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REVIEW ARTICLE

A review of the genetic background in complicated *WT1*-related disorders

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Abstract

The Wilms tumor 1 (*WT1*) gene was first identified in 1990 as a strong candidate for conferring a predisposition to Wilms tumor. The WT1 protein has four zinc finger structures (DNA binding domain) at the C-terminus, which bind to transcriptional regulatory sequences on DNA, and acts as a transcription factor. WT1 is expressed during kidney development and regulates differentiation, and is also expressed in glomerular epithelial cells after birth to maintain the structure of podocytes. *WT1*-related disorders are a group of conditions associated with an aberrant or absent copy of the *WT1* gene. This group of conditions encompasses a wide phenotypic spectrum that includes Denys–Drash syndrome (DDS), Frasier syndrome (FS), Wilms–aniridia–genitourinary–mental retardation syndrome, and isolated manifestations of nephropathy or Wilms tumor. The genotype–phenotype correlation is becoming clearer: patients with missense variants in DNA binding sites including C2H2 sites manifest DDS and develop early-onset and rapidly developing end-stage kidney disease. A deeper understanding of the genotype–phenotype correlation has also been obtained in DDS, but no such correlation has been observed in FS. The incidence of Wilms tumor is higher in patients with DDS and exon-truncating variants than in those with non-truncating variants. Here, we briefly describe the genetic background of this highly complicated *WT1*-related disorders.

Keywords WT1 · DNA binding · Denys-Drash syndrome · C2H2 · Frasier syndrome

Introduction

The *WT1* gene was isolated in 1990 as the causative gene of Wilms tumor and is located on chromosome 11p13 [1, 2]. The gene spans approximately 50 kb and contains 10 coding exons: exons 1–6 encode an N-terminus Gln/Pro-rich domain and exons 7–10 encode four consecutive (Cys)2-(His)2 zinc finger C-terminus domains (C2H2 site) (Fig. 1) [3]. This gene encodes a transcription factor that plays an important role in cell growth and differentiation, and its expression is restricted to a limited number of tissues, including gonads and kidney, as well as progenitor cells of various tissue types [4–7]. The protein is expressed as several isoforms. A major alternative splice donor site at the end of intron 9 results in the incorporation of three additional amino acids, lysine, threonine, and serine (KTS), between the third and fourth zinc fingers (Fig. 2a) [8]. The WT1 (–KTS) protein is believed to act primarily as a transcription factor, whereas the WT1 (+KTS) protein is involved in post-transcriptional processes [9–12]. *WT1* variants in the germline are known to cause Wilms tumor as well as renal glomerulosclerosis and gonadal dysplasia.

Nephrotic syndrome (NS) is the most common glomerular disease in children and adults, characterized by massive proteinuria, hypoalbuminemia, and edema. Based on the response to glucocorticoid therapy, NS is classified into steroid-sensitive nephrotic syndrome or steroid-resistant nephrotic syndrome (SRNS). Focal segmental glomerulosclerosis (FSGS) is the most common histopathological finding in SRNS [13]. To date, more than 50 genes have been shown to cause monogenic SRNS and/or FSGS [14, 15]. Although detection rates vary by region and criteria, comprehensive genetic testing has identified podocyte-related gene variants in approximately 20%–30% of SRNS patients [14–18]. It has also been shown that there are differences in the distribution and frequency of variants by ethnic background and region. According to several studies conducted in Western countries, the three most common causative

