TRA1-60 の陽性を確認した。また得られたクローンの mRNA の PCR を行い、OCT4, SOX2, DNMT3b, REX1, c-MYC, GDF3, KLF4, SAL4f, NANOG の発現を確認した。今後、得られた iPS 細胞を用いて、疾患解析を行っていく。

D 考察

人工多能性幹細胞(iPS 細胞)は、希少疾患やモデルマウスのない疾患の解析において有用と考えられている。小児の希少難治性腎・泌尿器疾患群は症例数も少なく、また小児という特殊性から、病態解析に十分な検体を得ることが困難である場合が少なくない。非侵襲的に iPS 細胞が樹立できれば、現時点で重要な解析手段となることはもちろん、将来的に新たな解析法が可能となるまでの患者情報の保存手段としての側面も挙げられる。

iPS 細胞を用いた疾患解析としては、疾患表現型を認める細胞へ分化誘導し、解析を行う手段が一般的である。我々は、神経症状を有する Galloway-Mowat 症候群に対して、神経分化誘導を行い、その解析を行う方針を考えている。また、最近、間葉系細胞から腎臓構成細胞への分化誘導法が報告されており、この方面からの解析も検討していく。

E 結論

Alport 症候群、4p monosomy の患者由来の血液細胞から iPS 細胞を樹立した。

F 健康危険情報

なし

G 研究発表

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H 知的財産権の出願・登録状況

1) 特許取得

なし

2) 実用新案登録

なし

3) その他

なし

III. 研究成果の刊行に関する一覧表

IV. 研究成果の刊行物

研究成果の刊行に関する一覧表

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Right hypoplastic kidney

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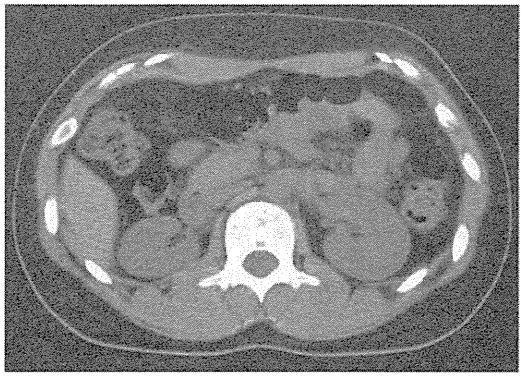

Figure 1 | Computed tomography showing right renal hypoplasia.

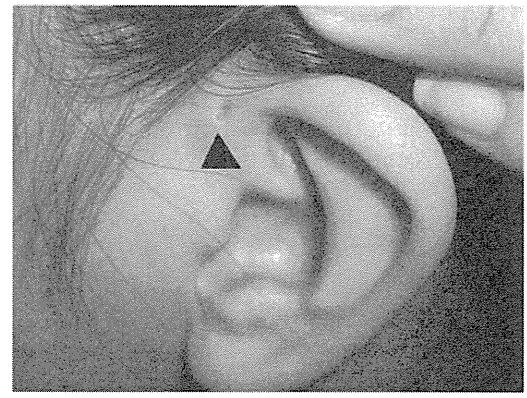

Figure 2 | A preauricular pit (left ear, arrowhead).

A 21-year-old woman was referred to our hospital because of proteinuria and mild renal dysfunction. Her serum creatinine concentration was 1.10 mg/dl and estimated glomerular filtration rate was 53.9 ml/min per 1.73 m^2. The urinary analysis revealed 2+ protein. Abdominal computerized tomography (CT) showed right renal hypoplasia (Figure 1). In addition, she had mixed hearing disturbance and a history of surgeries for correction of left cervical branchial fistulae and bilateral preauricular pits (Figure 2, left ear). Her temporal bone CT presented bilateral inner ear malformation. On the basis of these findings, she was suspected to have branchio-oto-renal (BOR) syndrome, although she has no family history on renal dysfunction and hearing disturbance. The BOR syndrome is an autosomal dominant disorder, which is characterized by the association of branchial anomalies (preauricular pits and branchial fistulae or cysts), otic anomalies affecting the outer, middle, and/or inner ear, which frequently lead to hearing disturbance (sensorineural, conductive, or mixed), and a wide spectrum of renal anomalies ranging from mild hypoplasia to lethal bilateral renal aplasia. The prevalence is approximately 1 in 40,000. The BOR syndrome is associated with several genetic mutations in *EYA1*, *SIX1*, *SALL1*, and *SIX5*. Because of disagreement towards conducting gene analysis from the patient's family, we performed gene analysis of only the patient with informed consent. DNA sequencing analysis revealed a heterozygous mutation, c.880 C>T, p.R294X, in exon 10 of the *EYA1* gene. The BOR syndrome should be taken into consideration on the differential diagnosis in young adult patients with renal dysfunction and hearing disturbance.

