UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

DOTTORATO DI RICERCA IN MEDICINA CLINICA E SPERIMENTALE

Ciclo XXXVI

PRECISION MEDICINE for CHRONIC KIDNEY DISEASE:

DIAGNOSTIC YIELD and CLINICAL IMPACT of a NEPHROPATHY PANEL STUDY

Candidato:
Dott.ssa Silvia Giovanella

Relatore:
Prof. Riccardo Magistroni

Correlatore:
Prof. Gianni Cappelli

Coordinatore del Corso di Dottorato:
Prof. Marco Vinceti

TABLE OF CONTENTS

1. INTRODUCTION

1.1. CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is a worldwide public health problem, with adverse outcomes of kidney failure, cardiovascular disease, and premature death. CKD affects approximately 9-13% of the world's population, corresponding to 700 million individuals(1, 2).

Following the National Kidney Foundation (NKF-KDOQI) guidelines, 2002, and the latest modification "Kidney Disease: Improving Global Outcomes (KDIGO)" of 2014, CKD is defined as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min/1.73 m^2 for at least 3 months or the presence of kidney damage markers such as albuminuria, histology or structural anomalies and electrolyte imbalance.

The 2012 KDIGO (3) recommends that CKD is classified based on cause, GFR category, and albuminuria category. It grades CKD into six categories based on GFR (G1 to G5 with G3 split into 3a and 3b) and three levels of albuminuria (A1, A2, and A3).

Albuminuria is a marker of kidney damage; albumin normally didn't leak through the glomeruli filters. Albumin in the urine can indicate kidney disease, even if GFR is above 60.

Considering the cause of CKD, the GFR category, the albuminuria category and other risk factors (comorbidity), it could be possible to predict the risk for the outcome of CKD. In Figure 1 of KDIGO guidelines, the green box indicates a low risk of developing CKD, in yellow a moderate increase, in orange a high risk and in red a very high risk. Therefore, the increase in albuminuria indicates a worse prognosis for the same GFR. Normal or high kidney function, in the presence of more than 300mg/g of albuminuria, can predict a high risk of CKD.

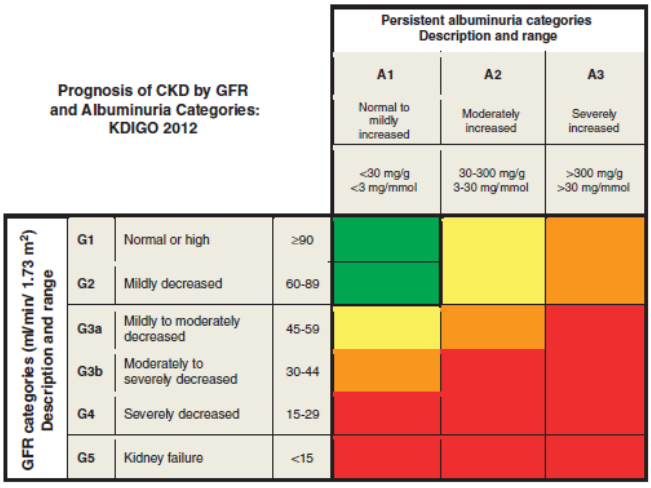


Figure 1. KDIGO guidelines concerning the prognosis of CKD by GFR and Albuminuria (3). Green box indicates a low risk of developing CKD, in yellow a moderate increase, in orange a high risk and in red a very high risk.

The final stage of CKD, the G5 category, is considered the end-stage kidney disease (ESKD). In this stage renal replacement therapy (RRT) is required, such as hemodialysis or peritoneal dialysis as well as kidney transplantation (treatment of choice).

In 2010, about 2,618 million people worldwide received RRT (4) with a prediction of 5,4 million people in 2030(5). RRT impacts the quality of life and life expectancy (6) and results in substantial healthcare costs(7). The ESKD is burdened with a high rate of morbidity and mortality, is fatal if not adequately treated and results in a lowered life expectancy life, with a 5-year survival for dialysis patients of 55.6 percent(6).

So, it is crucial to implement screening and medical investigation strategies for individuals at high risk of developing kidney failure (KF)(8), to mitigate the life burden, and evaluate the prognosis and the transmission risk to the offspring.

It is known that the main causes of ESKD are diabetic nephropathy (19%), glomerulonephritis (17%), and hypertensive nephropathy (16%), but in the 20% of RRT cases, the etiology remains undetermined(9). However, it has recently been reported that approximately 10% of adult ESKD can be attributable to genetic causes (10, 11) and that 10-29% of adult patients with ESKD reported a positive family history of CKD(12, 13).

It is therefore of growing importance to identify effective methods of diagnosis.

1.2. GENETIC CONDITIONS OF CKD

Recent studies have shown that a genomic approach can detect the underlying cause of CKD in about 30% of pediatric and 5-30% of adult cases(14), identifying more than 600 monogenic genes correlated to kidney diseases(15). Indeed, in the pediatric population, genetic diseases are the main cause of early-onset CKD, since 70% of cases have an onset before 25 years(16). Recently, it was recognized as a high prevalence also in the adult population, with 10-15% of ESKD cases attributable to genetic causes(10, 11).

Nowadays exist several approaches to genetic analysis with different diagnostic sensitivity and cost-effectiveness(17). The approaches mainly employed in genetic kidney disease are the panel strategy and whole exome sequencing (WES).

Recently, some research centers have begun to use whole genome sequencing (WGS) as a comprehensive analysis strategy, but the high cost and burden of analysis have not yet allowed for its wide adoption in clinical/diagnostic settings. The analysis of these methods will be addressed deeply in Chapter 4.

CKD is a genetically complex disease associated with monogenic, polygenic and environmental risks. It is possible that the high variability in phenotype expressed in several forms of monogenic disease could be attributed to polygenic factors(18).

Currently, the heritable forms of CKD can be distinguished in two different traits: the monogenic and the polygenic. A monogenic trait is expressed as a result of the effect of a gene, while polygenic as the effect of multiple genes.

a. MONOGENIC KIDNEY DISEASE

Mendelian kidney disease is a monogenic disease due to one or two pathogenic variants in a single gene correlated to the disease phenotype. The variant could be truncating, such as nonsense, frameshift and splicing site alteration or non-truncating as a missense variant. Often, truncating variants are mainly associated with a worse prognosis compared to a missense variant.

For example, in the case of polycystic kidney disease, the cystic phenotype is caused in 80% of cases by variants in PKD1 and 15% in PKD2. The patients with truncating PKD1 variants have an estimated mean age at ESKD of 52.5 years old compared to 70.8 years of patients with non-truncating PKD1 variants(19).

The severity and variability of the phenotype are also influenced by the penetrance and expressivity of the variant. Penetrance refers to the percent by which a genotype is expressed in phenotype while

expressivity is related to individual variability. It is also possible for a family member not to express the familial phenotype but to carry the variant, in this extreme case we refer to incomplete penetrance.

The presence of high phenotypic variability may be associated with the presence of modifier genes, mosaicism, or epigenetic modifications that affect the expression of the mutated gene.

The case of twins with the same pathogenetic variant on PAX2 related to Renal Coloboma Syndrome is reported in the literature. One showed the renal phenotype resulting in kidney transplantation while the other showed only the ocular phenotype resulting in blindness(20).

The phenotypic variability and the incomplete penetrance are two important issues to consider when assessing prognosis as well as in pregnancy counseling (21).

b. MULTIFACTORIAL DISEASE

Polygenic diseases are influenced by many effects, such as variants predisposition and environmental effects.

The predisposing genetic factors are analyzed by the employment of genome-wide association studies (GWAS) methods. These studies involve screening the entire genome of a large number of individuals to identify genetic markers that are statistically associated with the trait or disease of interest. The studies promise to uncover new biological insights correlated to kidney health and the outcome of kidney diseases and highlight possible therapeutic targets in personalized medicine. Indeed, GWAS studies have identified several genetic loci associated with CKD risk, including variants in genes related to inflammation, blood pressure regulation, and kidney function. Until now, GWAS studies provide evidence of genetic basis in specific nephropathy diseases such as IgA nephropathy, membranous glomerulonephritis, and diabetic nephropathy(22).

Two important risk variants for the development of CKD were identified in the APOL1 gene. These variants were found only in Africans or with African ancestry individuals. The high frequency of these variants is due to their ability to protect against trypanosomes, a parasite that causes sleeping sickness; for which a heterozygote advantage model has been proposed to explain its prevalence, especially in such populations(23). There is currently no universally accepted explanation for why such variants lead, in the presence of other environmental factors as well, to an increased risk of kidney failure(24).

In this thesis, only monogenic diseases will be explored.

1.3. MONOGENIC KIDNEY DISEASE

The main genetic kidney diseases with monogenic etiology can be classified into five phenotype categories: cystic diseases, glomerulopathies, Congenital Anomalies of the Kidney and Urinary Tract (CAKUT), tubulopathies, and nephrocalcinosis/nephrolithiasis.

1.3.1. CYSTIC KIDNEY DISEASE

Cystic kidney diseases are multisystemic disorders that can develop due to genetic or non-genetic causes in children and adults, and they may lead to KF. The most common cystic disease is autosomal dominant polycystic kidney disease (ADPKD) caused by PKD1 or PKD2 pathogenic variants, while the recessive form, ARPKD, is caused by PKHD1 gene variants.

Other cystic forms, less frequent, are medullary cystic kidney disease (MCKD) due to MCKD1 and MCKD2 mutations, and juvenile nephronophthisis (JNPHP), a recessive condition caused by NPHP1-NPHP5 genes.

<u>POLYCYSTIC KIDNEY – AUTOSOMAL DOMINANT</u>

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic inherited kidney disease, with an incidence range between 1:400 and 1:2000(25, 26). ADPKD represents the fourth global and the leading genetic cause of kidney failure, responsible for about 10% of KF cases(27).

The disease is characterized by the formation of bilateral renal cysts that slowly grow causing a progressive increase in kidney volume. The cysts induce severe morphological and functional alteration of the organ (Figure 2), and the result is KF. On average, about half of the individuals experience kidney failure in the sixth decade of life(28). This genetic defect also affects other organs, causing hepatic cysts, pancreatic cysts, seminal vesicle cysts or cerebral aneurysms(29).

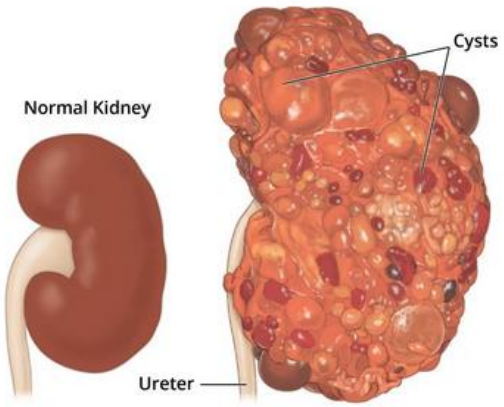


Figure 2. Normal kidney (left) and the enlarged cystic kidney (right).

ADPKD is associated with hypertension, abdominal pain and palpable abdominal mass, hematuria from cyst bleeding episodes and urinary tract infections.

The cystic phenotype is caused in 80% of cases by pathogenic variants on PKD1 and in the 15% on PKD2, other responsible genes were recently identified, such as GANAB and DNAJB11(30, 31).

The differential diagnosis for ADPKD with early onset is ARPKD, renal cysts due to HNF1b variants, tuberous sclerosis complex, von Hippel-Lindau syndrome, and acquired cystic kidney disease.

In patients with few renal cysts and several liver cysts, a genetic test could be useful in distinguishing ADPKD and the liver form, polycystic liver disease, caused mainly by PRKCSH, SEC63, ALG8, SEC61B, GANAB or LRP5(32).

POLYCYSTIC KIDNEY – AUTOSOMAL RECESSIVE

Autosomal recessive polycystic kidney disease (ARPKD) is a rare, genetic hepatorenal fibrocystic syndrome. ARPKD occurs in 1:20000 live births, however, in isolated or inbred populations, the prevalence is higher(33).

ARPKD is characterized by cystic dilatation and ectasia of renal collecting tubules, and a ductal plate malformation of the liver resulting in congenital hepatic fibrosis. The kidney is also enlarged, but the shape is preserved. Renal failure will develop around 5 to 20 years of age(29).

ARPKD is generally associated with hepatic disease, including portal hypertension, varices, and splenomegaly.

PKHD1 is the most frequently identified causative gene, encoding the ciliary protein fibrocystin, expressed in the kidney or liver (Figure 3). A recently identified gene is DZIP1L, encoding a zinc finger protein.

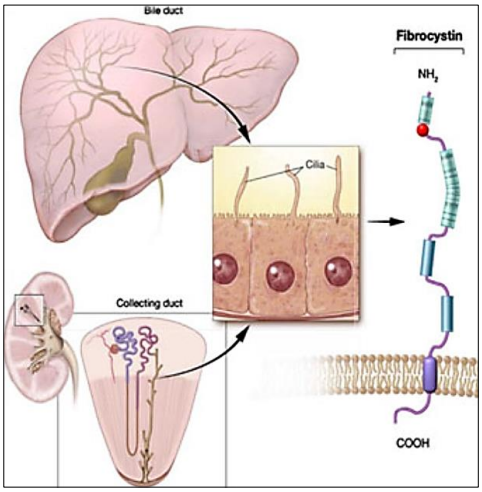


Figure 3. Fibrocystin (polycystin) molecule expressed in the cilia of both the liver's bile ducts and the kidney's collecting ducts.

NEPHRONOPHTHISIS

Nephronophthisis is a genetic renal ciliopathy characterized by the reduced ability of the kidneys to concentrate solutes, chronic tubulointerstitial nephritis, sporadic presence of cysts, and progression to ESKD. The disease is categorized into three clinical subtypes: infantile, juvenile and late-onset. The frequency varies among the Countries, Ala-Mello et al. (34) reported an incidence in Europe of 1: 61 800 live births.

Juvenile Nephronophthisis (JNPHP) is the most common reason for genetic ESKD in children, determining 15% of kidney failure in childhood (35).

More than 25 genes have been identified and related to the pathogenesis of this disease; the majority of these encode ciliary proteins that cluster to distinct subcellular localizations (Figure 4). Pathogenic variants (mainly homozygous deletions) to the NPHP1 gene, which codes for nephrocystin-1, represent the most common cause, found in about 20% of cases (36). Mutations in the gene INVS, coding for inversin, are frequently responsible for infantile NPHP, while variants in NPHP3, NPHP4, and NEK8 rise to late-onset nephronophthisis.

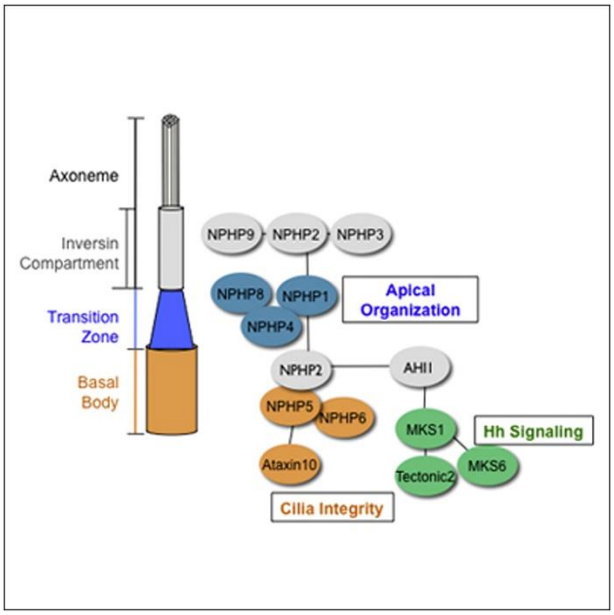


Figure 4. The Nephrocystins are expressed along the ciliary axoneme (37).

1.3.2. GLOMERULAR DISEASE

Glomerular diseases are characterized by structural glomerular abnormalities, deposition of material within the glomerular tuft, glomerular basement membrane, or podocyte cytoplasm. In some cases, the patterns of injury overlap other hereditary or nonhereditary disorders. Therefore, a genetic test could be informative about the diagnosis and the treatment.

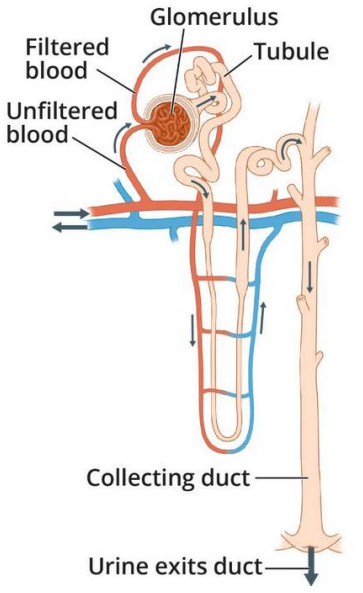


Figure 5. Nephron structure with glomerulus, tubule and collecting duct. The glomerulus is the filtering unit of the kidney.

Glomerulopathies account for a significant fraction of ESKD(38). The most important symptoms include proteinuria, hematuria, hypoproteinemia, edema, and a reduced glomerular filtration rate. Glomerular proteinuria is a hallmark of glomerular disease and results from increased permeability of the glomerular filtration barrier. The normal excretion levels of protein into the urine range between 0 and 150mg/d. Proteinuria is clinically characterized by a protein loss in the urine of more than 150mg/d. Proteinuria with a value of 3- 3.5g is defined in the “nephrotic range”(38).

Histologically, the most typical finding is a focal segmental glomerulosclerosis (FSGS, Figure 6); but it is also possible to find a picture of membranoproliferative glomerulonephritis or glomerulosclerosis mesangial diffuse.

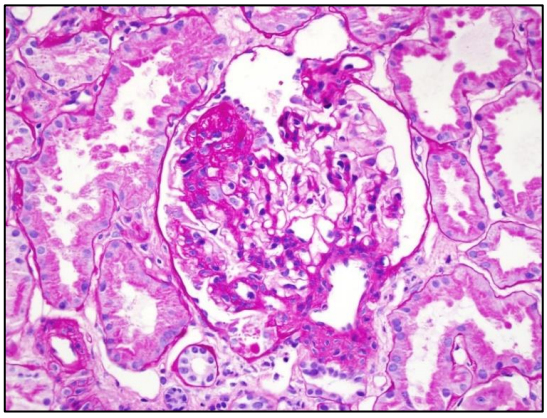


Figure 6. Glomerulus with histological features of focal segmental glomerulosclerosis.

Genetic forms of glomerular disease are predominantly caused by genetic defects in the structural molecules or regulatory factors of the glomerular filtration barrier. Currently, more than 80 genes are known to be involved in genetic glomerular diseases.

Figure 7 shows the podocyte adhered to the glomerular basement membrane (GBM), which expressed integrin, collagen IV and laminin that regulate an important signal transmission between the matrix and the glomerulus cells.

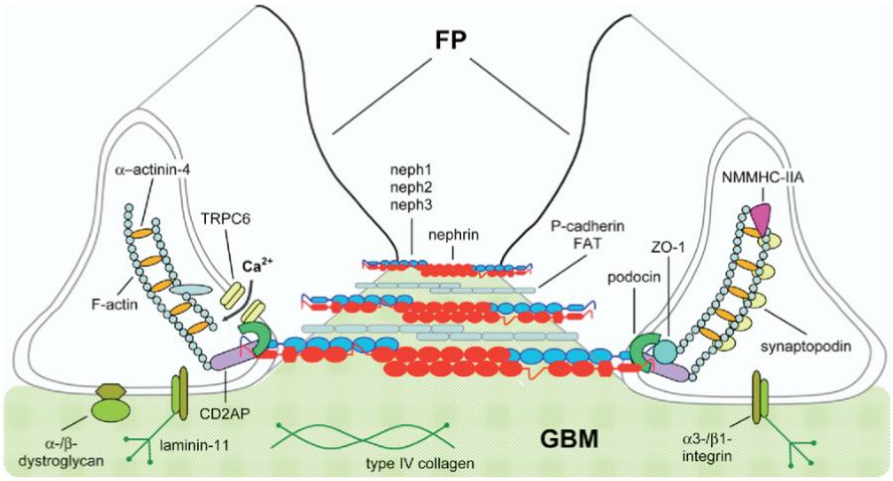


Figure 7. Schematic representation of podocyte foot processes (FP) and the protein involved. GBM, glomerular basement membrane (38).

These proteins are involved in the development of major forms of hereditary glomerular diseases, such as Alport syndrome and hereditary podocytopathies as focal segmental glomerulosclerosis and collapsing glomerulopathy.

Whereas another hereditary glomerulopathy, Fabry disease, is due to the accumulation of sphingolipids at the glomerular side.

ALPORT SYNDROME

Alport syndrome is a genetic condition characterized by kidney disease, hearing impairment, and eye abnormalities (lenticonus or retinopathy). In Europe, Alport syndrome accounts for 0.6% of patients in KF. Gibson et al (39) performed a study on the frequency of predicted pathogenic variants on collagen IV, using a population database. It resulted in a heterozygous predicted pathogenic COL4A3 or COL4A4 variants in 1:106. Other studies must be performed to understand the actual incidence of this disease that could be not so rare.