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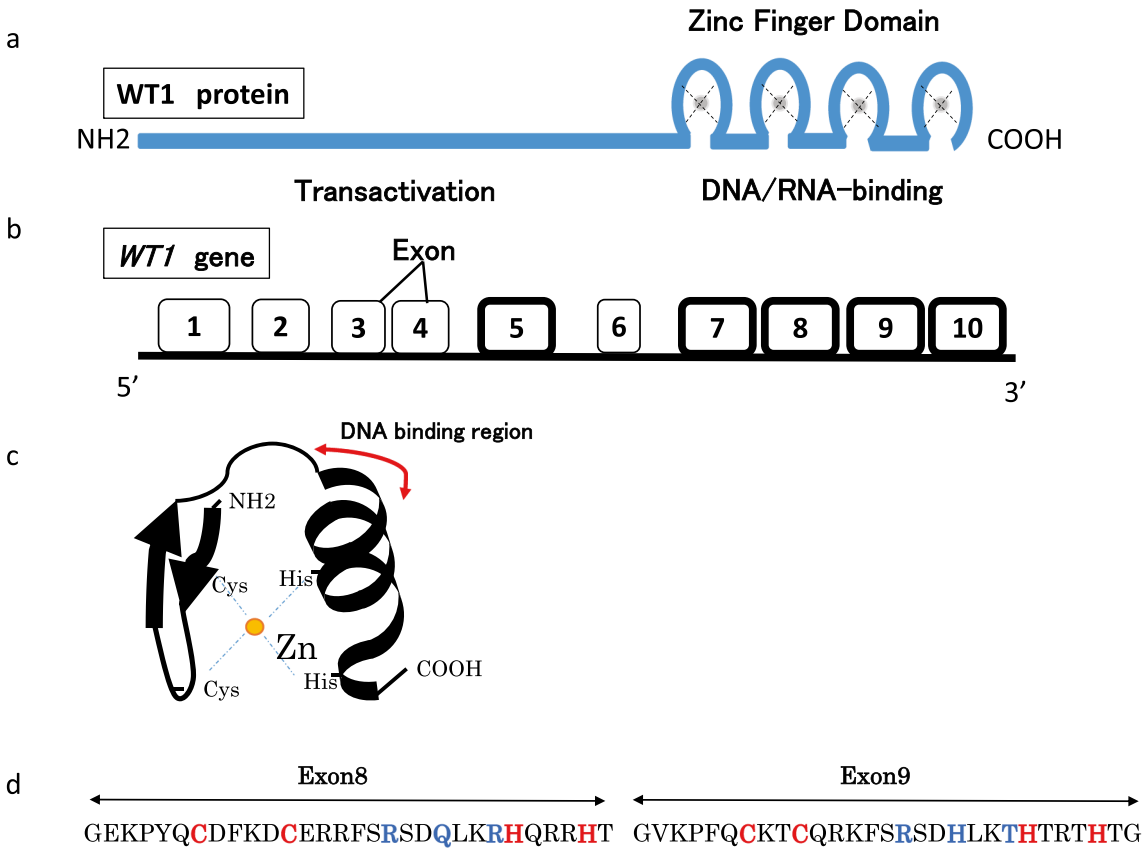

Fig. 1 Schematic representations of the *WT1* gene and protein. (**a**) Known functional domains of the WT1 protein include DNA/RNA binding (zinc finger) domain. (**b**) *WT1* comprises 10 exons. The inclusion of exon 5 leads to the insertion of 17 amino acid residues into the regulatory domain of *WT1*. (**c**) C2H2-type zinc fingers contain a short beta hairpin and an alpha helix, where a single zinc atom is held in place by C2H2 residues in a tetrahedral array. (**d**) The sequence-recognition amino acids at the protein–DNA interface in exons 8 and 9 are shown in blue. The Cys2–His2 structural amino acids that coordinate the zinc ions and hydrophobic core are shown in red

genes are *NPHS1*, *NPHS2*, and *WT1* [14, 16, 17]. In Asian countries, *WT1* is the most frequently detected causative gene [19–21]. Despite the high rate at which *WT1* is the causative gene in diseases that result in proteinuria, there are no definitive and detailed findings on the clinical manifestations of cases that do not meet the criteria for established syndromes such as Denys–Drash syndrome (DDS) and Frasier syndrome (FS). Actually, DDS has been asserted to be caused by missense variants in *WT1* only in exon 8–9 DNA binding sites and C2H2 sites [22]; however, many nephrologists still believe that many more variants including truncating variants in exons 8–9 can cause DDS.

Today, genetic testing is widely available and allows the risk stratification, management, and follow-up of patients with proteinuria. In order to treat patients with *WT1* variants, it is necessary to understand the possible symptoms depending on the location of the variant. In this review, we present the function and structure of the *WT1* gene, followed by a long-established and well-known syndrome to organize our knowledge and the association between its variants and clinical manifestations. And finally, we describe our recent findings on the correlation between genotype and phenotype, particularly in renal manifestations.

