

Original Article

Clinical features and genetic findings in Chinese children with distal renal tubular acidosis

Fang Zhou^{1,2}, Jianhua Mao¹, Qing Ye³, Xiujuan Zhu¹, Yingying Zhang¹, Yuhong Ye¹, Haidong Fu¹, Huijun Shen¹, Zhihong Lu¹, Yonghui Xia¹, Aimin Liu¹, Qiang Shu¹, Lizhong Du¹

¹Department of Nephrology, The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang Province, China; ²Department of Internal Medicine, Hangzhou Children's Hospital, Hangzhou, Zhejiang Province, China; ³Department of Zhejiang Key Laboratory for Neonatal Diseases, The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang Province, China

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Abstract: Distal renal tubular acidosis (dRTA) is characterized by metabolic acidosis due to uric acid dysfunction. The aim of this study was to demonstrate the genetic diagnosis of Chinese children with dRTA by whole-exome sequencing. From Jan. 2010 to Sept. 2015, 16 children with dRTA were recruited to investigate the possibility of genetic diagnosis and to examine any genotype-phenotype relationships in these patients. Sanger sequencing was used to confirm mutations identified by whole-exome sequencing. Clinical and biological features in the patients included hyperchloremic metabolic acidosis, impaired growth, hypokalemia, nephrocalcinosis, nephrolithiasis, hypercalciuria, hypocitraturia, and rickets or osteomalacia. Seventeen mutations in the solute carrier family 4 member 1 (*SLC4A1*), ATPase H⁺ transporting VO subunit a4 (*ATP6VOA4*), ATPase H⁺ transporting V1 subunit B1 (*ATP6V1B1*), WNK lysine deficient protein kinase 1 (*WNK1*) and the claudin 16 (*CLDN16*) were identified in 15 patients, and 14 of these mutations are novel. Only 1 patient was negative for any mutations. Our results demonstrate the existence of *SLC4A1*, *ATP6V1B1*, *ATP6VOA4*, *WNK1* and *CLDN16* mutations in Chinese children with dRTA and indicate that compound heterozygosity at 2 or more different but related genes can be responsible for its pathogenesis. This study also indicates that whole-exome sequencing is a labor and cost-effective means of analyzing dRTA-associated genes.

Keywords: Distal renal tubular acidosis, genetic mutations, whole-exome sequencing, Chinese children, compound heterozygosity

Introduction

Primary distal renal tubular acidosis (dRTA) is caused by genetic defects that result in uric acid dysfunction in the presence of hyperchloremic metabolic acidosis [1]. The main clinical features of dRTA are polydipsia, loss of appetite, vomiting, diarrhea and/or constipation, and polyuria [2]. Chronic acidosis and secondary alterations such as polyuria, polydipsia, and vomiting can affect growth, leading to failure to thrive. In general, dRTA has a better prognosis if diagnosed early and subjected to continued alkaline treatment. Untreated, dRTA causes rickets and/or growth retardation in children and osteomalacia in adults and gradual deterioration of renal function with time.

Distal RTA can be transmitted as either an autosomal dominant or an autosomal recessive

trait. The autosomal dominant phenotype typically appears in a mild form during adolescence [3]. The autosomal recessive phenotype usually results in growth retardation, and may or may not lead to deafness. The parents of children with this variant are unaffected. Autosomal recessive dRTA is associated with mutations in solute carrier family 4 member 1 (*SLC4A1*), ATPase H⁺ transporting VO subunit a4 (*ATP6VOA4*), and ATPase H⁺ transporting V1 subunit B1 (*ATP6V1B1*) [4]. However, in approximately 20% of patients with dRTA, no mutations were found in these genes, suggesting that defects in other transporters or channels might also be involved in dRTA pathogenesis.

Next generation sequencing (NGS) provides >95% exon coverage including 85% of known Mendelian disorders causing mutations [5]. Whole-exome sequencing is useful in identify-

