abstract

The renal prognosis of patients with Wilms’ tumor, aniridia, genitourinary anomalies, and mental retardation syndrome (WAGR) is poor. However, the renal histology and its mechanisms are not well understood. We performed renal biopsies in 3 patients with WAGR syndrome who had heavy proteinuria. The complete deletion of one WT1 allele was detected in each patient by constitutional chromosomal deletion at 11p13 using G-banding, high-resolution G-banding, and fluorescence in situ hybridization. The patients exhibited proteinuria at the ages of 6, 10, and 6 years and were diagnosed as having focal segmental glomerulosclerosis (FSGS) at the ages of 7, 16, and 19 years, respectively. They exhibited normal or mildly declined renal function at the time of biopsy. Re-examination of a nephrectomized kidney from 1 patient revealed that some glomeruli showed segmental sclerosis, although he did not have proteinuria at the time of nephrectomy. The other 2 patients did not develop Wilms’ tumor and thus did not undergo nephrectomy, chemotherapy, or radiotherapy, thereby eliminating any effect of these therapies on the renal histology. In conclusion, complete deletion of one WT1 allele may induce the development of FSGS. Our findings suggest that haploinsufficiency of the WT1 could be responsible for the development of FSGS.
Miller et al first described WAGR syndrome (Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation). Children with WAGR syndrome invariably have a constitutional chromosomal deletion at 11p13, the region where the WT1 gene is located. Patients with Denys-Drash syndrome (DDS) usually have a germline missense mutation, which is predicted to result in an amino acid substitution in the eighth or ninth which is predicted to result in an amino acid substitution in the eighth or ninth exon of WT1. Little et al suggested that the severe nephropathy associated with DDS, which frequently leads to early renal failure, might result from the dominant-negative action of altered WT1. By contrast, because of the less severe genital anomalies and apparent lack of nephropathy associated with WAGR, a reduced WT1 dosage during embryogenesis is thought to have a less pronounced effect on development, especially on renal system development. Breslow et al reviewed nearly 6000 patients enrolled in 4 clinical trials administered by the US National Wilms Tumor Study Group between 1969 and 1995. Of 22 patients with DDS, 13 (59%) developed renal failure; of 46 patients with WAGR, 10 (22%) developed renal failure. The cumulative risks of renal failure at 20 years were 62% and 38%, respectively. These findings suggest that nephropathy is not uniquely associated with WAGR and is not associated with missense mutations in WT1 and that patients with the WAGR syndrome should be followed up closely throughout life for signs of nephropathy.

The renal prognosis of patients with WAGR is poor. However, the renal histology and its mechanisms are not well understood. We therefore performed renal biopsies to reveal the renal pathology in 3 patients with WAGR syndrome who had heavy proteinuria.

**CASE REPORTS**

**Patient 1**

Patient 1 was a male diagnosed with bilateral microphthalmos at 1 month of age. Wilms’ tumor developed bilaterally at 3 years of age. He also had undescended testes and mental retardation. Previous analysis of G-banded metaphase chromosomes revealed a deletion of chromosome 11p13-15.1 in one allele; the diagnosis of atypical WAGR syndrome was therefore made. Because of a large tumor in the right kidney after the first chemotherapy treatment, the right kidney was nephrectomized. A diagnosis of nephroblastoma (nephroblastic type) was made. At the same time, the contralateral left kidney was biopsied, but no tumor was detected. The nephrectomized kidney revealed that there were no immature glomeruli, and a few glomeruli showed segmental sclerosis (Fig 1 A and B). The patient did not have proteinuria at the time of nephrectomy although microalbuminuria could have been detected. The patient then underwent a second session of chemotherapy and radiotherapy treatment with left kidney protection. He developed heavy proteinuria at 6 years of age. The left kidney was biopsied (open biopsy) at age 7 years. Renal biopsy findings were consistent with focal segmental glomerulosclerosis (FSGS) (Fig 1 C and D). At the time of biopsy, the patient’s height was 169.2 cm, his weight was 21.7 kg (–0.7 SD), and blood pressure was 120/80 mm Hg. Biochemical data were as follows: total protein, 6.8 g/dL; albumin, 4.3 g/dL; BUN, 25.0 mg/dL; creatinine, 1.20 mg/dL; 24-hour CrCl, 91.0 mL/min/1.73 m²; early morning urinary protein, 3+ (as measured by using a dipstick test); urinary protein to urinary creatinine ratio, 2.7 (milligram/milligram); daily urinary protein, 3.1 g; and urinary β-2 microglobulin, 0.064 mg/dL. At the latest follow-up (24 years of age), his renal function was stable (BUN: 25.0 mg/dL; creatinine: 1.20 mg/dL) with ACEI treatment, and he had not developed Wilms’ tumor.