Renal development

The kidneys develop from the intermediate mesoderm and are formed in three stages: anterior, middle, and posterior kidneys. Most of the anterior and middle kidneys later degenerate, and the kidneys that function in adult mammals are the posterior kidneys. In mammals, the kidney, or postrenal gland, arises from the most caudal part of the mesonephric duct (Wolff's duct), in a process in which the ureteric bud emerges and mesenchyme assembles around it. The ureteric bud invades the metanephric mesenchyme and the metanephric mesenchyme cells condense around the ureteric bud tip to form the cap mesenchyme. The cap mesenchyme contains the nephrogenic progenitor cells that give rise to

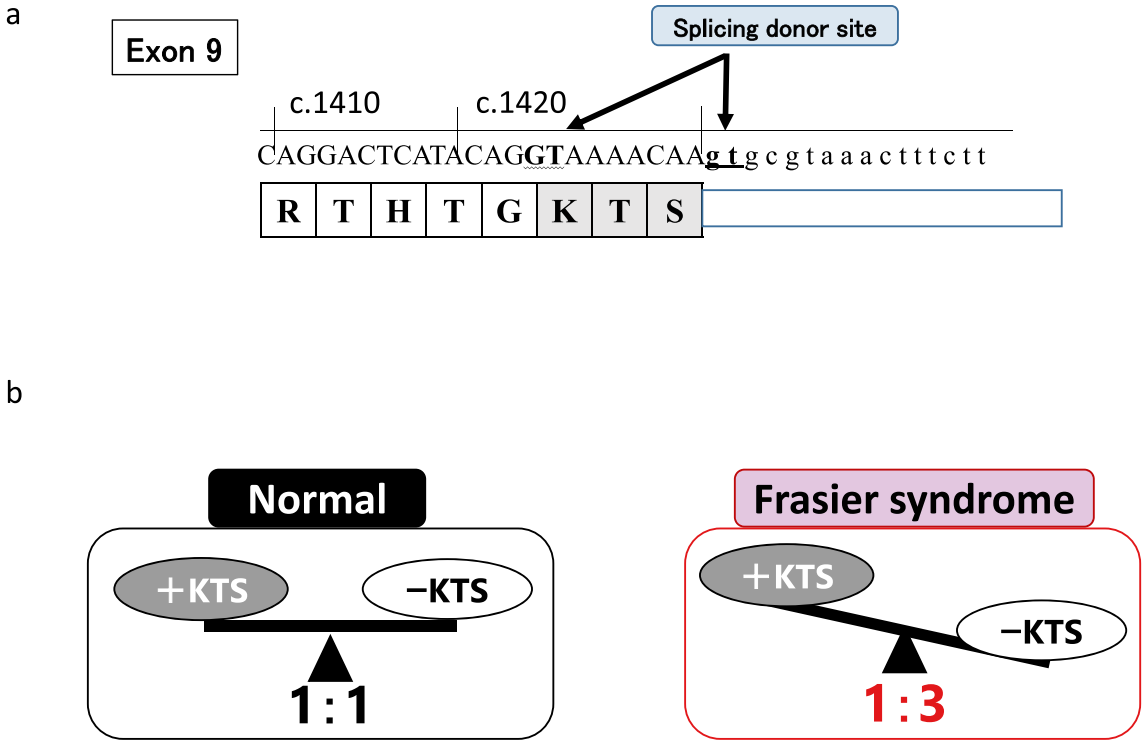

Fig. 2 (**a**) Alternative splicing at the end of exon 9 produces the tripeptide KTS, which is inserted between zinc fingers 3 and 4. (**b**) Schematic representation of the balance of + KTS and − KTS isoforms in normal cases and its imbalance in Frasier syndrome

all epithelial cells of the nephron. The interaction of the ureteric bud with the posterior renal mesenchyme results in a posterior kidney with millions of nephrons. Mesenchymal cells aggregate around the ureteric bud, which epithelializes into an S-shaped body, giving rise to the glomerulus and the proximal and distal tubules. The ureteric bud undergoes a series of branching steps to become the collecting duct and ureter [23].