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Focal Segmental Glomerulosclerosis in Patients With Complete Deletion of One *WT1* Allele

Kazumoto Iijima, Tomonosuke Someya, Shuichi Ito, Kandai Nozu, Koichi Nakanishi, Kentaro Matsuoka, Hirofumi Ohashi, Michio Nagata, Koichi Kamei and Satoshi Sasaki

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Focal Segmental Glomerulosclerosis in Patients With Complete Deletion of One *WT1* Allele

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KEY WORDS
deletion, focal segmental glomerulosclerosis, WAGR syndrome, *WT1*

ABBREVIATIONS
ACEI—angiotensin-converting enzyme inhibitor
BUN—blood urea nitrogen
CrCl—creatinine clearance
DDS—Denys-Drash syndrome
DMS—diffuse mesangial sclerosis
FSGS—focal segmental glomerulosclerosis
WAGR—Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation

Each author contributed to the study as follows: Dr Iijima, patient management and manuscript writing; Dr Someya, patient management; Dr Ito, patient management; Dr Nozu, genetic analysis; Dr Nakanishi, genetic analysis; Dr Matsuoka, pathological analysis; Dr Ohashi, genetic analysis; Dr Nagata, pathological analysis; Dr Kamei, patient management; and Dr Sasaki, patient management.

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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. *Pediatrics* 2012;129:e1621–e1625

Miller et al[1] 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 exon of *WT1*. Little et al[2] 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.[3] Breslow et al[4] 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 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[5]; the diagnosis of atypical WAGR syndrome was therefore made.[6] 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 107.3 cm (−2.9 SD), weight was 21.7 kg (−0.7 SD), and blood pressure was 120/80 mm Hg. Biochemical data were as follows: total protein, 6.5 g/dL; albumin, 3.3 g/dL; blood urea nitrogen (BUN), 12.9 mg/dL; creatinine, 0.43 mg/dL; 24-hour creatinine clearance (CrCl), 72.2 mL/min/1.73 m^2; early morning urinary protein, 3+ (as measured by using a dipstick test); urinary protein to urinary creatinine ratio, 3.6 (milligram/milligram); and urinary β-2 microglobulin, 0.44 mg/dL (normal range: <0.23 mg/dL). His renal function gradually deteriorated despite angiotensin-converting enzyme inhibitor (ACEI) treatment. At 14 years of age, he underwent a preemptive living-related renal transplantation from his father.

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, 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 m^2; 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 2C), and she was therefore diagnosed with WAGR syndrome. The patient developed proteinuria at

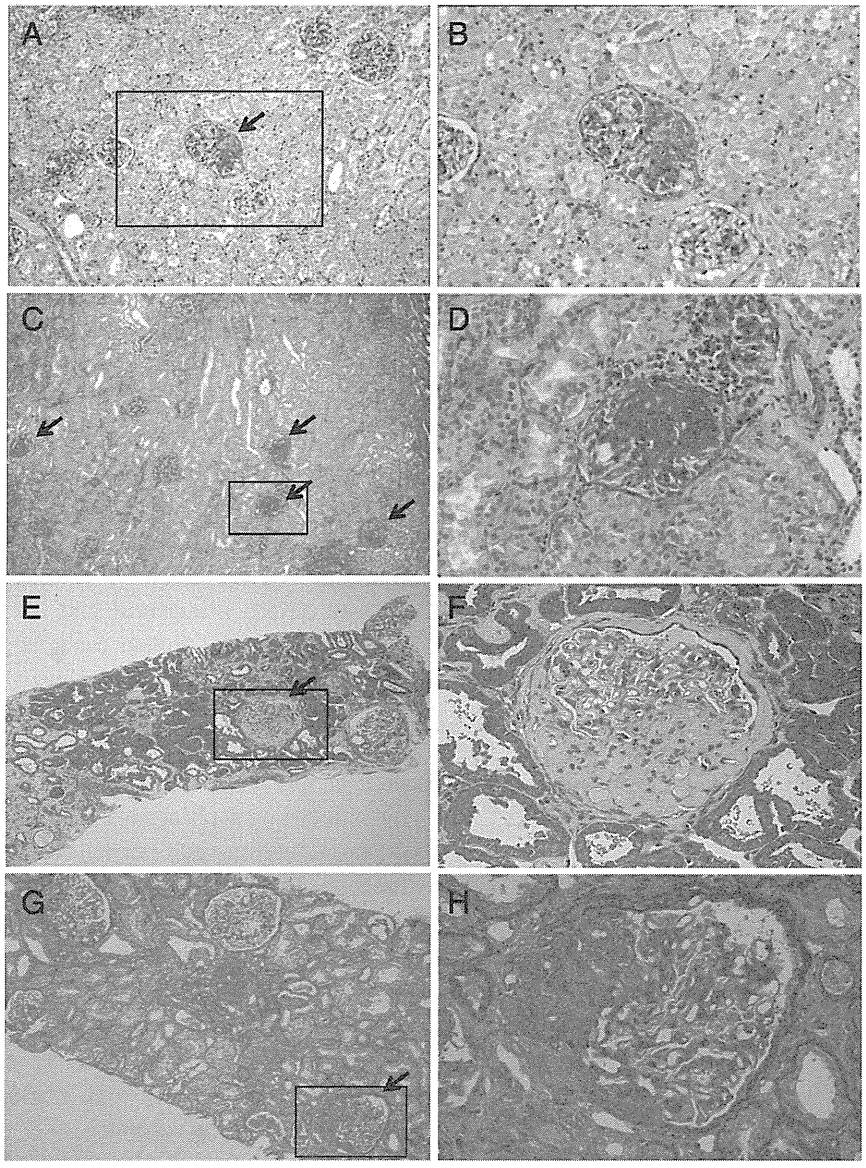

FIGURE 1

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 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, 2.3 g/dL; BUN, 15.0 mg/dL; creatinine, 0.65 mg/dL; estimated glomerular filtration rate, 100.7 mL/min/1.73 m^2). We were unable to obtain her parents' consent for renal biopsy, and they chose to start drug treatment. However, treatment with prednisolone and ACEI was not effective, and her renal function gradually deteriorated. Therefore, she underwent renal biopsy at age 19 years. At the time of biopsy, her height was 144.5 cm, weight was 72.5 kg, and blood pressure was 130/83 mm Hg. Biochemical data were as follows: total protein, 5.5 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 m^2; 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.[7] 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

