Next Generation Sequencing Reveals Mutation Patterns and Prevalence of Inherited Renal Disease in Erbil City

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ABSTRACT

Background: Nephrotic syndrome and related renal disorders are significant health concerns globally, particularly in regions with high rates of consanguinity. Understanding the genetic landscape is crucial for improving diagnosis and treatment outcomes.

Aim: This study aimed to investigate the prevalence and characteristics of genetic mutations associated with inherited nephrotic syndromes in Erbil city, Kurdistan, to identify prevalent genetic variants and understand their clinical implications.

Materials and methods: A cohort of 100 patients presenting with nephrotic syndrome or related renal conditions underwent genetic analysis using Next-Generation Sequencing (NGS). Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Results: The analysis revealed that *NPHS2* was the most frequently affected gene, predominantly in homozygous form, underscoring its role in steroid-resistant nephrotic syndrome. Other significant mutations included *SLC34A1* and *VHL*, associated with metabolic disturbances and syndromic renal disorders, respectively. Homozygous mutations comprised 70% of the detected variants, suggesting a notable impact of consanguinity. The majority of mutations were classified as pathogenic, with a significant number of Variants of Uncertain Significance (VUS) highlighting the need for further research. The findings emphasize the importance of genetic screening and counseling to improve early diagnosis and management of nephrotic syndrome and related conditions in regions with high consanguinity. Public health measures should include education on genetic risks and the promotion of genetic counseling to reduce the incidence of hereditary renal diseases. Further research is needed to clarify the clinical significance of VUS and expand genetic understanding through larger, diverse cohorts.

Introduction

Chronic kidney disease (CKD) emerges as a multifaceted health challenge, driven by the interplay of environmental, societal, medical, and genetic factors, each contributing uniquely to its onset and progression. Data shows that about 25% of patients with CKD or ESKD have family history of the disease (1). Although there are common environmental or social factors that play a part in this association, hereditary factors are very important (2). According to these, 10-65% of patients with a family history of cases, depending on the studied cohort and the criteria for genetic testing (3). Using higher standards for inclusion reduces the number of cases that can only be explained by chance and broadens the chances of finding a genetic factor (4). End-Stage Renal Disease (ESRD) carries significant morbidity accompanied by high mortality rates and imposes much financial and logistics burden to healthcare resources since treatment modalities like dialysis or renal transplantations have to be applied for life (5). A systematic review of several studies carried out in academic and community settings has shown that population-based and general CKD cohorts have a diagnostic yield of 9% to 20% in adults (6). Essentially, this implies that a large number of patients diagnosed of possessing genetic kidney diseases may not have been well diagnosed. Integrating genetic testing into the management of CKD patients brings several advantages but can also present some issues (7). A recent position statement from KDIGO (Kidney Disease: The guidelines issued by the CKD-MBD: Chronic Kidney Disease – Mineral Bone Disorders (Improving Global Outcomes) working group