The earliest expression of Wt1 in the kidney was identified in the intermediate mesoderm and subsequently in the metanephric and cap mesenchyme [24, 25]. In the metanephric mesenchyme, Wt1 is required for the production of ureteric bud branching signals and for the nephrogenic progenitor cell response to ureteric bud-derived nephrogenic signals, demonstrating that this gene is crucial for the cell interactions during kidney formation [26, 27]. In later stages, Wt1 is involved in the control of mesenchymal–epithelial transition and has an essential role in the development and maintenance of podocytes [28–30].

Structure

The Wilms tumor 1 (*WT1*) gene is located on chromosome 11p13 and encodes a transcriptional DNA binding protein. *WT1* contains 10 exons, which encode a proline/glutamine-rich transcriptional regulation region (exons 1–6) and four C2H2-zinc fingers (ZFs) (exons 7–10). The C2H2-zinc finger motif is employed by a diverse array of transcription factors that play important roles in cellular signal transduction [31] (Fig. 1).

Transcription factor plays an important role in cellular development and cell survival [4]. WT1 recognizes and binds to the DNA sequence 5′-GCG(T/G)GGGCG-3′ [31, 32] and regulates the expression of numerous target genes. WT1 also binds to the promoters and enhancers of several podocyte-specific genes [33–35].

The variants associated with DDS are predominantly missense variants in the first three ZFs, most often clustered in ZF2 and ZF3, that alter either the C2H2 structural amino acids that coordinate the zinc ions or the sequence-recognition amino acids at the protein–DNA interface (Fig. 1c, d). Because ZF2 and ZF3 correspond to exons 8 and 9, respectively, variants at these positions alter transcription factor activity and cause clinical symptoms.

There are at least 36 potential mammalian WT1 isoforms. This diversity is created through a combination of alternative transcription start sites, translation start sites, splicing, and RNA editing. Alternative splice sites at the end of exon 9 lead to the insertion of three amino acids (KTS) after the glycine between zinc fingers 3 and 4 [36]. This alternative splice site is highly conserved during evolution and is found in all vertebrates. The relative abundance of these

splice forms is constant; developmental abnormalities are associated with altered ratios of WT1 (+KTS) and WT1 (−KTS) isoforms with the standard ratio being almost 1:1 [37, 38] (Fig. 2). The +KTS and −KTS isoforms perform distinct biological functions and differ in their nucleic acid binding properties. The WT1 (−KTS) isoform binds DNA sequence-specifically and appears to function primarily in transcriptional regulation; more than 30 putative target genes of it have been identified. The WT1 (+KTS) splice variant binds mRNA and plays a role in mRNA metabolism or splicing [39]. The unbalanced production of these isoforms causes FS.

Clinical features and genetic background in *WT1*-related syndromes

WT1 is essential for normal urogenital development, and pathogenic variants in the *WT1* gene have been shown to be associated with syndromes such as DDS, FS, and Wilms–aniridia–genitourinary–mental retardation (WAGR) syndrome.

DDS (OMIM#194,080) is a disorder known to involve Wilms tumor, genital anomalies, and nephropathy. Its mode of inheritance is autosomal dominant. Familial cases have been reported, albeit rarely [40]. This syndrome was first described by Denys et al. in 1967 [41]. In 1970, Drash et al. reported two unrelated children with a syndrome comprising pseudohermaphroditism, Wilms tumor, hypertension, and degenerative renal disease [42]. In 10 independent cases of DDS, missense variants in the C2H2-zinc finger domains of one *WT1* gene allele were found in 1991 [43]. Nine of these variants were found within exon 9 (C2H2-zinc finger 3), while the tenth was in exon 8 (C2H2-zinc finger 2). These variants directly affect DNA sequence recognition, that is, DNA binding ability. A small number of cases of DDS associated with variants other than those in exons 8 and 9 have been reported [44, 45]. Lipska et al. presented clinical information on 24 patients with missense variants in DNA binding sites. The median age of onset was 0.9 and the median age of developing ESKD was 2.5 years. Other clinical manifestations included pathological findings of diffuse mesangial sclerosis (DMS) in 74% (17/23), Wilms tumor in 54% (13/24), genital abnormalities in 43% (9/21), and urinary tract malformation in 4% (1/24) [46]. Heterozygous *WT1* missense variants (mainly zinc finger domains) in DDS lead to a severe phenotype compared with deletions in WAGR syndrome [25]. The mutant WT1 protein dimerizes with the wild-type protein and acts in a dominant-negative manner, which may explain the more severe phenotype compared with haploinsufficiency [47].

