Genetic Analysis of Two Female Patients with Incomplete Denys-Drash Syndrome

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Abstract. Denys-Drash syndrome (DDS) is characterized by genital anomaly, early onset nephropathy and high risk for developing Wilms’ tumor (WT). Recently, mutations in exon 8 or 9 of the Wilms’ tumor suppressor gene (WT1) have been found in the majority of DDS patients studied. We analyzed these two exons of the WT1 gene in genomic DNA from two female patients with DDS by using polymerase-chain reaction (PCR) and direct sequencing. The patients were accompanied with normal external genitalia, early onset renal failure between 6 and 12 months of age, and unilateral Wilms’ tumor. Genomic DNA was isolated from peripheral blood leucocytes of the patients. Amplification of exons 8 and 9 of the WT1 gene by PCR was performed, and direct sequencing of the PCR product was performed using an automatic DNA sequencer. Two heterozygous missense mutations were found in these patients, including a missense mutation in exon 9 at codon 388 replacing the wild-type Cys with Phe, and a previously described mutation in exon 9 at codon 398 replacing the wild-type Leu with Pro. Cys388Phe is a novel mutation in the WT1 gene in the DDS. These cases are considered to be “incomplete DDS” with nephropathy and Wilms’ tumor and without genital anomaly, the validity of which has been confirmed by mutation analysis.

Key words: WT1, Denys-Drash syndrome, Renal failure, Genitourinary abnormality

DENYS-Drash syndrome (DDS) is a rare developmental disorder characterized by progressive nephropathy leading to end-stage renal failure, pseudohermaphroditism in males, and an increased risk for Wilms’ tumor [1, 2]. The WT1 gene, encoding a zinc finger transcriptional factor, is involved in gonadal and renal development. Recent genetic studies revealed that mutations in exon 8 or 9 of the gene, which encode zinc fingers (ZF) 2 and 3, respectively, were found in the majority of patients with DDS. Nephropathy, the most consistent feature of DDS, usually develops between two months and two years of age, presents with progressive renal failure or nephrotic syndrome, and reveals pathological findings of focal or diffuse mesangial sclerosis [3, 4]. In this study, we employed a direct sequencing of the polymerase-chain reaction (PCR) product to determine accurately the diagnosis of DDS in female patients with normal external genitalia, renal failure and unilateral Wilms’ tumor.

Materials and Method

DNA analysis

Genomic DNA was extracted from peripheral blood leucocytes according to previously published protocols [5]. Informed consent was obtained from the patients and their parents. Amplification of ex-
ons 8 and 9 of the WT1 gene using PCR was performed on 10 ng genomic DNA templates in a total volume of 100 µl using an automatic thermocycler (Takara Co., Japan) with primers exon 8 (5' CCT TTA ATG AGA TCC CCT TTT CCA G 3' AAC ACA GCT GCC AGC AAT GAG) and exon 9 (5' CCT CAC TGT GCC CAC ATT G 3' CCC TCT CAT CAC AAT TTC ATT CC). A 186-bp PCR product for exon 8 and a 222-bp PCR product for exon 9 were obtained after 35 cycles. For direct DNA sequencing, half of the PCR mixture was used for dideoxy sequencing using an Applied Biosystems DNA sequencer 373A (Applied Biosystems, Foster City, CA, USA). The same primers as used for PCR were used for the sequencing.

Results

Clinical data

Two female patients with normal external genitalia were examined. The clinical details of these patients are given Table 1. Both patients had healthy parents. Case 1 patient did not have any siblings. Case 2 patient had a healthy younger brother. We could analyze only the patients' DNA. These patients developed renal failure between 6 and 12 months of age, the histopathological diagnosis being mesangial sclerosis. They developed unilateral Wilms' tumor. Clinical profile of the two patients analyzed are given in Table 1. Case 2 patient had been received renal transplant and case 1 patient had been started on hemodialysis at 1 year age.

Mutation analysis of the WT1 gene in germline DNA

We found that case 1 was heterozygous for a G to T transition at nt 1163 in exon 9 of the WT1 gene.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>External genitalia</th>
<th>Nephrotic syndrome</th>
<th>WT</th>
<th>Karyotype</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 y.o.</td>
<td>♀</td>
<td>Normal Female</td>
<td>+ (early onset)</td>
<td>+</td>
<td>46XX</td>
<td>Cys388Phe†</td>
</tr>
<tr>
<td>2</td>
<td>13 y.o.</td>
<td>♀</td>
<td>Normal Female</td>
<td>+ (early onset)</td>
<td>+</td>
<td>46XX</td>
<td>Leu398Pro</td>
</tr>
</tbody>
</table>

† Novel mutation
WT: Wilms tumor

Discussion

We found two constitutional mutations in the WT1
As expected, these mutations occurred in the ZF-coding exons of the gene, and were thus expected to disrupt the normal DNA regulatory function of proteins [7-9].

