Clinical Kidney Journal

Clinical Kidney Journal, 2024, vol. 17, no. 1, 1–5

https://doi.org/10.1093/ckj/sfad250
Advance Access Publication Date: 9 November 2023
Editorial Comment

EDITORIAL COMMENT

Replacing a kidney biopsy by exome sequencing in undetermined kidney diseases—not yet ready for prime time!

Roser Torra [1], Andreas Kronbichler[2] and Ingeborg M. Bajema[3]

[1]Inherited Kidney Diseases, Nephrology Department, Fundació Puigvert, Institut d'Investigacions Biomèdiques (IIB-Sant Pau), Department of Medicine, Universitat Autonoma de Barcelona (UAB), Spain, [2]Department of Internal Medicine IV, Nephrology and Hypertension, Medical University Innsbruck, Innsbruck, Austria and [3]Department of Pathology and Medical Biology, University of Groningen, University Medical Center, Groningen, The Netherlands

Correspondence to: Roser Torra; E-mail: rtorra@fundacio-puigvert.es; Twitter handle: @torra_roser

The diagnosis of kidney diseases traditionally relies on clinical features, laboratory tests and imaging. In many cases, a kidney biopsy is necessary to determine the underlying pathology. However, kidney biopsies are invasive and carry a risk of complications such as bleeding. In some cases, a renal biopsy may not yield a definitive diagnosis. Undetermined kidney disease (UKD) is a relatively new term for which KDIGO has already indicated the need for further clarification, but it unequivocally refers to a group of patients that are lacking a final diagnosis in spite of various efforts to obtain one. UKD forms a challenge for nephrologists but recent studies have shown that monogenic disease-causing variants may explain around 25% of these nephropathies [1]. This editorial discusses a study published in this issue of *Clinical Kidney Journal* [2] that investigated the effectiveness of exome sequencing (ES) in getting closer to a diagnosis of patients with UKD, and the implications of this approach for routine nephrological healthcare.

Inherited kidney diseases assumably account for around 10%–15% of renal diseases in adult patients and for a majority of renal diseases in paediatric patients on kidney replacement therapy (KRT) [3]. However, it is well known that this figure may be higher as many patients in the different registries reach the need for KRT without a diagnosis or with one that raises doubts [4]. In many cases, inaccurate diagnoses serve to disguise a suboptimal diagnostic workup. There are two fundamental ways in which genetics can explain the family aggregation of chronic kidney disease (CKD): (i) pathogenic variants in Mendelian genes are rare, but they exert an enormous disease-causing effect—examples include inherited kidney diseases such as autosomal dominant polycystic kidney disease, Alport syndrome, autosomal dominant tubulointerstitial kidney disease and tubulopathies among many others; and (ii) common genetic variants exert a very slight effect on the phenotype but are very frequent and may account for around 20% of heritability of CKD. Between 10% and 29% of adults with chronic kidney failure note a positive family history for nephropathy, across different ethnicities and aetiologies [5–7]. Furthermore, glomerular filtration rate has a heritability of approximately 30%–60% in the general population [8–10], and other indices of kidney function, such as albuminuria and electrolyte excretion, show a similar significant heritability [11–13]. Genomic approaches have emerged as a promising tool for diagnosing kidney diseases. Genomic approaches can identify mutations in genes that are responsible for the development of kidney diseases and can provide valuable insights into the pathogenesis of these diseases. In addition, in the near future, genomic approaches will help to identify patients who are at increased risk of developing kidney diseases, which can aid in the development of personalized screening and prevention strategies.

If there is a high suspicion of an inherited kidney disease, the key question is to what extent and in which scenarios genetic testing can replace kidney biopsy. A proposed simplified