FS (OMIM#136,680) is a disorder known to involve genital anomalies and progressive glomerulopathy. FS results from variants that reduce the +KTS to −KTS ratio by disrupting the splice donor of the +KTS isoform (Fig. 2b). Developing Wilms tumor in FS is less common than DDS. Its mode of inheritance is autosomal dominant and familial cases of FS have been reported [48]. In 1987, Moorthy et al. suggested that some of the patients reported to have DDS in fact had a different disorder, for which they suggested the designation "FS" [49]. The first confirmed case of FS was reported by Frasier et al. in 1964 [50]. In 1997, Barbaux et al. identified variants in the donor splice site of intron 9 of the *WT1* gene with a predicted loss of the so-called +KTS isoform in three patients with FS [51]. Subsequently, Lipska et al. revealed clinical information on 19 patients with intron 9 variants. In this group, unlike in patients with DNA binding site variants, the pathological findings were focal segmental glomerulosclerosis in 88% (15/17) and DMS in 6% (1/17) [46].

WAGR syndrome (OMIM#194,072) is a disorder known to involve Wilms tumor, aniridia, genital anomalies, and impaired intellectual development syndrome. In 1964, Miller et al. first described the association of aniridia, hemihypertrophy, and other congenital anomalies with Wilms tumor [52]. In 1988, Puissant et al. reported a patient with WAGR and a de novo reciprocal translocation, 46,XY,t(5;11)(q11;p13) [53]. WAGR syndrome is a contiguous gene deletion syndrome involving an interstitial de novo 11p13 microdeletion of variable size, which explains the variability of clinical signs [54]. The affected genes include *PAX6* and *WT1*. *BDNF* deletion can be included among the causative variants and is associated with the WAGRO phenotype (OMIM# 612,469). WAGRO is a specific phenotype of WAGR, with the additional feature of obesity, and is associated with haploinsufficiency of the *BDNF* gene.

Clinical features and genetic background in *WT1*-related disorders

CAKUT (congenital anomalies of the kidney and urinary tract) phenotype

WT1 is required for ureter induction, formation of the nephron, and differentiation and maintenance of the glomerulus. Mice carrying *Wt1* variants lack kidney, exhibit renal dysplasia, or develop renal failure [55]. It was reported that 11% (7/61) of patients with a *WT1* variant had CAKUT, including duplex kidney, horseshoe kidney, malrotation, vesicoureteral reflex, and pelviureteric junction stenosis [46]. Three of the patients had intron 9 variants, two had truncation variants, and the rest had one DNA binding site variant and one other missense variant each.

Wilms tumor

Wilms tumor is the most common renal malignancy in pediatric populations. Approximately 9%–17% of all Wilms tumors are associated with a predisposing syndrome [56], the most common of which are WAGR, DDS, Beckwith–Wiedemann syndrome, isolated hemihypertrophy, and Perlman syndrome [57]. The risk of developing malignancy varies by syndrome. WAGR and DDS are classified as at high risk (> 20%) of developing Wilms tumor, while Frasier syndrome is classified as at moderate risk (5%–20%).

According to a review of 150 cases published in 1994, patients with DDS have a likelihood of developing Wilms tumor as high as 95%, with median age at occurrence of 12 months [58]. Among children with DDS and Wilms tumor, 20% have bilateral masses [58, 59].

Lipska et al. reported that Wilms tumors developed in 23 out of 61 patients with *WT1*-related steroid-resistant nephrotic syndrome. Of them, 22 were carriers of exonic *WT1* variants who were cumulatively followed up for 199 years (that is, one case per 9 years at risk). Overall, 13 of 24 (54%) with missense variants, 7 of 9 (78%) with truncating variants, 2 of 7 (29%) with other missense variants, and 1 of 19 (5%) with intron 9 variants developed Wilms tumor.

