

RESEARCH

Exonic *WT1* pathogenic variants in 46,XY DSD associated with gonadoblastoma

Sneha Arya^{1,*}, Sandeep Kumar^{1,*}, Anurag R Lila¹, Vijaya Sarathi², Saba Samad Memon¹, Rohit Barnabas¹, Hemangini Thakkar³, Virendra A Patil¹, Nalini S Shah¹ and Tushar R Bandgar¹

¹Department of Endocrinology, Seth G S Medical College & KEM Hospital, Mumbai, India

²Department of Endocrinology, Vydehi Institute of Medical Sciences and Research Centre, Bangalore, India

³Department of Radiology, Seth G S Medical College & KEM Hospital, Mumbai, India

Correspondence should be addressed to A R Lila: anuraglila@gmail.com

*(S Arya and S Kumar contributed equally to this work)

Abstract

Objective: The literature regarding gonadoblastoma risk in exonic Wilms' tumor suppressor gene (*WT1*) pathogenic variants is sparse. The aim of this study is to describe the phenotypic and genotypic characteristics of Asian-Indian patients with *WT1* pathogenic variants and systematically review the literature on association of exonic *WT1* pathogenic variants and gonadoblastoma.

Design: Combined retrospective-prospective analysis.

Methods: In this study, 46,XY DSD patients with *WT1* pathogenic variants detected by clinical exome sequencing from a cohort of 150 index patients and their affected relatives were included. The PubMed database was searched for the literature on gonadoblastoma with exonic *WT1* pathogenic variants.

Results: The prevalence of *WT1* pathogenic variants among 46,XY DSD index patients was 2.7% (4/150). All the four patients had atypical genitalia and cryptorchidism. None of them had Wilms' tumor till the last follow-up, whereas one patient had late-onset nephropathy. 11p13 deletion was present in one patient with aniridia. The family with p.Arg458Gln pathogenic variant had varied phenotypic spectrum of Frasier syndrome; two siblings had gonadoblastoma, one of them had growing teratoma syndrome (first to report with *WT1*). On literature review, of >100 exonic point pathogenic variants, only eight variants (p.Arg462Trp, p.Tyr177*, p.Arg434His, p.Met410Arg, p.Gln142*, p.Glu437Lys, p.Arg458*, and p.Arg458Gln) in *WT1* were associated with gonadoblastoma in a total of 15 cases (including our two cases).

Conclusions: *WT1* alterations account for 3% of 46,XY DSD patients in our cohort. 46,XY DSD patients harboring exonic *WT1* pathogenic variants carry a small but definitive risk of gonadoblastoma; hence, these patients require a gonadoblastoma surveillance with a more stringent surveillance in those harboring a gonadoblastoma-associated variant.

Key Words

- ▶ 46
- ▶ XY DSD
- ▶ *WT1* gene
- ▶ gonadoblastoma
- ▶ Frasier syndrome

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Introduction

Wilms' tumor suppressor gene (*WT1*), located on chromosome 11p13, encodes a zinc-finger protein. It contains ten exons and spans about 50 kb (1). Exons 1–6 encode a proline/glutamine-rich transcriptional-regulation region, and exons 7–10 encode the four zinc

fingers of the DNA-binding domain (2). Pathogenic alterations of this transcription factor cause various disorders of urogenital development. There is a broad phenotypic spectrum with *WT1* pathogenic variants. Microdeletions (including the *PAX6* gene) lead to

WAGR syndrome (OMIM 194072), a complex phenotype associated with Wilms' tumor, aniridia, genitourinary anomalies and mental retardation (3). Exonic variants are responsible for Denys-Drash syndrome (DDS, OMIM 194080), comprising XY gonadal dysgenesis, predisposition to nephropathy, and Wilms' tumors (4). Five percent of patients with sporadic Wilms' tumors have *WT1* pathogenic variants, whereas >90% of DDS patients harbor exonic *WT1* pathogenic variants (5). Donor splice-site mutations (downshifting of the ratio of +KTS (lysine, threonine, and serine) to -KTS isoforms) of intron 9 are responsible for Frasier syndrome (FS, OMIM 136680), comprising of XY gonadal dysgenesis and predisposition to gonadoblastoma and nephropathy (6). Wilm's tumor is not typically seen in patients with FS. Very few exonic pathogenic variants have been described with FS to date (7). While the risk of Wilm's tumor is well described, the literature regarding gonadoblastoma risk in exonic *WT1* pathogenic variants is sparse (8). In this study, we describe the phenotypic and genotypic spectrum of *WT1* pathogenic variants from a large cohort of Asian-Indian 46,XY DSD. Additionally, a systematic literature review was done to describe characteristics of patients having gonadoblastoma associated with exonic *WT1* pathogenic variants.

