ORIGINAL ARTICLE

The role of genomic disorders in chronic kidney failure of undetermined aetiology ≤50 years

Jeff Granhøj[1,2], Katja Venborg Pedersen[1], Mads Malik Aagaard[1],
Jesper Aagaard Graakjaer[1], Dorte Launholt Lildballe[3,4,6], Henrik Birn[4,5,6]
and Maria Rasmussen [1,2]

[1]Department of Clinical Genetics, Lillebaelt Hospital–University Hospital of Southern Denmark, Vejle,
Denmark, [2]Department of Regional Health Research, University of Southern Denmark, Odense, Denmark,
[3]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark, [4]Department of Clinical
Medicine, Aarhus University, Aarhus, Denmark, [5]Department of Renal Medicine, Aarhus University Hospital,
Aarhus, Denmark and [6]Department of Biomedicine, Aarhus University, Aarhus, Denmark

Correspondence to: Maria Rasmussen; E-mail: maria.rasmussen5@rsyd.dk

ABSTRACT

Background. Genomic disorders caused by copy number variations (CNVs) are prevalent in patients with kidney disease; however, their contribution to chronic kidney failure (KF) of undetermined aetiology (uKF) is unclear. We screened patients with uKF aged 50 years or younger to establish the prevalence of causative CNVs.

Methods. We enrolled patients with an onset of KF ≤50 years from suspected undetermined aetiology for initial review of medical records to exclude patients with clear-cut clinical or histopathological kidney diagnoses or patients with already established genetic kidney diseases. Next, we performed single nucleotide polymorphism (SNP) array–based CNV screening. All the detected CNVs were systematically classified and evaluated as possible causes of the patient's kidney disease. Patients with CNVs not explaining the kidney phenotype were additionally screened for causal variants in 540 genes using whole-genome sequencing.
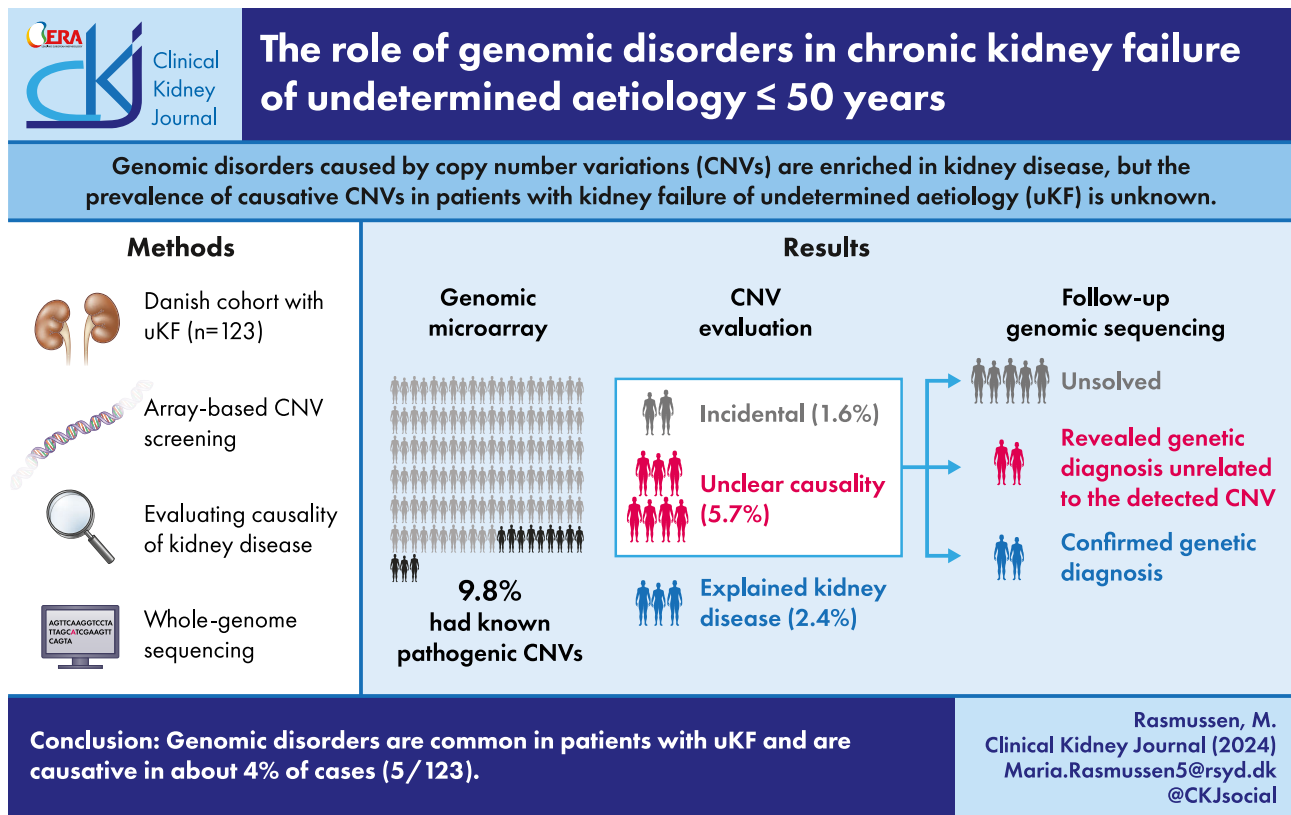
Results. We enrolled 172 patients, of whom 123 underwent SNP-array. Pathogenic CNVs corresponding to known genomic disorders were identified in 12 patients (9.8%). The identified genomic disorders provided a causative kidney diagnosis in three patients, all of whom had reached KF by age 18 years. The remaining nine patients had CNVs with unclear kidney disease causality. Subsequently, whole-genome sequencing provided a causative genetic diagnosis in an additional four patients, including two diagnostic sequence variants unrelated to the detected CNVs.

