Germline Wilms tumor suppressor gene (*WT1*) mutation leading to isolated genital malformation without Wilms tumor or nephropathy

Birgit Köhler, MD, Valerié Schumacher, PhD, Dagmar l'Allemand, MD, Brigitte Royer-Pokora, PhD, and Annette Grüters, MD, PhD

Mutations of the Wilms tumor suppressor gene (*WT1*) have been described only in patients with syndromes associated with urogenital malformation and Wilms tumor or nephropathy. We present a male patient with an isolated genital malformation caused by a *WT1* mutation. (J Pediatr 2001;138:421-4)

Wilms tumor suppressor gene (*WT1*), one of the earliest acting transcription factors in urogenital differentiation and development, is highly expressed in the urogenital ridge and is required for development of the bipotential gonad and the primordial kidney.[1] The *WT1* gene, cloned in 1990, is located on chromosome 11p13 and consists of 10 exons. *WT1* mutations have been described only in patients with syndromes associated with urogenital malformation and Wilms tumor or nephropathy. *WT1* was found to be deleted in patients with Wilms tumor, aniridia, genitourinary malformation, and mental retardation syndrome.[2,3] *WT1* mutations were identified in Denys-Drash syndrome, which is the most severe disease caused by *WT1* abnormalities. In males, DDS is characterized by ambiguous genitalia and nephropathy, with or without Wilms tumor. In females, it is characterized by normal external genitalia and nephropathy, with or without Wilms tumor.[4] Furthermore, Frasier syndrome, which consists of genital malformation, nephropathy, and gonadoblastoma, was also found to be caused by *WT1* mutations.[5] Subsequently, severe hypospadias and Wilms tumor, in the absence of other signs of DDS, WAGR, or Frasier syndrome, were described in 7 patients with *WT1* mutations.[6-10] In this report we present a boy with isolated genital malformation in whom a heterozygous *WT1* mutation in the germline was identified. No other associated signs of syndromes, nephropathy, or Wilms tumor occurred until the age of 18 years.

From the Children's Hospital, Philipps University, Marburg, Germany; the Charité Children's Hospital, Pediatric Endocrinology, Humboldt University, Berlin, Germany; and the Institute of Human Genetics, Heinrich Heine University, Düsseldorf, Germany.

Drs. Köhler and Schumacher contributed equally to the work.

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Reprint requests: Annette Grüters, MD, PhD, Charité Children's Hospital, Augustenburger Platz 1, 13353 Berlin, Germany.

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Case Report

The patient is an 18-year-old who was born with severe hypospadias and bilateral cryptorchidism. Karyotyping showed a normal male 46 XY. Congenital adrenal hyperplasia and defects of testosterone biosynthesis were ruled out by extensive biochemical tests and molecular genetic analysis of the 5α-reductase and androgen receptor gene (Table). Mullerian structures (a rudimentary uterus and an upper vaginal pouch) were visualized by ultrasonography. Laparotomy revealed both testes to be located high, retroperitoneally below the kidneys. There were no renal abnormalities (Fig 1).

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In early infancy, the patient had surgical correction of hypospadias, and both testes were brought into the scrotum (Fig 1). Pubertal development was completely normal. At the age of 18 years, the patient has normal external masculinization with a normal penis size and a normal prostate but rather low testicular volumes of 10 mL on each side.

AMH	Anti-müllerian hormone
DAX1	Dosage-sensitive sex reversal—adrenal hypoplasia congenita critical region on the X chromosome, gene 1
DDS	Denys-Drash syndrome
PCR	Polymerase chain reaction
SRY	Sex-determining region on the Y chromosome
SSCP	Single-stranded conformational polymorphism
WAGR	Wilms tumor, aniridia, genitourinary malformation, and mental retardation
WT1	Wilms tumor suppressor gene

No hormonal abnormalities were observed except for moderate elevations in follicle-stimulating hormone (32 mU/mL) and luteinizing hormone lev-