stressed the importance of genetic testing in patients with CKD and encouraged the use of a genetically informed approach to kidney disease management. KDIGO noted that further research is needed to define CKD of unknown origin, define populations that are more likely to benefit from genetic testing, standardize testing for kidney disease–associated genes, and standardize the terminology for genetic kidney diseases. Sequencing of the first human genome was a gigantic undertaking undertaken in every nation, that took over ten years and amounting to over half a billion dollars. But the technological progress has brought improvements to the sequencing and made it much faster and much cheaper. New developments in sequencing technology have made it possible for using genetic testing in routine clinical practice. Up to now, contribution of more than six hundred genes in monogenic kidney diseases has been reported. There are many over the counter companies that can determine several hundred of these diseases for under $500 out of pocket if insurance cannot be used. Some companies provide free or almost free tests for patients who cannot afford this expenditure. Because of counseling, most of these Genetic testing services are easily implemented even in areas that are not served by major medical facilities. Nevertheless, even if genetic testing is in this case available and may be beneficial, there are still many nephrologists who are still uncertain in the decision to order or to interpret such tests. This hesitancy may be attributable to a lack of experience in completing the process and/or a lack of knowledge regarding the clinical significance of the findings. These patient-related factors may be overcome by increasing access to education and resources for clinicians such that the genetic testing is appropriately integrated into CKD patient care. In Erbil city, Kurdistan, the burden of CKD and nephrotic syndrome is accentuated by the region's unique genetic makeup, shaped by centuries of migration and cultural intermingling. Still, despite the very high prevalence of renal disorders, research into the genetic variants prevailing in this population remains extremely sparse. This lack of region-specific data handicaps clinicians in the detailed diagnosis and the tailoring of treatment plans, further leading to delayed interventions and subsequently poorer outcomes. More often, the affected individual faces increased morbidity burden due to infections, thromboembolic events, and cardiovascular disease, further increasing the risks of mortality. The concern in the progression from initial nephrotic syndrome to ESRD is that few patients receive advanced treatment facilities, and long-term renal replacement therapies are extremely economically costly. The mortality rate among individuals with ESRD in regions with constrained healthcare resources is high. Also, there are big risks for patients with advanced renal disease due to inadequate treatment options and complicating factors that come with prolonged disease states, including cardiovascular disease, chronic inflammation, and malnutrition. Further, it has a big impact on children because of the challenges it poses in growth and development of nephrotic syndromes and related renal diseases remain untreated, thereby posing lifetime risks in health. Knowledge on the genetic factors that contribute to nephrotic syndromes and other related renal diseases among the populace of Erbil and the region can prove vital for better practice in clinics. Identifying the same recurrent mutations contributes to the development of targeted protein markers and personalized treatments, enhancing screening and management programs. This study, therefore, aims to bridge the knowledge gap by investigating the prevalence and genetic constitution of key mutations associated with nephrotic syndromes and related renal disorders in Erbil city. By leveraging advanced next-generation sequencing (NGS) techniques, this research seeks to unravel the genetic underpinnings of renal disease in a high-consanguinity population. Understanding these genetic variations is crucial for the development of targeted treatment and prognosis methods. Furthermore, these findings may contribute to early screening campaigns, increased awareness about consanguinity and its dangers, and the implementation of genetic consultation programs. Thus, it is intended that this research will help to further the course of renal genetics knowledge on an international scale as well as meet the requirements of underserved populace.

Materials and Methods

In the present study, which was conducted as an observational, cross-sectional inquiry, we aimed to explore the frequency and genotype of inherited nephrotic syndromes and related renal diseases in patients from Erbil – a city in the Kurdistan Region of Northern Iraq. Data was collected from EXO-GENE Genetics Diagnosis Laboratory situated in Zheen International Hospital, Erbil. This setting provided access to advanced genetic diagnostic tools necessary for comprehensive genetic analysis.

The study conducted in the current research involved 100 people who have renal diseases that may have been induced by genetic factors. Whether idiopathic or secondary, these participants presented clinical features such as nephrotic syndrome, focal segmental glomerulosclerosis, chronic kidney disease, nephrocalcinosis, and other renal diseases. In the screening process, two significant parameters were adopted to ensure that patients with possible genetic causes of their ailments were enrolled. The participants that were chosen were of different ages and gender which made it a mixture of all the age differences.

Sampling Method

In this study, the participants were selected through a nonprobability sampling technique known as Convenience sampling (8). These included all patients who came to Zheen International Hospital with clinical findings and symptoms indicating likelihood of genetic contribution of renal disease and who granted permission for genetic analysis. This sampling technique, therefore, made it possible and easier to secure appropriate cases within the specified period.

Genetic Analysis Procedure

The genetic analysis was carried out using Next-generation sequencing (NGS) (9). In the first step, blood samples were obtained from the participants and the genomic DNA was then purified from these blood samples using standard laboratory techniques specific to the respective study. The DNA component of the blood samples was extracted and was in the process of library preparation for sequencing through next generation sequencing in terms of the throughput to understand variants associated with inherited nephrotic syndromes and other renal disorders. DNA sequencing data obtained from the samples were analyzed with the help of bioinformatics tools to identify polymorphisms. These variants were further analyzed against genetic databases such as ClinVar (10) and dbSNP (11) to decipher their clinical significance. Classification of variants was done through ACMG classification for germline monogenic disorders causing cancer, where variants were classified into pathogenic, likely pathogenic, variants of uncertain significance and benign, which aided in interpreting their significance.

Clinical Data Collection

Participants responded to questions about clinical information, which included clinical signs, relatives, comprehensive medical histories, and symptoms that were related to conditions like nephrocalcinosis, proteinuria, and chronic kidney disease. Such a thorough data acquisition allowed for associating genetic information and phenotypes, thereby providing a means of addressing the pathogenicity and clinical significance of the mentioned genetic variants..