Kidney biopsy may appear normal or with non-specific marks; these include FSGS, tubular atrophy, and interstitial fibrosis. Electron microscopy, a gold standard for Alport diagnosis, can reveal longitudinal splitting and multi-lamellation of the GBM lamina densa (40).

Alport syndrome occurs due to pathogenic variants on type 4 collagen (Figure 8). In 80% of cases, Alport syndrome is inherited in an X-linked pattern and caused by COL4A5 gene. However, it can be inherited as an autosomal recessive or dominant form in the case of variants in COL4A3 or COL4A4 (40, 41).

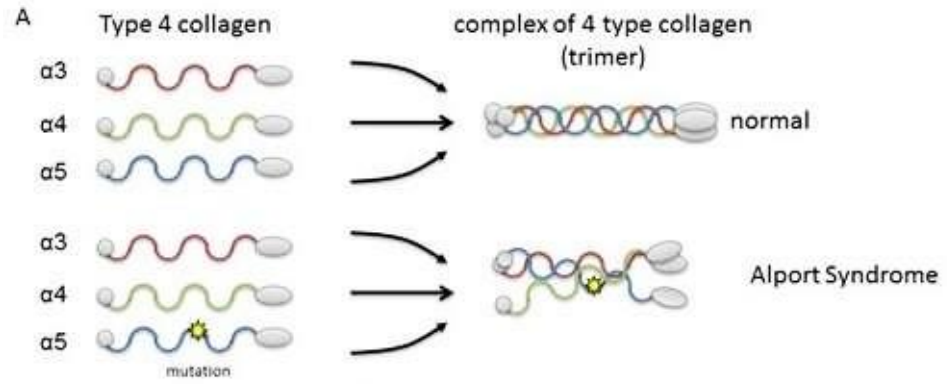


Figure 8. Schematic representation of type 4 collagen alpha 3, alpha 4 and alpha 5. On the right normal helix and the altered helix in Alport syndrome due to a variant on alpha 5.

Women in heterozygosity for COL4A5 show mild hematuria, often associated with proteinuria. Hardly ascribable to Alport, however being an X-linked form, they may transmit the variant to their son who will develop a severe form of Alport associated with deafness and early KF.

It, therefore, appears pivotal to carry out genetic counseling to detect these cases (41).

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal Segmental Glomerulosclerosis (FSGS) is defined by the presence of proteinuria and segmental glomerular sclerosis, due to a podocyte injury and depletion. It can be distinguished from minimal change disease by hematuria, hypertension and kidney failure(42).

Primary FSGS is usually a progressive disorder with <5% spontaneous remission and a 50% ESKD in 5–8 years from the histological diagnosis(43).

According to the etiology, FSGS has been classified into immunological, genetic and secondary forms. The genetic forms are associated with pathogenic variants in genes expressed in the interaction between podocytes and the GBM(44).

Recently, mutations in more than 50 genes were identified, most of which are characterized by onset in childhood(45).

NHPS1 is an essential component of the glomerular slit diaphragm; mutations in this gene account for 40–60% of the congenital nephrotic syndrome(46). NPHS2 is a transmembrane protein involved in the recruitment of nephrin at the slit diaphragm; homozygous or compound heterozygous mutations in NPHS2 are associated with childhood-onset FSGS(47). Proteins of the cytoskeleton complex can be involved in the etiology of FSGS, such as ACTN4, MYH9, IFN2 and ANLN. Moreover, mutations in structural components of GBM as type IV collagenic genes (COL4A3, COL4A4, COL4A5) and LAMA5 can also lead to FSGS(45).

Genetic tests are recommended for early-onset forms, to limit immunosuppression exposure due to the frequent steroid-resistant FSGS-associated and to determine the risk of recurrent kidney transplantation (KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases).

FABRY DISEASE

Fabry disease (FD) is an X-linked metabolic disorder caused by mutations in the α-galactosidase A (GLA) gene encoding the lysosomal AGAL enzyme. Loss of enzymatic AGAL activity and cellular accumulation of sphingolipids may lead to podocyturia and renal loss of function with increased cardiovascular morbidity and mortality(48). The incidence reported is 1:100,000, however, it could be underestimated(49).

Males affected by FD are hemizygotes and they show α-GalA mutations with no or very little residual α-galactosidase activity. In addition to the characteristic angiokeratoma, the patients develop corneal opacity, neuropathic pain, intolerance to heat, inability to sweat, micro-albuminuria and increased intima-media thickness. Later in life, the patients develop progressive kidney disease, cardiac symptoms and cerebrovascular disease. Recently, it was observed that also a significant portion of female FD heterozygotes develop complications.

FD patients can benefit from an enzyme replacement therapy using a recombinant enzyme. Therefore, it is relevant to perform genetic testing in the early stages to provide a better prognosis.

Histologically, in electron microscopy, it could be possible to observe inclusion with dense and pale material, called “zebra bodies”, either on endomyocardial or kidney biopsies (Figure 9)(49).

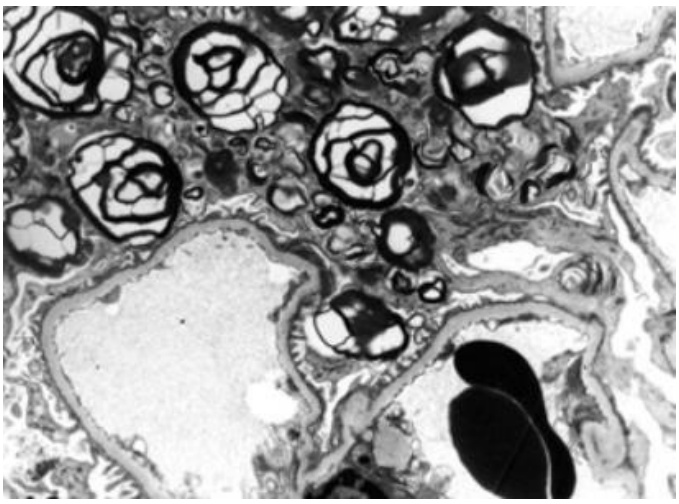


Figure 9. Zebra bodies in kidney biopsy (electron microscopy).

1.3.3. CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT

The spectrum of congenital anomalies of the kidney and urinary tract (CAKUT) is extremely broad and ranges from mild, asymptomatic malformations such as a double ureter or minimal ureteral pelvic obstructions to severe, life-threatening pathologies like bilateral renal agenesis or renal dysplasia (Figure 10). Renal agenesis/hypoplasia and dysplasia account for a significant portion of these anomalies, and a genetic contribution to its cause is being increasingly recognized.

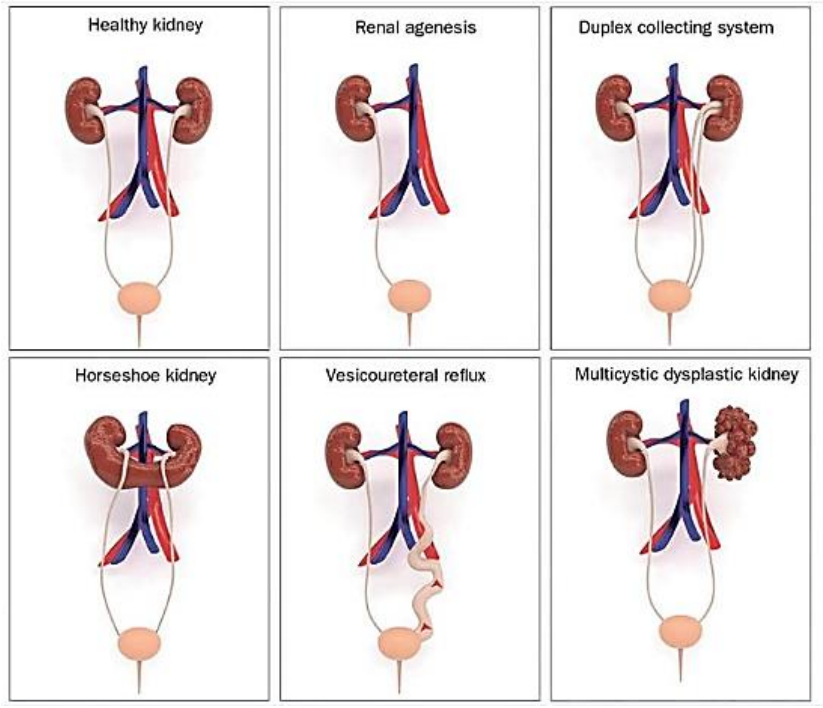


Figure 10. Schematic representation of healthy kidney (upper left) and the broad spectrum of congenital anomalies of the kidney and urinary tract.

The frequency is estimated at 3:1,000 births(50). The 2016 European Society for Paediatric Nephrology (ESPN)/European Renal Association (ERA) registry reported that 30% of children in ESKD are affected by a form of CAKUT(51).

In some cases, CAKUT may be diagnosed antenatally with ultrasound, in others it can be found incidentally in the presence of impaired kidney function or frequent urinary tract infections.

Genetically, more than 150 genes are associated with CAKUT, however many others could be identified. Many of these genes are transcription factors that are important in embryonic development. The most common are: PAX2, HNF1B, EYA1, SALL1, GATA3, PBX1(52). The monogenic forms of CAKUT are mostly explained by single pathogenic variants (10-15% of cases).

Pathogenic copy number variants (CNVs) are a second important cause of monogenic CAKUT, reported in 4.1% of 2842 CAKUT cases by Verbitsky et al(53, 54).

1.3.4. NEPHROCALCINOSIS AND NEPHROLITHIASIS

Kidney stones (also known as nephrolithiasis) are highly prevalent, affecting approximately 10% of adults worldwide. Kidney stone formation results from an imbalance of inhibitors and promoters of crystallization, and calcium-containing calculi account for over 80% of stones. In the case of nephrocalcinosis the calcifications are placed in the renal parenchyma while the nephrolithiasis is placed in the collecting system (figure 11). Commonly the main symptom is renal colic caused by a ureteral obstruction.

There are many etiologies of nephrolithiasis including genetic predisposition, diet, environment, and lifestyle. The advent of high-throughput sequencing techniques has enabled a monogenic cause of kidney stones to be identified in up to 30% of children and 10% of adults who form stones, with about 35 different genes implicated(55). Among these, the most frequent are SLC4A1, with a dominant transmission, AGXT, GRHPR, and HOGA1 associated with hyperoxaluria phenotype, OCRL1 and CLCN5 with Dent disease and CYP24A1 with hypercalcemia.

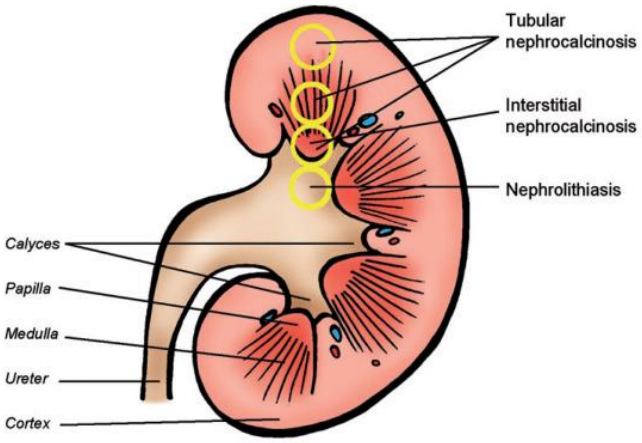


Figure 11. Localization of the calcifications: renal parenchyma for nephrocalcinosis, collecting system for nephrolithiasis.

PRIMARY HYPEROXALURIA

Hyperoxaluria is characterized by an increased urinary excretion of oxalate. The disease spectrum includes recurrent kidney stones, nephrocalcinosis and urinary tract infections. Calcium oxalate is deposited in various organs. Liver-kidney and isolated kidney transplantation are the treatments of choice for this disease(56).

However, the disease is often diagnosed only in ESKD or even when the disease recurs on the transplanted kidney(57).

Primary hyperoxaluria type 1 (PH1) is the most common and severe form, accounting for 80% of PH cases. It is caused by a defect in the Vitamin B6-dependent hepatic peroxisomal enzyme, Alanine Glyoxalate Aminotransferase (AGT) (Figure 12), this deficiency results in increased blood levels of oxalate, with affected subjects at high risk of recurrent nephrolithiasis. The phenotype is associated with pathogenic variants in AGXT(58). PH1 is accounting for 1-2% of pediatric ESKD cases(59).

Primary hyperoxaluria type 2 (PH2) represents about 10% of cases. Dysfunction of the enzyme glyoxalate/hydroxypyruvate reductase (GRHPR) leads to increased urinary excretion of L-glyceric acid and oxalate (Figure 12).

Primary hyperoxaluria type 3 (PH 3) is related to a genetic defect in the HOGA1 gene which codes for the mitochondrial 4-hydroxy 2-oxoglutarate aldolase (Figure 12)(60).

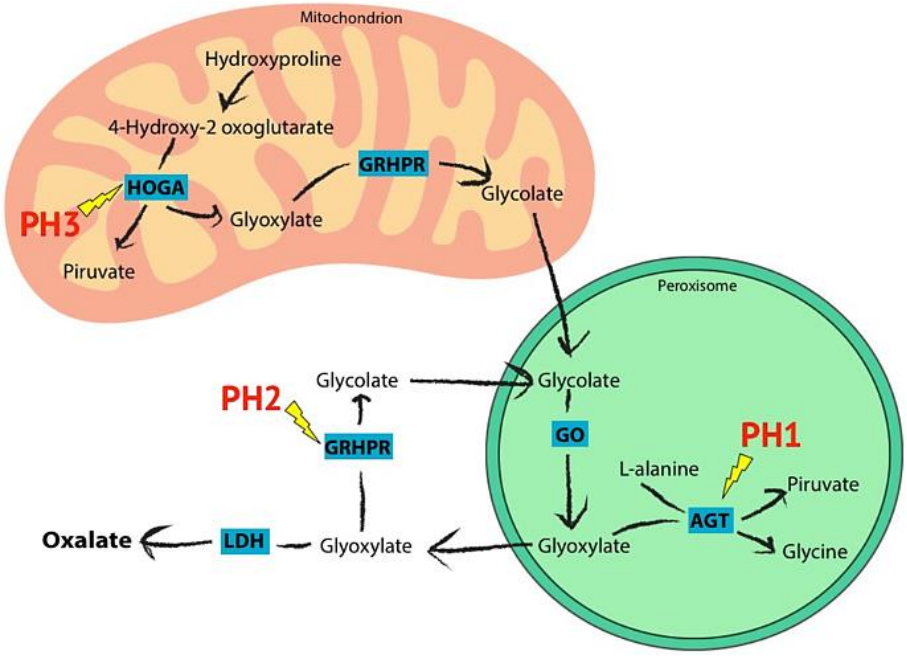


Figure 12. Molecular basis of the three types of hyperoxaluria: PH1, PH2 and PH3 (60).

DENT SYNDROME

Dent Syndrome is characterized by proximal tubular dysfunction and proteinuria of low molecular weight, associated with nephrocalcinosis and nephrolithiasis, hypercalciuria, hypophosphatemia and hyperphosphaturia, aminoaciduria, glycosuria, and microhematuria. Thirty to 80 percent of males face ESKD between the third and fifth decades of life(61).

50-60% of patients have a mutation in the CLCN5 gene, which codes for the Cl-/H+ exchanger of the chlorine channel, in 15%, a mutation in the OCRL gene, that regulates membrane trafficking, while in 25% of cases, the genetic cause remains unknown(61).

CYSTINURIA

Cystinuria is one of the most frequent monogenic causes of kidney stones, responsible for 6-10% of pediatric nephrolithiasis(62). It is associated with pathogenic variants in SLC7A9 or SLC3A1 genes, which code for cystine transport proteins(63). These variants lead to ineffective reabsorption of cystine at the proximal tubule, with a subsequent increase of cystine in the urine, which precipitates and forms stones.

1.3.5. TUBULOPATHY

Hereditary tubulopathies represent a heterogeneous group of rare diseases whose clinical diagnosis is complicated by the high phenotypic variability. Common features include polyuria, polydipsia, irritability, growth failure, nephrocalcinosis and blood pressure anomalies. Growth failure is related to chronic acidosis that results in protein catabolism and growth hormone deficiency(64).

Blood pressure effects vary. In tubulopathies that result in water or salt retention hypertension is observed (e.g. Liddle syndrome), in those with salt and water wasting, the effect is hypo- or normotension (e.g. Bartter Syndrome). Extra-renal manifestations may include hearing loss, ophthalmologic involvement and developmental delay(64).

To date, more than 50 genes have been related to tubulopathies(65), most of which are directly involved in the processes of reabsorption or secretion of water and solutes.

The diagnostic yield is about 50% for pediatric or young adult patients(66). The most frequently involved genes are SLC12A3, CLCNKB, SLC12A1 and ATP6V0A4.

RENAL TUBULAR ACIDOSIS

Renal tubular acidosis (RTA) is a class of disorders characterized by metabolic acidosis due to the inability of renal tubules to maintain acid-base balance. The chemical imbalance is a consequence of low tubular bicarbonate ($HCO3^-$) reabsorption in the proximal convoluted tubule or impairment in the urinary hydrogen ion (H^+) excretion, particularly in the distal nephron(67, 68).
There are four major forms of RTA. The hypokalemic RTAs include distal (type 1) and proximal (type 2). The hyperkalemic RTAs include hypoaldosteronism (type 4) and voltage-dependent RTA. Type 3 RTA is a very rare disease caused by carbonic anhydrase II deficiency.
Patients with RTA show low arterial pH, and low serum bicarbonate with hyperchloremia but normal serum anion gap. The dyselectrolytemia can include hypokalemia or hyperkalemia (frequently) and hypercalciuria (rarely). Adults with RTA are often asymptomatic or may have muscular weakness related to hypokalemia, nephrocalcinosis or recurrent renal stones(69). The diagnosis of RTA requires pH urine analysis and urinary ammonium excretion. If the urine anion gap is zero or positive, the urinary excretion of $NH4^+$ is relatively low and a renal cause for the acidosis is likely(70).
The classic form of RTA is distal RTA (dRTA, RTA type 1), which is characterized by impaired hydrogen ion secretion in the distal nephron. Patients with distal RTA present hypocitraturia and frequently develop renal calcifications and calcium-containing kidney stones. Nephrocalcinosis is an extremely common feature (90% of confirmed dRTA) because higher pH leads to the precipitation of calcium phosphate(71).
Inherited dRTA results from mutations in the coding genes for the renal apical membrane H-ATPase proton pump (ATP6V1B1, ATP6V0A4) or the basolateral membrane anion exchanger AE1 (SLC4A1)(72). Pathogenic variants could also be associated with sensorineural hearing loss because of the involvement of these genes in hearing(72).

BARTTER SYNDROME

Bartter syndrome is a rare inherited tubulopathy characterized by salt loss with hypokalemia, polyuria, hyperchloremic metabolic alkalosis and hyperaldosteronism. It can manifest prenatally with polyhydramnios and impaired intrauterine growth.

Pathogenic variants lead to a loss of salts at the tubular tract with activation of the renin-angiotensin system and subsequent metabolic alkalosis, with hypokalemia and hypochloremia. Altered reabsorption of salts also leads to reduced calcium reabsorption with hypercalciuria and possible nephrocalcinosis.
Four types can be distinguished(73):

- Type 1: variants in SCL12A1 gene, which codes for the cotransporter $Na^+/K^+/2Cl^-$, located in the ascending tract of the loop of Henle;

- Type 2: variants in KCNJ1, which encodes for an ATP-dependent potassium channel;
- Type 3: variants in CLCNKB, a gene coding for a chlorine voltage-dependent;
- Type 4: variants in BSND gene, coding for a subunit of the chlorine channel.

GITELMAN SYNDROME

Gitelman syndrome (GS) is associated with salt loss with hypomagnesemia, hypokalemia, metabolic alkalosis, hypocalciuria, and activation of the renin-angiotensin-aldosterone system. It generally occurs in late childhood or early adulthood with muscle weakness, cramps, tetany, paresthesia, thirst, nocturia and asthenia(74). Classically, it is caused by variants in the SLC12A3 gene that codes for a thiazide-sensitive NaCl cotransporter expressed in the apical membrane of the cells lining of the distal convoluted tubule. Progression to renal insufficiency is extremely rare in GS(75).

ADTKD

Autosomal dominant tubulointerstitial kidney disease (ADTKD) is a recently defined entity that includes rare kidney diseases characterized by tubular damage and interstitial fibrosis in the absence of glomerular lesions, with progression to end-stage renal disease. Patients with ADTKD-UMOD often develop gout at an early onset(76).
The features and the progression to KF are variable, the age of ESKD varies from 20 to 75 years old(76).

Regarding the gene related, three types can be distinguished:

- ADTKD-UMOD
- ADTKD-MUC1
- ADTKD-REN

Recently, other genes were associated with ADTKD, such as HNF1B, SEC61A1 and DNAJB11.

For ADTKD-MUC1, the causative mutations are in 90% of cases frameshift variants in a variable number of tandem repeats (VNTR), that are not detectable using the standard NGS sequencing, but it requires long-read technology. It is thought that MUC1fs deposition results in accelerated apoptosis of renal tubular cells, tubulo-interstitial fibrosis, and progressive chronic kidney disease(77).

