Genetic Factors Associated with Congenital Anomalies of the Kidney and Urinary Tract Associated and a Spectrum of Extrarenal Disorders

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Abstract

Background. Several genes, including those of the renin-angiotensin system (RAS), act in concert to guide mammalian renal development from early nephrogenesis to definitive nephron formation. Aberrant forms of genes involved in nephrogenesis alter kidney development, leading to congenital anomalies of the kidney and urinary tract.

Methods. We analyzed the main genes involved in nephrogenesis, PAX2, RET and RAS-related genes, in 17 children with renal dysmorphism.

Results. A total of 17 cases were reviewed; pathogenic variants were identified in four and incidental variants in three. Two of three patients with PAX2 abnormalities manifested renal coloboma syndrome. No eye lesion was present in the third, who exhibited a frame-shift mutation from heterozygous insertion of T in exon 11; facial and skeletal abnormalities were detected. In analysis of RAS-related genes, an angiotensinogen gene (AGT) mutation (M268T) was identified in a patient exhibiting dwarf kidney, facial and skeletal abnormalities, mental retardation, and pituitary hyperplasia. Another patient exhibited an angiotensin II receptor type 1 (AGTR1) gene mutation (p.L191L). A renal specimen showed tubular dysgenesis; extrarenal abnormalities included skull ossification defects. RET abnormality was detected in two patients with heterozygous mutations. One exhibited Hirschsprung disease and right renal agenesis. The other exhibited oligomeganephronia and hypothryoidism but not multiple endocrine neoplasia nor medullary thyroid carcinoma (MTC); the etiology of these abnormalities is unclear, as is the patient’s risk for MTC. Follow-up is necessary.

Conclusions. Gene aberration was detected in seven of 17 patients (41%). Unidentified genetic causes may contribute to pathophysiology in the remaining 10 patients.

Key words: gene mutation, renal dysmorphism, extrarenal abnormalities

Introduction

Most of the kidney in adult mammals develops from the metanephros, which begins differentiation upon extension of the ureteric bud (UB) from the caudal Wolffian duct to the dorsal metanephric mesenchyme 1. During nephrogenesis, a network of several genes, including those that encode the renin-angiotensin system (RAS), direct branching of the UB along with the formation and action of the mature nephrons 2. Aberrant forms of genes involved in nephrogenesis can alter kidney development, leading to congenital anomalies of the kidney and urinary tract (CAKUT). CAKUT is a spectrum of structural renal malformations, including hydronephrosis, renal agenesis, renal hypodysplasia, multicystic dysplastic kidney, ureteropelvic junction obstruction, vesicoureteral reflux (VUR), ureter duplex, megaureter, and posterior urethral valves 3. CAKUT can lead to chronic renal failure in children, and the prevalence of these malformations.
The PAX2 gene, a member of the PAX family, controls other genes through directed synthesis of the protein paired box and alterations in DNA binding. During the embryonic stage, PAX2 is involved in the development of the eyes, ears, central nervous system, and urogenital system. PAX2 abnormality may result in renal coloboma syndrome (RCS). PAX2 is expressed in the mesoderm, causing formation of the metanephros, Wolffian duct, and UB during nephrogenesis. When PAX2 expression is reduced, the number of nephrons decreases and subsequent renal hypoplasia occurs. Abnormality of PAX2 also impairs myelination in visual, auditory and central nervous system structures, resulting in related morphologic and functional abnormalities. The RAS genes are important in maintaining physiological functions, including regulation of blood pressure and balance of electrolytes; these genes also contribute to organogenesis, including formation of the metanephric kidney. RAS gene aberrations induce diverse clinical phenotypes, including abnormal renal tubule formation, ureteropelvic junction stenosis, megaloureter, multicystic dysplastic kidney, and posterior urethral valves. HNF1β is a transcription factor that has been linked to proximal tubular differentiation in mouse studies. HNF1β mutations in humans are associated with a broad spectrum of diseases, including renal cysts, diabetes, and maturity onset diabetes of the young–type 5, as well as hepatic, genital, and pancreatic abnormalities that lead to a variety of renal and extrarenal manifestations. SALL1 functions in anal, limb, and ear development as well as renal development; its mutations cause Townes-Brock syndrome.