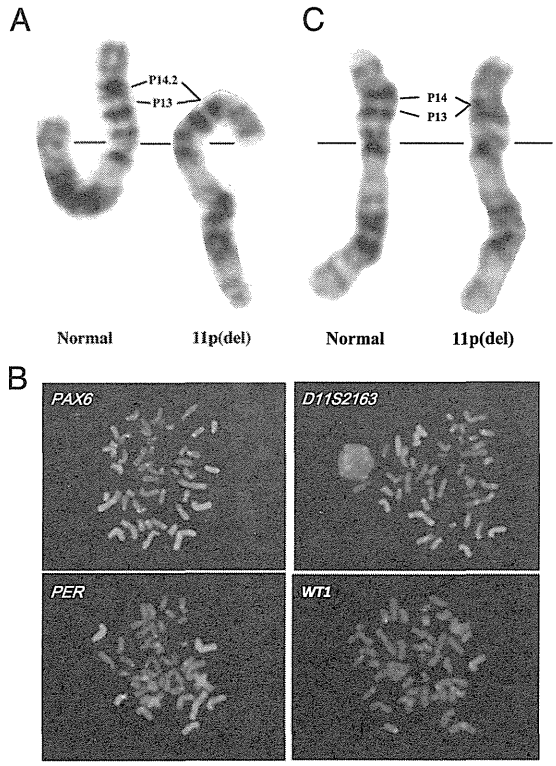

FIGURE 2

High-resolution G-banding of chromosome 11 and fluorescence in situ hybridization (FISH) in patient 2 and G-banding of chromosome 11 in patient 3. A, Patient 2 had deletion of chromosome 11p13-p14.2 in one allele. B, FISH using P1-derived artificial chromosome clones (1083G3 for *PAX6*; 65P5 for *D11S2163*; 685F3 for *PER*; and 104M13 for *WT1*) as probes was performed for patient 2, as previously reported.[6] Each FISH signal for *PAX6, D11S2163, PER, and WT1* was observed in only one chromosome 11 homolog, indicating heterozygous deletion of the WAGR region of 11p. C, Patient 3 had deletion of chromosome 11p13-p14 in one allele.

histology. 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.[8] 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.[9] *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.[9,10] Orloff et al[11] 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.[12] 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.[12] 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.

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BRIEF REPORT

Alport-like glomerular basement membrane changes with renal-coloboma syndrome

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Abstract
Background Autosomal dominant mutations in paired box gene 2 (*PAX2*), on chromosome 10q24, are responsible for renal coloboma syndrome (RCS). The role of *PAX2* in glomerular basement membrane (GBM) formation and maintenance remains unknown.
Case-diagnosis We report a case of a 13-year-old Japanese girl who had both optic disk coloboma and renal insufficiency. Her father and sister also had both coloboma and renal dysfunction. Renal pathological findings revealed a basket-weave pattern of the GBM, which was compatible with Alport syndrome, but type IV collagen α5 staining was normal. The patient's findings of coloboma and renal dysfunction suggested that she had RCS, and genetic analysis revealed a *PAX2* heterozygous mutation in exon 2 (c.76dup, p.Val26Glyfsx27) without any mutations of *COL4A3*, *COL4A4*, and *COL4A5,* which are responsible for autosomal and X-linked Alport syndrome.
Conclusions *PAX2* mutations may result in abnormal GBM structure.

Keywords Renal-colombia syndrome · PAX2 · Glomerular basement membrane · Type IV collagen · Podocyte

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Introduction

Renal-coloboma syndrome (RCS, OMIM 120330) is a rare autosomal dominant disorder associated with paired box gene 2 (*PAX2*, 10q24) heterozygous mutations [1]. PAX2 is a nuclear transcriptional factor and is highly conserved among species [2]. In the fetal period, PAX2 is expressed in the otic and optic vesicles, spinal cord, hindbrain, mesonephros, and metanephros in the embryonic kidney. PAX2 is one of the central regulators for early-stage kidney development, but the precise mechanisms of PAX2 for kidney development have not been fully clarified. RCS is characterized by ocular and renal abnormalities. Renal malformations include hypoplasia, dysplasia, vesicoureteral reflux (VUR), multicystic dysplastic kidney, and horseshoe kidney [3]. Renal histopathological findings in RCS have been reported, including oligomeganephronia that is induced by a reduction in nephron number in the RCS kidney. However, there are no previous reports of obvious glomerular basement membrane (GBM) changes as evaluated by electron microscopy. We report here for the first time remarkable GBM changes with RCS due to *PAX2* mutation, which are similar to those found in Alport syndrome.