Materials and methods

This study is a part of the research project titled 'genotype-phenotype correlation in Indian patients with 46,XY DSD' approved by the Institutional Ethics Committee II of Seth GS Medical College (EC/OA-47/2014), Mumbai. Consecutive index patients with 46,XY DSD having atypical or female external genitalia registered between January 2005 and August 2020 were included in the study. Patients presenting with isolated hypospadias (distal and mid) or isolated cryptorchidism (unilateral/bilateral) with typically male genitalia were excluded. Genetic analysis was done after obtaining the written informed consent. Wherever available, family members of the index cases with pathogenic variations were offered genetic analysis for the observed pathogenic variant.

Demographic characteristics (age at presentation and evaluation, family history of DSD, and sex assignment), clinical findings (external genitalia development grading by Sinnecker score), hormonal profile, ultrasonogram of abdomen and pelvis, and histopathology of gonads were recorded (9).

Genomic DNA was isolated from peripheral blood leukocytes by standard techniques. Molecular screening of the *WT1* gene was performed by next-generation sequencing. Targeted gene capture was done using a custom capture kit. The libraries were sequenced to mean > 80–100X coverage on the Illumina sequencing platform. The coverage for the *WT1* gene was 100X. We followed the GATK best practices framework for the identification of variants in the sample using Sentieon (v201808.01). All the variants were confirmed by Sanger sequencing. The observed variants were classified as per American College of Medical Genetics and Genomics guidelines. Sequence variants were designated using the reference sequences of *WT1* isoform D, NM_024426.4 (cDNA), and NP_077744.3 (protein). Splicing effects of exonic variants associated with gonadoblastoma were tested using *in silico* splicing prediction tools, Netgene2 and NNsplice, and were compared with wild type.

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone (T) levels were measured by chemiluminescence immunoassay (ADVIA Centaur XP Immunoassay Systems, Siemens). Human chorionic gonadotropin (hCG)-stimulated T values were evaluated after three daily doses (1500 IU) of i.m. hCG. Anti-Mullerian hormone (AMH) levels were measured by ELISA. Intra-assay and inter-assay coefficients of variation were less than 10% for all the hormonal assays.

Systematic review of literature

The PubMed database (English language) was searched in September 2021 using the keywords '*WT1* mutation AND gonadoblastoma' OR 'Denys-Drash syndrome' OR 'Frasier syndrome'. A total of 413 publications were screened. Cross-references of selected publications and review articles were used to find additional articles. After exclusion for various reasons (as detailed in Fig. 1), 12 articles (13 patients) had histologically proven gonadoblastoma associated with exonic pathogenic variants in *WT1* and were thus included. Patients' data were tabulated to include details of phenotype, genotype, and characteristics of associated tumors and/or nephropathy.

Statistical analysis

The categorical data were expressed as actual numbers and percentages. Quantitative data were expressed as range.