Received: 28.7.2023; Editorial decision: 1.9.2023

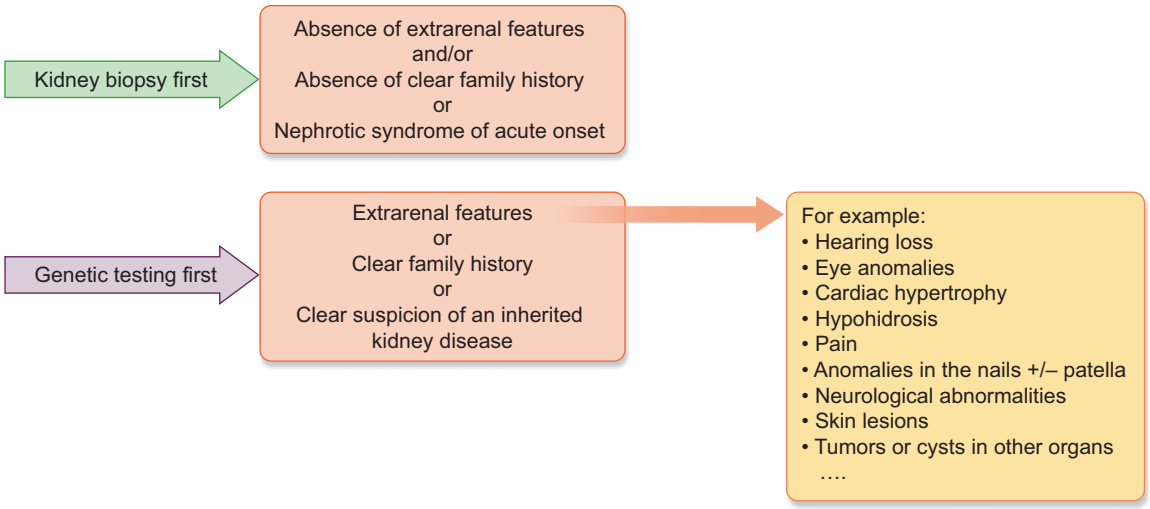

Figure 1: Diagnostic workup of a proteinuric kidney disease in case a genetic background is considered. Two different scenarios are considered state-of-the-art when a genetic cause of kidney disease is considered: **(a)** an approach favouring a diagnostic biopsy first; and **(b)** scenarios when a genetic testing should be performed first. A synthesis of different features is required to choose one of the options, i.e. the acuity of onset of symptoms (i.e. nephrotic syndrome) or a confirmed case of a genetic kidney disease in a closely related family member.

algorithm is highlighted in Fig. 1. The most common genetic finding in UKD or adult focal segmental glomerulosclerosis (FSGS) (without African ancestry) are pathogenic DNA variants in *COL4A3* or *COL4A4*. Considering the recent evidence of these pathogenic variants being present in 1/106 individuals [14], the real ability of these variants to cause kidney disease by themselves is on hold. Biased cohorts towards severely affected individuals have been reported but the name 'autosomal dominant Alport syndrome' is being questioned as these patients do not have a syndrome, as they have no extrarenal involvement and moreover, many of them do not even have haematuria and/or albuminuria. In addition, the true implications of *COL4A3* and *COL4A4* risk variants have still not been deciphered. Variants in both risk genes, which are as common as present in 1:600 Icelanders who carry a *COL4A3* variant, are not always disease-causing [15]. There remains the possibility that detection of a variant is misleading, and overlaps with a distinct kidney disease, which might lead to undertreatment of the individual patient. Also, variants in *COL4A3* or *COL4A4* may be hypothesized to exert a modifying effect or a predisposition towards CKD similarly to *APOL1*. Most patients with variants in *COL4A3* or *COL4A4* reach the need for KRT with very mild proteinuria, opposite to the autosomal recessive or X-linked Alport forms. Therefore, the proposal to replace kidney biopsies in those with a positive molecular diagnosis of variants in these genes might be a bit preliminary. The phenotypic presentation of patients with *COL4A3* and *COL4A4* variants differs, and different histopathology lesions have been reported. Most will have a 'thin basement membrane' pattern on electron microscopy, but apart from that, findings indicative of minimal change disease (MCD) and FSGS have been described [16]. The single reported case with MCD had a complete foot process effacement on electron microscopy, indicating that this case indeed might exhibit coexistence of a collagenopathy and 'idiopathic' MCD, the latter with a potential benefit from specific therapy. Prognosis differs between the lesions, and more specific therapies such as sparsentan, a dual endothelin and angiotensin receptor antagonist, are tested in patients with FSGS and underlying disease-causing variants. In such cases, the overall recommendations need to be followed, and a stringent proteinuria reduction $\leq$1.5 g/g creatinine with conservative measures is recommended, as this has a beneficial effect on long-term kidney outcomes [17]. Hence, it may be incorrect to assign the kidney disease uniquely to these *COL4A3* and *COL4A4* variants, and a kidney biopsy in such scenarios is desirable to make a final diagnosis.

Whole-exome sequencing (WES) is a genomic approach that can be used to diagnose UKD. WES involves sequencing the exons, or coding regions, of all protein-coding genes in the human genome. In addition to WES, targeted gene panel sequencing can also be used to attempt to make a diagnosis for a patient with UKD. This involves sequencing specific genes or genomic regions that are known to be associated with the development of kidney diseases.