Case report

Patient 1

A 6-year-old Japanese girl visited our hospital because of mild proteinuria. We found that she also had bilateral optic disk coloboma (Fig. 1a). At the age of 13 years, laboratory findings showed that her blood urea nitrogen (BUN) level was 26 mg/dl, serum creatinine (SCr) level was 0.97 mg/dl, and creatinine clearance (CrCl) was decreased to 59.1 ml/min/1.73 m^2. Urinary protein was slightly increased (0.3 g/day), especially urinary β2 microglobulin (1,000 μg/l, normal range <230 μg/l), but she had no hematuria. Ultrasonography revealed left renal atrophy (kidney size 64× 35 mm), but the right kidney size was normal (81× 37 mm). Her eye and renal abnormalities were compatible

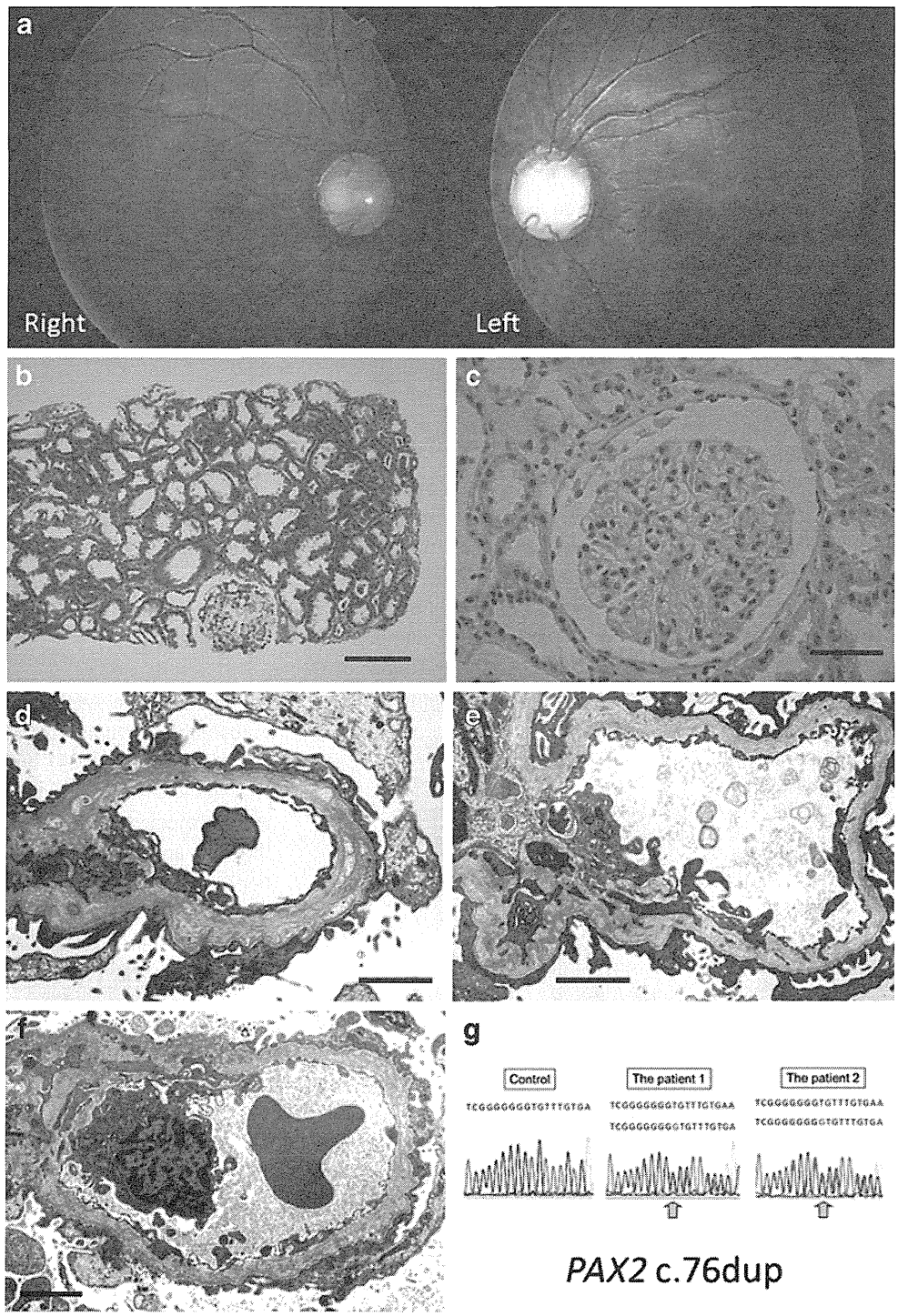

Fig. 1 Fundus photographs from patient 1 (**a**): bilateral optic discs are enlarged. Renal pathological findings in patient 1 (**b**–**c**): Light microscopy shows that the number of glomeruli is small (**b**), periodic acid-methenamine-silver (PAM) staining, original magnification ×100, *scale bar*=100 μm), but hypertrophy or proliferative lesions cannot be seen in the glomeruli (**c**), periodic acid-Schiff (PAS) staining, original magnification ×400, *scale bar*=30 μm). Electron microscopy shows thickening, which is compatible with a basket-weave appearance in the glomerular basement membrane (GBM) in patient 1 (**d**) and patient 2 (**e**). GBM findings are similar to a genetically confirmed case of Alport syndrome by *COL4A3* homozygous mutation (**f**) (original magnification ×5,000, *scale bar*=2 μm). Genetic analysis (**g**) shows that both patients have a *PAX2* mutation (c.76dup)

with RCS, but the precise cause of her renal insufficiency was unknown.

