Next-Generation Sequencing-Based Genetic Diagnostic Strategies of Inherited Kidney Diseases

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Keywords

Inherited kidney diseases · Next-generation sequencing · Variants interpretation · Genetic diagnosis

Abstract

Background: At least 10% of adults and most of the children who receive renal replacement therapy have inherited kidney diseases. These disorders substantially decrease their life quality and have a large effect on the health-care system. Multisystem complications, with typical challenges for rare disorders, including variable phenotypes and fragmented clinical and biological data, make genetic diagnosis of inherited kidney disorders difficult. In current clinical practice, genetic diagnosis is important for clinical management, estimating disease development, and applying personal treatment for patients. **Summary:** Inherited kidney diseases comprise hundreds of different disorders. Here, we have summarized various monogenic kidney disorders. These disorders are caused by mutations in genes coding for a wide range of proteins including receptors, channels/transporters, enzymes, transcription factors, and structural components that might also have a role in extrarenal organs (bone, eyes, brain, skin, ear, etc.). With the development of next-

generation sequencing technologies, genetic testing and analysis become more accessible, promoting our understanding of the pathophysiologic mechanisms of inherited kidney diseases. However, challenges exist in interpreting the significance of genetic variants and translating them to guide clinical managements. Alport syndrome is chosen as an example to introduce the practical application of genetic testing and diagnosis on inherited kidney diseases, considering its clinical features, genetic backgrounds, and genetic testing for making a genetic diagnosis. **Key Messages:** Recent advances in genomics have highlighted the complexity of Mendelian disorders, which is due to allelic heterogeneity (distinct mutations in the same gene produce distinct phenotypes), locus heterogeneity (mutations in distinct genes result in similar phenotypes), reduced penetrance, variable expressivity, modifier genes, and/or environmental factors. Implementation of precision medicine in clinical nephrology can improve the clinical diagnostic rate and treatment efficiency of kidney diseases, which requires a good understanding of genetics for nephrologists.

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Introduction

Inherited kidney disorders represent a significant risk for the development of end-stage renal disease (ESRD), most of which have manifestations beginning from childhood. Recent advances in molecular genetics have made great impacts on better understanding of the molecular mechanisms of different inherited kidney diseases, some of which were not considered as genetic caused previously.

Many syndromic kidney diseases such as Alport syndrome (AS), thin basement membrane nephropathy (TBMN), autosomal dominant polycystic kidney disease (ADPKD), and a set of tubular transporter disorders are caused by pathogenic single-nucleotide variants, small frameshift insertions or deletions in the disease-causing genes. In addition, chromosomal abnormalities or copy number variants (CNVs) can cause syndromes with kidney disorders, such as Down syndrome, Turner syndrome, or Williams syndrome. The inherited kidney disorders with their genetic causes and clinical manifestations are summarized in Table 1.

In an era of precision medicine, understanding the genetic background in individuals with suspected inherited kidney diseases has important diagnostic and prognostic implications. In this review, we introduced some types of inherited kidney diseases, overviewed the application of next-generation sequencing (NGS) technologies on clinical diagnosis of inherited kidney diseases, especially in finding novel pathogenic genes, summarized the guidelines and standards for interpreting variants, and used AS as an example to specifically discuss the conditions and challenges of genetic diagnosis of inherited kidney diseases.

Inherited Renal Structural Abnormality

Ciliopathies

Ciliopathies are a class of pediatric developmental disorders that are caused by impaired function or structure of cilia [1, 2]. Cilia is microtubule-based organelles projecting from the cytoplasmic membrane of the cell. Abnormalities of cilia can cause diseases in many organs, of which the most often affected are kidney, eye, ear, liver, lung, and brain. The diseases associated with renal ciliopathies includes PKD, nephronophthisis (NPHP), renal cystic dysplasia [2] and some multisystem ciliopathies, including Joubert syndrome and related diseases, Bardet-Biedl syndrome, and Meckel-Gruber syndrome [3].

Autosomal Dominant PKD

ADPKD is one of the most common genetic diseases among all races. Over 90% of patients with ADPKD are caused by heterozygous mutations in *PKD1* or *PKD2* gene, encoding the polycystin-1 or polycystin-2 proteins, respectively. Polycystin-2 regulates protein trafficking and polycystin-1 expression. Polycystin-1 and 2 form a heteromeric complex with fibrocystin and PKHD1, which mediates Ca^{2+} signaling in the primary cilium in response to the fluid flow. However, the exact mechanism on how *PKD1/2* mutations cause ADPKD is unclear [4].

Currently, there are >2,300 variants of *PKD1* and *PKD2* indexed in the ADPKD mutation database (https://pkdb.mayo.edu/cgi-bin/v2_display_mutations.cgi?apkd_mode=PROD). *PKD1* mutations account for nearly 85% of affected families [5]. Genetic analysis is a helpful method for a quick and believable diagnosis. However, as the *PKD1* gene is large and has a high guanine-cytosine content with a high degree of pseudo-homology, obtaining a genetic diagnosis of ADPKD is challenging. Specialized screening of *PKD1* is required, for example, *PKD1*-specific long-range PCR and whole-genome sequencing (WGS) can have high technical sensitivity for genetic testing. Besides, capture panel-based approaches have been developed for genetic diagnosis of ADPKD with high efficiency [6]. In addition, novel genes such as *GANAB* and *DNAJB11* are found to cause ADPKD in several families [7–9].