Conclusions. Genomic disorders were prevalent in this cohort with uKF, and causative CNVs were identified in 5 of 123 patients. Further studies combining the analysis of CNVs and sequence variants are needed to clarify the causal role of genomic disorders in kidney disease.

GRAPHICAL ABSTRACT

The role of genomic disorders in chronic kidney failure of undetermined aetiology ≤ 50 years

Genomic disorders caused by copy number variations (CNVs) are enriched in kidney disease, but the prevalence of causative CNVs in patients with kidney failure of undetermined aetiology (uKF) is unknown.

Methods
- Danish cohort with uKF (n=123)
- Array-based CNV screening
- Evaluating causality of kidney disease
- Whole-genome sequencing

Results

Genomic microarray

9.8% had known pathogenic CNVs

CNV evaluation
- Incidental (1.6%)
- Unclear causality (5.7%)
- Explained kidney disease (2.4%)

Follow-up genomic sequencing
- Unsolved
- Revealed genetic diagnosis unrelated to the detected CNV
- Confirmed genetic diagnosis

Conclusion: Genomic disorders are common in patients with uKF and are causative in about 4% of cases (5/123).

Rasmussen, M.
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Maria.Rasmussen5@rsyd.dk
@CKJsocial

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KEY LEARNING POINTS

What was known:
- Genomic disorders caused by copy number variations (CNVs) are more prevalent in patients with chronic kidney disease than controls, but the extent to which they cause kidney failure remains unclear and the prevalence of causative CNVs in patients with chronic kidney failure of undetermined aetiology (uKF) is unknown.

This study adds:
- We performed CNV screening in 123 patients with uKF ≤50 years. Microarray genotyping identified genomic disorders in 9.8%, but only provided a causative diagnosis in three patients. Follow-up whole-genome sequencing improved the diagnostic yield and uncovered alternative genetic explanations in some patients with CNVs not fully explaining their kidney phenotype.

