



CLINICAL RESEARCH ARTICLE

Sequence variants in the renin–angiotensin system genes are associated with isolated multicystic dysplastic kidney in children

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BACKGROUND: Multicystic dysplastic kidney (MCDK) is a common form of congenital cystic kidney disease in children. The etiology of MCDK remains unclear. Given an important role of the renin–angiotensin system in normal kidney development, we explored whether MCDK in children is associated with variants in the genes encoding renin–angiotensin system components by Sanger sequencing.

METHODS: The coding regions of renin (*REN*), angiotensinogen (*AGT*), *ACE*, and angiotensin 1 receptor (*AGTR1*) genes were amplified by PCR. The effect of DNA sequence variants on protein function was predicted with PolyPhen-2 software.

RESULTS: 3 novel and known *AGT* variants were found. 1 variant was probably damaging, 1 was possibly damaging and one was benign. Out of 7 *REN* variants, 4 were probably damaging and 3 were benign. Of 6 *ACE* variants, 3 were probably damaging and 3 benign. 3 *AGTR1* variants were found. 2 variants were possibly damaging, and one was benign.

CONCLUSION: We report novel associations of sequence variants in *REN*, *AGT*, *ACE*, or *AGTR1* genes in children with isolated MCDK in the United States. Our findings suggest a recessive disease model and support the hypothesis of multiple renin–angiotensin system gene involvement in MCDK.

Pediatric Research (2021) 90:205–211; <https://doi.org/10.1038/s41390-020-01255-y>

IMPACT:

- Discovery of novel gene variants in renin–angiotensin genes in children with MCDK.
- Novel possibly damaging gene variants discovered.
- Multiple renin–angiotensin system gene variants are involved in MCDK.

INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) account for 30–50% of cases of end-stage renal disease (ESRD) in children.¹ Despite the high risk of ESRD from CAKUT, the gene variants causing the majority of CAKUT are unknown. Multicystic dysplastic kidney (MCDK) is the most common and severe type of cystic kidney dysplasia in children. MCDK occurs in 1 in 1000 to 1 in 4300 live births.^{2,3} MCDK consists of numerous non-communicating cysts with no or minimal functional renal tissue among cysts. MCDK is unilateral in most cases but may involve both kidneys. If bilateral, MCDK gives rise to Potter syndrome (limb deformities, typical facial appearance, and pulmonary hypoplasia). Histological examination of MCDK reveals the presence of immature epithelium and persistence of connective tissue.^{2,3} MCDK is considered to result from either in utero urinary tract obstruction or from abnormal inductive cross-talk between the ureteric bud and surrounding metanephric mesenchyme during embryonic kidney organogenesis.^{4,5} Although MCDK is considered to be a non-heritable developmental anomaly, available evidence indicates that gene variants may play an important role in the pathogenesis of MCDK.

Variants in *HNF1B* and *PAX2* were found in patients with MCDK and other variable renal phenotypes.^{6,7} Furthermore, MCDK is associated with variants in the *CHD1L*, *ROBO2*, *HNF1B*, and *SALL1* genes.⁸

Pharmacological antagonism of the renin–angiotensin system or mutations in the renin (*REN*), angiotensinogen (*AGT*), *ACE*, and angiotensin 1 receptor (*AGTR1*) genes in animals or humans result in a broad spectrum of CAKUT that include hydronephrosis, renal medullary hypodysplasia, abnormal ureteric bud budding, renal tubular dysgenesis (RTD, OMIM 267430), duplex renal collecting system and defective urinary concentrating ability.^{9–18} Since CAKUT is the leading cause of renal failure in children,^{1,19} it is essential to identify the molecular mechanisms that cause different forms of CAKUT when renin–angiotensin system signaling is disrupted. The role of the renin–angiotensin system genes in isolated MCDK in children has not been determined. Here, we followed a candidate gene approach by hypothesizing that the renin–angiotensin system could be involved in the etiology of MCDK. We report novel associations of *REN*, *AGT*, *ACE*, and *AGTR1* gene variants with isolated MCDK in children.

