Advances in Brief

Inherited WTI Mutation in Denys-Drash Syndrome

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Abstract

Patients with the Denys-Drash syndrome (Wilms' tumor, genital anomalies, and nephropathy) have been demonstrated to carry de novo constitutional mutations in WTI, the Wilms' tumor gene at chromosome 11p13. We report three new cases, two carrying a previously described WTI exon 9 mutation and one with a novel WTI exon 8 mutation. However, unlike patients in previous reports, one of our three patients inherited the affected allele from his phenotypically unaffected father. This observation indicates that the WTI exon 9 mutation affecting 394Arg demonstrated in over one-half of the patients with the Denys-Drash syndrome may exhibit incomplete penetrance. Consequently, familial studies in patients affected by this syndrome are recommended.

Introduction

Wilms' tumor, or nephroblastoma, is a renal malignancy that affects approximately 1 in 10,000 children under 16 years of age. Cytogenetic observations in patients with Wilms' tumor, aniridia, genitourinary malformations, and mental retardation (the WAGR syndrome) demonstrated chromosome 11p13 germ-line deletions, providing the first indication that this chromosome segment is involved in Wilms' tumorigenesis (1). Molecular analysis of the homozygously deleted region chromosome 11p13 in two Wilms' tumors led, in 1990, to the independent identification of WTI, a Wilms' tumor suppressor gene, by three groups (2-4). Occasionally, the presence of Wilms' tumor in a patient is associated with genital anomalies and nephropathy: the Denys-Drash syndrome (5, 6). The clinical overlap between this syndrome and the WAGR syndrome suggested that the DDS could also arise as a consequence of a deletion of one or more genes localized at chromosome 11p13 (7). Recently, constitutional WTI mutations have been demonstrated in 23 of 25 reported individuals with DDS, providing evidence of a direct role for WTI in this syndrome (8-10). Based on two families evaluated, it was suggested that the DDS follows a dominant mode of inheritance with high penetrance and that it is extremely unlikely that parents of individuals with the DDS carry the mutations found in the affected children (8). Subsequent analysis of four additional families seemed to confirm this hypothesis (10). We report here the presence of the same WTI exon 9 mutation in a phenotypically abnormal male and his phenotypically unaffected father. This finding demonstrates that WTI mutations causing the DDS can indeed be inherited and need not be expressed phenotypically.

Materials and Methods

Patients. The clinical and cytogenetic features of three patients with DDS are summarized in Table 1. D7, the father of patient D5, is at present a healthy 42-year-old male. Physical examination did not reveal any abnormalities; in particular he has bilaterally descended testes of normal volume and a normal penis; he does not have hypospadias. In 1985, he donated his left kidney for transplantation to his son, patient D5.

Genomic DNA Preparation. Constitutional DNA from all three patients and their parents and several family members of patient D5 were isolated from peripheral leukocytes as described previously (11). Isolation of the constitutional DNAs of patient D5 and his father (D7) were repeated following the initial results in order to confirm the analyses.

PCR Amplification. All WTI exon sequences from genomic DNA were amplified by PCR, and the PCR products were analyzed for SSCP (12) in order to detect possible deletions, insertions, or point mutations. In the analysis of WTI exon 8 we used primers (A-2)8 and (S-2)8, and in the analysis of WTI exon 9 primers (A-2)9 and (S-2)9 which have been described previously (13). PCR was carried out in a GeneAmp PCR system 9600 (Perkin-Elmer) with a final volume of 100 μl, containing 250 mmol/liter each of dATP, dGTP, and dTTP; 20 mmol/liter Tris base-0.09 M boric acid-2.5 msi EDTA running buffer at 30 W at 4°C for 4—6 h, and at 5 W at room temperature for 4—6 h. The DNA samples were denatured at 95°C for 3 min and immediately placed on ice before being run on 6% polyacrylamide nondenaturing gels containing 10% glycerol. Electrophoresis was performed in 0.09 M Tris base-0.09 M boric acid-2.5 μM EDTA running buffer at 30 W at room temperature for 4—6 h, at 30 W at 4°C for 4—6 h, and at 5 W at room temperature for 16 h. The gels were dried and exposed to XAR-5 film (Kodak) for 16—96 h without an intensifying screen at room temperature.

Sequencing. The mechanisms used for direct sequencing of biotinylated PCR products have recently been described (10, 14). Briefly, PCR amplification with one primer biotinylated at the 5' end and one nonbiotinylated primer was performed when the PCR product was to be used for sequencing. The final volume remained 100 μl, 1 mCi [32P]dCTP was omitted, the concentration of the nucleotides became 250 mmol/liter for each deoxynucleotide triphosphate, and the amount of primer used was decreased to 5—15 pmol. Adsorption of a 50-μl aliquot of the biotinylated PCR product on streptavidin-coated magnetic Dynabeads (Dynal, Merseyside, United Kingdom) was performed utilizing a magnetic particle concentrator (Dynal) as recommended by the manufacturer. Following denaturation in 100 μl of 0.15 M NaOH, biotinylated and nonbiotinylated strands were separated on the magnetic particle concentrator and both strands were sequenced using a Sequenase (United States Biochemical) kit according to the instructions of the manufacturer.