Potential impact:
- Genomic disorders are common in patients with uKF ≤50 years and are causative in approximately 4% of cases.

INTRODUCTION

Copy number variations (CNVs) are structural variations in the genome resulting from deletions or duplications that can cause genomic disorders by changing normal gene dosage or disrupting gene structure [1]. Chromosomal microarray identifies known genomic disorders in 4% of non-syndromic children with chronic kidney disease (CKD) [2, 3], and CNVs are particularly prevalent in patients with congenital anomalies of the kidney and urinary tract (CAKUT) [4–7], a major cause of childhood-onset kidney failure (KF) [8]. The contribution of CNVs to CKD in adults remains unclear. Recently, known genomic disorders were identified in 1.1% of 6679 adults with all-cause CKD, demonstrating an excess burden of genomic disorders compared with 0.65% in 30 746 controls [2]. However, interpreting the causative, contributive or incidental nature of the genomic

disorders was complicated by the proportion of patients aged over 50 years and the proportion with diabetes. In comparison, 0.9% of 2794 adult kidney transplant recipients aged 18–50 years were homozygous for *NPHP1* (*Nephrocystin-1*) deletions on chromosome 2q13 [9] causing nephronophthisis. Interestingly, in these patients, CKD of unknown cause was the most common diagnosis, with only 12% being clinically diagnosed with nephronophthisis. This suggests that causative genomic disorders are underdiagnosed and that the clinical utility of CNV screening may be improved in younger patients with unexplained KF. Moreover, previous chromosomal microarray-based studies have been limited by their inability to detect smaller DNA sequence variants, which may clarify the role of the identified genomic disorders. Therefore, we used single nucleotide polymorphism (SNP)-array to screen for genomic disorders in a cohort of patients with KF of undetermined aetiology (uKF) ≤50 years of age. Additionally, we performed whole-genome sequencing in patients with genomic disorders that did not fully explain their kidney phenotypes.

MATERIALS AND METHODS

Study design and cohort recruitment

This was a national cross-sectional study examining the prevalence of causative genomic disorders in a cohort with uKF. We defined KF as either estimated glomerular filtration rate <15 mL/min or renal replacement therapy (kidney transplantation or dialysis) for ≥3 months and family history of KF as one or more first- or second-degree relative with reported KF. Similar to previous studies [10, 11], we defined uKF as the absence of (i) a plausible clinical diagnosis, (ii) a specific histopathological diagnosis or (iii) a specific structural kidney diagnosis (e.g. polycystic kidney disease). Patients with non-specific kidney morphology (e.g. renal hypodysplasia), non-specific kidney histology (e.g. focal segmental glomerulosclerosis) or hypertensive kidney disease were considered to have uKF.

Patients were recruited between February 2020 and May 2023. Most patients were identified from the Danish Nephrology Registry (*n* = 149/172, 87%) comprising all patients treated with chronic dialysis or kidney transplantation in Denmark since 1990 [12]. Adult patients living in the Region of Southern Denmark or the Regions of Central and Northern Jutland and registered with KF ≤50 years of age and primary renal disease (PRD) codes [13] consistent with uKF (Supplementary data, Table S1) were invited by letter to participate in the study (*n* = 574). A total of 265 patients (46.2%) responded to the letter invitation by either declining (*n* = 116) or accepting (*n* = 149). In addition, patients were recruited by responding to study advertisement in a newsletter from the Danish Kidney Association (*n* = 5) or by referral from clinical departments informed about inclusion/exclusion criteria of the project (*n* = 18).

The 172 patients enrolled in the study underwent initial eligibility assessment in which medical records and any kidney biopsy results were reviewed to rule out specific causes of kidney disease. Inclusion criteria for genetic screening were (i) onset of uKF ≤50 years or (ii) CKD of undetermined cause and a family history of onset of KF ≤50 years, and (iii) aged >18 years old at inclusion. Additionally, we excluded patients with genetic kidney disease that were already established by molecular genetic screening in the clinical setting.