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Received: 7 August 2020 Revised: 6 October 2020 Accepted: 22 October 2020

Published online: 10 November 2020

MATERIALS AND METHODS

Patients and samples

This study protocol was approved by the IRB committee of Tulane University School of Medicine (IRB#:150438-4). Written informed consent was obtained from the parents or guardians of the children who served as subjects of the investigation and, when appropriate, assent from the subjects themselves. Peripheral blood specimens were obtained from 10 unrelated patients with MCDK diagnosed by renal ultrasonography (US) (mean age 8.5 ± 1.1 years) and 20 pooled healthy race-, age- and sex-matched controls (4 white females, 5 white males, 5 black females and 6 black males) after obtaining appropriate consent. The control group consisted of patients who had renal imaging studies performed for renal system diseases other than CAKUT (eg, asymptomatic microscopic hematuria, mild proteinuria). 6 patients with MCDK were females and 4- males. Renal function was calculated from serum creatinine levels using a modified Schwartz equation (length in cm × 0.413/serum creatinine in mg/dL).²⁰

DNA preparation

DNA was isolated from patients' blood using an automated Qiagen EZ1 instrument with their EZ1 Blood Extraction kit (Qiagen Inc., Valencia, CA) and was quantified by the Qubit® 2.0 Fluorometer with the Qubit® dsDNA BR assay kit (Invitrogen™, Eugene, OR, USA). DNA was purified with Diffinity RapidTip (Diffinity Genomics) and both strands of DNA were sequenced using the dideoxy chain termination method using a 373A sequencer. The coding sequences of the *REN*, *AGT*, *ACE*, and *AGTR1* genes were amplified by PCR: renin (*REN*, ENSG00000143839, 9 exons), angiotensinogen (*AGT*, ENSG00000135744, 5 exons), *ACE* (ENSG00000159640, 26 exons), and angiotensin 1 receptor (*AGTR1*, ENSG00000144891) (1 exon). Primer sequences and conditions for PCR amplification were obtained from Dr. Marie Gubler (Hopital Necker-Enfants Malades, Paris, France).¹⁰ The DNA sequences of patients were compared to DNA sequences of controls using Sequencher5.1 by direct sequence analysis.

Prediction of deleterious sequence variants

Prediction of deleterious sequence variants was performed with PolyPhen-2 (Polymorphism-Phenotypingv2) software (<http://genetics.bwh.harvard.edu/pph2>). PolyPhen-2 predicts the potential effect of amino acid substitutions on the function and structure of proteins by comparing evolutionary conservation using a Bayesian approach.²¹ The projection scores are assigned in the range of 0–1, where 0 is considered to be benign and 1 being a deleterious variant. Score 0.0–0.15 variants are predicted to be

benign; 0.15–0.84 variants are possibly deleterious; 0.85–1.0 variants are probably deleterious (more likely to be harmful).

Ensembl variant effect predictor

Conservation of amino acid residues corresponding to respective DNA nucleotide sequence variants identified in patients with MCDK and the consequence of DNA nucleotide variants on the amino acid sequence of respective proteins (e.g., missense, stop lost, frameshift) was predicted with Ensembl Variant Effect Predictor (<https://useast.ensembl.org/>).²² Gene variant positions and alleles identified in MCDK patients were uploaded according to the default Variant Effect Predictor format on the Ensembl genome browser (<https://useast.ensembl.org/>). Corresponding chromosome, variant location on the whole gene sequence, allele, strand, and identifier were entered in respective columns.

Variant filtering on the basis of population frequency

Analysis of rare alleles (minor allele frequency <1%) was performed using the Genome Aggregation Database (gnomAD) (<https://gnomad.broadinstitute.org/>). The v2 data set on the gnomAD website covers 125,748 exome sequences and 15,708 whole-genome sequences from unrelated persons sequenced as part of different studies. The gnomAD database v2 was queried for the known and novel variants in *AGT*, *REN*, *ACE*, and *AGTR1* genes detected in children with MCDK.