1.4. GENETIC TESTING APPROACHES

To confirm or identify the presence of genetic kidney disease, genetic testing is required. Obtaining a differential diagnosis is critical in determining the most appropriate treatment and follow-up for the patient and identifying the risk of disease transmission to offspring.

There are several approaches to genomic analysis, each with different diagnostic sensitivities and cost-effectiveness(17). The most common approaches are based on 3 main technologies, including targeted gene panels, whole exome sequencing (WES) and whole genome sequencing (WGS). Sanger technology remains a validation system for next-generation sequencing (NGS) and the analysis of small specific genes.

it is still quite debated which method is the most effective. Sanger and targeted panels are certainly more widespread methods in clinical diagnostics, more cost-effective and the results more easily analyzed. However, they have a small number of analyzable genes, and introducing new genes into an existing panel requires redesign and revalidation of the panel. Genome-wide methods, on the other hand, are less spread, more expensive, and require high expertise in variant analysis and evaluation but allow the entire exome or genome to be examined simultaneously.

SANGER TECHNOLOGY

Sanger sequencing is a “first-generation” DNA sequencing method, based on selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. This analysis is limited to single DNA fragments of up to 1000 base pairs and is not efficient for large or multi-gene analyses.

This technology is a rapid and cost-effective method, allowing for population screening for a single disease. It has high coverage and high sensitivity for detecting single nucleotide polymorphisms.

It is useful for the validation of the variants identified in NGS and for the analysis of small genes correlated to targeted diseases. In genetic kidney disease is used for the identification of the GLA gene (429 amino acid) in suspected Fabry’s disease for specific identification of the variant position for the determination of the best therapeutical approach.

TARGETED PANEL TECHNOLOGY

The targeted panel can assess multiple genes in parallel, saving time and reducing costs. It is focused on a select set of genes that have known or suspected associations with the disease under study. Employing a panel technology is an effective approach to establish a genetic diagnosis in case of a

clearly defined phenotype and the aim is to define the molecular diagnosis between phenocopies(78). Starting from a defined phenotype and applying the test on a limited number of genes, the approach is simplified compared to WES or WGS approaches. Furthermore, using a specific genes panel helps prevent the discovery of incidental findings, as there is currently no consensus on how to report them.

On the other hand, the targeted panel cannot be easily extended to include new genes. This means that the panel's content might become quickly outdated over time as new genetic associations with the condition are identified. Furthermore, in consideration of all the possible clinical presentations, a significant number of different panels should be maintained by the analytical laboratory increasing the complexity and cost of this solution.

Currently, targeted panels vary in the number of genes included in the analysis, with the largest panels able to analyze around 2000 genes. The clinical impact largely depends on the genes included and therefore, whether a differential diagnosis can be made.

WHOLE EXOME SEQUENCING

WES is a technology approach that can capture the majority of coding regions. It is estimated that up to 85% of all pathogenic mutations fall within this region. WES is mainly applied in cases of unclear clinical suspicion. This technology allows the analysis of targeted genes but also allows for re-analysis as genomic components are newly identified, through the application of WES-based virtual panels.

However, WES exhibits low coverage compared to other methods and often requires Sanger sequencing to increase the depth of analysis(17). WES does not achieve complete coverage of the exome in some areas, such as GC-rich regions and homology sequences(79). The GC content can reduce the efficiency of nucleotide hybridization, causing lower sequencing coverage (80, 81) and requiring modified *Polymerase* Chain Reaction (PCR) conditions and the design of exon-specific primers.

WES technology is a cost-effective option compared to WGS (82) and the data generated can be easily analyzed and stored.

WHOLE GENOME SEQUENCING

The most advanced technological approach is represented by WGS, which allows for the investigation of the entire genome, including regulatory regions and non-coding variants.
It has good coverage, an improved mappability compared to WES and no GC-bias, reducing the rate of false-negative variant calls(80).

WGS approach overcomes the pseudogene sequence similarity and the duplicated regions(83). For example, in the context of diagnosing ADPKD, the most frequent genetic kidney disease(84), it is highly impactful. Indeed, the PKD1 gene shows six pseudogenes with a sequence similarity of 97%. Mallawaarachchi et al.(83) report that the diagnostic yield of WGS in ADPKD patients is 80%.

However, the short-read technology still largely adopted in next-generation systems, is struggling to detect complex structural variants such as large inversions, deletions, or translocations. The technological advancement of long-read technology could overcome this limitation(85).

Despite its potential, WGS is still not widely used in clinical practice due to its higher costs and less developed analytical tools compared to more established sequencing methods like WES. However, with ongoing advancements and cost reductions, WGS may become more commonly employed in the future, especially for cases where its unique benefits are crucial, such as diagnosing complex genetic conditions like PKD.

Furthermore, large-scale WGS projects aimed at the collection of a large amount of genomic data (one million sequenced genomes in the European program Beyond 1 Million Genomes) will help to establish a complete repertoire of genomic variability permitting better identification of rare pathogenic variants in the coding and non-coding domain.

Currently, studies evaluating the diagnostic performance of WGS in the field of kidney diseases are few. Various non-nephrological studies, report a debated increase in the effectiveness of WGS. Bertoli-Avella (86) et al. in a study about 1007 patients, mainly with neurological diseases, demonstrate that 29.6% of the unsolved WES cases could benefit from WGS. However, the interpretation of these data is not unique, Biskup et al. (87)responding to the above-mentioned study, report that the variants claimed as WGS results, could be identified using a well-established WES technology, and only the 1.4% can be attributable uniquely to WGS.

In other studies, the incremental rate varies from 2-9%(88, 89). Ewans et al. (88)report an additional 9% yield of WGS with 13 new diagnoses attributable to WGS in WES unsolved cases. These additional diagnoses detected were related to unknown gene-disease association, insufficient sequencing coverage and CNVs detection.

COMPARATIVE GENOMIC HYBRIDIZATION (Array CGH)

Array CGH is based on the use of differentially labeled tests and reference genomic DNA samples that are simultaneously hybridized to DNA targets arrayed on a glass slide or other solid platform. It allows for a high-resolution evaluation of DNA copy number alterations associated with chromosome abnormalities. The primary advantage of aCGH is the ability to simultaneously detect aneuploidies,

deletions, duplications, and/or amplifications of any locus represented on an array. This method is of particular use in diagnosing forms of CAKUT in which CNVs are associated(54).

1.5. PRECISION MEDICINE

A genetic diagnosis has important implications for the nephropathic patient: it allows to definition of a more accurate prognosis and a more suitable therapy, in some cases avoiding invasive medical investigations (renal biopsy) or unnecessary and potentially toxic therapy, as well as allows to evaluate the risk of transmitting the disease to the offspring and to consider alternative solutions for pregnancy planning (pre-implantation diagnosis).

TAILORED THERAPY

A genetic diagnosis can suggest the identification of a more effective therapeutic choice and a plan for follow-up and visits to monitor disease progression. New therapeutic treatments or enrollment in clinical trials may require a genetic confirmation of the diagnosis as criteria for the administration. An example is the administration of Tolvaptan for a polycystic kidney. According to AIFA guidelines, Tolvaptan can be considered as a treatment option and administered to a patient with ADPKD without a positive family history, only if the patient presents a positive genetic diagnosis; the positivity of the genetic test ensures that these patients have access to the therapy.

The identification of a diagnosis in unclear CKD (uCKD), can ensure a treatment. About 10-40% of uCKD can obtain a genetic diagnosis thanks to a genetic test(90). AGXT pathogenic variants in patients with recurrent stones, granted access to a new biological therapy called Lumasiran.
A genetic diagnosis may also prevent the patient from receiving an unnecessary and potentially harmful therapy, such as the administration of immunosuppressive drugs in patients with genetic forms of nephrotic syndrome or with Alport syndrome, which in most cases are steroid-resistant.

AVOIDING INVASIVE INVESTIGATIONS

A genetic diagnosis can prevent investigation by renal biopsy, which is not always diagnostic, especially in cases of Alport. As kidney biopsy is expensive and has serious risks, the identification of variants in some genes, including UMOD and APOL1 has the potential to obviate or supplement a kidney biopsy which may not provide a definitive diagnosis. Moreover, Jayasinghe et al. reported that, compared to kidney biopsy, WES is a lower-risk and more cost-effective approach for children with a glomerular disease(91).

EXTRA-RENAL MANIFESTATIONS SURVEILLANCE

Genetic kidney diseases can affect organs beyond the kidneys. It's crucial to tailor the surveillance and management of these manifestations to the specific genetic condition. Regular follow-ups and

clinical assessments are key to identifying and addressing these extra-renal complications in a timely and effective manner.

Pathogenic variants, causative of renal phenotypes, have been associated with other features. Patients with variants on HNF1b may display pancreatic changes such as diabetes, and hypomagnesemia(92), as well as, patients with Alport syndrome may manifest lenticonus and hearing loss(93). The identification of a variant may therefore also have implications for patient surveillance, and it is critical to create a multidisciplinary team to follow these patients.

GENETIC COUNSELING

Genetic counseling holds an important role for the patient and family members. Genetic counseling can provide an assessment of the risk of disease transmission to offspring as well as allow consideration of preimplantation diagnosis in the context of family planning counseling.

It is important to provide pre- and post-genetic testing counseling. Ensuring, on the one hand, greater awareness when signing consent and evaluating results-return options, and on the other hand, a better understanding of the results and the implications for the patients and their families (94).

2. ABSTRACT

Chronic kidney disease (CKD) is a global public health issue affecting 9-13% of the population. Despite advances in understanding the underlying causes, 20% of cases remain unexplained. The application of a genomic approach holds the potential to uncover the CKD etiology in a relevant portion of pediatric and adult patients, with estimated diagnostic rates ranging from 5-30%. However, there is a lack of consensus in the scientific community on the optimal diagnostic algorithm. Genetic approaches include targeted panels, whole exome sequencing (WES), and whole genome sequencing (WGS). While WGS offers a comprehensive analysis, its employment is less widespread due to high costs and computational burden. Identifying a genetic diagnosis carries significant implications for tailored therapy, avoiding invasive investigations, monitoring extra-renal manifestations, and conducting genetic counseling for family planning. Genetic testing is a crucial component of precision medicine in managing CKD.

From this perspective, we conducted a retrospective study to assess the diagnostic yield and clinical impact of a Nephropathy panel (NES) covering 44 genes for genetic kidney diseases. The study, named DECIDE, involved Italian and Spanish centers encompassing pediatric and adult patients. Clinical presentation was classified into cystic disease, glomerulopathy, CAKUT, tubulopathy, nephrocalcinosis/nephrolithiasis (NC/NL), unknown CKD, and at risk with negative phenotype. The NES panel's diagnostic yield was calculated. The genotype-phenotype correlation was assessed using Kaplan-Meier analyses. Results from alternative technologies were collected for negative cases.
Employing machine learning, the sensitivity and specificity were defined, and an algorithm with the model-defined predictive features was developed to predict the performance of the test in the patient's clinical setting.

Of 809 patients, 692 index cases were analyzed. Cystic kidney disease was the most common presentation (371 patients), followed by glomerulopathy (184 patients). The other 5 clinical presentations made a minor contribution: CAKUT (45), NC/NL (36), tubulopathy (22), unknown CKD (22), and 12 showed a negative renal phenotype but they were at risk of disease development. A total of 252 diagnostic variants were identified, resulting in a 36% yield. Cystic kidney disease had the highest yield (49%, 183 positive cases), followed by tubulopathy (32%, 7), glomerulopathy (28%, 52), CAKUT (13%, 6) and NC/NL (11%, 4). No diagnostic variants were found in cases of unknown CKD or at risk with negative phenotypes. Eight genes accounted for 95% of the diagnosis: PKD1 (47%), PKD2 (21%), COL4A5 (9%), COL4A3 (7%), COL4A4 (4%), PKHD1 (4%), SLC12A3 (2%), CYP24A1 (2%).

Diagnostic results confirmed clinical indications in 70% of cases, defined the diagnosis in 23%, and altered clinical suspicion in 7%.

In 25 cases with negative NES results, further investigations led to only three diagnoses (one with WES, two with panels).

The analysis of the clinical predictors of a positive result highlighted a relevant strength of the family history (OR 4.7) and the cystic phenotype (OR 6.0) in achieving a definitive diagnosis. This evidence permitted the elaboration of a diagnostic algorithm to identify which cases would benefit most from the test.

In conclusion, the results showed the potential value of the NES panel in diagnosing genetic kidney disease, particularly in cystic disease or glomerulopathy cases. However, 64% of patients remain undiagnosed, leading to the proposal of a national project to explore WGS as a potential solution. This proposal will aim to improve diagnostic capabilities, pivotal in advancing personalized therapy and understanding of genetic kidney diseases.

3. METHODS

The study, named DECIDE (Diagnostic EffiCacy kIdney Disease European), was conducted in five European centers: three from Italy, the University Hospital of Modena, the Bambino Gesù Children's Hospital in Rome, and the University Hospital of Parma; and two Spanish, the University Hospital Jimenez Diaz Foundation in Madrid and the Virgen de las Nieves University Hospital in Granada. Modena assumed the role of coordinating center.

The centers adopted as their primary diagnostic approach, the same diagnostic panel. The NES (Nephropathies Solution) panel was developed by SOPHiA GENETICS in collaboration with the Bambino Gesù Children's Hospital in Rome. It covers 44 genes related to the main genetic kidney diseases (Table 1).

GENES	DISEASE	CATEGORY
AGXT	Primary Oxaluria type 1	Nephrolithiasis/Nephrocalcinosis
AQP2	Nephrogenic diabetes insipidus	Tubulopathy
ATP6V0A4	Renal tubular acidosis	Tubulopathy
ATP6V1B1	Renal tubular acidosis with deafness	Tubulopathy
AVPR2	Nephrogenic diabetes insipidus	Tubulopathy
BSND	Bartter syndrome, type 4a	Tubulopathy
CASR	Hypocalcemia	Tubulopathy
CEP290	Joubert syndrome	Nephronophthisis
CLCN5	Dent Disease	Nephrolithiasis/Nephrocalcinosis
CLCNKB	Bartter syndrome, type 3	Tubulopathy
COL4A3	Alport syndrome	Glomerulopathy
COL4A4	Alport syndrome	Glomerulopathy
COL4A5	Alport syndrome	Glomerulopathy
CRB2	Focal segmental glomerulosclerosis	SRNS
CTNS	Cystinosis	Nephrolithiasis/Nephrocalcinosis
CUBN	Megaloblastic anemia-1, Finnish type	Tubulopathy
CYP24A1	Hypercalcemia, infantile	Nephrolithiasis/Nephrocalcinosis
DSTYK	CAKUT	CAKUT
EMP2	Nephrotic syndrome	SRNS
EYA1	Branchiootic syndrome	CAKUT
FN1	Glomerulopathy with fibronectin deposits	Glomerulopathy
FOXC1	Dysgenesis	CAKUT
GRHPR	Hyperoxaluria, primary type II	Nephrolithiasis/Nephrocalcinosis
HNF1b	ADTKD	ADTKD
KANK2	Nephrotic syndrome, type 16	SRNS
KCNJ1	Bartter syndrome, type 2	Tubulopathy

LAMB2	Nephrotic syndrome, type 5	SRNS
NPHS2	Nephrotic syndrome, type 2	SRNS
NR3C2	Pseudohypoaldosteronism type I	Pediatric Hypertension
OCRL	Dent disease 2	Nephrolithiasis/Nephrocalcinosis
PAX2	Focal segmental glomerulosclerosis	SRNS
PHEX	Hypophosphatemic rickets	Rickets
PKD1	ADPKD	Cystic kidney
PKD2	ADPKD	Cystic kidney
PKHD1	ARPKD	Cystic kidney
SIX1	Branchiootic syndrome 3	CAKUT
SLC12A1	Bartter syndrome, type 1	Tubulopathy
SLC12A3	Gitelman syndrome	Tubulopathy
SLC34A1	Hypercalcemia infantile	Nephrolithiasis/Nephrocalcinosis
SLC4A1	Renal tubular acidosis	Tubulopathy
SLC4A4	Renal tubular acidosis	Tubulopathy
TTC21B	Nephronophthisis	Nephronophthisis
UMOD	ADTKD	ADTKD
WT1	Denys-Drash syndrome	SRNS

Table 1: List of genes included in the panel and the diseases related.

The study was performed retrospectively on a nephropathic population undergone to the genetic panel, in a period between 2017 and the beginning of 2023. The population comprised pediatric and adult patients, with or without a family history of nephropathy and with a variable clinical presentation. Each center involved obtained approval from its local ethics committee.

Each enrolled subject has undergone a genetic test with an NES panel, the results and the interpretation of the results were provided during a previous genetic/nephrology counseling.

Inclusion criteria:

- Patients between the ages of 0 and 100;
- A genetic report obtained by the NES panel analysis;
- Patients with a clinical presentation comprised of the following categories:
    - Cystic kidney disease - the presence of renal cysts by radiologic examination with or without a positive family history, large and/or hyperechoic kidney >95° percentile;
    - Glomerulopathy - urine abnormalities and suspect for a mendelian condition (compatible syndromic condition, positive family history, not conclusive renal biopsy examination);

- CAKUT - congenital abnormalities of kidney and urinary tract, hyperechoic hypo-dysplastic kidneys (<3° percentile);
- Tubulopathy - dysfunction in specialized channels and transporters (glycosuria, aminoaciduria, polyuria, bicarbonaturia, increased beta2-microglobinuria, proteinuria, hypercalciuria, acidosis, etc.);
- Nephrocalcinosis/Nephrolithiasis - renal stones, renal calcifications, nephrocalcinosis;
- At risk with negative phenotype - the absence of kidney function abnormalities, positive family history (e.g. kidney donation assessment);
- Unknown CKD - undiagnosed CKD or late referral in the context of high suspicion of a genetic condition.

Exclusion criteria:

- Subjects who refused to participate in the study and did not sign the consent for using their data for scientific purposes;
- Prenatal analysis will not be collected.

3.1. DATA COLLECTION

All the centers collected clinical and genetic data from patients who complied with the inclusion criteria of the study.

a) CLINICAL DATABASE:
- Clinical presentation (main and secondary);
- Sex (male or female);
- Age of exam execution;
- Age at onset of the disease (first diagnosis or first identification of the kidney symptoms);
- Kidney function (for eGFR>15ml/min, only native kidney);
- Presence of hypertension;
- Age of kidney failure;
- Family history of nephropathy and Inheritance (AD, AR, X-linked).

b) GENETIC DATABASE
- Variants: gene, cDNA position, protein position;
- ACMG classification (C5=pathogenic, C4=likely pathogenic and C3=uncertain significance);

- Copy number variations;
- Zygosity (homozygous, heterozygous, hemizygous).

In addition, only diagnostic variants from further investigations were collected. Specifically, cDNA and protein positions of variants were collected from analyses such as disease-specific panels, Sanger (GLA gene), WES, and aCGH.

In the Modena cohort, data about the correlation between the clinical suspicion and the genetic results was assessed in terms of confirmation, definition and alteration. Data about the clinical implications of the genetic results were collected.

3.2. DATA ANALYSIS

DESCRIPTIVE ANALYSIS OF THE VARIANTS

The clinical data were collected according to the data collection protocol by the participating centers. The variant data were obtained by the analysis of the SOPHiA platform. Sequencing data were processed for single nucleotide variants (SNVs), indels, and CNVs via the SOPHiA DDM platform based on SOPHiA Artificial Intelligence (AI). According to this analysis, the variants were defined as truncating, frameshift, missense, splicing, indels, and CNV. These potentially functional variants were defined as missense, nonsense, splice site, or indel variants with a minor allele frequency of less than 1% in gnOMAD (gnOMAD v3.11 is a database of genetic variation that spans 76,156 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies).
Variants were classified, following the ACMG guidelines(95), into 5 classes: pathogenic (C5), likely pathogenic (C4), uncertain significance (VUS or C3), likely benign (C2), benign (C1).

Statistical analysis was performed using SPSS software v26.0 and R v4.0. Quantitative data were shown as
median and interquartile range (IQR). Categorical variables are expressed as percentages. The Kruskal Wallis test with Bonferroni correction was used for continuous variables and the chi-square test for categorical variables. The level of statistical significance was set at a value of $P<0.05$.

DIAGNOSTIC YIELD

The diagnostic yield of the test was calculated as the number of diagnostic variants found to the total variants and expressed in percentage. The yield was calculated for each clinical presentation and the total population. An assessment of the diagnostic yield for each group involved was considered.

As diagnostic variants, we considered pathogenic or likely pathogenic variants correlated to the patient's phenotype.

VUS RE-EVALUATION

The frequency of the variants, identified by the NES panel, was compared to the expected frequency in the general population. The enrichment of pathogenetic variants was expressed in terms of odds ratio and formally assessed through the Fisher test.

GENOTYPE/PHENOTYPE CORRELATION

We evaluated genotype-phenotype correlation separately for each clinical presentation. We compared the age at ESKD by Kaplan-Meier analysis with Log-rank testing comparing the test's results (Diagnostic, C3, Negative).

In the case of ADPKD and Alport syndrome a Kaplan-Meier survival analysis was evaluated for the type of variants (truncating vs non truncating or AD vs AR).