SNP-array genotyping

Genomic DNA was isolated from peripheral venous blood samples using the Tecan Freedom EVO®-HSM system (Männedorf, Switzerland). All SNP-array analyses were performed using the HumanCytoSNP-12 DNA Analysis BeadChip v2.1 platform (Illumina, San Diego, CA, USA) comprising 301 232 SNP markers and allowing a lower CNV size detection limit of approximately 50 kb. DNA amplification, labelling and hybridization were performed according to manufacturer's protocol, and the beadchips were scanned on Illumina Nextseq 550. Genome wide CNVs were called using Bluefuse Multi v4.5 and KaryoStudio v1.4.3 (Illumina). Additionally, each dataset was manually inspected for CNVs potentially missed by the software. Only datasets with a LogRDev ≤0.3 in KaryoStudio and passing intensity probe quality controls filters in GenomeStudio v2011 (Illumina) were used for further analysis. Detected CNVs were mapped to the human reference genome Hg38/CRCh38 and annotated using Bluefuse Multi, KaryoStudio and Varseq v2.2.3 (Golden Helix, Bozeman, MT, USA). CNVs were classified according to the joint consensus recommendation of American College of Medical Genetics guidelines and the Clinical Genome Resource [14] and evaluated as possible causes of kidney disease by comparing the associated phenotypes reported in the literature or publicly available databases (see Supplementary Methods) with the phenotype of the patient. CNVs overlapping genomic disorders with well-established causal relation to kidney disease were considered causative. The causality was considered "unclear" in CNVs overlapping genomic disorders previously reported in cohorts with kidney disease if (i) the reported kidney disease did not match the patient phenotype, (ii) it did not include a known kidney disease–associated gene or (iii) only detecting one pathogenic variant associated to well-known kidney disease with autosomal recessive inheritance. CNVs overlapping genomic disorders with no direct relation to kidney disease were considered incidental findings. Detection of only benign or likely benign CNVs were reported as "normal."

Single whole-genome sequencing

A detailed description is provided in the Supplementary Methods. Briefly, genomic DNA was used to construct a whole-genome library (xGen DNA Lib Prep EZ UNI, Integrated DNA Technologies, Coralville, IA, USA), which was sequenced on NovaSeq 6000 (Illumina, San Diego, CA, USA) with paired-end 2*150 base pairs and dual indexing to a mean read coverage of 30×. Raw data were aligned to the human reference genome (hg38/GRCH38). Single-nucleotide variations, minor nucleotide insertions/deletions and CNVs were called using an in-house bioinformatics pipeline and annotated in VarSeq 2.3.0 (Golden Helix, Bozeman, MT, USA). Data analysis was restricted to 540 genes associated with kidney disease or hypertension (Supplementary data, File S1). Variants were classified according to the ACMG criteria for sequence variants [15], with only C4 (likely pathogenic) and C5 (pathogenic) variants explaining the patient's kidney phenotype being considered diagnostic.