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Results

PCR/SSCP analysis used to search for deletions, insertions, or base pair mutations within the \( WT1 \) exons demonstrated (Fig. 1) a mobility shift in exon 9 (encoding zinc finger 3) of one allele from patients D1 and D5, and in exon 8 (encoding zinc finger 2) of one allele from patient D10. Constitutional DNA from the parents of D1 (D2 and D3) and D10 (D8 and D9) demonstrated a normal (homozygous) SSCP pattern (Fig. 1). A homozygous SSCP pattern was also found for the mother (D6), three paternal aunts (S1D7, S2D7, S3D7), and brother (BD5) of D5, but the father (D7) of D5 showed a heterozygous SSCP pattern similar to the one demonstrated for his son (Fig. 1). The heterozygous SSCP pattern found in the father was confirmed on a second blood sample taken 3 months after the first one. In addition, very numerous tandem repeat studies were performed on samples from D5, D6, and D7 and were found to be consistent with the presumed paternity (data not shown). DNA of a (the only) paternal uncle of D5 could not be obtained.

Sequence analysis of constitutional DNA revealed a \( 1129A \) to T transition converting amino acid residue \( 377 \)His to Tyr in patient D10 (Fig. 2A), and a \( 1180C \) to T transition converting amino acid residue \( 394 \)Arg to Trp in patients D1 and D5 (Fig. 2B). Sequence analysis of D7 revealed the same \( 1180C \) to T transition found in patient D5 (Fig. 2B). The described mutations were confirmed by sequence analysis of at least five additional samples from any given specimen.

Discussion

We report three patients with the DDS who were found to have constitutional \( WT1 \) mutations which alter the amino acid sequence of the protein domains that are critical for DNA sequence recognition by zinc finger proteins. The \( WT1 \) exon 9 mutations found in patients D1 and D5 affect amino acid residue \( 394 \)Arg, which has been implicated in hydrogen bonding interactions with a guanidine residue in the cognate DNA recognition sequence (15). This mutation has now been reported in 14 of 25 (=55%) individuals with the DDS. The \( WT1 \) exon 8 mutation found in patient D10 affects amino acid residue \( 377 \)His and disrupts one of the four zinc binding ligands of zinc finger 2; this mutation has not been described previously. In addition to these two \( WT1 \) missense mutations, an exon 9 missense mutation affecting \( 366 \)Arg in two patient, an exon 7 missense mutation affecting \( 330 \)Cys in one patient, an insertion of G at position 821 in exon 6 resulting in the immediate generation of a stop codon in one patient, and finally, an intron 9 mutation, which prevents splicing at one of the alternative splice donor sites of exon 9, in another patient (8—10). The \( WT1 \) zinc finger region, encoded by exons 7–10, and especially exon 9, seems to be a “hot spot” for constitutional mutations causing the DDS.

The identification of constitutional \( WT1 \) zinc finger mutations in patients with the DDS, as well as the described \( WT1 \) exon 9 mutation in a phenotypically normal father of a child with this syndrome, raises the question of the origin of the mutations. A recessive mode of inheritance, resulting from the inheritance of a mutant \( WT1 \) allele from each parent, seems unlikely given the fact that in the 23 patients reported with a constitutional \( WT1 \) mutation, only one abnormal allele has been observed in each patient (8–10). Although false negative results have been reported for SSCP analysis, it is considered a very sensitive assay (8). It seems unlikely that the SSCP assay would have failed to reveal a second mutation in all 23 patients analyzed. The data are more consistent with an autosomal dominant mode of inheritance for this disorder. Because all cases reported to date have occurred sporadically, the \( WT1 \) allelic penetrance has been assumed to be very high (8), leading to the suggestion that it would be extremely unlikely for a parent of a child with the DDS to carry a nonpenetrant mutation (8). This hypothesis has been supported by the analysis of constitutional DNA from both parents of each of the previously reported cases (8, 10). None of the parents carried the mutant allele of their affected child. However, our results demonstrate that a phenotypically unaffected parent can have and transmit a \( WT1 \) exon 9 mutation to his affected son.

The presence of the \( WT1 \) exon 9 missense germline mutation in an unaffected parent suggests either that \( WT1 \) mutations exhibit incomplete penetrance [in contrast to what was previously assumed (8)], possibly related to somatic mosaicism (16) or genomic imprinting (17). Insofar as the latter possibility is concerned, the recent demonstration of equivalent expression of paternally and maternally inherited \( WT1 \) alleles in Wilms'
Fig. 2. Sequence analysis of \textit{WTI} exon 8 (coding zinc finger 2) in patient D10 and of \textit{WTI} exon 9 (coding zinc finger 3) in patients D1, D5, and D7 (the father of patient D5). Sequencing was performed as described in "Materials and Methods." The nucleotide numbering system is based on the coding sequence only and considers the A of the ATG initiator as +1. The numbering system for the amino acids considers the initiator methionine as +1. (A) \textit{WTI} exon 8 sequence analysis of constitutional DNA of patient D10 shows a 377C to T point mutation which converts 377His to Tyr. (B) \textit{WTI} exon 9 sequence analysis demonstrates a 394A to C transition which converts 394Arg to Trp in patients D1, D5, and D7 (the father of D5).
tumors and normal tissues heterozygous for the *WT1* gene suggests that *WT1* is not subject to transcriptional imprinting (18). We therefore believe that another cause of incomplete penetrance is the more likely explanation for our findings. Unfortunately, we have been unable to assess the possibility of somatic mosaicism in this individual; sperm analysis is not feasible because D7 has had a vasectomy performed and has thus far not consented to providing us with a skin biopsy for fibroblast culture and analysis.

This report demonstrates that *WT1* mutations in patients with DDS may be inherited from a phenotypically normal parent, the carrier of the mutation. Accordingly, familial studies of affected patients are indicated. It is expected that such studies will improve the genetic counseling provided to individuals affected by this syndrome and their families and enhance our understanding of the role of *WT1* in genitourinary development and Wilms' tumorogenesis.

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**References**


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