RESULTS

Summary of clinical features

The mean age of children with MCDK was 8.5 ± 1.1 years (Table 1). Six were females and four were males. Two patients were Caucasian and eight were African-American. All patients had normal kidney function at the time of collection of a blood sample and normal blood pressure. Self-reported family history was negative for known MCDK or other known kidney anomalies in all children. 9 of 10 cases of MCDK were isolated and one was associated with Turner's syndrome. All cases of MCDK were unilateral with a left vs. right MCDK ratio of 1:1 (Table 1). In all cases, the contralateral kidney underwent appropriate compensatory hypertrophy. In only one case contralateral kidney demonstrated the presence of mild hydronephrosis. All patient's parents were phenotypically normal with the self-reported absence of known structural anomalies of the renal system or kidney disease. The mean age of children in the control group was 9.7 ± 0.9 years (Table 2). Self-reported family history was negative for known kidney anomalies in all children in the control group.

Table 1. Clinical characteristics of children with MCDK.

Subject	Sex	Age (year)	Race	Height (cm)	Weight (Kg)	Patient phenotype
3	Male	10	White	140	32	Left MCDK Aspergers
14	Female	9	White	139	31	Left MCDK
16	Female	9	Black	124	21	Right MCDK
22	Female	20	Black	148	41	Right MCDK Turner syndrome
26	Male	11	Black	140	35	Left MCDK
36	Female	12	Black	136	33	Right MCDK Left hydronephrosis
39	Male	7	Black	117	20	Right MCDK
41	Female	5	Black	115	21	Left MCDK
42	Male	4	Black	107	21	Right MCDK
43	Male	8	Black	121	22	Left MCDK

Table 2. Clinical characteristics of children in the control group.

Subject	Sex	Age (year)	Race	Height (cm)	Weight (kg)	Patient phenotype
1	Female	12	White	142	34	Mild proteinuria
2	Female	4	Black	115	20	Microscopic hematuria
4	Male	13	Black	156	42	Mild proteinuria
5	Male	9	Black	136	30	Microscopic hematuria
6	Male	7	Black	125	25	Microscopic hematuria
8	Male	12	White	150	48	Microscopic hematuria
10	Male	4	White	110	32	Microscopic hematuria
11	Female	15	Black	162	60	Mild proteinuria
13	Male	6	White	120	20	Mild proteinuria
28	Female	14	Black	161	64	Microscopic hematuria
29	Female	11	White	142	36	Microscopic hematuria
30	Male	7	Black	128	26	Mild proteinuria
31	Male	16	Black	175	68	Mild proteinuria
32	Male	9	Black	138	35	Microscopic hematuria
33	Male	12	White	151	55	Microscopic hematuria
34	Male	11	White	146	38	Microscopic hematuria
35	Female	7	White	128	25	Microscopic hematuria
40	Female	8	Black	131	27	Microscopic hematuria
45	Female	5	White	112	20	Mild proteinuria
46	Female	10	Black	143	37	Microscopic hematuria

Gene sequence variants

To determine whether MCDK is linked to variants in the renin–angiotensin system genes, we performed Sanger sequencing of exons and exon-intron junctions of renin (*REN*), angiotensinogen (*AGT*), *ACE*, and angiotensin 1 receptor (*AGTR1*) genes.¹⁰ A total of 19 variants (9 novel and 10 known) in *REN*, *AGT*, *ACE*, and *AGTR1* genes were found in 10 patients with MCDK (Table 3). 8 were predicted to be benign and 11- possibly or probably pathogenic by PolyPhen-2 analysis (Table 3). None of the known or novel variants identified in children with MCDK were detected in the control group. 2 novel and one known *AGT* variants were found. The *AGT* variants C1128T and T1311C were identified as “probably damaging” with PolyPhen-2 scores of 0.938 and 1.0 (Table 2). 7 heterozygous *REN* variants (6 novel and one known) were found. 4 were predicted to be probably damaging with scores of 0.993–1.0 and 3 were benign. The most striking variation seems to be the 10 base insertion in *REN* identified in patients 26 and 41 (Table 2), which will lead to a frameshift, probably to a premature stop and most likely represents a loss of function mutation. The fact that this variation has been found in two unrelated patients supports the hypothesis that MCDK might be associated with heterozygous *REN* mutations.

