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INVITED REVIEW

TRIM28 variants and Wilms' tumour predisposition

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Abstract

TRIM28 **was recently identified as a Wilms' tumour (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 tumour components or anaplasia does not exclude the presence of a germline or somatic** ***TRIM28*** **variant. In children with WT,** ***TRIM28*** **acts as a classical tumour suppressor gene, with both alleles generally disrupted in the tumour. Therefore, loss of TRIM28 (KAP1/TIF1beta) protein expression in tumour 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 tumourigenesis 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.**

Keywords: TRIM28; KAP1; TIF1beta; Wilms' tumour; nephroblastoma; cancer predisposition; embryonic kidney development

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Background

Wilms' tumour (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 tumour 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 tumour 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 tumour [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).

Histological features of *TRIM28*-mutated tumours

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 tumours. 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 Tumour 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 tumour 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 tumours 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 tumours from 46 patients [12–16]. Out of the 51 tumours, 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 tumours appear to be the predominant subtype among *TRIM28*-mutated tumours, the presence of other tumour 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 tumours. All reported nephrogenic rests were perilobar rests.

TRIM28 acts a tumour suppressor in patients with WT

TRIM28 acts as a classical tumour suppressor gene in WT patients, where disruption of both alleles appears to be required to initiate tumour development. In ten *TRIM28*-mutated tumours in which immunohistochemistry (IHC) was performed (Figure 2), including seven with a germline variant, tumour cells had lost expression of TRIM28, in contrast to the surrounding non-malignant 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 tumours, 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 tumours. Eight tumours (72%) did not reveal any driver gene mutation [14,15]. One tumour revealed a *TP53* mutation, which was likely related to its diffuse anaplastic histology [13,21]. In the study by Diets *et al*, two tumours revealed somatic mutations in *DICER1*, *AMER1* (individual 3), and *NF1* (individual 4) [15].