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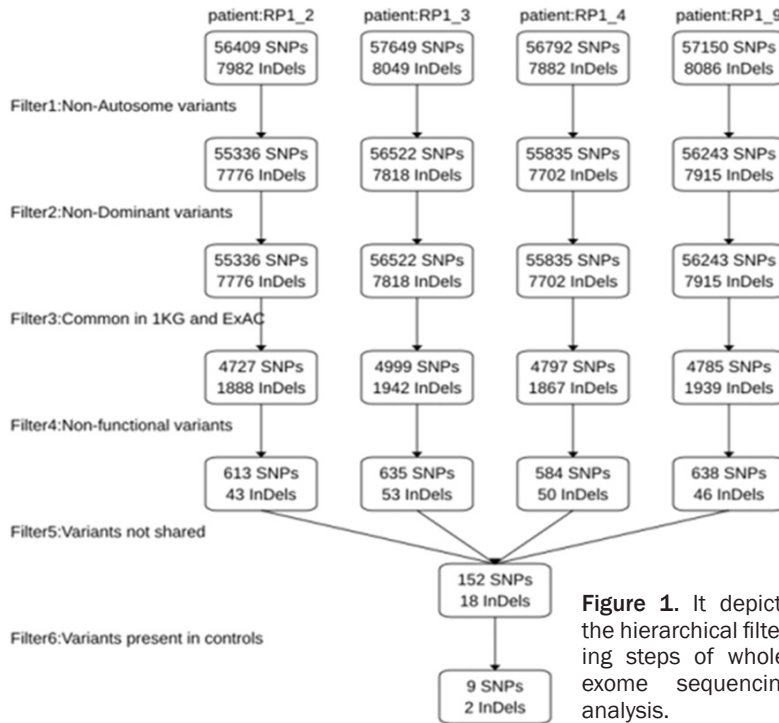


Figure 1. It depicts the hierarchical filtering steps of whole-exome sequencing analysis.

ease was considered to be a secondary form of dRTA such as Sjören's syndrome, obstructive uropathy, chronic active viral hepatitis or drug-induced nephropathy were excluded. All patients or their parents signed an informed consent form to allow participation in the study and publication of their results for scientific purposes, and the Children's Hospital of Zhejiang University School of Medicine approved the study (CH-200912001). The clinical trial registration number was ZJU-CH-20020130. Clinical findings and laboratory results were collected for all 16 children, and their family histories were summarized. Biochemical analyses

included the measurement of serum magnesium, phosphate, calcium, potassium, and sodium levels, as well as the measurement of urinary calcium and protein.

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Materials and methods

Subjects and clinical assessment

Sixteen children clinically diagnosed with dRTA were recruited from the Department of Nephrology at The Children's Hospital of Zhejiang University School of Medicine from January 2010 to September 2015. The disease was diagnosed based on the following criteria: (1) hyperchloremic metabolic acidosis with a normal anion gap (AG; blood AG 12 ± 2 mmol/L, blood Cl > 105 mmol/L, blood pH < 7.35 or $\text{HCO}_2 \leq 22$ mmol/L); (2) inability to lower the urinary pH (urine pH > 6.4), even in severe metabolic acidosis; (3) reduced urine titratable acid (TA) and NH_4^+ levels by urine TA assay (urine TA < 10 mmol/L, urine $\text{NH}_4^+ < 25$ mmol/L); and (4) the results of ammonium chloride loading tests in patients with a blood pH > 7.35 . Ammonium chloride (0.1 g/kg) was administered orally for 3 days. On the 3rd day, the blood carbon dioxide combining power and blood and urinary pH values were determined. Patients whose dis-

DNA extraction

Genomic DNA was extracted from 5 mL of the peripheral blood of patients with dRA using a QIAamp Blood DNA Mini Kit (Qiagen, Milano, Italy) according to the manufacturer's instructions. DNA concentrations were determined using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). DNA samples were stored at -20°C until use.

Whole-exome sequencing

Exome sequencing was performed in two pools to optimize the results. Samples were pooled based on the clinical features of the patients. The first pool was comprised of two dRTA siblings without deafness, and the second included dRTA twin sisters with associated deafness. An array capture was used to enrich the relevant human genes (SeqCap EZ Human Exome Library v2.0, Roche®, Basel, Switzerland), and these genes were sequenced on the Illumina HiSeq 2000 platform (Illumina, Inc, USA).

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Table 1. Clinical and biochemical features of children with clinically diagnosed distal renal tubular acidosis