Patient 2

Patient 2 is patient 1's elder sister, and she had left renal atrophy detected soon after birth. At the age of 4 months, eye abnormalities including optic disk coloboma and macular hypoplasia were found. At the age of 10 months, mild proteinuria (0.4 g/day) and VUR were detected, and at the age of 5 years, she received a surgery for VUR. However, her proteinuria did not disappear and her renal function deteriorated. She had a renal biopsy performed at the age of 6 years, and electron microscopy showed that the glomeruli had diffuse thickening accompanied by a basket-weave formation of the GBM, which was compatible with Alport syndrome. She was diagnosed with Alport syndrome on the basis of renal histological findings; however, hematuria never appeared during her course, and genetic analysis was not performed at that time.

Family history

The patients' father also had coloboma and renal failure, but his funduscopic findings and cause of his renal dysfunction were unknown because he died after receiving four renal transplantations. The paternal grandfather had left renal atrophy, but his renal function was preserved and he did not have coloboma. Others in the family showed no renal disorder. None of their relatives had hearing loss.

Renal histology and genetic analysis

To confirm the cause of renal insufficiency, we performed renal biopsy in patient 1. Light microscopy findings showed that the kidney was oligonephronic but not enlarged (Fig. 1b, c). Immunofluorescent staining showed no significant abnormalities. Electron microscopy showed thickening and thinning of the GBM (Fig. 1d), similar to patient 2 (Fig. 1e). The patients' GBM findings are similar to a genetically confirmed case of Alport syndrome (Fig. 1f). The eye abnormality, oligonephronic kidney, and family history indicated that patient 1 suffered from RCS. To confirm this diagnosis, we performed genetic analysis of *PAX2* for both patients and their paternal grandfather after obtaining informed consent. We detected a c.76dup heterozygous mutation in exon 2 of *PAX2* (Fig. 1g) in the siblings but not the grandfather. This frameshift mutation induces an amino acid change from valine to glycine and introduces a premature stop codon (p.Val26Glyfsx27). To exclude the possibility of complicating Alport syndrome, we performed type IV collagen staining of glomeruli for patient 1 and observed that the α5 chain [α5 (IV)] staining pattern was normal. Furthermore, we sequenced the genes *COL4A4* (2q35-q37), *COL4A3* (2q36-q37), and *COL4A5* (Xq22), which are responsible for autosomal recessive, dominant, and X-linked Alport syndrome. No mutations or significant variants were detected in either patient. Based on the presence of a heterozygous *PAX2* mutation in both patients with normal immunohistochemistry for α5 (IV) and the absence of significant sequence variation in any of the genes encoding type IV collagen proteins found in the GBM, we concluded that the GBM changes resulted from *PAX2* haploinsufficiency in our patients.

Discussion

We identified that our patients had a *PAX2* heterozygous mutation in exon 2 (c.76dup, p.Val26Gly fsx27). Although there is no genotype–phenotype correlation in RCS, this is the most frequent mutation of *PAX2* [4]. This frameshift mutation leads to haploinsufficiency of the PAX2 protein. The $Pax2^{1Neu\ +/-}$ mutant mouse is a model of RCS that has a heterozygote 1-bp insertion in *PAX2* [5], [6], and it has been reported that heterozygous mutations of *PAX2* induce apoptotic cells in the fetal kidney and reduce branching of the ureteric bud. As a result, *PAX2* heterozygous mutations induce renal hypoplasia [7]. Oligomeganephronia is induced by renal hypoplasia and *PAX2* mutation [8], and there are few reports regarding the association with oligomeganephronia and GBM changes [9]. Although the number of glomeruli in patient 1 was decreased, glomerular enlargement was not observed. The causes of our patients' renal insufficiency are unknown, but reducing renal mass may induce this condition.

Laminin, type IV collagen α3 (α3 [IV]) chain, α4 (α4 [IV]) chain, and α5 (IV) are major components of the GBM. Laminin is produced by both podocytes and endothelial cells, and α3 (IV), α4 (IV), and α5 (IV) originate only from podocytes [10]. A host of transcription factors, especially WT1 and PAX2, play a significant role in modulating podocyte maturation. Although PAX2 is essential for embryonic renal formation, a decrease in PAX2 and increase in WT1 in the embryonic kidney are also necessary for further differentiation of podocytes [11]. Therefore, *PAX2* mutation may result in abnormal GBM production in podocytes, but further investigations are required to clarify this issue.

In conclusion, this is the first report of Alport-like GBM changes in RCS due to *PAX2* mutation. It is unknown whether *PAX2* haploinsufficiency leads to GBM changes, as observed in the siblings in this study. Our observations may lead to an improved understanding of the pathogenesis of RCS.