ADPKD is usually characterized by massive enlargement of the kidneys secondary to cyst growth and development [10]. The most frequent complication is progressive renal failure which leads to ESRD at 40–59 years of age. Hypertension is very common in ADPKD and is found in most individuals with enlarged kidneys or decreased glomerular filtration rate. Other complications include acute and chronic pain, gross hematuria, cyst infection, and nephrolithiasis. The most common extrarenal manifestation is liver cysts, which present in >90% of patients with ADPKD over 35 years of age. Other manifestations include intracranial aneurysms, development of cysts in pancreas, seminal vesicles, and ovaries [11]. *PKD1* and *PKD2* mutations have different prognostic implications. Patients with *PKD2* mutations develop renal cysts, hypertension, and ESRD at a later age than patients with *PKD1* mutations. Variability in disease severity within families and appearance of extrarenal manifestations have been reported [12], suggesting other genetic and environmental factors can modify the severity of this disease [13]. For example, a second mutation in *PKD1* or *PKD2* and digenic heterozygous alleles in both *PKD1* and

Table 1. Genetic mutations involved in major inherited kidney disorders

Disease	Variants	Inheritance	Main extrarenal manifestations	Renal phenotypes
Chromosomal abnormalities and CNVs involved in kidney disorders				
Down syndrome	Trisomy 21	Not inherited	Hydronephrosis and obstructive lesions	Multicystic kidney and renal agenesis
Turner syndrome	Monosomy X	Not inherited	Short stature, heart abnormalities, ovarian hypofunction or premature ovarian failure, hypertension, and webbed neck	Horseshoe kidney and duplex kidney
Williams syndrome	7q11.23 deletion	AD	Short stature, abnormal weight gain, facial abnormalities, heart abnormalities, mental retardation, relative sparing of language, and bladder diverticulae	Asymmetric kidney, solitary kidney, reflux, and nephrocalcinosis
DiGeorge syndrome	22q11.2 deletion	AD	Micrognathia, immunodeficiency, congenital heart disease, feeding and growing problems, enamel hypoplasia, mild skeletal anomalies, and facial dysmorphisms	Absent, dysplastic, multicystic kidney, obstructive uropathy, and reflux
Ciliopathies				
ADPKD	<i>PKD1</i> , <i>PKD2</i> , <i>GANAB</i> , and <i>DNAJB11</i>	AD	Liver cysts; seminal vesicles, arachnoid membrane, cerebral aneurism, and cysts in pancreas	Polycystic kidney, progressive renal insufficiency, and ESRD
ARPKD	<i>PKHD1</i> and <i>DZIP1L</i>	AR	Oligohydramnios phenotype (potter facies, severe respiratory insufficiency, limb contractures), hypertension, periportal fibrosis, and bile duct dilation/proliferation	Enlarged echogenic kidney and polycystic kidney dysplasia
Juvenile NPHP	<i>NPHP1</i> /3/4/5/6/7/8/9	AR	Growth retardation and chronic iron-resistant anemia	NPHP, polydipsia, polyuria, and ESRD at median age of 13
Infantile NPHP	<i>NPHP2</i>	AR	Oligohydramnios phenotype (potter facies, severe respiratory insufficiency, and limb contractures), and hypertension	Enlarged hyperechogenic kidney, tubulointerstitial nephritis, cortical microcysts, hyperkalemia metabolic acidosis, and ESRD at median age of 1
JSRD (types 1–7, types 9–11, types 14–16, types 18–22)	<i>INPP5E</i> , <i>TMEM216</i> , <i>AHI1</i> , <i>NPHP1</i> , <i>CEP290</i> , <i>TMEM67</i> , <i>RP</i> - for type 9; <i>XL</i>) <i>GRIP1L</i> , <i>CC2D2A</i> , <i>OFD1</i> , <i>TTC21B</i> , <i>TMEM237</i> , <i>CEP41</i> , <i>TMEM138</i> , <i>TCTN3</i> , <i>ZNF423</i> , <i>TMEM231</i> , <i>CSPP1</i> , and <i>PDE6D</i>	AR (except for type 9: XL)	Cerebellar vermis hypoplasia, abnormal eye movements; hypotonia, developmental delay, congenital hepatic fibrosis, and polydactyly	Renal cystic disease, typically NPHP
BBS	<i>BBS1</i> - <i>BBS14</i>	AR	Obesity, cognitive deficits, hypogonadism, retinal degeneration, and polydactyly	Fibrocystic renal disease
MKS	<i>MKS1</i> -13 and <i>TXNDC15</i>	AR	Occipital encephalocele, polydactyly, and hepatic fibrosis	Renal cystic dysplasia
CAKUT				
RCS	<i>PAX2</i>	AD	Optic nerve coloboma, cystic malformation of optic nerve, loose joints, and mild hearing loss	Multiple kidney cysts