ID	Gender	Age at onset	Age at diagnosis	Blood pH value	Serum bicarbonate (mmol/L)	Serum potassium (mmol/L)	SCr (μmol/L)	eGFR (ml/min)	Serum chloride (mmol/L)	Urine pH	Rickets	Nephrocalcinosis	SNHL
1	Female	5Y	7Y	7.284	13.2	2.6	50.6	124.9	116	7.5	Yes	Yes	No
2	Male	3Y	3Y3M	7.262	11.4	1.9	60.4	73.0	115	8.0	Yes	Yes	No
3	Female	1Y	2Y1M	7.275	12.9	2.5	40.0	93.1	118	7.5	Yes	Yes	No
4	Female	5M	3Y5M	7.256	13.6	2.7	52.4	82.3	116	7.5	Yes	Yes	No
5	Female	11M	1Y11M	7.371	13.2	2.7	32.2	94.4	116	7.5	Yes	Yes	No
6	Male	9Y	9Y	7.388	14.5	1.9	54.4	108.1	121	8.0	Yes	Yes	No
7	Female	13Y	14Y1M	7.321	14.2	2.8	56.6	117.7	116	7.5	Yes	Yes	Yes
8	Female	13Y	13Y1M	7.312	15.2	1.7	38.0	181.2	115	8.0	Yes	No	No
9	Female	1Y8M	2Y1M	7.319	16.1	2.5	24.0	159.3	127	8.0	No	Yes	No
10	Female	7Y	8Y9M	7.334	15.8	2.3	44.0	124.7	114	7.5	Yes	Yes	No
11	Female	3Y1M	3Y3M	7.294	16.2	2.7	58	65.9	123	7.5	Yes	Yes	No
12	Male	3Y	6Y6M	7.279	17.6	3.0	44.7	118.4	116	7.0	Yes	Yes	No
13	Female	1Y	5Y	7.245	16.8	2.6	45.8	109.1	118	7.5	Yes	Yes	No
14	Female	7M	7M	7.256	15.8	2.2	36.2	68.5	116	7.5	Yes	No	No
15	Female	28D	46D	7.320	15.0	2.9	24.6	84.6	116	8.0	No	No	No
16	Female	4M	1Y2M	7.257	16.3	2.4	31.0	77.4	117	7.5	Yes	Yes	Yes

Date filtering

The following initial steps were performed to prioritize the high-quality variants: (i) Variants within intergenic, intronic, and untranslated regions (UTRs) and synonymous mutations were excluded from downstream analysis; (ii) Variants with a quality score <20 were excluded; (iii) Only conservation scores (phyloP) >3 were considered upon comparison of humans and 43 other vertebrates. After the initial selection, the remaining genes were filtered by function. PolyPhen-2 software (<http://genetics.bwh.harvard.edu/pph2/>) was used to predict the possible impact of variants. The final set of selected variants was visually inspected using the Integrative Genomics Viewer. Thirteen polymorphic variants previously described in public databases were investigated and compared with the variations found in the current exome. The selected mutations investigated in this study were not found in previous exome sequences (<http://evs.gs.washington.edu/EVS/>). **Figure 1** depicts the hierarchical filtering steps of whole-exome sequencing analysis.

Sanger sequencing validation

Sanger sequencing was used to confirm the NGS data. DNA from all patients and their parents were subjected to polymerase chain reaction (PCR), and polyacrylamide gel electrophoresis was used to determine the size of the

amplification products. Products were purified using the QIAquick PCR Purification Kit (Qiagen, Milano, Italy) and sequenced with both forward and reverse primers using the ABI BigDye Terminator Cycle Sequencing Kit v. 3.1 on an ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were aligned with reference sequences, and mutations were identified using Sequencher DNA Sequence Analysis Software (<http://www.genecodes.com>). All primers were designed using the online tool Primer3 (<http://sourceforge.net/projects/primer3/>). The primers for exon 12 of ATP6V1B1 were 5'-TTGACCCCTCGGAATGTAGG3'- and 5'-CCGGACCCTCTCTCCTT-AC3'-(product size: 238 bp). The primers for exon 13 of ATP6V1B1 were 5'-ATGCAAATCGTGGAGCTGTG3' and 5'-ATGAATCAGGGCAA-GACGGT3' (product size: 264 bp).

Mapping and protein structure prediction

Protein and DNA sequence alignments were performed using ClustalW (<http://www.genome.jp/tools-bin/clustalw>) and MultAlin (<http://multalin.toulouse.inra.fr/multalin/>), respectively. The predicted effects of amino acid substitutions on the biological function of the protein were evaluated using both PolyPhen-2 and Provean software (<http://genetics.bwh.harvard.edu/pph2/> and <http://provean.jcvi.org>, respectively).