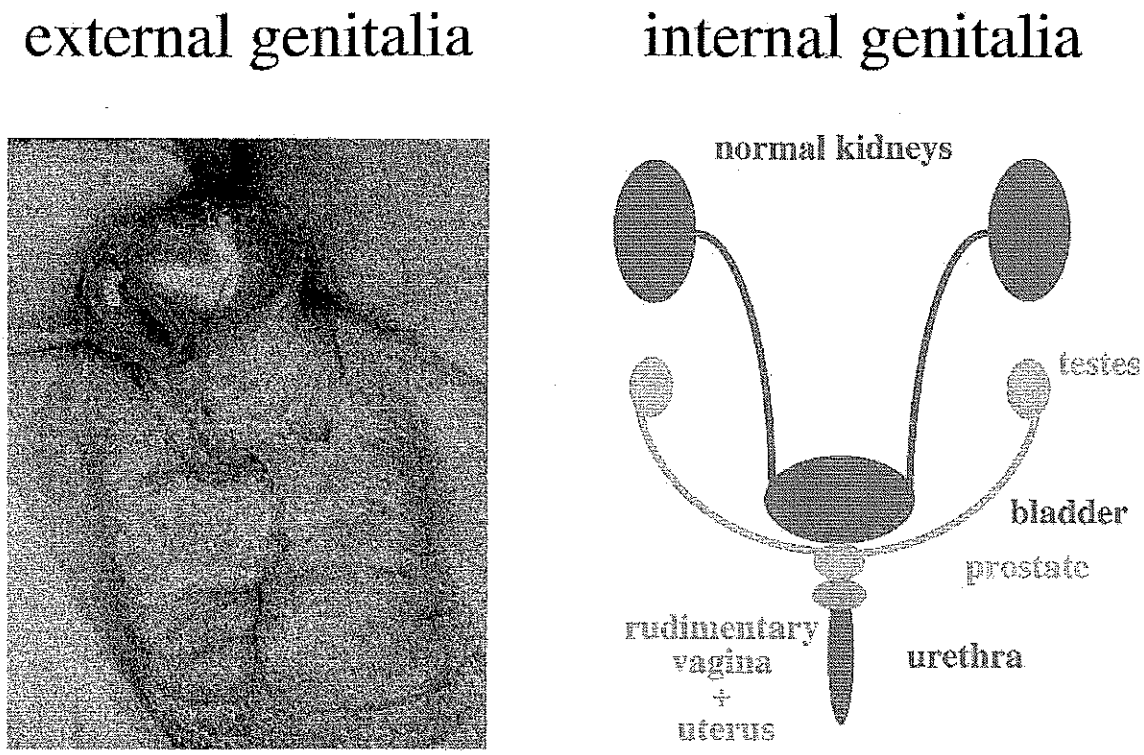

Fig 1. External (after surgery at age of 2 years) and internal genitalia of the patient.

Table. Endocrine investigations of the patient

At birth	Patient	Reference range	At the age of 18 y	Patient	Reference range
17-Hydroxyprogesterone (ng/mL)	2.1	2-20	Testosterone (ng/mL)	4.4	1.30-6.10
Cortisol (μg/dL)	9.6	5-25	Dihydrotestosterone	30	16-110
Testosterone-like substance (ng/mL)	0.13	0.1-0.5	Dehydroepiandrosterone sulfate (ng/mL)	1596	608-3280
			Follicle-stimulating hormone (U/L)	32	1.4-5.1
			Luteinizing hormone (U/L)	13	3.4-7.5

Mutations of the 5α-reductase and androgen receptor gene were excluded by PCR-SSCP analysis.
Radioimmunoassays were used for all hormone measurements.

els (13 mU/mL), which are indicative of disturbed testicular function (Table).

Repeated ultrasound studies and regular urine analysis until now did not reveal any evidence for renal failure or Wilms tumor. At present, the patient is in his final year of college.