Several genetic studies have been conducted to identify the genetic basis of suspected inherited kidney disease. An algorithm to perform WES was recently proposed by a specialist centre in Italy. Patients (mainly children and adolescents) were referred when they had a treatment-refractory disease course, familial history of kidney disease and/or parental consanguinity, or extra-renal involvement, or evidence of congenital abnormalities of the kidney and urinary tract (CAKUT) and CKD stage $\geq$2, or at least two cysts in each kidney (ultrasound), or persistent hyperechoic kidneys or nephrocalcinosis (ultrasound) or persistent metabolic abnormalities. WES was performed in 476 patients, which led to a molecular diagnosis in 319 (67%) individuals. The suspected clinical diagnosis was confirmed in 229 (72%) patients, while revision of diagnosis occurred in 90 (28%) and reclassification in 68 (21%) patients. The highest to the lowest agreement between suspected and confirmed molecular diagnosis was reported for ciliopathies (87%), tubulopathies (86%), syndromic CKD (78%), metabolic kidney diseases (72%), collagenopathies (60%), podocytopathies (54%), CAKUT (47%) and CKD of unknown origin (18%). More importantly, WES had a significant impact on care of these patients, as it led to additional workup/changed surveillance in a majority of patients,

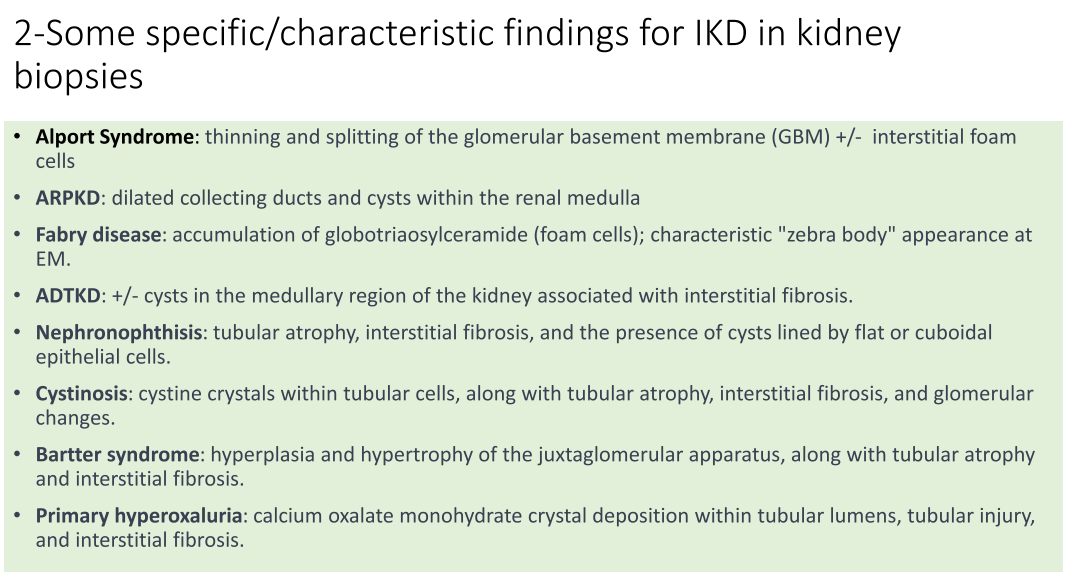

Figure 2: Some specific/characteristic findings for inherited kidney diseases in kidney biopsies. Difference in histologic presentation of some inherited kidney diseases is presented. Notably, a synthesis of light microscopic and electron microscopic changes is required to confirm a suspected diagnosis in many circumstances.

