney	MS-MLPA tumor	Clinical score[a]	Unilateral/bilateral/multifocal + BWSp features
WESK054	pUPD 11p15	pUPD 11p15	pUPD 11p15	5	Multifocal + hemihypertrophy, helical ear pits
WESK058	pUPD 11p15	n.a.	n.a.	5	Unilateral + hemihypertrophy, diastasis recti, macroglossia
WESK145	pUPD 11p15	n.a.	pUPD 11p15	1	Unilateral, no BWSp features
WESK056	IC1 GOM	n.a.	n.a.	4	Bilateral + hemihypertrophy
WESK129	IC1 GOM	n.a.	n.a.	7	Multifocal + hemihypertrophy, macroglossia, ear creases
WESK130	IC1 GOM	Failed	IC1 GOM	5	Bilateral + facial nevus flammeus, umbilical hernia
WESK117	IC1 GOM	n.a.	n.a.	4	Bilateral + macroglossia, birth weight >2SDS above mean
WESK039	IC1 GOM	n.a.	n.a.	3	Unilateral + hemihypertrophy
WESK062	Normal	pUPD 11p15	pUPD 11p15	4	Bilateral + hemihypertrophy
WESK055	Normal[b]	IC1 GOM	IC1 GOM	6	Unilateral + hemihypertrophy, facial nevus flammeus, helical ear pits
WESK096	Normal[b]	IC1 GOM	IC1 GOM	4	Bilateral + hemihypertrophy
WESK128	Normal[b]	IC1 GOM	IC1 GOM	4	Bilateral + hemihypertrophy
WESK014	Normal	IC1 GOM	Failed	2	Multifocal, facial nevus flammeus
WESK088	Normal	IC1 GOM	n.a.	3	Multifocal, birth weight >2SDS above mean
WESK124	Normal	IC1 GOM	IC1 GOM	3	Unilateral + hemihypertrophy
WESK135	Normal	IC1 GOM	IC1 GOM	3	Bilateral + nephromegaly
WESK002	Normal	IC1 GOM	n.a.	2	Bilateral, no BWSp features
WESK046	Normal	IC1 GOM	IC1 GOM	2	Multifocal, no BWSp features
WESK073	Normal	IC1 GOM	n.a.	2	Multifocal, no BWSp features
WESK121	Normal	IC1 GOM	n.a.	2	Multifocal, no BWSp features
WESK003	Normal[b]	n.a.	n.a.	4	Multifocal + hemihypertrophy (clinical diagnosis only)
WESK009	Normal[b]	n.a.	n.a.	5	Multifocal + hemihypertrophy, umbilical hernia (clinical diagnosis only)
WESK022	Normal[b]	n.a.	n.a.	5	Multifocal + hemihypertrophy, facial nevus flammeus (clinical diagnosis only)
WESK025	Normal[b]	n.a.	n.a.	4	Bilateral + hemihypertrophy (clinical diagnosis only)

[a]According to Brioude et al. 2018 (consensus guideline); [b] And normal CDKN1C status; BWSp = Beckwith-Wiedemann Spectrum, MS-MLPA = methylation specific multiplex ligation-dependent probe amplification, pUPD 11p15 = paternal uniparental disomy of 11p15, IC1 GOM = gain of methylation at imprinting control center 1, n.a. = tissue not available for analysis.

6

TRIM28 mutations and Wilms tumor predisposition

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ABSTRACT

TRIM28 was recently identified as a Wilms tumor (WT) predisposition gene, with germline pathogenic variants identified in around 1% of isolated and 8% of familial WT cases. *TRIM28* variants are associated with epithelial WT, but the presence of other tumor components or anaplasia does not exclude the presence of a germline or somatic *TRIM28* variant. In children with WT, *TRIM28* acts as a classical tumor suppressor gene, with both alleles generally disrupted in the tumor. Therefore, loss of *TRIM28* (KAP1/TIF1beta) protein expression in tumor tissue by immunohistochemistry is an effective strategy to identify patients carrying pathogenic *TRIM28* variants. *TRIM28* is a ubiquitously expressed corepressor that binds transcription factors in a context-, species-, and cell-type-specific manner to control the expression of genes and transposable elements during embryogenesis and cellular differentiation. In this review, we describe the inheritance patterns, histopathological and clinical features of *TRIM28*-associated WT, as well as potential underlying mechanisms of tumorigenesis during embryonic kidney development.

Recognizing germline *TRIM28* variants in patients with WT can enable counselling, genetic testing, and potential early detection of WT in other children in the family. A further exploration of *TRIM28*-associated WT will help to unravel the diverse and complex mechanisms underlying WT development.

BACKGROUND

Wilms tumor (WT) is the most common renal malignancy of childhood, with a median age at diagnosis of 3 years, the majority of patients being diagnosed before the age of 7 years. Morphologically, WTs present with a triphasic histology composed of stromal, epithelial, and blastemal cells in variable proportions, but often two, or even only one, of these components predominate.[1]

WTs originate from a developmental arrest during nephrogenesis.[1–3] Manifestations of this developmental arrest include nephrogenic rests, which are embryonic remnants found in the surrounding kidney tissue of ±40% of WTs (±100% in bilateral cases) and are considered to be WT precursor lesions. Whereas intralobar rests are centrally located in the kidney and thought to arise in early nephrons, perilobar rests are located towards the periphery and thought to arise in a later stage of gestation.[1] Apart from nephrogenic rests, it was recently reported that WT precursor clones that genetically resemble the tumor can also exist within morphologically normal-appearing kidney tissue, a phenomenon referred to as clonal nephrogenesis.[4] For malignant transformation of these precursor clones or for nephrogenic rests to develop into WT, additional events are necessary.

Currently, approximately 40 different genes have been identified as possible drivers of WT development, with the most commonly mutated and established drivers being *WT1, WTX/AMER1, CTNNB1, SIX1, SIX2, DROSHA, DICER1, DCGR8,* and *TP53*.[5–7] However, given that a considerable proportion of WTs do not harbour mutations in any of these genes, the spectrum of driver mutations will likely be larger and also epigenetic mechanisms are thought to play an important role in WT development.[2,8]

A subset of WT patients has an underlying tumor predisposition syndrome. Whereas 1–2% of all WT cases are familial, most WT predisposition syndromes are caused by de novo (epi)mutations.[9,10] The most well-known examples include Beckwith–Wiedemann syndrome (BWS) and syndromes caused by germline *WT1* variants or deletions.[5,11] In recent years, novel WT predisposition genes (such as *TRIM28*, *CTR9*, and *REST*) have been identified, each in itself accounting for ≤1% of WT cases.[12] For many of these genes, the mechanisms by which they predispose to WT development are incompletely understood. Unravelling these mechanisms and the associated clinical and histopathological features will help to advance our understanding of WT pathogenesis. In this literature review, we will focus on one of the recently discovered WT predisposition genes, *TRIM28*. We describe the histopathological and clinical features of *TRIM28*-associated WT, as well as potential underlying mechanisms.

TRIM28 VARIANTS IN PATIENTS WITH WT

Pathogenic *TRIM28* variants have currently been reported in 46 patients with WT (**Table 1**), including 27 cases where the variant was detected in lymphocyte DNA, eight cases where the variant was detected in DNA derived from resected normal kidney tissue (lymphocyte DNA not available), and 11 cases where the variant was shown to be only present in the tumor.[12–16] Nineteen familial cases were reported in nine families.[12,14–16] *TRIM28* variants were considered to be germline events in 30 patients, based on their confirmation in heterozygosity in lymphocyte DNA (N = 27) or in kidney tissue in the case of familial WT (N = 3). In five patients, *TRIM28* variants were originally reported as germline variants[13,15] but may represent clonal nephrogenesis[4], since lymphocyte DNA for confirmation of germline status was not available and no other relatives were (known to be) affected. With one exception, the reported variants are truncating or splice site variants located throughout all protein coding domains of the *TRIM28* gene (**Figure 1**).

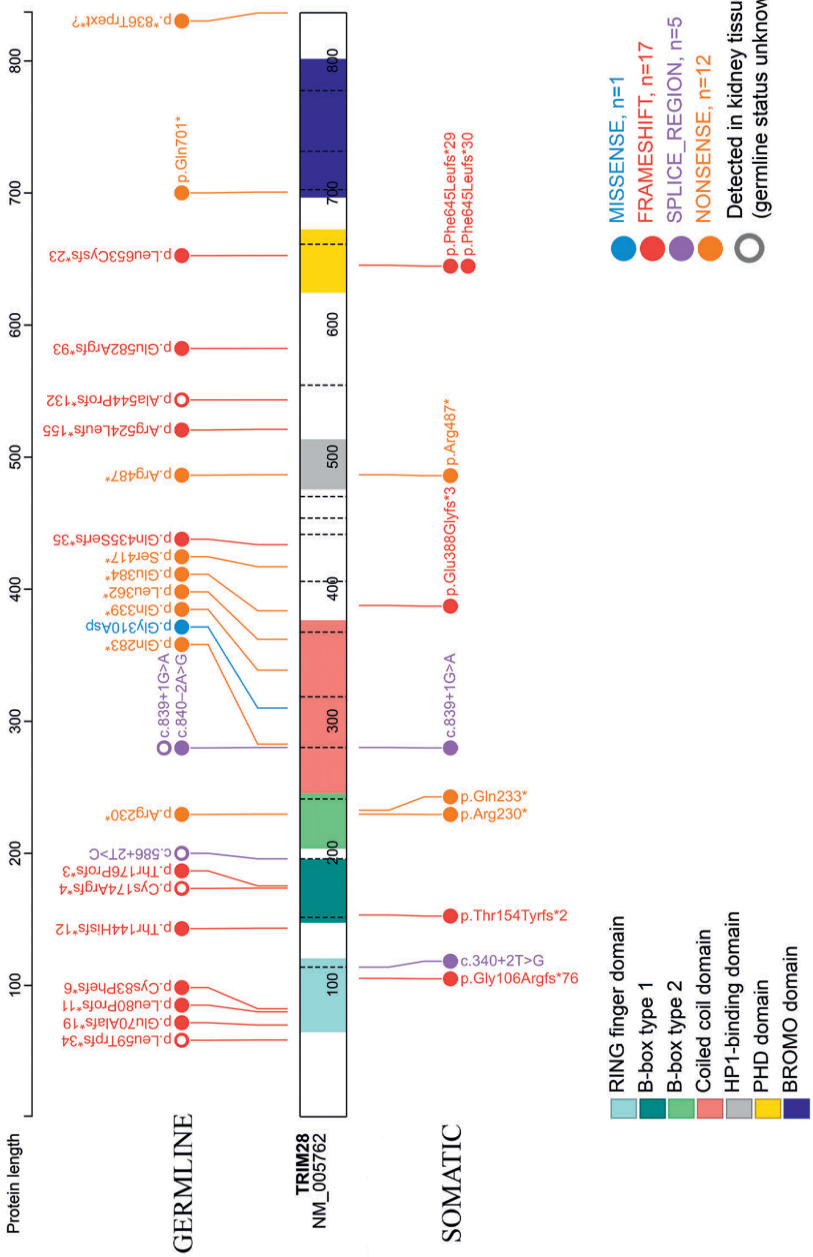

Figure 1. Schematic representation of the TRIM28 protein and reported germline and somatic variants in patients with Wilms' tumor. Variants identified in adjacent normal kidney tissue in non-familial cases (N = 5) are included in this figure as potential germline variants, marked as open circles. Protein annotations follow the recommendations of the Human Genome Variation Society (HGVS).

Table 1. Reported Wilms tumor patients with *TRIM28* variants in blood, kidney and/or tumor (N=46).

ID in original report (reference)	Mutation identified in:	Familial WT?	M/F	Age	Inheri-tance	Mutation	Histology	NR	LOH/IHC, other findings in tumor	FU
0477_01 [12]	Blood	Familial	F	24	Mat	p.Gly310Asp	Epithelial predominant [P]	NA	NA	NA
0477_02 [12]	Blood	Familial	M	84	Mat	p.Gly310Asp	Epithelial [P]	NA	NA	NA
0477_03 [12]	Blood	Familial	F	93	Mat	p.Gly310Asp	NA	NA	NA	NA
0498_01 [12] / 249 [14]	Blood & tumor	Familial	M	8	Mat	p.Glu583Argfs*93	Monomorphic epithelial [P]	NA	LOH	30
0498_02 [12] / 399 [14]	Blood & tumor	Familial	F	5	Mat	p.Glu583Argfs*93	Monomorphic epithelial	No	LOH	29
0498_03 [12]	Blood	Familial	F	6	NA	p.Glu583Argfs*93	Epithelial [P]	NA	NA	NA
0487_01 [12]	Blood	Familial	M	15	Mat	p.Thr144Hisfs*12	Epithelial predominant [P]	NA	NA	18
0487_02 [12]	Blood	Familial	M	18	NA	p.Thr144Hisfs*12	NA	NA	NA	NA
0506_01 [12] / 37 [14]	Blood & tumor	Familial	M	39	Mat	p.Thr176Profs*3[A]	Monomorphic epithelial [P]	No	CN-LOH, TRIM28 IHC loss	20
0506_02 [12] / 39 [14]	Blood & tumor	Familial	F	8	Mat	p.Thr176Profs*3[A]	L Monomorphic epithelial[P] R Monomorphic epithelial [P]	No	CN-LOH, TRIM28 IHC loss	20
7487_01 [12]	Blood	Isolated	F	118	Mat	p.Leu80Profs*11	Epithelial predominant with diffuse anaplasia [P]	NA	NA	3 †
1982 [12]	Blood	Isolated	M	11	DN	p.Leu653Cysfs*23	L Epithelial predominant [P] R Epithelial predominant [P]	NA	NA	15
6530 [12]	Blood	Isolated	M	15	DN	p.Glu70Alafs*19	Epithelial + blastemal [P]	NA	NA	5
1969 [12]	Blood	Isolated	M	118	DN	Splice, c.840–2A>G	Epithelial + blastemal [P]	NA	NA	10
7574 [12]	Blood	Isolated	M	13	DN	p.*836Trpext*?	Epithelial predominant [P]	NA	NA	NA
0902 [12]	Blood	Isolated	F	12	Mat	p.Ser417*	Epithelial predominant [P]	NA	NA	NA

Table 1. Continued.

ID in original report (reference)	Mutation identified in:	Familial WT?	M/F	Age	Inheri-tance	Mutation	Histology	NR	LOH/IHC, other findings in tumor	FU
0692 [12]	Blood	Isolated	F	13	NA	p.Arg487*	L NA R NA	NA	NA	36
6671 [12]	Blood	Isolated	F	10	NA	p.Arg230*	L Epithelial predominant [P] R Epithelial predominant [P]	NA	NA	5
0796 [12]	Blood	Isolated	F	61	NA	p.Leu362*	NA	NA	NA	28
0866 [12]	Blood	Isolated	F	90	NA	p.Gln435Serfs*35	Epithelial predominant [P]	NA	NA	22
0936 [12]	Blood	Isolated	M	8	NA	p.Glu384*	NA	NA	NA	NA
1 [15]	Blood & tumor	Familial	F	5	Mat	p.Cys83Phefs*6	L Epithelial type [P] R Epithelial type [P]	PLNR	CN-LOH, TRIM28 IHC loss	NA
2 [15]	Blood & tumor	Familial	F	18	Mat	p.Cys83Phefs*6	Epithelial type [P]	PLNR	CN-LOH, TRIM28 IHC loss	NA
3 [15]	Blood & tumor	Familial	M	69	Mat	p.Arg524Leufs*155	Mixed type [P]	PLNR	No LOH, TRIM28 IHC loss, mutations in *DICER1 & AMER1*	NA
4 [15]	Blood & tumor	Familial	M	7	Mat	p.Arg524Leufs*155	L Epithelial type [P] R Blastemal type [P]	PLNR	CN-LOH, TRIM28 IHC loss, *NF1* mutation	NA
5 [15]	Healthy kidney & tumor	Familial	F	6	NA	p.Gln283*	Epithelial type [P]	PLNR	NA	NA
6 [15]	Healthy kidney & tumor	Familial	F	7	NA	p.Gln283*	L Epithelial type [P] R Nephroblastomatosis [P]	NA	NA	NA

Table 1. Continued.

ID in original report (reference)	Mutation identified in:	Familial WT?	M/F	Age	Inheri-tance	Mutation	Histology	NR	LOH/IHC, other findings in tumor	FU
7 [15]	Both kidneys & tumor	Familial	M	6	Mat[B]	p.Gln339*	L Epithelial type [P] R Epithelial type [P]	PLNR	CN-LOH, TRIM28 IHC loss	NA
1 [16]	Blood & tumor	Familial	F	12	NA	p.Gln701*	L Epithelial type [P] R Epithelial type [P]	NA NA	CN-LOH CN-LOH	NA NA
2 [16]	Blood & tumor	Familial	F	14	NA	p.Gln701*	L Epithelial type [P] R Epithelial type [P]	NA NA	CN-LOH CN-LOH	8 4
8 [15]	Both kidneys & tumor	Isolated	M	17	NA	p.Cys152Glyfs*50	L Nephroblastomatosis [P] R Epithelial type [P]	PLNR	CN-LOH, TRIM28 IHC loss	NA
9 [15]	Healthy kidney & tumor	Isolated	F	7	NA	p.Leu59Trpfs*34	Epithelial type [P]	PLNR	NA	NA
11 [15]	Healthy kidney & tumor	Isolated	F	75	NA	p.Cys174Argfs*4	L Nephroblastomatosis [P] R Epithelial type [P]	PLNR	NA	NA
PAKVET [13]	Healthy kidney & tumor	NA	NA	13	NA	Splice, c.839+1G>A	Monomorphic epithelial	No	CN-LOH	NA
10 [15]	Healthy kidney & tumor	Isolated	F	40	Mosaic[C]	p.Ala544Profs*132	Epithelial type with diffuse anaplasia [P]	No	NA	NA
12 [15]	Tumor[D]	Isolated	F	8	Somatic	p.Met389Argfs*2	Epithelial type [P]	NA	NA	NA
PADWNP [13]	Tumor[D]	Isolated	NA	18	Somatic	p.Gln233*	Monomorphic epithelial	No	CN-LOH	NA
PAJMKN [13]	Tumor[D]	Isolated	NA	17	Somatic	p.Gly107Argfs*75[A]	Monomorphic epithelial	No	CN-LOH	NA
PAJMZF [13]	Tumor[D]	Isolated	NA	8	Somatic	p.Arg487*	Monomorphic epithelial	No	No LOH, promoter hypermethylation[F]	NA
PADDLL [13]	Tumor[D]	Isolated	NA	6	Somatic	p.Phe645Leufs*29	Monomorphic epithelial	No	NA	NA
PAJPER [13]	Tumor[D]	Isolated	NA	15	Somatic	Splice, c.839+1G>A and p.Arg487*	Monomorphic epithelial	No	NA	NA

Table 1. Continued.

ID in original report (reference)	Mutation identified in:	Familial WT?	M/F	Age	Inheri-tance	Mutation	Histology	NR	LOH/IHC, other findings in tumor	FU
PAKSJN [13]	Tumor[D]	Isolated	NA	91	Somatic	p.Arg230*	Monomorphic epithelial	No	NA	NA
PAJNYM [13]	Tumor[D]	Isolated	NA	10	Somatic	Splice, c.340+2T>G	Monomorphic epithelial	No	CN-LOH	NA
PAKYLT [6, 13]	Tumor[D]	Isolated	NA	NA	Somatic	Splice, c.839+1G>A	Anaplastic, epithelial	NA	CN-LOH, *TP53* mutation	NA
W117 [14]	Tumor[D]	Isolated	M	7	Somatic	p.Phe645Leufs*30	Monomorphic epithelial	No	No LOH, TRIM28 IHC loss, exon 1 hypermethylation	NA
WESK150 (This report)	Tumor[D]	Isolated	M	7	Somatic	p.Thr154Tyrfs*2	Epithelial type[P]	PLNR	CN-LOH, TRIM28 IHC loss	NA

M=male; F = female; Age = age at Wilms tumor diagnosis (months); DN = de novo, Mat = maternal, NR = nephrogenic rests; PLNR = perilobar nephrogenic rests; LOH = loss of heterozygosity, IHC = immunohistochemistry; CN-LOH = copy-neutral loss of heterozygosity; FU = duration of follow-up (years), [A]= protein annotation of original publication has been changed according to HGVS recommendations, [B]= assumed that mutation was inherited from mother, who was not tested but had bilateral Wilms tumor at age 8 years, [C]=based on variant allele frequency, [D]=absent in adjacent kidney tissue, [E]=not presumed to be responsible for silencing the wild-type allele, [P]= (presumably) after preoperative chemotherapy, † = patient deceased. Variants are described on transcript NM_005762.2.

Histological features of *TRIM28*-mutated tumors

The comparison of WT histology in *TRIM28*-mutated WTs is complicated by the use of two distinct histological classification systems: the Children's Oncology Group (COG) classification and the SIOP classification of renal tumors. The two classification systems apply to WTs treated with primary surgery and preoperatively treated WT, respectively.[17] Generally, preoperative chemotherapy is recommended in SIOP Renal Tumor Study Group (RTSG) protocols for all children aged ≥6 months at diagnosis[18], while in North American COG protocols it is only recommended for children with a known genetic predisposition and/or bilateral WT.[19] In most cases after preoperative chemotherapy, part of the tumor has become necrotic and because the undifferentiated, blastemal cells are more sensitive to chemotherapy, the initial composition of epithelium, stroma, and blastema may have shifted.[20] In the reviewed studies on *TRIM28*, it was frequently not specified whether tumors had been pretreated and/or which histological classification system had been used. Therefore, in this review, we will describe histology according to the terminology in the original reports.

Histological characterization was reported for 51 tumors from 46 patients.[12–16] Out of the 51 tumors, 44 (86%) were described as (monomorphic) epithelial (type or predominant) WTs, three (6%) as epithelial (type or predominant) with (diffuse) anaplasia, one as blastemal-type WT (2%), and two (4%) as 'epithelial and blastemal' WTs. Thus, although epithelial tumors appear to be the predominant subtype among *TRIM28*-mutated tumors, the presence of other tumor components (particularly blastema) or anaplasia does not exclude the presence of (germline or somatic) *TRIM28* variants.

The presence or absence of nephrogenic rests was specified for 24 patients with *TRIM28* variants. Nephrogenic rests were reported in 11 patients, including 7/10 (70%) with germline *TRIM28* variants, 3/5 (60%) patients with *TRIM28* variants that were confirmed in kidney tissue, and 1/9 (11%) patients with somatic *TRIM28* mutations in their tumors. All reported nephrogenic rests were perilobar rests.