Disclosure The authors declare that they have no competing financial interests and no funding sources to disclose.

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7 尿細管性アシドーシス

1 定義・概念

尿細管性アシドーシス(renal tubular acidosis：RTA)とは，糸球体障害がないか軽度の状態で，腎集合管における酸分泌または近位尿細管における重炭酸再吸収が障害され，アニオンギャップ(anion gap：AG)正常の代謝性アシドーシスを呈する疾患である．

2 病因・病態

1) I型RTA(遠位尿細管アシドーシス distal RTA：dRTA)

集合管では体内で産生された不揮発酸がH^+-ATPaseにより管腔に排泄される(図1)．尿pHが5.0のとき，管腔内のH^+は細胞内の250倍の濃度で存在する．dRTAの原因はH^+の排泄障害であり，著明なアシドーシス存在下でも尿pHが5.5～6.5以下になることはない．

a. 常染色体劣性 dRTA

集合管α介在細胞の管腔側膜に存在するH^+-ATPaseは二つのドメインからなり，細胞質に存在するV1ドメインはATPを消費して細胞膜に位置するV0ドメインを通じてH^+を管腔へと排泄している．V1ドメインはAからHまでの八つの，V0ドメインはa，c，c'，c''，dの五つのサブユニットから成っており，あわせて少なくとも13のサブユニットで構成されている[1]．B1サブユニット(*ATP6V1B1*遺伝子)とa4サブユニット(*ATP6V0A4*遺伝子)は腎と内耳に共通に発現しており，これらの異常により常染色体劣性dRTAを呈する[2,3]．難聴を伴うことがあり，B1サブユニット異常では特に頻度が高い[4]．ただし同じ家系内でも難聴を伴う患者と難聴を伴わない患者が存在する．

H^+-ATPaseは近位尿細管のエンドソーム膜にも発現している．エンドソーム内を酸性化する働きがあり，近位尿細管で再吸収された低分子蛋白のエンドサイトーシスにも寄与している[5]．アシドーシスの補正されていないdRTAでは尿細管性蛋白尿がみられるが，この原因として，エンドソーム内の酸性化障害が関与している可能性がある．

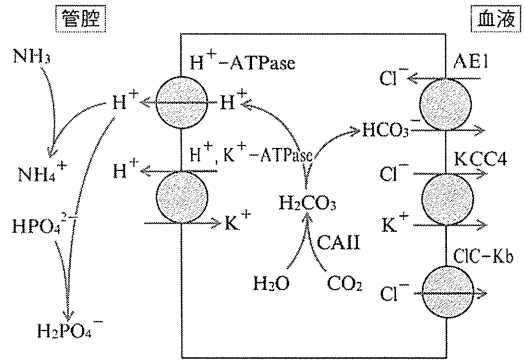

図1 集合管α介在細胞におけるH^+分泌

CA II：carbonic anhydrase II，AE1：anion exchanger 1，KCC4：potassium-chloride cotransporter，ClC-Kb：chloride channel

b. 常染色体優性 dRTA

集合管α介在細胞の基底側に存在するCl^-/HCO_3^-交換体(anion exchanger 1：AE1)(*SLC4A1*遺伝子)の異常は常染色体優性dRTAの原因となる[6]．

AE1は赤血球膜にも存在し，その細胞骨格構造維持に寄与しているため，遺伝性球状赤血球症やsouth-east Asian ovalocytocisの原因遺伝子としても報告されているが，これらの疾患にdRTAを合併することはまれである．

c. 二次性 dRTA

二次性dRTAの原因として頻度が高いのはSjögren症候群や全身性エリテマトーデス(systemic lupus erythematosus：SLE)などの自己免疫疾患である．そのほか腎移植後や腎間質の疾患でも生じうる．薬剤性としてはアムホテリシンB，リチウムやホスカルネットナトリウム水和物等がある．また閉塞性腎症や鎌状赤血球腎症でもdRTAを生じる．

d. 病態

dRTAでは排泄障害によって蓄積した酸を中和するために骨のハイドロキシアパタイトが利用されるため，無治療では骨吸収が亢進する．このためくる病，骨軟化症を呈し，尿中Ca排泄は増加する．また塩基の平衡を保つために近位尿細管におけるクエン酸再吸収が増加する．結果として高カルシウム尿症および低クエン酸尿症となり，腎石灰化および結石が生じる．腎石灰化の結果として多血症を呈することがあるがその機序は明らかでない．また，集合管においてH^+が分泌されない代わりにK排泄が亢進して低カリウム血症を呈する．これにはレニン-アンジオテンシン系(renin-angiotensin system：RAS)の亢進が関与しているとされる．アムホテリシンによるdRTAでは管腔側膜がH^+の濃度勾配を維持できず，細胞内へH^+の逆拡散(back diffusion)が起こると考えられている．

2) II型RTA(近位尿細管性アシドーシス proximal RTA：pRTA)

近位尿細管における重炭酸再吸収障害が原因である(図2)．Fanconi症候群の一症状として発症することが多い．一方，単独永続性の常染色体劣性pRTAは基底側に存在するNa^+-HCO_3^-共輸送体(*SLC4A4*遺伝子)の異常である[7]．その他，常染色体優性遺伝のpRTAもいくつか報告があるが，原因遺伝子はわかっていない．