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Table 2. Mutations by whole-exome sequencing and corroborated Sanger sequencing in 16 patients with clinically diagnosed dRTA

ID	Gene	AD/AR	Variations	Effects	Verification in 1000 people without renal disorders	SIFT_score	Polyphen2_HDIV_score
1	SLC4A1	AD/AR	c. 2102G>A (E17) ^Δ	p. G701D (HGMD 9854053)	0	0.001	1
			c. 1387G>A (E12) ^Δ	p. G463S	0	0.245	0.644
			c. 92T>C (E3) ^Δ	p. M31T	0.001	0.565	0.001
2	SLC4A1	AD/AR	c. 1766G>A (E14) ^Δ	p. R589H (HGMD 9312167)	0	0	0.999
3	SLC4A1	AD/AR	c. 2102G>A (E17) ^{ΔΔ}	p. G701D	0	0.001	1
			c. 92T>C (E3) ^{ΔΔ}	p. M31T	0.001	0.565	0.001
4	SLC4A1	AD/AR	c. 1765C>T (E14) ^Δ	p. R589C (HGMD 9312167)	0	0	1
			c. 1898C>T (E16) ^Δ	p. S633L	0	0.203	0.027
5	SLC4A1	AD/AR	c. 2102G>A (E17) ^{ΔΔ}	p. G701D (HGMD 9854053)	0	0.001	1
			c. 92T>C (E3) ^{ΔΔ}	p. M31T	0.001	0.565	0.001
6	SLC4A1	AD/AR	c. 2102G>A (E17) ^{ΔΔ}	p. G701D	0	0.001	1
			c. 92T>C (E3) ^{ΔΔ}	P. M31T	0.001	0.565	0.001
7 [#]	ATP6V1B1	AR	c. 130G>T (E2) ^Δ	P. V44L	0	0.011	0.422
	WNK1	AD	c. 6248C>T (E22) ^Δ	p. P2083L	0.013896	0.003	0.835
	ATP6VOA4	AR	c. 1030-5_1030-4insT(IVS11) ^Δ	Insertion	0	-	-
8	ATP6V1B1	AR	c. 904C>T (E9) ^Δ	p. R302W	0	0	0.998
	CLDN16	AR	c. 164G (E1) ^Δ	p. A56Lfs*16	0	-	-
9	ATP6VOA4	AR	c. 1009~1020 deletion (E11) ^Δ	No frame shift	0	-	-
10	WNK1	AD	c. 6650G>A (E24) ^Δ	p. R2217H	0	0	1
11	SLC4A1	AD/AR	c. 2102G>A (E17) ^{ΔΔ}	p. G701D	0	0.001	1
			c. 92T>C (E3) ^{ΔΔ}	p. M31T	0.001	0.565	0.001
12	SLC4A1	AD	c. 216G>T (E5) ^Δ	p. E72D	0.010	0.092	0.006
13	SLC4A1	AD	c. 1766G>A (E14) ^Δ	p. R589H	0	0	0.999
14	ATP6V1B1	AR	c. 1326~1343 deletion (E13) ^Δ	P. 442~448 deletion	0	-	-
			c. 1354 deletion T (E13) ^Δ	P. F452fs	0	-	-
15	ATP6VOA4	AR	c. 639+1G>A (E9) ^{ΔΔ}	splicing	0	-	-
16 [#]	-	-	-	-	-	-	-

[#]With sensorineural hearing loss. ^ΔHeterogeneous. ^{ΔΔ}Homozygous.

Results

Patients with dRTA

From January 2010 to September 2015, 33 children were diagnosed with primary dRTA; 16 of these patients were analyzed by NGS, and Sanger sequencing was used to confirm mutations in 15 of these. Among the 16 patients analyzed by NGS, most were sporadic cases (15/16, 93.8%), and only one patient had a family history (her mother also presented with renal tubular acidosis). All patients were non-consanguineous.

Clinical characteristics & biochemical findings

The clinical and biochemical features of the 16 patients with dRTA are shown in **Table 1**. All 16 patients presented with growth retardation,

anorexia, vomiting, and weakness. The average age of dRTA onset was 3 years and 10 months, with 5 infants aged <1 year at onset. The average age of diagnosis was 5 years and 1 month. Sensorineural hearing loss (SNHL) was observed in patients 7 and 16. No patients presented with tubular proteinuria or hematuria, hemolytic anemia due to dominant hereditary spherocytosis, overhydrated cation leak syndrome, or variable hemolytic anemia due to dominant hereditary stomatocytosis.