Table 1 (continued)

Disease	Variants	Inheritance	Main extrarenal manifestations	Renal phenotypes
BOR	<i>EYA1</i> , <i>SIX5</i> , and <i>SIX1</i>	AD	Preauricular pits, branchial fistulas, branchial cysts, ear malformations, and hearing loss	Renal anomalies, renal collecting system anomalies, and renal dysplasia
TBS	<i>SALL1</i>	AD	External ear anomalies, hearing loss, preaxial polydactyly, triphalangeal thumbs, and imperforate anus	Renal dysplastic, multicystic kidney, and renal failure
VUR	<i>SOX17</i> , <i>ROBO2</i>	AD (reduced penetrance)	Vesicoureteral reflux	Reflux nephropathy complicated by hypertension, proteinuria or renal failure, renal hypoplastic, and duplex renal system
Inherited glomerular diseases				
Inherited NS	Multiple genes	AD/AR	WT1-related Wilms tumor (WT) syndromes, Pierson's syndrome, etc.	SRNS
AS	<i>COL4A5</i> , <i>COL4A3</i> , and <i>COL4A4</i>	XL/AD/AR/digenic	Sensorineural hearing loss and ocular defects	Hematuria, proteinuria, progressive renal dysfunction, and ESRD
Nail-patella syndrome	<i>LMX1B</i>	AD	Poorly developed nails and kneecaps	Proteinuria, nephritis, and kidney failure
Renal tubulopathies and metabolic diseases				
dRTA	<i>SLC4A1</i> , <i>ATP6V0A4</i> , <i>ATP6V1B1</i> , <i>FOXI1</i> , and <i>WDR72</i>	AD/AR	Hyperchloremic nonanion gap metabolic acidosis, hypokalemia, growth deficiency, and sensorineural hearing loss	Nephrocalcinosis, nephrolithiasis, medullary cysts, and impaired renal function
RFS	<i>GATM</i> , <i>EHADH</i> , <i>HNF4A</i> , and <i>SLC34A1</i>	AD/AR	Failure to thrive, rickets, and hyperchloremic metabolic acidosis	Aminoaciduria, normoglycemic glycosuria, and polyuria with bicarbonaturia
Dent disease	<i>CLCN5</i> and <i>OCRL</i>	XL	–	LMW proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and ESRD
Cystinosis	<i>CTNS</i>	AR	Poor growth and hypophosphatemic/calipenic rickets	RFS, nephropathic cystinosis, and impaired glomerular function
Fabry disease	<i>GLA</i>	XL	Angiokeratomas, stroke, and myocardial infarction	Proteinuria and ESRD
Bartter's syndrome, types 1–4	<i>SLC12A1</i> , <i>KCNJ1</i> , <i>CLCNKB</i> , and <i>CLNCKA</i> , <i>BSND</i>	AR/digenic	Growth retardation, sensorineural deafness, and generalized muscle weakness	Renal salt wasting, hypokalemic metabolic alkalosis, and hypercalciuria

AD, autosomal dominant; ADPKD, autosomal dominant polycystic kidney disease; AR, autosomal recessive; ARPKD, autosomal recessive polycystic kidney disease; BBS, Bardet-Biedl syndrome; BOR, Branchio-Oto renal syndrome; CAKUT, congenital anomalies of the kidney and urinary tract; ESRD, end-stage renal disease; JSRD, Joubert syndrome and related diseases; LMW, low molecular weight; MKS, Meckel-Gruber syndrome; NPHP, nephronophthisis; RCS, renal coloboma syndrome; RFS, renal Fanconi syndrome; RTA, renal tubular acidosis; dRTA, distal renal tubular acidosis; SRNS, steroid-resistant nephrotic syndrome; TBS, Townes-Brocks syndrome; VUR, vesicoureteral reflux; XL, X-linked; CNVs, copy number variants.

PKD2 were reported as a genetic modifier associated with antenatal PKD [14]. Also, a *PKD1* pathogenic allele in combination with a mutation in *HNF1B* may cause severe early-onset PKD [15].

Autosomal Recessive PKD

Autosomal recessive PKD (ARPKD) is rarer than AD-PKD, caused by biallelic mutations in *PKHD1* encoding the fibrocystin protein [16]. *DZIP1L* is another pathogenic gene of ARPKD [17]. ARPKD can be identified either prenatally or neonatally by ultrasound based on the presence of enlarged echogenic kidney. In extreme cases, severe oligohydramnios leads to Potter's phenotype, which consists of pulmonary hypoplasia, characteristic face, and deformations [18]. Molecular diagnosis is necessary to confirm a genetic diagnosis and to enable future reproductive options for a family with severe cases [19].