ASSESSMENT OF CLINICAL PREDICTORS

A machine learning approach (PyCaret package - https://github.com/pycaret/pycaret/releases) was employed to determine the test's sensitivity and specificity and to identify clinical predictors that lead to a conclusive diagnosis. A ROC curve and a logistic regression were performed.

4. RESULTS

4.1. CHARACTERISTICS OF THE COHORT

The DECIDE project collected clinical and genetic data from a cohort of subjects that underwent genomic analysis with NES panel, from 2017 to 2023. In total, genomic data from 809 cases were collected.

The targeted tests were excluded, and only index cases were considered in the statistical analysis (Figure 13). Therefore, the subsequent analyses were performed on a cohort of 692 cases, of whom 85% had complete genomic and clinical data.

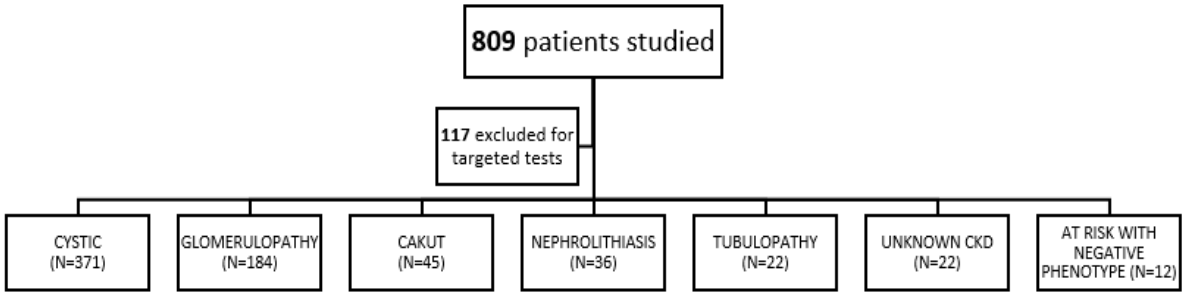


Figure 13. Flow chart of the DECIDE study.

The 692 cases were classified into seven clinical presentations before the test execution, as decided during the protocol definitions.

The leading clinical presentation was cystic kidney disease with 371 patients, followed by glomerulopathy with 184 patients. The other 5 clinical presentations made a minor contribution: 45 patients with CAKUT phenotype, 36 with nephrolithiasis/nephrocalcinosis, 22 with tubulopathy, 22 patients presented with unknown CKD before a genomic test and 12 showed a negative renal phenotype but they were at risk of disease development due to familial segregation analysis (Figure 14).

Among the 22 patients with unknown CKD, the majority were considered late referrals with an unclear underlying diagnosis.

Subjects at risk included primarily those apparent phenotype-negative individuals who were candidates for kidney donation in the context of a family history of CKD.

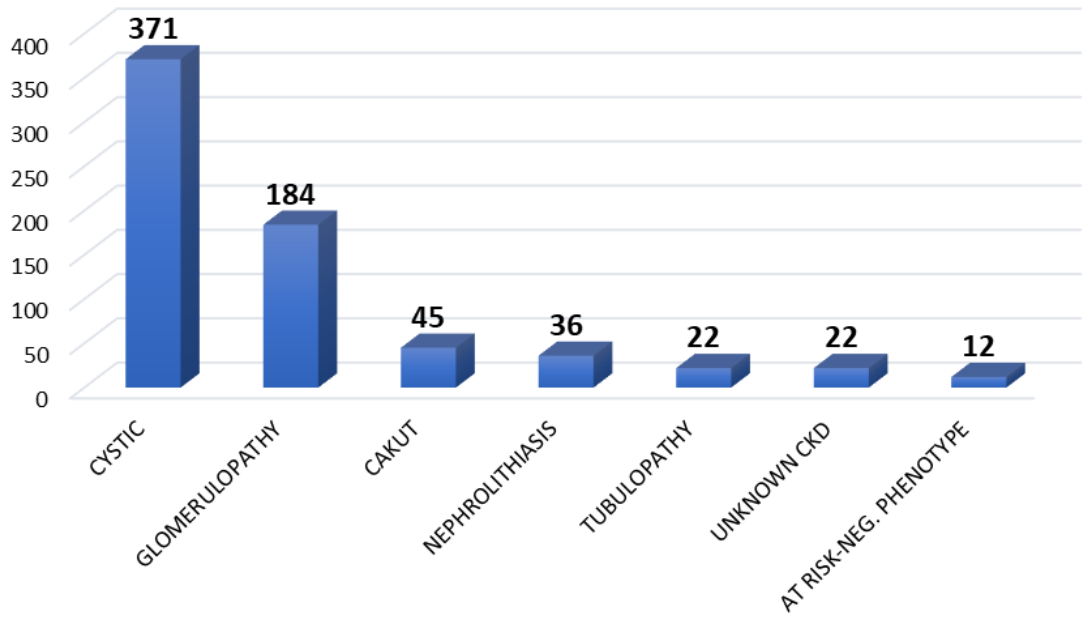


Figure 14. Distribution of the enrolled patients based on the clinical presentation.

CLINICAL CHARACTERIZATION

The clinical characteristics of the 692 index cases who underwent genomic analysis were shown in Table 2.

Cohort	ALL (N=693)	CYSTIC DISEASE (N=371)	GLOMERULOPATHY (N=184)	CAKUT (N=45)	NEPHROLITHIASIS (N=36)	TUBULOPATHY (N=22)	UNKNOWN CKD (N=22)	AT RISK-NEGATIVE PHENOTYPE (N=12)
Male : Female	1.2:1	1.4:1	1:1	1.3:1	1.6:1	0.8:1	2.7:1	0.9:1
Age at exam (yr)	50 (38-61)	50 (39-62)	55 (41-62)	37 (28-47)	48 (39-57)	46 (30-58)	51 (39-59)	41 (29-52)
Age at onset (yr)	38 (23-51)	40 (24-51)	40 (24-55)	23 (3-41)	38 (18-47)	35 (21-54)	33 (24-49)	---
eGFR (mL/min/1.73m2)	64 (41-95)	70 (43-98)	55 (38-88)	60 (38-79)	81 (55-99)	56 (41-81)	42 (18-50)	100 (90-109)
Hypertension (%)	66%	64%	79%	59%	50%	22%	83%	0%
Kidney failure (%)	19%	15%	26%	18%	11%	0%	64%	0%
Age of kidney failure (yr)	46 (32-59)	50 (42-60)	47 (29-61)	31 (23-50)	48 (33-72)	---	37 (26-45)	---
Family history (%)	57%	61%	55%	32%	73%	31%	58%	100%

Table 2. Clinical characteristics of the patients enrolled for the total population and for each clinical presentation.

The cohort was predominantly adult, only 10 cases were <18 years old at the time of the test. The median age at the exam was 50 years old (IQR 38-61 years old).

The percentage of male in the cohort was comparable to that female, except for the category of unknown CKD where male was 2.7 times female (p-value<0.05).

The younger group was CAKUT with a median age at the exam of 37 years old (IQR 28-47) and the age at onset of 23 (IQR 3-41) (p-value<0.05).

The patients with the most advanced chronic kidney disease at presentation are from the uCKD group, who have lower eGFR (42 (IQR 18-50), p-value<0.001), a higher percentage of hypertension (83%) and kidney failure events (64%, p-value<0.001).

Tubulopathy is less associated with hypertension, with only 22% of patients presenting with high blood pressure. Indeed, some types of tubulopathy are more often associated with hypo- or normotension due to salt and water wasting. None of the patients developed renal failure before testing (p-value<0.001).

Family history was available in 539 cases of which 310 (57%) showed a positive history of kidney disease.

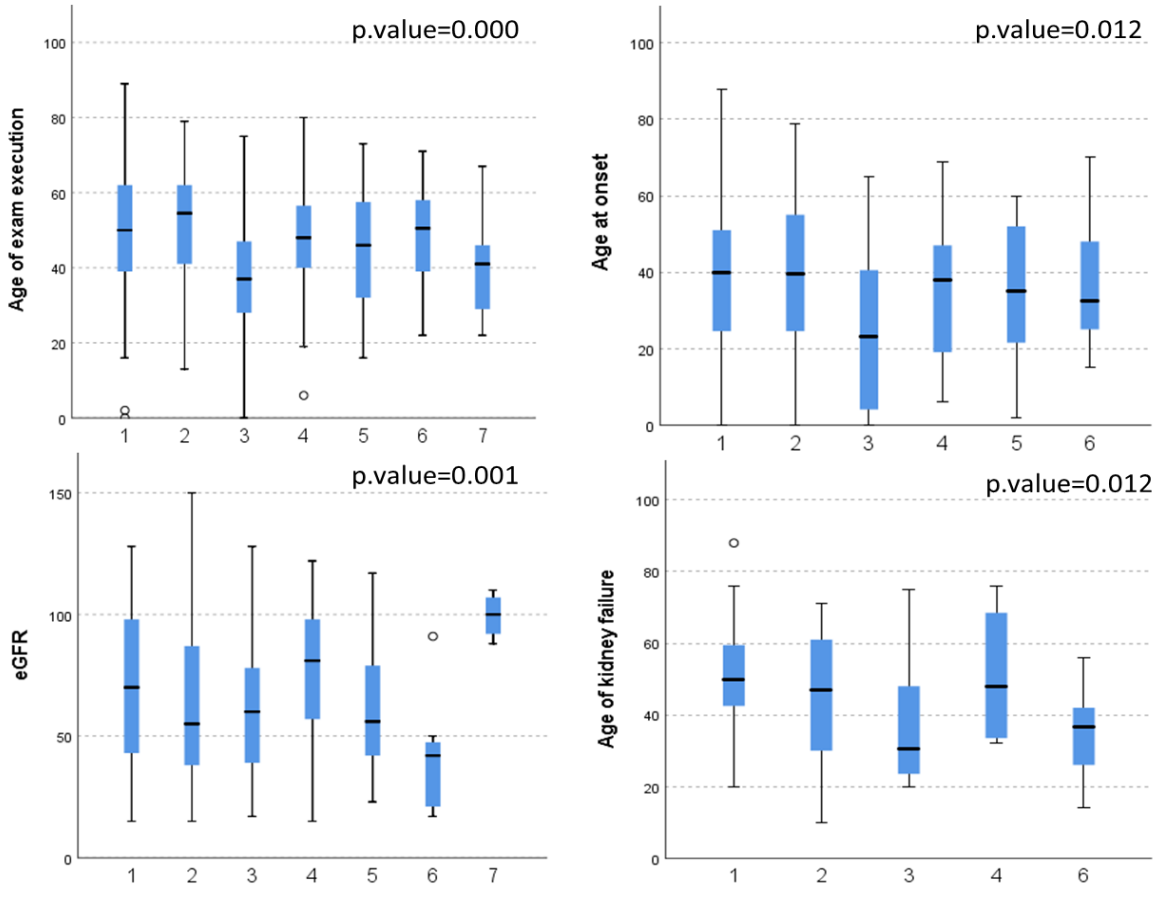


Figure 15. Kruskal-Wallis test for statistical analysis. Level of statistical significance set as P<0.05 1:Cystic kidney, 2: Glomerulopathy, 3: CAKUT, 4: Nephrolithiasis/Nephrocalcinosis, 5: Tubulopathy, 6: uCKD, 7: At risk with negative phenotype.

	Chi-square	Significance
Sex	6.1	0.013
Hypertension	52.7	0.000
Kidney failure	216.8	0.000
Family History	10.7	0.001

Table 3. Chi square test for categorical data. The level of statistical significance is set as P<0.05.

4.2. GENE PANEL ANALYSIS

NES panel covers 44 genes related to the main genetic kidney disease. 11 genes are correlated with autosomal dominant conditions, 26 genes with recessive conditions, 2 genes showed both modes of inheritance (COL4A3, COL4A4) and 5 with an X-linked mode (X chromosome location). Using the gnomAD pLI and Z-scores, we assessed respectively the intolerance to heterozygous loss-of-function and the intolerance to accept missense variants for our setting of the gene (Table 4). The score compares the predicted number of variants expected to be seen in the variant's population dataset with the observed amount of variation. The predicted constraint metrics, Z-score and pLI score, are frequently used to prioritize candidate genes when analyzing WES or WGS data. pLI closer to 1 is considered as extremely intolerant for loss of function variations (nonsense, frameshift, splicing alterations). Positive Z scores indicate increased constraint, so the transcript had fewer variants than expected. Negative Z scores were given to transcripts that had more variants than expected.

GENE	pLI	Z score	GENE	pLI	Z score	GENE	pLI	Z score	GENE	pLI	Z score
DSTYK	0,51	1,59	**AGXT**	0	-0,27	**CUBN**	0	-1,17	**SLC12A3**	0	-0,94
FOXC1	0,95	0,34	**AQP2**	0,02	0,55	**EMP2**	0	-0,44	**SLC34A1**	0	-0,48
HNF1B	1	1,73	**ATP6V0A4**	0	0,85	**EYA1**	0,1	1,2	**SLC4A4**	1	3,14
NR3C2	0,84	2,1	**ATP6V1B1**	0	0,23	**FN1**	0	1,65	**TTC21B**	0	-0,41
PAX2	0,67	1,49	**BSND**	0	0,07	**GRHPR**	0	0,74	**AVPR2**	0,56	1,01
PKD1	1	-4,32	**CASR**	0,05	3,12	**KCNJ1**	0	-0,28	**CLCN5**	0,99	2,53
PKD2	0	0,27	**CEP290**	0	0,47	**KANK2**	0,04	0,2	**COL4A5**	1	2,5
SIX1	0,69	1,49	**CLCNKB**	0	-0,56	**LAMB2**	0	0,77	**OCRL**	1	2,96
SLC4A1	0,85	1,66	**CRB2**	0	0,29	**NPHS2**	0	0,69	**PHEX**	1	1,71
UMOD	0	1	**CTNS**	0	-0,92	**PKHD1**	0	-0,98	**COL4A3**	0	1,99
WT1	1	1,78	**CYP24A1**	0	-1,1	**SLC12A1**	0	1,16	**COL4A4**	0	1,02

Table 4. pLI and Z-score for our setting of genes, extracted by gnomAD. In orange, the genes correlated to autosomal dominant conditions, in yellow those with autosomal recessive conditions, in light blue genes located on the X-chromosome and in green the two genes with both modes of transmission.

Differences in pLI and Z-score can be observed in the 4 groups of genes: dominant, recessive, X-linked and dominant/recessive (Table 5, Figure 16 and 17). The average pLI score for dominant genes is 0.59; PKD1, HNF1b and WT1 are extremely intolerant (pLI=1).

The genes on the X chromosome are more loss of function intolerance, with a pLI=0.91 and more constraint, with a positive z-score of 2.14.

In contrast, recessive genes show less intolerance to loss of function and missense variants.

	pLI	Z-score
Dominant genes	0.59	0.72
Recessive genes	0.05	0.29
X-linked genes	0.91	2.14
Dom/rec genes	0.00	1.51

Table 5. Mean of pLI and Z-score in dominant, recessive and x-linked genes.

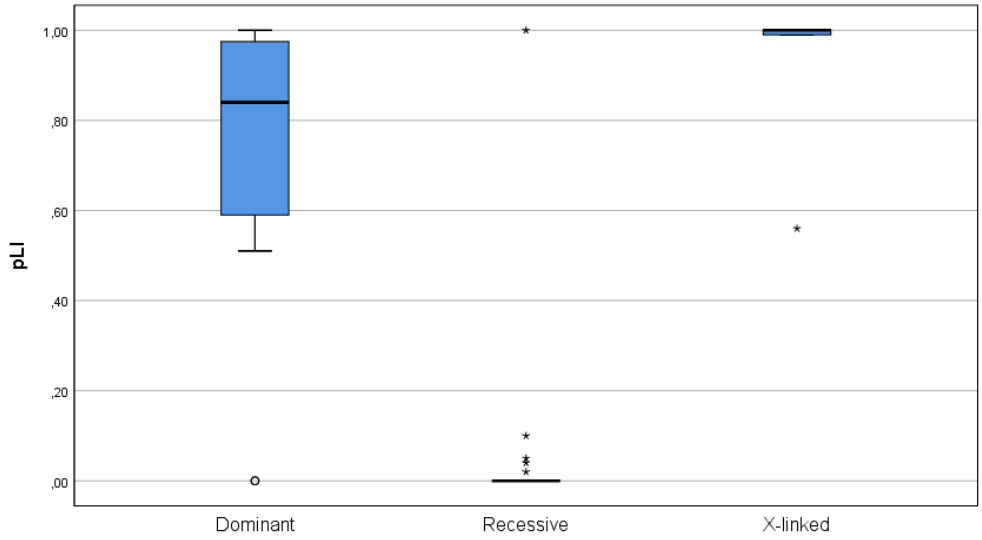


Figure 16. Boxplot of the pLI score distribution for dominant, recessive and x-linked genes.

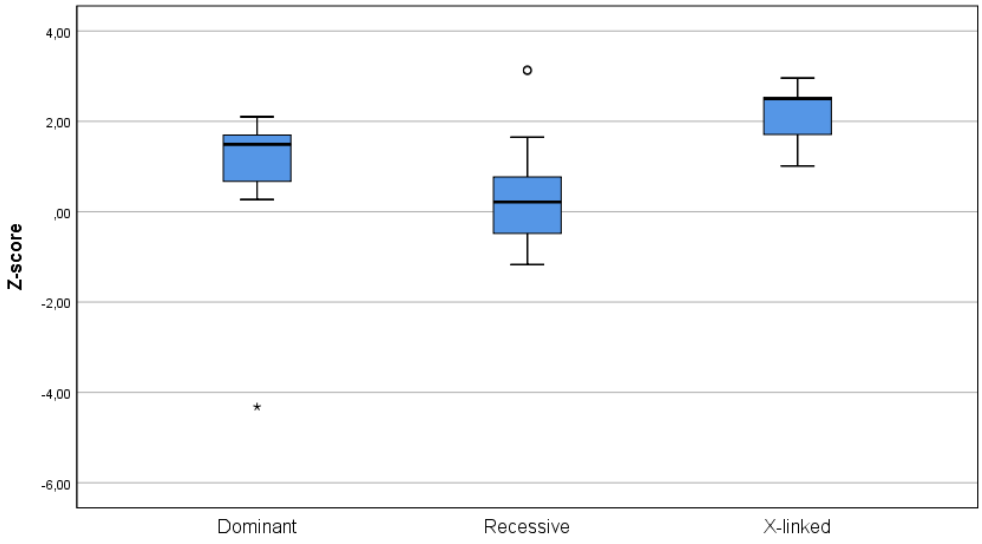


Figure 17. Boxplot of the Z-score distribution for dominant, recessive and x-linked genes.

4.2.1. ANALYSIS OF VARIANTS DETECTED IN THE GENETIC TESTING

A total of 1016 variants were collected during the project. In particular, 14.6% of the variants were classified as C5 for ACMG guidelines, 15.3% as C4 and the other 70.1% as C3 (Figure 18). Each gene of the panel had at least one variant.

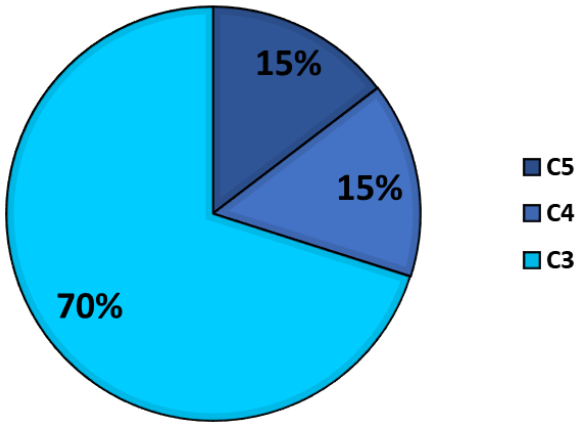


Figure 18. Distribution of the variants detected in genetic analysis. C5=Pathogenic (dark blue), C4=Likely Pathogenic (blue), C3=Variants of uncertain significance (light blue).

The largest number of variants falls in the PKD1 gene, primarily due to the prevalence of cystic population in our cohort, its size (47 191 bases) and a high polymorphism (Z score = -4.32).

Many of the variants are found in heterozygosity, except for 6 cases of compound heterozygosity and 3 cases of homozygosity. The compound heterozygosity was found in PKHD1 genes (2 cases), COL4A4 (2 cases), COL4A3 (1 case) and SLC12A3 (1 case).

The 3.5% of variants were classified as CNVs, with enrichment for CLCNKB gene duplications in the Modena cohort, found 16 times as C3 not correlated to phenotype; probably a polymorphism for this population.

Half of the genes in the panel do not have truncating variants. The PKD1 gene, with a total of 209 variants, exhibits a truncating variant percentage of 27% among all variants. In contrast, PKD2 shows a truncating variant rate of 74%, PAX2 has 50%, and HNF1b has 33%.

PKD2 shows more truncating variants than non-truncating variants, a difference also observed in the literature (96, 97).

4.3. DIAGNOSTIC YIELD OF THE PANEL

We separately evaluated the diagnostic yield of the NES panel in the seven clinical presentations.