Ethics

The study approved by the Danish National Committee on Health Research Ethics (1 906 020) and conducted in accordance with the Helsinki Declaration. All patients received genetic counselling and gave written informed consent before participation.
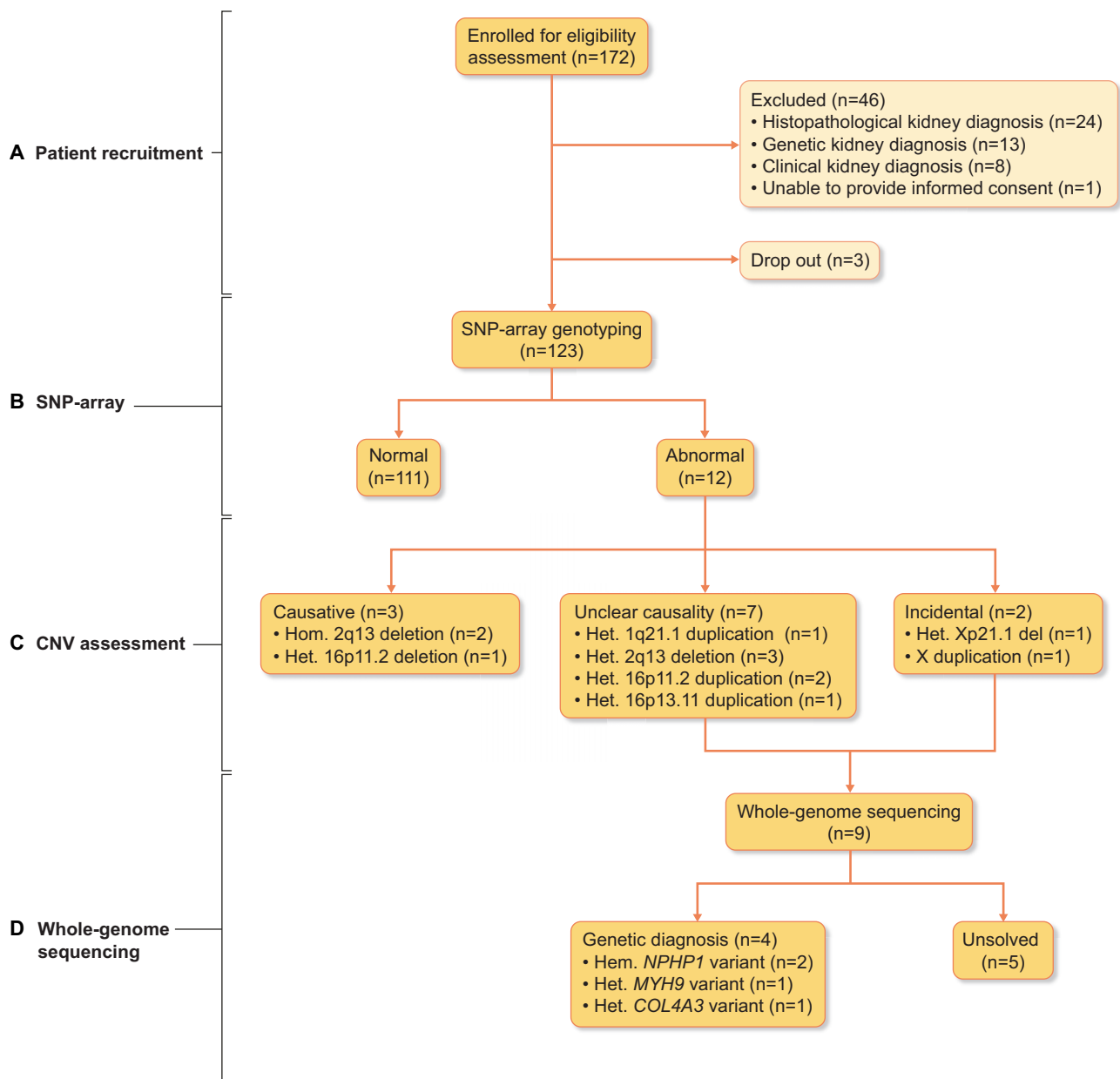
Figure 1: Study flow chart. (**A**) A total of 172 patients were enrolled for eligibility assessment based on inclusion and exclusion criteria. Of these, 46 patients were excluded, and another 3 patients dropped out due to not providing a blood sample for genetic analysis. (**B**) SNP-array genotyping were performed on 123 patients of which 111 were normal (90%) and 12 were abnormal (10%) due to identification of a pathogenic CNV. (**C**) The CNVs and corresponding genomic disorders were assessed for their causative, unclear causality or incidental relationship with the patient's kidney phenotype. (**D**) The patients carrying CNVs with unclear causality (*n* = 7) or considered incidental (*n* = 2) underwent whole-genome sequencing to screen 540 genes associated with kidney disease or hypertesion. A genetic diagnosis were obtained in four patients while five patients remained unsolved. Representative comparisons of CNV calls in SNP-array data vs whole-genome sequencing data are provided in Supplementary data, Figs S1 and S2. Hom, homozygous; Hem, hemizygous; Het, heterozygous.