Nephronophthisis

NPHP is a heterogeneous group of autosomal recessive cystic kidney disorders and is one of the most common reasons for ESRD in children and adolescents [1]. NPHP is characterized by reduced renal concentrating ability, chronic tubulointerstitial nephritis, cystic renal disease, and progression to ESRD before the age of 30 years old. Pathologically, NPHP presents with tubulointerstitial fibrosis, tubular dilatation, corticomedullary cyst formation, and tubular atrophy. There are 3 types of NPHP: infantile, juvenile, and adolescent form based on the age of onset for ESRD. The juvenile form is most common. To date, 8 genes (*NPHP1*, 3, 4, 5, 6, 7, 8, and 9) have been reported to cause the juvenile NPHP, whereas only *NPHP2* is reported as a causal gene for the infantile NPHP [20].

Nearly 10–20% of NPHP have extrarenal manifestations such as retinal disease, neurological disease, liver fibrosis, and skeletal dysplasia. NPHP is also a major clinical finding in several ciliopathy syndromes, including Joubert syndrome and related diseases, Bardet-Biedl syndrome, and Meckel-Gruber syndrome [21].

Congenital Anomalies of the Kidney and Urinary Tract

Congenital anomalies of the kidney and urinary tract (CAKUT) are disorders caused by defects during embryonic kidney development. Malformations such as ureteropelvic junction obstruction, kidney agenesis, multicystic dysplastic kidneys, kidney dysplasia, kidney hypoplasia, vesicoureteral reflux, and duplex collecting system are included in the spectrum of CAKUT [22]. CAKUT can be sporadic or familial, syndromic or nonsyndromic,

and the nonsyndromic form is more common. Isolated CAKUT can be associated with 500 syndromes. Syndromic CAKUT are usually associated with defects in the transcription factors that regulate critical steps in early renal development. For example, the process of the ureter budding toward the metanephric mesenchyme and mesenchyme induction/condensation.

Renal Coloboma Syndrome

Renal coloboma syndrome is an autosomal dominant condition characterized by optic nerve dysplasia and congenital kidney anomalies, usually presenting as renal hypodysplasia [23]. Histologically, kidneys exhibit reduced and enlarged glomeruli, a phenotype called oligomeganephronia. Consequences of the renal hypodysplasia include hypertension, proteinuria, and renal insufficiency that frequently progresses to ESRD. Renal coloboma syndrome is caused by mutations in the *PAX2* gene [24], which is a homeobox transcription factor directly regulating the action of glial cell line-derived neurotrophic factor.

Branchio-Oto Renal Syndrome

Branchio-Oto renal (BOR) syndrome is an autosomal dominant genetic disorder with various combinations of abnormalities in kidney, brachial arches, and external/inner ear [25]. The kidney abnormalities differ among patients. In mild cases, the kidney may be unusually shaped. In severe cases, there may be duplication of the kidney collection system and/or failure of kidney development [26]. The genetic causes of BOR syndrome are mutations in the *EYA1*, *SIX5*, or *SIX1*, of which the *EYA1* variants are the most common cause [27].

Townes-Brocks Syndrome

Townes-Brocks syndrome (TBS) is an autosomal dominant genetic disorder, characterized by the triad of imperforate anus, dysplastic ears, and thumb malformations. Renal impairment, including ESRD, may occur with or without structural abnormalities (mild malrotation, ectopia, horseshoe kidney, renal hypoplasia, polycystic kidneys, and vesicoureteral reflux). TBS is caused by pathogenic variants in *SALL1* gene, encoding Sal-like protein 1, a transcriptional repressor mainly involved in organogenesis [28]. Nonsense mutations are the most common form of pathogenic variants but deletion mutations have also been reported [29]. The highest expression of *SALL1* occurs in the kidney, followed by mild expression in other organs such as developing brain and limbs, which are all affected in TBS.

Inherited Renal Dysfunction

Glomerular Diseases

Inherited Nephrotic Syndrome

Inherited nephrotic syndrome (NS) is a group of disorders presenting with massive proteinuria. Clinically, NS mainly manifests as steroid-resistant nephrotic syndrome (SRNS). Focal segmental glomerulosclerosis (FSGS) are pathologically prevalent. Most cases of inherited NS present with an isolated glomerular disease, while some can be a syndromic disorder with extrarenal manifestations. Biallelic pathogenic variants in *NPHS1* (encoding nephrin) or *NPHS2* (encoding podocin) are the most common genetic cause in most of the inherited NS patients. More than 45 pathogenic genes have been associated with SRNS or inherited NS, of which some known podocyte genes can explain 57%–100% of familial and infant-onset NS [30]. There is significant phenotypic variability associated with inherited NS. Recessive mutations in *NPHS1*, *NPHS2*, *LAMB2*, and *PLCE1* are reported to cause severe clinical features of early-onset NS and progress to ESRD, during infancy or throughout childhood, while recessive mutations in *MYO1E* and *CD2AP* cause severe childhood-onset NS and progress to ESRD later [30]. Autosomal dominant inherited NS is rare, occurring mostly in juvenile and adult cases. The most common causative gene is *INF2* (up to 17%), other genes include *TRPC6* (up to 12%) and *ACTN4* (3.5%) [31]. Among syndromic forms, WT1-associated glomerulopathy is the most frequent one, followed by Pierson's syndrome caused by biallelic pathogenic variants in *LAMB2*. Once the clinical suspicion of inherited NS is raised, genetic testing should be performed by gene panel screening or whole-exome sequencing (WES) for possible pathogenic genes. Some inherited NS associated mutations have been proved to be viable therapeutic targets. For example, *TRPC6* inhibitors is feasible for treating diseases that are associated with TRPC over activation in vivo, which can be a candidate therapy for patients with specific *TRPC6* mutations [32].