As diagnostic variants, we considered only C5 and C4 variants that showed correlation with the phenotype. Consequently, 10% of the C5 and C4 variants were excluded. This exclusion encompassed examples such as heterozygous C5/C4 variants in a recessive gene. Clinical assessments were conducted before excluding the diagnostic role of the variant.

In one case, a C4 variant was reclassified as C3 during a multidisciplinary session following a reassessment of both literature evidence and the absence of the phenotype in our patients.

Overall, the yield resulted highest among patients with a clinical diagnosis of cystic kidney disease, with 49% diagnosed (183 positive cases), followed by tubulopathy with 32% (7), glomerulopathy 28% (52), CAKUT 13% (6) and nephrolithiasis/nephrocalcinosis 11% (4). No diagnostic variants were found in patients with unknown CKD and patients at risk with negative phenotype (Figure 19,20).

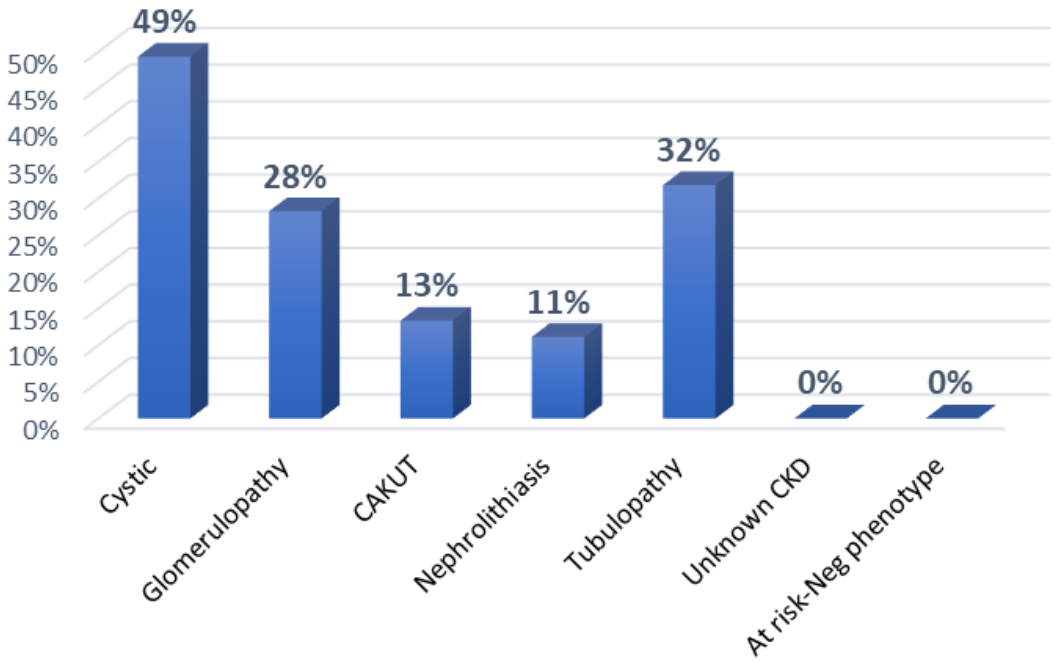


Figure 169. Distribution of diagnostic yield for each clinical presentation.

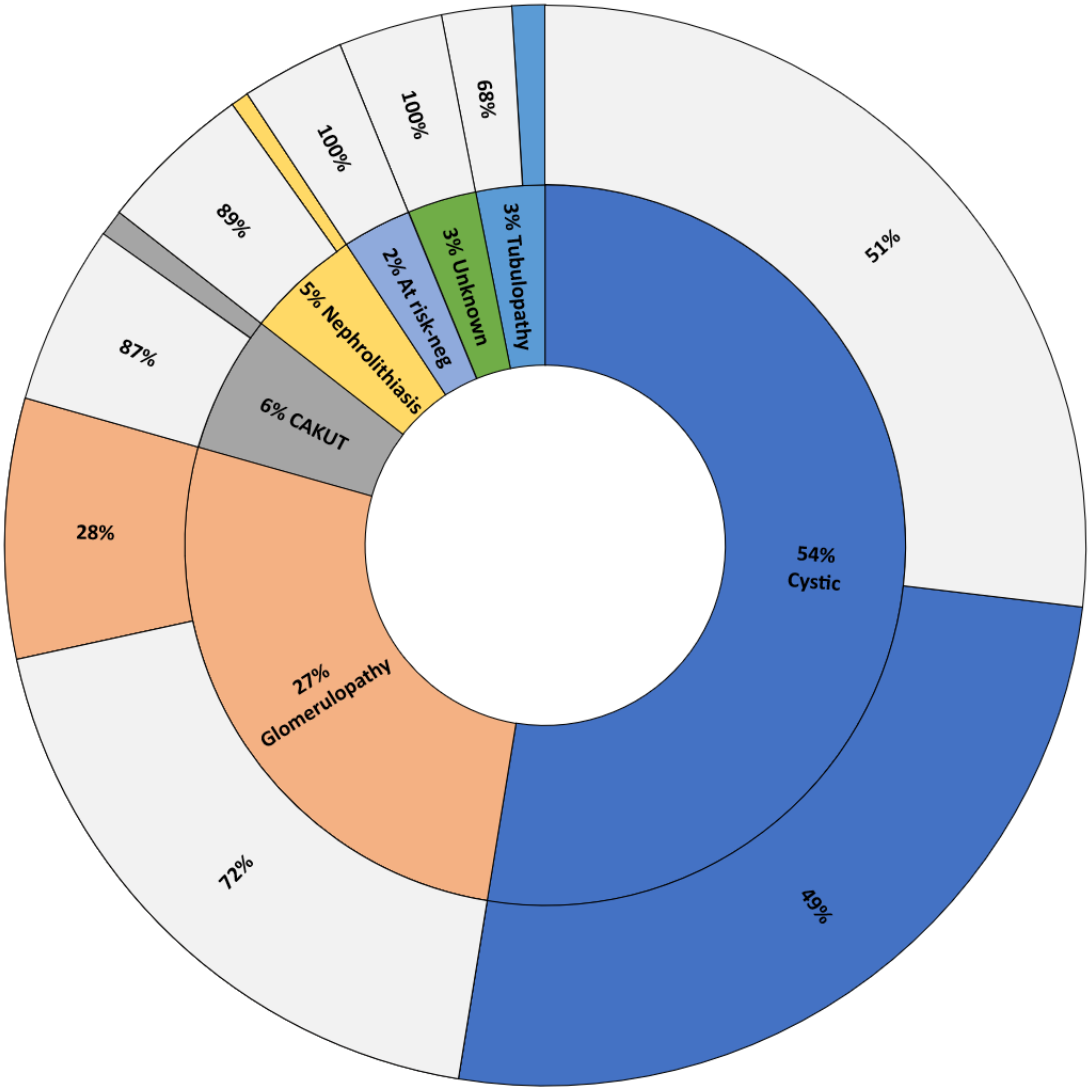


Figure 20. Chart of patient distribution and the diagnostic cases for each clinical presentation. In grey the non-diagnostic cases.

Therefore, we detected 252 diagnostic variants within our cohort of 692 subjects, resulting in an overall yield of 36%.

Among the 252 diagnostic variants identified, 153 were truncating variants (61%) and 99 non truncating variants (39%).

Nevertheless, some variants while correlated with the phenotype remain variants of uncertain significance by ACMG criteria. These variants, however, could be reevaluated if one or more conditions emerge, such as if they were reported as pathogenic by a reputable source in the literature (PP5) or a major segregation study was carried out (PP1). These variants are 9% of the total (Figure 21).

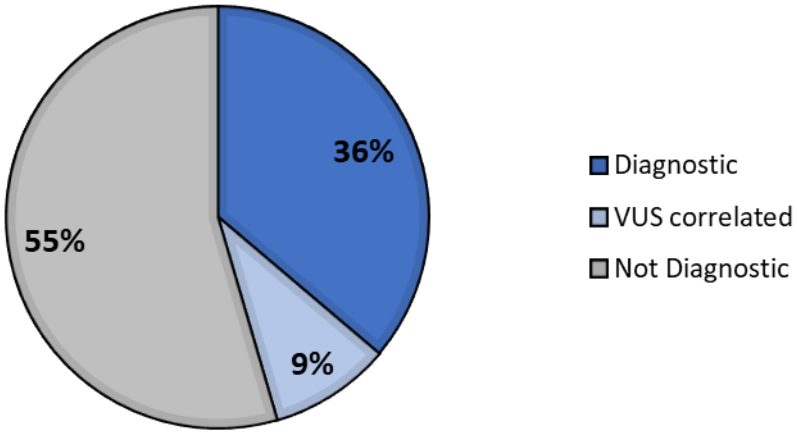


Figure 21. Distribution of the diagnostic and non-diagnostic variants. In blue the diagnostic variants, in light blue the variants of uncertain significance (VUS) correlated with phenotype and in grey the non-diagnostic variants.

Specifically, 55 variants (23%) classified C3 in the cystic disease category were evaluated as phenotype-related variants, mainly variants in PKD1.

Considering all variants related to the phenotype, in cystic disease and tubulopathy, variants classified as C5 predominate (43% and 71% respectively) while in the glomerulopathy and nephrocalcinosis categories, C4 variants prevail (54% and 75%).

4.3.1. DIAGNOSTIC YIELD AND GENETIC FINDINGS

In considering the diagnostic variants identified, 8 genes accounted for 95% of the diagnosis (Figure 22): PKD1 (47%), PKD2 (21%), COL4A5 (9%), COL4A3 (7%), COL4A4 (4%), PKHD1 (4%), SLC12A3 (2%), CYP24A1 (2%).

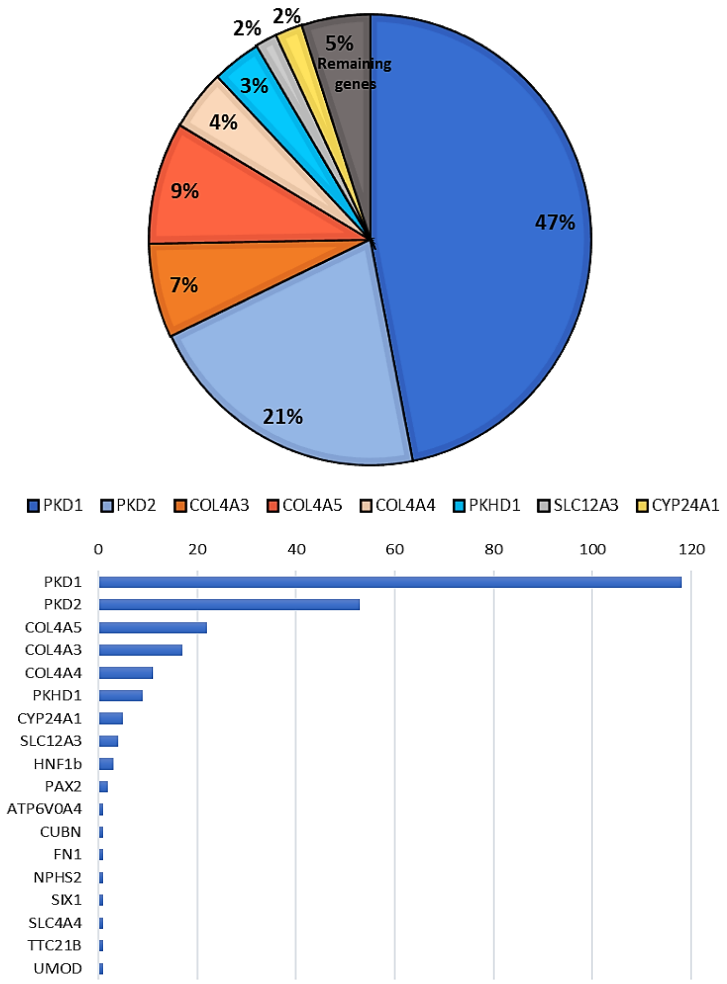


Figure 22. List of genes mainly related to definitive diagnosis.

The greatest diagnostic yield was within the cystic disease; variants in PKD1 and PKD2 accounted for 93% of the diagnostic findings in this category, therefore ADPKD was the diagnosis primarily identified in this group of patients.

The Alport syndrome was the pathology mainly identified in patients with a pattern of glomerulopathy, collagen IV genes COL4A3, COL4A4 and COL4A5 were identified in 93% of diagnoses.

The 50% of the CAKUT phenotype was due to diagnostic variants on HNF1b, followed by PAX2 and SIX1.

Nephrolithiasis and nephrocalcinosis in our cohort were 100% identified by diagnostic variants on the CYP24A1 gene, which although recessive, in the heterozygous form appears to give a mild phenotype.

Then in the tubulopathies we identified in 57% of cases a diagnosis of SLC12A3, mainly in the compound heterozygous form.

4.3.2. DISTRIBUTION OF DIAGNOSTIC YIELD IN THE CENTERS INVOLVED

We aimed to assess the distribution of the diagnostic yield in the 4 centers and the difference in the case mix (Figure 23). Modena and Granada presented all 7 clinical presentations, while Madrid had patients with a clinical presentation of cystic disease (90%), glomerulopathy and tubulopathy. The center of Parma collected data only from patients with cystic phenotypes.

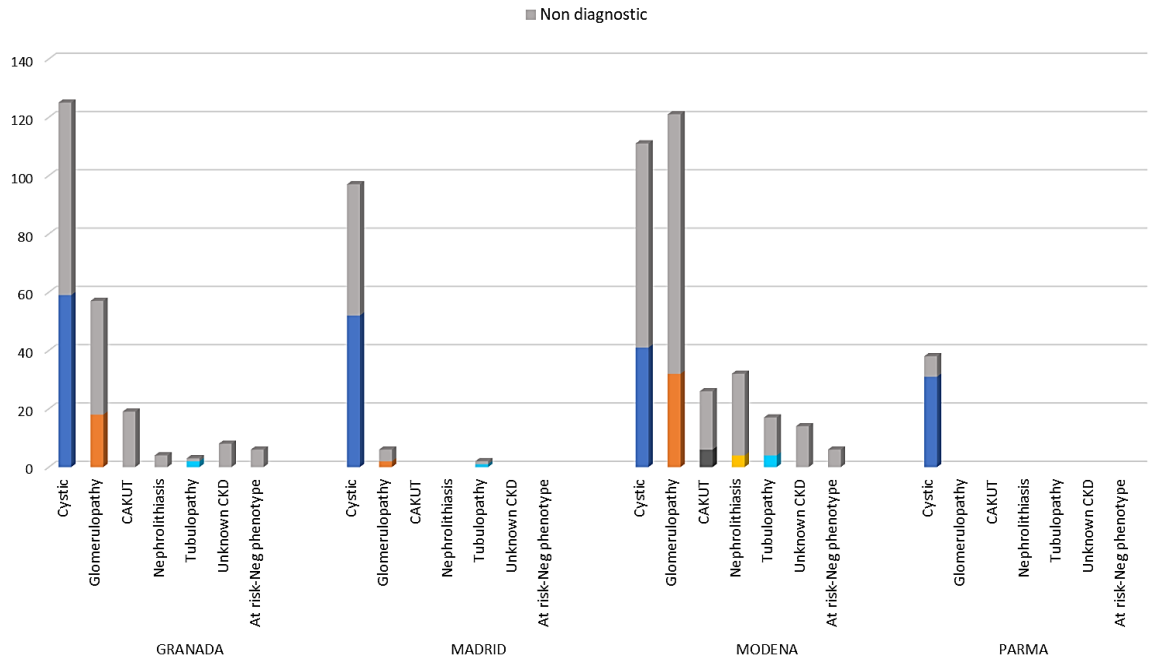


Figure 23. Distribution of the diagnostic yield in the 4 centers. Colors indicate the diagnostic cases, in grey the non-diagnostic.

The difference in case mix is related both to the population examined by the different centers as part of genetic counseling and to the centers' availability of other testing methods. For example, the Madrid center offers the WES method, which in some cases follows a preliminary panel analysis; in other cases, the patient undergoes WES directly.

The difference in diagnostic yield for patients with cystic phenotype in the 4 centers was statistically different (p.value<0.001). Parma showed a diagnostic yield of 82%, while the other three groups displayed a yield between 40 and 50%.

This difference may be related to the criteria for selecting patients for testing. In some centers, such as ours, in the presence of a clinical diagnosis of polycystic kidney, genetic testing is not performed unless it is needed for other clinical considerations (for example preimplantation diagnosis, living donation, prognostic evaluation for treatment, etc.). Instead, those who have an atypical cystic picture

and/or who do not have a family history of cystic kidney are genetically screened. This explains the relatively low diagnostic yield of a patient with cystic clinical presentation in our cohort. Because these patients are frequently phenotypically atypical cystic patients, they frequently result in PKD1-PKD2 negative cases. These cases often require enlarged specific cystic panels for differential diagnosis.

Furthermore, no diagnosis was found in the group of patients affected by CAKUT in the center of Granada, while in Modena 6 diagnoses on 26 patients (23%) were identified.

For tubulopathy disorder no comparison can be made since the small number of patients per center.

4.4. FURTHER INVESTIGATION AND ALGORITHM OPTIMIZATION

64% of patients were not diagnosed by NES panel investigation. In some cases, however, the center proceeded with additional investigations to genetically screen the disease (Table 6). In the Granada center it was possible to screen 6 patients with cystic phenotype for WES, of these only one patient benefited from the detection of an intronic variant on PKD1 (PKD1: c.10167+25_10167+43del).
On the other hand, 11 cases underwent pathology-specific extended panels. None of the patients with cystic phenotype reached a diagnosis, while two pathogenic variants were identified in one patient with FSGS and one patient with nephronophthisis, in INF2 (p.Glu220Lys) and NPHP1 (homozygous deletion), respectively.

Six cases of CAKUT were analyzed with a CGH array to detect possible large CNV. In only one case a variant, on TMEM231 (16q23.1 74601872_75887900), was found that could explain the phenotype.

Analysis	Clinical presentation	N°Test	Diagnostic	Non diagnostic
WES	Cystic disease	5	1 (PKD1 int)	4
Disease-specific panel	Cystic disease	8	0	8
	Nephronophthisis	1	1 (NPHP1)	0
	FSGS	2	1 (INF2)	1
Array CGH and Karyotype	CAKUT	6	1	5
GLA (Fabry)	Unknown CKD	3	0	3

Table 6. Additional investigations in negative cases considering the diagnostic and non-diagnostic results.

4.5. VARIANT DUPLICATION: AN EPIDEMIOLOGICAL EVALUATION OF VUS

C3 variants are challenging to evaluate, and only if there is significant familiarity or availability of functional studies it is possible to assess their pathogenicity.
According to the ACMG, if the variant is present at a significantly higher frequency in cases than in controls, with OR<5, the variant acquires a pathogenicity criterion such that the variant can be re-evaluated.
Thus, considering the large number of variants in our database, we evaluated the frequency of C3 variants that were correlated with the phenotype.
One variant was found repeated three times in the database, it was later found that the subjects belonged to the same family and that it was a hypomorphic variant.

From this analysis, we identified twice repeated variants in unrelated patients, 4 variants in PKD1, 1 in PKD2 and 1 in UMOD, in patients with cystic phenotype.
We then observed many duplicated, triplicated or even quadruplicated variants but that were not related to the phenotype. In particular, we observed that duplications of part of the gene or the entire CLCNKB gene are very frequent, with 16 cases in Modena and one case in the Granada cohort.
While considering the variants with a frequency greater than one in our cohort we found the variant COL4A3:p.Leu1474Pro, was recently described as hypomorphic (39). This variant in our cohort is reported as phenotype-related (Syndrome of Alport) in six cases while it is not in one case of nephrolithiasis. The variant is present in one case in compound heterozygosity.

This analysis did not provide statistically relevant data but did allow us to identify variants that are more likely to have a pathogenic role than variants, on the other hand, that are very frequent and unrelated to the phenotype. Valuable information at the time of referral and for a program of revaluation of the pathogenicity of these VUS.

4.6. SURVIVAL ANALYSIS

Kaplan Meier analysis was performed to evaluate renal survival in the seven clinical presentations. Renal function was correlated with the genetic test result, in particular, pathogenic variants (C4 and C5), variants of uncertain significance (C3), and cases of non-detectable mutation (NMD) were considered (Table 7). To evaluate the renal survival, the age of kidney failure was considered. We considered all the variants found, regardless of the correlation with phenotype.

Two categories, tubulopathy and patients at risk with negative phenotype, were excluded for the absence of kidney failure events, as well as nephrolithiasis because a renal failure occurred only in 4 patients.

Figure 24 showed the survival analysis for cystic disease, glomerulopathy, CAKUT and unknown CKD.

Cystic disease is the only category in which a statistical difference in renal function trends is observed by considering genetic test results. Patients with pathogenic variants (C5, C4) showed a worse prognosis compared to other test results (log rank=19.2, p.value=0.001).

In the glomerulopathy, there is no statistical difference between the three survival curves. They have a similar trend although the presence of pathogenic variants suggests a tendency to a worse prognosis (log rank=5.5, p.value=0.06).

In CAKUT and unknown CKD, the presence of pathogenic variants didn't lead to a worse prognosis (CAKUT: log rank=1.8, p.value=0.4; uCKD: log rank=3.1, p.value=0.2). However, in these cases, the pathogenic variants are very limited, with many genes not detected by the panel.