A prior study used targeted exome sequencing to screen 122 CAKUT patients and discovered that 5% of these patients harbored deleterious rare variants or novel mutations in the GDNF-GFRα1-RET pathway. Genetic causes of CAKUT are associated with a wide range of extrarenal complications; thus, genetic analysis can aid in understanding the pathophysiology of CAKUT. Our purpose in this study was to use a genetic approach to identify the etiology of CAKUT in patients. We analyzed PAX2, RET and multiple RAS genes (angiotensinogen, angiotensin II (Ang II), and Ang II receptor types 1 and 2) in 17 pediatric patients with abnormal renal formation who were referred to our department.

## Materials and Methods

### Subjects

Our subjects included 17 patients with congenital renal dysplasia who were referred to our department.

### Methods

Genomic DNA extraction, polymerase chain reaction (PCR), and determination of gene sequences

Approximately 5 mL of peripheral blood was collected from patients into tubes containing Na-EDTA; genomic DNA was extracted from the blood using NucleoSpin for Blood (TaKaRa Bio Inc, Shiga, Japan). Human genomic DNA (Clontech, code 636401, CA) was used as a control. Patient samples and control genomic DNA were diluted with sterile water to prepare 10 ng/μL solutions. PCR was performed using the diluted DNA solutions as templates; the reaction was performed in a PCR Thermal Cycler Dice Gradient (TaKaRa Bio Inc, Shiga, Japan). To determine the extent of deletions and to identify break points, PCR primers were designed to amplify fragments of approximately 200–300 bp, based on gene sequences registered in GenBank. Primers for amplifying PAX2 exon 11 were 5’-ATGTCTCCTCACCCGTGGATC-3’ (forward, F) and 5’-AGGCCCAGGCCTAACCTGCTAAA-3’ (reverse, R); angiotensinogen (AGT) primers were 5’-GATCTGGTTAGATGGCACTTA-3’ (F), and 5’-AAAGGTGGAGACTGGGGGTG-3’ (R); angiotensin receptor 1 (AGTR1) primers were 5’-GTTACTACGTATGACTGAG-3’ (F), and 5’-CCACATAATGCATTTCGTGCCT-3’ (R); angiotensin receptor 2 gene (AGTR2) primers were 5’-CTAATGATTCAAGGATGTCCT-3’ (F), and 5’-CATTTCCAGAACAGGTAGGGGA-3’ (R); RET exon4 primers were 5’-CACAGTCATCGCTGCAAACCTCGTC-3’ (F), and
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5'-GAGGTGATCCGCTTCCGTCCTTCTCCAA-3' (R). For PCR, the amplification protocol for PAX2 was 94°C for 30 seconds, 64°C for 30 seconds (annealing), and 72°C for 1 minute (extension), repeated for 30 cycles; the protocol for AGT, AGTR1 and AGTR2 was 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds, repeated for 35 cycles; and the protocol for RET was 98°C for 10 seconds, 63°C for 15 seconds, and 68°C for 1 minute, repeated for 30 cycles.

Direct sequencing

PCR products were enzymatically purified and templates for sequencing were prepared. The sequencing reaction was performed using the DNA templates and the dye terminator method of the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). Reaction products were purified by gel filtration, and sequence analysis was performed with a capillary-type sequencer, ABI3730xl (Applied Biosystems, CA, USA).

Results

Gene aberrations were detected in four of the 17 patients (PAX2, 3; RET, 1); incidental variants were detected in three patients (RAS genes, 2; RET, 1). Clinical features and gene mutations in these seven patients are summarized in Table 1. Unreported 5 cases are described below.