Alport Syndrome

Alport syndrome (AS) is an inherited kidney disorder characterized by hematuria, proteinuria, and progressive renal failure. Some patients may have sensorineural deafness, eye abnormalities, esophageal leiomyoma, and other extrarenal manifestations [33]. The pathogenic genes of AS are *COL4A3*, *COL4A4*, and *COL4A5*, which encode type IV collagen $\alpha 3$, $\alpha 4$, and $\alpha 5$ chain, respectively. There are 3 types of AS: X-linked AS (XLAS), autosomal domi-

nant AS (ADAS), and autosomal recessive AS (ARAS) [34]. XLAS is the most common type of AS, caused by pathogenic variants in *COL4A5* gene on the X chromosome. ARAS, which takes second place, is caused by biallelic variants in *COL4A3* or *COL4A4*. ADAS is the rarest form caused by a single variant in *COL4A3* or *COL4A4* [35]. An alternative genetic model that may apply to AS is digenic inheritance [36], where 2 pathogenic mutations occur respectively in *COL4A3*, *COL4A4*, or *COL4A5*. The genetic cause of AS can be complicated, in some cases, variants can appear in some podocyte genes, which are considered as modifier genes for the disease progression.

Different inheritance mode of AS leads to different symptoms. For XLAS, there is often a family history of hematuria (with or without proteinuria) or renal failure. Male patients with hemizygous mutations in *COL4A5* are severely affected, whereas heterozygous female carriers have less severe manifestations. These female carriers usually have a broad spectrum of symptoms from isolated hematuria to ESRD, depending on the variants type and the degree of the X chromosome mosaicism. It is observed in female patients that approximately 98% of them have hematuria and 73% have both hematuria and proteinuria [37]. ARAS shows symptoms similar to those in male XLAS patients and there is no gender difference [37–39]. As for ADAS, median ages for developing proteinuria and renal insufficiency are 17 and 70 years old, respectively. Both hearing loss and eye lesion were reported to occur quite rarely for ADAS [33].

Renal Tubulopathies and Metabolic Diseases

The kidney provides homeostasis by a complex transport function mostly performed by specialized proteins distributed along the renal tubules. Pathogenic variants in the genes encoding these proteins will impair their function and have consequences on the whole organism, leading to renal tubulopathies [40]. Renal tubulopathies have high genetic and phenotypic heterogeneity [41]. Over 50 disease-causing genes have been identified for renal tubulopathies to date [42]. The phenotypes include polyuria and polydipsia, impaired growth, rickets and osteopenia, ultimately, and renal insufficiency.

Renal Tubular Acidosis

Renal tubular acidosis (RTA) is a group of transport defects in the process of bicarbonate (HCO_3^-) reabsorption, or hydrogen ion (H^+) excretion, or both, which result in systemic acidosis and hypokalemia with a normal glomerular filtration rate. RTA is classified into 4 types: type I (also called distal RTA, dRTA) is the classical form;

type 2 (also called proximal RTA, pRTA) is caused by a failure of the proximal tubular cells to reabsorb filtered bicarbonate from the urine; type 3 is a type of combined pRTA and dRTA; type 4 has absolute hypoaldosteronism or aldosterone insensitivity, which is included in the RTA as it has a mild (normal anion gap) metabolic acidosis.

The genetic causes and mechanisms of dRTA have been extensively investigated [43, 44]. The pathogenic molecular basis for all known dRTA is that, mutations in important proteins involved in acid-base transport lead to impaired acid excretion and dysfunction of intercalated cells in the collecting tubules. For now, *ATP6V0A4* [45], *ATP6V1B1*, *FOXI1*, *WDR72*, and *SLC4A1* have been identified as the pathogenic genes of dominant or recessive dRTA [46]. Isolated pRTA is rare, which is inherited as autosomal recessive manner with mutations in *SLC4A4* encoding the $\text{Na}^+/\text{HCO}_3^-$ cotransporter. It is usually a part of generalized proximal tubular dysfunction, termed Fanconi syndrome, with global dysfunction of all carriers in the proximal convoluted tubule.

Renal Fanconi Syndrome

Renal Fanconi syndrome (RFS) refers to the generalized dysfunction of the proximal tubule, onsets between infancy and childhood, resulting in defective of reabsorption of all small molecules. The classic manifestations include glucosuria, amino acid urine, low molecular weight proteinuria, bicarbonaturia, and acidosis [47]. RFS only affects the proximal tubule but not other nephron segments in its isolated form. However, RFS is usually secondary to a systemic autosomal recessive or X-linked disease such as cystinosis, Dent disease, and Lowe syndrome. A set of causal genes have been identified, including *GATM*, *EHHADH*, *HNF4A*, and *SLC34A1* [48].