Clinical presentation	ACMG	N°patients	N° of kidney failure
Cystic	C3	81	10 (12%)
	C4+C5	139	27 (19%)
	NMD	53	3 (6%)
Glomerulopathy	C3	81	20 (25%)
	C4+C5	59	18 (31%)
	NMD	38	7 (18%)
CAKUT	C3	28	3 (11%)
	C4+C5	6	1 (17%)
	NMD	11	4 (36%)
Unknown	C3	10	7 (70%)
	C4+C5	3	1 (33%)
	NMD	9	6 (67%)

Table 7. Number and percentage of kidney failure regarding the ACMG classification, for each clinical presentation. NMD non-detectable mutation.

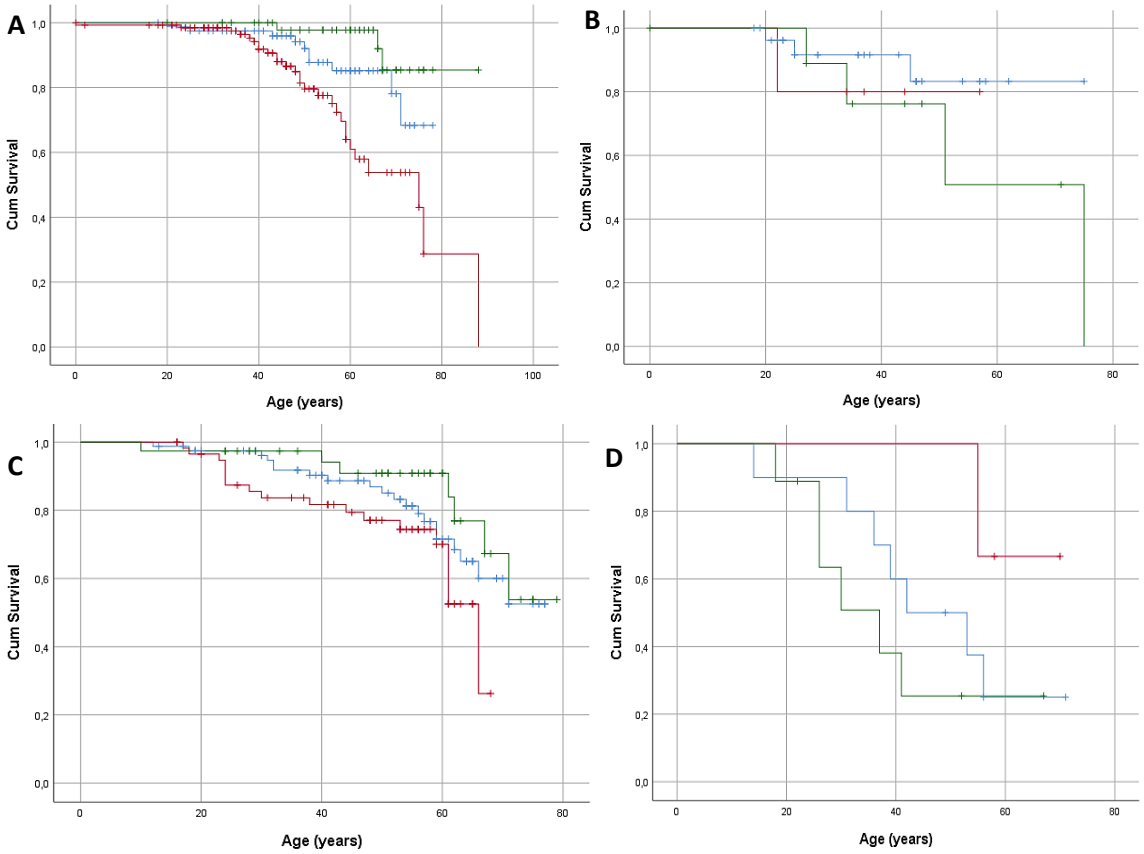
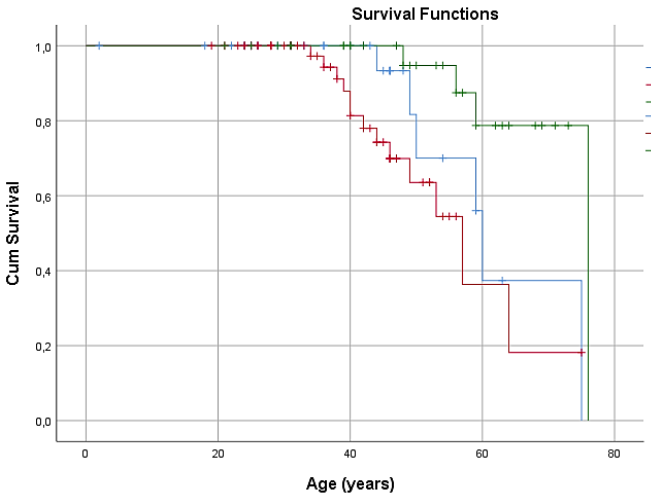


Figure 24. Kaplan Meier analysis of the renal survival. In red for C4+C5 variants, in blue for C3 and in green for the non-detectable mutations (NMD). A. Cystic disease, B. Glomerulopathy, C. CAKUT, D. Unknown CKD.

We then considered the two diseases most represented in our cohort and assessed the correlation between genes and phenotype.

For this analysis, we considered only diagnostic variants that lead to a definite diagnosis of ADPKD (Figure 25) or Alport syndrome (Figure 26).

In ADPKD, the percentage of patients in KF was higher in cases of the truncating variant in PKD1 (in red) compared to non-truncating PKD1 and variants in PKD2, with a log-rank of 11.7 and p-value=0.003.

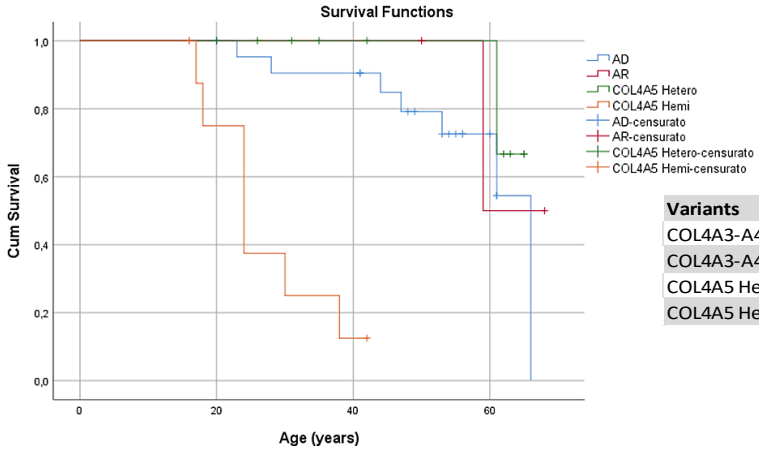


Variants	N°Patients	N°of kidney failure
PKD1 NT	31	6 (19%)
PKD1 T	55	13 (24%)
PKD2	30	4 (13%)

Figure 25. Kaplan Meier analysis of the renal survival. In red for PKD1 truncating variants, in blue for PKD1 non truncating variants and in green for PKD2 variants.

For Alport syndrome, we looked at the survival function for patients with COL4A5 variants in hemizygous (male) or heterozygous form (female) and patients with COL4A3 and COL4A4 in dominant or recessive form. Males with COL4A5 showed the worse prognosis, with 78% of patients already in KF at the time of the genetic test (log rank=34.6, p-value=0.0001). No statistical differences are observed between the forms of AD and AR. However, the forms in AD show a worse trend.

Surprisingly patients with a homozygous or compound heterozygous condition in the autosomal recessive form had a trend similar to COL4A5 in heterozygosity. However, only three patients with this genetic condition are included.



Variants	N°Patients	N°of kidney failure
COL4A3-A4 AD	22	7 (32%)
COL4A3-A4 AR	3	1 (33%)
COL4A5 Hetero	11	2 (18%)
COL4A5 Hemi	9	7 (78%)

Figure 26. Kaplan Meier analysis of the renal survival. In orange for COL4A5 in hemizygosity, in green for COL4A5 in heterozygosity, in blue for autosomal dominant forms, and in red for autosomal recessive.

4.7. CLINICAL PREDICTORS OF DIAGNOSTIC YIELD

Employing the collected data, we assessed which clinical factors were predictors of a genetic diagnosis (Table 8, Figure 27). Through univariate analysis, we identified 4 predictors of diagnosis in our cohort: family history (OR 4.7), early onset of the disease (OR 2.2), kidney failure (OR 1.6) and clinical presentation (OR 6.2 for cystic disease when compared to nephrolithiasis).

No differences emerged for the gender category.

The Table 8 shows the strength of the association of clinical factors with achieving a definitive genetic diagnosis.

	Odds Ratio	95% CI	p.value
Gender	0.85	0.60-1.19	0.34
Early onset	2.22	1.49-3.31	0.00
ESRD	1.63	1.06-2.49	0.02
Family History	4.74	3.18-7.21	0.00
Clinical diagnosis			
Cystic disease	6.21	2.34-21.48	0.00
Glomerulopathy	2.78	1.01-9.80	0.07
CAKUT	0.90	0.22-3.95	0.89
Nephrolithiasis	reference	reference	reference
Tubulopathy	2.95	0.66-14.05	0.15
uCKD	nd		
Negative Phenotype	nd		

Table 8. Prediction factors of a genetic diagnosis. For the clinical presentation, the nephrolithiasis was considered as reference.

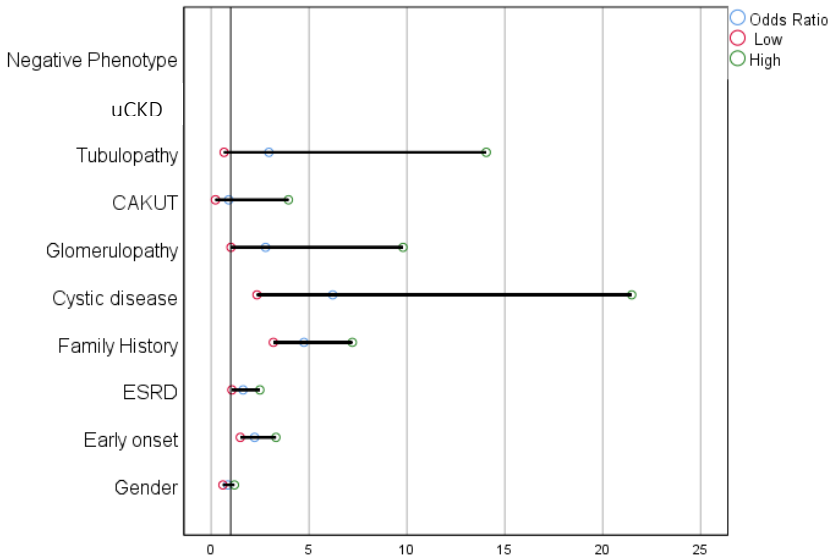


Figure 27. Odds ratio for each factor in blue. In red (low) and in green (high) the 95% of confidence interval.

Then we employed a machine learning approach to define the sensitivity and specificity of the test and again identify what the predisposing factors were.

The training set was on 485 cases and the test set on 208 cases.

We elaborated a ROC (Receiver Operating Characteristics) curve that plot the true positive rate (sensitivity) and the false positive rate (1-specificity) to evaluate the model performance in distinguishing patients with diagnostic results and patients without.

We considered the model with the best AUC (area under the curve), in this case the logistic regression, with an AUC of 0.78, the sensitivity of 0.46 and precision of 0.65 (Table 9).

	Model	Accuracy	AUC	Recall	Prec.	F1	Kappa	MCC	TT (Sec)
lr	Logistic Regression	0.7136	0.7816	0.4569	0.6484	0.5305	0.3367	0.3491	0.9750
lda	Linear Discriminant Analysis	0.6991	0.7783	0.4624	0.6131	0.5231	0.3119	0.3201	0.0320
gbc	Gradient Boosting Classifier	0.7153	0.7716	0.6477	0.6024	0.6229	0.3954	0.3971	0.1340
catboost	CatBoost Classifier	0.7176	0.7708	0.6088	0.6149	0.6080	0.3886	0.3911	2.3420
nb	Naive Bayes	0.6907	0.7687	0.7225	0.5577	0.6281	0.3713	0.3821	0.1150
lightgbm	Light Gradient Boosting Machine	0.7091	0.7633	0.6366	0.5946	0.6125	0.3807	0.3834	0.4770
qda	Quadratic Discriminant Analysis	0.6969	0.7507	0.7284	0.5639	0.6340	0.3827	0.3940	0.0620
rf	Random Forest Classifier	0.6929	0.7488	0.6088	0.5788	0.5883	0.3456	0.3493	0.6000
ada	Ada Boost Classifier	0.7010	0.7438	0.6366	0.5822	0.6065	0.3669	0.3691	0.2260
et	Extra Trees Classifier	0.6908	0.7267	0.6088	0.5776	0.5887	0.3429	0.3463	0.2050
dt	Decision Tree Classifier	0.6638	0.6620	0.6366	0.5326	0.5768	0.3026	0.3088	0.1210
knn	K Neighbors Classifier	0.6511	0.6427	0.4928	0.5241	0.5013	0.2355	0.2398	0.1590
dummy	Dummy Classifier	0.6372	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0530
svm	SVM - Linear Kernel	0.6100	0.0000	0.4150	0.3858	0.3479	0.1255	0.1346	0.0980
ridge	Ridge Classifier	0.7074	0.0000	0.4624	0.6331	0.5296	0.3272	0.3377	0.0990

Table 9. Possible models to adopt for calculating the area under the curve, the precision and the recall (sensitivity).

According to the F1 metric (that expresses a good compromise between precision and recall) we choose a threshold (t_f) of 0.32. T_f corresponds to the highest value of f_1 (Figure 28).

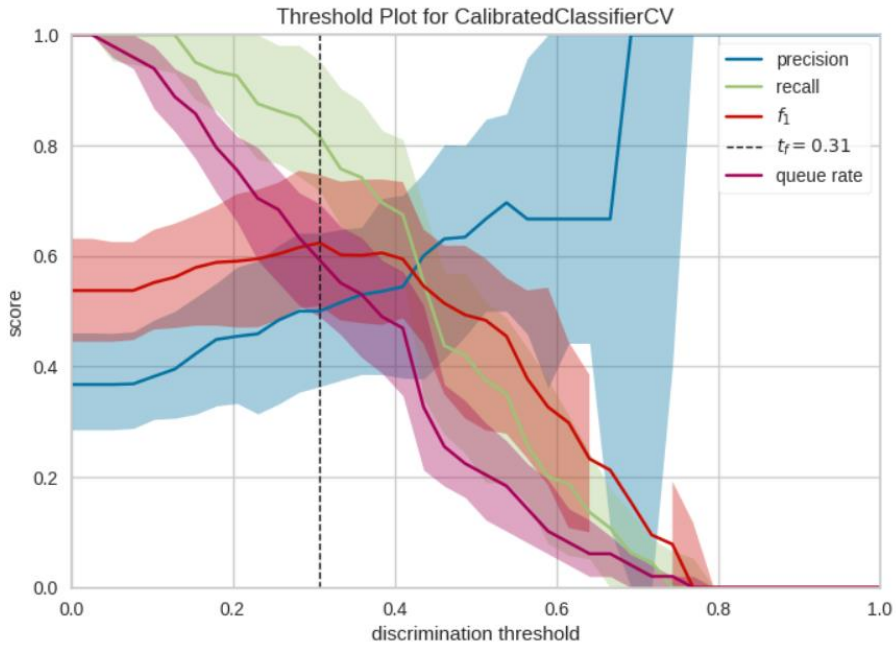


Figure 28. Identification of threshold (0.32), as the highest value of F1.

In the test set (208 cases) the model correctly predicted 54 cases, 44 incorrectly predicted as diagnostic, while in 22 cases didn't predict a diagnostic case (Table 10). Otherwise in 88 cases correctly predicted a non-diagnostic test. The true positive rate (TPR) of the test was 71% and the true negative rate (TNR) was 66%.

		Predicted label		
		0	**1**	
Diagnostic label	**0**	88	44	132
	1	22	54	76
		110	98	208

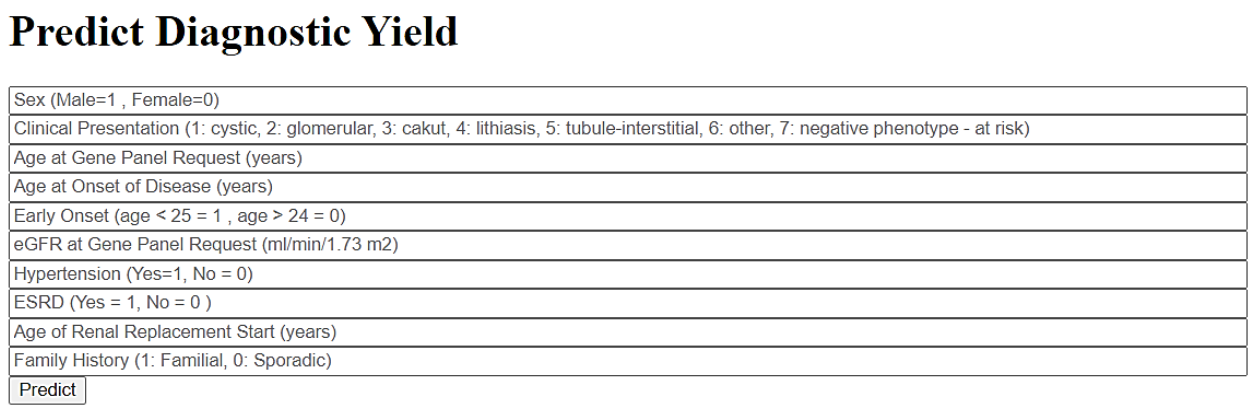
Table 10. Diagnostic label versus predicted label for the test set.

It is pivotal to identify under which conditions the algorithm incorrectly predicts a non-diagnostic test in understanding what is the best trade-off to keep between precision and recall. We then extracted the model's prediction data and analyzed the cases in which the test result was diagnostic but predicted to be negative. We observed that the cases in which the test was not predicted as positive were mainly cases in which there was no familiarity or cases in which familiarity was present, but the patient had a high onset age and a good eGFR at the time of the test. In particular,

the 50% of cases were without a family history, such as all the cystic patients (6 cases) with a pathogenic variant in PKD1. For the clinical presentation of glomerulopathy, the undetected diagnoses (10 cases) were all Alport syndrome The latter case, for example occurs in cases of Alport AD.

In order to employ these considerations in clinical practice, we developed an algorithm with the model-defined predictive features of diagnostic yield.

The algorithm allows input of the patient's clinical data, such as sex, age, filtrate, kidney failure, and predicts what is the probability of getting a diagnosis (Figure 29).



Sex	1 (male)
Clinical presentation	2 (glomerulopathy)
Age of Exam	42
Age at onset	41
Early onset	0
eGFR	104
Hypertension	1 (yes)
ESRD	0 (no)
Family History	1 (yes)

Prediction label=1

Prediction score= 0.40

Threshold=0.32

Figure 29. Example of the algorithm's operation. A prediction score of 0.40 results in a prediction label of 1.

The algorithm gives a prediction score as a result. If this parameter is more than the threshold fixed, the prediction label is 1 and the genetic test could be predictive of a definitive diagnosis.

4.8. CLINICAL IMPACT OF THE GENETIC TEST

A genetic diagnosis can potentially carry multiple clinical impacts, including the identification of a definitive diagnosis for the patient, tailoring treatment, family counseling implications in both transmission risk and preimplantation diagnosis.

In our cohort, the clinical impact was evaluated in the 82 Modena patients who received a diagnosis through genetic testing with NES panel.

The suspected clinical diagnosis was confirmed in 70% of the 82 patients, a diagnosis was defined in 23% of patients and in 7% of patients the genetic diagnosis changed the clinical suspect (Figure 30).

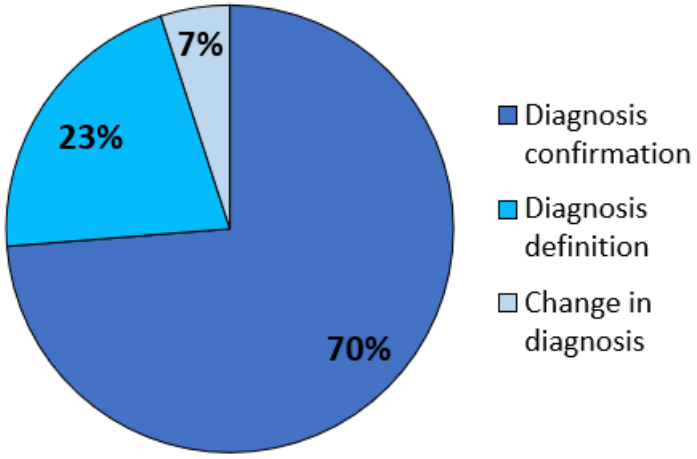


Figure 30. Pie chart of the definitive diagnosis: confirmed, defined and changed.