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

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

ID in original report [reference]	Mutation identified in:	Familial WT?	M/F	Age	Inheritance	Mutation	Histology	NR	LOH/IHC, other findings in tumour	FU
0477_01 [12]	Blood	Familial	F	24	Mat	p.Gly310Asp	Epithelial predominant†	NA	NA	NA
0477_02 [12]	Blood	Familial	M	84	Mat	p.Gly310Asp	Epithelial†	NA	NA	NA
0477_03 [12]	Blood	Familial	F	93	Mat	p.Gly310Asp	NA	NA	NA	NA
0498_01 [12] / 249 [14]	Blood & tumour	Familial	M	8	Mat	p.Glu583Argfs*93	Monomorphic epithelial†	NA	LOH	30
0498_02 [12] / 399 [14]	Blood & tumour	Familial	F	5	Mat	p.Glu583Argfs*93	Monomorphic epithelial	No	LOH	29
0498_03 [12]	Blood	Familial	F	6	NA	p.Glu583Argfs*93	Epithelial†	NA	NA	NA
0487_01 [12]	Blood	Familial	M	15	Mat	p.Thr144Hisfs*12	Epithelial predominant †	NA	NA	18
0487_02 [12]	Blood	Familial	M	18	NA	p.Thr144Hisfs*12	NA	NA	NA	NA
0506_01 [12] / 37 [14]	Blood & tumour	Familial	M	39	Mat	p.Thr176Profs*3[2]	Monomorphic epithelial†	No	CN-LOH, TRIM28 IHC loss	20
0506_02 [12] / 39 [14]	Blood & tumour	Familial	F	8	Mat	p.Thr176Profs*3[2]	L Monomorphic epithelial† R Monomorphic epithelial†	No	CN-LOH, TRIM28 IHC loss	20
7487_01 [12]	Blood	Isolated	F	118	Mat	p.Leu80Profs*11	Epithelial predominant with diffuse anaplasia†	NA	NA	3††
1982 [12]	Blood	Isolated	M	11	DN	p.Leu653Cysfs*23	L Epithelial predominant† R Epithelial predominant†	NA	NA	15
6530 [12]	Blood	Isolated	M	15	DN	p.Glu70Alafs*19	Epithelial + blastemal†	NA	NA	5
1969 [12]	Blood	Isolated	M	118	DN	Splice, c.840–2A>G	Epithelial + blastemal†	NA	NA	10
7574 [12]	Blood	Isolated	M	13	DN	p.*836Trpext*?	Epithelial predominant†	NA	NA	NA
0902 [12]	Blood	Isolated	F	12	Mat	p.Ser417*	Epithelial predominant†	NA	NA	NA
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† R Epithelial predominant†	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†	NA	NA	22
0936 [12]	Blood	Isolated	M	8	NA	p.Glu384*	NA	NA	NA	NA
1 [15]	Blood & tumour	Familial	F	5	Mat	p.Cys83Phefs*6	L Epithelial type † R Epithelial type †	PLNR	CN-LOH, TRIM28 IHC loss	
2 [15]	Blood & tumour	Familial	F	18	Mat	p.Cys83Phefs*6	Epithelial type†	PLNR	CN-LOH, TRIM28 IHC loss	NA
3 [15]	Blood & tumour	Familial	M	69	Mat	p.Arg524Leufs*155	Mixed type†	PLNR	No LOH, TRIM28 IHC loss, mutations in *DICER1* & *AMER1*	NA
4 [15]	Blood & tumour	Familial	M	7	Mat	p.Arg524Leufs*155	L Epithelial type† R Blastemal type†	PLNR	CN-LOH, TRIM28 IHC loss, *NF1* mutation	NA