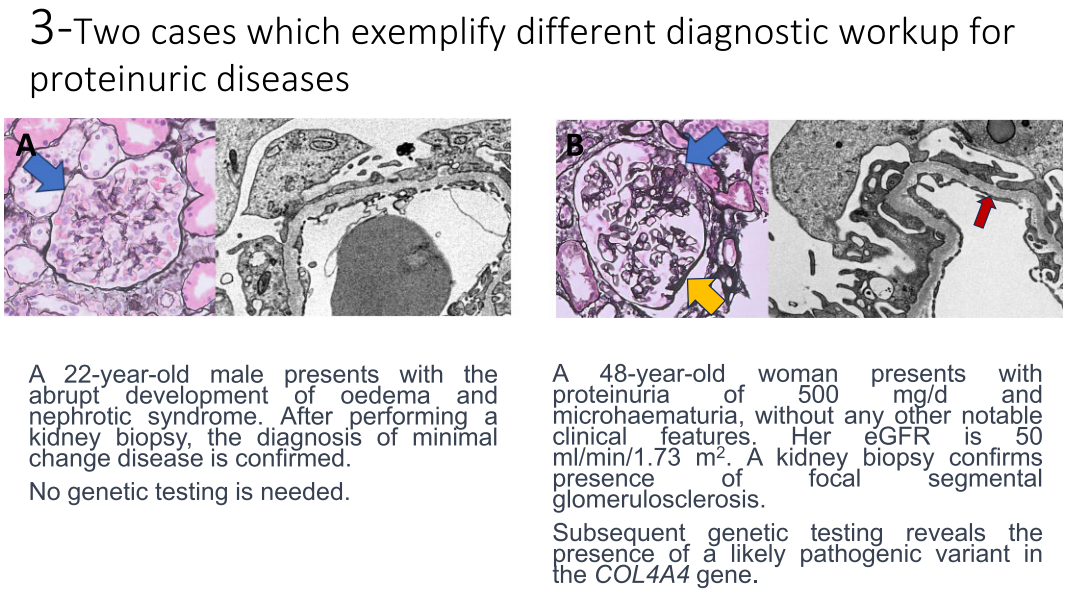

Figure 3: Two cases which exemplify different presentation forms of 'nephrotic' diseases presumably caused by a circulatory factor (primary) or due to a likely pathogenic variant. Electron microscopy is useful to estimate the extent of foot process effacement. In case A, a renal biopsy with at least 10 glomeruli is displayed. Apart from prominent podocytes, glomeruli show no architectural changes. No segmental lesions. Electron microscopy (EM) shows foot process effacement throughout the tissue. Glomerular basement membrane (GBM) has a normal width. In case B, a renal biopsy in which some glomeruli show FSGS is displayed. The example shows a segmental sclerotic lesion at (blue arrow) characterized by mesangial sclerosis and adhesion to Bowman's capsule. There is another adhesion indicated by the yellow arrow. EM shows variable amount of foot process effacement. GBM is slightly variable and has areas (red arrow) where the width is as low as 189 nm, in other areas it is within normal range.

tailored treatments in 29%, had kidney transplant implications in 12% and enabled specific reproductive counselling in 24%. Finally, as healthcare systems worldwide are facing financial strains, the early WES model was cost-effective, with a cost reduction of close to 1500€ per patient tested [18]. A number of parameters (resistance to treatment, family history and extrarenal involvement) were predictive of a genetic diagnosis. Findings from renal biopsies were not reported in detail and it is unknown how they could have contributed to the diagnostic outcome.

In this issue of the *CKJ*, Robert *et al.* [2] published a study of 230 adult patients with UKD investigating the usefulness of ES to resolve UKD in routine care. Genomic sequencing was performed using a targeted bioinformatic customized kidney gene panel (675 genes). Of the 230 patients, 28 monogenic renal disorders were detected in 75 patients (32.6%), which is in line

with previous cohorts of patients with UKD. Collagenopathies were the most common genetic kidney diagnosis, accounting for 46.7% ($n = 35$) of diagnoses, with *COL4A3* and *COL4A4* accounting for 80% of these cases.

The 230 patients in the study were defined to have UKD according to the following criteria of absence: no biopsy-proven diagnosis, no specific morphological diagnosis (such as PKD) and no plausible renal diagnosis (such as diabetic kidney disease). In this study, 69 patients had an inconclusive renal biopsy, and mention is made of 71 patients in whom a renal biopsy was impossible. This leaves us with 90 patients in whom a biopsy was not even considered for unknown reasons. The underlying kidney disease of all 230 patients is regarded as unsolved, and they are subdivided into four groups: unclassified nephropathy; undetermined nephropathy; undetermined tubulointerstitial nephritis; and undetermined vascular nephropathy. Although kidney biopsy findings of the 69 patients who had an inconclusive renal biopsy must have contributed to this classification, it is unclear how the patients with biopsies are distributed among these four groups. In any case, underlying disease was unsolved for all 230 patients at the beginning of the study, and the question was to what extent ES could solve some of the uncertain diagnosis. By performance of ES, the authors claim that 75 of the cases were solved, whereas 155 remain unsolved. Among the 75 solved patients, 24 had a previously inconclusive kidney biopsy and it would have been of interest to make a comparison of the renal biopsy findings in relation to the findings of ES [2].