1. PAX2 gene aberration

Patient 2 exhibited occult blood in the urine at 7 years of age. Imaging revealed right renal hypoplasia (Figure 1a). Extrarenal abnormalities included characteristic facial features (acrocephaly, hypertelorism, ectropium palpebrae spasticum, and saddle nose; Figure 1b) and a supernumerary rib (Figure 1c). Visual acuity and fields were normal; no abnormalities were detected in the retina or optic nerve. Family history was noncontributory. His most recent (15 years of age) creatinine clearance rate (Ccr) was 58.4 mL/min/1.73m². Gene analysis identified a heterozygous frame-shift mutation, with an insertion of T in exon 11 (nucleotide or NT position, 21 336 271-21 336 272; Figure 1d). The left kidney appeared normal by imaging, but abnormally few glomeruli (3.6/mm²), which were enlarged, were evident in a renal biopsy specimen; some were segmentally sclerotic (Figure 1e).

Patient 3 exhibited proteinuria at 6 years of age. Imaging revealed bilateral renal hypoplasia (Figure 2a). Extrarenal symptoms include strabismus, first noted at 2 years; at that time, she was diagnosed with optic nerve hypoplasia. Her father exhibited end-stage renal disease and visual impairment of unknown cause. Her most recent Ccr (14 years of age) was 38 mL/min/1.73m², demonstrating progression of renal insufficiency. Gene analysis identified a heterozygous frame-shift mutation, with C inserted in exon 2 (NT position, 21 258 147-21 258 148; Figure 2b). A similar mutation was identified in her father.

Table 1  Clinical features and mutated genes in patients with renal dysplasia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Physical growth delay</th>
<th>Mental retardation</th>
<th>Facial dysmorphism and skeletal anomaly</th>
<th>Renal dysplasia</th>
<th>Ocular complication</th>
<th>Other complication</th>
<th>Genetic defect</th>
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<tr>
<td>1</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>Bil. hypoplasia</td>
<td>Coloboma</td>
<td>—</td>
<td>PAX2</td>
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<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>Eurypenia, oxycephalus, saddle nose</td>
<td>R. hypoplasia</td>
<td>Ectopia palpebrar</td>
<td>Supernumerary rib</td>
<td>PAX2</td>
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<tr>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Bil. hypoplasia</td>
<td>Coloboma</td>
<td>—</td>
<td>PAX2</td>
</tr>
<tr>
<td>4</td>
<td>Short stature</td>
<td>+</td>
<td>Low-set ear, euryopia, oxycephalus, saddle nose</td>
<td>L. hypoplasia</td>
<td>Microphthalmia, amblyopia</td>
<td>Pituitary hyperplasia</td>
<td>AGT</td>
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<tr>
<td>5</td>
<td>Short stature</td>
<td>+</td>
<td>Narrow chest, thorax, slender arm and leg</td>
<td>Bil. hypoplasia</td>
<td>—</td>
<td>Renal tubular dysgenisis</td>
<td>AGTR1</td>
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<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Bil. hypoplasia</td>
<td>—</td>
<td>Hypothyroidism</td>
<td>RET</td>
</tr>
<tr>
<td>7</td>
<td>Short stature</td>
<td>—</td>
<td>—</td>
<td>R. agenesis</td>
<td>—</td>
<td>Hirschsprung’s disease</td>
<td>RET</td>
</tr>
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Abbreviations: bil. bilateral; R. right; L. left
Figure 1  Patient 2. Images, facial features, and histologic findings of the kidney (periodic acid-Schiff stain, original magnification, x40), and gene analysis findings.

The right kidney is hypoplastic (CT in a, indicated by circle), and a supernumerary right rib (radiograph in c, indicated by arrow) is present. Facial features are abnormal (acrocephaly, hypertelorism, ectropium palpebrae, and saddle nose; b). On histologic examination of the kidney, glomeruli are few (3.6/mm²) and enlarged; with some exhibiting segmental sclerosis (e). On PAX2 gene analysis, a frame-shift mutation caused by heterozygous insertion of thymine (T) is detected in exon 11 (d, indicated by circle).

Figure 2  Abdominal CT findings and PAX2 gene analysis in Patient 3.

Both kidneys are hypoplastic (a). Gene analysis shows a frame-shift mutation involving heterozygous insertion of cytosine (C) in exon 2 (b, indicated by circle).