At initial diagnosis, all patients presented with aciduria (urine pH>7.0) and metabolic acidosis (arterial pH 7.245-7.388), and they failed to acidify their urine below pH 7.0 after standard oral ammonium chloride loading, or both. All index cases presented with hypokalemia and hyperchloremia. Furthermore, after correction

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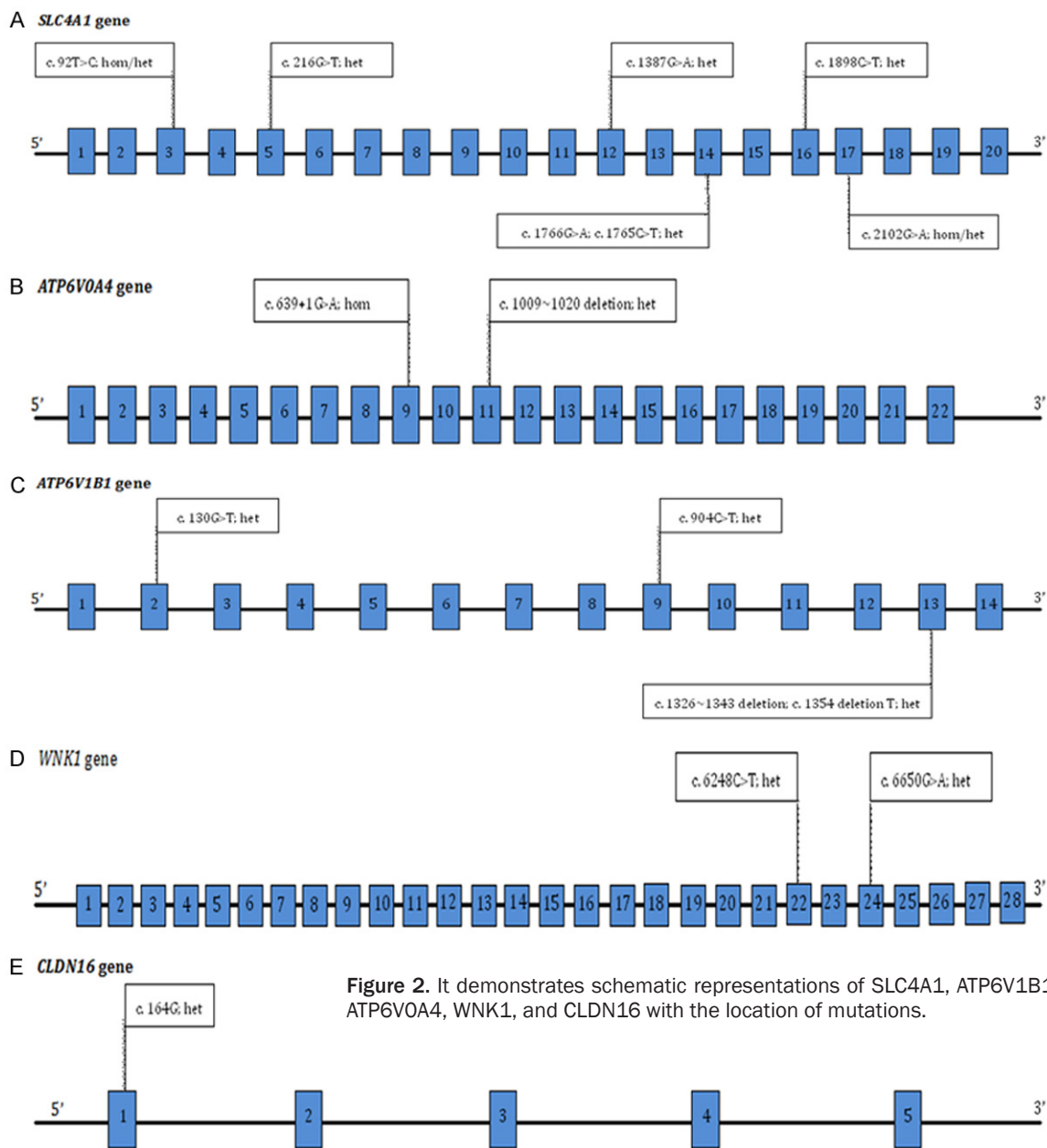


Figure 2. It demonstrates schematic representations of SLC4A1, ATP6V1B1, ATP6V0A4, WNK1, and CLDN16 with the location of mutations.

of their dehydration, all patients had normal serum phosphate, magnesium, calcium, and sodium levels. In all cases, alkali replacement resulted in improved in biochemical parameters.

Analysis of the glomerular filtration function

Serum creatinine varied from 24.0-60.4 $\mu\text{mol/L}$, and the estimated glomerular filtration rate (GFR) varied from 65.9-124.9 mL/min. Six of the patients were diagnosed with stage G2 chronic kidney disease with a GFR of 65.9-84.6 mL/min (**Table 1**). The time from onset to dis-

ease diagnosis ranged from several days to 3 years in these 6 patients.