Because DDS was originally described as associated with male pseudohermaphroditism, the diagnosis has been easily overlooked in phenotypically normal females with a 46,XX karyotype [10]. Females constitute a minority of reported cases of DDS, and are usually diagnosed only after the appearance of Wilms' tumor. There is no explanation for the difference in incidence between the sexes, and the comparatively low reported incidence in females probably represents underdiagnosis. Sex is determined chromosomally at conception, but for the first 6 weeks the fetus remains sexually undifferentiated, sex organ formation being completed at the 14th gestational week. This process involves a delicately orchestrated, well-timed interplay between several gene products including various sex hormones and their receptors the WT1, and others. The WT1 gene, and its products are expressed at high levels in those mesodermally derived tissues that experience mesenchymal-epithelial transition during development, including the genital ridge and developing mesothelium, kidney and gonads [7, 9, 11]. WT1 expression is linked with podocyte differentiation during nephrogenesis, but continues to be expressed in adult podocytes, sertoli cells and so on [11].

Alternative splicing at two sites creates four WT1 isoforms: the presence or absence of 17 amino acids encoded by exon 5, and the inclusion or exclusion of the three amino acids sequence of KTS between ZF 3 and 4. The +KTS and −KTS isoforms are functionally different, and a balance in the ratio of the isoforms is essential for normal development of the genitourinary system [12, 13]. Mutations in the WT1 gene are found in almost all cases of DDS. These are typically heterozygous missense mutations clustered in exons 8 and 9, which encode the ZF 3 and 4, and result in altered WT1 proteins that are defective in DNA binding. The severe phenotypes of DDS are thought to be caused by dominant-negative WT1 proteins. Functionally normal WT1 tissue levels are probably reduced below 50% by dimerization of normal and mutant WT1 proteins [3].

Both cases presented here had unilateral Wilms' tumor, which had been removed. The risks of tumorigenesis and the pattern of developmental disorder in the kidneys and gonads are influenced by the type of WT1 gene mutation. Inactivation of both copies of the gene can cause Wilms' tumor (the two-hit theory). Heterozygous loss of WT1 has been identified in DDS with complete (severe progressive glomerulonephropathy, gonadal dysgenesis and predisposition to Wilms' tumor) and incomplete (severe progressive glomerulonephropathy and either gonadal dysgenesis or predisposition to Wilms' tumor) forms of disease [11]. Both of our patients revealed nephropathy and Wilms' tumor. "Incomplete DDS" is applicable to patients like ours, whose diagnosis of manifest nephropathy with Wilms' tumor or genital anomaly, has been confirmed by mutation analysis [13].

Nephropathy, a consistent manifestation of DDS, usually occurs very early in infancy and results in end-stage renal failure before the age of 10 years in almost all cases. The most characteristic light microscopic findings of DDS nephropathy is diffuse mesangial sclerosis. In addition, irregular thickening and splitting of the glomerular basement membrane are typically observed by electron microscopy.
Furthermore, nephropathy, which is the invariant feature of DDS, is not a result of WT1 haplo-insufficiency because no nephropathy develops in WAGR (Wilms' tumor, aniridia, genital abnormalities and mental retardation) patients [11].

A variety of mutations have been reported in the DDS gene to date [3, 14]. Little and Wells reviewed 100 reports of intragenic WT1 mutations and examined accompanying clinical phenotypes [14]. They reported that Arg394Trp remains the most commonly reported DDS WT1 mutation, being present in 39.6% of the patients in their review [14].

Frasier syndrome, which is characterized by gonadal dysgenesis and nephropathy resulting in delayed renal failure, was the most important differential diagnosis in DDS [15, 16]. It was reported that intronic mutations lead to Frasier syndrome; this result provided the first clue that WT1 isoforms resulting from alternative splicing of exon 9 have different physiological roles in tumorigenesis and urogenital organogenesis [15]. The two cases in the present report are considered to be "incomplete DDS" with nephropathy and Wilms' tumor and without genital anomaly, the validity of which has been confirmed by mutation analysis.

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References