6 heterozygous *ACE* variants (all were known variants) were found. 3 variants were predicted to be probably damaging with scores of 0.74–1.0 and 3 variants were predicted to be benign. Probably damaging *ACE* variant C616T in exon 4 was identified in three unrelated patients with MCDK, whereas probably damaging *ACE* variant T3421C in exon 23 was present in two unrelated patients (Table 3). In addition to the 10 base insertion in *REN* identified in patients 26 and 41, both patients carry additional but different high PolyPhen-2 score changes in the *ACE* gene (Table 3). These findings support the hypothesis of multiple renin–angiotensin system gene involvement in MCDK and may explain why researchers missed the underlying heritability pattern until now. 3 heterozygous *AGTR1* variants (one novel and two known) were found. 2 variants were considered to be possibly deleterious with scores of 0.504 and 0.6 and one was predicted to be benign. Figure 1 shows chromatograms of sequence variants in

AGT, *REN*, *ACE* *AGTR1* in select patients with MCDK and chromatograms of respective controls.

To determine the significance of identified sequence variants, we examined the evolutionary conservation of amino acid residues corresponding to respective DNA nucleotide sequence variants identified in patients with MCDK using Ensembl Variant Effect Predictor. Comparison in silico revealed that respective amino acid residues are highly conserved in multiple mammals (select residues corresponding to respective DNA nucleotide sequence variants depicted in Fig. 1 are shown in Fig. 2). Thus, these residues are likely to be important for protein function. The consequence of probably pathogenic DNA sequence variants detected in patients with MCDK on the protein sequence identified with Ensembl Variant Effect Predictor showed respective *AGT*, *REN*, and *ACE* to be 100% missense variants and *AGTR1* variant C961T, predicted as possibly damaging by PolyPhen-2, as 100% synonymous variant (Fig. 2). *AGTR1* variant C961T was predicted as possibly damaging by PolyPhen-2 but was predicted to be synonymous (benign) by Ensembl Variant Effect Predictor. This discrepancy is likely observed, in part, due to different predictive features utilized by different in silico analysis tools. In addition, the PolyPhen-2 score for the C961T variant was relatively low at 0.504, thus reducing the likelihood of this variant being damaging.

The prevalence of known gene variants identified in this study in the general population was determined using the gnomAD database. *ACE* variants C616T, G815T, C1249T, A2360G, A2362G, and T3421C, *AGT* variant C1128T, *AGTR1* variants C961T and A1450G, *REN* variant A248C were found in the gnomAD database. *ACE* variant C616T has the highest allele frequency of 0.0009104 in the African group. The highest age distribution is 35–40 and 45–50 years of age. *ACE* variant G815T has a maximal frequency of 0.0006505 in the European group. The highest age distribution is 50–55 years of age. *ACE* variant C1249T has the top frequency of 0.0009186 in the European group. *ACE* variant A2360G has the highest prevalence of 0.00001962 in the African group. The highest age distribution is 45–50 and 60–65 years of age. *ACE* variant A2362G has the maximal allele prevalence of 0.0003155 in

Table 3. Sequence variants detected in the renin–angiotensin system genes.

