Letters to the Editor

Am. J. Hum. Genet. 64:1778, 1999

Evidence for the Genetic Heterogeneity of Nephropathic Phenotypes Associated with Denys-Drash and Frasier Syndromes

To the Editor:

The association of constitutional heterozygous mutations of the Wilms tumor 1 (WT1) gene with the majority of cases of both Denys-Drash syndrome (DDS [MIM 194080]) (Denys et al. 1967; Drash et al. 1970) and Frasier syndrome (FS [MIM 136680]) (Frasier et al. 1964) has been well described; however, the characteristic nephropathies connected with these syndromes—that is, diffuse mesangial sclerosis (DMS [MIM 256370]) and focal segmental glomerulosclerosis (FSGS [MIM 603278])—occur more commonly in early life, as disorders confined to the kidney. It is unclear how frequent WT1 gene mutations are in this population.

The WT1 gene encodes a transcription factor critical for the normal development and function of the urogenital tract, reflected by the knockout-mouse homologue of WT1, in which homozygous inactivation of Wt1 causes absent kidneys and malformation of the gonads (Kreidberg et al. 1993). Alternative splicing results in at least 4 different zinc-finger protein isoforms, and the possibility of RNA editing and use of an alternative initiation codon increases this number to 16 (Bruening and Pelletier 1996). A correct ratio of isoforms appears crucial for normal gene function. During renal development, maximum WT1 expression occurs in condensing mesenchyme and during the mesenchymal-epithelial switch. In the mature nephron, WT1 expression is confined to the podocytes, a highly specialized layer of epithelial cells in the glomerulus. Here it may have a role in the maintenance of these cells, thus affecting the integrity of the glomerular filter (Pritchard-Jones et al. 1990). Accumulating evidence supports a regulatory role for WT1 in kidney development, although little is known about either its targets or which genetic cascades are affected by its abnormal function.

A wide variety of WT1 mutations is seen in DDS—a triad of intersex, nephropathy due to DMS, and Wilms tumor—making genotype-phenotype correlation difficult even with the aid of computer programs (Jeanpierre et al. 1998*a*). In contrast, FS is caused by specific intronic point mutations that disrupt the exon 9 alternative splice-donor site, reversing the normal WT1 +/− KTS isoform ratio (Klamt et al. 1998). It is also associated with intersex, but there is no predisposition to Wilms, and the nephropathy typically results from FSGS. These conditions act as human disease models of the effects of WT1 gene mutations and provide further strong evidence of WT1's crucial role in both renal and gonadal development. How WT1 mutations affect glomerular development remains a matter of debate; some mutations may be mediated during the progression of nephrogenesis, perhaps through aberrant interactions of WT1 with genes and proteins important for this process, thereby causing abnormal glomerular differentiation. It is equally possible is that the problem could lie in the terminally differentiated, nondividing podocytes, particularly if silencing of the normal allele were to occur, as in some tissues (e.g., placenta and brain [Jinno et al. 1994]), and if the mutant WT1 protein were incapable of maintaining normal podocyte function. Further study of animal models with targeted gene mutations may shed light on this increasingly complex picture.

Jeanpierre et al. (1998*b*) demonstrated that 4 of their 10 patients with DMS but no other features of DDS had mutations in exons 8 and 9 of WT1; 3 of these 4 patients were female. One mutation, 1147T→C (F383L) in exon 9, was novel. Two other mutations, 1186G→A (D396N) in exon 9 and 1129C→T (H377Y) in exon 8, had been found in previously reported cases of DDS. The fourth mutation, (+4C→T) in intron 9, had been shown to cause FS (Barbaux et al. 1997; Kikuchi et al. 1998; Klamt et al. 1998). Schumacher et al. (1998) studied a broader spectrum of patients with early-onset nephrotic syndrome, to identify possible WT1 gene mutations; two of four patients who had DMS but lacked other features of either DDS or FS had mutations; one of them had a newly discovered mutation, 1135G→T (G379C) in exon 8, and the other had a mutation, 1180C→T (R394W) in exon 9, that previously had been reported described in a case of DDS; both patients were female and were reported to have other unspecified features consistent with this syndrome.