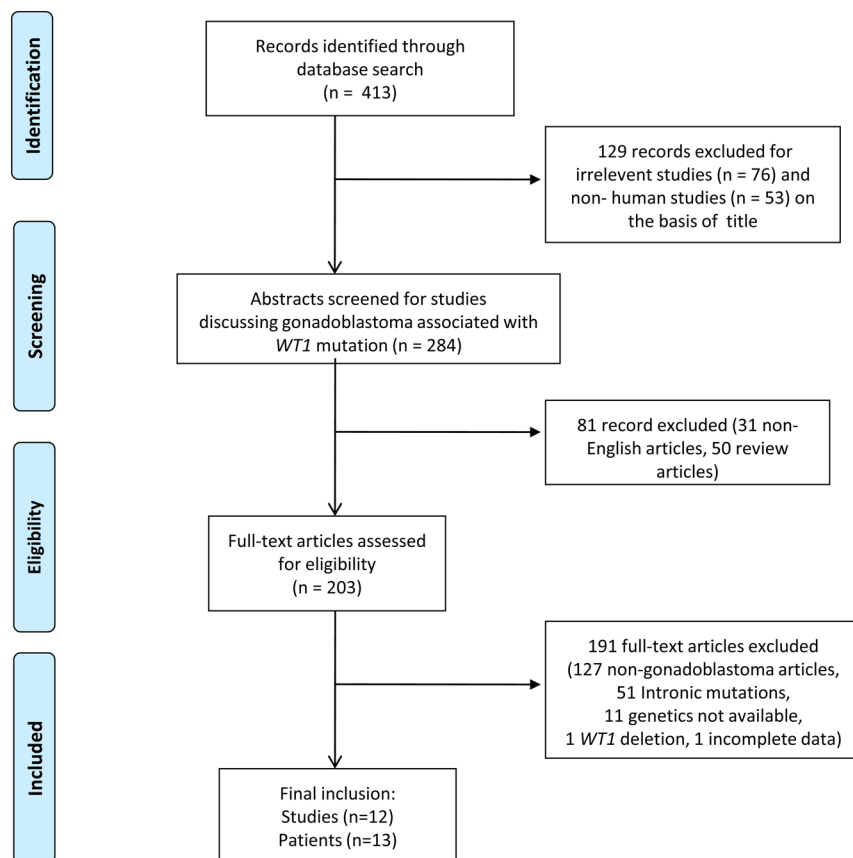


Figure 1
PRISMA flow chart for literature search of gonadoblastoma associated with *WT1* exonic pathogenic variants.

Results

Four (2.7%) of the 150 index patients of 46,XY DSD had pathogenic variants in *WT1*. Cryptorchidism (unilateral/bilateral) was noted in 82 of the 150 index patients, and all four (4/82, 4.9%) patients with *WT1* variants belonged to this group. An affected family member (P4b) with 46,XY DSD was also included for the phenotypic analysis.

The clinical findings, hormonal profile, and genetic data of patients from our cohort with pathogenic variants in *WT1* are summarized in Table 1. Three patients (P1–P3) presented at prepubertal age (0.5–7 years) with atypical genitalia (Sinnecker score 2–3) and cryptorchidism were reared as males. P1 had an additional finding of aniridia. None of them had proteinuria or Wilms’ tumor till the last follow-up. Ultrasonogram did not show gonadoblastoma, but gonadal histology was not available for these patients. Diagnostic laparoscopy for the non-localized gonads, orchidopexy with biopsy of the localized gonads, and male genitoplasty, as appropriate, have been planned in these three patients. The patient with aniridia (P1) had contiguous 11p13 deletion comprising of *WT1* and *PAX6* genes. P2 had a *de novo* pathogenic variant,

p.Arg462Trp. P3 was found to have a maternally inherited pathogenic variant, p.Arg458Gln.

P4a was a familial case with an affected sibling. He presented at 26 years with complaints of gynecomastia and atypical genitalia (Sinnecker score 3). At initial evaluation, he was hypertensive (170/100 mmHg) and had microalbuminuria (urine dipstick for albumin: trace). On follow-up at 28 years of age, his spot urine albumin/creatinine ratio: 2360 µg/mg (normal < 30 µg/mg) was suggestive of macroalbuminuria. His serum creatinine: 1.0 mg/dL (eGFR: 92.6 mL/min/1.73 m²) was normal. He underwent bilateral mastectomy and male genitoplasty with the laparoscopic bilateral gonadectomy and removal of Mullerian structures in view of non-functioning gonads and risk of malignancy. Histopathology of the left gonad was suggestive of gonadoblastoma, whereas the right gonad was dysgenetic. Patient was started on testosterone replacement therapy. This patient had an FS phenotype (46,XY gonadal dysgenesis, late-onset nephropathy, and gonadoblastoma). His younger sister (P4b) was 46,XY DSD, presented with primary amenorrhea at 17 years of age and had female external genitalia with normal breast development on examination. At presentation, her gonads were non-localizable on an ultrasonogram.

Table 1 Clinical, hormonal, and genetic profile of patients with *WT1* pathogenic variants.