METHODS

DNA was extracted from peripheral blood leukocytes with a DNA extraction kit (Quiagen). Polymerase chain reaction–single-stranded conformational polymorphism analysis was used to detect alterations in the coding exons and splice sites of the *WT1* gene. All 10 exons and parts of the introns containing the splice sites were amplified by PCR from genomic DNA. SSCP analysis was performed with 4 different electrophoresis conditions to maximize the sensitivity of the technique. Primer sequences and PCR and SSCP conditions were as previously described.[8] Direct sequencing was performed with an automatic LICOR sequencer (MWG Biotech).

RESULTS

A heterozygous point mutation, 1168 C→T, was found in exon 9, changing the codon for arginine into a stop codon (R390X). During translation, this mutation leads to a truncated protein in which the last zinc finger neces-

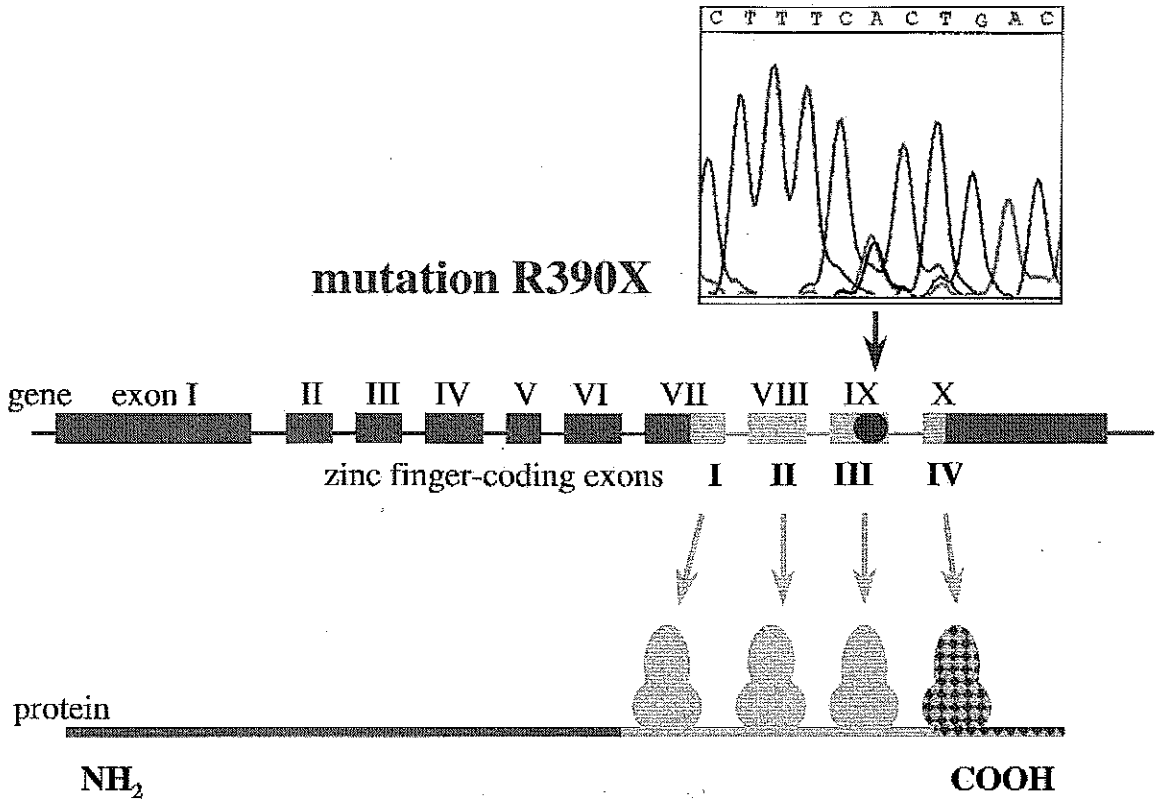

Fig 2. The *WT1* gene and protein including the mutation found in the patient.

sary for DNA binding of *WT1* is missing (Fig 2). Interrupted DNA binding to target genes of *WT1* fusion proteins with loss of the last zinc finger has been previously demonstrated by electrophoretic mobility shift assays.[11]

DISCUSSION

We report a boy with isolated male genital malformation caused by a *WT1* mutation. Like patients with DDS or WAGR syndrome, the patient had severe hypospadias and cryptorchidism at birth, but neither renal insufficiency nor Wilms tumor occurred during childhood and adolescence.