Statistics

Statistical analyses were performed using STATA 17 (StataCorp LLC, College Station, TX, USA), and a two-tailed P-value <.05 was considered statistically significant. Baseline characteristics were expressed as frequencies, percentages and medians with interquartile ranges. Fischer's exact test was performed for all comparisons of categorical variables. Wilcoxon rank-sum test was used to compare median age of KF between patients with and without CNVs.

RESULTS

Cohort characteristics

We initially enrolled 172 unrelated patients into the study for eligibility assessment. Forty-six were subsequently excluded and three dropped out (Fig. 1A). The baseline characteristics of the final cohort comprising 123 patients is shown in Table 1. The median age of KF was 37 (range 8–50) years, with 91% of the cohort having adult-onset KF. Most patients had PRD codes

Table 1: Cohort baseline characteristics ($n = 123$).

Cohort characteristics	
Sex	
Male	66 of 123 (53.6%)
Female	57 of 123 (46.3%)
PRD codes[a,b]	
Chronic kidney disease of unknown/unspecified aetiology	46 of 109 (42.2%)
Hypertensive kidney disease	12 of 109 (11.0%)
Glomerular kidney disease	36 of 109 (33.0%)
Congenital dysplasia/hypoplasia	5 of 109 (4.6%)
Tubulointerstitial kidney disease	6 of 109 (5.5%)
Familial nephropathy	4 of 109 (3.7%)
Age of KF[c]	
Median	37 years
Mean	35 years
Range	8–50 years
<18 years	11 of 122 (9.0%)
≥18 years	111 of 122 (91.0%)
Hypertension at time of kidney disease diagnosis	55 of 123 (44.7%)
Native kidney biopsy performed	50 of 123 (40.6%)
Family history of KF	24 of 123 (19.5%)

[a]PRD codes were only available from 109 patients recruited from the Danish Nephrology Registry.
[b]Similar PRD codes are pooled in phenotype groups: chronic kidney disease of unknown/unspecified aetiology (3555, 3564, 3691, 3708), hypertensive kidney disease (2359), glomerular kidney disease (1061, 1267, 1377, 3749), tubulointerstitial kidney disease (1884, 1897, 2005), congenital dysplasia/hypoplasia (1625) and familial nephropathy (2804, 3295, 3379).
[c]The total of patients with KF was $n = 122$, as one patients had uCKD not requiring renal replacement therapy and a family history of KF before age 50 years.

corresponding to uCKD or undetermined glomerular kidney disease. Notably, only 11% were registered with hypertensive kidney disease, although medical records revealed that 45% of the patients were hypertensive at the time of being diagnosed with kidney disease. A native kidney biopsy had been performed in 40%, and 20% of the patients reported a family history of KF.

Prevalence of genomic disorders in uKF cohort

We identified pathogenic CNVs corresponding to known genomic disorders in 12 of 123 patients (9.8%), while the remaining 111 SNP-arrays were normal (90.2%), with no rare CNVs of unknown significance detected (Fig. 1B). We further evaluated whether the identified CNVs explained the patient's kidney phenotype (Fig. 1C, Table 2). Three CNVs provided a causative kidney disease diagnosis, including two patients with homozygous *NPHP1* deletions (*NPHP1*-nephronophthisis) and one patient with a heterozygous 16p11.2 deletion (*TBX6*-associated CAKUT [7, 16, 17]). Seven patients had CNVs with an uncertain causal relationship to their kidney phenotype, including three patients with heterozygous *NPHP1* deletions and four patients carrying heterozygous microduplications previously associated with CKD [2, 3] or CAKUT [4, 6, 7]. Finally, two CNVs were incidental findings unrelated to the kidney disease, including a female carrier of a Xp21.1 deletion and a 47,XXY aneuploidy. We found no statistically significant differences when comparing patients with abnormal vs normal SNP-array (Table 3).