TRIM28 acts a tumor suppressor in patients with WT

TRIM28 acts as a classical tumor suppressor gene in WT patients, where disruption of both alleles appears to be required to initiate tumor development. In ten *TRIM28*-mutated tumors in which immunohistochemistry (IHC) was performed (**Figure 2**), including seven with a germline variant, tumor cells had lost expression of *TRIM28*, in contrast to the surrounding nonmalignant cells, that showed retained nuclear expression (**Table 1**).[14,15] Loss of heterozygosity (LOH) was found to be the most common mechanism for this second hit, which was confirmed in 17 out of 20 cases. In 13 of these 17 tumors, B-allele frequency and/or SNP array data were available, revealing that in all these cases LOH was caused by a somatic recombination event on the q-arm of chromosome 19, resulting in (copy-neutral) homozygosity of the mutated allele. The size of the LOH

region (if reported) varied from regions encompassing almost the entire chromosome arm (19q13.11–19q13.43)[13] to regions less than 0.5 Mb.[15]

Mutations in other known WT driver genes were assessed in whole exome sequencing (WES) data of 11 *TRIM28*-mutated tumors. Eight tumors (72%) did not reveal any driver gene mutation.[14,15] One tumor revealed a TP53 mutation, which was likely related to its diffuse anaplastic histology.[13,21] In the study by Diets et al, two tumors revealed somatic mutations in *DICER1*, *AMER1* (individual 3), and *NF1* (individual 4).[15]

Recently, Brzezinski et al observed that *TRIM28*-mutated tumors belong to a subgroup of WT with genomewide dysregulation of DNA methylation[22] and display a very distinct and recognizable DNA methylation pattern (Brzezinski, personal communication).

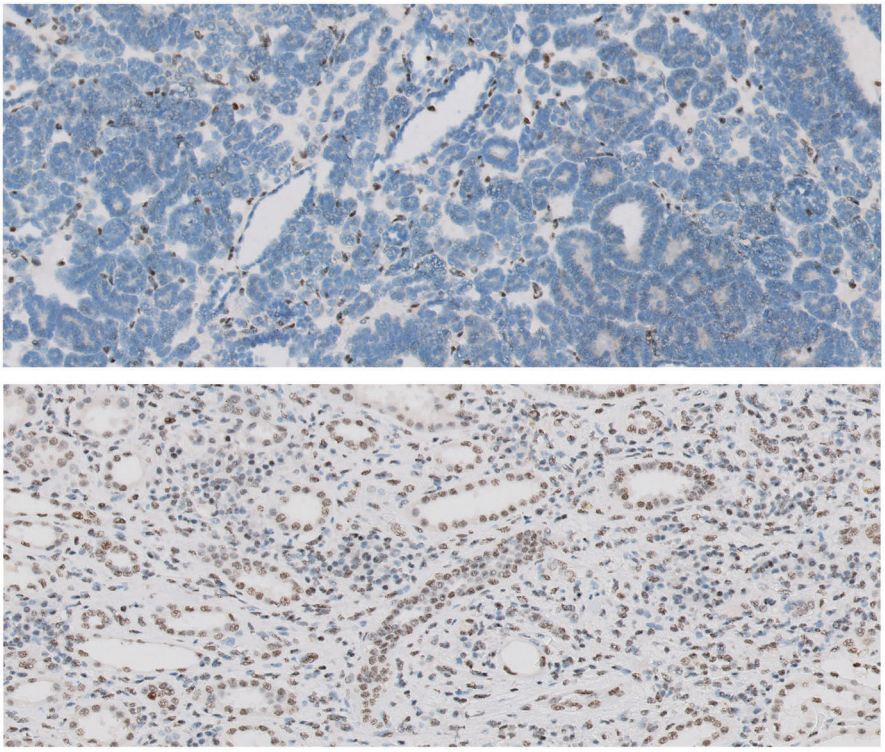

Figure 2. Loss of TRIM28 protein expression in TRIM28-mutated Wilms' tumor.
Top: immunohistochemical staining with anti-KAP1 antibody (ab10484) in an epithelial Wilms' tumor (WT) of a 7 month-old boy with a somatic TRIM28 mutation showing absent nuclear staining in tumor cells, with retained expression of KAP1 in non-tumoral cells. Bottom: retained expression of KAP1 in adjacent normal kidney tissue. The counterstaining with Mayer's haematoxylin (blue) appears more intense in the tumor, due to the fact that the tumor slice is slightly thicker and lacks KAP1 (brown) staining.

BIOLOGICAL FUNCTIONS OF TRIM28

TRIM28 (also known as KAP1 or TIF1beta) is a multidomain protein that is part of the tripartite motif (TRIM)-containing protein family. Proteins in this family are associated with a wide variety of physiological processes.[23] Although TRIM28 is ubiquitously expressed, its functions are context-, species-, and/or cell type-dependent.[24,25] TRIM28 is a central regulator of transcription that can either promote or repress chromatin accessibility. TRIM28 does not have a DNA-binding domain, but is indirectly recruited to genomic loci through its interaction with a variety of transcription factors that determine target specificity.[26] An important group of transcription factors is the large family of Krüppel-associated box-containing zinc-finger proteins (KRAB-ZFPs, also known as KRAB-ZNF proteins) that control transcriptional repression during embryogenesis and tissue differentiation.[27–29] These KRAB-ZFP–TRIM28 complexes subsequently recruit multiple chromatin-modifying proteins, including the histone deacetylase complex NuRD, heterochromatin protein 1 (HP1), and the histone H3 lysine 9 (H3K9me3)-specific methyltransferase SETDB1.[30] This transcriptional effect of TRIM28 appears to depend on the post-translational modifications of TRIM28.[24,31] Specifically, SUMOylated TRIM28 acts as a scaffold for heterochromatin inducing factors, whereas phosphorylated TRIM28 promotes chromatin accessibility and enables transcriptional elongation by releasing paused RNA polymerase II.[32] Targets of TRIM28-mediated transcriptional regulation include protein-coding as well as promoter regions, imprinting control regions, long non-coding RNAs (lncRNAs), and transposable elements.[25,33,34] Through this extensive protein–protein interaction network, TRIM28 is involved in a wide variety of cellular processes, including cell differentiation[24], stem cell maintenance[34], DNA damage repair[35], establishment of genomic imprints[36,37], apoptosis[38], and autophagy[39]. Therefore, it is perhaps not surprising that loss of TRIM28 is lethal in mouse embryos[37] and overexpression of TRIM28 is observed in many cancer types.[31]

TRIM28 and WT development

As is true for many of the recently discovered WT predisposition genes, much needs to be unravelled about how pathogenic *TRIM28* variants lead to WT development (**Figure 3**). WTs result from maldevelopment of the embryonic kidney and many WT predisposition genes are involved in the transcriptional regulation of nephrogenesis, WT1 being the most extensively studied. As yet, however, the exact mechanisms of WT development in the context of these germline variants are still not fully elucidated.[1,40]

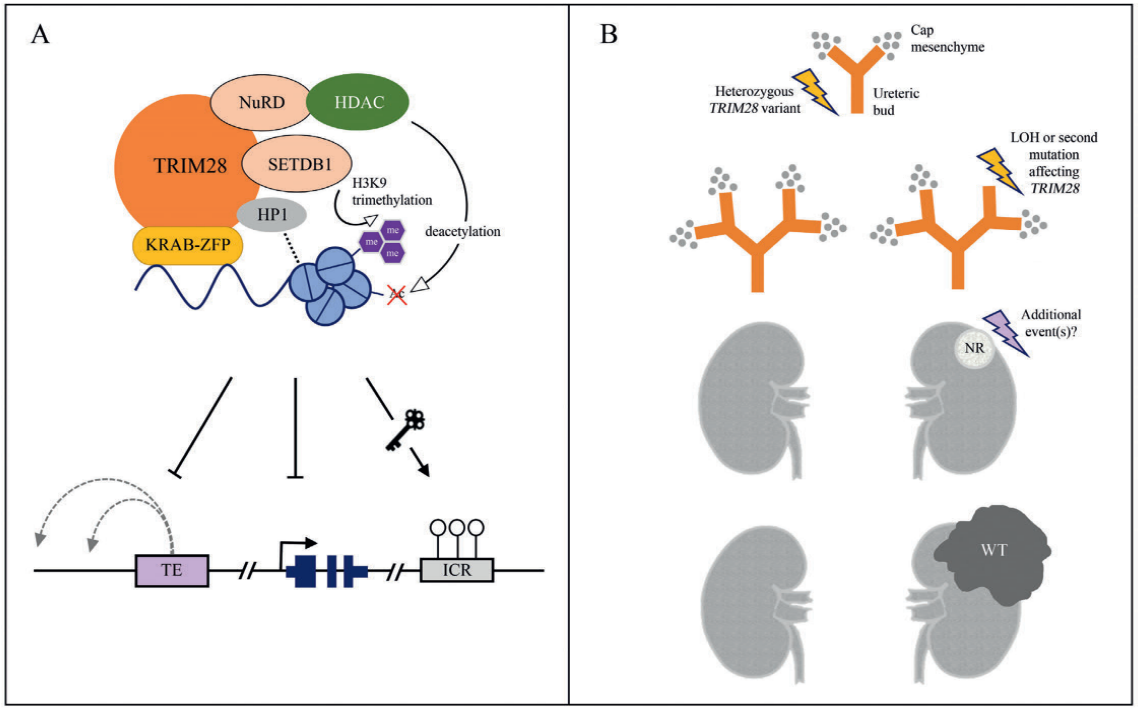

Figure 3. Model for *TRIM28*-mutated Wilms' tumor development. *TRIM28* is thought to act as a transcriptional corepressor during the early stages of kidney development, through its interaction with one of the Krüppel-associated box-containing zinc-finger proteins (KRAB-ZFPs). H3K9, histone H3 lysine 9; me, methyl group; Ac, acetyl group; TE, transposable element; ICR, imprinting control region; LOH, loss of heterozygosity; WT, Wilms' tumor. (A) The TRIM28–KRAB-ZFP complex acts as a scaffold for chromatin-modifying proteins that regulate local chromatin accessibility and gene expression, including SET domain bifurcated histone lysine methyltransferase 1 (SETDB1), the nucleosome remodelling and deacetylase complex (NuRD), histone deacetylases (HDACs), and heterochromatin protein 1 (HP1). Targeted transposable elements (TEs) and genes are repressed, whereas imprinting control regions (ICRs) are maintained. (B) Loss of TRIM28 in the embryonic kidney leads to a branching arrest which may cause nephrogenic rests (NRs) to persist in the postnatal kidney. Additional events are necessary for NRs to develop into WT.

When compared with germline *WT1* variants which are associated with intralobar nephrogenic rests, the identification of perilobar nephrogenic rests in patients with germline *TRIM28* variants suggests a relatively late disturbance of nephrogenesis, which is normally completed by 34–37 weeks of gestation.[41,42] The predominance of epithelial WT suggests that the arrested renal mesenchyme is somehow directed towards epithelial differentiation. In embryonic rat kidneys, Dihazi et al demonstrated that knockdown of TRIM28 indeed resulted in reduced ureteric bud branching or even branching arrest, which provides a potential model of how *TRIM28* mutations could lead to the formation of nephrogenic rests and WT (**Figure 3B**). In their study, TRIM28 protein was expressed in the ureteric bud, cap mesenchyme, and renal vesicle, but downregulated in comma- and S-shaped bodies, the subsequent stages that develop into the mature nephron.[43] Based on bioinformatics analysis of chromatin immunoprecipitation (ChIP) data previously generated by O'Geen et al[44], Dihazi et al identified 22 genes involved in kidney development among the ±7000 potential binding sites of TRIM28.[43] These included *WT1, BMP4, BMP7, GDNF,* and *RET*, which are known to play important roles in ureteric bud branching.[45] Of these genes, *BMP4*[25], *BMP7*[26], and *RET*[25,26] were also among the significantly upregulated genes in TRIM28 knockdown HEK293 cell lines[26] and/or TRIM28 knockout human ESCs.[25]

In WTs studied by Armstrong et al[13] and Halliday et al[14], pathogenic *TRIM28* variants were correlated to a specific gene expression pattern that had previously been labelled the S1 subtype, described as a postinduction gene expression pattern.[6] Compared with other WTs, *TRIM28*-mutated and S1-subtype WTs had 18 differentially expressed genes in common, including lower expression of *SIX2*[13]. *SIX2* is a homeobox protein, normally expressed in the cap mesenchyme, which is responsible for maintaining the undifferentiated state of blastemal cells.[46] Additionally, *TRIM28*-mutated WTs revealed an increased expression of four KRAB-ZFP genes, namely *ZNF728, ZNF676, ZNF208,* and *ZNF780A*. Presumably, these four KRAB-ZFPs play crucial roles in *TRIM28*-mediated silencing of specific genomic loci in the developing kidney. The overexpression of these genes may be explained by the fact that the expression of KRAB-ZFP genes appears to be controlled by a *TRIM28*-dependent auto-regulatory mechanism.[44] Finally, a large number of transposable elements across the genome were found to show differential expression, the majority of which were overexpressed.[13]

Transposable elements

TRIM28 is known to be involved in the silencing of a wide range of transposable elements (TEs), including LINE-1, LTRs, HERVs, and SVAs (**Figure 3A**).[25,34,47] TEs are repetitive DNA sequences that comprise about half of the human genome, most of them remnants of ancient proviral infections.[48] In recent years, it has been shown that specific TEs can be expressed and (retro)transpose themselves into new genomic locations, in germ cells, embryonic stem cells, and cancer cells.[49–52]

In cancer cells, TEs can disrupt protein coding or regulatory sequences of specific tumor suppressor genes.[52] Additionally, global hypomethylation of TEs has been associated with genomic instability in various adult cancer types.[51] Although WTs generally harbour few mutations or copy number changes compared with adult cancer, *TRIM28*-mutated WTs were recently shown to be part of a subgroup of WTs which are less stable genomically.[22]

In embryonic stem cells (ESCs), the expression of TEs was shown to correlate with changes in chromatin accessibility and DNA methylation, and it is thought that TRIM28-mediated TE silencing may have evolved to regulate germline competency and somatic lineage differentiation.[25,36,53] As in HEK293 cells[26], human ESCs with TRIM28 knockout showed an extensive number of differentially expressed TEs and KRAB-ZNF genes.[25] In contrast to TRIM28-deficient mouse ESCs[53], human ESCs with TRIM28 knockout retained self-renewal capacity and even displayed a growth advantage.[25] Yet TRIM28 knockout ESCs seemed less capable of producing primordial germ cells and cardiomyocytes, and it was suggested that specific cell lineages with a very narrow developmental window are affected by *TRIM28* loss.[25] We hypothesize that this balance between differentiation and proliferation is also disturbed in nephron progenitor cells that lack *TRIM28*, probably resulting in an extensively deregulated transcriptional landscape that blocks normal differentiation and favours tumorigenesis.

Maternal inheritance

A remarkable observation in the families identified thus far was that in all 15 patients with WT for whom parental inheritance could be established, the pathogenic *TRIM28* variant was inherited from the mother (three of whom were also diagnosed with WT).[12,15] The underlying cause of this maternal inheritance pattern is currently unknown. A recently proposed explanation is related to the *PEG3* imprinting control region (ICR), which is a paternally expressed ICR located in close vicinity to *TRIM28* on the tip of chromosome arm 19q.[12] *PEG3* was suggested to function as a tumor suppressor gene, which is inactivated by the somatic loss of the paternal 19q arm in the case of a germline *TRIM28* mutation on the maternal allele. Although this scenario requires further analysis, the LOH region in at least two published *TRIM28*-mutated tumors did not include *PEG3*.[12,15]

Another explanation for the maternal inheritance pattern could be that pathogenic *TRIM28* variants impair spermatogenesis and result in male subfertility or infertility, as was suggested by a recent study in mice with heterozygous loss of *TRIM28*.[54] This would prevent male carriers from passing the variant on to offspring. In published pedigrees of families with carriers of pathogenic *TRIM28* variants, all male carriers were affected with WT and none were reported to have children carrying the variant[12,15], although case 37[14] fathered a wildtype daughter (unpublished data, February 2021). Fertility assessment in male carriers, as well as determining the parental origin of

de novo *TRIM28* mutations, will help to clarify whether genomic imprinting or male infertility, or a combination of both, explains the maternal inheritance pattern.

TRIM28 interacts with other WT genes

Two WT-associated genes, *REST* and *AMER1*, have been reported to interact with *TRIM28*. The *REST* gene which, like *TRIM28*, was recently identified as a WT predisposition gene, encodes a KRAB-ZFP which binds to DNA targets and recruits *TRIM28* as a corepressor in the regulation of genes involved in neuronal development.[55] The *AMER1* gene, somatically mutated in ±18% of WTs, encodes the WTX protein which was demonstrated to be a binding partner of TRIM28.[56] Further research is needed to characterize the networks in which these genes, including *TRIM28*, are involved.

CLINICAL IMPLICATIONS

WT risk and age at diagnosis

Among the 30 patients with germline *TRIM28* variants (17 female, 13 male), ten (33%) had bilateral disease. Median age at WT diagnosis was 13 months (range 5–118 months), which is younger compared with general WT cohorts.[57] However, compared with WT patients with germline WT1 variants, where >95% of tumors are diagnosed before the age of 5 years[58], a relatively large proportion of patients with *TRIM28* variants presented at older ages. We found that 25/30 patients (83%) were diagnosed before the age of 7 years and 28/30 (93%) before the age of 8 years, which may encourage continuing surveillance until the age of 8 years (**Figure 4**). Additionally, based on two families in which all affected individuals were diagnosed before the age of 8 months, it is conceivable that other unidentified genetic factors play a role in the age of onset.[12,15]

Pedigrees from families with germline pathogenic *TRIM28* variants suggest a disease penetrance of ±67%, with 18 affected individuals out of a combined total of 27 (obligate) carriers.[12,15] Only one pedigree showed the presence of *TRIM28* variants in more than two generations. In this pedigree (ID_0477 in Mahamdallie et al[12]), four unaffected obligate carriers and six affected individuals were identified. Since reported families were identified based on the presence of multiple affected individuals, this estimated penetrance is likely biased, but certainly supports offering surveillance to children with germline *TRIM28* variants.

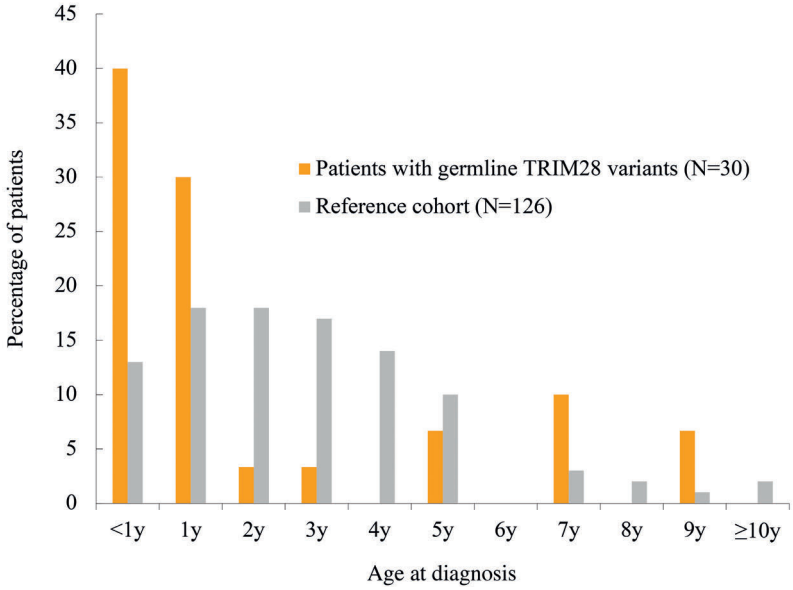

Figure 4. Age at Wilms' tumor diagnosis (in years) of patients with germline *TRIM28* variants (N = 30) versus an unselected reference cohort of patients with WT (N = 126). The reference cohort includes all patients diagnosed with WT in The Netherlands in a 5-year period.

Prognosis

In the reviewed studies, metastatic disease was not reported. Follow-up data were available for 13 patients with germline pathogenic variants in *TRIM28*, none of whom relapsed. The duration of follow-up ranged from 3 to 36 years, with a median of 20 years for patients with follow-up data. One patient with diffuse anaplastic WT died of an unspecified cause, 3 years after WT diagnosis.[12] It has been previously suggested that *TRIM28*-mutated WTs represent a subgroup of WTs with a low risk of metastases or relapse. This may be attributed to the fact that the majority are epithelial WTs, which are known to have a good outcome.[59,60] This information can be reassuring for families with young carriers of pathogenic *TRIM28* variants.

Additional phenotypes

Despite the involvement of *TRIM28* in a wide variety of cellular processes, there is no strong evidence suggesting that germline pathogenic *TRIM28* variants cause a phenotype other than WT predisposition in humans. Additional clinical findings were only documented in 4/33 patients, although phenotypic data may have been incompletely reported. For example, Mahamdallie et al only reported that patients had no other cancers[12] and no phenotypic data were available for the patient reported by Armstrong et al.[13] Patients with additional clinical findings included two unrelated patients with

autism and speech delay/intellectual disability[12] and two siblings with congenital heart defects, in one of them accompanied by oesophageal atresia and retinopathy.[15] For the two siblings, a different (genetic) cause of their congenital heart defect cannot be excluded, even though this was not identified with WES.[15] As mentioned previously, the male infertility observed in haploinsufficient mice[54] has not been documented in humans, but may warrant attention during the clinical follow-up of *TRIM28* mutation carriers.

Recommendations for the genetic analysis of *TRIM28* in patients with WT

To enable counselling, genetic testing, and early detection of WTs in young family members, it is important to recognize germline pathogenic *TRIM28* variants in patients with WT. Depending on local infrastructure and resources, some pediatric oncology centres may offer routine genetic testing to all patients, while others select those who are clinically suspected of having a genetic predisposition syndrome.[61] To identify patients with germline variants in *TRIM28*, we would recommend routine assessment of WTs for TRIM28 loss by IHC with the anti-KAP1 antibody (ab10484)[15], which is a relatively simple and inexpensive test. Even though the majority of *TRIM28*-mutated tumors are epithelial (predominant) WT, we would recommend including all WT subtypes in this assessment, as other histological subtypes have also been reported and an accurate distribution of *TRIM28* mutations among the different histological subtypes has not yet been determined. Subsequently, genetic analysis of *TRIM28* in blood-derived DNA can be performed in all patients who display loss of TRIM28 in the tumor.