Patients	Age at evaluation (year)	Sex of rearing	Presenting complaint	Tanner stage	Sinnecker score	Radioimaging gonads/Mullerian structures		FSH (IU/L) ^a	LH (IU/L) ^a	T (nmol/L) ^a	Stimulated T (nmol/L)	AMH (pmol/L) ^a	NP	WT	GB	Genetic	
						gonads/ Mullerian structures	Sinnecker score									Nucleotide change	Protein change
P1	2.5	M	Atypical genitalia and aniridia	P1B1	2	RGI, LG-, MS+	3.15 (0.1-0.9)	0.1 (0.1-0.2)	0.14 (0.3-1.0)	2.42	89.1 (282-1525)	A	A	NE	11p13 deletion		
P2	0.5	M	Atypical genitalia	P1B1	3	RG-, LG-, MS+	7.81 (0.1-0.9)	1.81 (0.1-0.2)	1.39 (0.3-1.0)	19.38	NA	A	A	NE	Exon 9	c.1384C>T	p.Arg462Trp
P3	7	M	Atypical genitalia	P1B1	2	RGS, LGI, MS-	1.83 (0.1-0.9)	0.29 (0.1-0.3)	0.14 (0.3-1.0)	3.43	NA	A	A	NE	Exon 9	c.1373G>A	p.Arg458Gln
P4a	26	M	Atypical genitalia, gynecomastia, and hypertension	P4B4	3	RGA, LGA, MS+	44.8 (0.6-5)	25.7 (1.5-6.3)	2.8 (9.8-33.3)	7.42	0.71 (13.6-146.4)	P	A	P	Exon 9	c.1373G>A	p.Arg458Gln
P4b	17	F	Primary amenorrhea	P5B5 ^b	5	RG-, LG-, MS+	47.0 (2.2-12.9)	27.0 (0.79-4.76)	2.7 (5.1-27.6)	NA	0.78 (23-128)	A	A	P	Exon 9	c.1373G>A	p.Arg458Gln

^aNormative data of hormonal value were taken from Fanelli *et al.* (doi: 10.1159/000486840); ^bEstradiol level: 370.4 pmol/L.

A, absent; AMH, anti-Mullerian hormone; F, female; FSH, follicle-stimulating hormone; GB, gonadoblastoma; LG-, left gonad not localized; LGI, left gonad inguinal; LGA, left gonad abdominal; LH, luteinizing hormone; M, male; MS-, Mullerian structures absent, MS+, Mullerian structures present; NE, not evident on routine evaluation; NP, nephropathy; P, present; RG-, right gonad not localized; RGI, right gonad inguinal; RGS, right gonad scrotal; RGA, right gonad abdominal; T, testosterone; WT, Wilms' tumor.

Six months later, she presented with abdominal pain and was found to have a right-sided pelvic mass (17 × 15 × 16 cm). Tumor markers including serum lactate dehydrogenase, α-fetoprotein (αFP), hCG, and cancer antigen (CA) 125 were elevated. The patient underwent exploratory laparotomy, and the right pelvic mass was excised along with left gonadectomy and bilateral salpingectomy. Histopathology of the right gonadal mass was suggestive of a malignant germ cell tumor and that of the left gonad showed a dysgerminoma. Following surgery, tumor markers normalized, and chemotherapy was started. While being on chemotherapy, she developed generalized lymphadenopathy. Biopsy from an enlarged supraclavicular lymph node showed a metastatic germ cell tumor. Based on the enlarging metastatic masses despite appropriate chemotherapy and normalization of tumor markers, a diagnosis of growing teratoma syndrome was made (Fig. 2). She remained normotensive and had a normal serum creatinine of 0.71 mg/dL (eGFR: 115 mL/min/1.73 m²) and no albuminuria (spot urine albumin/creatinine ratio: 29 μg/mg) till last follow up at 19 years of age. Hormonal evaluation at the presentation in both the siblings (P4a and P4b) was suggestive of primary gonadal failure. At the age of 45, their mother was found to have isolated microalbuminuria (spot urine albumin/creatinine ratio: 64 μg/mg), which was reconfirmed a month later. She was normotensive and had a normal serum creatinine of 0.8 mg/dL (eGFR: 82.4 mL/min/1.73 m²). Her menstrual cycles were regular, and serum gonadotropins were normal for age. None of the affected family members had Wilms' tumor until the last follow-up. These three familial patients had a missense pathogenic variant, p.Arg458Gln, in exon 9.

The details of patients with gonadoblastoma associated with *WT1* exonic pathogenic variants reported in the literature (*n* = 13) and in this study (one family) are summarized in Table 2 (4, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19). The age of presentation ranged from birth to 26 years. Gonadoblastoma was detected earliest at 7 months of age, ranging up to 28 years. Majority of the cases (12/15, 80%) were found to have gonadoblastoma after prophylactic gonadectomy, while in the rest (*n* = 3), gonadoblastoma was found on biopsy, autopsy, and imaging for an abdominal lump in one case each. Out of 15 patients, 9 had atypical external genitalia, and others had female external genitalia. Only 1 patient had Wilms' tumor, whereas nephropathy was observed in 12 of the 14 cases. Nephropathy was documented before 8 years of age in all the reported cases, whereas in our patient P4a, it was noted as microalbuminuria and hypertension at