Lehnhardt et al. reported that only patients with exon variants developed Wilms tumor [60]. A total of 12 of 36 (33%) with missense variants and 7 of 8 (88%) with truncating variants developed Wilms tumor [60].

We reported that 36% (47/129 cases) of patients with exon 8–9 *WT1* variants had Wilms tumor. This included 24 of 69 cases (35%) in the DNA binding site (DBS) group, 17 of 32 (53%) in the C2H2 group, and 6 of 28 (21%) in the Others group. Our group also reviewed 126 cases with *WT1* intron 9 variants, in which the prevalence of Wilms tumor was 3% (1/30 cases) [38]. Overall, patients with truncating variants developed Wilms tumor at a high frequency, and patients with missense variants at any position, albeit quite rarely for those with missense variants at locations other than exon 8 or 9, were at risk of developing Wilms tumor.

Gonadal development

WT1 is one of the key genes involved in the development of the gonads and adrenal glands [61]. According to Köhler et al. [62], patients with 46,XY disorders of sex development (DSD) with *WT1* variants identified by histological analysis of gonadal tissue showed a large spectrum of development, ranging from normal testes to varying degrees of gonadal dysgenesis, but patients with *WT1* pathogenic variants lacked a clear genotype–phenotype correlation.

Among patients with truncating pathogenic variants, which are nonsense, frameshift, and splice site variants other than intron 9 variants, the majority of 46,XY patients often have genital dysgenesis (46,XY DSD) and fewer have normal female external and internal genitalia (46,XY complete gonadal dysgenesis) [60]. Among patients with missense variants in exon 8 or 9 with or without DBS, the majority of 46,XY patients have genital anomalies/atypia (46,XY DSD), and 46,XY complete gonadal dysgenesis is rare [63]. Among patients with intron 9 variants, the diagnosis of 46,XY complete gonadal dysgenesis is observed in the majority of 46,XY patients, but partial forms also occur [60, 64].

Gonadoblastoma

A gonadoblastoma is a complex neoplasm composed of a mixture of gonadal elements, such as large primordial germ cells, immature Sertoli cells or granulosa cells of the sex cord, and gonadal stromal cells [65]. Classical gonadoblastoma occurs almost entirely in the dysgenetic gonads of individuals with disorders of sex development; however, a small number of cases arise in individuals with a normal peripheral karyotype and no evidence of a disorder of sex development [66]. Even in normal external male genitalia, testicular hypoplasia or dysplasia is possible and may lead to gonadoblastoma. Therefore, periodic examination and testing are necessary.

Gonadoblastoma has been identified in both FS and DDS, but the risk of it in FS is much higher than that in DDS [67]. However, the risk of gonadoblastoma should not be overlooked even in patients with DDS.

Deeper insights into genotype–phenotype correlations

Missense variants in exon 8 or 9

Based on crystallographic analysis [31], there are two types of disease-causing variants in exon8 or 9, which either destabilize the zinc finger structure or replace important base contact residues. We focused on the structure and classified the variants into three categories: DNA binding sites, C2H2 sites, and other sites. Genotype–phenotype correlations were evaluated in a systematic review of 174 cases with *WT1* exon 8 to 9 variants [22]. There were 95 DNA binding site variants, 38 C2H2 site variants, and 41 other site variants. The median age of developing ESKD was 0.90 in the DNA binding site group, 2.00 in the C2H2 site group, and 3.92 years in the other site group. We concluded that not only DNA binding sites but also C2H2 zinc finger structure sites are important for maintaining *WT1* transcriptional activity, and their mutation causes severe clinical symptoms (Table 1).

Recently, severe cases of fetal onset and early neonatal death due to *WT1* variants have been reported [68]. These patients have a heterozygous missense variant in *WT1* [NM_024426.6:exon9:c.1400G > A, p.Arg467Gln]. Five

Table 1 Genotype–phenotype association for *WT1*-related disorders

Type of variant		Exonic					Intronic	Gene
		missense variant				truncating variant	intron 9 variant	whole deletion
		exon8 or 9			Other than exon8 and 9			
		DNA binding site	C2H2 site	other				
nephropathy	time of onset	newborn-infancy	infancy	infancy	variable	(if it occurs) middle childhood	early childhood	(if it occurs) middle childhood
	ESKD	infancy	infancy-early childhood	early childhood	variable	(if it occurs) adolescence	adolescence	(if it occurs) adolescence
Wilms tumor		moderate			rare	high	rare	high
Genital abnormalities		moderate				moderate	moderate	high
Gonadoblastoma		rare				unknown	high	high

cases with the same missense variant were reported. The median age of onset was 0.08 years and the median age of ESKD was 0.2 years, which is extremely severe even for DDS in this genotype. The most common variant in DDS involves the same amino acid but with it changing to tryptophan (p.Arg467Trp). Different amino acid changes in the same DNA binding site may affect transcriptional activity.