DIRECTIONS FOR FUTURE RESEARCH

A further exploration of *TRIM28*-associated WT will help to unravel the diverse mechanisms that can lead to WT development. In vitro models suggest that loss of *TRIM28* leads to a loss of (epigenetic) transcriptional regulation. This may upregulate specific signalling pathways in the ureteric bud and metanephric mesenchyme, resulting in a disturbed balance between proliferation and differentiation, and in a branching arrest in the embryonic kidney. Further studies in embryonic kidney models are needed to determine exactly which signalling pathways are deregulated upon loss of *TRIM28*. This also includes the direct epigenetic impact of *TRIM28* deficiency, i.e. changes in DNA methylation and chromatin organization, in the developing kidney. Although we have gained many insights from mouse studies, additional studies are preferably conducted in human kidney models, given the recently described differences between human and mouse developmental programs during nephrogenesis.[42,62] For this purpose, organoid models may provide valuable opportunities. Organoid models can be established directly from tumor- and adjacent-kidney tissue of patients with germline pathogenic *TRIM28* variants.[63] Since such a model may not recapitulate the crucial effects of *TRIM28* loss during the earliest stages of nephrogenesis, *TRIM28*-deficient human pluripotent stem cells (hPSCs) could be an interesting alternative. We speculate that differentiation of

these hPSCs into kidney organoids will enable us to study the consequences of *TRIM28* loss during the earliest stages of nephrogenesis, which is not possible in patient derived organoids.[62] By additionally knocking out REST and AMER1, more insight into potential *TRIM28–REST* and *TRIM28–AMER1* regulatory effects may also be provided.

The role of TEs in human embryonic kidney and WT development warrants further investigation. In addition to the *TRIM28*-mediated transcriptional repression of TEs, recent evidence suggests that post-transcriptional repression of TEs is mediated by miRNAs[64], which is intriguing because miRNA processing genes (*DROSHA, DICER1, DIS3L2, DGCR8*) represent an important group of WT driver genes.[65]

Similar to some other WT predisposition genes[12], such as *WT1, IGF2*, and *DICER1, TRIM28* seems to promote WT development in both a germline and a somatic context. Given its role in early nephrogenesis and the high rate of germline variants, *TRIM28* mutations are considered early events. We speculate that the identified somatic mutations may have been present in a mosaic state in adjacent normal kidney tissue, as was demonstrated in one patient by Diets et al.[15] This could be further investigated by assessing multiple samples from adjacent normal kidney tissue of somatically *TRIM28*-mutated WT.

Finally, from a clinical perspective, it is relevant to collect more data on both healthy and affected carriers of pathogenic *TRIM28* variants. This will require international collaboration, and will help to improve the counselling of patients and their families.

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7

Wilms tumor surveillance in at-risk children: literature review and recommendations from the SIOP-Europe Host Genome Working Group and SIOP Renal Tumor Study Group

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ABSTRACT

Since previous consensus-based Wilms tumor (WT) surveillance guidelines were published, novel genes and syndromes associated with WT risk have been identified, and diagnostic molecular tests for previously known syndromes have improved. In view of this, the International Society of Pediatric Oncology (SIOP)-Europe Host Genome Working Group and SIOP Renal Tumor Study Group hereby present updated WT surveillance guidelines after an extensive literature review and international consensus meetings. These guidelines are for use by clinical geneticists, pediatricians, pediatric oncologists and radiologists involved in the care of children at risk of WT. Additionally, we emphasise the need to register all patients with a cancer predisposition syndrome in national or international databases, to enable the development of better tumor risk estimates and tumor surveillance programs in the future.

INTRODUCTION

Wilms tumor (WT) (nephroblastoma) is the most common childhood renal malignancy. Current treatment regimens include a combination of chemotherapy, surgery and, in some cases, radiotherapy, achieving survival rates of 90%.[1] Yet, advanced stage WT is still associated with significant morbidity and mortality. To enable the detection of smaller and lower-stage tumors[2,3], WT surveillance is offered to children with various cancer predisposition syndromes (CPS), with WT1-related syndromes and Beckwith-Wiedemann syndrome/spectrum (BWS/BWSp) being the most wellknown examples.[4-6]

In general, tumor surveillance is recommended if the benefits outweigh the costs and burden. This depends on many factors, including the tumor risk of the screened population and the consequences of early detection for prognosis and management. Worldwide, different countries use different arbitrary thresholds to determine whether WT surveillance is indicated in children with a specific CPS, varying between 1 and 5% estimated childhood WT risk.[4,5]

Since previous consensus-based WT surveillance guidelines[4] were published, novel genes and syndromes associated with WT risk have been identified.[7-9] For some previously known syndromes, molecular tests have improved, and/or larger series have been published, enabling better WT risk estimates. More recent surveillance recommendations are limited to BWSp[10] or targeted towards the North-American health-care culture.[5] Here, updated WT surveillance guidelines developed by the International Society of Pediatric Oncology (SIOP)-Europe Host Genome Working Group and SIOP Renal Tumor Study Group (RTSG) are presented.

Recommendations and the rationale behind them are discussed based on an extensive literature review and international consensus meetings, addressing all currently known WT predisposition genes and syndromes. Additionally, we discuss imaging modalities and surveillance interval and emphasise the need to prospectively register all patients with a CPS in national or international databases, to enable better risk estimates in the future. These guidelines are for use by clinical geneticists, pediatricians, pediatric oncologists and radiologists involved in the care of children at risk of WT.

METHODS

The international consensus group was comprised of 16 participants from the United Kingdom, The Netherlands, France, Germany and Japan, including pediatric oncologists, geneticists, a radiologist and an epidemiologist. Discussions occurred via video conferences and email communications. A preliminary meeting was held in June 2020 after which the identified CPS were divided into four groups, discussed in smaller meetings with eight participants each. Various patient/parent representatives were contacted and requested to comment on their experiences regarding the practical and emotional burden, recommended risk threshold and duration and willingness to travel for WT surveillance.

Based on the discussions during the meetings and the input from patient/parent representatives, recommendations were developed which were discussed in a final consensus meeting with all participants in November 2020.

A PubMed search was conducted using the keywords "Wilm*" or "Nephroblastoma*" or the MeSH term "Wilms Tumor" in combination with synonyms for the various WT predisposition genes and syndromes (**Supplemental Table 1**). Articles of interest were selected based on title/abstract screening, prioritizing cohort studies, larger series and previous literature reviews with information on WT occurrences for each gene or syndrome. Additionally, the PubMed search was combined with the keywords "Surveillance" or "Screening" to explore the evidence supporting (or against) surveillance.

For the majority of the reviewed genes and syndromes, our literature review identified only a limited number of studies which were mainly case reports or small case series. In order to grade the recommendations that were established during the consensus meetings, we used the following scale which was adapted from the recently published European Reference Network PTEN cancer surveillance guideline[11]: (i) strong evidence, consistent evidence and new evidence unlikely to change recommendation and expert consensus; (ii) moderate evidence, expert consensus or majority decision but with inconsistent evidence or significant new evidence expected and (iii) weak evidence, inconsistent evidence AND limited expert agreement.

CPS with an estimated childhood WT risk of more than 5% were primarily selected as those where surveillance should be offered.[12] For syndromes with an estimated WT risk between 1 and 5%, additional cancer risks were taken into account when deciding on whether to recommend surveillance. As accurate tumor risk estimates require large, unbiased cohorts with long-term follow-up, we estimated cumulative WT risks by calculating the percentage of reported patients with WT among the total number of reported individuals with a given CPS, acknowledging that such estimates are prone

to selection bias. The recommended duration of surveillance was based on the age at which approximately 90-95% of reported WTs have been diagnosed, in accordance with previous guidelines on WT surveillance[4,5] and other CPS.[13]

AIM AND POTENTIAL BENEFITS OF SURVEILLANCE

WT surveillance aims to improve survival and to reduce treatment-related toxicity for WT patients with a genetic and/or epigenetic predisposition, by enabling the detection of smaller and lower stage tumors. There are no studies directly comparing survival rates or morbidity between screened and unscreened patients. Owing to the generally good prognosis of WT, the effects of WT surveillance on overall survival rates may be small.

Diagnosing lower stage tumors can, however, avoid the need for toxic treatment such as anthracyclines or radiotherapy, reducing direct and late side-effects. It has been retrospectively demonstrated that children with BWS or hemihypertrophy undergoing WT surveillance had significantly lower stage WT compared with children not participating in a surveillance program[2,3] and that WTs in patients with Wilms tumor, aniridia, genitourinary anomalies and range of developmental delays (WAGR) syndrome are significantly smaller if they are surveillance-detected than symptomatic tumors.[14] Analysis of a registry-based cohort could provide stronger unbiased evidence in the future.

Diagnosing smaller tumors can also enable nephron-sparing surgery (NSS). The SIOP-RTSG 2016 UMBRELLA protocol recommends NSS for children with a genetic predisposition if feasible depending on the size and location of the tumor.[15] Several studies have demonstrated that NSS can be safely performed in children with a WT predisposition syndrome with unilateral or bilateral WT.[16-18] In patients with WAGR syndrome and WT, mortality was more frequently caused by end-stage renal disease (ESRD) than the tumor itself.[19] Therefore, NSS is believed to be particularly relevant for patients with a risk of developing renal failure (such as *WT1*-related conditions), where it may prevent or delay the need for dialysis or renal transplantation.

COSTS AND BURDEN OF SURVEILLANCE

In 2001, a cost-benefit analysis was performed to estimate the costs per life-year saved for WT and hepatoblastoma surveillance in a hypothetical cohort of children with BWS.[20] The costs were considered to be reasonable in comparison to other population-based cancer surveillance programs at the time.[20] An update of this study is warranted, which would ideally also address additional benefits such as decreased toxicity and the feasibility of NSS. False-positive or incidental findings detected by surveillance have been reported in children with BWSp. Choyke et al. reported two resected renal lesions, which were suspected to be cystic WT, but proved to be infected renal cysts upon

histological examination.[2] In one of these patients, a radical nephroureterectomy had been performed. Zarate et al. identified renal or liver abnormalities in 25 of 63 (40%) children with BWSp undergoing surveillance.[21] Such findings can trigger unnecessary interventions and investigations, leading to additional costs, and may cause anxiety in patients and their guardians.

The practical and emotional burden associated with cancer surveillance ranges from logistical issues to anxiety around surveillance visits. Based on input from the International WAGR Syndrome Association (IWSA) and the UK BWS Support Group, surveillance visits can be stressful for some parents while reassuring for others, and anxiety similarly varies from child to child. Both groups reported that not undergoing surveillance can also be stressful for parents. Practicalities such as time and transport can be an issue but are less important when surveillance visits can be combined with regular hospital visits for other indications. Overall, both groups emphasised that the benefits of surveillance outweigh the practical and emotional burden, and they would not object to surveillance of longer duration than that being proposed here.

GENERAL RECOMMENDATIONS: HOW TO SCREEN

Surveillance should be offered after parents have received counselling about WT risk in their child by a clinical geneticist or genetic counsellor. Renal ultrasonography is the recommended screening modality, which avoids radiation exposure (unlike computed tomography [CT] imaging) and does not require anaesthesia in young children (unlike magnetic resonance imaging [MRI]). Although CT or MRI may have a higher resolution for discriminating between different tumor types and nephrogenic rests, ultrasonography is believed to be equally effective for initial WT detection based on expert consensus. Guidelines on how to perform renal ultrasound surveillance are provided in **Table 1**.

Table 1. Guidelines for renal/abdominal ultrasonography in children at risk of Wilms tumor, adapted from Scott et al., 2006[4] and updated.

Equipment	High-frequency probes and pediatric settings. Linear (>10 MHz) in infants, curvilinear (>6 MHz) and linear (>10 MHz) probes in toddlers and children.
Preparation	Fasting and bladder preparation are not required.
Target organ	Kidney only, except for patients with BWS/BWSp, lateralised overgrowth or SGBS, who require a full abdominal ultrasound including adrenal glands and liver to check for other abdominal tumors, including neuroblastoma, hepatoblastoma and adrenocortical carcinoma.
Technique	Appropriate focal point and time gain settings. The whole renal parenchyma should be imaged in longitudinal and transverse planes with the child both supine and prone.
Normal variants	Foetal lobulations, dromedary hump, column of Bertin, duplex or bifid collecting systems.
Suspicious lesions	Solitary or multiple cystic or solid parenchymal lesions with or without sonographic signs of expansile growth. A solid lesion is more likely to represent malignancy than a simple cystic anechoic lesion.

BWS/BWSp: Beckwith-Wiedemann Syndrome/Spectrum, SGBS: Simpson-Golabi-Behmel Syndrome.

Surveillance can be undertaken at a local center but should be performed by someone with experience of pediatric ultrasonography with screen-detected lesions managed at a specialist center. For certain syndromes (specified in **Table 1**), we recommend replacing renal ultrasonography by full abdominal ultrasonography because of additional abdominal tumor risks.

Previous surveillance guidelines have recommended scans every 3-4 months[4-6,10] as WTs are known to have a high growth rate with the shortest reported estimated doubling time being 11 days.[22] We recommend a surveillance interval of 3 months because in clinical practice, surveillance visits can be delayed, and the consensus group agreed that an interval of ≥4 months risks higher tumor stage at diagnosis. A recent clinical report demonstrated that growth rate varies between tumors, and this may depend on their molecular characteristics.[23] Whether growth rate also varies between different underlying predisposition syndromes is a relevant research question to address in preclinical models.

GENERAL RECOMMENDATIONS: WHEN TO SCREEN

Surveillance recommendations for all identified CPS associated with an increased risk of WT development are presented in **Table 2** and discussed in more detail in the following sections. We recommend initiating surveillance at birth or as soon as a CPS is diagnosed. As molecular confirmation of a CPS can take some months, surveillance can be initiated based on the clinical suspicion of a CPS, while awaiting test results.

If WT surveillance is indicated, we recommend continuing surveillance until a child's 7th birthday regardless of the underlying CPS diagnosis. By the age of 7 years, 90% of sporadic WTs[3], 94% of WTs in children with BWS[3] and >95% of WTs in children with *WT1*-related syndromes[14,19,24] have been diagnosed, and this age has been previously recommended by other groups.[5,10] For other CPS, the number of reported patients with WT was too small to determine this percentage.

Table 2. Summary of cancer predisposition genes/syndromes with a reported risk of Wilms tumor (WT) development and surveillance recommendations.

Syndrome/gene		Estimated % of patients with this condition with WT	WT surveillance recommended? If yes: 3 monthly from birth until 7th birthday	Evidence*
WT1 mutations	Exonic missense variants	~50%	**Yes, renal US**	Strong
	Exonic truncating variants	~80%	**Yes, renal US**	Strong
	Intron 9 variants	~2%	No	Moderate
WAGR syndrome (11p13 deletion encompassing *WT1*)		~55%	**Yes, renal US**	Strong
Beckwith-Wiedemann syndrome/spectrum (BWS/BWSp)	LOM IC2	<1%	No	Moderate
	GOM IC1	~21%	**Yes, full abdominal US [A]**	Strong
	Paternal UPD 11p15	~8%	**Yes, full abdominal US [A]**	Strong
	CDKN1C mutation	~1%	**Yes, full abdominal US [A]**	Moderate
	Classical BWS with negative tests	~5%	**Yes, full abdominal US [A]**	Moderate
Lateralized overgrowth with ≥1 BWS feature		Unknown	**Yes, full abdominal US [A]**	Moderate
Lateralized overgrowth without additional BWS features		Unknown	No	Moderate
Perlman syndrome (*DIS3L2*) **(recessive)**		~64%	**Yes, renal US**	Strong
PIK3CA-related overgrowth (*PIK3CA*) **(somatic mosaic)**		1-5%	No	Moderate
Simpson-Golabi Behmel syndrome (*GPC3/GPC4*)		~3%	**Yes, full abdominal US [A]**	Moderate
TRIM28 mutations		>50% penetrance	**Yes, renal US**	Moderate
REST mutations		>50% penetrance	**Yes, renal US**	Moderate
CTR9 mutations	Truncating/splicing variants	Appears high	**Yes, renal US**	Moderate
	Missense variants	WT not reported	No	Moderate
HACE1 mutations		Unknown	No	Moderate
KDM3B mutations		Appears low	No	Moderate
FBXW7 mutations		Unknown	No	Moderate
NYNRIN mutations **(recessive)**		Unknown	No [B]	Moderate
Fanconi anemia	FANC-D1 (*BRCA2*) **(recessive)**	~20%	**Yes, renal US**	Strong
	FANC-N (*PALB2*) **(recessive)**	~40%	**Yes, renal US**	Strong
	Other subtypes	WT not reported	No	Moderate
Mulibrey Nanism (*TRIM37*) **(recessive)**		~6-8%	**Yes, renal US**	Moderate

Table 2. Continued.

Syndrome/gene		Estimated % of patients with this condition with WT	WT surveillance recommended? If yes: 3 monthly from birth until 7th birthday	Evidence*
Mosaic variegated aneuploidy (MVA)	*BUB1B* variants **(recessive)**	~50%	**Yes, renal US**	Moderate
	TRIP13 variants **(recessive)**	~20%	**Yes, renal US**	Moderate
	CEP57 variants **(recessive)**	WT not reported	**Yes, renal US**	Moderate
	MVA with unknown cause	WT not reported	**Yes, renal US**	Moderate
9q22.3 microdeletion syndrome		10-20%	**Yes, renal US**	Moderate
2p24.3 duplication (encompassing *MYCN*)		Unknown	No	Moderate
Osteopathia striata with cranial sclerosis (OSCS) (*WTX*) **(X-linked)**		Unknown, but appears >5%	**Yes, renal US**	Moderate
2q37 deletion syndrome	Extending to 2q37.1	10-20% (3 cases)	**Yes, renal US**	Moderate
	More distal deletions	WT not reported	No	Moderate
Bloom syndrome (*BLM*) **(recessive)**		~3%	No	Moderate
DICER1 syndrome (*DICER1*)		<2%	No [C,D]	Moderate
Li Fraumeni syndrome (*TP53*)		Low	No [C]	Moderate
Neurofibromatosis type 1 (*NF1*)		<1%	No [C]	Moderate
Hyperparathyroidism-jaw tumor syndrome (*CDC73*)		<5%	No [C]	Moderate
Constitutional mismatch repair deficiency (*MSH2, MSH6, MLH1, PMS2*) **(recessive)**		~3%	No [C]	Moderate
Bohring-Opitz syndrome (*ASXL1*)		~7%	**Yes, renal US**	Moderate
Trisomy 13		<1%	No	Moderate
Trisomy 18		~1%	No	Moderate

BWS/BWSp: Beckwith-Wiedemann Syndrome/Spectrum; GOM: gain of methylation; US: ultrasound; WAGR: Wilms tumor, aniridia, genitourinary anomalies and range of developmental delays. A: Additional risk of other abdominal tumors. B: Surveillance can be considered in a research setting. C: In these syndromes, cancer surveillance is recommended for other cancer types (beyond the scope of this guideline), but does not include 3- monthly renal or abdominal US. D: To enable early detection of cystic nephromas, the SIOP-Europe Host Genome Working Group and CanGene-CanVar Clinical Guideline Working Group recommend 6-monthly renal US until the child's 6th birthday (manuscript under review). *Evidence: (i) strong evidence: consistent evidence and new evidence unlikely to change recommendation and expert consensus. (ii) moderate evidence: expert consensus or majority decision but with inconsistent evidence or significant new evidence expected. (iii) weak evidence: inconsistent evidence AND limited expert agreement.

Among patients with *WT1*-related syndromes, >90-95% of WTs are diagnosed before the age of 5 years, although patients with nephrogenic rests progressing to (metachronous) tumors after the age of 5 years have been reported.[14,25] Although we have previously suggested screening patients with WT1-related syndromes until the age of 5 years[4] and to prolong surveillance only for patients with a prior diagnosis of WT/nephroblastomatosis[14], the consensus group agreed to recommend surveillance until the 7th birthday for all CPS including *WT1*-related syndromes. Factors that influenced this decision were that nephroblastomatosis may not be identified on ultrasound, to maintain consistency with other WT predisposition genes/syndromes and in response to patient/parent representatives' views.

CONSIDERATIONS FOR SPECIFIC WT PREDISPOSITION GENES AND SYNDROMES

WT1 pathogenic variants

WT surveillance is recommended for children with germline pathogenic variants in *WT1*, except for intron 9 mutations. *WT1* was the first known WT predisposition gene.[26-28] Germline *WT1* aberrations are present in an estimated 2e11% of patients with WT[29-33], usually occurring de novo in isolated (non-familial) cases. The exact percentage may vary between different geographic WT cohorts.[34] In addition to an increased risk of WT, *WT1* pathogenic variants are associated with renal disease (glomerulosclerosis) which can lead to renal failure and disorders of sexual development (DSD). There is considerable overlap in the phenotypic spectrum of patients previously referred to as having Denys-Drash syndrome (exon 8 or 9 mutations) or Frasier syndrome (intron 9 mutations)[35], although genotype-phenotype correlations exist.[24,36-38] Notably, WT can also be the first manifestation of a pathogenic *WT1* variant in children with an otherwise unremarkable medical and family history.

Based on data extracted from five studies (**Supplemental Table 2**), WTs were reported in ~50% of patients with exonic missense mutations, ~80% of patients with exonic truncating mutations and ~2% of patients with intron 9 mutations.[36-40] Patients included in these studies were identified because of the presence of nephrotic syndrome or DSD. A subset of these patients, particularly those with exonic missense variants, had ESRD in infancy and underwent prophylactic bilateral nephrectomies, potentially leading to an underestimate of WT risk in these studies. This is not the case for patients with intron 9 mutations, who typically develop ESRD at older ages.[41] Although patients with intron 9 mutations are frequently diagnosed with DSD, which is associated with a high risk of gonadoblastoma[41], ultrasound or MRI surveillance is not reliable for the early detection of gonadal neoplasms.[42] Therefore, combined with the low risk of WT, renal or abdominal ultrasound surveillance is not recommended for patients with intron 9 mutations.

In series of patients with *WT1* variants and WT, the age at tumor development varied from 0 to 4.5 years, with medians between 9 months and 1.6 years.[24,25,29,31,32,36-38,43] A risk of later-onset metachronous WT has been reported.[25]

WAGR syndrome

WT surveillance is recommended for all children with WAGR syndrome. WAGR syndrome is caused by the contiguous deletion of *WT1* and *PAX6* genes at 11p13. The diagnosis of WAGR syndrome is usually established early because of aniridia, frequently accompanied by other ophthalmologic abnormalities, genitourinary anomalies and developmental delay.