Whole-genome sequencing clarifies the role of genomic disorders in uKF

Next, we performed whole-genome sequencing in nine patients with CNVs of uncertain kidney disease significance (Fig. 1D), providing a causative diagnosis in four additional patients (Table 4). In patients F257 and F441, we re-identified the heterozygous *NPHP1* deletions and found additional variants in the remaining *NPHP1* gene. In patient F520, we found a known pathogenic variant in *MYH9* that fully explained the phenotype of thrombocytopenia, proteinuric kidney disease and hearing loss. In patient F429, we identified a likely pathogenic *COL4A3* variant consistent with slowly progressive proteinuric kidney disease. We failed to identify a genetic explanation in the remaining five patients. Additionally, we screened patient F67 and re-identified the 16p11.2 deletion along with a previously reported hypomorphic haplotype on the other gene allele [7, 16] (Table 4, Supplementary data, Fig. S1), solidifying the diagnosis of *TBX6*-associated CAKUT.

DISCUSSION

We performed SNP-array-based CNV screening in a cohort with uKF ≤50 years to explore the role of genomic disorders. Using stand-alone SNP-array, we found pathogenic CNVs associated with known genomic disorders in 9.8% of the cohort, but could only provide a causative diagnosis in three out of 123 patients (2.4%). In comparison, Verbitsky *et al.* [2] identified genomic disorders in 1.1% of adults with all-cause CKD, with 17q12 deletions being one of the most common genomic disorders. Besides the differences in sample size, the higher prevalence reported in our study may be explained by the accumulation of genomic disorders in younger patients with KF compared with earlier stages of CKD and older patients. Additionally, we did not find any patients with 17q12 deletions, which may have been due to the exclusion of patients with diabetic nephropathy in our study. However, most identified genomic disorders in our cohort had an unclear causal relationship with the patient's kidney phenotype and required additional analysis to identify a genetic cause. Excess of heterozygous *NPHP1* deletions have also been observed in kidney transplant recipients when compared with kidney transplant donors [9], indicating the presence of undetected pathogenic variants on the remaining *NPHP1* allele. Indeed, we found that two out of the three heterozygous *NPHP1* deletion carriers had additional *NPHP1* variants. Thus, *NPHP1*-nephronophthisis was the most frequent genetic diagnosis in our cohort, with half presenting with KF in adulthood.

Whole-exome and whole-genome sequencing is gradually replacing traditional microarray-based methods for CNV screening and becoming first-tier screening tests as they allow for simultaneous detection of both CNVs as well as sequence variants, producing an overall higher diagnostic yield [18] and a cost-saving screening strategy [19]. However, there is a discrepancy between CNVs detected by microarray versus whole-exome/whole-genome sequencing, as the latter is often restricted to CNVs containing specific genes of interest. Our study evaluate all CNVs previously associated to genomic disorders and followed up with whole-genome sequencing in selected patients. Indeed, we identified microduplications of 1q21.1, 16p11.2 and 16p13.11, which have been previously detected by microarray in large cohorts with CKD [2, 3] or CAKUT [4, 6, 7]. However, in contrast to deletions of *NPHP1* and 16p11.2, they were not

Table 2: Genomic disorders identified by SNP-array in patients with uKF.