In recent years, advances in molecular biology have shed light on the complexity of sexual differentiation, especially the importance of transcription factors.

WT1 is one of the earliest transcription factors expressed in the urogenital ridge. A defective *WT1* protein leads to a differentiation defect in both the gonads and the kidneys.

In one study, steroidogenic factor 1 and *WT1* were shown to synergize in the promotion of anti-müllerian hormone. A mutant *WT1* protein fails to synergize with steroidogenic factor 1 and leads to reduced AMH activation.[12] This finding explains the persisting müllerian remnants and undescended testes in our patient with a loss-of-function mutation in the *WT1* gene, which is comparable to the phenotype of males with AMH mutations. Furthermore, it was shown that *WT1* activates the dosage-sensitive sex reversal–adrenal hypoplasia congenita critical region on the X chromosome, gene 1 (DAX1) and itself is activated by the sex-determining region on the Y chromosome (SRY). A disturbed *WT1-DAX1* or *WT1-SRY* interaction in early development most likely results in partial sex reversal with the phenotype of severe hypospadias in boys with *WT1* mutations.[13,14] Because the phenotype of male patients with *WT1* mutations is similar to that of patients with defects of testosterone biosynthesis or androgen receptor defects, it is also possible that there is ineffective androgen production or action in those patients during early male development as a result of direct disturbed interaction of *WT1* and genes controlling testosterone biosynthesis or the androgen receptor. In one in vitro study, it was demonstrated that *WT1* represses the androgen receptor promotor.[15] The hypothesis of *WT1* repressing the genes of enzymes involved in testosterone production has yet to be proven.

WT1 is also a major factor in kidney development. However, our patient had neither nephropathy nor Wilms tumor until the age of 18 years. The lack of nephropathy might be explained by the genotype of this patient presenting a nonsense mutation. Mainly, patients with missense or splice-site mutations show nephropathy. Only in very few patients with nonsense mutations has nephropathy been observed.[7]

The mutation R390X in this patient was found in earlier studies in patients with isolated Wilms tumor, in patients with Wilms tumor associated with urogenital malformation, and in an 18-year-old boy with acute promyelocytic leukemia.[7] The absence of a Wilms tumor in early infancy in this patient might be due to a missing "second hit" in the *WT1* gene, leading to loss of the second allele. This explanation was proposed for some patients with *WT1* germline mutations or deletions, as in WAGR syndrome, who did not develop a Wilms tumor.[16] However, development of Wilms tumor has been described in patients with heterozygous mutations. Therefore additional mechanisms for tumor development must be postulated, such as haploinsufficiency of the *WT1* gene leading to failure of suppression of tumor growth–promoting genes and the insulin-like growth factor I receptor gene or the insulin-like growth factor II gene.[17,18] Because there are few reports of Wilms tumors in adults, the patient must be followed up by means of ultrasonography also in adulthood.[19] The important clinical conclusion of this case is that *WT1* mutations may be the cause of isolated hypospadias and cryptorchidism in children. The search for mutations in the *WT1* gene should be considered in the workup of patients with severe hypospadias even in the absence of Wilms tumor or nephropathy. Further investigations of the interaction of the different mutated *WT1* proteins with target genes, known for playing a role in initiation of tumor growth, are needed to predict which patients will develop a tumor in later life.

Note added in proof: At the age of 20 years, the patient developed proteinuria, and subsequent biopsy of the kidney revealed glomerulosclerosis.

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