Based on data extracted from four published cohorts of patients with WAGR syndrome (**Supplemental Table 3**), WTs were reported in ~55% of all patients.[44-47] Reported ages at WT diagnosis varied from 0.3 to 25 years (median ages: 15-23 months).[14,19,32,45,46,48] Similar to patients with *WT1* variants, patients with WAGR syndrome are at risk of developing metachronous tumors[14] and renal failure.[19]

Beckwith-Wiedemann spectrum

Surveillance by full abdominal ultrasound is recommended once every 3 months for all molecular subtypes of BWSp, except for IC2 (KCNQ1OT1:TSS-DMR) loss of methylation (IC2 LOM).

BWSp is the most frequently diagnosed WT predisposition syndrome, affecting 1 in 10,500 children in Western populations.[49] BWSp is considered an overgrowth syndrome with a highly variable phenotype which can include (lateralised) overgrowth, macroglossia, abdominal wall defects and hyperinsulinism leading to neonatal hypoglycemia.[10] BWS is molecularly characterised by genetic and/or epigenetic changes at the 11p15.5 imprinted region, which are frequently mosaic. In 2018, the European Cooperation in Science and Technology (COST) funded European Network for Congenital Imprinting Disorders published a consensus document in which the novel term BWSp was introduced. BWSp includes patients with classical BWS as well as patients with 'atypical BWS' (not meeting the criteria for a clinical diagnosis) or 'isolated lateralised overgrowth' with a BWS-associated molecular (epi)genetic alteration at the 11p15.5 imprinted region.[10]

Maternal IC2 LOM, the most prevalent molecular subtype, is associated with an estimated WT risk of only ~0.2%, and therefore, surveillance is not recommended.[10,50,51] Patients with a gain of methylation at the maternal IC1 locus (H19/IGF2 DMR) comprise only 5% of all patients with BWSp but have an estimated ~21% cumulative risk of WT development.[10,50,51]

This risk is estimated to be ~8% in patients with a paternal uniparental disomy of 11p15.5.[10,50,51] Pooled WT risk estimates and implications for surveillance are described for the major molecular subtypes in **Supplemental Table 4**, which was adapted from the study by Maas et al. and updated to include the more recently published study by Cöktü et al.[10,50,51]

Lateralised overgrowth

Full abdominal ultrasound is recommended once every 3 months for patients with lateralised overgrowth (LO) and ≥1 additional feature of BWSp. LO, also known as hemihypertrophy or hemihyperplasia, is defined as overgrowth of one side of the body compared with its contralateral side. This may be restricted to (part of) a limb or the face, with a pragmatic definition that it should be apparent 'from the end of the bed'.[52] The incidence is estimated to be 1:13,000 to 1:86,000 live births.[53]

If a syndromic diagnosis can be established based on molecular testing or clinical criteria, tumor surveillance should be initiated accordingly. Robust data are lacking for remaining patients (i.e. isolated LO and no detectable molecular finding). Two studies have estimated the overall tumor risk to be around 10%, with WT and neuroblastoma being the most common tumor types[54,55], although it is likely that this includes patients with low-level mosaic BWSp aberrations.

Therefore, for all patients with LO, we recommend careful assessment by a clinical geneticist and molecular testing which should include 11p15.5 analysis in germline DNA. Baseline abdominal ultrasonography is advised to assess the presence of organomegaly, which is an additional BWSp feature and therefore an indication for initiating WT surveillance. For significant isolated LO, we advise trying to establish the underlying (epi) genetic cause by testing overgrown tissues and initiating surveillance while awaiting test results. Further research focussing on this group of patients is necessary to clarify WT risks.

Other overgrowth syndromes

WT surveillance is recommended for Perlman syndrome (renal ultrasound) and Simpson Golabi Behmel syndrome (SGBS) (full abdominal ultrasound). Although WTs are reported in a subset of patients with *PIK3CA*-related overgrowth spectrum (PROS), surveillance is currently not recommended by the consensus group (see paragraph on PROS). In patients with other overgrowth syndromes (e.g. Sotos, Proteus, Malan, Thauvin Robinet Faivre and Weaver syndrome), WTs were only sporadically reported or not at all, and surveillance is therefore not recommended.

Perlman syndrome is an autosomal recessive syndrome associated with a 64% risk of WT development in children surviving the neonatal period, in addition to polyhydramnios, macrosomia, facial dysmorphism, renal dysplasia, multiple congenital anomalies and

frequently neurodevelopmental delay.[56-58] More than half of the children with Perlman syndrome die within the first year of life because of respiratory insufficiency, sepsis and/or renal failure.[59] In 2012, biallelic inactivating variants in *DIS3L2* were identified as the cause of Perlman syndrome.[60] *DIS3L2* appears to play a role in normal kidney development, and the mechanism by which Perlman syndrome increases WT risk may be due to increased IGF2 expression as demonstrated in mouse models.[61]

SGBS is an X-linked disorder due to pathogenic *GPC3* variants or deletions, which may involve *GPC4*, or a multi-exon duplication of *GPC4*.[62,63] Affected males have pre- and post-natal overgrowth, distinctive facial features, variable levels of intellectual disability and congenital anomalies.[64-66] Older studies reported WT risks between 5 and 15%[67-73], but these studies did not always include molecular analysis and cases may have been misdiagnosed. A 2019 literature review identified 152 patients with *GPC3* variants and found an overall tumor risk of 8.5%, including 5 WTs (5/152 = 3%), with the most common tumor type being hepatoblastoma.[74] Therefore, full abdominal ultrasonography is recommended once every 3 months for children with SGBS.

PROS covers a range of disorders now known to be caused by somatic mosaic *PIK3CA* mutations, including CLOVES syndrome (congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal anomalies), Klippel-Trenaunay syndrome, megalencephaly-capillary malformation syndrome and fibroadipose hyperplasia.[75-77] Although WT risk estimates vary between different reports, currently available data suggest that the WT risk is less than 5%.[76,77] Other tumor types have not been reported in relation to PROS, and therefore, surveillance is not recommended.

Novel genes

WT surveillance is recommended for all children with germline pathogenic variants in *TRIM28* or *REST*, as well as children with truncating *CTR9* variants.

TRIM28 was recently identified as a novel WT predisposition gene, with heterozygous germline pathogenic variants currently reported in ≥30 patients with WT.[7,78-81] Pedigrees from families with *TRIM28* variants suggest a WT penetrance of >50%.[7,80] Although the median age at WT diagnosis was young (13 months), only 83% of WTs were diagnosed before the age of 7 years[7,78-81], and further research is needed to determine whether longer surveillance is indicated for this group. Although there is some evidence that WT risk may be preferentially associated with maternally inherited familial *TRIM28* mutations[7], until definitive evidence is available, surveillance should be offered irrespective of the inheritance pattern.

REST pathogenic variants were identified in familial WT pedigrees by Mahamdallie et al., in 2015.[82] Heterozygous germline variants have currently been reported in 19 patients with WT from 14 families.[82,83] Additionally, a de novo deletion encompassing *REST* was

recently identified in a patient with diffuse hyperplastic perilobar nephroblastomatosis[84]. The *REST* gene encodes the RE1-silencing transcription factor which, similar to *TRIM28*, is thought to play an important role during embryonic development.[8] Pedigrees from families with *REST* variants suggest a disease penetrance of >50%.[82]

Inactivating heterozygous *CTR9* variants were identified in three WT families by Hanks et al.[85] in 2014 and reported in an additional family by Martins et al.[86] These four families included a total of nine patients with WT. In a recently presented conference abstract, missense *CTR9* variants were reported in 11 patients with neurodevelopmental disorders but no tumors (Meuwissen et al., P08.021.C at the European Society of Human Genetics Virtual Conference 2020.2). This suggests that only truncating variants are associated with an increased risk of WT development.

Other genes that have been associated with WT predisposition in the last decade include *HACE1, KDM3B, FBXW7* and *NYNRIN*.[7,87,88] Based on current evidence, we would not recommend standard surveillance for patients with *HACE1, KDM3B* or *FBXW7* variants, given that only few (≤5) patients have been reported to develop WT and there are no families with multiple affected relatives. *NYNRIN* pathogenic variants seem to predispose to WT development in a recessive manner, with biallelic variants identified in two affected siblings and a third unrelated patient.[7] We suggest that WT surveillance can be considered in a research setting for patients with biallelic (likely) pathogenic *NYNRIN* variants, with the aim to collect more data regarding these patients' WT risk.

Other syndromes

Other syndromes for which WT surveillance is recommended include Fanconi anaemia type D1, Fanconi anaemia type N, Mulibrey nanism, mosaic variegated aneuploidy (MVA), osteopathia striata with cranial sclerosis (OSCS), Bohring-Opitz syndrome, 9q22.3 deletions and 2q37.1 deletions (**Table 2**).

Fanconi anaemia types D1 (biallelic pathogenic *BRCA2* variants) and type N (biallelic pathogenic *PALB2* variants) are associated with estimated WT risks of around 20% and 40%, respectively.[89-93] We did not identify reports of WT in children with Fanconi anaemia because of other molecular causes, although these patients are at risk for a range of other malignancies which are beyond the scope of this guideline.[94]

Mulibrey nanism, caused by biallelic pathogenic *TRIM37* variants, has mainly been reported in Finnish patients and is associated with an estimated WT risk of 6-8%.[95,96]

MVA can be caused by biallelic *BUB1B, TRIP13* or *CEP57* pathogenic variants, while in some patients, the cause remains unknown.[97-100] WTs have been reported in approximately 50% of patients with *BUB1B* variants[101,102], 20% of patients with *TRIP13* variants[99] and, to our current knowledge, none of the reported patients with *CEP57*

variants or MVA because of an unknown cause.[100,103] Because of the limited number of reported patients, we recommend WT surveillance for all patients with cytogenetically confirmed MVA.

OSCS is an X-linked condition caused by germline loss-of-function variants affecting the *AMER1 (WTX)* gene. Currently, WT has been reported in four female heterozygotes[104,105], and bilateral nephrogenic rests were reported at autopsy in a male patient with OSCS.[106] Although two published OSCS cohorts, including 17 and 22 liveborn patients, respectively, did not report childhood tumors[107,108], we consider WT surveillance to be justifiable based on the well-established role of *AMER1/WTX* in WT development.[109]

Bohring-Opitz syndrome is assumed to be genetically heterogeneous, with a subset of patients harbouring germline heterozygous nonsense variants in *ASXL1*.[110] WT or nephroblastomatosis has been reported in 3 of 43 (7%) reported patients with a clinical or molecular diagnosis of Bohring-Opitz syndrome.[111,112] Therefore, WT surveillance is recommended for patients with Bohring-Opitz syndrome.

Among 44 published cases of 9q22.3 microdeletion syndrome, seven patients with WT (16%) were reported.[113] Although these deletions all encompass *PTCH1* and cause a clinical phenotype which overlaps with that of Gorlin syndrome[114], WTs have not been observed in patients with Gorlin syndrome (caused by *PTCH1* or *SUFU* pathogenic variants)[113], and WT surveillance is only recommended for patients with 9q22.3 deletions.

2q37 Deletion syndrome has been reported in around 115 patients[115], with the minimal critical region limited to a single gene (*HDAC4*) on 2q37.3.[116] WTs were reported in three of these patients, who all had deletions encompassing 2q37.1 (including *DIS3L2*, mutations in which cause Perlman syndrome [discussed previously]).[117] We suggest that WT surveillance can be considered in cases where the deletion includes 2q37.1.

Constitutional 2p24.3 duplication (involving *MYCN*) has been reported in less than 100 patients overall,with four reported cases of WT or nephroblastomatosis.[118-120] Two WT cases occurred within one family, where an (unknown) additional genetic factor may have played a role.[120] Until more evidence emerges in the future, we would currently not recommend standard WT surveillance.

Until recently, only three patients with WT had been reported in unrelated families with hyperparathyroidism jaw tumor syndrome (HP-JT), out of a total of >40 reported families (>100 patients).[121,122] In 2019, Mahamdallie et al. identified a germline *CDC73* mutation in a father and his daughter who were both affected with WT but had no additional phenotypic features of HP-JT.[7] We would not currently recommend standard WT surveillance, in line with previously published HP-JT surveillance guidelines.[123,124]

WTs have also been reported in patients with Bloom syndrome, *DICER1* syndrome, Li Fraumeni syndrome, neurofibromatosis type 1, constitutional mismatch repair deficiency, trisomy 13 and trisomy 18. For these syndromes, the estimated WT risk was considered too low to recommend targeted WT surveillance, although cancer surveillance for other tumor types is warranted in some of these conditions (but outside the scope of this guideline). Considerations and references for these syndromes are listed in **Supplemental Table 5**.

OTHER CONSIDERATIONS FOR CHILDREN DIAGNOSED WITH WT/ NEPHROBLASTOMATOSIS

In children with WT/nephroblastomatosis who have been diagnosed with a CPS, surveillance of the remaining kidney(s) by 3-monthly renal ultrasonography is warranted until the 7th birthday, or longer if indicated by the follow-up guidelines for the treated tumor.

For all patients with bilateral WT/nephroblastomatosis, we recommend surveillance of the remaining kidney(s) by 3-monthly renal ultrasonography until the 7th birthday and genetic testing to exclude germline genetic/epigenetic aberrations. While awaiting test results, siblings may be offered a single ultrasound examination. Recent evidence suggests that bilateral WT may frequently be due to postzygotic (mosaic) events.[125,126] If germline testing is negative, we therefore recommend that renal tissue from the resected kidney is tested, where possible, to exclude or diagnose a mosaic WT susceptible condition. The consensus opinion was that 3 monthly surveillance for siblings is not recommended if no germline genetic diagnosis is identified in the proband.

FAMILIAL WT

Familial WT is defined as the presence of ≥2 patients with WT within one family, who are at least third degree relatives of each other (**Figure 1**). The WT diagnosis of both patients should be confirmed in their medical records. If the causative gene is not identified after germline genetic testing, WT surveillance until the 7th birthday is recommended for first and second degree relatives of presumed mutation carriers.

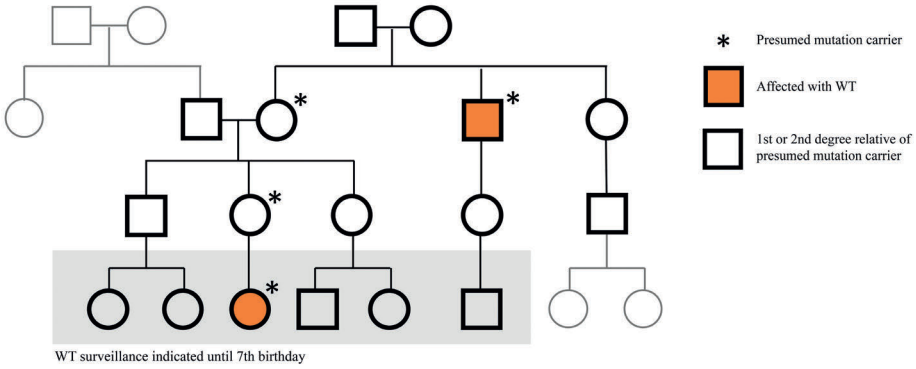

Figure 1. Example of a familial Wilms tumor (WT) pedigree where the causative gene is not identified.

FUTURE PERSPECTIVES

The development of this guideline has demonstrated an urgent need for more robust data to enable better (Wilms) tumor risk estimates for children with a CPS. We strongly advise clinical geneticists, pediatricians, pediatric oncologists, radiologists and epidemiologists to collaborate in the establishment of national or international CPS registries. Parent support organizations can play an important role in catalysing the development and/or awareness of such a registry. Several international registries already exist which can be used by clinicians, after local ethical approval and informed consent from parents have been obtained. This includes the DECIPHER database where any patient with a rare genomic variant (single nucleotide variant or copy number variant) can be registered (https://decipher.sanger.ac.uk/)[127], or the CPS registry established by the Heidelberg Hopp Childhood Tumor Center and Hannover Medical School, in which patients diagnosed with all types of CPS can be included (http://www.krebs-praedisposition.de/en/registries/cps-registry/). This CPS registry includes a self-registration option where (German or English speaking) parents can register their child's data. Additionally, the IWSA has designed a CoRDS (Coordination of Rare Diseases at Sanford) registry where (parents of) patients with WAGR syndrome can register their data for research purposes (https://wagr.org/wagr-syndromepatient-registry), and other CPS-specific registries may be realised in the future. Linking such registries to international WT/cancer registries can provide additional insight into tumor risks.

With the rise of genomic sequencing and advances in other molecular techniques in children with cancer, we expect that more children will be diagnosed with a CPS, novel CPS may be identified and known CPS may be further subdivided into molecular subtypes in the future. Therefore, WT surveillance guidelines will require continuous discussion and may be subject to change when new evidence emerges.

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Supplemental Table 1. Search terms used to identify relevant studies on currently known Wilms tumor predisposition genes and syndromes.

Topic	Search terms: (Wilm*[tiab] OR Nephroblastoma*[tiab] OR "Wilms Tumor"[Mesh]) AND
WT1 mutations	(WT1[tiab] OR "WT1 Proteins"[Mesh] OR "Frasier Syndrome"[Mesh] OR "Frasier syndrome"[tiab] OR "Denys-Drash Syndrome"[Mesh] OR "Denys-Drash"[tiab] OR "Denys Drash"[tiab] OR "Drash syndrome"[tiab] OR Pseudohermaphroditism[tiab] OR "Meacham syndrome"[tiab] OR "Meacham Winn Culler syndrome" [Supplementary Concept])
WAGR syndrome	(WAGR*[tiab] OR aniridia*[tiab] OR "WAGR Syndrome"[Mesh] OR "11p13 deletion"[tiab] OR "11p deletion"[tiab])
Beckwith-Wiedemann Syndrome/ Spectrum	("Beckwith-Wiedemann Syndrome"[Mesh] OR "Beckwith-Wiedemann"[tiab] OR "Wiedemann-Beckwith"[tiab] OR "Exomphalos-Macroglossia-Gigantism"[tiab] OR hemihypertroph*[tiab] OR hemihyperplasia*[tiab] OR "lateralized overgrowth"[tiab] OR 11p15[tiab] OR 11p15.5[tiab] OR "IGF2 protein, human" [Supplementary Concept] OR IGF2[tiab] OR "IGF-II"[tiab] OR "somatomedin A"[tiab] OR "insulinlike growth factor II"[tiab] OR "insulin-like growth factor 2"[tiab] OR "H19 long non-coding RNA" [Supplementary Concept] OR H19[tiab])
Lateralized overgrowth	hemihypertroph*[tiab] OR hemihyperplasia*[tiab] OR "lateralized overgrowth"[tiab]
Perlman syndrome	("Perlman syndrome"[tiab] OR "DIS3L2 protein, human" [Supplementary Concept] OR DIS3L2[tiab] OR "Nephroblastomatosis, fetal ascites, macrosomia and Wilms tumor"[Supplementary Concept])
PIK3CA-related overgrowth spectrum	("PIK3CA protein, human" [Supplementary Concept] OR PIK3CA*[tiab] OR p110alpha[tiab] OR phosphoinositide-3-kinase[tiab] OR "Congenital Lipomatous Overgrowth, Vascular Malformations, and Epidermal Nevi"[Supplementary Concept] OR CLOVES[tiab] or "Congenital lipomatous overgrowth"[tiab] OR "Klippel-Trenaunay"[tiab])
Sotos syndrome	("Sotos Syndrome"[Mesh] OR Sotos[tiab] OR Soto[tiab] OR NSD1[tiab] OR "nuclear receptor binding SET domain protein 1"[tiab] OR "Cerebral Gigantism"[tiab] OR "Cerebral Gigantisms"[tiab] OR "NSD1 protein, human" [Supplementary Concept])
Simpson-Golabi Behmel syndrome	("Simpson-Golabi-Behmel syndrome" [Supplementary Concept] OR SGBS*[tiab] OR Simpson[tiab] OR "Bulldog syndrome"[tiab] OR "Mental Retardation-Overgrowth Syndrome"[tiab] OR "Golabi-Rosen syndrome"[tiab] OR "Dysplasia gigantism"[tiab] OR GPC3[tiab] OR GPC4[tiab] OR "GPC3 protein, human" [Supplementary Concept] OR MXR7[tiab] OR "glypican 3"[tiab] OR "GPC4 protein, human" [Supplementary Concept] OR "glypican 4"[tiab])
TRIM28 mutations	("TRIM28 protein, human" [Supplementary Concept] OR TRIM28[tiab] OR "Tripartite motif-containing protein 28"[tiab] OR "Krab-associated protein 1"[tiab] OR kap1[tiab] OR kap-1[tiab] OR "transcriptional intermediary factor 1-beta"[tiab] OR "transcription intermediary factor 1-beta"[tiab] OR "transcription intermediary factor 1 beta"[tiab] OR "transcriptional intermediary factor 1 beta"[tiab] OR tif1b[tiab] OR tif1beta[tiab] OR tif1-beta[tiab])
REST mutations	("RE1-silencing transcription factor" [Supplementary Concept] OR "REST gene"[tiab] OR "RE1-silencing transcription factor"[tiab] OR "Neuron-restrictive silencer factor"[tiab] OR NRSF[tiab] OR "repressor element silencing transcription factor"[tiab])
CTR9 mutations	("CTR9 protein, human"[Supplementary Concept] OR CTR9[tiab] OR SH2BP1[tiab] OR "SH2 domain binding protein 1"[tiab])
HACE1 mutations	("HACE1 protein, human" [Supplementary Concept] OR HACE1[tiab] OR "HECT domain and ankyrin repeat containing E3 ubiquitin-protein ligase 1"[tiab])

Supplemental Table 1. Search terms used to identify relevant studies on currently known Wilms tumor prediposition genes and syndromes.