病態の中心は重炭酸喪失によるアニオンギャップ正常の代謝性アシドーシスである．その他，重炭酸が多量に失われるのに伴ってNaとKの喪失も大きい．結果として循環血液量は減少し，RASが亢進することにより低カリウム血症が助長される．一方，Henleの太い上行脚以降でのNa再吸収の亢進によりCaの再吸収も亢進するため，アシドーシスによって骨吸収は亢進しているが，結果として尿中Ca排泄は正常である．また尿中クエン酸排泄は低下しないため，腎石灰化や尿路結石を生じることはない．

3) III型RTA(hybrid RTA)

I型とII型の両方の要素をもったRTAである．炭酸脱水酵素(carbonic anhydrase II：CA II)の変異は大理石骨病の一因であるが，CA IIは近位尿細管における重炭酸再吸収および集合管におけるH^+排泄の両方に寄与している(図1，図2)．CA IIは破骨細胞にも発現しており，酸を排泄して骨ミネラルを溶解する働きがある．また脳内のoligodendrocyteやastrocyteにも発現があり，その欠乏は脳の成熟に異常をきたす．このためCA II異常による大理石骨病はIII型RTAおよび脳内石灰化，精神発達遅滞を呈する．

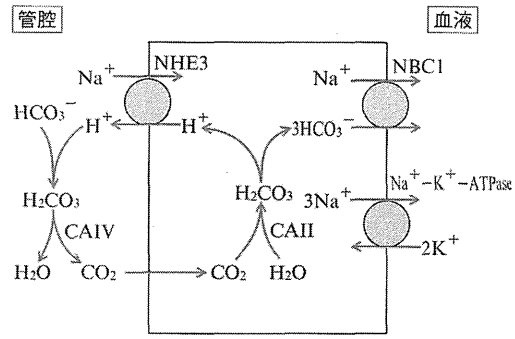

図2　近位尿細管におけるH^+の分泌とHCO_3^-の再吸収

NHE：sodium-proton exchanger，NBC：sodium-bicarbonate cotransporter

4) IV型RTA(高カリウム血症を伴うRTA)

アルドステロンは集合管主細胞においてNaの再吸収を促進し，Kを排泄している．またα介在細胞にも作用してH^+-ATPaseおよびCA IIの発現を増強し，H^+の排泄に寄与する．したがってアルドステロンの欠乏または作用不全はNa^+の喪失による循環血液量低下，高カリウム血症，代謝性アシドーシスを招く．

a. アルドステロン欠乏

先天性のものとしては21-hydroxylase欠損による先天性副腎過形成やアルドステロン合成酵素欠損がある．後天性のものとしては，Addison病，糖尿病性腎症，間質性腎炎等による低レニン性低アルドステロン症があげられる．

b. アルドステロン作用不全

集合管におけるアルドステロンに対する反応性が低下し，血中アルドステロン濃度は異常高値となるため，偽性低アルドステロン症(pseudohypo-aldosteronism：PHA)とよばれる．PHA type Iには常染色体優性遺伝を示すミネラルコルチコイド受容体(mineralocorticoid receptor)の異常と常染色体劣性遺伝を示す上皮型ナトリウムチャネル(epithelial sodium channel：ENaC)の異常がある．臨床上比較的よく遭遇するのは続発性のPHAであり，腎盂腎炎や尿路奇形がおもな原因である．

3 臨床徴候

1) I型RTA(dRTA)

常染色体劣性dRTAでは新生児期，乳児期よりアシドーシスによる成長障害(体重増加不良)，哺乳障害を認める．また集合管でH^+が排泄されない代わりにNa，Kの排泄が亢進しており，尿濃縮力障害による多飲多尿，夜間尿を呈する．低カリウム血症による筋力低下，四肢麻痺，不整脈がみられることもある．尿中Ca排泄は亢進し，クエン酸排泄は低下するため，腎石灰化や尿路結石を呈する．また低カリウム血症の持続により高率に腎嚢胞を認める．アシドーシスが遷延するとくる病，骨軟化症をきたしうる．感音難聴は*ATP6V1B1*変異のほとんどの患者が10歳未満で発症する．*ATP6V0A4*変異では難聴はないか，認めたとしても10歳以降とされていたが，その後の解析では少なくとも1/3程度の症例は10歳未満で感音難聴を呈することが判明した[4]．

常染色体優性dRTAでは成人後に尿路結石を呈し，ほかに症状がないことが多い．ほとんどの患者で赤血球膜の異常も認めない．

2) II型RTA(pRTA)

pRTAの主症状は成長障害である．dRTAと異なり腎石灰化や尿路結石を呈することはない．大量のアルカリ投与およびRASの亢進により尿中にKが失われ，低カリウム血症を呈する．Fanconi症候群の一部分症状として発症することが多く，その場合は低リン血症，低尿酸血症，アミノ酸尿，糖尿，低分子蛋白尿を呈する．低リン血症が遷延した場合のみ，くる病，骨軟化症を呈しうる．単独永続性のpRTAでは白内障，緑内障，帯状角膜変性などの眼症状を呈する．

3) III型RTA(hybrid RTA)