In the present study, besides genetic variants leading to collagenopathies, identified variants led to tubulointerstitial kidney disease in 16% ($n = 12$), ciliopathies in 12% ($n = 9$) and podocytopathies in 10.7% ($n = 8$), respectively. In line with reports of previous cohorts, the diagnostic yield of ES in the present study was especially high in patients with a family history of kidney disease, reaching 56.8% when the patient had both first- and second-degree family history of renal disease. Moreover, a molecular diagnosis was more common in female as opposed to male patients (54.5% versus 47%) [2]. The results emphasize that ES has a particularly high yield under certain clinical conditions, but that it does not provide the final answer in many other situations.

The study also underscores the importance of accurate diagnosis in UKD patients and the use of genetic testing to improve the diagnosis and decrease the percentage of patients reaching the need of KRT without diagnosis. It deserves a note of attention that in a group of 230 patients with a median age of 47.5 years and UKD, only 69 (30%) had a renal biopsy taken. It remains unknown whether a renal biopsy could have been diagnostic in the remaining 70%. In fact, it may be questioned why so many young patients in this cohort were deprived of the chance of getting a histological diagnosis. To emphasize the usefulness of ES, the risks and side-effects of renal biopsies are highlighted, but their usefulness is thereby underestimated. Misdiagnosis can lead to inappropriate treatment, and delays in correct diagnosis can lead to disease progression and worse outcomes. The use of ES in routine nephrological healthcare may therefore have significant clinical benefits for patients with CKD, but taking a renal biopsy in time, i.e. before extensive scarring precludes a correct diagnosis, should remain a consideration in spite of fear of rare complications. It needs to be emphasized that clinically meaningful complications, defined as bleeding requiring a transfusion, surgical/radiological intervention to stop the bleeding or to coil an arteriovenous fistula, or death, are extremely rare, especially in experienced centres [19]. In addition, the correct work-up of the renal biopsy should be a continuous point of attention. As mentioned by Robert *et al.*, peforming electron microscopy has become more and more difficult in France, and this is the technique by which renal diseases with a genetic background can be diagnosed in particular. The importance of electron microscopy has recently been exemplified by a report of four cases from a large US institution, and underlined that thorough histologic assessment was required to make a diagnosis [20]. There are several findings in a kidney biopsy that, although not pathognomonic, clearly suggest a diagnosis of an inherited kidney disease (Fig. 2).

We therefore conclude that genetic testing should become a regular diagnostic tool for the diagnosis of kidney diseases, in particular in those patients who have a family history of renal disease or who are therapy resistant. However, only in patients with a high suspicion of an inherited kidney disease could genetic testing eventually replace a kidney biopsy (Fig. 3). As mentioned above, we propose that patients with proteinuria and without extra-renal features or a positive family history should always undergo a diagnostic kidney biopsy, as this procedure not only helps making a final diagnosis, but also highlights potential differential diagnosis and helps understanding the prognosis of an individual. Electron microscopy is deemed necessary in cases with 'inconclusive' biopsy reports and in cases of diagnostic uncertainties.

CONFLICT OF INTEREST STATEMENT

R.T. is president-elect of ERA. She has consulted for AstraZeneca, Otsuka, Chiesi, Takeda, Sanofi, Alnylam, Amicus and Kyowa, and her research is funded by Fundació la Marató de TV3 202036-30, Instituto de Salud Carlos III PI 22/0031, RICORS40, Instituto de Salud Carlos III, Funded by EU-Next Generation and Mechanism of recuperation and resilience (MRR). A.K. consulted for CSL Vifor, Otsuka, GlaxoSmithKline, Catalyst Biosciences, Walden Biosciences and Delta4, and received research grants from CSL Vifor and Otsuka. I.M.B. consulted for Boehringer-Ingelheim, Novartis, Catalyst Biosciences and Toleranzia.