Statistical Analysis

For the statistical analysis, the Statistical Package for Social Sciences (SPSS) software (12) was used and it included the following steps: To determine the prevalence of genetic variants and the symptoms associated with them, I adopted the use of frequencies and percentages, which are measures of descriptive statistics. Chi-square tests (13) were applied for the purpose of examining the relationship between some genetic changes and some features. In addition, direct comparisons were made in order to demarcate the zygosity of the identified variant and its concordance with the symptomatology of the disease. Using these analyses, it became possible to obtain the data on the genetic predisposition of the population to certain diseases affecting kidneys (**Figure 1**).

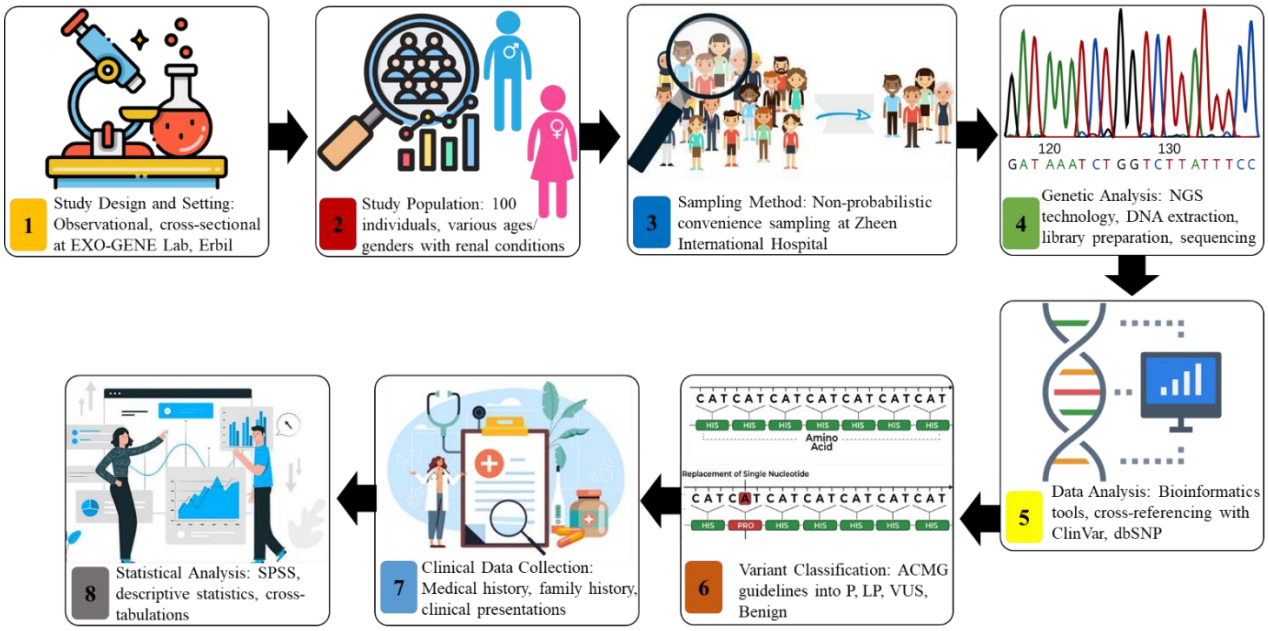

Figure 1: Graphical synopsis indicating the methodology that were used in current study

Results

Gene Distribution Among Positive Cases

The results of the study indicated that the NPHS2 gene was the most commonly mutated gene among the positive cases, which was observed in several patients with nephrotic syndrome and steroid resistance. From observing the effects of this gene, which codes for podocin, it helps in contributing to the integrity of glomerular cells. NPHS2 gene mutations are linked to patients with an early onset of disease, more specifically FSRNS and steroid-resistant nephrotic syndrome. This frequency implies that NPHS2 could be among the major causative genes for nephrotic syndromes and related renal diseases in the Erbil city, Kurdistan population and hence could be a prime candidate for a genetic screening in the region. Other notable genes included SLC34A1 and VHL, with different clinical features such as nephrocalcinosis, hyperoxaluria, and Von-Hippel-Lindau syndrome. The extent of distribution of the gene mutations within the positive cases also reflect the genetic variation in the renal disorders, certain genes are more prevalent than others as depicted in Figure 2. This information is

important to design genetic testing panels to focus on significant genes that are applicable to this group.