2. RAS gene aberration

Patient 4 was born at 39 weeks and 2 days of gestation; birth height and weight were 44 cm and 2204 g. He exhibited unusual facial features when he was 1 year old. He also exhibited a left dwarf kidney with small cysts (Figure 3a). Extrarenal abnormalities included unusual facial features (acrocephaly, microphthalmia, saddle nose, and low-set ears; Figure 3b); mental retardation (IQ, 42); pituitary hyperplasia; and foramen magnum stenosis (Figure 3c). His abdomen was flat, upper and lower limbs were slender and elongated, stature was small (15 years of age): height, 148 cm (-2.5 SD); body weight, 35 kg (-2.0 SD). Family history was noncontributory. Current renal function was stable. Gene analysis identified a homozygous mutation of M268T (T/T→C/C) in exon 2 of AGT (Figure 3d).

Patient 5 was born at 37 weeks and 1 day of gestation; birth weight was 2374 g. Tachypnea and cyanosis were evident after birth, and no urination occurred during the first 24 hours of life. Renal function abnormalities and bilateral dwarf kidneys were detected (Figure 4a) when he was referred to our department. Notably, his mother was not prescribed angiotensin inhibitors or receptor antagonists during pregnancy. His present height (4 years old) was 90 cm (-3.3 SD), and
body weight was 10.9 kg (-2.6 SD). Extrarenal abnormalities included mental retardation and skeletal defects such as a small abdomen, slender upper and lower limbs, and areas of skull thinning (Figure 4b). Urinalysis revealed low specific gravity and excessive urinary β₂-microglobulin (19420 μg/L). A renal biopsy specimen obtained at 3 years of age showed marked cystic dilation of renal tubules, mainly in distal tubules (Figure 4c). Gene analysis identified a heterozygous mutation, p.L191L (C/C→T/C), in exon 5 of AGTR1 (Figure 4d).

Figure 3  Patient 4. CT findings, facial features, renal histologic findings, and AGT gene analysis. The left kidney is hypoplastic (a). Abnormal facial feature such as acrocephaly, microphthalmia, saddle nose, and low-set ears, are noted (b). In magnetic resonance imaging of the head (c), pituitary hyperplasia (indicated by circle) and foramen magnum stenosis (indicated by arrow) are evident. Gene analysis (d) shows homozygous mutation of M268T in exon 2 in the patient, while the father and mother each show a heterozygous G/G mutation.

Figure 4  Patient 5. Imaging findings of the kidneys (a, b), renal histologic findings (c), and AGTR1 gene analysis (d). Bilateral dwarf kidneys are seen to contain small cysts (a). The left kidney shows dilation of the renal pelvis. Ossification of the skull is partially defective (b, indicated by arrow). On histologic examination of the kidney, renal tubules are markedly dilated; findings are suggestive of renal tubular dysgenesis (c). AGTR1 gene analysis discloses a heterozygous mutation, p.L191L, in exon 5 (d).
3. RET gene aberration

Patient 6 had no adverse perinatal events and a normal birth weight. Renal dysfunction was detected at 1 year of age (blood urea nitrogen (BUN), 24 mg/dL; serum creatinine (s-Cr), 0.6 mg/dL). Increased serum thyroid-stimulating hormone (TSH; 12.3 mU/mL) was noted, but free thyroid hormone concentrations (FT3, FT4) were normal. No auto-antibodies, including anti-thyroid receptor or anti-thyroid peroxidase antibodies, were detected. Currently, (23 years of age), TSH was normalized because of thyroxine administration.

No imaging or laboratory findings have suggested medullary thyroid cancer (MTC). Renal ultrasonography at 21 years of age showed both kidneys to be small. Irregular parenchymal echoes were seen; overall echogenicity was increased (Figure 5a). Ccr was 61.3 mL/min/1.73m², indicating mild renal insufficiency. On histologic examination of a renal biopsy specimen, there were few glomeruli (5/mm²), which were enlarged (oligomeganephronia; Figure 5b). Gene analysis identified a heterozygous mutation of T278N (C/C→C/A) in exon 4 (Figure 5c).