REFERENCES

1. Hays T, Groopman EE, Gharavi AG. Genetic testing for kidney disease of unknown etiology. *Kidney Int* 2020;**98**:590–600. https://doi.org/10.1016/j.kint.2020.03.031
2. Robert T, Raymond L, Dancer M *et al.* Beyond the kidney biopsy: genomic approach to undetermined kidney diseases. *Clin Kidney J* 2023
3. Devuyst O, Knoers NV, Remuzzi G *et al.* Rare inherited kidney diseases: challenges, opportunities, and perspectives. *Lancet* 2014;**383**:1844–59. https://doi.org/10.1016/S0140-6736(14)60659-0
4. Torra R, Furlano M, Ortiz A *et al.* Genetic kidney diseases as an underrecognized cause of chronic kidney disease: the key role of international registry reports. *Clin Kidney J* 2021;**14**:1879–85. https://doi.org/10.1093/ckj/sfab056
5. Skrunes R, Svarstad E, Reisæter AV *et al.* Familial clustering of ESRD in the Norwegian population. *Clin J Am Soc Nephrol* 2014;**9**:1692–700. https://doi.org/10.2215/CJN.01680214
6. Connaughton DM, Bukhari S, Conlon P *et al.* The Irish Kidney Gene Project—prevalence of family history in patients with kidney disease in Ireland. *Nephron* 2015;**130**:293–301. https://doi.org/10.1159/000436983

7. McClellan WM, Satko SG, Gladstone E *et al*. Individuals with a family history of ESRD are a high-risk population for CKD: implications for targeted surveillance and intervention activities. *Am J Kidney Dis* 2009;**53**:S100–6. https://doi.org/10.1053/j.ajkd.2008.07.059
8. Fox CS, Yang Q, Cupples LA *et al*. Genomewide linkage analysis to serum creatinine, GFR, and creatinine clearance in a community-based population: the Framingham Heart Study. *J Am Soc Nephrol* 2004;**15**:2457–61. https://doi.org/10.1097/01.ASN.0000135972.13396.6F
9. Arpegård J, Viktorin A, Chang Z *et al*. Comparison of heritability of cystatin C- and creatinine-based estimates of kidney function and their relation to heritability of cardiovascular disease. *J Am Heart Assoc* 2015;**4**:e001467. https://doi.org/10.1161/JAHA.114.001467
10. Gorski M, van der Most PJ, Teumer A *et al*. 1000 genomes-based meta-analysis identifies 10 novel loci for kidney function. *Sci Rep* 2017;**7**:45040. https://doi.org/10.1038/srep45040
11. Moulin F, Ponte B, Pruijm M *et al*. A population-based approach to assess the heritability and distribution of renal handling of electrolytes. *Kidney Int* 2017;**92**:1536–43. https://doi.org/10.1016/j.kint.2017.06.020
12. Lieske JC, Turner ST, Edeh SN *et al*. Heritability of urinary traits that contribute to nephrolithiasis. *Clin J Am Soc Nephrol* 2014;**9**:943–50. https://doi.org/10.2215/CJN.08210813
13. Zanetti D, Rao A, Gustafsson S *et al*. Identification of 22 novel loci associated with urinary biomarkers of albumin, sodium, and potassium excretion. *Kidney Int* 2019;**95**:1197–208. https://doi.org/10.1016/j.kint.2018.12.017
14. Gibson J, Fieldhouse R, Chan MMY *et al*. Prevalence estimates of predicted pathogenic *COL4A3-COL4A5* variants in a population sequencing database and their implications for Alport syndrome. *J Am Soc Nephrol* 2021;**32**:2273–90. https://doi.org/10.1681/ASN.2020071065
15. Barua M, Paterson AD. Population-based studies reveal an additive role of type IV collagen variants in hematuria and albuminuria. *Pediatr Nephrol* 2022;**37**:253–62. https://doi.org/10.1007/s00467-021-04934-y
16. Groen in 't Woud S, Rood IM, Steenbergen E *et al*. Kidney disease associated with mono-allelic *COL4A3* and *COL4A4* variants: a case series of 17 families. *Kidney Med* 2023;**5**:100607. https://doi.org/10.1016/j.xkme.2023.100607
17. Troost JP, Trachtman H, Nachman PH *et al*. An outcomes-based definition of proteinuria remission in focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 2018;**13**:414–21. https://doi.org/10.2215/CJN.04780517
18. Becherucci F, Landini S, Palazzo V *et al*. A clinical workflow for cost-saving high-rate diagnosis of genetic kidney diseases. *J Am Soc Nephrol* 2023;**34**:706–20. https://doi.org/10.1681/ASN.0000000000000076
19. Poggio ED, McClelland RL, Blank KN *et al*. Systematic review and meta-analysis of native kidney biopsy complications. *Clin J Am Soc Nephrol* 2020;**15**:1595–602. https://doi.org/10.2215/CJN.04710420
20. Yamashita M, Lin MY, Hou J *et al*. The continuing need for electron microscopy in examination of medical renal biopsies: examples in practice. *Glomerular Dis* 2021;**1**:145–59. https://doi.org/10.1159/000516831

Received: 28.7.2023; Editorial decision: 1.9.2023