Dent Disease

Dent disease is a rare, X-linked proximal renal tubular disorder that occurs almost exclusively in males. The most frequent signs are low molecular weight proteinuria and hypercalciuria. Other signs include nephrocalcinosis, nephrolithiasis, and slowly progressive renal failure. In most affected males, progressive kidney problems lead to ESRD in their early to mid-adulthood. Dent disease can result from loss-of-function (LoF) mutations in *CLCN5*, leading to Dent-1 (60%) [49]; or *OCRL*, leading to Dent-2 (15%) [50]. The remaining patients have a Dent disease phenotype with neither of these mutations. CIC-5, encoded by *CLCN5*, is involved in the acidification of early endosomes and protein reuptake by proximal tubular cells. Podocytes and parietal epithelial cells

also express CIC-5, suggesting its active involvement in the onset of glomerular damage in Dent disease. Different pathogenic *CLCN5* variants can reduce CIC-5 function at different degrees, which may cause phenotypic variability [51].

Cystinosis

Cystinosis, a rare autosomal recessive disorder, is a lysosomal storage disease characterized by the accumulation of free cystine in lysosomes, leading to intracellular crystal formation. It is caused by mutations in the *CTNS* gene that codes for cystinosis, the lysosomal membrane-specific transporter for cystine [52]. There are 3 distinct types of cystinosis, each with slightly different symptoms: nephropathic cystinosis [53], intermediate cystinosis [54], and ocular, nonnephropathic cystinosis [55]. Infants affected by cystinosis initially exhibit RFS at 6–12 months of age and can develop to ESRD around 6–10 years of age. Cystine crystals may also present in the cornea and conjunctiva, leading to tearing and photophobia. Other extrarenal manifestations include growth retardation, hypothyroidism, hypergonadotropic hypogonadism, diabetes mellitus, a distal vacuolar myopathy, and dysphagia.

Fabry Disease

Fabry disease is a rare X-linked lysosomal storage disorder that results from deficiency of lysosomal enzyme α -galactosidase A (α -gal A) encoded by the *GLA* gene [56]. Glycosphingolipids are components of plasma membrane that are degraded in the lysosome. Deficient activity of α -gal A leads to accumulation of glycosphingolipids with terminal α -galactosyl residues derived mostly from the turnover of cells in the kidneys, heart, lungs, nerve system, and skin. Fabry disease is a complex, multisystem disorder characterized by chronic pain and acroparesthesia, gastrointestinal disturbances, angiokeratomata, progressive renal impairment, cardiomyopathy, and stroke. Renal failure is a major complication of Fabry disease in most adult male patients. Diagnosis of Fabry disease can be made by measurement of plasma or leukocyte α -gal A activity, skin biopsy, or sequencing of the defective gene. Female with a pathogenic mutation in the *GLA* gene is necessary to confirm the diagnose of Fabry disease since these patients often have normal α -gal A activity. Hundreds of mutations of *GLA* gene have been identified. Missense or nonsense mutations are the most frequent, and small/large deletions or insertions also appear. Mutations leading to complete LoF of the gene product are associated with classic forms of the disease,

Fig. 1. Paradigm of calling and interpreting genomic variations for patients with inherited kidney diseases.

whereas mutations resulting in amino acid change might occasionally be associated with late-onset disease and milder phenotypes [57]. Recombinant enzyme replacement therapy with intravenous agalsidase- α or agalsidase- β has been applied in clinical practice since 2001 and has overall good efficacy. In addition, oral pharmacological

chaperone migalastat can selectively and reversibly bind to the active center of mutant α -gal A enzyme, so as to significantly improve the enzyme activity. Gene therapy for Fabry disease is a promising therapeutic option, whose safety is currently evaluated under several clinical studies [58, 59].

NGS Technologies and Genetic Diagnosis of Inherited Kidney Diseases

NGS Technologies and Their Clinical Applications

NGS technologies refer to the deep, high-throughput, in-parallel DNA sequencing technologies. They are capable of sequencing and finding multiple candidate genes in rare disorders both for basic research and clinical practice [60]. NGS can be implemented within different applications, including WGS, WES, RNA sequencing, targeted sequencing, panel sequencing, methylation sequencing, and a set of array-based sequencing. So far, development in NGS technologies and bioinformatics analysis have greatly benefit the researches of rare diseases, as pathogenic gene identified by NGS technologies is essential for downstream clinical managements such as precise diagnosis, carrier testing, genetic counseling, and personalized therapy.

Genetic Diagnosis of Inherited Kidney Diseases

Over the last few years, diagnostics of inherited kidney diseases were improved with the accumulation of genetic knowledge on kidney pathophysiology. Our knowledge on the genetic cause of an inherited disease will not only help patients and their families to make reproductive options or other life-changing decisions but also improve our understanding of genotype-phenotype correlations, disease progression, and eventually the development of more personized therapeutics [61]. However, some patients with inherited kidney diseases have highly genetic heterogeneity that are difficult for genetic analysis. Genes that have guanine-cytosine-rich sequence or with duplicated tracts, such as *COL4A3*, *COL4A4*, *COL4A5*, and *PKD1*, as well as those with CNVs, are also making difficulties for genetic analysis. A paradigm of calling and interpreting genomic variants for patients with inherited kidney diseases are shown in Figure 1.