Discussion

PAX2 encodes a critical transcription factor that is expressed in the nephron progenitors. PAX2 mutations are most commonly associated with RCS (also called papillorenal syndrome), an autosomal dominant disorder that is characterized by optic nerve malformations (optic nerve coloboma, optic nerve dysplasia) and renal defects (oligomeganephronia, hypodysplasia with or without VUR, and renal cysts). Other, less common extrarenal manifestations include sensorineural hearing loss and brain malformations. PAX2 gene aberration was detected in three patients in our study. Patient 1, who was previously described, manifested P130H as a novel mutation; however, most clinical features of RCS were present. Patient 3 also developed common clinical findings of RCS; this represents a paternally-inherited case with a frame-shift mutation induced by heterozygous insertion of cytosine (C) in exon 2. PAX2 abnormalities associated with RCS include five types of frame-shift mutation in exons 2 or 5; two types of missense mutations in exon 3; and observation of chromosomal translocation of PAX 2 introns; among these, 619insG in exon 2 has been reported most frequently. The frame-shift mutation in exon 2 (in our Patient 3) has not been reported previously. On the other hand, no ocular abnormalities were observed in Patient 2, but skeletal malformations were present, including abnormal facial features and a supernumerary rib. During prenatal development, PAX2 first is expressed in the future midbrain and hindbrain, where it acts in migration and proliferation of cranial neural crest (CNC) cells. PAX2 also acts in craniofacial morphogenesis and skeletal
structure by promoting differentiation of CNC-derived tissues. A frame-shift mutation from heterozygous insertion of thymidine (T) into exon 11 was detected in Patient 2, where the abnormal differentiation of CNC-derived tissues may have caused facial dysmorphism and skeletal abnormalities instead of the typical RCS phenotype associated with PAX2 gene aberrations. The degree of optic nerve coloboma varies, both between patients and between each eye in an individual, even in patients with identical PAX2 mutations. Moreover, a recent study revealed that identical twins with PAX2 mutations exhibited different eye and kidney abnormalities. Consistent with our study, point mutations within the coding region of PAX2 may not represent the only cause of RCS. Analysis of the PAX2 gene may be indicated in patients with both facial dysmorphisms and urinary tract abnormalities. PAX2 gene aberrations may be associated with oligomeganephronia, as shown in renal biopsy specimens. Taken together, these findings indicate that multiple factors may exert additive effects on the progression of kidney and eye dysfunctions.

The RAS system plays a critical role in blood pressure and fluid/electrolyte homeostasis. In the kidney, RAS components are expressed in both temporal- and spatial-dependent manners during UB branching and nephron formation; notably, Ang II participates in nephron formation. In Patient 4, we observed hypoplasia of the left kidney, mental retardation, and pituitary hyperplasia. The causal relationship between the AGT gene abnormality and the pituitary defect in this child is unclear; however, RAS genes play a role in maintenance of cerebral microvascular circulation and differentiation, as well as maturation of nerve cells. Yosypiv et al. reported large low-set ears, limb-position defects, arthrogryposis, and skull ossification defects as extrarenal consequences of AGT gene aberration. Ang II is involved in bone metabolism; importantly, abnormality of the AGT gene encoding the precursor of Ang II in Patient 4 would likely result in synthesis of an incomplete form of Ang II, which may be responsible for the patient’s skeletal abnormalities. In Patient 5, renal biopsy showed that renal tubules were markedly dilated, consistent with renal tubular dysgenesis (RTD). RTD is characterized by anuria immediately after birth due to hypoplasia of proximal tubules; skull ossification defects are also common. Early death from pulmonary hypoplasia is likely. RTD inheritance is autosomal dominant and involves various abnormalities of RAS genes. In Patient 5, anuria and tachypnea were noted immediately after birth; however, the survival of the patient suggests that some functional activity remains in the defective mutant protein encoded by AGTR1. At present, this patient and Patient 4 exhibit similar mental retardation phenotypes. Further, Takeshita et al. reported mental retardation associated with AGTR2 gene aberration, suggesting that the RAS system is important for neuronal development. Since the AGTR1 mutation in patient 5 may serve as a synonymous substitution, the exact cause of the neuronal defects is still unknown. However, Patient 5 exhibited RTD and skeletal abnormalities characterized by gene mutations in the RAS system. Other genes such as REN (renin), which causes RTD, may be involved.