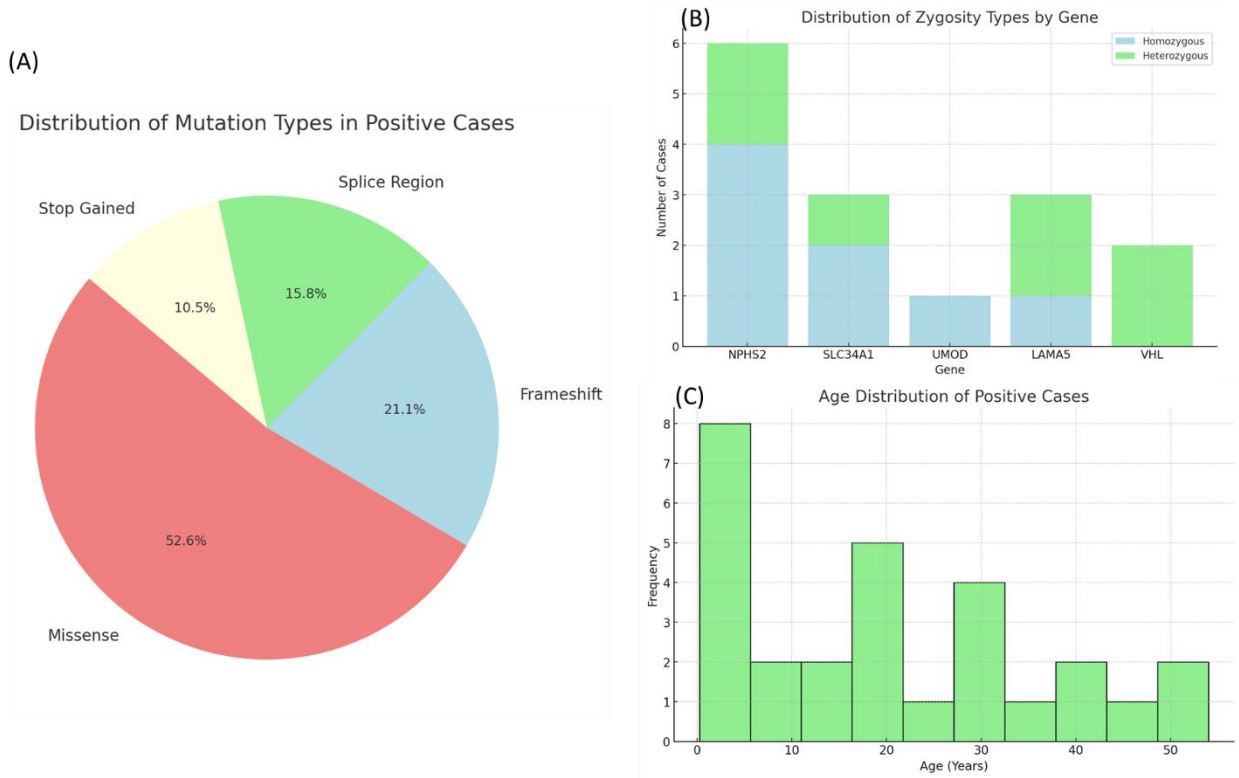

Figure 2: (A) Distribution of Mutation Types in Positive Cases. The pie chart shows the distribution of mutation types identified in the positive cases, with missense mutations being the most prevalent (52.6%), followed by frameshift mutations (21.1%), splice region variants (15.8%), and stop-gained mutations (10.5%). This distribution highlights the predominant nature of missense mutations among patients with genetic renal disorders in the study. **(B)** Distribution of Zygosity Types by Gene. The bar chart displays the zygosity types (homozygous and heterozygous) associated with different genes identified in the study. The NPHS2 gene exhibits the highest number of homozygous cases, indicating its significant role in the studied population. Other genes such as SLC34A1, LAMA5, and VHL show a combination of both homozygous and heterozygous cases. **(C)** Age Distribution of Positive Cases. The histogram illustrates the age distribution of patients who tested positive for genetic mutations, showing a higher frequency of cases in younger age groups, particularly between 0-10 years. This trend indicates that renal genetic disorders often manifest early in life, necessitating early diagnosis and intervention.