Patient number	Gene	Exon	Normal	Variant	Location	Type	Patient phenotype	PP2Score	Prediction	Gender	Race
3	<i>AGTR1</i>	4B	C	T	961	HET	Left MCDK Aspergers	0.504	Possibly damaging	Male	White
	<i>AGT</i>	2	C	T	1128	HET		1	Probably damaging		
	<i>AGT</i>	2	T	C	1311 ^a	HET		0.938	Possibly damaging		
	<i>AGT</i>	3	C	G	364 ^a	HOM		0.001	BENIGN		
14	<i>REN</i>	1	C	T	134 ^a	HET	Left MCDK	1	Probably damaging	Female	White
	<i>REN</i>	2	A	C	248	HET		0	BENIGN		
16	<i>ACE</i>	23	T	C	3421	HET	Right MCDK	1	Probably damaging	Female	Black
	<i>ACE</i>	16–17	A	G	2362	HET		0.074	BENIGN		
	<i>AGTR1</i>	4C	A	G	1450	HET		0.001	BENIGN		
22	<i>ACE</i>	8	C	T	1249	HET	Right MCDK	1	Probably damaging	Female	Black
	<i>AGTR1</i>	4B	T	A	960 ^a	HET		0.6	Possibly damaging		
	<i>REN</i>	2	G	A	290 ^a	HET		1	Probably damaging		
	<i>REN</i>	3	G	A	375 ^a	HET		0.148	BENIGN		
26	<i>ACE</i>	16–17	G	A	2362	HET	Left MCDK	0.074	BENIGN	Male	Black
	<i>ACE</i>	23	T	C	3421	HET		1	Probably damaging		
	<i>REN</i>	4	G	A	446 ^a	HET		0.993	Probably damaging		
	<i>REN</i>	7	CTGACTGTGC		873 ^a	INSERTION					
36	<i>AGTR1</i>	4B	C	T	961	HET	Right MCDK	0.504	Possibly damaging	Female	Black
39	<i>ACE</i>	16	G	T	2362	HET	Right MCDK	0.074	BENIGN	Male	Black
	<i>ACE</i>	4	C	T	616	HET		1	Probably damaging		
	<i>ACE</i>	5	G	T	815	HET		0.42	BENIGN		
41	<i>ACE</i>	4	C	T	616	HET	Left MCDK Turner syndrome	1	Probably damaging	Female	Black
	<i>ACE</i>	16	G	A	2362	HET		0.074	BENIGN		
	<i>REN</i>	7	CTGACTGTGC		873 ^a	INSERTION					
	<i>AGTR1</i>	4C	A	G	1450	HET		0.001	BENIGN		
42	<i>AGTR1</i>	4B	C	T	961	HET	Right MCDK	0.504	Possibly damaging	Male	Black
43	<i>REN</i>	5	G	A	655 ^a	HET	Left MCDK	0.243	BENIGN	Female	Black
	<i>ACE</i>	4	C	T	616	HET		1	Probably damaging		
	<i>ACE</i>	16	A	G	2360	HET		0.074	BENIGN		

PP2 PolyPhen-2, HET heterozygous, HOM homozygous.
^aIndicates novel variants identified in this study.

the Latino group. The highest age distribution is 40–50 years of age. *ACE* variant T3421C shows the highest frequency of 0.0008754 in the East Asian group. The peak age distribution is 45–50 years. *AGT* variant C1128T shows the highest frequency of 0.0008013 in the African group. The peak age distribution is 60–65 and 70–75 years. *AGTR1* variant C961T shows the highest frequency of 0.0001063 in the European group. The peak age distribution is 50–60 years. *AGTR1* variant A1450G shows that the highest frequency of 0.0008754 in the East Asian group. No specific age groups are identified as the highest distribution. *REN* variant A248C shows the highest frequency of 0.00002891 in the East Asian group. No specific age groups are identified as the highest distribution. No population frequency for novel renin–angiotensin system gene variants identified in this study was found in the gnomAD database. From the data in OMIM (<https://www.omim.org>) and NCBI (<https://www.ncbi.nlm.nih.gov>) websites, the known renin–angiotensin system gene variants identified in this study are found in renal dysplasia.

DISCUSSION

The present study discovered nine novel sequence variants in the renin–angiotensin system genes in six out of ten (60%) children with MCDK. From total 19 variants in *AGT*, *REN*, *ACE*, and *AGTR1* detected in children with MCDK. 8 were projected to be benign

and 11- possibly or probably pathogenic by PolyPhen-2 analysis. MCDK is one of the most frequent types of congenital anomalies of the kidney and urinary tract (CAKUT). MCDK usually is identified sporadically, but families with autosomal-dominant inheritance have been described.²³ This observation supports the possibility that genetic factors contribute to MCDK. However, the possible role of the renin–angiotensin system in the etiology of MCDK is unknown.