Topic	Search terms: (Wilm*[tiab] OR Nephroblastoma*[tiab] OR "Wilms Tumor"[Mesh]) AND
KDM3B mutations	("KDM3B protein, human" [Supplementary Concept] OR KDM3B[tiab] OR 5qNCA[tiab] OR "lysine-specific demethylase 3B" OR LOC51780[tiab] OR JMJD1B[tiab] OR "jumonji domain containing 1B"[tiab])
FBXW7 mutations	("F-Box-WD Repeat-Containing Protein 7"[Mesh] OR "FBXW7 protein, human" [Supplementary Concept] OR FBXW7[tiab] OR "F-Box-WD Repeat-Containing Protein 7"[tiab] OR "SEL-10"[tiab] FBW7[tiab] OR CDC4[tiab] OR "F-box and WD-40 domain protein 7"[tiab])
NYNRIN mutations	(NYNRIN[tiab] OR "NYN Domain And Retroviral Integrase Containing"[tiab] OR KIAA1305[tiab] OR "NYN Domain And Retroviral Integrase Catalytic Domain-Containing Protein"[tiab] OR "cousin of GIN1"[tiab] OR CGIN1[tiab])
Bloom syndrome	("Bloom Syndrome"[Mesh] OR Bloom*[tiab] OR "Congenital Telangiectatic Erythema"[tiab] OR "Congenital Telangiectatic Erythemas"[tiab] OR BLM[tiab] OR "Bloom syndrome protein" [Supplementary Concept])
DICER1 syndrome	("DICER1 protein, human" [Supplementary Concept] OR dicer1[tiab] OR "dicer protein"[tiab] OR "Dcr-1 homolog"[tiab] OR HERNA[tiab])
Li Fraumeni syndrome	("Li-Fraumeni Syndrome"[Mesh] OR "Li-Fraumeni"[tiab] OR "Genes, p53"[Mesh] OR p53[tiab] OR TP53[tiab])
Neurofibromatosis type 1	("Neurofibromatosis 1"[Mesh] OR neurofibromatosis[tiab] OR Recklinghausen*[tiab] OR "Molluscum Fibrosum"[tiab] OR "Watson syndrome"[tiab] OR "pulmonic stenosis"[tiab] OR "café-au-lait"[tiab] OR "café au lait"[tiab] OR NF1[tiab] OR "Neurofibromin 1"[Mesh])
Mulibrey Nanism	("Mulibrey Nanism"[Mesh] OR "Mulibrey nanism"[tiab] OR "Muscle-Liver-Brain-Eye Nanism"[tiab] OR "Muscle Liver Brain Eye Nanism"[tiab] OR "Perheentupa Syndrome"[tiab] OR "TRIM37 protein, human" [Supplementary Concept] OR TRIM37[tiab] OR "tripartite motif-containing 37"[tiab] OR "MUL protein"[tiab])
Fanconi anemia	("Fanconi Anemia"[Mesh] OR Fanconi[tiab] OR FANC*[tiab] OR BRCA2[tiab] OR "Genes, BRCA2"[Mesh] OR PALB2[tiab] OR "Fanconi Anemia Complementation Group N Protein"[Mesh])
Mosaic variegated aneuploidy	("Mosaic variegated aneuploidy syndrome" [Supplementary Concept] OR "mosaic variegated aneuploidy"[tiab] OR "Instability mitotic non disjunction syndrome"[tiab] OR "MVA Syndrome"[tiab] OR "Chromosomal mosaicism due to mitotic instability"[tiab] OR "TRIP13 protein, human" [Supplementary Concept] OR TRIP13[tiab] OR "BUB1B protein, human" [Supplementary Concept] OR BUB1B[tiab] OR "CEP57 protein, human" [Supplementary Concept] OR CEP57[tiab] OR "premature chromatid separation"[tiab])
Hyperparathyroid-jaw tumor syndrome	("Hyperparathyroidism 2" [Supplementary Concept] OR Hyperparathyroid*[tiab] OR "Hpt-Jt"[tiab] OR "CDC73 protein, human" [Supplementary Concept] OR CDC73[tiab] OR parafibromin[tiab] OR HRPT2[tiab])
Bohring-Opitz syndrome	("ASXL1 protein, human" [Supplementary Concept] OR ASXL1[tiab] OR "additional sex combs like 1"[tiab] OR "Bohring syndrome" [Supplementary Concept] OR Bohring*[tiab] OR "Opitz trigonocephaly-like syndrome"[tiab] OR "C-like syndrome"[tiab])

Supplemental Table 1. Search terms used to identify relevant studies on currently known Wilms tumor predisposition genes and syndromes.

Topic	Search terms: (Wilm*[tiab] OR Nephroblastoma*[tiab] OR "Wilms Tumor"[Mesh]) AND
Constitutional mismatch repair deficiency	("Turcot syndrome" [Supplementary Concept] OR Turcot[tiab] OR "mismatch repair deficiency"[tiab] OR "MMR deficiency"[tiab] OR CMMRD[tiab] OR "Brain Tumor-Polyposis Syndrome 1"[tiab] OR "Mismatch Repair Cancer Syndrome"[tiab] OR MSH2[tiab] OR "MSH2 protein, human" [Supplementary Concept] OR MSH6[tiab] OR PMS2[tiab] OR "PMS2 protein, human" [Supplementary Concept] OR MLH1[tiab] OR "MLH1 protein, human" [Supplementary Concept] OR EPCAM[tiab] OR "EPCAM protein, human" [Supplementary Concept])
Trisomy 18	("Trisomy 18 Syndrome"[Mesh] OR "trisomy 18"[tiab] OR "trisomy E"[tiab] OR "Edwards syndrome"[tiab] OR "chromosome 18 trisomy"[tiab] OR "chromosome 18 duplication"[tiab] OR "chromosome 18 duplications"[tiab])
Trisomy 13	("Trisomy 13 Syndrome"[Mesh] OR "trisomy 13"[tiab] OR Patau*[tiab] OR "Bartholin-Patau"[tiab] OR "Bartholin Patau"[tiab] OR "chromosome 13 trisomy"[tiab] OR "chromosome 13 duplication"[tiab] OR "chromosome 13 duplications"[tiab])
9q22.3 deletion	(9q22*[tiab] OR "9q22.3 Microdeletion" [Supplementary Concept])
MYCN duplication	("MYCN protein, human" [Supplementary Concept] OR MYCN[tiab] OR bHLHe37[tiab] OR NMYC[tiab] OR "N-myc"[tiab] OR "V-myc"[tiab])
Osteopathia striata cranial sclerosis	("Osteopathia striata cranial sclerosis" [Supplementary Concept] OR "osteopathia striata"[tiab] OR "Hyperostosis Generalisata with Striations"[tiab])
2q37 deletion	(2q37*[tiab] OR "Chromosome 2q37 deletion syndrome" [Supplementary Concept] OR "brachydactyly-mental retardation syndrome"[tiab] OR "Albright hereditary osteodystrophy-like syndrome"[tiab])

Supplemental Table 2. Cohort studies used to estimate the percentage of patients with *WT1* mutations who develop Wilms tumor (WT).

	Patients with WT / total (%)						
	Chernin 2010[1] [A]	Köhler 2011[2] [B]	Lipska 2014[3] [C]	Lehnhardt 2015[4] [D]	Sun 2020[5] [E]	Pooled	Surveillance recommended?
Exonic missense	8/18	0/2	15/24	12/17	4/16	39/77 (**50.6%**)	Yes
Exonic truncating	4/4	2/3	7/9	7/8	2/3	22/27 (**81.5%**)	Yes
Intron 9	0/18	0/1	1/19	0/9	0/7	1/54 (**1.9%**)	**No**

[A] Excluding 12 patients who had prophylactic bilateral nephrectomies or died before the age of 2 years. [B] Patients who had prophylactic bilateral nephrectomies were already excluded in this report. [C] Excluding 7 patients who had prophylactic bilateral nephrectomies. [D] Excluding 19 patients who had prophylactic bilateral nephrectomies. [E] Excluding 4 patients who had prophylactic bilateral nephrectomies prior to (potential) WT development.

References (Supplemental table 2):

1. Chernin G, Vega-Warner V, Schoeb DS, et al. Genotype/phenotype correlation in nephrotic syndrome caused by WT1 mutations. Clin J Am Soc Nephrol. 2010;5(9):1655-1662.
2. Köhler B, Biebermann H, Friedsam V, et al. Analysis of the Wilms' tumor suppressor gene (WT1) in patients 46,XY disorders of sex development. J Clin Endocrinol Metab. 2011;96(7):E1131-1136.
3. Lipska BS, Ranchin B, Iatropoulos P, et al. Genotype-phenotype associations in WT1 glomerulopathy. Kidney Int. 2014;85(5):1169-1178.
4. Lehnhardt A, Karnatz C, Ahlenstiel-Grunow T, et al. Clinical and molecular characterization of patients with heterozygous mutations in wilms tumor suppressor gene 1. Clin J Am Soc Nephrol. 2015;10(5):825-831.
5. Sun S, Xu L, Bi Y, et al. Early diagnosis of WT1 nephropathy and follow up in a Chinese multicenter cohort. Eur J Med Genet. 2020;63(11):104047.

Supplemental Table 3. Cohort studies used to estimate the percentage of patients with WAGR syndrome who develop Wilms tumor (WT).

	Patients with WT / total (%)					
	Muto 2002[1 A]	Fischbach 2005[2 B]	Van Heyningen 2007[3 C]	Marakhonov 2019[4 D]	Pooled	Surveillance recommended?
WAGR syndrome	4/8	31/54	27/53	10/17	72/132 (**54.5%**)	Yes

[A] One patient was still at risk (age 1 year). [B] Some patients were potentially still at risk (age at data collection is not specified). [C] Six patients were potentially still at risk (age 4-7 years). [D] All patients were five years or older.

References (Supplemental table 3):

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4. Marakhonov AV, Vasilyeva TA, Voskresenskaya AA, et al. LMO2 gene deletions significantly worsen the prognosis of Wilms' tumor development in patients with WAGR syndrome. Hum Mol Genet. 2019;28(19):3323-3326.

Supplemental Table 4. Cohort studies used to estimate the percentage of patients with Beckwith-Wiedemann Spectrum (BWSp) who develop Wilms tumor (WT).

	Patients with WT / total (%)									
	Weksberg 2001[1 A]	Gaston 2001[2 A]	Bliek 2004[3 B]	Brioude 2013[4 C]	Ibrahim 2014[5 D]	Mussa 2016[6E]	Maas 2016[7F]	Cöktü 2020[8G]	Pooled	Surveillance recommended?
LOM IC2	0/35	0/45	0/27	0/257	0/288	0/190	2/114 (**1.8%**)	1/208	3/1164 (**0.2%**)	**No**
GOM IC1	1/3	4/11	6/9	8/35	3/28	7/31	6/19 (**31.6%**)	0/31	35/167 (**21.0%**)	Yes
Paternal UPD 11p15	5/21	3/11	4/13	10/81	1/99	3/87	3/44 (**6.8%**)	3/64	32/420 (**7.6%**)	Yes
CDKN1C mutation	0/5	0/2	n.a.	0/34	1/21	0/10	n.a.	0/6	1/78 (**1.3%**)	Yes [H]
Classical BWS with negative molecular tests	4/17	1/24	2/17	n.a.	3/201	n.a.	4/52 (**11.5%**)	n.a.	14/311 (**4.5%**)	Yes

[A-E] Numbers adapted from Maas et al. 2016, who ensured that patient data were not used more than once if incorporated in multiple publications.

[A] Age of patients at the end of follow-up is not specified; patients may still have been at risk of developing WT.

[B] Only patients from France included (Dutch patients included in Maas et al. 2016). Patients were followed until 5th birthday, unless they developed tumors before this age.

[C] Median age at the end of data collection was 7.48 years; 66.8% of the patients were over 5 years old.

[D] Age of patients at the end of follow-up is not specified; patients may still have been at risk of developing WT. All WTs were diagnosed prior to referral for BWS testing.

[E] Median follow-up was 8.9 years and 120 patients (37.7%) were over 8 years old at last follow-up.

[F] Study with the most complete follow-up: all but three patients were at least 5 years of age when last data were gathered.

[G] Birth years of included patients ranged from 1978 to 2018, median age at last follow-up not specified.

[H] Based on overall tumor risk, estimated to be 6.9% by Maas et al. 2016 (one WT, two neuroblastoma, one ganglioneuroma and one acute lymphoblastic leukemia).

References (Supplemental table 4):

1. Weksberg R, Nishikawa J, Caluseriu O, et al. Tumor development in the Beckwith-Wiedemann syndrome is associated with a variety of constitutional molecular 11p15 alterations including imprinting defects of KCNQ1OT1. Hum Mol Genet. 2001;10(26):2989-3000.
2. Gaston V, Le Bouc Y, Soupre V, et al. Analysis of the methylation status of the KCNQ1OT and H19 genes in leukocyte DNA for the diagnosis and prognosis of Beckwith-Wiedemann syndrome. Eur J Hum Genet. 2001;9(6):409-418.
3. Bliek J, Gicquel C, Maas S, Gaston V, Le Bouc Y, Mannens M. Epigenotyping as a tool for the prediction of tumor risk and tumor type in patients with Beckwith-Wiedemann syndrome (BWS). J Pediatr. 2004;145(6):796-799.
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5. Ibrahim A, Kirby G, Hardy C, et al. Methylation analysis and diagnostics of Beckwith-Wiedemann syndrome in 1,000 subjects. Clin Epigenetics. 2014;6(1):11.
6. Mussa A, Russo S, De Crescenzo A, et al. (Epi)genotype-phenotype correlations in Beckwith-Wiedemann syndrome. Eur J Hum Genet. 2016;24(2):183-190.
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Supplemental Table 5. Additional considerations and references for patients with Bloom syndrome, DICER1 syndrome, Li Fraumeni syndrome, neurofibromatosis type 1, constitutional mismatch repair deficiency (CMMRD), trisomy 13 and trisomy 18.

Condition	Estimated % of patients with this condition with Wilms tumor (WT)	Other considerations
Bloom syndrome	2.9% based on 8 cases identified among 277 individuals in the Bloom Syndrome Registry (1954-2018).[1]	Higher risks of other cancrers.
DICER1 syndrome	Low, based on 5 families reported in which 6/22 individuals with a pathogenic *DICER1* variant from these families developed WT.[2] Three cases of WT were identified in seven families with *DICER1* variants, with a single WT in each family.[3] No reports of WT in a study of 89 carriers of *DICER1* variants.[4]	Current renal screening recommendation for DICER1 syndrome includes six monthly abdominal US until 8 years and then annually until 12 years. This screening is advised to identify cystic nephromas, renal sarcomas, cysts and WT.[5]
Li Fraumeni syndrome	Low, based on 12 cases reported in total in association with Li Fraumeni syndrome.[6]	Screening is not currently included in international guidelines.[7]
Neurofibromatosis type 1	<1% based on some single case reports. Older paper reports three cases of NF1 in series of 342 children with WT.[8]	-
Constitutional mismatch repair deficiency (CMMRD)	WT has been observed in CMMRD, but is limited to single case reports, often with other malignancies.[9,10]	Far higher risks of other cancers. Screening is not recommended in the CMMRD or WT guidance to date[11,12], although the 2017 AACR recommendations mentioned that reports of patients with neuroblastomas, WT and, more commonly, lymphomas may contribute to future recommendations including abdominal US.[13]
Trisomy 13	<1%, based on only 2 reported cases.[14,15] Nephroblastomatosis was identified in the kidneys of an aborted fetus with trisomy 13.[16]	Intensive treatment is controversial because of the poor prognosis of Patau syndrome.[17]
Trisomy 18	±1% (with hepatoblastoma risk of ±2%) bassed on 12 WT reported/registered between 1981-2014, among an estimated 1340 children surviving ≥1 year with trisomy 18 in the United States in this period.[18] In 2018, Farmakis et al. reviewed the literature and SOFT registry and identified a total of 21 WT and 44 hepatoblastoma cases.[19]	Intensive treatment is controversial because of the poor prognosis of Edwards syndrome.[19]

References (Supplemental table 5):

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3. Foulkes WD, Bahubeshi A, Hamel N, et al. Extending the phenotypes associated with DICER1 mutations. Human mutation. 2011;32(12):1381-1384.
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14. Olson JM, Hamilton A, Breslow NE. Non-11p constitutional chromosome abnormalities in Wilms' tumor patients. Medical and pediatric oncology. 1995;24(5):305-309.
15. Sweeney H, Pelegano J. Wilms tumor in a child with trisomy 13. Journal of pediatric hematology/oncology. 2000;22(2):171-172.
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8

Renal cell carcinoma in young *FH* mutation carriers: case series and review of the literature

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ABSTRACT

Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) is an autosomal dominant syndrome caused by heterozygous pathogenic germline variants in the fumarate hydratase (*FH*) gene. It is characterized by cutaneous and uterine leiomyomas and an increased risk of developing renal cell carcinoma (RCC), which is usually adult-onset. HLRCC-related RCC tends to be aggressive and can metastasize even when the primary tumor is small. Data on children and adolescents are scarce. Herein, we report two patients from unrelated Dutch families, with HLRCC-related RCC at the ages of 15 and 18 years, and a third patient with an *FH* mutation and complex renal cysts at the age of 13. Both RCC's were localized and successfully resected, and careful MRI surveillance was initiated to monitor the renal cysts. One of the patients with RCC subsequently developed an ovarian Leydig cell tumor. A review of the literature identified 10 previously reported cases of HLRCC-related RCC in patients aged younger than 20 years, five of them presenting with metastatic disease. These data emphasize the importance of recognizing HLRCC in young patients to enable early detection of RCC, albeit rare. They support the recommendations from the 2014 consensus guideline, in which genetic testing for *FH* mutations, and renal MRI surveillance, is advised for HLRCC family members from the age of 8–10 years onwards.

INTRODUCTION

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant syndrome caused by heterozygous germline variants in the fumarate hydratase (*FH*) gene, associated with an increased risk of developing renal cell carcinoma (RCC). The first report describing a family with HLRCC was published in Finland in 2001, and over 300 affected families from various countries have been described since.[1–4]

Other clinical manifestations of HLRCC include multiple cutaneous leiomyomas in 73–100% of *FH* mutations carriers and uterine leiomyomas in ± 75% of female carriers.[4–6] Additionally, germline mutations in the *FH* gene have been identified in a small percentage of patients with paragangliomas and pheochromocytomas.[7, 8]

The *FH* gene, located on chromosome region 1q42.1, is a tumor suppressor gene that encodes the enzyme fumarate hydratase (fumarase), which plays a role in both the tricarboxylic acid (TCA) cycle in mitochondria, as well as the response to DNA double strand breaks in the nucleus.[3, 9] Somatic inactivation of the second allele can be demonstrated in most, but not all, HLRCC-related tumors.[2, 10–12] Biallelic germline mutations are rare and cause a syndrome known as fumarase deficiency, characterized by early onset, severe encephalopathy.[5] In patients with fumarase deficiency, mutations are usually missense or inframe duplications that do not necessarily result in complete loss of enzyme activity.[13] More than 200 distinct variants spread over the entire coding region of the *FH* gene have been published in the Leiden Open (source) Variation Database system (LOVD)[13] and so far, a clear correlation between the type or location of the *FH* mutation and cancer risk has not been observed.[5]

The absolute risk of developing RCC is estimated to be 10–15%, with a median age of onset of 40–41 years.[4, 14] RCC can be the first manifestation of HLRCC. Histologically, loss of staining for FH and positive staining for 2-succino-cysteine (2SC), which accumulates in the setting of FH deficiency, can support the diagnosis of HLRCC-related RCC.[14, 15]

In adults, HLRCC-related RCC is known to be aggressive and can metastasize even when the primary tumor is small. Data on children and adolescents are scarce. We herein report three young patients from unrelated Dutch families, aged 15, 18 and 13 years respectively, as well as the results of a systematic literature review on HLRCC-related RCC in patients younger than 20 years. This review contributes to existing recommendations for genetic testing, tumor surveillance and resection in children and adolescents.

METHODS

Patients were evaluated at the Princess Máxima Center for Pediatric Oncology (case 1 and 3) and Radboud University Medical Center Nijmegen (case 2). Genetic, radiological and histopathological studies were reviewed. All patients as well as the parents in case 1 and 3, gave informed consent for inclusion of their clinical data in this manuscript. For the literature review, databases of PubMed an Embase were searched for HLRCC-related renal tumors occurring in patients < 20 years (**Supplementary Table 1**).

After removing duplicates, the search yielded 1221 articles (**Supplementary Figure 1**). Any report (manuscript or conference abstract), written in English, Dutch, German, French or Spanish, describing a HLRCC-related renal tumor in a patient younger than 20 years of age, was eligible for inclusion. After title/abstract screening, a total of 86 reports were eligible for full text screening, during which 77 articles were excluded based on full text not being available, only including patients ≥ 20 years old, only reviewing or describing previously reported patients, or lack of germline genetic testing to confirm the diagnosis of HLRCC.