5 [15]	Healthy kidney & tumour	Familial	F	6	NA	p.Gln283*	Epithelial type†	PLNR	NA	NA
6 [15]	Healthy kidney & tumour	Familial	F	7	NA	p.Gln283*	L Epithelial type† R Nephroblastomatosis†	PLNR	NA	NA
7 [15]	Both kidneys & tumour	Familial	M	6	Mat‡	p.Gln339*	L Epithelial type† R Epithelial type†	PLNR	CN-LOH, TRIM28 IHC loss	NA

(Continues)

Table 1. Continued

ID in original report [reference]	Mutation identified in:	Familial WT?	M/F	Age	Inheritance	Mutation	Histology	NR	LOH/IHC, other findings in tumour	FU
1 [16]	Blood & tumour	Familial	F	12	NA	p.Gln701*	L Epithelial type†	NA	CN-LOH	NA
							R Epithelial type†	NA	CN-LOH	NA
2 [16]	Blood & tumour	Familial	F	14	NA	p.Gln701*	L Epithelial type†	NA	CN-LOH	8
							R Epithelial type†	NA	CN-LOH	4
8 [15]	Both kidneys & tumour	Isolated	M	17	NA	Splice, c.586+2T>C	L Nephroblastomatosis†	PLNR	CN-LOH, TRIM28 IHC loss	NA
							R Epithelial type†			
9 [15]	Healthy kidney & tumour	Isolated	F	7	NA	p.Leu59Trpfs*34	Epithelial type†	PLNR	NA	NA
11 [15]	Healthy kidney & tumour	Isolated	F	75	NA	p.Cys174Argfs*4	L Nephroblastomatosis†	PLNR	NA	
							R Epithelial type†			
PAKVET [13]	Healthy kidney & tumour	NA	NA	13	NA	Splice, c.839+1G>A	Monomorphic epithelial	No	CN-LOH	NA
10 [15]	Healthy kidney & tumour	Isolated	F	40	Mosaic§	p.Ala544Profs*132	Epithelial type with diffuse anaplasia†	No	NA	NA
12 [15]	Tumour‖	Isolated	F	8	Somatic	p.Met389Argfs*2	Epithelial type†	NA	NA	NA
PADWNP [13]	Tumour‖	Isolated	NA	18	Somatic	p.Gln233*	Monomorphic epithelial	No	CN-LOH	NA
PAJMKN [13]	Tumour‖	Isolated	NA	17	Somatic	p.Gly107Argfs*75	Monomorphic epithelial	No	CN-LOH	NA
PAJMZF [13]	Tumour‖	Isolated	NA	8	Somatic	p.Arg487*	Monomorphic epithelial	No	No LOH, promoter hypermethylation¶	NA
PADDLL [13]	Tumour‖	Isolated	NA	6	Somatic	p.Phe645Leufs*29	Monomorphic epithelial	No	NA	NA
PAJPER [13]	Tumour‖	Isolated	NA	15	Somatic	Splice, c.839+1G>A and p.Arg487*	Monomorphic epithelial	No	NA	NA
PAKSJN [13]	Tumour‖	Isolated	NA	91	Somatic	p.Arg230*	Monomorphic epithelial	No	NA	NA
PAJNYM [13]	Tumour‖	Isolated	NA	10	Somatic	Splice, c.340+2T>G	Monomorphic epithelial	No	CN-LOH	NA
PAKYLT [6,13]	Tumour‖	Isolated	NA	NA	Somatic	Splice, c.839+1G>A	Anaplastic, epithelial	NA	CN-LOH, *TP53* mutation	NA
W117 [14]	Tumour‖	Isolated	M	7	Somatic	p.Phe645Leufs*30	Monomorphic epithelial	No	No LOH, TRIM28 IHC loss, exon 1 hypermethylation	NA
WESK150 (this report)	Tumour‖	Isolated	M	7	Somatic	p.Thr154Tyrfs*2	Epithelial type†	PLNR	CN-LOH, TRIM28 IHC loss	NA