The renin–angiotensin system is composed of substrate angiotensinogen (AGT) produced in the liver and converted by aspartyl protease renin to generate decapeptide Angiotensin I (Ang I). Ang I is cleaved by zinc metalloproteinase ACE to angiotensin II (Ang II), the active effector octapeptide in the renin–angiotensin system.¹⁸ Ang II exerts its actions through two distinct receptors, *AGTR1* and *AGTR2*. *AGTR1* propagates Ang II-induced vasoconstriction and cell proliferation, whereas *AGTR2* causes vasodilation. The major components of the renin–angiotensin system are expressed early during kidney development in humans.^{10,18,24} Crucial role of the renin–angiotensin system in kidney formation and function in humans is evident from the observation that *AGT*, *REN*, *ACE*, and *AGTR1* variants are linked to renal tubular dysgenesis, an autosomal-recessive disease characterized by poorly differentiated proximal tubules, oligohydramnios due to anuria of the fetus, reduced arterial blood pressure and neonatal death from

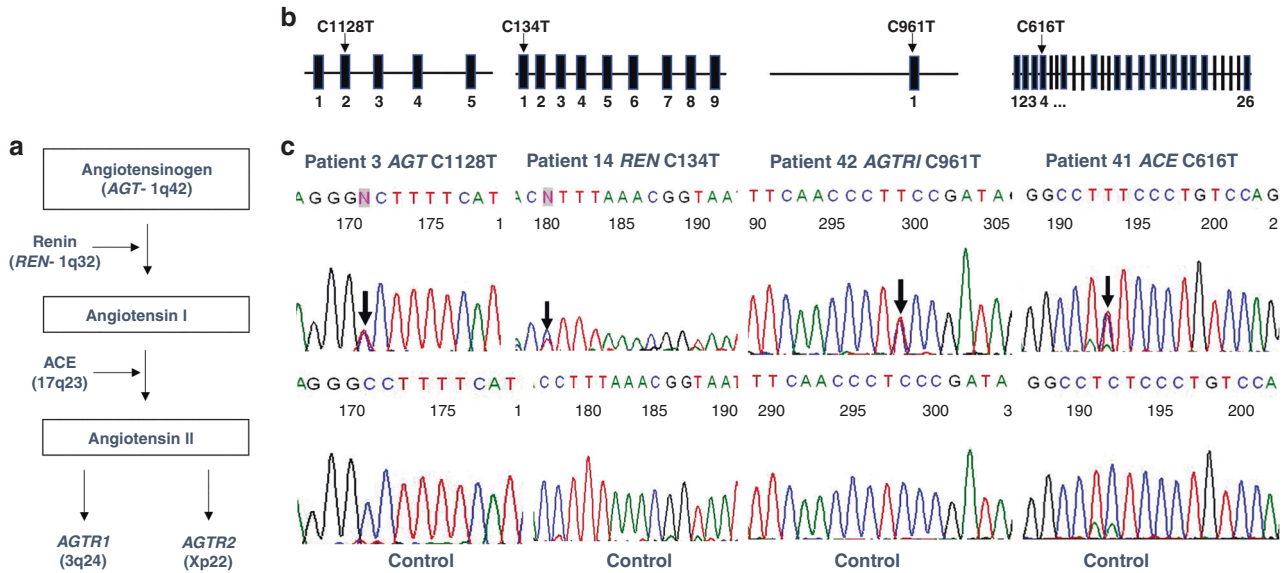


Fig. 1 Identification of variants in angiotensinogen (AGT), renin (REN), angiotensin-converting enzyme (ACE), and Angiotensin II receptor type 1 (AGTR1) in children with multicystic dysplastic kidney (MCDK). **a** Components and cascade of the renin-angiotensin system. The chromosomal location of each gene is shown. **b** Gene map of human AGT, REN, ACE, and AGTR1 depicting non-synonymous variants (arrows) identified in select MCDK patients shown in panel C (boxes represent exons, numbers represent exon numbers). **c** Chromatograms in upper panels show representative possibly pathogenic sequence variants in AGT, REN, ACE, AGTR1 in patients with MCDK (arrows) as determined by PolyPhen-2. Chromatograms in lower panels show sequencing traces of healthy controls.