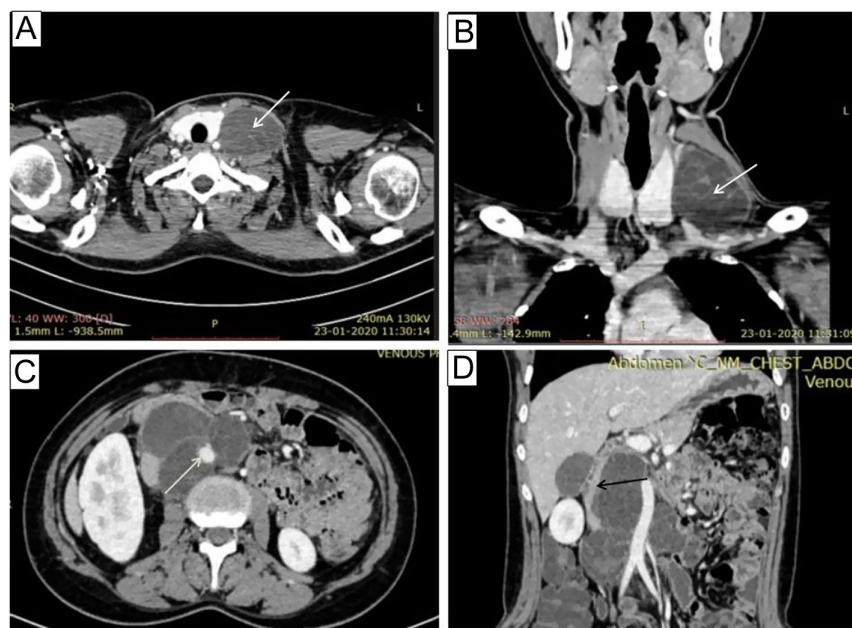


Figure 2

(A) Axial and (B) coronal contrast-enhanced CT images at the level of the neck showing a well defined enlarged left supraclavicular lymph node (white arrow) with cystic areas and few internal septae. Similar morphology lymph nodes are also seen in the para-aortic and inter-aortocaval locations in axial (C) and coronal (D) images of the abdomen. Note the lifting of the aorta (grey arrow - C) and rightward displacement of the inferior vena cava (black arrow - D) and resultant altered axis of the right kidney.

26 years. All patients had a karyotype of 46,XY except for one patient with 47,XXY. Twelve out of the 14 index cases had pathogenic variants in DNA-binding domain (exons 7, 8, and 9). The most common exonic pathogenic variant found was p.Arg462Trp ($n=5$) followed by p.Arg434His ($n=2$) and p.Met410Arg ($n=2$); others were p.Tyr177*, p.Glu437Lys, p.Arg458*, p.Arg458Gln, and p.Gln142*. On *in silico* splicing prediction analysis, these missense *WT1* variants associated with gonadoblastoma were not found to be different from the wild type.

Discussion

This study reports *WT1* pathogenic variations in 4 (2.7%) of the 150 Asian-Indian index patients with 46,XY DSD and describes an increased gonadoblastoma risk for the first time with p.Arg458Gln, a missense variant in exon 9, and a widely variable FS phenotypic spectrum in this family.

The prevalence (~3%) of *WT1* pathogenic variations in our cohort of 46,XY DSD patients is similar to that reported (0.7–5%) from various larger ($n \geq 40$) cohorts from the rest of the world (20, 21, 22, 23). A typical characteristic in patients with gonadal dysgenesis is impalpable gonad(s). In our study, all four patients with *WT1* variants had unilateral or bilateral cryptorchidism. Similarly, Kohler *et al.* reported universal occurrence of cryptorchidism in 46,XY DSD patients with *WT1* pathogenic variants. None of the patients in our series had nongonadal manifestations

at presentation except P1, who had detectable aniridia. Hence, all 46,XY DSD patients with suspected gonadal dysgenesis should undergo a formal ophthalmology examination to identify aniridia, the presence of which can guide the appropriate genetic test (11p13 deletion). Wilms' tumors were absent in all patients at presentation and during the follow-up. Nephropathy was seen in one patient, detected in the third (P4a) decade. Hence, *WT1* genetic analysis should be considered in 46,XY DSD patients with gonadal dysgenesis irrespective of the other features of *WT1*-associated syndromes.

The phenotype in P1 (atypical genitalia and aniridia) was consistent with WAGR syndrome, the characteristic phenotype of 11p13 deletion. In a large cohort of WAGR syndrome ($n=54$), 24, 14, and 11 patients had all the four, three, and two classical components, respectively. The most common feature was aniridia (98.1%), whereas Wilms' tumor was observed in 57.4% (3). Nevertheless, up to 45% of patients with aniridia harboring 11p13 deletion comprising of *WT1* and *PAX6* may develop Wilms' tumor (24). Hence, 11p13 deletion patients not manifesting Wilms' tumor at diagnosis should be monitored for its development. In our case, Wilms' tumor was not found till the last follow-up (2.5 years of age).