The RET gene, located at q.11.2 of chromosome 10, is a proto-oncogene encoding receptor tyrosine kinase. The RET protein, which complexes with glial cell-derived neurotrophic factor (GDNF) and its receptor, GFRα1, is involved in cell proliferation and differentiation in the kidney, nervous system, and genital organs. During kidney development, signal transduction through the GDNF-RET system is essential for sprouting of the UB, followed by branching of the UB through GDNF-RET interactions with several genes. RET mutations can result in either loss-of-function or gain-of-function mutations. Heterozygous mice (GDNF+/-) exhibit significantly fewer nephrons that display structural changes on histological analysis, such as enlargement of podocytes with increased cytoplasmic vacuolization and marked thickening of the glomerular basement membrane. Similar findings were also recorded in Patient 6, indicating that RET mutation may contribute to renal dysplasia. Additionally, loss of RET gene function is involved in development of the extrarenal pathology, Hirschsprung disease (HSCR). Conversely, gain-of-function mutations are involved in multiple endocrine neoplasia (MEN) syndromes: MTC and pheochromocytoma. In Patient 6, renal dysplasia (loss-of-function) and hypothyroidism (possible gain-of-function) may have occurred simultaneously as a result of the heterozygous T278N mutation. However, MTC did not develop. Since this mutation affects the extracellular cadherin-like domain of RET, it may influence binding between GDNF and GFRα1. Epithelial cells in the thyroid follicle arise from thyroid stem cells; abnormal
rearrangement of the RET/PTC gene inhibits this differentiation process and can cause carcinogenesis\(^3\). The resulting incompletely differentiated follicular cells might exhibit impairment of thyroid hormone secretion, with increased TSH required for adequate secretion. MEN and MTC are possible consequences of gain-of-function mutations in the RET gene. However, one patient with an RET gene mutation showed hypothyroidism accompanying Hashimoto disease; this patient also exhibited cystic renal lesions\(^3\). A patient with simultaneous MEN and HSCR also has been reported\(^4\), implying the presence of a gene mutation that results in both loss and gain of function. Patient 7, who was previously described\(^4\), had a p.S811F mutation in exon 14. Reduction of RET gene expression to 30% of normal levels led to HSCR-like neural defects in the distal large intestine; however, no renal consequences were observed in a mouse model. In a RET(−/−) mouse model, neural elements in the distal large intestine were lost and marked renal hypoplasia was observed\(^4\), suggesting that the RET mutation in our patient substantially interfered with a functioning protein product. On analysis of molecular structure, we found that a large phenylalanine side chain—resulting from substitution for serine—most likely interfered with ATP processes that include sufficient cAMP production, resulting in marked enzyme inactivation. Renal pathology is not sufficient to elucidate the mechanism of congenital development of a unilateral kidney or of lateral renal differences in patients with two kidneys. However, differences in gene expression may contribute to the severity of disease, including renal size differences. Furthermore, fusion at the embryonic stage, and the existence of VUR after birth, suggests kidney atrophy on the side of the lesion.

**Conclusion**

We investigated abnormalities in PAX2, RET and RAS genes, which are frequent causes of renal and urinary tract malformations. Gene aberration was detected in seven of 17 patients. Since unidentified genetic causes might contribute to pathophysiology in the remaining 10 patients, extensive gene analysis using whole-exon sequencing may be necessary.

**Authors’ contributions**

This study was authored by a special student of the Kindai University Graduate School, Faculty of Medicine. This manuscript is not under consideration for publication elsewhere, in any language, except as an abstract.

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**Informed Consent**

The study was performed following approval by the Ethics Committee of Kindai University Faculty of Medicine (approval number 26-204). Written informed consent was acquired from patients or the patients’ parents/guardians. Use of renal specimens for evaluation of renal histologic findings, and for reproduction in figures, was approved by patients or patients’ parents and/or guardians.

**Conflict of interests**

The authors declare that they have no competing interests involving this work.

**References**