In clinical practices, the first step for clinicians and researchers to make a genetics evaluation includes a review of the family history, personal history, and some kidney functional examination results [62]. Then, the clinicians should choose proper genetic sequencing technology for the patients based on the initial examination. If one is suspected to have a specific kind of kidney disease, applying gene panels for a targeted sequencing is an efficient option. However, if no causal variant is identified, a subsequent more comprehensive approach is in need for genetic diagnosis and future data reanalysis. The causative genetic mutation identified in the affected individual needs to be verified in his/her families by NGS technologies or Sanger sequencing.

Transforming genetic discoveries into targeted treatments is also important and challenging. For most inherited kidney diseases, treatment remains essentially unchanged and mainly supportive; however, identification of the disease causal genes and the advent of gene editing technologies [63] may provide opportunities for the future treatment of some particular kinds of inherited kidney diseases.

Clinical Interpretation of Genomic Variations

It is still a challenge to interpret the large list of genomic variation, especially those with unknown significance, which can lead to overinterpretation and even false diagnosis, unless stringent criteria are applied. To improve the accuracy of genetic diagnosis, universally accepted guidelines for variant interpretation have been developed by the American College of Medical Genetics (ACMG) [64]. It provides standards and guidelines for the interpretation of genomic variations based on evidence-based recommendations on aspects including the use of literature, database, and in silico predictors, as well as criteria for variant interpretation and reporting. These recommendations are proved to be very useful in the clinical context.

The ACMG recommends that “variant” identified in Mendelian disorders be classified into the following 5 categories: (1) pathogenic (P), (2) likely pathogenic (LP), (3) uncertain significance (VUS), (4) likely benign, or (5) benign (B). Criteria for pathogenic variants can be divided into very strong (PVS1), strong (PS1~4), moderate (PM1~6), or supporting evidence (PP1~5). Evidence for benign variants can be divided into independent (stand-alone, BA1), strong (BS1~4), or supporting evidence (BP1~6). For a given variation, the genetic researchers should select proper criteria based on the observed evidence.

There are several kinds of variants in the genome with different pathological significance [65]. Nonsense variants, frameshift variants, and exonic deletions/insertions (indel) are very “strong” variants, generally assumed to cause loss of function of the gene, which lead to reduced or absent protein function and nonsense-mediated RNA decay. Variants that affect splice sites may cause exon skipping and shortening or inclusion of introns, usually leading to a defected protein product.

A CNV is defined as a segment of DNA (at least 1 kb in size) that differs in copy number compared with a representative reference genome. It is one of the main causes of many genetic disorders. CNVs can be detected by most genomic microarrays, including array-based comparative

Fig. 2. Pedigree of 3 families with AS. XL-AS, X-linked Alport syndrome; LP, likely pathogenic; AD-AS, autosomal dominant Alport syndrome; VUS, uncertain significance; AR-AS, autosomal recessive Alport syndrome; P, pathogenic.

genomic hybridization (aCGH) and single-nucleotide polymorphism-based platforms. Now, NGS-based CNV analysis is increasingly used in clinical testing through genome, exome, or gene panel sequencing, but WES is less capable compared with WGS, where all regions of the genome are covered. Most CNVs are unique, requiring further investigations to determine their potential clinical significance. ACMG has provided standards and guidelines for interpretation and reporting of postnatal constitutional CNVs detected by cytogenetic microarray (e.g., chromosomal microarray) in 2011 [66]. Subsequently, ACMG and the Clinical Genome Resource (ClinGen) provided a set of technical standards for the interpretation and reporting of constitutional CNVs in 2019 [67]. In this guideline, CNV classification categories were changed to the 5-tier classification system recommended in the ACMG/AMP sequence variant interpretation guidelines.

Application of NGS in Identifying Novel Genes of Inherited Kidney Diseases

After applying the genetic sequencing and variant interpretation, there are still big chances that no pathogenic genes or variants are found and thus no genetic diagnosis can be made. In these cases, some novel pathogenic genes might play a role. However, the pathophysiological importance of those novel genes has not been comprehensively addressed, so it is indisputable to functionally test the identified novel variants (i.e., measurable outcomes in cell lines or animal models).

In 2011, Bredrup et al. [68] identified the *WDR19* (*NPHP13*) gene as the pathogenic gene in patients with

NPHP using WES. This gene encodes intraflagellar transport (IFT) 144, a member in the IFT complex A that drives retrograde ciliary transport. They collected 3 unrelated families, each with different clinical manifestations: Jeune syndrome plus NPHP-like nephropathy, Sensenbrenner syndrome plus NPHP-like nephropathy, and nephropathy. WES was applied to these patients and found pathogenic variants in the shared gene *WDR19*. They demonstrated the ciliary pathogenesis by showing the absence of IFT144 in the cilia, as well as disrupted ciliary abundance and morphology in patient's fibroblasts. Their work identified a similar molecular cause to seemingly different yet clinically overlapping disorders, which broaden the research field of inherited nephropathy.