P2, who presented in infancy with atypical genitalia, had p.Arg462Trp, a hot-spot most common (39.6%) pathogenic variant described with DDS (5). This genetic variant exhibits a broad phenotypic spectrum as reviewed by Mazen *et al.*, where out of 15 patients, 14 (93.3%) patients had nephropathy, 4 (26.6%) had

Table 2 Review of reported cases of gonadoblastoma with *WT1* exonic point pathogenic variants.

Case no.	Author, year of publication	Age at presentation (years)	Karyotype	Exon /type of pathogenic variant	Pathogenic variant (previously described using P19544)	External genitalia phenotype	Internal genitalia	GB/age at diagnosis (years)	WT/age at diagnosis if P or last follow-up if A (years)	NP/age at diagnosis if P or last follow-up if A (years)
1.	Pelletier <i>et al.</i> 1991	NA	46,XY	9/missense	p.Arg462Trp (p.Arg394Trp)	Atypical	RG: dysgenetic LG: dysgenetic	P/NA	P/NA	P/NA
2.	Pelletier <i>et al.</i> 1991	NA	46,XY	8/missense	p.Arg434His (p.Arg366His)	Female	MS: P RG: dysgenetic LG: streak MS:A NA	P/NA	A	P/NA
3.	Jeanpierre <i>et al.</i> 1998	3	46,XY	9/missense	p.Arg462Trp (p.Arg394Trp)	Atypical	Single dysgenetic abdominal gonad with GB	P/4.8	A/6	P/3.1
4.	Koshaka <i>et al.</i> 1999	At birth	46,XY	9/nonsense	p.Arg458* (p.Arg390*)	Atypical	MS:P RG: abdominal, GB LG: abdominal, GB	P/NA	A/NA	P/8
5.	Fukuzawa <i>et al.</i> 2002	0.9	46,XY	7/missense	p.Met410Arg (p.Met342Arg)	Female	MS: P RG: dysgenetic, GB	P/8.8	A/8.8	P/0.9
6.	Auber <i>et al.</i> 2003	NA	46,XY	9/missense	p.Arg462Trp (p.Arg394Trp)	Atypical	MS: A Streak abdominal gonad with GB	P/3	A/NA	P/NA
7.	Maesaka <i>et al.</i> 2006	NA	46,XY	7/missense	p.Met410Arg (p.Met342Arg)	Female	MS:P RG: abdominal, GB LG: abdominal testes	P/8	A/NA	P/1
8.	Regev <i>et al.</i> 2008	At birth	46,XY	1/nonsense	p.Tyr177* (p.Tyr109*)	Atypical	MS:P RG: abdominal, GB LG: abdominal testes	P/1	A/6	NA
9.	Nso Roca <i>et al.</i> 2009	NA	46,XY	8/missense	p.Arg434His (p.Arg366His)	Atypical	MS: P NA	P/NA	A/NA	P/0.7
10.	Tanteles <i>et al.</i> 2011	1	47,XXY	9/missense	p.Arg462Trp (p.Arg394Trp)	Female	RG: abdominal, GB LG: abdominal, GB	P/NA	A/NA	P/1
11.	Patel <i>et al.</i> 2013	At birth	46,XY	8/missense	p.Glu437Lys (p.Glu369Lys)	Female	MS: NA RG: abdominal, GB LG: abdominal, GB	P/0.6	A/1.1	P/0.9
12.	Mazen <i>et al.</i> 2017	0.4	46,XY	9/missense	p.Arg462Trp (p.Arg394Trp)	Atypical	MS:P Single dysgenetic abdominal gonad with GB	P/0.75	A/4	A/4
13.	Souza <i>et al.</i> 2018	At birth	46,XY	1/nonsense	p.Gln142*	Atypical	MS: P One gonad dysgenetic, other with GB	P/4	A/9	P/4
14.	This study (P4a)	26	46,XY	9/missense	p.Arg458Gln (p.Arg390Gln)	Atypical	MS: NA RG: abdominal, sysgenetic LG: abdominal, GB	P/28	A/28	P/26
15.	This study (P4b)	17	46,XY	9/missense	p.Arg458Gln (p.Arg390Gln)	Female	MS: P RG: abdominal, GB LG: abdominal, GB	P/17.5	A/19	A/19

A, absent; DDS, Denys-Drash syndrome; FS, Frasier syndrome; GB, gonadoblastoma; GD, gonadal dysgenesis; LG, left gonad; MS, Mullerian structures; NA, not available; NP, nephropathy; P, present; RG, right gonad; WT, Wilms' tumor.