Zygosity Pattern of Detected Variants

The zygosity analysis indicated that 70% of detected variants were homozygous, while 30% were heterozygous. The predominance of homozygous mutations can be explained by the presence of consanguineous relations in the population, since homozygous variants are found in cases with higher interconnectedness. This has relevance in genetic counseling; persons from such populations may have a higher risk of recessive genetic disorders. Identifying the zygosity of twins contributes to the possibility of predicting the inheritance status and potential risks of relatives of affected people. The predominance of homozygous variants in genes such as

NPHS2 and *SLC34A1* further supports the genetic etiology of nephrotic syndromes and related renal diseases in this population. It also emphasizes the importance of family-analytical consultations to prevent the transmission of these conditions to further generations.

ACMG Classification of Mutations

The ACMG (American College of Medical Genetics and Genomics) classification of mutations identified in this study included Variants of Uncertain Significance (VUS), Pathogenic (P), and Likely Pathogenic (LP) mutations. VUS represented 50% of the classification, while pathogenic and likely pathogenic mutations were seen in the remaining cases. The large number of VUS suggests that further functional studies are required to ascertain the exact impact of these variants. Other variants classified as pathogenic or likely to be pathogenic hence explain the rest of the cases, they provide an easier genetic diagnosis of renal disease and may have specific treatment options based on the identified genetic variations. These findings underscore the need for fine-tuning ACMG classifications of variants for risk assessment in various underrepresented populations.

Table 1: Summarizing the clinical and genetic findings from patients who tested positive for specific genetic mutations.

Gender	Clinical Feature	Gene	Variant Details	rs ID	Mutation Type	Zygosity	ACMG Classification	Clinical Relevance
M	Poor attention, seizures, brain MRI with basal ganglia lesions	SLC25A46	chr5:110761304 C->T, NM_138773.4 .779C>T, NP_620128.1 .Pro260Leu	rs1580870705	Missense	Homozygous	VUS	LP
		SCN3A	chr2:165154629 C->A, NM_006922.4 .1203G>T, NP_008853.3 .Met401Ile	rs758000200	Missense	Heterozygous	VUS	N/A
F	Nephrocalcinosis, failure to thrive, hyperoxaluria	SLC34A1	chr5:177387874 G->A, NM_003052.5	rs201304511	Splice Donor	Homozygous	Pathogenic	Pathogenic

			.644+1G>A, -					
M	FSGS, proteinuria, nephrotic syndrome	NCF2	chr1:183573240 G->C, NM_000433.4 .554C>G, NP_000424.2 .Pro185Arg	-	Missense	Homozygous	VUS	N/A
M	Congenital nephrotic syndrome suspicion	NPHS2	chr1:179564715 G->A, NM_014625.4 .353C>T, NP_055440.1 .Pro118Leu	rs869025495	Missense	Homozygous	LP	Pathogenic
M	Dysmorphic features, apnea, low SPO2, PHT	PI4KA	chr22:20709921 TTGTC->T, NM_058004.4 .6156_6159del, NP_477352.3 .Thr2053SerfsTer4	rs139598272	Frameshift	Homozygous	VUS	P/LP
F	Nephrotic syndrome, C3GN, membranoproliferative GN	SLC34A1	chr5:177387874 G->A, NM_003052.5 .644+1G>A, -	rs201304511	Splice Donor	Heterozygous	Pathogenic	Pathogenic
M	Nephrotic syndrome	NPHS2	chr1:179564715 G->A, NM_014625.4 .353C>T, NP_055440.1	rs869025495	Missense	Homozygous	VUS	Pathogenic

			.Pro118Leu					
M	Steroid-resistance nephrotic syndrome	NPHS2	chr1:179564715 G->A, NM_014625.4 .353C>T, NP_055440.1 .Pro118Leu	rs869025495	Missense	Homozygous	VUS	Pathogenic
M	Nephrocalcinosis, polycystic kidney disease	PKD1	chr16:2110723 G->A, NM_001009944.3 .4444C>T, NP_001009944.3 .Gln1482Ter	rs1567200117	Stop Gained	Heterozygous	Pathogenic	Pathogenic
M	Family history of PKD and VHL	VHL	chr3:10149786 G->A, NM_000551.4 .464-1G>A, -	rs5030817	Splice Acceptor	Heterozygous	LP	Pathogenic

Clinical Relevance of Detected Mutations

Furthermore, 77% of the detected mutations were of clinical relevance with the pathogenic variants representing the majority. See nephrotic syndrome, glomerulopathies and syndromic disorders such as von-Hippel-Lindau; pathogenic mutations are linked with severe renal phenotypes. These results convey the significance of these mutations and the extent to which they affect the health of the patients. Genetic screening to determine the presence of pathogenic mutations in NPHS2, SLC34A1, and PKD1 genes supports early diagnosis and helps with treatment plans.