In the 57 patients who experienced confirmation of clinical suspicion, the phenotype included a cystic disease, validated by variants in PKD1, PKD2, or PKHD1. Additionally, there were suspicions of Alport syndrome characterized by proteinuria, microhematuria, and hearing loss, or suspicions of Gitelman syndrome confirmed by compound heterozygosity variants in SLC12A3 (Figure 28).

Regarding the 19 cases in which a diagnosis was defined, 12 received an Alport diagnosis. These patients had either an undefined histologic picture or had achieved KF without a definitive diagnosis. These diagnoses had relevant implications for the family as well, with at least 10 subjects with Alport syndrome being identified without the need for biopsy and young female subjects with COL4A5 variants at risk of transmission to a possible son.

Furthermore, in one patient with three inconclusive biopsies and relevant familiar history, a frameshift on HNF1b was found with a final diagnosis of HNF1b-ADTKD.

Surprisingly, in 6 cases, genetic diagnosis changed the initial clinical suspicion:

1. Patient, with a significant family history of KF, with an initial diagnosis of IgAN, resistant to steroid therapy was reclassified as affected by Alport Syndrome. This diagnosis is led to the exclusion of the patient from an IgAN clinical trial focused on a complement inhibitor;
2. Patient with a family history of interstitial nephritis progressed to KF, was reclassified as affected by Alport Syndrome, with extension of that diagnosis to other family members;
3. Young patient in ESKD with CKD of unknown origin (small kidneys), showed homozygosity on TTC21B;
4. Patient showing glomerulosclerosis, with significant family history of KF with evidence of mesangial glomerulonephritis and multicystic kidney phenotypes. Patient and her family reported a pathogenic variant in PAX2. The diagnosis of Renal Coloboma Syndrome led to the identification of coloboma in one apparently negative subject of the family and renal syndrome in a newborn with growth failure.
5. Patient with histological diagnosis of tubulopathy and important family history of KF, reported a variant in HNF1b. This diagnosis had implication for clinical follow up of the entire family, especially for the management of diabetes and hypomagnesemia risk.
6. Patients with late referral and multicystic kidney. The genetic test identified two variants in NPHS2 (gene deletion + missense variant). The multicystic diagnosis was probably related to a misinterpretation of the acquired cysts in ESKD.

A Sankey diagram (Figure 31) represents the primary diagnosis of the 82 patients that achieved a definitive diagnosis, such as ADPKD and Alport syndrome, and the reclassification of the phenotype.

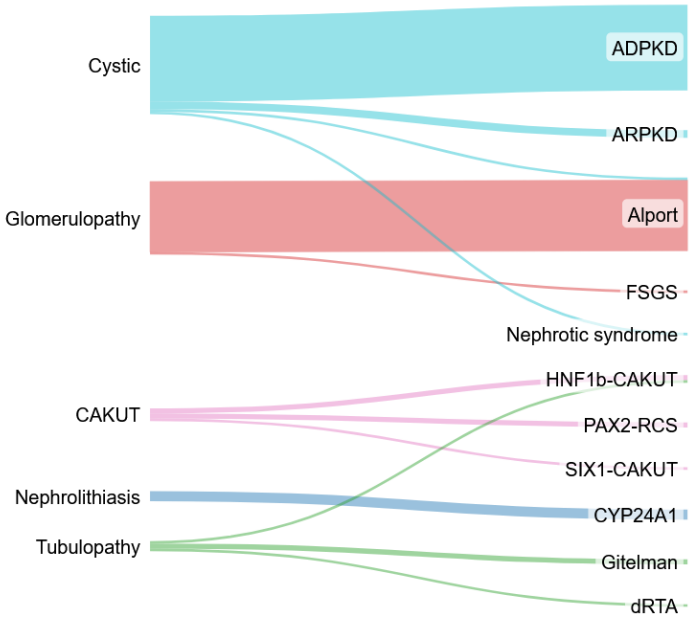


Figure 31. A Sankey diagram for clinical diagnosis is on the left and genetic diagnosis is on the right. FSGS, Focal Segmental Glomerulosclerosis; RCS, Renal Coloboma Syndrome; dRTA, distal Renal Tubular Acidosis.

For the 82 patients of our cohort, we assessed the potential implications in clinical management (Figure 32). In the majority of cases, 77%, the diagnosis led to indications about the clinical follow-up and surveillance, such as for diabetes (HNF1b), aneurysm (ADPKD), coloboma (PAX2) and hearing loss with two indications of cochlear implants (COL4A5 and ATP6V0A4).

In 18 cases the patients were eligible to be included in a national registry of the disease and 6 cases were evaluated for enrollment in clinical trials.

In 10 cases a diagnostic finding has avoided unnecessary renal biopsy and futile immunosuppressive treatments.

Then in 3 cases the diagnosis implied evaluation by living familiar transplantation and in 6 cases reproductive counseling was conducted.

To conclude, in 4 cases a change in the disease treatment was applied: ACE inhibitor addition, assessment for dapagliflozin in a patient with Alport, exclusion of a patient with Alport from a clinical trial for IgAN and treatment for urinary acidosis in a patient with ATP6V0A4 pathogenic variant.

However, in several cases, tailored therapy was not possible because the disease was at an advanced stage. However, the family members will still benefit from these findings.

Indeed, in 21 cases (26%), family members were also studied.

The familial variant was found in two apparently negative subjects. In one of these cases, the subject had a child born with renal impairments due to the pathogenic variant of the family (21).

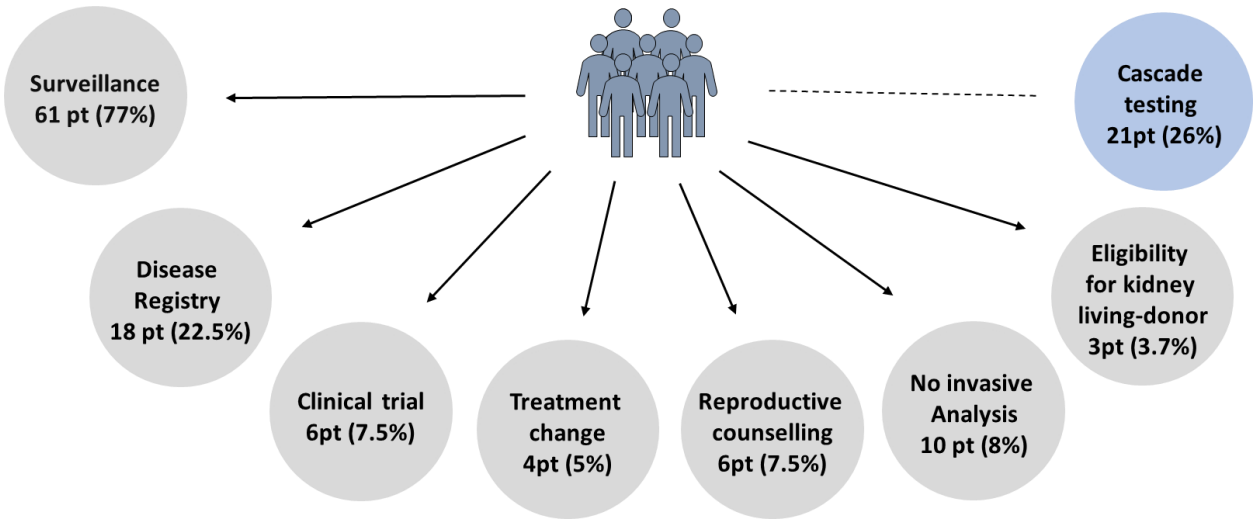


Figure 32. Clinical management for the 82 patients that received a definitive genetic diagnosis.

5. DISCUSSION

20% of CKD cases miss a known etiology. The application of a genomic approach holds the potential to uncover the CKD etiology in a relevant portion of pediatric and adult patients (5-30%). However, there is a lack of consensus in the scientific community on the optimal diagnostic algorithm.

Targeted panels allow rapid, cost-effective analysis, but an update with new findings requires a redesign and re-validation. On the other hand, WES and WGS allow broad-spectrum screening even in unclear clinical suspicion. However, these methods require significant computational burden, expertise in VUS analysis, and carry ethical implications related to secondary findings, variants unrelated to the primary clinical indication. These features pose limitations to the widespread adoption of these approaches in clinical routine.

In this perspective, we conducted a retrospective European study to assess the diagnostic yield of a Nephropathy panel covering 44 genes, employed in a routine setting.

A total of 809 patients underwent NES panel from 2017 to 2023, for suspected genetic kidney disease. The data analysis was conducted on 692 index cases.

The leading clinical presentation was cystic kidney disease with 371 patients, followed by glomerulopathy with 184 patients, CAKUT with 45, nephrolithiasis/nephrocalcinosis with 36, tubulopathy with 22, 22 patients with undefined cause of CKD and the other 12 patients showed a negative renal phenotype but they were at risk of disease development.

The younger group was CAKUT with a median age at exam execution of 37 years old (IQR 28-47) and the age of onset of 23 (IQR 3-41).

The patients with the most advanced chronic kidney disease at presentation are from the uCKD group, who have lower eGFR, 42ml/min (IQR 18-50), a higher percentage of hypertension (83%) and high rate of kidney failure events (64%) in the follow-up.

Family history was available in 539 cases of which 310 (57%) showed a positive history of kidney disease. A higher percentage compared to the literature, is estimated to 30% in patients with kidney disease (12). This is probably related to the selection bias of these patients who were referred to a tertiary-level outpatient clinic devoted to patients with a suspected genetic condition or to genetic services.

A total of 1016 variants were collected during the project. In particular, 14.6% of the variants were classified as C5 for ACMG guidelines (95), 15.3% as C4 and the other 70.1% as C3. Many of the variants were found in heterozygosity, except for 6 cases of compound heterozygosity and 3 cases of homozygosity.

Overall, 252 variants were classified as diagnostic, for a total range of 36%. Additionally, 9% of the variants were classified as C3 correlated to the phenotype, these variants may be re-classified if future evidence will emerge.

The 36% diagnostic yield appears to be in line with the literature. The diagnostic rate for a disease-specific target panel applied to an adult population is reported to be from 7 to 22% (98-106) while considering broader non-specific panels the percentage is between 12 and 50% (107-113).

In our cohort, cystic kidney disease had the highest yield (49%, 183 positive cases), followed by tubulopathy (32%, 7), glomerulopathy (28%, 52), CAKUT (13%, 6) and nephrolithiasis (11%, 4). No diagnostic variants were found in cases of unknown CKD or for patients at risk but with negative phenotypes.

Eight genes accounted for 95% of the diagnosis: PKD1 (47%), PKD2 (21%), COL4A5 (9%), COL4A3 (7%), COL4A4 (4%), PKHD1 (4%), SLC12A3 (2%), CYP24A1 (2%). The same genes were reported as most frequently associated with genetic kidney disease, especially in an adult population by the available literature as well. In the study by Groopman et al (114) conducted on about 3315 subjects using WES, 60% of the pathogenic variants were detected in the first 5 genes we identified (PKD1, PKD2, COL4A5, COL4A4 and COL4A3). In this study, they reported that the diagnoses were mainly related to the following genes: PKD1 (24%), COL4A5 (14%), COL4A3 (8.6%), PKD2 (7%), COL4A4 (6.7%), UMOD (3%), TRPC6 (2.5%), INF2 (2%).

The greatest diagnostic yield was within the cystic disease; variants in PKD1 and PKD2 accounted for 93% of the diagnostic findings in this category, therefore ADPKD was the diagnosis primarily identified in this group of patients.

Alport syndrome was the pathology mainly recognized in patients with a pattern of glomerulopathy, collagen IV genes COL4A3, COL4A4 and COL4A5 were identified in 93% of diagnoses.

Thus, this targeted approach may constitute an effective first-tier analysis for patients with a specific clinical presentation, such as ADPKD or Alport syndrome suspicion.

Considering the prognosis of patients, the study showed that the presence of pathogenic variants results in a statistically significant worse prognosis only in the population with cystic phenotype. In patients with genetically confirmed ADPKD, the presence of a truncating variant in PKD1 leads to a worse prognosis than other pathogenic variants, as did the detection of a hemizygous variant in COL4A5 for Alport syndrome. The same findings were reported by Pei et al (19) in a study conducted on 220 patients affected by ADPKD and by Savige et al (39) for patients with Alport syndrome. The latter reported a similar trend in hemizygous and autosomal recessive patients, in contrast to what we observed in our study. The reason for this apparent divergence requires further investigations, but the small number of cases in our cohort could have a role.

The diagnostic results confirmed a previous suspected clinical diagnosis in 70% of cases, defined the diagnosis in 23%, and modified the previous clinical orientation in 7%. The reclassification of the initial clinical diagnosis after the test was reported in the literature with a range from 10 to 45% (101, 112, 114, 115).

The identification of a diagnosis or the confirmation of a clinical suspicion can have a large clinical impact, including tailoring treatment, surveillance and family counseling implications in both transmission risk and pre-implantation diagnosis. In our cohort, the 77% of patients have been addressed to proper surveillance and follow-up, the 7% were enrolled in a clinical trial and 22% included in disease registries. For the 26% of patients, the family members were studied with cascade testing. This allowed a prompter indication of diagnosis avoiding additional investigations such as a renal biopsy, as well as providing eligibility assessment for living donations in the same family.

Familial counseling permitted the screening of two apparently negative subjects, which showed familial pathogenic variants. In one of these cases, the subject had a child born with renal impairments due to the PAX2 pathogenic variant of the family (21).

Although 36% of the patients have received a definitive diagnosis, 64% remain undiagnosed. Further investigations (WES, targeted panels, CGH-array) were conducted in 25 patients and only 3 cases received a definitive diagnosis (12%). The epidemiological assessment of C3 variants' frequency didn't provide statistically relevant data due to an absence of higher frequency in cases than in controls.

We have therefore developed using a machine learning approach, an algorithm based on the predictors of diagnosis to provide in which clinical contexts the employment of this approach results most appropriate. The family history and the cystic phenotype showed a greater impact in achieving a definitive diagnosis. A prediction value higher than the threshold (0.32) indicates more likely that the test is diagnostic.

Nevertheless, considering a threshold > 0.32 the precision of the test increases but the recall decreases. Surely, increasing the precision, most of the tests that will be performed will have a positive result, reducing the number of tests that need to be performed and thus no less importantly the costs to the health care system. But by increasing precision, it decreases recall and it is possible that diagnostic tests could be predicted as negative.
The algorithm should only be an indication for the clinician to consider whether to propose the patient for genetic testing via NES panel or consider other types of strategies such as WES.

In addition to developing an algorithm to maximize the potential of the panel, we are also evaluating alternative strategies to provide an answer for those cases that are still not diagnostic.

In particular, we have submitted a national proposal to be allowed to screen WGS patients who tested negative for NES but with a strong suspicion of genetic disease. We will prioritize the patients we screen based on family history and early onset.

Briefly, the NES panel certainly achieved a good diagnostic goal, aligned with the diagnostic yield reported in the literature in the field of genetic kidney disease.
It is a targeted panel, with 44 genes, but it covers the diagnosis for the most frequent diseases, such as ADPKD and Alport.
It allows for a rapid approach, avoiding the computational burden due to WES and WGS analysis and a high depth of call (200x at least). It is also able to identify CNVs (3.5% of the variants identified), outperforming Sanger technology for PKD1 analysis.
Therefore, it could be considered a valuable first-tier tool for routine clinical approaches.

A reassessment of the panel from an epidemiological perspective could probably improve its application and performance as a first screening approach, without the need for infrastructure and laboratories capable of coping with high amounts of data and a large number of variants of uncertain significance.

6. CONCLUSION

Identifying a diagnosis in the context of kidney disease is pivotal to providing targeted therapy, new treatment options with enrollment to clinical trials, and family counseling at the same time as avoiding invasive investigations and futile treatments.

Currently, methods are available that allow increasingly advanced investigations, from disease-specific panels to WGS with long-reads technology. However, higher costs and computational burden make it difficult to deploy these technologies as routine methods.

In this study we assessed the diagnostic yield of a panel with a limited number of genes related to the principal genetic kidney diseases; the clinical presentations covered were cystic diseases, glomerulopathy, CAKUT, nephrolithiasis, tubulopathy and CKD of unknown origin.

The diagnostic yield was 36% with the highest rate in cystic diseases (49%). The yield was comparable to the average of the diagnostic sensitivities reported in the literature, both for panels and WES methods. A comparison with WGS studies is difficult because this approach is still rare in this specialty. One article reports an analysis in WGS on a polycystic population. Mallawaarachchi et al.[116] report that the diagnostic yield was 80% in this population. A similar yield was reported by Bullich et al. [117] in a cohort of clinically ADPKD patients, screened with a panel of 140 genes. No studies were conducted on non-disease-specific populations, in the field of kidney diseases.

In our cohort, the primary diagnosed diseases were ADPKD and Alport; indeed, we observed that the polycystic and collagen IV genes accounted for the 87% of the diagnosis.

To increase the panel precision, we elaborated an algorithm based on the predictive factors of diagnosis. This algorithm has been experimentally implemented at the center of Modena and it will be shared with the other centers involved.

Although the patients in our cohort have been well clinically characterized and have a higher percentage of positive family history than reported in the literature for CKD, 64% remain undiagnosed.

In the effort to identify new strategies to improve our diagnostic capability, we aim to explore the role of WGS analysis. The proposal, named ORIENTING, aims to undergo WGS for 100 patients with important family history and early onset of disease. This screening could improve diagnostic capabilities, pivotal in advancing personalized therapy and understanding of genetic kidney diseases.

7. BIBLIOGRAPHY

1. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. PLoS One. 2016;11(7):e0158765.
2. Cockwell P, Fisher LA. The global burden of chronic kidney disease. Lancet. 2020;395(10225):662-4.
3. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney-international; 2013.
4. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. Lancet. 2015;385(9981):1975-82.
5. Bello AK, Levin A, Lunney M, Osman MA, Ye F, Ashuntantang G, et al. Global Kidney Health Atlas: A report by the International Society of Nephrology on the Global Burden of End-stage Kidney Disease and Capacity for Kidney Replacement Therapy and Conservative Care across World Countries and Regions. 2019.
6. Nordio M, Limido A, Maggiore U, Nichelatti M, Postorino M, Quintaliani G, et al. Survival in patients treated by long-term dialysis compared with the general population. Am J Kidney Dis. 2012;59(6):819-28.
7. Sundström J, Bodegard J, Bollmann A, Vervloet MG, Mark PB, Karasik A, et al. Prevalence, outcomes, and cost of chronic kidney disease in a contemporary population of 2·4 million patients from 11 countries: The CaReMe CKD study. Lancet Reg Health Eur. 2022;20:100438.
8. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet. 2013;382(9888):260-72.
9. Neild GH. Primary renal disease in young adults with renal failure. Nephrol Dial Transplant. 2010;25(4):1025-32.
10. Torra R, Furlano M, Ortiz A, Ars E. Genetic kidney diseases as an underrecognized cause of chronic kidney disease: the key role of international registry reports. Clin Kidney J. 2021;14(8):1879-85.
11. Devuyst O, Knoers NV, Remuzzi G, Schaefer F, Association BotWGfIKDotERAaEDaT. Rare inherited kidney diseases: challenges, opportunities, and perspectives. Lancet. 2014;383(9931):1844-59.
12. Connaughton DM, Bukhari S, Conlon P, Cassidy E, O'Toole M, Mohamad M, et al. The Irish Kidney Gene Project--Prevalence of Family History in Patients with Kidney Disease in Ireland. Nephron. 2015;130(4):293-301.
13. Freedman BI, Volkova NV, Satko SG, Krisher J, Jurkovitz C, Soucie JM, et al. Population-based screening for family history of end-stage renal disease among incident dialysis patients. Am J Nephrol. 2005;25(6):529-35.
14. Connaughton DM, Hildebrandt F. Personalized medicine in chronic kidney disease by detection of monogenic mutations. Nephrol Dial Transplant. 2020;35(3):390-7.
15. Rasouly HM, Groopman EE, Heyman-Kantor R, Fasel DA, Mitrotti A, Westland R, et al. The Burden of Candidate Pathogenic Variants for Kidney and Genitourinary Disorders Emerging From Exome Sequencing. Ann Intern Med. 2019;170(1):11-21.
16. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. Nat Rev Nephrol. 2016;12(3):133-46.
17. Knoers N, Antignac C, Bergmann C, Dahan K, Giglio S, Heidet L, et al. Genetic testing in the diagnosis of chronic kidney disease: recommendations for clinical practice. Nephrol Dial Transplant. 2022;37(2):239-54.
18. Atlas Khan , Ning Shang J, Nestor G, , Chunhua Wen[g] , Hripcsak G, et al. Polygenic risk affects the penetrance of monogenic kidney disease. medRxiv. 2023.
19. Hwang YH, Conklin J, Chan W, Roslin NM, Liu J, He N, et al. Refining Genotype-Phenotype Correlation in Autosomal Dominant Polycystic Kidney Disease. J Am Soc Nephrol. 2016;27(6):1861-8.