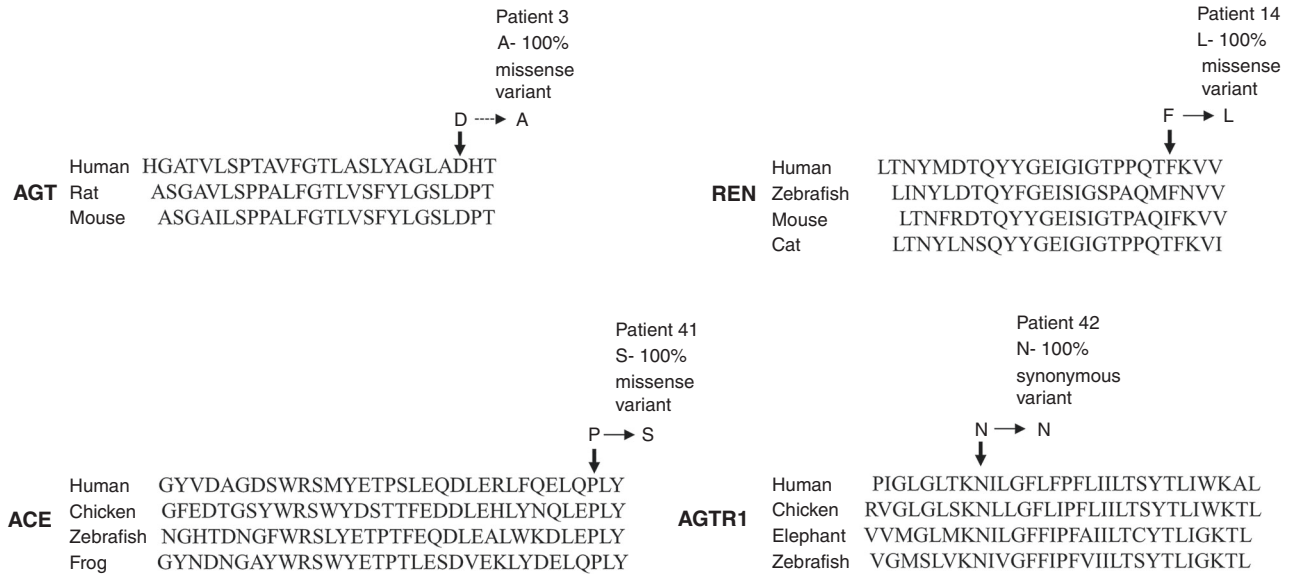


Fig. 2 Identification of the conservation of amino acid residues corresponding to respective DNA nucleotide sequence variants identified in patients with MCDK. Comparison of amino acid sequences of the human angiotensinogen (AGT), renin (REN), angiotensin-converting enzyme (ACE), and angiotensin II AT1 receptor (AGTR1) proteins among species shows respective protein residues (vertical arrows) to be conserved in different species. D- aspartic acid (position 152 with respect to human AGT protein), F- phenylalanine (position 30 with respect to human renin protein), N- asparagine (position 191 with respect to human AGTR1 protein), P- proline (position 206 with respect to human ACE protein). The consequence of DNA sequence variants identified in patients with MCDK on the protein sequence identified with Ensembl Variant Effect Predictor (horizontal arrows). Amino acid changes: AGT- from D to A (alanine); REN- F to L (leucine); ACE- P to S (serine); AGTR1- N to N.

pulmonary hypoplasia.¹⁰ Our present findings demonstrate that AGT, REN, ACE, and AGTR1 variants are associated with MCDK in children. Other studies also demonstrate alterations in the renin-angiotensin system component expression or presence of renin-angiotensin system gene variants in cystic kidney disease. In this regard, the abnormal expression of AGT in renal cysts is reported in the mouse model of autosomal-recessive polycystic

kidney disease (ADPKD).²⁵ Bi-allelic loss of function variants in ACE was reported in unrelated children with cystic renal disease.²⁶ However, the role of the renin-angiotensin system in the pathogenesis of MCDK and the molecular mechanisms by which disrupted renin-angiotensin system signaling may be causally linked to human MCDK are unknown. Renin, AGT, ACE, AGTR1, and Ang II are localized in kidney cysts in human autosomal-dominant

polycystic kidney disease (ADPKD) and autosomal-recessive PKD (ARPKD).^{27–29} In ADPKD, renin is expressed predominantly in cysts of distal tubule origin, while AGT is present mainly in cysts arising from the proximal tubule. It has been proposed that overactivity of an autocrine/paracrine renin–angiotensin system may contribute to cyst formation in human ADPKD.^{27–30}