Mutation detection

SLC4A1 mutations: Analysis of the coding regions and flanking intronic sequences of SLC4A1 showed the presence of 7 different mutations (c. 2102G>A, c. 92T>C, c. 1387G>A, c. 1766G>A, c. 1765C>T, c. 1898C>T, and c. 216G>A) in 8 families (**Table 2**; **Figure 2**). All are missense mutations, and 4 (c. 92T>C, c. 1387G>A, c. 1898C>T, and c. 216G>A) are novel.

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The missense mutation c. 2102G>A in exon 17 was identified in 5 of the 9 patients with *SLC4A1* gene variations in the current study, and the missense mutation c. 92T>C in exon 3 was identified in 5/9 dRTA patients. This mutation caused a methionine to threonine substitution that was predicted to be deleterious by the PolyPhen-2 and Provean software.

ATP6V1B1 mutations: Analysis of the coding regions and flanking intronic sequences of *ATP6V1B1* showed the presence of 4 different mutations (c. 130G>T, c. 904C>T, c. 1326-1343 deletion, and c. 1354 deletion T) in 3 families (**Table 2; Figure 2**). Two are previously reported missense mutations, and the others (the c. 1326-1343 deletion without frameshift and the c. 1354 deletion T with frameshift) are novel.

ATP6VOA4 mutations: Analysis of the coding regions and flanking intronic sequences of *ATP6VOA4* showed the presence of 3 different mutations (c. 1030-5_1030-4insT, c. 1009-1020 deletion, c. 639+1G>A) in 3 families (**Table 2; Figure 2**). All are novel mutations.

WNK lysine deficient protein kinase 1 (WNK1) mutations

Analysis of the coding regions and flanking intronic sequences of *WNK1* showed the presence of 2 different mutations (c. 6650G>A, c. 6248C>T) in 2 patients (**Table 2; Figure 2**). The former causes an arginine to histidine substitution; the latter causes a proline to leucine substitution. Both are novel mutations.

Claudin 16 (CLDN16) mutations

Analysis of the coding regions and flanking intronic sequences of *CLDN16* showed the presence of 1 mutation (c. 164G) in 1 patient, which is novel (**Table 2; Figure 2**). There were also mutations in *ATP6V1B1* (c. 904C>T in exon 9, p. R302W) and *CLDN16* (c. 164G in exon 1, p. A56Lfs*16) in this patient.

Discussion

In this study, we have genetically analyzed a large cohort of Chinese children with dRTA. We identified 7 *SLC4A1* mutations, 4 *ATP6V1B1* mutations, 3 *ATP6VOA4* mutations, 2 *WNK1* mutations, and 1 *CLDN16* mutation in 15/16 patients with clinically diagnosed dRTA, while no genetic mutations were found in the remain-

ing patient. Remarkably, 4 novel mutations were observed in each of the *SLC4A1* and *ATP6V1B1* genes, 3 novel mutations were identified in the *ATP6VOA4* gene, and 1 novel mutation was identified in the *CLDN16* gene by NGS and confirmed by Sanger sequencing.

SLC4A1 in dRTA

Distal RTA (dRTA) is characterized by the failure of the kidney to appropriately produce acidic urine in the presence of systemic metabolic acidosis or after acid loading, due to hydrogen ion secretion failure in the distal nephron [6]. Both autosomal dominant and autosomal recessive inheritance patterns may be observed in families with primary dRTA, and the spectrum of clinical severity is wide. Karet [6] reported that age of onset was much younger in recessive patients, with index cases often diagnosed in infancy. Hypokalemia was more severe, and all recessive patients had growth retardation. Nephrocalcinosis was also a common finding in this subset, even in neonates. In contrast, patients with dominant inheritance were diagnosed at older ages, and none had radiological evidence of nephrocalcinosis. According to the literature, the autosomal dominant form of primary dRTA is commonly caused by mutations in *SLC4A1*, though *SLC4A1* mutations have also been detected in cases of the autosomal recessive primary dRTA without deafness [7, 8]. In the current study, we identified 7 different mutations in *SLC4A1* (c. 2102G>A, c. 92T>C, c. 1387G>A, c. 1766G>A, c. 1765C>T, c. 1898C>T, c. 216G>A) in 9/16 patients, and a missense mutation in exon 17 (c. 2102G>A), which has been reported as deleterious (HGMD 98540-53), was found in 5 of the 9 patients.