20. Iatropoulos P, Daina E, Mele C, Maranta R, Remuzzi G, Noris M. Discordant phenotype in monozygotic twins with renal coloboma syndrome and a PAX2 mutation. Pediatr Nephrol. 2012;27(10):1989-93.
21. Giovanella S, Pasini A, Ligabue G, Testa F, Mori G, Tagliafico E, et al. PAX2/Renal Coloboma Syndrome Expresses Extreme Intrafamilial Phenotypic Variability. Nephron. 2023;147(2):120-6.
22. Tin A, Köttgen A. Genome-Wide Association Studies of CKD and Related Traits. Clin J Am Soc Nephrol. 2020;15(11):1643-56.
23. Cooper A, Ilboudo H, Alibu VP, Ravel S, Enyaru J, Weir W, et al. renal risk variants have contrasting resistance and susceptibility associations with African trypanosomiasis. Elife. 2017;6.
24. Friedman DJ, Pollak MR. APOL1 Nephropathy: From Genetics to Clinical Applications. Clin J Am Soc Nephrol. 2021;16(2):294-303.
25. Chebib FT, Torres VE. Autosomal Dominant Polycystic Kidney Disease: Core Curriculum 2016. Am J Kidney Dis. 2016;67(5):792-810.
26. Solazzo A, Testa F, Giovanella S, Busutti M, Furci L, Carrera P, et al. The prevalence of autosomal dominant polycystic kidney disease (ADPKD): A meta-analysis of European literature and prevalence evaluation in the Italian province of Modena suggest that ADPKD is a rare and underdiagnosed condition. PLoS One. 2018;13(1):e0190430.
27. Spithoven EM, Kramer A, Meijer E, Orskov B, Wanner C, Abad JM, et al. Renal replacement therapy for autosomal dominant polycystic kidney disease (ADPKD) in Europe: prevalence and survival--an analysis of data from the ERA-EDTA Registry. Nephrol Dial Transplant. 2014;29 Suppl 4(Suppl 4):iv15-25.
28. Gabow PA, Johnson AM, Kaehny WD, Kimberling WJ, Lezotte DC, Duley IT, et al. Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease. Kidney Int. 1992;41(5):1311-9.
29. Cystic kidney disease. 2023.
30. Allison SJ. DNAJB11: another player in ADPKD. Nat Rev Nephrol. 2018;14(8):476.
31. Porath B, Gainullin VG, Cornec-Le Gall E, Dillinger EK, Heyer CM, Hopp K, et al. Mutations in GANAB, Encoding the Glucosidase IIα Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. Am J Hum Genet. 2016;98(6):1193-207.
32. Boerrigter MM, Bongers EMHF, Lugtenberg D, Nevens F, Drenth JPH. Polycystic liver disease genes: Practical considerations for genetic testing. Eur J Med Genet. 2021;64(3):104160.
33. Bergmann C. Genetics of Autosomal Recessive Polycystic Kidney Disease and Its Differential Diagnoses. Front Pediatr. 2017;5:221.
34. Ala-Mello S, Sankila EM, Koskimies O, de la Chapelle A, Kääriäinen H. Molecular studies in Finnish patients with familial juvenile nephronophthisis exclude a founder effect and support a common mutation causing mechanism. J Med Genet. 1998;35(4):279-83.
35. Luo F, Tao YH. Nephronophthisis: A review of genotype-phenotype correlation. Nephrology (Carlton). 2018;23(10):904-11.
36. Wolf MT, Hildebrandt F. Nephronophthisis. Pediatr Nephrol. 2011;26(2):181-94.
37. Wolf MT. Nephronophthisis and related syndromes. Curr Opin Pediatr. 2015;27(2):201-11.
38. Möller CC, Pollak MR, Reiser J. The genetic basis of human glomerular disease. Adv Chronic Kidney Dis. 2006;13(2):166-73.
39. Gibson J, Fieldhouse R, Chan MMY, Sadeghi-Alavijeh O, Burnett L, Izzi V, et al. Prevalence Estimates of Predicted Pathogenic. J Am Soc Nephrol. 2021;32(9):2273-90.
40. Watson S, Padala SA, Hashmi MF, . JSB. Alport syndrome. 2023.
41. Savige J, Ariani F, Mari F, Bruttini M, Renieri A, Gross O, et al. Expert consensus guidelines for the genetic diagnosis of Alport syndrome. Pediatr Nephrol. 2019;34(7):1175-89.
42. Korbet SM. Clinical picture and outcome of primary focal segmental glomerulosclerosis. Nephrol Dial Transplant. 1999;14 Suppl 3:68-73.
43. Bose B, Cattran D, Registry TG. Glomerular diseases: FSGS. Clin J Am Soc Nephrol. 2014;9(3):626-32.
44. Tato AM, Carrera N, García-Murias M, Shabaka A, Ávila A, Mora Mora MT, et al. Genetic testing in focal segmental glomerulosclerosis: in whom and when? Clin Kidney J. 2023;16(11):2011-22.

45. Lepori N, Zand L, Sethi S, Fernandez-Juarez G, Fervenza FC. Clinical and pathological phenotype of genetic causes of focal segmental glomerulosclerosis in adults. Clin Kidney J. 2018;11(2):179-90.
46. Kestilä M, Lenkkeri U, Männikkö M, Lamerdin J, McCready P, Putaala H, et al. Positionally cloned gene for a novel glomerular protein--nephrin--is mutated in congenital nephrotic syndrome. Mol Cell. 1998;1(4):575-82.
47. Hinkes B, Vlangos C, Heeringa S, Mucha B, Gbadegesin R, Liu J, et al. Specific podocin mutations correlate with age of onset in steroid-resistant nephrotic syndrome. J Am Soc Nephrol. 2008;19(2):365-71.
48. Desnick RJ, Wasserstein MP, Banikazemi M. Fabry disease (alpha-galactosidase A deficiency): renal involvement and enzyme replacement therapy. Contrib Nephrol. 2001(136):174-92.
49. Germain DP. Fabry disease. Orphanet J Rare Dis. 2010;5:30.
50. Sanna-Cherchi S, Caridi G, Weng PL, Scolari F, Perfumo F, Gharavi AG, et al. Genetic approaches to human renal agenesis/hypoplasia and dysplasia. Pediatr Nephrol. 2007;22(10):1675-84.
51. Harambat J, Bonthuis M, Groothoff JW, Schaefer F, Tizard EJ, Verrina E, et al. Lessons learned from the ESPN/ERA-EDTA Registry. Pediatr Nephrol. 2016;31(11):2055-64.
52. Kagan M, Pleniceanu O, Vivante A. The genetic basis of congenital anomalies of the kidney and urinary tract. Pediatr Nephrol. 2022;37(10):2231-43.
53. Knoers NVAM. The term CAKUT has outlived its usefulness: the case for the defense. Pediatr Nephrol. 2022;37(11):2793-8.
54. Verbitsky M, Westland R, Perez A, Kiryluk K, Liu Q, Krithivasan P, et al. The copy number variation landscape of congenital anomalies of the kidney and urinary tract. Nat Genet. 2019;51(1):117-27.
55. Singh P, Harris PC, Sas DJ, Lieske JC. The genetics of kidney stone disease and nephrocalcinosis. Nat Rev Nephrol. 2022;18(4):224-40.
56. Bhasin B, Ürekli HM, Atta MG. Primary and secondary hyperoxaluria: Understanding the enigma. World J Nephrol. 2015;4(2):235-44.
57. Spasovski G, Beck BB, Blau N, Hoppe B, Tasic V. Late diagnosis of primary hyperoxaluria after failed kidney transplantation. Int Urol Nephrol. 2010;42(3):825-9.
58. Purdue PE, Takada Y, Danpure CJ. Identification of mutations associated with peroxisome-to-mitochondrion mistargeting of alanine/glyoxylate aminotransferase in primary hyperoxaluria type 1. J Cell Biol. 1990;111(6 Pt 1):2341-51.
59. Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al. Primary Hyperoxaluria Type 1. 1993.
60. Belostotsky R, Seboun E, Idelson GH, Milliner DS, Becker-Cohen R, Rinat C, et al. Mutations in DHDPSL are responsible for primary hyperoxaluria type III. Am J Hum Genet. 2010;87(3):392-9.
61. Devuyst O, Thakker RV. Dent's disease. Orphanet J Rare Dis. 2010;5:28.
62. Eisner BH, Goldfarb DS, Baum MA, Langman CB, Curhan GC, Preminger GM, et al. Evaluation and Medical Management of Patients with Cystine Nephrolithiasis: A Consensus Statement. J Endourol. 2020;34(11):1103-10.
63. Policastro LJ, Saggi SJ, Goldfarb DS, Weiss JP. Personalized Intervention in Monogenic Stone Formers. J Urol. 2018;199(3):623-32.
64. Kermond R, Mallett A, McCarthy H. A clinical approach to tubulopathies in children and young adults. Pediatr Nephrol. 2023;38(3):651-62.
65. Downie ML, Lopez Garcia SC, Kleta R, Bockenhauer D. Inherited Tubulopathies of the Kidney: Insights from Genetics. Clin J Am Soc Nephrol. 2021;16(4):620-30.
66. Tanudisastro HA, Holman K, Ho G, Farnsworth E, Fisk K, Gayagay T, et al. Australia and New Zealand renal gene panel testing in routine clinical practice of 542 families. NPJ Genom Med. 2021;6(1):20.
67. Soares SBM, de Menezes Silva LAW, de Carvalho Mrad FC, Simões E Silva AC. Distal renal tubular acidosis: genetic causes and management. World J Pediatr. 2019;15(5):422-31.
68. Rodríguez Soriano J. Renal tubular acidosis: the clinical entity. J Am Soc Nephrol. 2002;13(8):2160-70.

69. Melvi LE. Renal tubular acidosis. BMJ Best Practice. 2020.
70. Holliday MA, Barratt TM, Avner ED. Pediatric nephrology. Third edition ed.
71. Lopez-Garcia SC, Emma F, Walsh SB, Fila M, Hooman N, Zaniew M, et al. Treatment and long-term outcome in primary distal renal tubular acidosis. Nephrol Dial Transplant. 2019;34(6):981-91.
72. Alexander TR, Law L, Gil-Pena H. Hereditary Distal Renal Tubular Acidosis. In: GeneReviews, editor.2019.
73. Konrad M, Nijenhuis T, Ariceta G, Bertholet-Thomas A, Calo LA, Capasso G, et al. Diagnosis and management of Bartter syndrome: executive summary of the consensus and recommendations from the European Rare Kidney Disease Reference Network Working Group for Tubular Disorders. Kidney Int. 2021;99(2):324-35.
74. Blanchard A, Bockenhauer D, Bolignano D, Calò LA, Cosyns E, Devuyst O, et al. Gitelman syndrome: consensus and guidance from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2017;91(1):24-33.
75. Knoers NV, Levtchenko EN. Gitelman syndrome. Orphanet J Rare Dis. 2008;3:22.
76. Kidd K, Vylet'al P, Schaeffer C, Olinger E, Živná M, Hodaňová K, et al. Genetic and Clinical Predictors of Age of ESKD in Individuals With Autosomal Dominant Tubulointerstitial Kidney Disease Due to. Kidney Int Rep. 2020;5(9):1472-85.
77. Bleyer AJ, Kmoch S. Autosomal dominant tubulointerstitial kidney disease: of names and genes. Kidney Int. 2014;86(3):459-61.
78. Riedhammer KM, Braunisch MC, Günthner R, Wagner M, Hemmer C, Strom TM, et al. Exome Sequencing and Identification of Phenocopies in Patients With Clinically Presumed Hereditary Nephropathies. Am J Kidney Dis. 2020;76(4):460-70.
79. Xue Y, Ankala A, Wilcox WR, Hegde MR. Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: single-gene, gene panel, or exome/genome sequencing. Genet Med. 2015;17(6):444-51.
80. Sims D, Sudbery I, Ilott NE, Heger A, Ponting CP. Sequencing depth and coverage: key considerations in genomic analyses. Nat Rev Genet. 2014;15(2):121-32.
81. Barbitoff YA, Polev DE, Glotov AS, Serebryakova EA, Shcherbakova IV, Kiselev AM, et al. Systematic dissection of biases in whole-exome and whole-genome sequencing reveals major determinants of coding sequence coverage. Sci Rep. 2020;10(1):2057.
82. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med. 2018;20(10):1122-30.
83. Mallawaarachchi AC, Lundie B, Hort Y, Schonrock N, Senum SR, Gayevskiy V, et al. Genomic diagnostics in polycystic kidney disease: an assessment of real-world use of whole-genome sequencing. Eur J Hum Genet. 2021;29(5):760-70.
84. Chapman AB, Devuyst O, Eckardt KU, Gansevoort RT, Harris T, Horie S, et al. Autosomal-dominant polycystic kidney disease (ADPKD): executive summary from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2015;88(1):17-27.
85. Mantere T, Kersten S, Hoischen A. Long-Read Sequencing Emerging in Medical Genetics. Front Genet. 2019;10:426.
86. Bertoli-Avella AM, Beetz C, Ameziane N, Rocha ME, Guatibonza P, Pereira C, et al. Successful application of genome sequencing in a diagnostic setting: 1007 index cases from a clinically heterogeneous cohort. Eur J Hum Genet. 2021;29(1):141-53.
87. Battke F, Schulte B, Schulze M, Biskup S. The question of WGS's clinical utility remains unanswered. Eur J Hum Genet. 2021;29(5):722-3.
88. Ewans LJ, Minoche AE, Schofield D, Shrestha R, Puttick C, Zhu Y, et al. Whole exome and genome sequencing in mendelian disorders: a diagnostic and health economic analysis. Eur J Hum Genet. 2022;30(10):1121-31.
89. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. Nat Rev Genet. 2018;19(5):325.
90. Hays T, Groopman EE, Gharavi AG. Genetic testing for kidney disease of unknown etiology. Kidney Int. 2020;98(3):590-600.

91. Jayasinghe K, Wu Y, Stark Z, Kerr PG, Mallett AJ, Gaff C, et al. Cost-Effectiveness of Targeted Exome Analysis as a Diagnostic Test in Glomerular Diseases. Kidney Int Rep. 2021;6(11):2850-61.
92. Mirshahi UL, Bhan A, Tholen LE, Fang B, Chen G, Moore B, et al. Framework From a Multidisciplinary Approach for Transitioning Variants of Unknown Significance From Clinical Genetic Testing in Kidney Disease to a Definitive Classification. Kidney Int Rep. 2022;7(9):2047-58.
93. Kashtan CE, Ding J, Garosi G, Heidet L, Massella L, Nakanishi K, et al. Alport syndrome: a unified classification of genetic disorders of collagen IV α345: a position paper of the Alport Syndrome Classification Working Group. Kidney Int. 2018;93(5):1045-51.
94. Deans ZC, Ahn JW, Carreira IM, Dequeker E, Henderson M, Lovrecic L, et al. Recommendations for reporting results of diagnostic genomic testing. Eur J Hum Genet. 2022;30(9):1011-6.
95. Sue Richards NA, Sherri Bale, David Bick, Soma Das, Julie Gastier-Foster, Wayne W. Grody, Madhuri Hegde, Elaine Lyon, Elaine Spector, Karl Voelkerding, and Heidi L. Rehm. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. 2015.
96. Carrera P, Calzavara S, Magistroni R, den Dunnen JT, Rigo F, Stenirri S, et al. Deciphering Variability of PKD1 and PKD2 in an Italian Cohort of 643 Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD). Sci Rep. 2016;6:30850.
97. Orisio S, Noris M, Rigoldi M, Bresin E, Perico N, Trillini M, et al. Mutation Analysis of PKD1 and PKD2 Genes in a Large Italian Cohort Reveals Novel Pathogenic Variants Including A Novel Complex Rearrangement. Nephron. 2023.
98. Trautmann A, Lipska-Ziętkiewicz BS, Schaefer F. Exploring the Clinical and Genetic Spectrum of Steroid Resistant Nephrotic Syndrome: The PodoNet Registry. Front Pediatr. 2018;6:200.
99. Bullich G, Domingo-Gallego A, Vargas I, Ruiz P, Lorente-Grandoso L, Furlano M, et al. A kidney-disease gene panel allows a comprehensive genetic diagnosis of cystic and glomerular inherited kidney diseases. Kidney Int. 2018;94(2):363-71.
100. Gefen AM, Sethna CB, Cil O, Perwad F, Schoettler M, Michael M, et al. Genetic testing in children with nephrolithiasis and nephrocalcinosis. Pediatr Nephrol. 2023.
101. Obeidova L, Seeman T, Fencl F, Blahova K, Hojny J, Elisakova V, et al. Results of targeted next-generation sequencing in children with cystic kidney diseases often change the clinical diagnosis. PLoS One. 2020;15(6):e0235071.
102. Ashton EJ, Legrand A, Benoit V, Roncelin I, Venisse A, Zennaro MC, et al. Simultaneous sequencing of 37 genes identified causative mutations in the majority of children with renal tubulopathies. Kidney Int. 2018;93(4):961-7.
103. Lieberman KV, Chang AR, Block GA, Robinson K, Bristow SL, Morales A, et al. The KIDNEYCODE Program: Diagnostic Yield and Clinical Features of Individuals with CKD. Kidney360. 2022;3(5):900-9.
104. Rush ET, Johnson B, Aradhya S, Beltran D, Bristow SL, Eisenbeis S, et al. Molecular Diagnoses of X-Linked and Other Genetic Hypophosphatemias: Results From a Sponsored Genetic Testing Program. J Bone Miner Res. 2022;37(2):202-14.
105. Schönauer R, Baatz S, Nemitz-Kliemchen M, Frank V, Petzold F, Sewerin S, et al. Matching clinical and genetic diagnoses in autosomal dominant polycystic kidney disease reveals novel phenocopies and potential candidate genes. Genet Med. 2020;22(8):1374-83.
106. Bao M, Cai J, Yang X, Ma W. Genetic screening for Bartter syndrome and Gitelman syndrome pathogenic genes among individuals with hypertension and hypokalemia. Clin Exp Hypertens. 2019;41(4):381-8.
107. Rheault MN, McLaughlin HM, Mitchell A, Blake LE, Devarajan P, Warady BA, et al. COL4A gene variants are common in children with hematuria and a family history of kidney disease. Pediatr Nephrol. 2023.
108. Chen J, Lin F, Zhai Y, Wang C, Wu B, Ma D, et al. Diagnostic and clinical utility of genetic testing in children with kidney failure. Pediatr Nephrol. 2021;36(11):3653-62.

109. Domingo-Gallego A, Pybus M, Bullich G, Furlano M, Ejarque-Vila L, Lorente-Grandoso L, et al. Clinical utility of genetic testing in early-onset kidney disease: seven genes are the main players. Nephrol Dial Transplant. 2022;37(4):687-96.
110. Bleyer AJ, Westemeyer M, Xie J, Bloom MS, Brossart K, Eckel JJ, et al. Genetic Etiologies for Chronic Kidney Disease Revealed through Next-Generation Renal Gene Panel. Am J Nephrol. 2022;53(4):297-306.
111. Oh J, Shin JI, Lee K, Lee C, Ko Y, Lee JS. Clinical application of a phenotype-based NGS panel for differential diagnosis of inherited kidney disease and beyond. Clin Genet. 2021;99(2):236-49.
112. Mansilla MA, Sompallae RR, Nishimura CJ, Kwitek AE, Kimble MJ, Freese ME, et al. Targeted broad-based genetic testing by next-generation sequencing informs diagnosis and facilitates management in patients with kidney diseases. Nephrol Dial Transplant. 2021;36(2):295-305.
113. Schrezenmeier E, Kremerskothen E, Halleck F, Staeck O, Liefeldt L, Choi M, et al. The underestimated burden of monogenic kidney disease in adults waitlisted for kidney transplantation. Genet Med. 2021;23(7):1219-24.
114. Groopman EE, Marasa M, Cameron-Christie S, Petrovski S, Aggarwal VS, Milo-Rasouly H, et al. Diagnostic Utility of Exome Sequencing for Kidney Disease. N Engl J Med. 2019;380(2):142-51.
115. Connaughton DM, Kennedy C, Shril S, Mann N, Murray SL, Williams PA, et al. Monogenic causes of chronic kidney disease in adults. Kidney Int. 2019;95(4):914-28.

116. Mallawaarachchi, A.C., et al., Genomic diagnostics in polycystic kidney disease: an assessment of real-world use of whole-genome sequencing. Eur J Hum Genet, 2021. **29**(5): p. 760-770.
117. Bullich, G., et al., A kidney-disease gene panel allows a comprehensive genetic diagnosis of cystic and glomerular inherited kidney diseases. Kidney Int, 2018. **94**(2): p. 363-371.

8. ACKNOWLEDGEMENTS

I would like to acknowledge Prof. Riccardo Magistroni, my supervisor, for the guidance and advice that carried me through the three years of my doctorate. I am also grateful to Prof. Gianni Cappelli, my co-supervisor, and Prof. Gabriele Donati, the head of the Nephrology unit, for their support in allowing me to undertake this significant doctoral project.

I would also like to thank my colleagues, Dott.ssa Giulia Ligabue and Dott. Marco Ferrarini for their assistance with methodologies, constructive discussions and unwavering support.

Additionally, I thank the collaborating centers whose contribution enabled the realization of the DECIDE project by providing patient data and expertise.

I would like to give special thanks to my husband and family for their constant support and encouragement throughout the challenges, defeats and professional achievements.