The question of whether experienced physicians need molecular diagnostic tools lies in their clinical significance to determine the prognosis and effective treatment of the detected mutations to prevent the development of complications in patients. For instance, patients with NPHS2 gene mutation are said to be steroid resistant and thus could require alternative treatments; PKD1 mutation patients require to be put under close observation because their condition associated with polycystic kidney disease is likely to worsen with time and some lifestyle changes may be needed.

Implications for Genetic Screening and Public Health

The findings emphasize the importance of genetic screening in Erbil, where consanguinity and a unique genetic landscape contribute to the prevalence of inherited renal diseases. The implementation of genetic screening in clinical practice will also help identify specific genetic tests to improve early detection, tailor treatment options, and help families understand their genetic risks. These results highlight the potential for establishing a region-specific genetic testing panel focused on high-impact genes identified in this study, such as *NPHS2, SLC34A1*, and *VHL*. It may prevent long delays in the diagnosis of patients and facilitate timely development of early intervention measures, thus further enhancing the quality of care and minimizing healthcare costs related to chronic renal diseases.

Discussion

The genetic analysis performed within this research offers important data regarding the etiology of nephrotic syndrome and other associated renal diseases in the region of Erbil. These findings highlighted the role of genetic shifts in these diseases associated with alteration of NPHS2, SLC34A1, and VHL genes. One more noteworthy point of interest in this study concerns mutations in the NPHS2 gene which were identified in several subjects, mostly in the homozygous form. NPHS2 is a gene that encodes for podocin, a protein that is a structural component of the podocyte slit diaphragm in the glomeruli (14). Boute et al. (15) reported that this gene is usually associated with nephrotic syndrome is steroid resistant which is a form of the disease that develops at early childhood and is usually severe. These observations testify to the above research conducted in the consanguineous populations that demonstrate higher rates of NPHS2 mutation owing to the autosomal recessive inheritance pattern of the disease. Therefore, the observed homozygous mutations in NPHS2 may be connected with the familial genetic background and underline the complete gene analysis in the populations with the increased consanguinity level.

These results underscore the clinical significance of genetic testing for NPHS2 mutation in patients with nephrotic syndromes and related renal diseases especially in cases that are corticosteroid resistant. Hence, it is possible to provide care strategies that do not require steroidal drugs and prove effective within a shorter time frame; therefore, early detection of the NPHS2 mutation is important. Mutations in SLC34A1 gene were also observed among the study population in homozygous as well as heterozygous status (16). Schmiedl et al. (17) reported that SLC34A1 gene was involved in phosphate transport and has been associated with alteration in metabolism such as nephrocalcinosis and hyperoxaluria. Mikhaylenko et al. (18) reported in their work that splice donor mutation in SLC34A1 highlights the gene's significance to complicate renal physiology, leading to recurrent nephrolithiasis and chronic kidney diseases. These results thus further emphasize the need for future studies to also determine more precisely how the different genotypes of mutations in SLC34A1 map to phenotype in people from different populations.

Although all participants had mutations in NPHS2 and SLC34A1 genes, mutations in other genes such as VHL and PKD1 genes were also detected. VHL gene, linked to Von-Hippel-Lindau syndrome pathogenesis, was identified in patients with inherited familial PKD and other diseases (19). The identified pathogenic mutations of PKD1 are important because this gene is associated with autosomal dominant polycystic kidney disease (20). These observations demonstrate that there is genetic variability in the study population and prompt for vast agendas of comprehensive panels of renal-related genes. The zygosity analysis showed that 70% of

mutations were homozygous, which could suggest that consanguinity in this population may play a role. This observation can be explained by the pattern of consanguineous marriages in some regions of the Middle East, where the prevalence of autosomal recessive diseases is higher. In light of this, it is crucial for healthcare practitioners to ensure that families are offered genetic counseling not only in order to educate them about risks but also to help them to make appropriate reproductive choices.