Wilms' tumor, and 2 (13.3%) had gonadoblastoma (18). As per our literature review, p.Arg462Trp was also the most common (5/14, 35.7%) variant reported in patients with gonadoblastoma and exonic *WT1* pathogenic variants. However, the limited follow-up did not allow this patient's (P2) complete phenotypic characterization as he did not manifest either with early nephropathy or Wilms' tumor at the last follow-up (6 months of age). p.Arg462Trp, being the most common exonic *WT1* pathogenic variant associated with gonadoblastoma, P2 has been advised to undergo bilateral orchidopexy with biopsy and subsequent close surveillance for gonadoblastoma development.

A lack of definitive genotype–phenotype correlation in patients with *WT1* pathogenic variants has been observed. Notably, affected XY patients with p.Arg458Gln variant (P3, P4a, and P4b) had variable phenotype. P3 and P4a had partial gonadal dysgenesis, whereas P4b had complete gonadal dysgenesis. P4a had late-onset nephropathy, whereas P3 and P4b had no nephropathy till the last follow-up. P4a and P4b had gonadoblastoma, whereas gonadal histology of P3 was not available (though his ultrasonogram was normal at the last follow-up). Interestingly, P4b had spontaneous normal breast development. This may be due to the production of estrogens (serum estradiol: 370.4 pmol/L) by streak gonads, or pre-existing gonadoblastoma, or increased sensitivity of breast tissue to estrogens (25). p.Arg458Gln has been reported previously in a Hispanic girl (karyotype not described) who developed steroid-resistant nephrotic syndrome at 10 years of age (26). Another 46,XY DSD patient with atypical genitalia (raised as female) inherited this variant from an unaffected father, showing incomplete penetrance (20). Similarly, the mother of patients 4a and 4b, who also had the same variant, had no overt manifestations

but had only microalbuminuria on evaluation at 45 years of age. Notably, each of the three family members with p.Arg458Gln had different phenotypes of FS: the mother had type 3 FS (female external genitalia with 46,XX with no gonadoblastoma risk), the elder sibling had type 2 FS (male external genitalia with 46,XY and gonadoblastoma risk in one-third), and the younger sibling had type 1 FS (female external genitalia with 46,XY with gonadoblastoma risk in two-thirds) (27). These observations emphasize the wide variability in the phenotype of patients with p.Arg458Gln, even among the members of the same family.

Notably, the P4b had no localization of gonads at initial evaluation but presented with a large gonadoblastoma 6 months later. This patient also had growing teratoma syndrome after surgery and chemotherapy, suggesting an aggressive behavior, which probably explains the rapid development and progression of gonadoblastoma at diagnosis. On the other hand, this may also undermine the limitations of the commonly recommended modalities for gonadoblastoma surveillance such as ultrasonogram to identify pre-malignant lesion or small-sized malignant germ cell tumor (28). This patient is the first case of growing teratoma syndrome with *WT1* pathogenic variants, to the best of our knowledge. The association of the gonadoblastoma in two of our three 46,XY DSD patients with p.Arg458Gln suggests a high risk of gonadoblastoma with this variant, suggesting a need for regular monitoring. Additionally, the rapid development and progression of gonadoblastoma in one of our patients may suggest frequent monitoring at 4–6 monthly intervals. Based on the occurrence of gonadoblastoma in P4a and P4b, P3 having the same variant was advised left-sided orchidopexy with biopsy. Further plan is to monitor the child for the development of gonadoblastoma with monthly