Distal RTA with SNHL

The autosomal recessive form of primary dRTA can also be caused by mutations in *ATP6V1B1* and *ATP6VOA4*, which are commonly accompanied by SNHL or deafness [9, 10]. In the current study, we identified 4 different mutations (c. 130G>T, c. 904C>T, c. 1326-1343 deletion, c. 1354 deletion T) in *ATP6V1B1* in 3 families, and 3 different mutations (c. 1030-5_1030-4insT, c. 1009-1020 deletion, c. 639+1G>A) in *ATP6VOA4* in 3 families. One 13-year-old female who presented with SNHL harbored compound heterogeneous mutations in *ATP6V1B1* (c. 130G>T), *ATP6VOA4* (c. 1030-5_1030-4insT),

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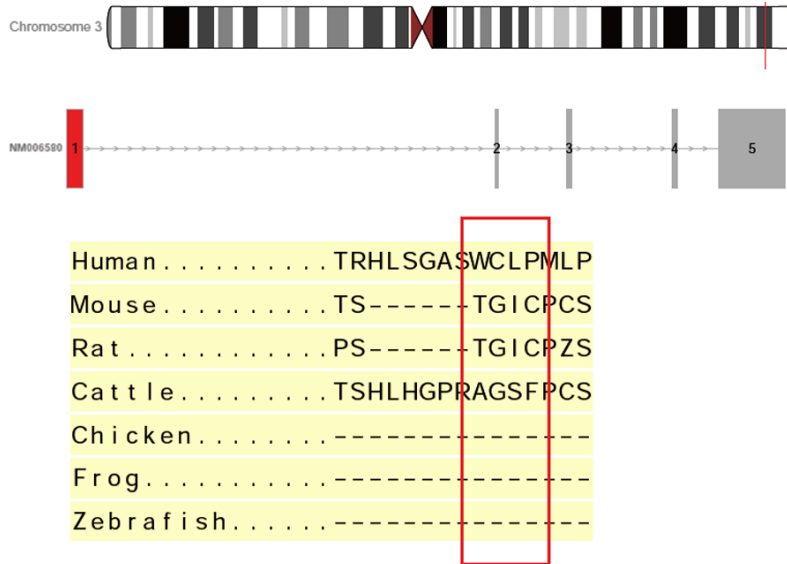


Figure 3. It shows conservative analysis of WNK1 mutation chr12.1005523 (c. 6650G>A, E24).

and *WNK1* (c. 6248C>T). Interestingly, no significantly pathogenic mutation(s) were found in a 4-month-old girl who presented with SNHL at disease onset. Follow-up will be necessary to identify pathogenic mutation(s) in this patient in the future.

WNK1

Wilson et al. [11] reported that mutations in two members of the WNK family of serine-threonine kinases cause pseudohypoaldosteronism type II (PHA II; OMIM no. 145260). PHA II is an autosomal dominant disorder characterized by hyperkalemia, hypertension (despite normal glomerular filtration), and renal tubular acidosis resulting from impaired renal K⁺ and H⁺ excretion. Deletion of the first intron of *WNK1* results in excessive expression of the ubiquitous full-length kinase (L-*WNK1*) in the distal convoluted tubule (DCT) and ubiquitous ectopic expression of kidney specific-*WNK1*. Increased L-*WNK1* expression in the DCT results in increased Na-Cl cotransporter activity and therefore hypertension and hypervolemia. However, the mechanisms underlying metabolic acidosis and hyperkalemia, remain uncertain [12]. In the present study, 2 different mutations (c. 6650G>A, c. 6248C>T) in *WNK1* were identified in 2 patients, and a 10-year-old boy with a heterogeneous c. 6650G>A mutation in *WNK1* presented with dRTA, hyperkalemia, and hyperten-

sion. The relationship between *WNK1* mutation and this phenotype remains unclear because to the best of our knowledge, no missense mutations in *WNK1* have been shown to cause any isolated tubular disorders. Both *WNK1* mutations identified in this study were predicted to be pathogenic by in silico analysis. Based on conservative analysis of the c. 6650G>A, E24 *WNK1* mutation, the results are listed in **Figure 3**. It is possibility that these alterations are insignificant, and that the phenotypes observed were due to large deletions in either *ATP6VOA4* or *ATP6V1B1*

that could not be detected by either whole-exome sequencing or Sanger sequencing. According to previous reports, no causative genes have been identified in approximately 20% of patients with dRTA. For that reason, further functional analysis to identify the causal relationships between missense mutations in *WNK1* and the onset of isolated tubular acidosis will be necessary.