CASE PRESENTATION

Case 1

A 15-year old female presented with a large right-sided abdominal mass. Her family history included uterine and cutaneous leiomyomas and a confirmed *FH* mutation in mother's family (**Figure 1a**). Physical examination revealed small, cutaneous lesions of the lower legs, suggestive for leiomyomas. On MRI using a customized HLRCC-protocol (**Table 1**), the mass was mostly cystic with peripheral solid nodules (**Figure 1b, c**). The nodules showed strong enhancement after contrast administration and restricted diffusion on diffusion-weighted imaging (DWI). In the left kidney, multiple cystic lesions were observed without solid components. Brain MRI and total body FDG-positron emission tomography (FDG-PET) did not reveal signs of metastatic spread. Right-sided nephrectomy revealed an RCC with a maximum diameter of 20 cm (T2N0M0, four lymph nodes sampled), with tumor cells lining the cysts. There was no spread beyond the kidney and resection margins were free of tumor. Solid areas consisted of vital epithelial tumor with a predominantly tubular, partially papillary growth pattern of strongly eosinophilic cells with mild to moderate nuclear atypia (**Figure 1d, e)** and diffuse 2SC staining (**Figure 1f**). Prominent nucleoli were seen only in rare areas with papillary architecture, without perinucleolar halos. Germline genetic testing by MLPA confirmed the presence of the familial heterozygous deletion of the *FH* gene (c.(?_1)_(*1_?)del) in the patient and her 18-year old healthy sister, a deletion which has been previously reported in other patients with HLRCC.[3, 6, 16–18]

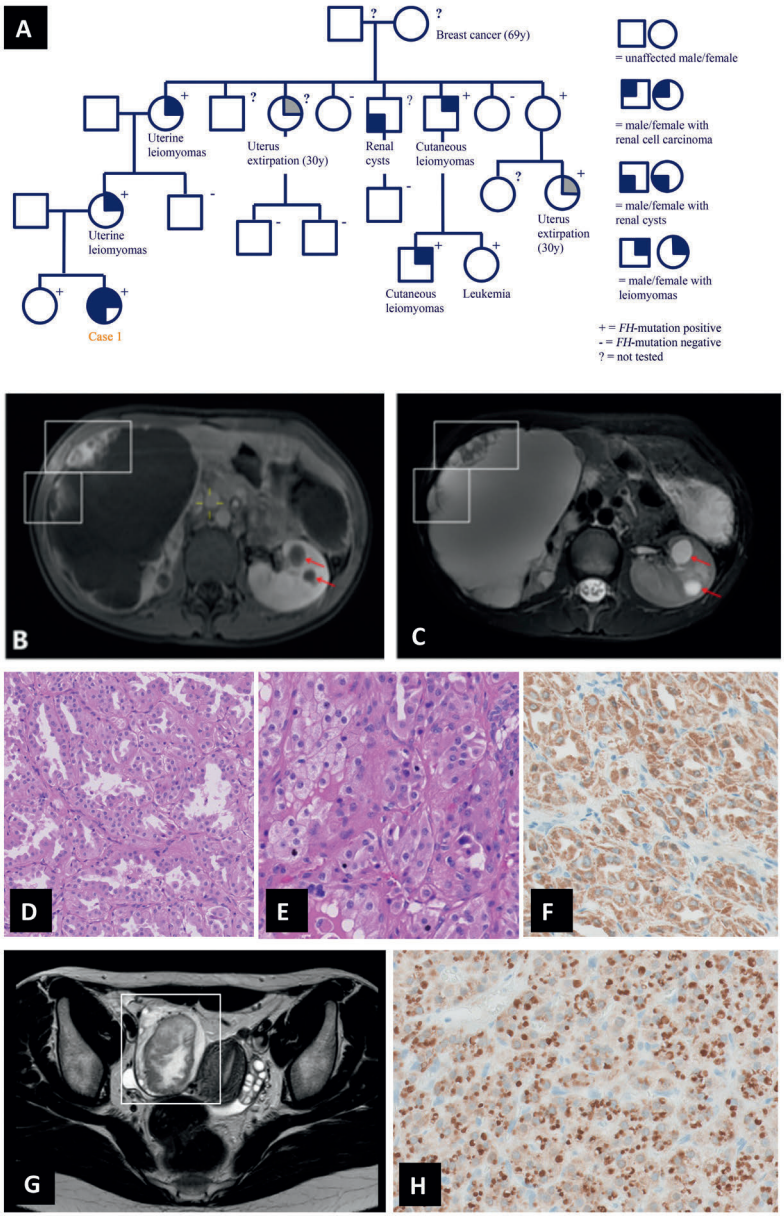

Figure 1. Case 1 (female, 15 years, renal cell carcinoma and Leydig cell tumor): a family pedigree; **b–c** contrast enhanced T1W-MRI (**b**) and abdominal T2W-MRI (**c**) showing large right-sided kidney mass, which is mostly cystic with peripheral solid nodules (boxes). In the left kidney, multiple cystic lesions (arrows) are observed without solid components; **d–f** histology of the renal tumor: vital epithelial tumor with a predominantly tubular, partially papillary growth pattern (**d**) of strongly eosinophilic cells with mild to moderate nuclear atypia (**e**), and diffuse 2SC staining (**f**). **g** T2W-MRI of the pelvic region showing a right-sided ovarian lesion (box) with both solid and cystic components. **h** Ovarian Leydig cell tumor showing diffuse 2SC staining.

The left kidney is monitored with MRI's at 3 and 6 months after diagnosis, then every 6 months for 3 years, and yearly thereafter. Whereas the kidney appeared unchanged, the patient developed an ovarian lesion (**Figure 1g**) after a follow-up of 30 months, at the age of 18, which was successfully resected and histologically characterized as a Leydig cell tumor; a well demarcated lesion with uniform cells showing large, round nuclei, prominent nucleoli and lack of necrosis, nuclear atypia or mitotic figures. The tumor showed diffuse 2SC staining (**Figure 1h**). Whole exome sequencing (Illumina NovaSeq platform) was performed on the Leydig cell tumor, but a second hit in the *FH* gene was not identified.

Table 1. Scan parameters for renal MRI surveillance in patients with HLRCC.

Parameter	T2w-fat suppression	3D-T2	DWI	T1 pre/post
Pulse sequence	2-D MultiVane with spectral fat saturation	3-D turbo spin-echo with variable flip angle	2-D single-shot spin-echo with spectral fat saturation	2-D ultrafast spoiled gradient echo with fat suppression
Repetition time (ms)	2450	449	1611	3.89
Echo time (ms)	100	90	79	1.82
Flip angle (degree)	90	-	90	10
Slice orientation	axial	axial	axial	axial
Slice thickness (mm)	3	0.9-1.15	5	4
Slice gap (mm)	3	0	0	2
Echotrain length	30	85	1	1
Field of view (mm^2)	400	400	450	380
B-values (s/mm^2)	-	-	0, 150, 1000	-

Case 2

An 18-year old female, carrier of an *FH* mutation (c.1330delA; p.Arg444fs; NM_000143.3), was referred for a suspect lesion in the left kidney, observed on renal MRI surveillance. The mutation was derived from her asymptomatic father and had been previously identified in a distant adult cousin with cutaneous leiomyomas (**Figure 2a**). This mutation has not been previously reported. Subsequent CT imaging with contrast administration showed a 9 mm cystic lesion, with an area of increased density suspect for nodular enhancement **(Figure 2b)**. A chest X-ray did not reveal signs of lung metastases. A partial nephrectomy was performed; the resected cyst showed focal papillary proliferations with a lining of atypical epithelial cells with some prominent nucleoli. The nucleoli were not significantly enlarged, strongly eosinophilic or surrounded by halos. No necrosis or strong mitotic activity were present. 2SC immunohistochemical staining was positive (**Figure 2c**), and the lesion was characterized as an early stage of HLRCC-related RCC. A second hit analysis was not performed. The patient is doing well after a follow-up of 45 months.

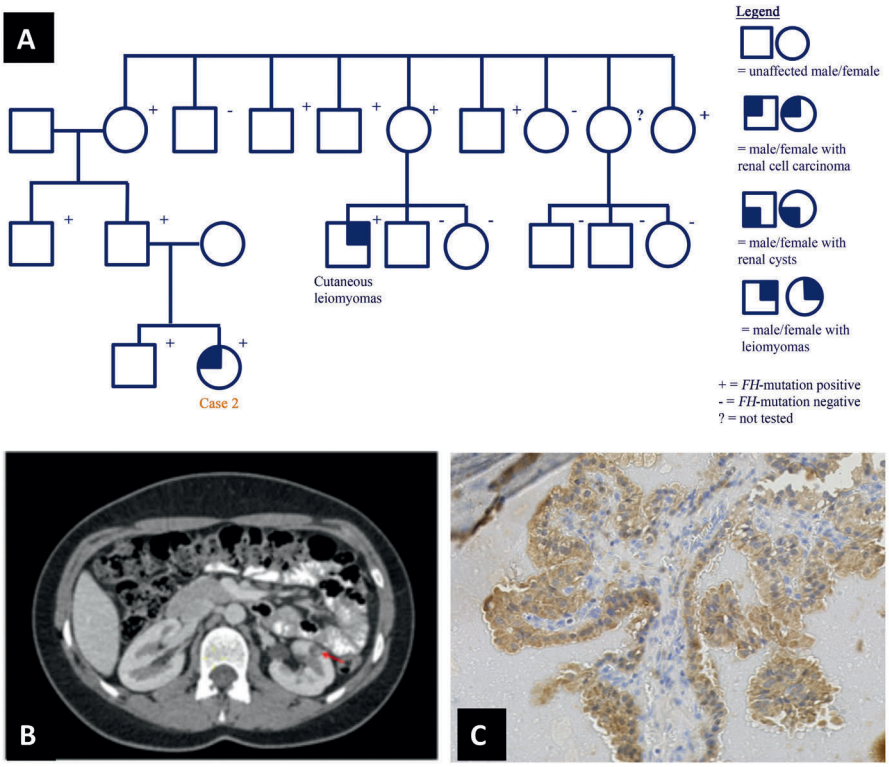

Figure 2. Case 2 (female, 18 years, renal cell carcinoma): a family pedigree; data are missing on the presence of leiomyomas in *FH* mutation carriers; **b** abdominal CT after contrast administration, showing a 9 mm cystic lesion in the left kidney, with an area of increased density (arrow) suspect for nodular enhancement. **c** Tumor cells showing diffuse 2SC staining.

Case 3

A 13-year old female and her 8-year old sister were referred for ultrasound screening because of a recently confirmed *FH* mutation (c.1210G>T; p.Glu404*; NM_000143.3). The *FH* mutation was initially detected in the girls' mother who had cutaneous leiomyomas (**Figure 3a**), and this specific mutation was previously published in a case series.[19] In the 13-year old girl, the ultrasound identified two lesions in the right kidney which required further assessment, and the suspicion of RCC was discussed with the family. Subsequent MRI demonstrated two complex cystic lesions with variable hemorrhagic content in the right kidney with a maximum diameter of 7.1 cm and 2.2 cm respectively (**Figure 3b–d**). No nodular enhancement was detected. An international review of the MRI scans agreed with this interpretation. After 18 months follow-up, the cysts had grown in size but no solid components appeared, with MRI's performed at 3, 6, 12 and 18 months after the initial referral.

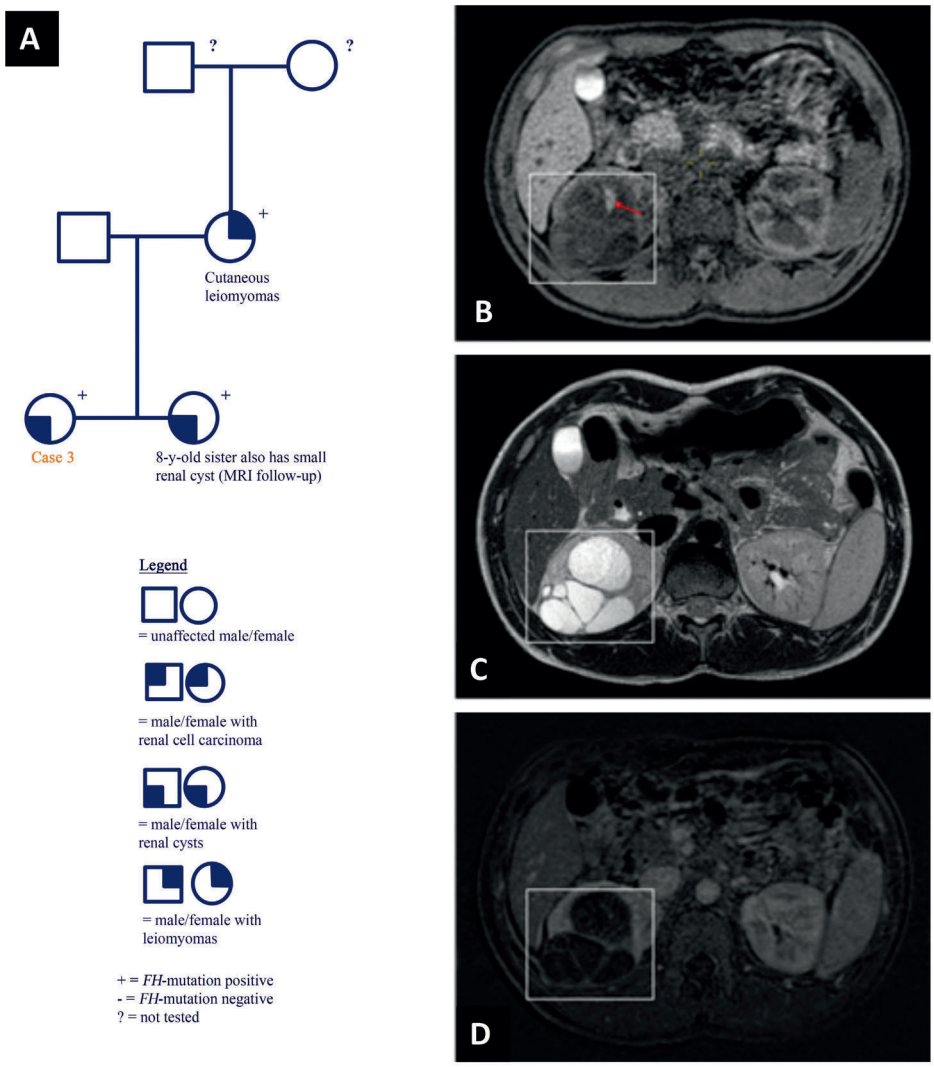

Figure 3. Case 3 (female, 13 years, complex renal cysts): a family pedigree; **b–d** abdominal T1 MRI (**b**) showing multilocular cysts (box) in the right kidney with area suspect for hemorrhagic content (arrow). Abdominal T2W MRI (**c**) and subtraction MRI (**d**) showing no nodular enhancement.

LITERATURE REVIEW

The literature review revealed 10 additional patients with HLRCC-related RCC diagnosed between 10 and 18 years of age (**Table 2**).[12, 18, 20–25] Additionally, a Wilms tumor was identified in a 2-year-old female patient who later developed cutaneous and uterine leiomyomas at the age of 25. She was confirmed to carry a germline c.1189G>A (p.Gly397Arg; NM_000143.3) mutation in the *FH* gene. Since no tissue from the Wilms tumor was available, FH expression could not be evaluated and the causal relationship remains uncertain.[26] This particular mutation has been described in other patients with HLRCC, including the 11-year old patient with HLRCC-related RCC in **Table 2**.[22]

In two of the described young patients with RCC, histology was not further specified.[24, 25] Among the other patients, two tumors were described as HLRCC-associated RCC with a variety of histological patterns[12], whereas four tumors were described as papillary type 2 RCC[18, 21–23], one as tubulopapillary RCC[27] and one as a collecting duct tumor.[20] Although most patients were symptomatic at presentation, an 11-year old male patient was diagnosed with localized RCC at his first surveillance visit.[22] Overall, five out of ten patients presented with metastatic disease[12, 18, 20, 21, 23], two had localized disease[12, 22], and disease stage was not reported for the remaining three. Follow-up data were available of five patients, of whom four died within 2 years after diagnosis.[20, 21, 23, 28] The one patient with localized disease and follow-up data, showed no evidence of disease after 3 years.[22] The exact mutation was specified in 5/10 cases, including single nucleotide deletions in exon 8 in two patients[21, 28], a missense mutation in exon 8[22], a missense mutation in exon 7[23], and a duplication in exon 10.[27]

Table 2. HLRCC-related renal cell carcinoma (RCC) before the age of 20 years (confirmed *FH* mutation).

#	References	*FH* mutation	Age	Sex	Presentation	Histology	Disease stage	Outcome (FU)
1	Alam et al. 2005[17,a]	*NA*	16	F	Symptomatic	Collecting duct tumor	Metastatic	Died (2years)
2	Merino et al. 2007[12,a]	*NA*	17	F	*NA*	HLRCC-associated RCC	Localized	*NA*
3			18	F	*NA*		Metastatic	*NA*
4	Al Refae et al. 2007[21,a]	c.1293del (exon 8)	17	M	Symptomatic	Papillary type 2 RCC	Metastatic	Died (15 months)
5	Alrashdi et al. 2010[22,a,b]	c.1189G>A (exon 8)	11	M	Surveillance	Papillary type 2 RCC	Localized	NED (3 years)
6	Gardie et al. 2011[18,a,c]	c.1123del (exon 8)	17	M	*N.A.*	Papillary type 2 RCC	Metastatic	Died (2 years)
7	Van Spaendonck-Zwarts et al. 2012[23,a]	c.1002T>G (exon 7)	18	F	Symptomatic	Papillary type 2 RCC, focally showing prominent nucleoli surrounded by a clear halo	Metastatic	Died (8 months)
8	Nix et al. 2012[24,d] (*meeting abstract)*	*NA*	10*	*NA*	*NA*	RCC, not specified	*NA*	*NA*
9	Toubaji et al. 2013[25] (*meeting abstract)*	*NA*	18	*NA*	*NA*	RCC, not specified	*NA*	*NA*
10	Bhola et al. 2018[27]	c.1430-1437dup (exon 10)	15	F	Symptomatic	Tubulo-papillary carcinoma	*NA*	*NA*
11	This report	Whole gene deletion	15	F	Symptomatic	HLRCC-associated RCC	Localized	Second tumor (Leydig cell tumor), 2 years after initial diagnosis
12	This report	c.1210G>T (exon 8)	18	F	Surveillance	HLRCC-associated RCC	Localized	NED (4 years)

Mutations are described using NM_000143.3. FU = follow-up time since diagnosis, NED = no evidence of disease, NA = not available. [a]Previously included in literature review by Van Spaendonck-Zwarts et al.[23] [b]Follow-up data reported in Van Spaendonck-Zwartset al.[23] [c]Follow-up data reported in Van Spaendonck-Zwarts et al.[23] and Wong et al.[28] [d]This 10-year old patient is also referred to in Menko et al.[14] (describes personal communication with Dr. Linehan).

DISCUSSION

Including the two new cases in this report, a total of 12 RCC's have been reported to date in *FH* mutation carriers younger than 20 years of age. Its aggressive nature, as illustrated by our literature review, emphasizes the importance of early genetic testing and surveillance.

Recently, a large, national series of French patients with HLRCC was published, in which 34 (19%) out of 182 *FH* mutation carriers developed RCC.[4] In this study, *FH* mutation carriers were identified through the two national laboratories accredited for *FH* germline testing. It is remarkable that none of the tumors in the French cohort occurred before the age of 20 years, illustrating that this early manifestation of HLRCC is rare and our literature review is likely to be influenced by a publication bias. Nevertheless, it may well be that *FH* germline testing is not always performed when RCC occurs in young patients from families that are not yet diagnosed with HLRCC. Notably, these patients may not yet have developed the typical clinical features of HLRCC. In these patients the young age at diagnosis of RCC and characteristics of the tumor can trigger awareness for an underlying syndrome.

Tumor characteristics typically associated with HLRCC, include papillary type 2 RCC and prominent nucleoli surrounded by a clear halo.[12] Yet, a recent review on histopathological features of FH-deficient RCC, concluded that a complex architecture with multiple histological patterns was more characteristic than the presence of perinucleolar halos. Moreover, histological patterns other than papillary type 2 RCC predominated in 40% of cases.[29] Interestingly, focused genetic testing in 212 RCC's registered in the Children's Oncology Group, revealed three FH-deficient RCC's that were initially classified as RCC-NOS, in patients aged 17–18 years.[30] Since germline genetic data are lacking for these patients, a diagnosis of HLRCC could not be confirmed. Yet, these studies demonstrate the value of FH/2SC-immunostaining and genetic testing in unclassified or morphologically complex RCC, in both children and adults. Currently, HLRCC-associated RCC is recognized as a separate category in the World Health Organization (WHO) classification of renal tumors.[31]

Leydig cell tumors, as identified in case 1, have been previously described in three patients with HLRCC, including two males with testicular Leydig cell tumors and a female with bilateral steroid cell tumors and metastatic RCC.[32,33] With this fourth patient, we provide further evidence for an association between HLRCC and Leydig cell tumors. The three previously reported patients each had a different missense mutation in *FH*, and in contrast to the Leydig cell tumor of case 1, loss of the wild-type *FH* allele was demonstrated in the two testicular tumors.[31] Immunostaining for FH or 2SC was not performed in the previously reported cases, while in our patient, both the RCC and the Leydig cell tumor showed 2SC positivity, as expected in FH-deficient tumors.

In the past, in The Netherlands, it was advised to start genetic testing of HLRCC family members at the age of 20 years, but this changed based on evidence of early-onset RCC in this syndrome, including an 18-year old Dutch female from a known HLRCC family who presented with metastatic RCC and died 8 months after diagnosis.[23] Five out of the 12 young patients in our case series and literature review, presented with symptoms, of whom three died of disease.[20, 21, 23, 27] International recommendations for genetic testing and renal tumor surveillance were published in a 2014 consensus guideline, following discussions during the Fifth Symposium on Birt–Hogg–Dubé syndrome and Second Symposium on HLRCC.[14] Based on the report of a 10-year old patient[14, 24], the guideline recommends to offer *FH* mutation testing to children of affected families from the age of 8–10 years onwards, and if positive to start annual renal MRI screening (**Box 1**).[14, 34]

MRI is preferred over abdominal ultrasound, because of the low sensitivity of ultrasound to detect small lesions.[14] MRI is also considered superior to CT-imaging because radiation is avoided, which is particularly relevant in this young age category, and because of a better soft tissue resolution to identify small nodules that may be present in cyst walls. A specific HLRCC MRI-protocol (**Table 1**) is recommended, using 1–3 mm slices through the kidneys. If solid lesions are detected, a surgical resection with wide surgical margins is warranted, independent of the size of the lesions, unlike other hereditary renal cancer syndromes where surgical intervention is only recommended for tumors that exceed 3 cm.[14]

It is unclear to what extent renal cysts have the potential to undergo malignant transformation. In 2006, Lehtonen et al. observed a higher prevalence of renal cysts in *FH* mutation carriers compared to the general population, but they did not find RCC to be more frequent in *FH* mutation carriers with renal cysts, compared to those without renal cysts.[35] Since then, three reports have been published suggesting that renal cysts may represent a potential preneoplastic lesion of the HLRCC-related renal cell carcinoma, based on the presence of atypical cells[12, 36] or 2SC uptake[37] in the lining of resected cysts. Therefore, we recommend to intensify surveillance if renal cysts are detected in *FH* mutation carriers, using shorter intervals between scans (**Box 1**).

A potential downside of early surveillance is the anxiety it may cause to patients and their families, particularly when a suspicious lesion requires further assessment, as illustrated by case 3 in this report. The risk and benefit of surveillance needs to be balanced in individual cases, in fair communication with the parents (shared decision making), and requires referral to expert centers with multidisciplinary teams.

Overall, our findings suggest that the incidence of HLRCC-related RCC is low but not negligible in patients younger than 20 years of age, emphasizing the importance of early genetic testing and renal surveillance in HLRCC family members. These data support the recommendations from the 2014 consensus guideline on HLRCC, in which genetic

testing for *FH* mutations, and renal MRI surveillance, is advised from the age of 8–10 years onwards.

Box 1

Recommended schedule for renal surveillance in FH mutation carriers:

- Yearly MRI scans from the age of 8-10 years onwards
- If renal cysts are detected, closer monitoring is indicated:
    - o 1st year: at 3, 6 and 12 months after detection of cysts, if no solid nodules appear:
    - o 2nd-4th year: every 6 months, if no solid nodules appear:
    - o 5th year and onwards: yearly MRI scans
- If solid nodules are detected, perform brain MRI and total body FDG-positron emission tomography (FDG-PET) for staging (repeat 1x after 3 months)

References: Menko et al.[14] and personal communication with Dr. W.M. Linehan.