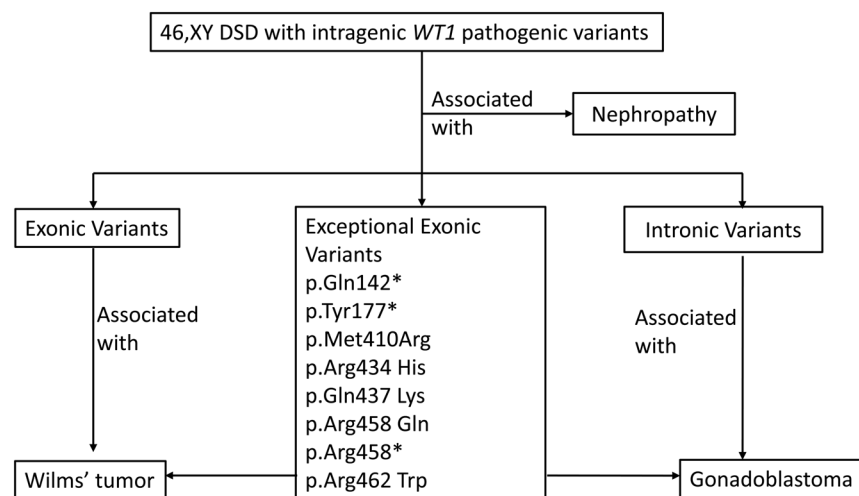


Figure 3 Schematic presentation of 46,XY DSD patients with intragenic *WT1* pathogenic variants and associated syndromic features.

self-examination, and annual monitoring with ultrasonogram of gonads, and tumor markers (serum α FP and hCG). Parents were also counseled about the limitations of these surveillance modalities (29).

In patients with intron 9 splice-site variants in *WT1*, those with type 1 and type 2 FS are at high risk for gonadoblastoma (27). The risk for gonadoblastoma seems to be lower in patients with nonsense or missense *WT1* variants and partial gonadal dysgenesis, but current data are insufficient for guiding management (8). To date, more than 100 exonic point variants in *WT1* have been reported with different phenotypes (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=WT1>, accessed 21/09/2021). In the literature review, we found only 13 patients having gonadoblastoma with one of the seven exonic point variants in *WT1*, p.Arg462Trp, p.Arg458*, p.Arg434His, p.Tyr177*, p.Met410Arg, p.Gln142*, and p.Gln437Lys. Here, we report the association of p.Arg458Gln, the eighth exonic variant, with gonadoblastoma. In patients with these eight variants, we suggest stringent regular surveillance for gonadoblastoma development. We also recommend shared decision making with patient/parents for prophylactic gonadectomy after weighing the adequacy of residual gonadal function to preserve fertility and avoid the need for hormone replacement and the potential for gender dysphoria against the risk of production of opposite sex hormones and missing the early diagnosis of germ cell neoplasia due to the inherent limitations of surveillance methods (Fig. 3). Although the gonadoblastoma has been reported with only eight variants, we caution against assuming the absence of gonadoblastoma risk in 46,XY DSD patients with other exonic *WT1* variants.

The intron 9 splice-site variants, the most common variants in FS patients, cause a reduction in the ratio of +KTS to -KTS *WT1* isoforms (<2:1). The +KTS *WT1* isoform upregulates SRY expression, which in turn upregulates SOX9 expression and thus, plays an active role in Sertoli cell differentiation and homeostasis. Decreased expression of +KTS *WT1*, SRY, and SOX9 affects Sertoli cell maturation and thus, their ability to support germ cell differentiation. This may result in the arrest of spermatogenesis and malignant transformation of germ cells (30). The molecular physiology of how the exonic missense variants in *WT1* lead to gonadoblastoma is not clear. In human genetic disorders, a few exonic missense pathogenic variants are reported to be associated with splicing defects (31). However, on *in silico* analysis, no such association with splicing defects was noted for the exonic *WT1* variants associated with gonadoblastoma. Hence, further cDNA and mRNA studies are warranted to evaluate the effect of

p.Arg462Trp, p.Arg434His, p.Gln437Lys, p.Met410Arg, and p.Arg458Gln on splicing defects.

The study was limited by a small number of participants, the retrospective phenotypic data collection, and a shorter duration of the follow-up. Nonetheless, this is the first report of *WT1* pathogenic variants in 46,XY DSD cohort from India that reports a few novel observations in patients with *WT1* pathogenic variants.

In conclusion, *WT1* alterations account for 3% of patients with 46,XY DSD in our cohort. We report the first case of *WT1*-associated growing teratoma syndrome. Wide phenotypic variation may be present despite the same genotype. This study and the systematic review demonstrate a small but definitive gonadoblastoma risk in 46,XY DSD patients with exonic *WT1* variants suggesting a need for gonadoblastoma surveillance in them. Of >100 exonic point pathogenic variants reported to date, only eight variants (p.Arg462Trp (most common), p.Tyr177*, p.Arg434His, p.Gln437Lys, p.Arg458*, p.Met410Arg, p.Gln142*, and p.Arg458Gln) in *WT1* were associated with gonadoblastoma. We suggest that more stringent gonadoblastoma surveillance should be considered in 46,XY DSD patients with these variants.

Declaration of interest

The authors declare that there is no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

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