We tested the hypothesis that WT1 gene mutations

Table 1

Results and Clinical Status

Case	Karyotype	Nephropathy				Syndromal Tumor?	Genital Status (Internal or External)	Other Renal or Extrarenal Defects?	Family History?	*WT1* Gene Mutation?	Age at Last Follow-up
		Onset of Proteinuria	ESRF/ Dialysis?	Nephrectomy?	Transplant?						
DMS:											
1	46,XX	Day 1	Yes	No	Yes	No	Female	Psychomotor	Yes	No; exons 1–10	10 years
2	46,XX	Day 1	Yes	Yes, right and left	Yes	No	Female	No	No	No; exons 1–10	4 years
3	46,XX	Day 1	Yes	Yes, right and left	No	No	Female	No	Yes	No; exons 1–10	8 years
4	46,XY	Day 1	Yes	No	No	No	Male	No	No	No; exons 1–10	(Died at age $3\frac{1}{2}$ years)
5	46,XY	>1 year	Yes	Yes, right and left	Yes	No	Male	No	No	No; exons 1–10	13 years
6	46,XY	>1 year	Yes	Yes, right and left	Yes	No	Male	No	No	No; exons 1–10	$11\frac{1}{2}$ years
7	46,XY	>1 year	No	No	No	No	Male	No	No	No; exons 1–10	3 years
8	46,XX	Day 1	No	No	No	No	Female	Psychomotor, hypothyroidism	No	No; exons 1–10	(Died at age 7 mo)
9	46,XY	6 mo	Yes	No	Yes	No	Male	Pulmonary stenosis	No	No; exons 1–10	$14\frac{1}{2}$ years
10	46,XX	>1 year	No	No	No	No	Female	Psychomotor	No	No; exons 1–10	(Died at age $3\frac{1}{2}$ years)
11	46,XY	18 mo	Yes	No	No	No	Male	No	No	No; exons 1–10	$5\frac{1}{2}$ years
12	46,XX	Day 1	Yes	No	No	No	Female	Psychomotor	?	No; exons 2–10	(Died at age 2 mo)
13	Unknown	Day1	Yes	No	No	No	Female	No	Yes	No; exons 2–10	(Died at age 7 mo)
14	46,XY	Day1	No	No	No	No	Female	No	No	Exon 4 polymorphism	(Died at age 3 mo)
15	46,XX	Day1	No	No	No	No	Female	Psychomotor	No	No; exons 2–10	(Died at age 10 mo)
16	46,XY	Day 1	No	No	No	No	Male	Cryptorchidism?	No	*Afl*III polymorphism	(Died at age 2 mo)
17	Unknown	Day 1	No	No	No	No	Unknown	No	No	No; exons 2–10	(Died at age 10 wk)
18	Unknown	Day 1	No	No	No	No	Unknown	No	No	No; exons 2–10	(Died at age 8 mo)
19	46,XX	1 year	Yes	No	No	No	Female	Left kidney dysplastic	No	No; exons 5, 8, 9	$2\frac{1}{2}$ years
20	46,XY	9 mo	No	No	No	No	Male	no	No	No; exons 5, 8, 9	14 mo
21	46,XY	>2 years	Yes	Yes, right and left	Yes	Wilms	Female	No	No	Exon 9: 1180C→T (R394W)	13 years
22	46,XY	1 year	Yes	Yes, right and left	Yes	Wilms	Ambiguous	No	No	Exon 9: 1180C→T (R394W)	$11\frac{1}{2}$ years
FSGS:											
23	46,XX	4 mo		No	No	No	Female	No	No	Exons 1–10	7 years
24	46,XX	>1 year	?	?	?	No	Female	No	Yes	Exons 1–10	Lost to follow-up
25	46,XY	>1 year	?	?	?	No	Male	No	Yes	Exons 1–10	Lost to follow-up
26	46,XX	Day 1	No	No	No	No	Female	No	Yes	Exons 1–10	$3\frac{1}{2}$ years
27	46,XY	18 mo	Yes	No	Yes	No	Male	No	Yes	Exons 1–10	13 years
28	46,XY	>1 year	Yes	?	Yes	No	Male	No	No	Exons 1–10	7 years
29	46,XX	1 year	Yes	Yes, right and left	No	No	Female	Spondyloepiphyseal dysplasia	No	Exons 1–10	(Died at age 6 years)
30	46,XY	5 years	8 years	No	Yes	Gonadoblastoma	Female	No	?	Intron 9 +5G→A	16 years

occur in cases of DMS and of congenital/early-onset FSGS occurring in the absence of other features of DDS or FS. Our intent was to identify how common mutations of the WT1 gene were in this population and to begin establishing the boundaries of the DDS/FS spectrum of disease.