CLDN16

In 1972, Michelis et al. [13] reported clinical data from two siblings with dRTA, including decreased bicarbonate threshold, hypomagnesaemia, nephrocalcinosis, convulsions, and polyuria. Subsequently, similar cases were described indicating that the patients also had hypercalciuria, progressive renal function decline and sometimes, severe ocular defects. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive tubular disorder that is frequently associated with progressive renal failure [14]. The primary defect is related to impaired tubular reabsorption of magnesium and calcium in the thick ascending limb of the loop of Henle. Mutations in *CLDN16* (FHHNC type 1) and *CLDN19* (FHHNC type 2), which encode the renal tight junction proteins claudin-16 and claudin-19 [15], were identified as the underlying genetic causes. Incomplete dRTA is often

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observed in both types of FHHNC, but the pathogenic mechanism is unknown. Manz et al. [16] reported that polyuria, hypomagnesemia, hypercalciuria, hyposthenuria, advanced nephrocalcinosis, and low GFR in a pair of female siblings, followed for over 10 years. Acid loading revealed incomplete dRTA in these patients. Weber et al. [17] found that 17 of 20 patients with *CLDN16* mutations presented with incomplete dRTA. Hampson et al. [18] documented the case of a 7-year-old boy with renal impairment, partial dRTA and defective urinary concentrating ability who harbored 2 heterozygous mutations in *CLDN16*. One patient was identified as having compound mutations in both *ATP6V1B1* (c. 904C>T in exon 9, p. R302W) and *CLDN16* (c. 164G in exon 1, p. A56Lfs*16).

In this study, we identified a frameshift mutation in *CLDN16* that decreased the protein length from 305 amino acids to 70. This *CLDN16* mutation was judged as “VUS: unidentified” by *in silico* analysis. The role of this mutation in Patient 8 will require further study.

Oligogenic inheritance

Considering the complex phenotypic heterogeneity of many human disorders, clear distinctions between monogenic and complex traits cannot always be drawn. The determination of a single mutation inherited in a simple Mendelian pattern is sometimes not sufficient to predict exact clinical phenotypes. Rather, it could be argued that all genetic traits are complex, due to a genetic background with multiple variations capable of modifying the phenotype [19]. According to Chang et al. [20], few multi-genic, recessively inherited Mendelian diseases present as heterozygous mutations in different genes. Gómez et al. [21] reported a compound heterozygous patient with dRTA with a known mutation in *ATP6V1B1* (p. G609R) and a pathogenic variation in *SLC4A1* (p. E508K) confirming that compound heterozygosity in two different but functionally related genes can be responsible for dRTA. In the present study, we identified 2 cases with this phenomenon: case 7 harbored 3 mutations from 3 different genes: a c. 130G>T mutation in *ATP6V1B1*, a c. 1030-5_1030-4insT mutation in *ATP6VOA4* and c. 6248C>T in the *WNK1*; and case 8 harbored a c. 904C>T mutation in *ATP6V1B1* and a c. 164G deletion in *CLDN16*. These cases deser-

ve further observation and follow-up study to clarify the importance of the combined effects of 2 or more different but functionally related genes in disease pathogenesis.

Whole-exome sequencing is a rapid and useful tool for genetic diagnosis, which will hopefully result in discovery of many novel mutations. An increasing number of studies have used this technique to identify disease-causing mutations for Mendelian disorders [21, 22]. Our results demonstrate that despite the limitations of the technique and the pooling methodology, NGS is a potent tool that offers reliable results with lower effort and cost than Sanger sequencing [9, 23-25].

Conclusion

In this report, we present the genetic analysis of a Chinese cohort of patients with dRTA, as well as a new diagnostic strategy to facilitate dRTA genetic testing in China. Putative dRTA mutations were found in 94% of the index cases (15/16), genetically confirming the clinical diagnosis. This demonstrates that whole-exome sequencing is labor and cost effective for the analysis of dRTA genes. Our results demonstrate the presence of *SLC4A1*, *ATP6V1B1*, *ATP6VOA4*, *WNK1* and *CLDN16* mutations in Chinese children with dRTA for the first time and show that compound heterozygosity at 2 or more different but related genes (oligogenic inheritance) can be responsible for the development of dRTA.

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Disclosure of conflict of interest

None.

Address correspondence to: Jianhua Mao, Department of Nephrology, The Children's Hospital of Zhejiang University School of Medicine, 57 Zhugan Lane, Hangzhou 310003, Zhejiang Province, China. E-mail: maojh88@zju.edu.cn

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