M, male; F, female; Age, age at Wilms' tumour 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); NA, Not available.

†(Presumably) after preoperative chemotherapy.

‡Assumed that mutation was inherited from mother, who was not tested but had bilateral Wilms' tumour at age 8 years.

§Based on variant allele frequency.

‖Absent in adjacent kidney tissue.

¶Not presumed to be responsible for silencing the wild-type allele.

Variants are described on transcript NM_005762.2 according to the Human Genome Variation Society (HGVS) recommendations. ††Patient deceased. The protein annotation of the original publication has been changed according to HGVS recommendations.

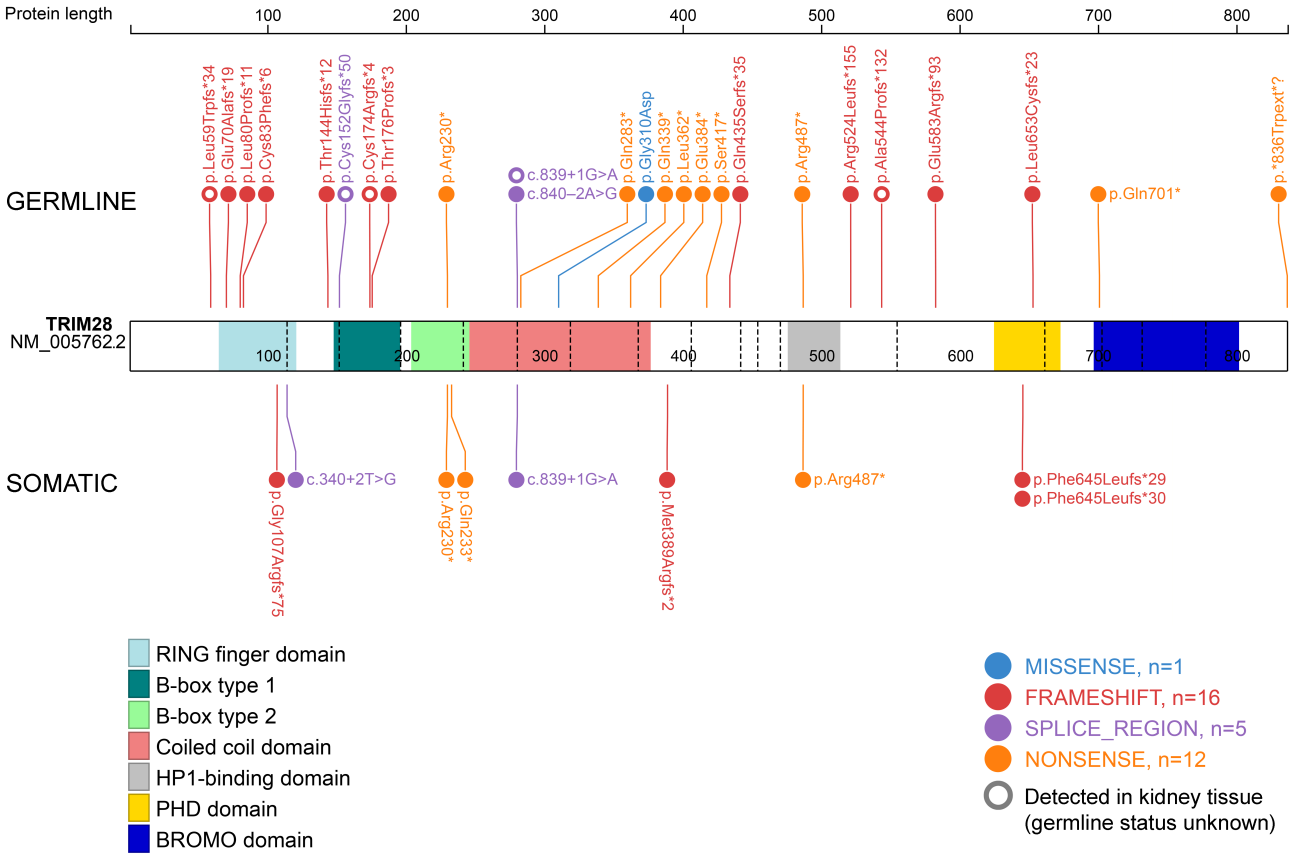

Figure 1. Schematic representation of the TRIM28 protein and reported germline and somatic variants in patients with Wilms' tumour. Variants identified in adjacent normal kidney tissue in non-familial cases (N = 5) are included in this figure as potential germline variants, marked as open circles. Protein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

Biological functions of TRIM28

TRIM28 (also known as KAP1 or TIF1beta) is a multi-domain 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].