A series of 30 patients, 22 with DMS and 8 with FSGS, were screened for mutations of WT1 (table 1). The diagnosis of DMS or FSGS was established on the basis of either renal biopsy or postmortem renal histological findings. The majority of surviving patients with DMS were followed-up with yearly renal ultrasounds, to exclude Wilms tumor. Twenty-seven of the 30 patients had documented karyotype analysis. Congenital malformations were investigated by clinical examination and the appropriate investigations. None of the patients with psychomotor abnormalities were given a diagnosis of Galloway-Mowat syndrome. Abnormalities of the internal gonads were examined by pelvic ultrasound, and in some cases, by laparotomy. Patient 14 had an equivocal report with regard to karyotype—and, therefore, possible intersex status—but no other features of DDS. Two patients with DMS (patients 21 and 22) had DDS with known 1180C→T (R394W) mutations in exon 9 and were used as positive controls. Genomic DNA was obtained from blood of 23 patients and from paraffin-section material in 7 patients, by standard phenol-chloroform extraction methods. The two most common DDS mutations located in exon 9 (i.e., 1180C→T [R394W], 60%; and 1186G→A [D396N], 15%) abolish an *Rsr*II site in zinc finger 3 (Little et al. 1993). Heterozygous loss of the site was observed in patients 21 and 22, as expected, whereas patients 1–20 showed normal restriction-enzyme digests. We used SSCP to analyze exons 1–10 after PCR amplification, using oligonucleotides that had also been published by Baird et al. (1992*a*) and radiolabeling them with [^{32}P]. Products were electrophoresed, at 4°C, on both 5% and 10% nondenaturing polyacrylamide gels. In addition, exons 5, 8, and 9 were directly sequenced on an ABI 377 automated sequencer. PCR products were purified either on spin columns (Promega Biotec) or by gel extraction (Qiagen Gel Extraction Kit). Samples (2–5 μl) of the purified product were sequenced by use of a Thermo-Sequenase dye terminator cycle sequencing kit and 5 pmol of the original primer.

No WT1 mutations were detected in either the 20 patients with isolated DMS or the 7 patients with isolated FSGS, although patient 14 demonstrated a newly discovered polymorphism in codon 178 C→T in exon 4, which conserves a threonine, and patient 16 a demonstrated previously reported (Groves et al. 1992) polymorphism, an A→G transition in codon 313 (arginine) in exon 7, which destroys an *Afl*III restriction-enzyme recognition site. Both DDS patients showed the expected (as reported by Baird et al. 1992*b*) 1180C→T mutations in exon 9; in addition, a +5G→A mutation in intron 9 was detected during this study in patient 30, who had classic features of FS (previously reported by Klamt et al. 1998).

In conclusion, when a larger, more generalized population of cases with DMS is examined, mutations of WT1 are both less frequent than initial data have suggested and absent in isolated FSGS. This confirms the genetic heterogeneity of DMS and FSGS, despite the uniform renal histological findings seen throughout this group of conditions. Supportive evidence comes from linkage studies of familial FSGS, which show that the candidate gene for this condition lies on chromosome 1q25-q31 (Fuchshuber et al. 1995). Isolated DMS and FSGS may therefore also result from abnormalities of other glomerular genes, perhaps downstream of WT1 and mimicking the effects of WT1 mutations seen in DDS and FS.

We propose that, if WT1 gene mutations are present in isolated renal DMS or FSGS, this lends strong support to a diagnosis of DDS or FS, and the spectrum of DDS should be broadened to include occasional cases in which the characteristic nephropathy does occur alone. In this situation, WT1 mutations appear more common in phenotypic females, which affirms the less critical role of WT1 in female gonadal development, as has been suggested by experimental data (Nachtigal et al. 1998). This finding also correlates with cases of FS in which individuals with characteristic WT1 mutations in intron 9 and with a 46,XX karyotype develop nephropathy but have no obvious gonadal abnormality (Klamt et al. 1998). Karyotype analysis remains the most important first-line investigation in phenotypic females with isolated renal DMS or FSGS; however, WT1 mutation analysis should also be considered in isolated DMS and FSGS, since the presence of characteristic WT1 gene mutations may be important in the determination of Wilms tumor risk, especially in 46,XX individuals who may not demonstrate any other clues for an underlying diagnosis of DDS.

Acknowledgments

A.B.K. is funded by a National Kidney Research Fund Twistington Higgins Fellowship. We would like to thank the renal units throughout the United Kingdom, which kindly provided patients and clinical information for this study.

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Electronic-Database Information

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nim.nih.gov/Omim (for FS [MIM 136680], DDS [MIM 194080], DMS [MIM 256370], and FSGS [MIM 603278])

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0002-9297/99/6406-0032$02.00