**Patient 2**

Patient 2 was a male with aniridia, bilateral undescended testes, hypospadias, grade III to IV bilateral vesicoureteral reflux, and mental retardation. High-resolution G-banding revealed deletion of chromosome 11p13-p14.2 in one allele (Fig 2A), and fluorescence in situ hybridization showed heterozygous deletions of PAX6, D11S2163, PER, and WT1 (Fig 2B), indicating WAGR syndrome. He had a single febrile urinary tract infection at 2 years of age and underwent an antireflux operation at 4 years of age, which resolved his vesicoureteral reflux. A dimercaptosuccinic acid radionuclide scan showed several defects in his right kidney. His proteinuria was detected at 10 years of age by the school urinary screening program. His proteinuria gradually increased, and he underwent renal biopsy (right kidney) at age 16 years. Renal biopsy findings were consistent with FSGS (Fig 1 E and F). At the time of biopsy, the patient’s height was 169.2 cm, his weight was 67.4 kg, and blood pressure was 128/78 mm Hg. Biochemical data were as follows: total protein, 6.8 g/dL; albumin, 4.3 g/dL; BUN, 25.0 mg/dL; creatinine, 1.20 mg/dL; 24-hour CrCl, 91.0 mL/min/1.73 m²; early morning urinary protein, 3+ (as measured by using a dipstick test); urinary protein to urinary creatinine ratio, 2.7 (milligram/milligram); daily urinary protein, 3.1 g; and urinary β-2 microglobulin, 0.064 mg/dL. At the latest follow-up (24 years of age), his renal function was stable (BUN: 25.0 mg/dL; creatinine: 1.20 mg/dL) with ACEI treatment, and he had not developed Wilms’ tumor.

**Patient 3**

Patient 3 was a female with aniridia and mental retardation. G-banding revealed deletion of chromosome 11p13-p14 in one allele (Fig 2G), and she was therefore diagnosed with WAGR syndrome. The patient developed proteinuria at
the age of 6 years and nephrotic syndrome with normal renal function at age 15 years (urinary protein to urinary creatinine ratio, 10.6 [milligram/milligram]; total protein, 5.6 g/dL; albumin, 2.5 g/dL; BUN, 30.0 mg/dL; creatinine, 1.40 mg/dL; 24-hour CrCl, 44.65 mL/min/1.73 m²; early morning urinary protein, 3+ (as measured by using a dipstick test); daily urinary protein, 5.89 g; and urinary β-2 microglobulin, 0.495 mg/dL. Renal biopsy findings were consistent with FSGS (Fig 1 G and H). To date, she has not developed Wilms’ tumor.