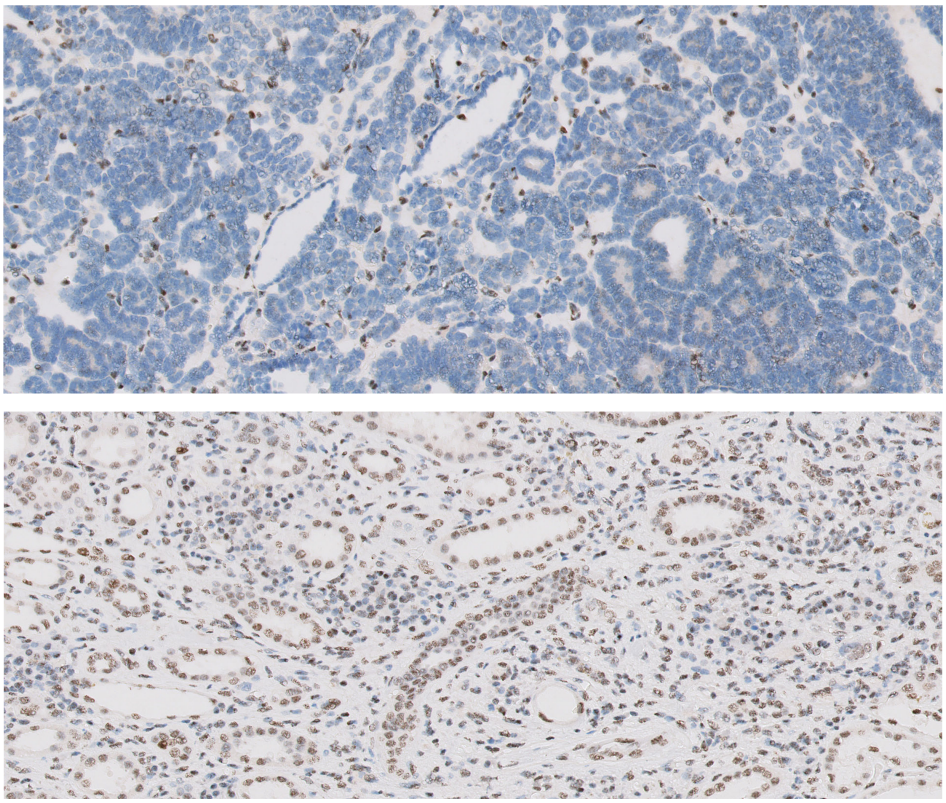

Figure 2. Loss of TRIM28 protein expression in *TRIM28*-mutated Wilms' tumour. Top: immunohistochemical staining with anti-KAP1 antibody (ab10484) in an epithelial Wilms' tumour (WT) of a 7-month-old boy with a somatic *TRIM28* mutation showing absent nuclear staining in tumour cells, with retained expression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

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 post-induction 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)