The primary cilium is another factor that plays an important role in PKD. Variants in ciliary proteins cause aberrant cilia signaling and cyst formation.³¹ Absence of polycystin 1 (PC1) and polycystin 2 (PC2) function causes ADPKD in humans,²⁶ while lack of fibrocystin results in ARPKD.³² In regard to the cross-talk between primary cilia and the renin–angiotensin system in PKD, mice lacking PC1 and cilia show increased number of renal cysts, increased kidney prorenin, kidney, and urinary AGT contents.³³ Functionally, cAMP responses to Ang II administration in the collecting duct are higher in cells without cilia compared to cells with cilia, indicating increased activity of the local renin–angiotensin system in the absence of cilia. Thus, enhanced renin–angiotensin system activity may contribute to cyst expansion in PKD by activating cAMP.³⁴ The paired homeobox 2 gene (*PAX2*) is another candidate responsible for cyst formation. Given that increased levels of *PAX2* expression are observed in a number of cystic kidney diseases and are accompanied by enhanced proliferation of epithelial kidney cells, increased expression of *PAX2* may be an important contributor to the development of renal cysts.³⁵ Since angiotensin II induces *PAX2* expression in the mesenchymal cells of the fetal kidney,³⁶ disruption of renin–angiotensin system signaling may contribute to MCDK by dysregulation of *PAX2*. An important mechanism contributing to kidney cyst expansion is tubular epithelial cell proliferation. Given that Ang II, acting via the *AGTR1*, promotes tubular epithelial cell proliferation,³⁷ increased activity of the renin–angiotensin system may promote cyst growth in PKD by stimulation of epithelial cell proliferation.

In view of the urinary tract “obstructive” theory for MCKD’s occurrence,^{4,5} reduced or disrupted renin–angiotensin system signaling likely has a role in MCKD. This probability is supported by the findings that deletion of *Agt*, *Ren*, *Ace*, or Ang II receptor *Agtr1a;Agtr1b* in mice causes small medulla and dilation of the renal pelvis.^{15,17} In *Agtr1a;Agtr1b*-knockout mice, these renal structural anomalies are due to functional urinary tract obstruction.¹¹ Identification of possibly and probably pathogenic variants in *AGT*, *REN*, *ACE*, and *AGTR1* in this study suggests that reduced renin–angiotensin system activity may contribute to cyst formation in MCKD, in part, via early in utero urinary tract obstruction. Additional work is needed to investigate the expression of the renin–angiotensin system constituents in the kidney tissue of MCKD cases to examine whether aberrant quantitative and/or spatial/cellular expression renin–angiotensin system components may be involved in cystogenesis in MCKD.

In summary, the current study describes novel associations of variants in the genes encoding AGT, renin, ACE and *AGTR1* with isolated MCKD in children. These findings support the hypothesis of multiple renin–angiotensin system genes are involved in MCKD and highlight a crucial contribution of the renin–angiotensin system to cyst formation in children with MCKD.

ACKNOWLEDGEMENTS

This work was supported by NIH Grant DK-071699 to Ihor V. Yosypiv.

AUTHOR CONTRIBUTIONS

Each author has met the Pediatric Research authorship requirements. Both I.V.Y. and R.S. had substantial contributions to conception and design, acquisition of data, analysis, and interpretation of data. RS drafted the article. I.V.Y. and R.S. revised the

article for important intellectual content. R.S. and I.V.Y. approved the submitted version to be published.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Patient consent: Informed consent was obtained from the parents or guardians of the children who served as subjects of the investigation and when appropriate, assent from the subjects themselves.

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