Intron 9 variants

Genotype–phenotype correlations were evaluated in a systematic review of 126 cases with *WT1* intron 9 variants [38]. Patients included 3 with +1G > A, 2 with +2 T > C, 1 with 3 + G > T, 66 with +4C > T, 51 with +5G > A, 2 with +5G > T, and 1 with +6 T > A. The median age of onset of proteinuria was 4 years. Furthermore, the median age of developing ESKD was 16 years. There were no significant differences in the renal survival period among the genotypes.

Missense variants other than in exon 8 or 9

Because missense variants in *WT1* at locations other than exon 8 or 9 are so rare, no comprehensive reports on such cases have been published and the details remain unclear. At the time of writing, 28 pathogenic variants at locations other than exon 8 or 9 that are associated with nephropathy have been reported in the Human Gene Mutation Database (HGMD) Professional v2024.1 (https://portal.biobase-international.com/hgmd/pro/start.php). Their locations are as follows: 9 variants in exon 1, 3 variants in exon 2, 1 variant in exon 4, 2 variants in exon 6, 12 variants in exon 7, and 1 variant in exon 10. In many cases, the details of clinical symptoms are unknown, but the severity is known to vary.

Truncating variants

A genotype–phenotype association study in *WT1*-related disorders with a large number of cases was reported in 2014 [46]. In that study, 61 patients with *WT1*-related steroid-resistant nephrotic syndrome in the PodoNet cohort were evaluated. Seven truncating variants were identified. The median age of onset of nephropathy was 12.3 years and the median age of developing ESKD was 16.5 years.

Meanwhile, in 2015, a retrospective genotypic, phenotypic, and therapeutic analysis of 53 patients with *WT1* variants from Germany, Austria, and Switzerland was performed [60]. A total of 8 of 53 (15%) patients had a truncating variant. The median age of onset of nephropathy was 9.7 years, 73% of patients required RRT, and the median age of developing ESKD was 16.5 years.

In both cohorts, cases presenting with nephropathy were collected. In fact, 31 truncating variants have been reported in HGMD v2024.1 to date, 22 of which were associated with clinical manifestations of Wilms tumor only. The presence of a truncating variant is associated with a higher rate of Wilms tumor than nephropathy. Since all variants are located before the DNA binding site of exon 9, the renal symptoms may be milder because dominant-negative effects on DNA binding may be rare.

Future challenges

Patients with renal symptoms who have missense variants in exons 8 or 9 and patients with Wilms tumor who have truncating variants are relatively well reported. However, there are still few reports of patients with variants in other positions, and a clear genotype–phenotype correlations have not been established. Since genetic testing is now easily available, it is expected that more and more patients will be found

with only one clinical symptom. It is necessary to follow such patients over a long period and carefully monitor their progress to accumulate data on the types of clinical symptoms they present. Future work will consider the functional analysis of these data to elucidate the mechanisms by which pathological variants in the *WT1* gene cause their respective symptoms. We believe that such research will eventually lead to gene therapy.

Conclusion

As genetic testing proliferates, an increasing number of patients with *WT1* variants are being identified. It has become clear that discrepancies in symptom manifestation in kidney disorders arise based on the variant's location. Beyond renal complications, urogenital anomalies and tumorigenesis can occur, underscoring the importance of a wider awareness of such cases as *WT1*-related disorders beyond the field of nephrology. Against this background, recent progress has deepened our understanding of genotype–phenotype correlations in *WT1*-related disorders.

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Authors contribution C.N. drafted and K.N. critically revised the work.

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Declarations

Conflict of interest The authors have declared that there are no conflicts of interest.

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