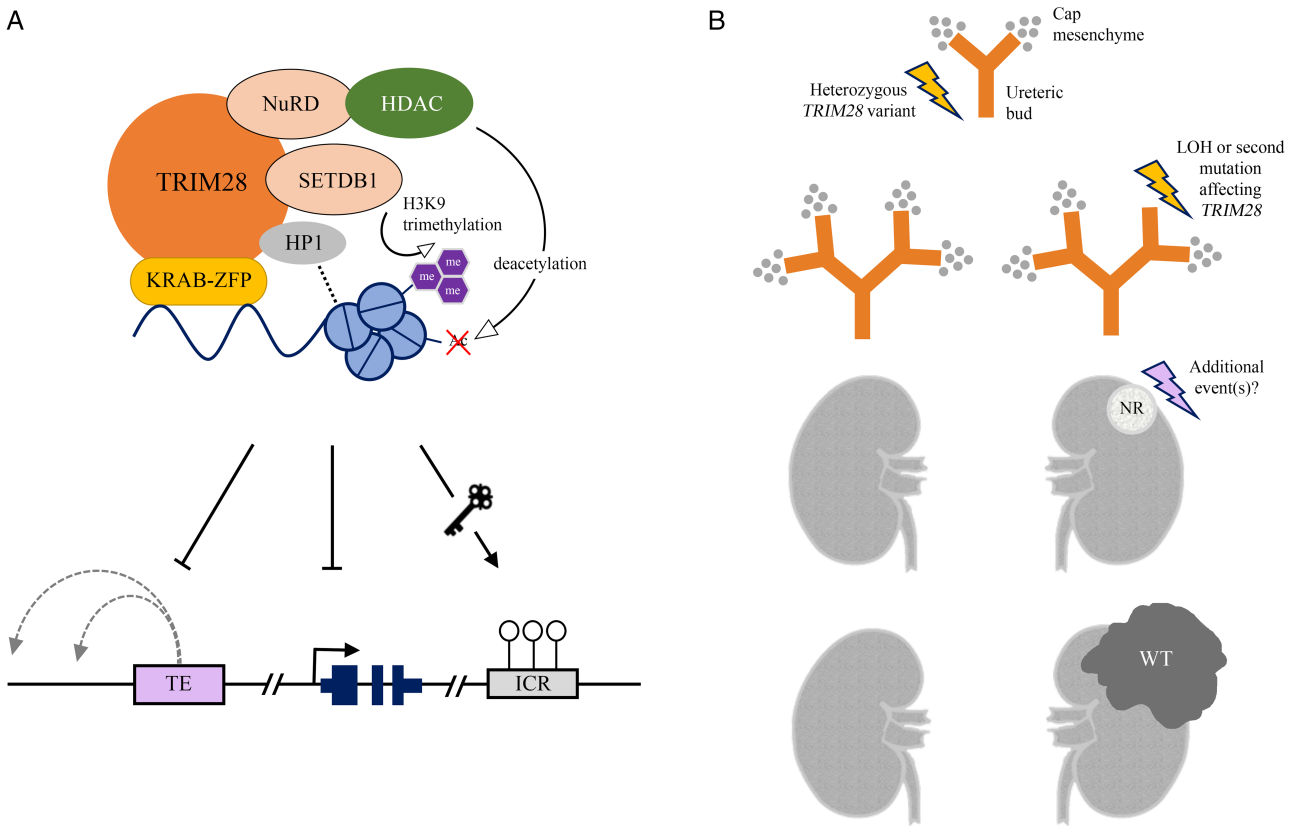

Figure 3. Model for *TRIM28*-mutated Wilms' tumour development. TRIM28 is thought to act as a transcriptional corepressor during the early stages of kidney development, through its interaction with one of the Krüppel-associated box-containing zinc-finger proteins (KRAB-ZFPs). H3K9, histone H3 lysine 9; me, methyl group; Ac, acetyl group; TE, transposable element; ICR, imprinting control region; LOH, loss of heterozygosity; WT, Wilms' tumour. (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.

[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 tumour 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 tumourigenesis.

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 tumour 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 tumours 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 tumours 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

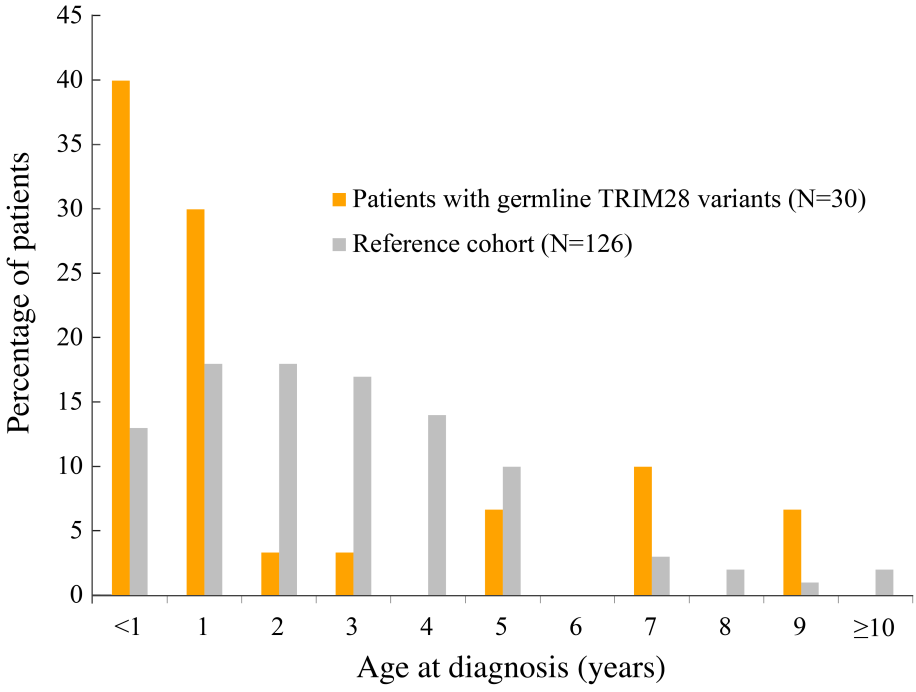

Figure 4. Age at Wilms' tumour diagnosis (in years) of patients with germline *TRIM28* variants (*N* = 30) versus an unselected reference cohort of patients with WT (*N* = 126). The reference cohort includes all patients diagnosed with WT in The Netherlands in a 5-year period.

~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.

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 paediatric 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 tumours 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 tumour.

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 tumour- 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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Author contributions statement

RPK was responsible for conceptualization. JAH wrote the original draft. All the authors wrote, reviewed and edited the final paper.

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