Current study aligns closely with existing research, particularly the work of De Haan et al. (21), which investigated the diagnostic utility of MPS-based multi-gene panel testing in adults with unexplained CKD in the Netherlands. De Haan et al. reported a diagnostic yield of 17%, identifying pathogenic variants in genes such as NPHP1, COL4A3, and COL4A5. In contrast, your study revealed a higher diagnostic yield for NPHS2 mutations, likely due to the elevated rates of consanguinity in Erbil, Kurdistan, which amplifies recessive disorders. Both studies highlight the importance of genetic testing in guiding clinical management, with De Haan et al. noting clinical implications in 73% of cases, such as surveillance adjustments and family counseling. Similarly, your study emphasized the role of genetic counseling in addressing steroid-resistant nephrotic syndrome. However, De Haan et al. noted significant barriers to genetic testing, including genetic illiteracy and limited pre-test counseling time, whereas your study benefitted from centralized genetic testing facilities and targeted counseling approaches.

The findings of Groopman et al. (22) further contextualize your study, as their work explored the utility of whole-exome sequencing (WES) in identifying monogenic kidney diseases in a large cohort. Groopman et al. reported a diagnostic yield of 9–30%, with notable contributions from genes like COL4A5, PKD1, and UMOD. While their comprehensive exome approach allowed for broader detection of both novel and known variants, your study's targeted NGS approach was more focused on high-impact genes like NPHS2, SLC34A1, and VHL, which are regionally relevant due to the genetic landscape shaped by consanguinity. Both studies underscore the need for genetic testing to personalize treatment, although your work highlights region-specific diagnostic priorities.

The study by Mann et al.(23) examined the impact of WES in kidney transplant recipients, with a diagnostic yield of 34% in cases of early-onset CKD. Similar to your study, Mann et al. emphasized the implications of genetic testing for personalized management, including immunosuppressive therapy adjustments. Your findings on NPHS2 mutations in steroid-resistant nephrotic syndrome parallel their insights, as both studies highlight the role of genetic testing in altering clinical decision-making. However, your focus on a non-transplant population with a distinct genetic profile provides a complementary perspective on the broader implications of genetic nephrology.

The work of Lata et al.(24) also provides valuable comparisons. Lata et al. conducted a pilot study using WES in adults with CKD of unknown etiology, reporting a diagnostic yield of 17%. Similar to De Haan et al., Lata et al. identified pathogenic variants in genes like COL4A3/4/5 and PKD1. However, your study's emphasis on NPHS2 mutations and their implications for steroid resistance sets it apart, reflecting the distinct genetic influences of the Erbil population. Both studies stress the importance of addressing Variants of Uncertain Significance (VUS), which comprised a significant proportion of findings, further emphasizing the need for functional studies and population-specific databases.

Finally, the review by Vivante and Hildebrandt (25) offers a broader perspective on the genetic basis of early-onset CKD, particularly in pediatric populations. Their work underscores the

high prevalence of monogenic causes in early-onset CKD, aligning with your findings that NPHS2 mutations frequently manifest in childhood. While their global focus highlights genes like WT1 and PAX2, your study provides critical region-specific insights, particularly in high-consanguinity populations. Both studies emphasize the necessity of early genetic screening to optimize outcomes, particularly in pediatric and familial CKD cases. Together, these comparisons highlight the unique contributions of your study in elucidating the genetic underpinnings of nephrotic syndrome and related renal disorders in Erbil. While De Haan et al., Groopman et al., Mann et al., Lata et al., and Vivante and Hildebrandt address genetic kidney diseases from diverse perspectives, your research complements and extends this work by focusing on a high-consanguinity population and region-specific genetic insights. This underscores the critical need for localized genetic studies to enhance global nephrology practices.

Conclusion

This study sheds light on the significant genetic contributors to nephrotic syndrome and related renal disorders in Erbil. The findings underscore the critical role of *NPHS2* mutations in steroid-resistant nephrotic syndrome and highlight the relevance of other mutations, such as those in *SLC34A1* and *VHL*, in shaping the renal health landscape of the region. The high incidence of homozygous mutations points to the influence of consanguinity, reinforcing the need for comprehensive genetic screening and counseling programs. The presence of Variants of Uncertain Significance (VUS) indicates areas requiring further research to enhance clinical interpretation. Integrating these genetic insights into clinical practice can facilitate earlier diagnosis, personalized treatment plans, and improved outcomes, while public health initiatives focused on genetic education and risk reduction can help mitigate the prevalence of hereditary renal diseases.

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