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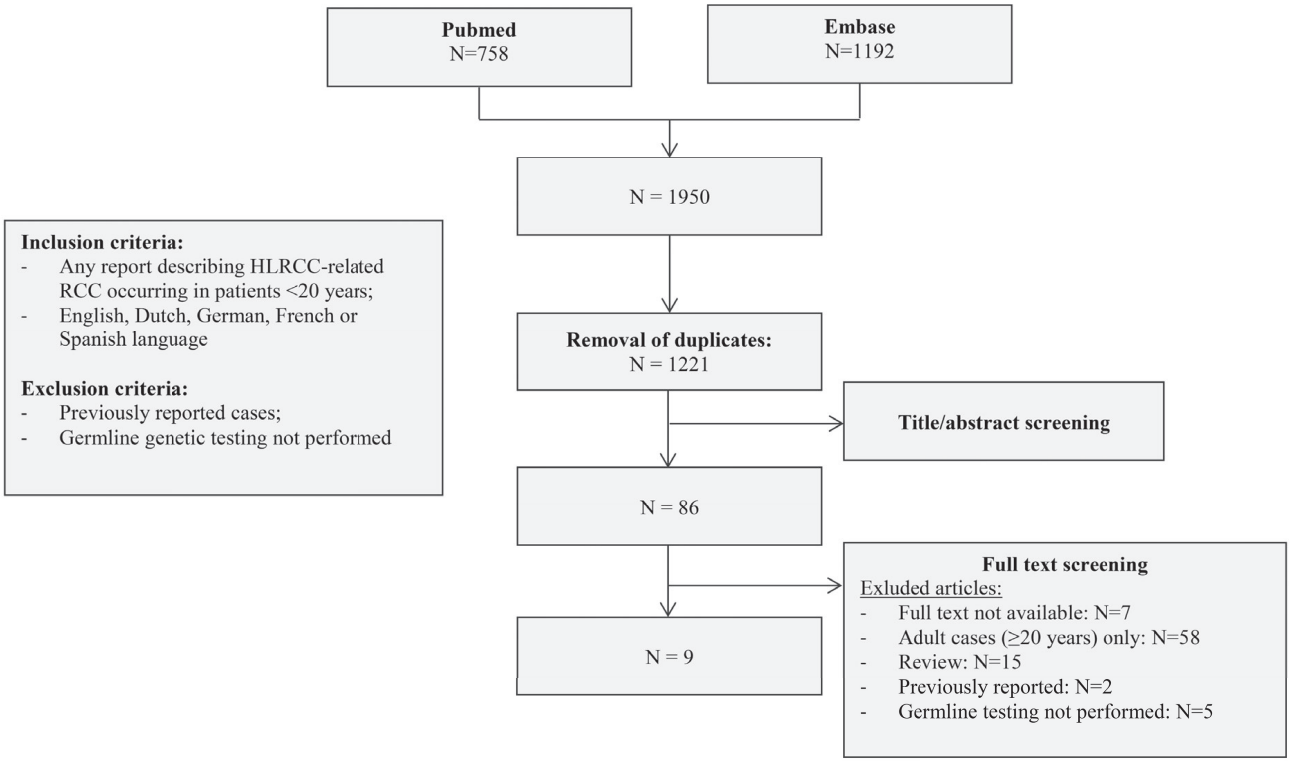

Supplementary Figure 1. Flowchart of literature search (Updated April 4th, 2019); N = number of reports.

Supplementary Table 1. Search terms used in the literature search

Pubmed:

Carcinoma*[Title/Abstract] OR Cancer[Title/Abstract] OR Tumor[Title/Abstract] OR Tumour[Title/Abstract]	OR	hypernephroma*[Title/Abstract] OR RCC[Title/Abstract] OR Grawitz[Title/Abstract] OR "Carcinoma, Renal Cell"[Mesh]
AND		
Renal[Title/Abstract] OR Nephroid[Title/Abstract] OR Kidney[Title/Abstract] OR Papillary[Title/Abstract] OR "Collecting duct"[Title/Abstract] OR Hypernephroid[Title/Abstract]		
AND		
HLRCC[Title/Abstract] OR Leiomyom*[Title/Abstract] OR "fumarate hydratase"[Title/Abstract] OR "Fumarate Hydratase"[Mesh] OR "FH mutation"[Title/Abstract] OR Reed's[Title/Abstract] OR "Leiomyomatosis"[Mesh]		

Embase:

Carcinoma*:ab,ti OR Cancer:ab,ti OR Tumor:ab,ti OR Tumour:ab,ti	OR	hypernephroma*:ab,ti OR RCC:ab,ti OR Grawitz:ab,ti OR 'renal cell carcinoma'/exp
AND		
Renal:ab,ti OR Nephroid:ab,ti OR Kidney:ab,ti OR Papillary:ab,ti OR 'Collecting duct':ab,ti OR Hypernephroid:ab,ti		
AND		
HLRCC:ab,ti OR Leiomyom*:ab,ti OR 'fumarate hydratase':ab,ti OR 'fumarate hydratase'/exp OR 'FH mutation':ab,ti OR Reed*:ab,ti OR 'leiomyomatosis'/exp		

9

Discussion and future perspectives

The aim of this thesis was to provide novel insights into relevant clinical, prognostic and underlying (epi)genetic factors in children with renal tumors, with a focus on Wilms tumor (WT). The findings presented here have potential implications for WT treatment stratification, genetic counseling and testing of children with WT. Based on the findings in this thesis, we encourage the consideration of age in the design of future SIOP-RTSG protocols. In a unique unselected national cohort of children with WT, this thesis demonstrates that the rate of predisposing factors is higher than what has been previously reported, and identifies an important role for heterozygous *DIS3L2* variants. Moreover, this thesis provides recommendations for WT registration and therapy, as well as consensus-based surveillance guidelines for all currently known WT predisposition genes and syndromes, which can be used by clinical geneticists, pediatricians, pediatric oncologists and radiologists. Below, the implications of our findings for I) Wilms tumor treatment and outcome and II) (Epi)genetic predisposition to pediatric renal tumors, and suggestions for future research will be discussed.

I. WILMS TUMOR TREATMENT AND OUTCOME

Improving treatment stratification

Despite efforts to reduce toxicity, WT treatment regimens are still associated with direct and late side-effects. During the writing of this thesis, the Renal Tumor Study Group of the International Society of Pediatric Oncology (SIOP-RTSG) launched the SIOP-RTSG UMBRELLA 2016 protocol, which includes updated recommendations for the diagnosis and treatment of childhood renal tumors.[1] Compared to previous protocols, criteria for treatment stratification were refined, with the aim to reduce toxicity without compromising effectiveness. For example, tumor volume ≤500mL is now an additional criterion to allow omission of doxorubicin in the postoperative treatment of a subset of patients with stage II-III intermediate-risk WT.[1]

Because of the two different worldwide approaches to WT treatment, staging and histological classification, outcomes of SIOP-RTSG and Children's Oncology Group – Renal Tumor Group (COG-RTG) protocols cannot be directly compared. Yet, results from COG-RTG protocols indicate that a carefully selected subgroup of children with WT can be treated with nephrectomy only, avoiding the side effects of chemo- and radiotherapy alltogether.[2, 3] To select patients for treatment with nephrectomy only, age <2 years was one of the criteria, since older age had previously been associated with an increased risk of relapse.[4-7]

Based on the findings in this thesis, we encourage the consideration of age in the design of future SIOP-RTSG protocols. Although we were unable to identify optimal age cutoffs for risk stratification, we demonstrated that age is an independent prognostic factor for event-free survival in patients treated with preoperative chemotherapy, when correcting for stage, histological classification and tumor volume.[8] We would specifically

recommend to exclude older patients from reduced-treatment strategies, similar to the COG-RTG strategy. Notably, adverse molecular aberrations which are thought to reflect genomic instability, such as gain of 1q[9, 10], appear to be more prevalent in WTs of older patients.[10-12] Therefore, the ongoing SIOP-RTSG UMBRELLA study will clarify whether age retains prognostic significance when combined with the presence or absence of these molecular aberrations. Currently, in 2021, more than 1000 patients have already been registered in UMBRELLA. After the results of UMBRELLA become available, novel strategies for treatment stratification, including molecular aberrations and/or age, will be implemented in SIOP-RTSG protocols.

Treatment of children with a WT predisposition syndrome

Specific treatment recommendations for children with a WT predisposition syndrome are provided in SIOP-RTSG[1] and COG[13] protocols and they mainly involve the encouragement of nephron-sparing surgery (NSS). Patients with a WT predisposition syndrome are more likely to develop bilateral/second tumors and renal failure, which can be the result of bilateral surgeries as well as underlying genetic factors. Therefore, if the presence of a WT predisposition syndrome is apparent at the time of WT diagnosis, NSS is recommended, where feasible, to preserve as much functional renal tissue as possible. Several studies have demonstrated that NSS can be safely performed in children with a WT predisposition syndrome with unilateral or bilateral WT, depending on the size and location of the tumor(s).[13-15] Currently, compared to children with nonsyndromic WT, postoperative treatment regimens are similar for children with a WT predisposition syndrome. This thesis shows that children with Wilms tumor, Aniridia, Genito-urinary anomalies and Range of developmental delays (WAGR) syndrome can be successfully treated with existing WT protocols.[16] However, apart from WT, they are confronted with many other health problems which require specialized care. We demonstrate that in contrast to nonsyndromic WT, mortality in children with WAGR syndrome is more frequently due to comorbidity and/or toxicity than to the tumor itself.[16] Previous studies have indicated that specific health problems, such as obesity, can be related to the size of the deletion and the involvement of specific genes such as *BDNF*.[17] Further research is needed to clarify these genotype-phenotype correlations, and to assess whether children with WAGR syndrome and WT are more vulnerable to develop specific toxicities.

II. (EPI)GENETIC PREDISPOSITION TO PEDIATRIC RENAL TUMORS

(Epi)genetic predisposing factors in children with WT

With a comprehensive and stepwise approach of diagnostic genetic testing and research-based whole exome sequencing (WES), in a unique national unselected cohort of children with WT, we have determined the prevalence of (epi)genetic predisposing factors, including mosaic aberrations and clinical diagnoses, to be at least 33%. This

level of (epi)genetic predisposition is higher than the 5-24% that was reported in previous studies.[18-21] Below, various explanations for this higher prevalence will be explored.

Role of postzygotic mosaicism

This thesis demonstrates that many WT predisposing factors are present in a mosaic state, and that mosaic alterations can be easily missed by standard diagnostic tests. The most common postzygotic, mosaic alterations which we identified in children with WT, are 11p15 methylation defects, which cause Beckwith-Wiedemann Spectrum (BWSp).[22, 23] These epigenetic alterations are by definition somatic events, which can occur at different stages during embryonic development.

Depending on the level of mosaicism and their mosaic distribution, these methylation defects may be undetectable in blood. Methylation specific multiplex ligation-dependent probe amplification (MS-MLPA), which is the current diagnostic standard in the Netherlands, can only detect methylation defects which are present in at least ~20% of cells of the examined tissue type (methylation change of ≥10%). In children who are clinically suspected of BWSp, including children with WT, initial investigations are usually performed using blood-derived DNA. We demonstrate that additional analysis of resected normal kidney tissue-derived DNA increases the yield of BWSp testing from 1-8% in previous reports.[18-20], to 16% in this thesis. In the future, currently available molecular techniques may be refined to increase the sensitivity of BWSp testing in blood-derived DNA.[24]

Postzygotic mosaicism has also been demonstrated for several genetic alterations affecting WT predisposition genes, including *WT1*[25, 26] and *TRIM28*.[27-28] Postzygotic mutations can sometimes be detected by germline WES if a low variant allele frequency (VAF) detection threshold is applied, or by sequencing kidney tissue-derived DNA. When postzygotic mosaicism is demonstrated in healthy kidney tissue, it is not always clear whether the (epi)genetic alteration affects only the kidney(s), or also additional tissue types. In 2019, the concept 'clonal nephrogenesis' was introduced by Coorens et al. who demonstrated that in kidneys with WT, the surrounding normal kidney tissue can contain precursor clones which genetically resemble the tumor.[29] Specifically, the morphologically normal renal cortex was found to share somatic mutations with the tumor, which were absent in blood and renal pelvis, suggesting that WTs can arise from clonal expansions during embryonic development.[29] Interestingly, *H19* hypermethylation, which is one of the 11p15 methylation defects causing BWSp, was identified in many of the clonal expansions, suggesting an association between clonal nephrogenesis and this methylation defect.[29]

Overall, mosaic alterations affecting WT predisposition genes are much more frequent than previously thought. This challenges the classic definition of cancer predisposition. It appears that many WT predisposing aberrations are not inherited, but are only present

in a subset of an individual's cells and may sometimes even be limited to a single tissue type or organ. Theoretically, one kidney may be prone to WT development, while the other kidney is not. Even within a single kidney, a subset of cells may carry predisposing (epi)genetic alterations which are undetectable in other cells. It can be argued whether the term 'cancer predisposition' still applies to such localized, mosaic alterations. This raises questions about the implications for individual patients: to what extent do mosaic alterations increase the risk of metachronous tumors in the remaining kidney(s)? Further research is needed to answer this question. Currently, we recommend stricter follow-up for patients with mosaic alterations, compared to patients without a predisposition, because we cannot exclude the presence of the predisposing alteration in the remaining kidney tissue.

Heterozygous DIS3L2 variants

Based on our findings, *DIS3L2* is an important WT predisposition gene which can act as a tumor suppressor, with one allele disrupted in the germline, and a second somatic hit in the tumor. Until recently, germline genetic testing did not include the *DIS3L2* gene unless a child was suspected of Perlman syndrome. Perlman syndrome, a congenital overgrowth syndrome with a 64% risk of WT development, is caused by biallelic, germline *DIS3L2* variants.[30,31] Although heterozygous *DIS3L2* variants had been previously suggested to cause an increased WT risk[25, 32-34], they were not considered prevalent among children with WT. Yet, the findings in this thesis demonstrate that germline, heterozygous *DIS3L2* variants are a recurrent finding in children with WT.

The presence of heterozygous *DIS3L2* variants in unaffected parents and population databases implies a reduced penetrance. Based on the frequency of *DIS3L2* exon 9 deletions in the gnomAD population database (0.05%) and the incidence of WT (~1 in 10,000, which are not all *DIS3L2*-related), we expect the risk of WT development to be <1% for carriers of heterozygous *DIS3L2* aberrations. At the same time, these aberrations do account for at least 4% of all WTs. Following the two-hit model of tumor suppressor genes[35], we hypothesize that the impact of biallelic *DIS3L2* loss may be limited to a very specific time window in embryonic kidney development. Compared to other WT predisposition genes such as *WT1*, the biallelic loss of *DIS3L2* may be more easily tolerated during other stages of kidney development. Further research is needed to clarify these differences. We suggest that the WT risk for carriers of heterozygous *DIS3L2* variants can be studied by performing segregation analysis in family members of children with *DIS3L2*-associated WT.

Recently identified genes

Unlike *DIS3L2,* in which relatively common variants appear to cause a low WT risk, several WT predisposition genes have been recently identified, in which rare variants cause a high WT risk. One of these genes is *TRIM28*[28, 36-39], which is associated with a WT penetrance of >50% in heterozygous carriers.[27, 28, 36] Other recently identified

genes which are associated with a high WT risk include *REST*[40] (autosomal dominant) and *CTR9*[41] (autosomal recessive). In contrast to *DIS3L2* variants, the identification of germline *TRIM28, REST* or *CTR9* variants warrants counseling and genetic testing of family members, to enable potential early detection of WT in other children in the family. As neither *TRIM28, REST* nor *CTR9* variants seem to cause recognizable phenotypic features, they may be easily missed if not included in standard diagnostic gene panels for children with WT.

Standard genetic testing for children with WT

Based on this thesis, we recommend standard genetic counseling and germline genetic testing for children with WT. By doing so, we expect the detection of (epi)genetic predisposing factors to improve, and this may reveal that the prevalence of predisposing factors is even higher than reported in this thesis. Depending on local infrastructure and resources, standard genetic testing may not be feasible in all pediatric oncology clinics and settings. In that case, decision-support algorithms can be used to prioritize children for genetic testing. The MIPOGG tool is an example of a decision-support algorithm which has been recently developed for various childhood cancer types, including WT.[20] Using such a tool reduces the rate of genetic referrals, although our findings indicate that some diagnoses are missed with this approach. 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 as suggested in this thesis, which includes (mosaic) BWSp testing and a WES-based panel analysis. As WES interpretation is continuously evolving, the yield of WES-based panels, including splicing variants and small copy number variants (CNVs), is likely to increase in the future.

Geographic differences

This thesis describes (epi)genetic aberrations in a Dutch population of children with WT, and thus, does not account for the differences in (epi)genetic predisposing factors which appear to exist between different geographical populations. In two studies, 11p15 epimutations were found to be much less common in WTs from Japanese children compared to children from Western countries or New Zealand.[42, 43] In accordance with these findings, the prevalence of BWSp among children with WT is lower whereas the relative contribution of constitutional *WT1* mutations or deletions to (bilateral) WT development is higher in Japan.[44] These differences are intriguing, and further studies may address what causes these differences by studying evolutionary events[45] as well as potential environmental influences during embryonic development.[42]

Are there additional WT predisposition genes which remain to be identified?

Our exome-wide trio-analysis approach did not yield strong candidate WT predisposition genes outside the gene panel. This illustrates the complexity of searching for novel WT predisposition genes. In contrast to unsolved familial WT pedigrees, where a monogenic

cause is suspected[36], epigenetic factors and postzygotic mosaicism play an important role in isolated (non-familial) WT. Moreover, yet to be identified WT predisposition genes may exhibit reduced penetrance, as demonstrated for *DIS3L2*. Finally, future large-scale genetic and epidemiological studies may reveal oligogenic and/or multifactorial inheritance mechanisms involved in WT predisposition.

Surveillance in children at-risk

Within an international expert panel, we developed updated WT surveillance guidelines following an extensive literature review and international consensus meetings, addressing all currently recognized WT predisposition genes and syndromes. For the majority of the reviewed genes and syndromes, our literature review identified only a limited number of studies which were mainly case reports or small case series. Therefore, most recommendations were based on expert consensus. Below, the main discussion points will be addressed, demonstrating where more data are needed.

Lateralized overgrowth without a molecular diagnosis

Children with lateralized overgrowth (LO), also known as hemihypertrophy or hemihyperplasia, may be at risk for WT development. If a syndromic diagnosis can be established based on molecular testing or clinical criteria, tumor surveillance should be initiated accordingly. Robust data to inform recommendations for remaining patients (i.e. isolated LO and no detectable molecular finding) are lacking. Two studies have estimated the overall tumor risk to be around 10%, with WT and neuroblastoma being the most common tumor types[46, 47], although it is likely that this includes patients with low-level mosaic BWSp aberrations. A more recent study reported no tumors in a cohort of 48 children with isolated LO in whom molecular BWSp testing was negative in blood, without specifying the period of follow-up.[48] Yet, to truly determine the risk of WT development in children with isolated LO, these patients need to be followed at least until the age of seven years, with negative molecular tests in both blood as well as the overgrown tissue. Such a study has not yet been performed, but could provide valuable data to inform WT surveillance programs in the future.

Syndromes with only few reported patients

Other discussions within the consensus group concerned syndromes with only few reported patients, where WT risk estimates could not be reliably determined. Examples include mosaic variegated aneuploidy (MVA)[49-52], osteopathia striata with cranial sclerosis (OSCS)[53, 54], Bohring-Opitz syndrome[55, 56], 9q22.3 deletion syndrome[57], 2q37.1 deletion syndrome[58] and 2p24.3 duplication syndrome.[59-61] In this thesis, we strongly advise clinicians and/or parents to register patients with these syndromes in national or international databases, to enable the development of better tumor risk estimates and tumor surveillance programs in the future. We propose several international registries which already exist and can be used for this purpose.[62]

Surveillance for non-Wilms renal tumors in childhood

Surveillance recommendations for non-Wilms renal tumors are not extensively discussed in this thesis, as they have been either previously developed by other groups[63-65], or predisposing factors have not been identified. In the case of renal cell carcinoma (RCC) predisposition, surveillance is often not warranted until adulthood, and genetic testing of family members at risk can be postponed until they reach the age of 18 years. In this thesis, we describe twelve patients with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), identified in our own institution or in the literature, who presented with RCC before the age of 20 years.[66] The youngest reported patient developed RCC at the age of 10 years.[67] Given the aggressive nature of HLRCC-related RCC, this supports existing recommendations to offer genetic testing and renal MRI surveillance to children in HLRCC families from the age of 8-10 years onwards[67], but also raises questions on how to prepare children and their families for the implications of genetic testing. Otherwise healthy children who are diagnosed with HLRCC may experience anxiety as a result of genetic testing and MRI surveillance. It is therefore important to offer careful pre-test counseling and psychological support where necessary.

GAPS OF KNOWLEDGE AND FUTURE DIRECTIONS

During the writing of this thesis, several gaps of knowledge were identified that need to be addressed in the future (Table 1). This includes directions for future research in the field of WT treatment stratification, predisposing factors, risk and surveillance as well as non-Wilms renal tumors and psychosocial aspects of genomic sequencing.

THIS THESIS IN A BROADER PERSPECTIVE

As the repertoire of genomic techniques continues to expand, many childhood cancer hospitals are implementing techniques such as WES in standard clinical care. As described in this thesis, clinical and genomic markers can be combined to improve treatment stratification. Moreover, comprehensive genomic studies can increase the detection of (epi)genetic predisposing factors in children with cancer.

Combined, the high rate of germline and mosaic predisposing factors identified in children with WT, raises the question whether we are missing predisposing factors in other childhood cancer types. Similar to WT, the increased detection of mosaic alterations will likely lead to higher rates of identified (epi)genetic predisposition in other embryonal tumors. This has already been demonstrated for retinoblastoma, where mosaic *RB1* pathogenic variants, undetectable by Sanger sequencing, were identified by next-generation deep sequencing in 30% of patients with bilateral retinoblastoma and 6% of those with unilateral retinoblastoma.[68] Mosaicism has also been reported for *SMARCB1* pathogenic variants which cause predisposition to rhabdoid tumors (including malignant rhabdoid tumor of the kidney)[69], and for *DICER1* pathogenic variants which

cause a predisposition to pleuropulmonary blastoma, cystic nephroma, WT and many other tumor types.[70]

In addition to (low-level) mosaic variants, variants located in intronic and/or repeat-rich regions can also escape detection by standard diagnostic approaches. Techniques such as whole genome sequencing, RNA sequencing and optical genome mapping have already been proven effective in identifying such hidden variants.[71, 72] A more widespread application of these techniques will likely further increase the yield of genetic testing in children with cancer.