**DISCUSSION**

The current study demonstrated that 3 patients with atypical WAGR syndrome developed heavy proteinuria with FSGS, suggesting that the nephropathy seen in this syndrome is responsible for the FSGS lesion. Patient 1 had possible bilateral Wilms’ tumor and underwent unilateral nephrectomy, chemotherapy, and radiotherapy. Therefore, it is possible that the treatment of the remaining kidney for bilateral tumor or nephrogenic rest might account for the development of FSGS. However, the kidney nephrectomized after the first chemotherapy session but before radiotherapy treatment already showed segmental sclerosis in a few glomeruli, suggesting that radiotherapy was not the main cause of FSGS. Chemotherapeutic drugs such as adriamycin may induce FSGS as well as tubulointerstitial inflammation and fibrosis. However, there were no tubulointerstitial lesions, suggesting that chemotherapy might not have been the main cause of FSGS. Nevertheless, it is possible that surgical renal ablation caused FSGS in patient 1. Patients 2 and 3 did not develop Wilms’ tumor during the course of clinical observation, and thus they did not undergo nephrectomy, chemotherapy, or radiotherapy, thereby eliminating any effect of these therapies on renal

**FIGURE 1**

Renal histology. A, C, E, and G, Low magnification. B, D, F, and H, High magnification. Arrows show glomeruli with segmental glomerulosclerosis. A and B, Nephrectomized right kidney from patient 1. Patient 1 had no proteinuria at the time of nephrectomy. However, a few glomeruli exhibited segmental glomerulosclerosis although there were no immature glomeruli. C and D, Renal biopsy of left kidney from patient 1. Twenty-eight of 50 glomeruli showed segmental glomerulosclerosis. There were no tubulointerstitial lesions. E and F, Renal biopsy from patient 2. Two of eight glomeruli showed segmental glomerulosclerosis with interstitial fibrosis. G and H, Renal biopsy from patient 3. Ten of 30 glomeruli showed segmental glomerulosclerosis with interstitial fibrosis. All 3 patients exhibited FSGS (not otherwise specified).
The possibility of reflux nephropathy, however, could not be ruled out in patient 2. The perihilar variant with glomerular hypertrophy is particularly common in the secondary FSGS such as reduced renal mass–induced FSGS. However, all 3 patients exhibited FSGS (not otherwise specified) without glomerular hypertrophy, suggesting that surgical renal ablation (patient 1) and reflux nephropathy (patient 2) may not have been the main cause of FSGS in these 2 patients. These findings suggest that the complete deletion of one WT1 allele might have a pathogenetic role in the development of nephropathy.

The spectrum of glomerular diseases associated with WT1 mutations has been reviewed. WT1 mutations can cause syndromic and nonsyndromic glomerular disease. The syndromic forms include DDS (early-onset nephrotic syndrome with diffuse mesangial sclerosis [DMS]); 46,XY disorders of sex development and Wilms’ tumor; and Frasier syndrome (disorders of sex development, FSGS, and gonadoblastoma), which is caused by a mutation in the intron 9 splice site of WT1 leading to the loss of the +KTS isoform of the protein. Mutations associated with both syndromic and nonsyndromic glomerular disease tend to cluster in exons 8 and 9 of WT1, which encode zinc fingers 2 and 3. Orloff et al reported that single-nucleotide polymorphisms in WT1 may modulate the development of FSGS by altering WT1 function. The current study suggests that complete deletion of one WT1 allele may also induce the development of nephropathy.

Reduced expression levels of Wt1-induced glomerulopathies (crescentic glomerulonephritis or DMS) depending on gene dosage derived by combining Wt1-knockout mice and an inducible WT1 yeast artificial chromosome transgenic mouse model. Eleven percent of mice heterozygous for the WT1 mutation showed severe proteinuria and DMS with tubular cysts, protein casts, and severe interstitial inflammation, although nephrogenesis was not delayed. These findings indicate that the expression level of WT1 plays an important role, not only during nephrogenesis but also in the homeostasis of normal kidney function. These findings also support our conclusion that complete deletion of one WT1 allele in atypical WAGR syndrome could induce glomerulopathy without delayed nephrogenesis, although the reason for the discrepancy in histologic findings between man (FSGS) and mouse (DMS) is unclear.

CONCLUSIONS

Besides dominant-negative missense mutations in the eighth or ninth exon of WT1 and mutations at the donor splice site of intron 9, complete deletion of one WT1 allele may induce the development of FSGS. The findings in this study also suggest that haploinsufficiency of WT1 could be responsible for the development of FSGS.

REFERENCES


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