Genetic Diagnosis of AS

Expert consensus guidelines for the genetic diagnosis of AS were published in 2018 [69]. Here we discuss the application of the genetic diagnosis of AS using 3 cases as examples, each variant was estimated based on the ACMG criteria. The pedigrees for each case are shown in Figure 2.

Case 1: LP

The proband is a 25-year old man with hematuria (34.5 million RBC/mL) and proteinuria (0.53 g/24 h). Genetic testing by WES identified a nonsynonymous SNV in *COL4A5*: c.G3196A (p.Gly1066Ser) in the proband inherited from his mother. Based on the results of immunofluorescent staining and electron microscopy observation of absence of collagen IV in the patient's kidney biopsy, this mutation shows damaging effect on the gene

product (PS3). It is absent in population databases 1,000 Genomes Project and in extremely low frequency in Exome Aggregation Consortium (ExAC, frequency = 0.00001142) (PM2). According to the *in silico* prediction using multiple lines of computational evidence, this mutation has a deleterious effect on COL4A5 protein (PP3). Besides, it has been reported as a susceptible pathogenic variant in different AS patients [70], which supports the PP5 criteria: reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation. One strong evidence (PS3), 1 moderate evidence (PM2), and 2 supporting evidences (PP3, PP5) classify this variant as “likely pathogenic.”

Case 2: VUS

The case is a 19-year old girl with persistent hematuria (180 RBC/mL) and proteinuria (4.12 g/24 h). WES discovered a heterozygous nonframeshift deletion in COL4A3: c.1833_1844del, p.A613_P616del in the proband inherited from her father. This variant has not been recorded in the 1,000 Genome Database, ExAC, and Chinese Gene Mutation Database (PM2). And as a deletion mutation, it affects protein length due to in-frame deletions in a nonrepeat region (PM4). Besides, this mutation is co-segregation with disease in multiple affected family members in the AS pathogenic gene (PP1). Two moderate evidences (PM2, PM4) and 1 supporting evidence (PP1) classify this variant as “VUS.”

Case 3: Digenic

Mencarelli et al. [36] reported a family with COL4A3/COL4A4 mutation combinations. In this family, individuals with 2 heterozygous mutations had more severe phenotypes (chronic renal failure, sensorineural hearing loss, and preemptive renal transplantation) than those with a single heterozygous mutation (hematuria). The first mutation was COL4A4: c.4698delT, p.Cys1566Trpfs*37. This is a frameshift variant in the pathogenic gene of AS, leading to premature stop of the transcript (PVS1). The deletion of T is expected to result in a truncated protein product (PM4). Besides, the mutation and the other COL4A3 mutation of the proband were inherited independently (*in trans*), validated by testing of his offsprings (PM3). One very strong evidence (PVS1) and 2 moderate evidences (PM3 and PM4) classify this variant as “pathogenic.”

The second mutation of the proband was in COL4A3: c.G1558C, p.Gly520Arg. This mutation was classified as “likely pathogenic” because of 3 moderate

evidences: (1) this is a novel missense mutation which is absent in population databases (1,000 Genomes Project, or ExAC) (PM2). (2) The missense change at this amino acid residue p.Gly520Asp has been considered to be pathogenic before (PM5). (3) This mutation and the above COL4A4 mutation of the proband were inherited independently (*in trans*), validated by testing of his offspring (PM3).

Genetic testing is an important criterion for AS diagnosis [71]. Several points should be noted for the genetic diagnosis of AS. To reach a genetic diagnosis of AS, genetic testing is needed for both the patient and the family, especially those without extrarenal manifestations. In individuals with suspected AS, all 3 AS genes (COL4A3, COL4A4, and COL4A5) should be examined for pathogenic variants by panel sequencing, Sanger sequencing or WES, which can identify most of the pathogenic COL4A variants. Where causative pathogenic variants cannot be demonstrated, CNV should be considered by reexamining the NGS data or with multiplex ligation-dependent probe amplification. The remaining few changes include deep intronic splicing variants or somatic mosaicism. When no pathogenic variants are found, clinical diagnosis should be reviewed, some podocyte genes such as ACTN4, NPHS2, or MYO1E and genes in which mutations produce similar clinical features to AS (e.g., FSGS, complement pathway disorders, and MYH9-related disorders) should be examined.

Conclusion

In summary, genetic diagnosis is very important for the diagnosis of hereditary kidney diseases. Proper means and appropriate samples for genetic testing should be selected based on the phenotypes in patients. After performing NGS and bioinformatics analysis, a set of candidate variants will be generated, clinical interpretation should be made in combined with patient’s phenotypes, possible inheritance patterns, and consensus guidelines to interpret the pathogenicity. Incorporation of WES and WGS with traditional tools such as renal histopathology will help unravel the complex phenotypes and empower both accurate genetic diagnosis, and novel gene discovery for patients with inherited kidney diseases.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J.Z. wrote the draft and prepared the figures. C.Z. provided valuable modifications into the article. E.G. and Q.Z. revised the manuscript.

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