In the detection as well as the interpretation of germline and mosaic variants, the analysis of tumor tissue provides crucial information. First of all, specific predisposing variants can be recognized or suspected based on the tumor's morphology, by immunohistochemical staining or by more advanced techniques such as mutational signature analysis, RNA sequencing/transcriptome profiling and methylation profiling. Moreover, the relevance of novel variants which are detected in the germline, can be assessed by applying these techniques to determine the impact of the variant in tumor tissue. Therefore, when assessing a patient for possible (epi)genetic predisposing factors, germline (epi)genetic testing is ideally combined with (or even preceded by) molecular tumor profiling.

Although the aim of all these efforts is to inform patients and families about potential cancer risks, this thesis also raises the question how much patients and families actually want to know. The risks associated with some of the identified variants, such as the *DIS3L2* variants described in this thesis, are likely to be low. The identification of these variants may be scientifically relevant, while having little or no clinical consequences. Therefore, along with scientific developments in the field of cancer genomics, we need to study the psychosocial consequences and carefully consider whether and how to communicate genomic findings to patients and families.

Table 1. Renal tumors in children and the role of (epi)genetic predisposition: domains for future research.

Domain	Gaps of knowledge / future directions
WT treatment stratification	· Prognostic significance of age when combined with molecular aberrations in the tumor; · Identification and validation of novel treatment stratifiers (e.g. blastemal volume); · Feasibility of further treatment reduction in selected patients; · Differences in treatment toxicity in children with WT predisposition syndromes (e.g. WAGR syndrome) compared to other children with WT.
WT predisposing factors	· Prevalence of mosaic alterations in known WT predisposition genes; · Feasibility of BWSp testing on healthy resected kidney tissue; · Geographical differences in the type and prevalence of predisposing factors among children with WT; · Identification of additional WT predisposition genes which may exhibit reduced penetrance, oligogenic and/or multifactorial inheritance mechanisms; · Yield of implementing additional techniques such as whole genome sequencing, mutational signature analysis, RNA sequencing/transcriptome profiling and methylation profiling in standard clinical care.
WT risk and surveillance	· WT risk for children with very rare syndromes associated with WT development; · WT risk for children with isolated lateralized overgrowth without a molecular diagnosis; · WT risk for carriers of heterozygous *DIS3L2* variants; · Optimal duration of surveillance.
Non-Wilms renal tumors	· Predisposing factors in children with rare non-Wilms renal tumors; · Prevalence of mosaic alterations in known non-Wilms renal tumor predisposition genes; · Overlap in underlying (epi)genetic pathways and biological relationship between various renal tumors; · Optimizing guidelines for surveillance in non-Wilms renal tumor predisposition syndromes.
Genomic sequencing: psychosocial aspects	· Pre-test counseling of children and their families about (germline) genomic sequencing; · Communication of genomic sequencing results to children and their families; · Impact of diagnosing a cancer predisposition syndrome in children and their families; · Psychosocial consequences of high-risk versus low-risk cancer predisposing aberrations.

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10

Summary & Samenvatting

SUMMARY

Childhood renal tumors account for ~5% of all childhood cancer diagnoses. Among them, Wilms tumor (WT) is by far the most common. Although current WT treatment regimens lead to ~90% overall survival rates, they are associated with short- and long-term side effects, and still, not all children with WT can be cured. Currently, risk adapted treatment protocols have been implemented to further improve survival and reduce treatment toxicity. This is the focus of the first part of this thesis. Moreover, (epi) genetic predisposition is known to play an important role in WT. Insight into (epi)genetic predisposing factors can lead to a better understanding of tumor initiating processes and provide novel clues for therapy. Additionally, insight into (epi)genetic predisposition is needed to design strategies for genetic counseling and testing of children with WT, and to inform surveillance recommendations for children at risk of WT development. These topics are addressed in the second part of this thesis.

In the first part of this thesis, we describe the rationale behind WT treatment recommendations in the current International Society of Pediatric Oncology Renal Tumor Study Group (SIOP-RTSG) 2016 UMBRELLA protocol. The UMBRELLA protocol implements the findings from a previous trial that showed evidence for the feasibility of omitting anthracyclines in the majority of stage II en III intermediate-risk WT patients. In the future, treatment-related toxicity can potentially be further reduced by including age and/or molecular markers to stratify treatment intensity. We studied the prognostic significance of age in 5631 patients who were registered in SIOP 93-01 and SIOP 2001 protocols, and demonstrated that age is indeed an independent prognostic factor for event-free survival in patients treated with pre-operative chemotherapy. However, the significance of age combined with prognostic molecular markers needs to be prospectively validated in patients registered in the UMBRELLA protocol.

We also describe the clinical characteristics and outcome of 43 children with Wilms tumor, Aniridia, Genito-urinary anomalies and Range of developmental delays (WAGR) syndrome, identified through SIOP-RTSG registries. We observed a relatively high number of bilateral disease and absence of metastatic and diffuse anaplastic tumors. We demonstrated that children with WAGR syndrome can be successfully treated with existing WT protocols. Compared to children with nonsyndromic WT, mortality was more frequently associated with comorbidity and/or toxicity than to the tumor itself.

In the second part of this thesis, we describe the role of (epi)genetic predisposition in a Dutch, nationwide cohort of children with WT. (Trio-) whole exome sequencing (WES) was offered in all cases where no genetic predisposition had been identified by targeted diagnostic testing. We determined the prevalence of (epi)genetic predisposing factors, including mosaic aberrations and clinical diagnoses, to be at least 33%, which is higher than the 5-24% that had been reported in previous studies. Beckwith-Wiedemann

spectrum (BWSp) was diagnosed in 16% of all patients, compared to only 1-8% in earlier reports. This higher frequency was partly due to the fact that we performed BWSp testing on resected healthy kidney tissue in addition to blood-derived DNA. One of the most remarkable findings was the important contribution of heterozygous *DIS3L2* variants, which had not been previously reported in children with WT. We demonstrated that *DIS3L2* is a recurrent WT predisposition gene which can act as a tumor suppressor with reduced penetrance. Based on our findings, we propose a diagnostic strategy which includes (mosaic) BWSp testing and/or WES-based panel analysis for all children with WT.

In 2018, *TRIM28* was identified as a novel WT predisposition gene. To provide more insight into *TRIM28*-associated WT, we reviewed histopathological and clinical features as well as potential underlying mechanisms. *TRIM28* is a ubiquitously expressed corepressor that controls the expression of genes and transposable elements during embryogenesis and cellular differentiation. Based on the reviewed literature, we observed that heterozygous *TRIM28* variants are associated with a WT penetrance of >50%, and reported tumor types mainly include epithelial WT. Assessing TRIM28 protein expression by immunohistochemistry in tumor tissue is an effective strategy to identify patients carrying pathogenic *TRIM28* variants.

Following an extensive literature review and based on international consensus meetings, we developed WT surveillance guidelines on behalf of the SIOP Europe Host Genome Working Group and SIOP-RTSG, addressing all currently recognized WT predisposition genes and syndromes.

Apart from WT surveillance, renal surveillance for non-Wilms renal tumors is warranted in several cancer predisposition syndromes. In the final chapter of this thesis, we report on young patients with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) caused by germline pathogenic variants in the *FH* gene. These patients are at risk of developing aggressive HLRCC-related renal cell carcinoma (RCC). In our case series and literature review, we identified twelve unique patients with HLRCC who presented with RCC before the age of 20 years, which supports the recommendation to offer genetic testing and renal MRI surveillance from the age of 8-10 years onwards in children (suspected of) carrying *FH* pathogenic variants.

Overall, this thesis provides novel insights into relevant clinical, prognostic and underlying (epi)genetic factors in children with renal tumors. Future research can be directed into further improving WT treatment stratification. This is also the aim of the ongoing UMBRELLA study in which the prognostic significance of various molecular aberrations in the tumor is studied, in combination with age. Moreover, future research should aim to expand our knowledge on (epi)genetic predisposing factors and particularly the role of mosaic predisposing factors, both in WT, non-Wilms renal

tumors and other childhood cancer types. Finally, future research may be directed into refining recommendations for WT surveillance and genetic counseling, by determining the WT risk associated with rare and novel predisposing factors, as well as the impact of diagnosing a cancer predisposition syndrome in children and their families.

SAMENVATTING

Niertumoren vormen ongeveer 5% van alle kinderkanker diagnoses. Hiervan zijn Wilms tumoren verreweg het meest voorkomend. Hoewel tegenwoordig een overleving van ~90% wordt bereikt, gaat de behandeling van Wilms tumoren nog altijd gepaard met bijwerkingen op de korte en lange termijn, en kunnen nog niet alle kinderen met een Wilms tumor worden genezen. Om de overleving verder te verbeteren en bijwerkingen te verminderen, wordt de behandeling in de huidige behandelprotocollen zoveel mogelijk toegespitst op het individuele risico van de patiënt. Hierover gaat het eerste deel van dit proefschrift. Daarnaast is bekend dat erfelijke aanleg bij Wilms tumoren een belangrijke rol speelt. Het in kaart brengen van (epi)genetische factoren kan ons helpen om beter te begrijpen hoe de ziekte ontstaat en kan nieuwe aanknopingspunten bieden voor de behandeling. Bovendien is kennis over (epi)genetische factoren nodig om te bepalen wanneer genetische diagnostiek is geïndiceerd, en om screeningsadviezen op te stellen voor kinderen die een erfelijke aanleg voor Wilms tumoren blijken te hebben. Deze onderwerpen komen in het tweede deel van dit proefschrift aan bod.

In het eerste deel van dit proefschrift beschrijven we hoe de behandeladviezen in het huidige International Society of Pediatric Oncology Renal Tumor Study Group (SIOP-RTSG) 2016 UMBRELLA-protocol tot stand zijn gekomen. Het UMBRELLA-protocol implementeert de bevindingen van een eerdere studie, waarin werd aangetoond dat anthracyclines kunnen worden weggelaten bij de meeste patiënten met een intermediate-risk, stadium II of III Wilms tumor. In de toekomst kan de behandeling mogelijk nog verder worden toegespitst op de individuele patiënt, door ook leeftijd en moleculaire markers in de tumor mee te nemen in de risicostratificatie. Zo onderzochten we de prognostische waarde van leeftijd bij diagnose in een cohort van 5631 kinderen met Wilms tumoren, die geregistreerd stonden in de SIOP 93-01- en SIOP 2001 database. We toonden aan dat leeftijd inderdaad een onafhankelijke prognostische factor is bij kinderen die werden behandeld met pre-operatieve chemotherapie. Het belang van leeftijd in combinatie met prognostische moleculaire markers zal echter nog prospectief worden gevalideerd bij patiënten die momenteel worden geregistreerd in het UMBRELLA-protocol.

We beschrijven ook de klinische kenmerken en uitkomsten van 43 kinderen met het WAGR syndroom, die met SIOP-RTSG protocollen werden behandeld voor een Wilms tumor. De term WAGR is een afkorting van de meest voorkomende kenmerken: Wilms tumor, Aniridie, Genitaal- en urinewegafwijkingen en ontwikkelingsachterstand (in het Engels: “Range of developmental delays”). Deze 43 kinderen hadden relatief vaak bilaterale tumoren en géén van hen had uitgezaaide of diffuus anaplastische (hoog-risico) tumoren. We toonden aan dat kinderen met het WAGR syndroom succesvol kunnen worden behandeld met bestaande behandelprotocollen. Vergeleken met kinderen met

niet-syndromale Wilms tumoren, was mortaliteit vaker geassocieerd met comorbiditeit en/of bijwerkingen van de behandeling, dan met de tumor zelf.

In het tweede deel van dit proefschrift beschrijven we de rol van (epi)genetische aanleg in een Nederlands, landelijk cohort van kinderen met Wilms tumoren. (Trio-) whole exome sequencing (WES) werd aangeboden aan alle kinderen bij wie géén (epi)genetische aanleg werd vastgesteld met standaard, gerichte diagnostiek. (Epi)genetische aanleg, waaronder mozaïekafwijkingen en klinische diagnoses, werd bij 33% van alle kinderen aangetoond. Dit percentage is hoger is dan de 5-24% zoals in eerdere onderzoeken werd gevonden. Het Beckwith-Wiedemann spectrum (BWSp) werd bij 16% van alle patiënten vastgesteld, vergeleken met slechts 1-8% in eerdere studies. Dit verschil is mede te verklaren doordat we naast DNA uit bloed, ook DNA uit gezond nierweefsel hebben getest op mozaïek BWSp. Eén van de meest opvallende bevindingen was het relatief grote aandeel van heterozygote *DIS3L2*-varianten, wat niet eerder bij kinderen met Wilms tumoren werd beschreven. We toonden aan dat het *DIS3L2*-gen zich kan gedragen als een tumorsuppressor-gen met verminderde penetrantie. Op basis van onze bevindingen doen we een voorstel voor een nieuwe, diagnostische aanpak waarbij BWSp-diagnostiek en/of WES panel analyse standaard wordt aangeboden aan alle kinderen met Wilms tumoren.

In 2018 werd aangetoond dat pathogene varianten in het *TRIM28* gen een verhoogd risico op Wilms tumoren veroorzaken. Om hier meer inzicht in te krijgen, hebben we de klinische kenmerken en mogelijke onderliggende mechanismen op een rij gezet. *TRIM28* is een belangrijke corepressor die de expressie van genen en transposons tijdens de embryonale ontwikkeling en celdifferentiatie reguleert. Op basis van een literatuurstudie laten we zien dat de penetrantie van heterozygote *TRIM28*-varianten waarschijnlijk hoger dan 50% is, en dat kinderen met deze aanleg voornamelijk epitheliale Wilms tumoren ontwikkelen. Door middel van immuunhistochemie (eiwitkleuring) in tumorweefsel kunnen tumoren met verlies van het TRIM28 eiwit worden herkend. Dit is een effectieve strategie om kinderen met pathogene *TRIM28*-varianten op te sporen.

Namens de SIOP Europe Host Genome Working Group en SIOP-RTSG hebben we een internationale consensus richtlijn voor Wilms tumor screening opgesteld, waarin alle op dit moment bekend Wilms tumor genen en –syndromen aan bod komen.

Behalve voor Wilms tumoren, is tumorscreening soms ook al op kinderleeftijd geïndiceerd voor andere, zogenaamde ‘non-Wilms’ tumoren in de nier. In het laatste hoofdstuk van dit proefschrift beschrijven we jonge patiënten met hereditaire leiomyomatosis en niercelcarcinoom (HLRCC), veroorzaakt door pathogene varianten in het *FH*-gen. Deze patiënten hebben een verhoogd risico op het ontwikkelen van een agressieve vorm van niercelcarcinoom. Hoewel deze tumoren meestal pas op volwassen leeftijd ontstaan, laten we zien dat ook tieners al HLRCC-gerelateerde niercelcarcinomen

kunnen ontwikkelen. Dit ondersteunt de aanbeveling om genetische diagnostiek en tumorscreening vanaf de leeftijd van 8-10 jaar aan te bieden aan kinderen die (mogelijk) drager zijn van een pathogene *FH*-variant.

Concluderend draagt dit proefschrift bij aan de huidige kennis over klinische, prognostische en (epi)genetische factoren bij kinderen met niertumoren. Toekomstig onderzoek kan zich richten op het verfijnen van de Wilms tumor behandeling door een nog betere risicostratificatie. Dit is het doel van de UMBRELLA-studie waarin de prognostische waarde van moleculaire markers in de tumor wordt onderzocht, gecombineerd met klinische factoren zoals leeftijd bij diagnose. Daarnaast kan toekomstig onderzoek zich richten op een het verbreden van onze kennis over (epi) genetische aanleg en met name de rol van mozaïeken, zowel bij Wilms tumoren, non-Wilms niertumoren en andere vormen van kinderkanker. Ten slotte kan toekomstig onderzoek bijdragen aan beter onderbouwde screeningsadviezen voor kinderen met een aanleg voor Wilms tumoren, door het tumorrisico bij zeldzame en/of nieuwe (epi) genetische factoren te bepalen, en door meer aandacht te besteden aan de impact die het vinden van een Wilms tumor aanleg heeft op kinderen en hun families.

APPENDICES

List of Abbreviations
Curriculum Vitae
PhD Portfolio
List of Publications
Dankwoord / Acknowledgements

LIST OF ABBREVIATIONS

BWSp	Beckwith-Wiedemann Spectrum
CCSK	Clear cell sarcoma of the kidney
CNV	Copy number variant
COG	Children's Oncology Group
CPS	Cancer predisposition syndrome
EFS	Event-free survival
ESRD	End-stage renal disease
FH	Fumarate hydratase
HLRCC	Hereditary leiomyomatosis and renal cell cancer
HR	Hazard ratio
IHC	Immunohistochemistry
ILNR	Intralobar nephrogenic rest
IQR	Interquartile range
LO	Lateralized overgrowth
LOH	Loss of heterozygosity
MET	Mesenchymal-to-epithelial transition
MRI	Magnetic resonance imaging
MRTK	Malignant rhabdoid tumor of the kidney
MS-MLPA	Methylation specific multiplex ligation-dependent probe amplification
MVA	Mosaic variegated aneuploidy
NGS	Next generation sequencing
NR	Nephrogenic rest
NSS	Nephron-sparing surgery
NWTS	National Wilms tumor studies
OS	Overall survival
OSCS	Osteopathia striata with cranial sclerosis
PLNR	Perilobar nephrogenic rest
PROS	*PIK3CA*-related overgrowth spectrum
RCC	Renal cell carcinoma
RTSG	Renal Tumor Study Group
SGBS	Simpson Golabi Behmel syndrome
SIOP	International Society of Pediatric Oncology
SNV	Single nucleotide variant
UPD	Uniparental disomy
VUS	Variant of unknown significance
WAGR	Wilms tumor, aniridia, genitourinary anomalies and range of developmental delays
WES	Whole exome sequencing
WGS	Whole genome sequencing
WT	Wilms tumor

CURRICULUM VITAE

Janna Hol was born in Venlo, The Netherlands, on January 3rd 1991. She graduated from College Den Hulster in Venlo (*cum laude*) in 2008. That same year, she started her medical training at the Utrecht University Medical Center (UMCU) where she obtained her medical degree in 2015. During medical training, she traveled abroad for several internships and research projects, visiting hospitals in Buenos Aires, Paris and London.

From 2015-2016, she worked as a resident in pediatrics at Tergooi Hospital (Blaricum), after which she started her PhD project at the Princess Máxima Center for Pediatric Oncology (Utrecht) supervised by prof. dr. M.M. van den Heuvel-Eibrink (pediatric oncologist), dr. M.C.J. Jongmans (clinical geneticist) and dr. R.P. Kuiper (molecular biologist). She combined her work as a PhD student with positions in the Works Council (Ondernemingsraad) and the PriMá PhD Group of the Princess Máxima Center, representing and supporting other PhD students. Internationally, she is part of the SIOP Renal Tumor Study Group (RTSG) - Epidemiology, Genetics and Clinical Outcomes (ECO) panel as a Young Investigator.

During her research projects, she was fascinated by the field of genetics, as well as the psychosocial implications of genetic testing. This motivated her to choose a career in genetics. As of April 2021, she works as a resident in clinical genetics at the Erasmus Medical Center (Rotterdam), where she will start her training to become a clinical geneticist in 2022.

PHD PORTFOLIO

Name:	Janna A. Hol
PhD period:	June 2016 – February 2021
Research school:	Cancer, Stem Cells & Development (CS&D) Utrecht University, Graduate School of Life Sciences (GSLS)
Department:	Pediatric Oncology (Princess Máxima Center for Pediatric Oncology)
Supervisor:	Prof. dr. M.M. van den Heuvel-Eibrink
Co-supervisors:	Dr. M.C.J. Jongmans, Dr. R.P. Kuiper

1. PhD Training

	Year
Courses	
BROK, UMC Utrecht	2016
This Thing Called Science (GSLS)	2017
Introductory Biostatistics for Researchers (GSLS)	2017
NGS in DNA Diagnostics Course (MolMed)	2017
The Art of Presenting Science (GSLS)	2019
Human Disease Genetics (CS&D)	2019
Advanced Molecular Pathology (CTO)	2019
Seminars and Workshops	
Máxima Research Meetings & Seminars	2017
Research retreat Princess Máxima Center	2017, 2019
Conferences	
48th SIOP Congress, Dublin	2016
SIOP-RTSG Meeting, Mallorca (oral presentation)	2017
49th SIOP Congress, Washington DC (oral presentation)	2017
6th UK/Dutch Clinical Genetics Societies Meeting, Utrecht (poster presentation)	2018
SIOP-RTSG Meeting, Copenhagen (oral presentation)	2018
ESHG Congress, Milan (poster presentation)	2018
IWSA Conference on Wilms Tumor in WAGR Syndrome, Ann Arbor	2017, 2018
50th SIOP Congress, Kyoto	2018
51st SIOP Congress, Lyon (oral presentation)	2019
7th UK/Dutch Clinical Genetics Societies Meeting, Cambridge (oral presentation)	2020

SIOP-RTSG Meeting, Rio de Janeiro (oral presentation)	2020
SIOP-RTSG / COG Renal Tumor Biology Meeting (Virtual)	2020

2. Teaching activities

Supervising master student (Medicine)	2017-2018
Supervising master student (SUMMA)	2018-2019
Supervising master student (Medicine)	2019-2020

3. Other activities

PriMá PhD Group	2019-2020

4. Awards

SIOP Young Investigator Award (Washington DC)	2017
ESHG poster award (Virtual Conference)	2021

LIST OF PUBLICATIONS

This thesis

- **Hol JA**, Kuiper RP, van Dijk F, Waanders E, Van Peer SE, Koudijs MJ, Bladergroen R, Van Reijmersdal SV, Morgado LM, Bliek J, Lombardi MP, Hopman S, Drost J, De Krijger RR, Van den Heuvel-Eibrink MM, Jongmans MCJ. Prevalence of (epi) genetic predisposing factors in a 5-year unselected national Wilms tumor cohort: a comprehensive clinical and genomic characterization. *Submitted*

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