CA II異常による大理石骨病では易骨折性，dRTAとpRTAの両方の要素をもったRTA，脳内石灰化，および精神発達遅滞を呈する．

4) IV型RTA(高カリウム血症を伴うRTA)

高カリウム血症を呈することが特徴である．代謝性アシドーシスはほかのタイプのRTAほど重症ではない．ENaCの異常では乳児期からNa喪失の程度が強く，脱水や体重増加不良を呈する．

表1 尿細管性アシドーシスの病型分類

	I型(dRTA)	II型(pRTA)	IV型(高カリウム血症を伴うRTA)
血清K	低下	正常～低下	上昇
アシドーシス存在下の尿pH	>5.5	<5.5	<5.5
アシドーシス補正後の$FEHCO_3^-$	<5%	>15%	<10%
尿中クエン酸	低下	正常～増加	正常
腎結石/石灰化	+	−	−
HCO_3^-必要量	少量	大量	少量

4 診断と検査法(表1)

アニオンギャップ(AG)正常の代謝性アシドーシスが本疾患の特徴である．下痢など消化管からの重炭酸の喪失でも同様の所見になることに留意する．pRTAではアシドーシス補正後の重炭酸排泄分画[$FE\ HCO_3^-$=(血清クレアチニン〈Cr〉)/尿中Cr)×(尿中HCO_3^-/血中HCO_3^-)×100(%)]が15%以上になること，dRTAではアシドーシス存在下での尿pHが高いこと(5.5以上)が診断の要点である．

1) AG

RTAではAG正常(12±2 mEq/L)の代謝性アシドーシスを呈する．尿AG(UAG)は尿中(Na+K−Cl)で算出され，通常は−20～−50 mEq/Lである．RTAではNH_4^+の排泄障害または尿中HCO_3^-の増加のため尿中(Na+K)に比較して尿中Clが少なくなり，UAGは正となる．一方消化管からのHCO_3^-の喪失による代謝性アシドーシスの場合は腎からのNH_4^+の排泄は保たれるため，UAGは負のままである．

2) 尿酸性化能

dRTAではアシドーシス存在下でも尿pHは5.5以下にならないが，pRTAでは5.5以下になる．

a. 塩化アンモニウム負荷試験

アシドーシスが軽微な場合(incomplete type)に診断確定のために行う．塩化アンモニウム0.1 g/kgを30～60分かけて投与し，内服直前と，内服後1時間ごとに尿pHを測定する．内服後2～3時間後に採血を行い，十分アシドーシスに傾いていることを確認する．正常者あるいはpRTAでは尿pHは5.5以下になるが，dRTAでは5.5以下にならない．すでに高度のアシドーシスが存在する場合にはこの試験

は不要かつ禁忌である．

b．フロセミド負荷試験

塩化アンモニウム負荷試験が嘔吐などのために施行できない場合や，dRTAの病型分類のために行われることがある．フロセミド0.5～1 mg/kg静注前後で尿pHを測定する．分泌不全型(H^+-ATPaseまたはAE1の異常)のdRTAでは尿pHは5.5以下にならないが，勾配不全型(アムホテリシンB等でみられるback diffusion)の場合は尿pHは5.5以下になる．

3）アシドーシス補正後の $FEHCO_3^-$

pRTAでは$FEHCO_3^-$は15％以上となるが，dRTAでは5％未満である．十分にアシドーシスを補正してから検査する．

4）その他

Fanconi症候群の存在はpRTAの，腎石灰化/結石の存在はdRTAの診断の参考所見である．

5　治療法

1）I型RTA(dRTA)

アルカリの補充が基本である．腎石灰化や低カリウム血症に対処するため，クエン酸製剤を用いる．通常2～4 mEq/kg/日の補充が必要である．ただし急性期や幼少児ではこれより多量(4～8 mEq/kg/日)のアルカリを必要とすることが多い．成人では1～2 mEq/kg/日の補充で十分である．

2）II型RTA(pRTA)

重炭酸の喪失が大きいためdRTAに比べて大量のアルカリが必要となる(5～15 mEq/kg/日)．クエン酸製剤(Kを含む)を用いても低カリウム血症が増悪するため，カリウム製剤の投与が必要になる．サイアザイド系利尿薬がアルカリの必要量を減らすのに有効とされる．Fanconi症候群によるpRTAで低リン血症が存在する場合には中性リン酸塩(リンとして20～100 mg/kg/日)や活性化ビタミンD製剤を用いる．

3）IV型RTA

アルドステロン欠乏に対しては，原疾患に応じてグルココルチコイドまたはミネラルコルチコイドの補充で対応する．PHA type Iでは塩化ナトリウム3～10 g/日を補充するが，成長に伴って減量または中止できる傾向にある．続発性PHAでは適宜Na補充，K制限を行う．

6　管理と予後

dRTA，pRTAともにアシドーシスの補正が基本である．dRTAでは血中HCO_3^-濃度のモニタリングのほか，低分子蛋白尿もコントロールのよい指標になる．早期から十分なアルカリ投与を行うことにより，成長障害や腎石灰化を防ぐことができる．腎石灰化は腎不全の原因となりうる．単独永続性のpRTAでは完全にアシドーシスを補正することは難しいが，成長障害の改善は期待できる．

dRTA，pRTAともに乳児期一過性で完治する孤発例がある[8,9]．

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