Family ID	Sex	KF age (years)	Clinical phenotype	Genomic region	CNV type	Start (Mb)	End (Mb)	Size (Mb)	Zygosity	Causality
F66	M	17	Presented with chronic uKF and small kidneys, no KB or FH	2q13	Del	110.10	110.22	0.12	Hom	Causative
F406	F	15	uCKD accompanied by growth stagnation, fatigue, polyuria/polydipsia, nocturnal enuresis, inconclusive KB, no FH	2q13	Del	110.10	110.22	0.12	Hom	Causative
F67	M	18	uCKD with solitary hypodysplastic right kidney, no KB or FH	16p11.2	Del	29.63	30.18	0.55	Het	Causative
F110	M	48	Medical history and KB consistent with hypertensive kidney disease, father with late-onset KF	1q21.1	Dup	145.43	149.09	3.66	Het	Unclear
F595	M	46	Nephrectomized due to Wilms tumor in childhood, progressive CKD with proteinuria, no KB or FH	2q13	Del	109.97	110.63	0.66	Het	Unclear
F257	F	30	Presented with uKF and small kidneys, no KB or FH	2q13	Del	110.10	111.64	0.54	Het	Unclear
F441	F	40	Presented with uKF and small kidneys, no KB or FH	2q13	Del	110.09	110.22	0.13	Het	Unclear
F422	F	31	uKF following pre-eclampsia, regained normal renal function, no KB or FH, pulmonic sarcoidosis	16p11.2	Dup	28.81	29.13	0.32	Het	Unclear
F434	F	17	uCKD with small kidneys, no KB or FH, perceptive hearing loss	16p11.2	Dup	28.81	29.01	0.20	Het	Unclear
F520	M	20	uCKD with proteinuria, no KB or FH, idiopathic thrombocytopenic purpura, hearing loss	16p13.11	Dup	15.03	16.19	1.16	Het	Unclear
F429	F	48	uCKD with proteinuria and hypertension, small kidneys, no KB, brother with KF	Xp21.1	Del	32.62	33.00	0.38	Het	Incidental
F507	M	50	KB with FSGS, no FH	X[a]	Dup	0.093	155.12	155.02	Het	Incidental

Genomic coordinates are based on UCSC genome build hg38. SNP-array genotypes and lists of protein-coding genes located in the detected CNVs are provided in Supplementary data, Table S3. Causality is an assessment of whether the identified CNV and corresponding genomic disorder provides an explanation for the kidney phenotype: "Causative" refers to CNVs overlapping genomic disorders with a well-established causal relation to kidney disease; "Unclear" refers to CNVs overlapping genomic disorders previously reported in cohorts with kidney disease in which the causal relation is considered unclear or not fully explaining the kidney phenotype; "Incidental" refers to CNVs overlapping genomic disorders that are considered incidental findings unrelated to kidney disease.

[a] Aneuploidy with gain of an entire chromosome X.

Del, deletion; Dup, duplication; F, female; FH, family history of KF; Hom, homozygous; Het, heterozygous; KB, kidney biopsy; M, male; uCKD; chronic kidney disease of undetermined aetiology.

Table 3: Comparison: of characteristics in patients with normal versus abnormal SNP-array ($n = 123$).

Variable	Abnormal SNP-array ($n = 12$)	Normal SNP-array ($n = 111$)	P-value
Male sex, n (%)	6 of 12 (50.0%)	60 of 111 (54.0%)	1.000[b]
Age of KF[a], median (IQR)	30.5 (17.5–47.0)	37.5 (28.0–45.0)	.407[c]
Childhood-onset KF, n (%)	3 of 12 (25.0%)	8 of 111 (7.2%)	.075[b]
Hypertension at initial kidney disease diagnosis, n (%)	4 of 12 (33.3%)	51 of 111 (45.9%)	.545[b]
Kidney biopsy, n (%)	4 of 12 (33.3%)	46 of 111 (41.4%)	.760[b]
Family history of KF, n (%)	2 of 12 (16.7%)	22 of 111 (19.8%)	1.000[b]

[a]$n = 110$ in the group with normal SNP-array, as one patients had uCKD not requiring renal replacement therapy at inclusion and a family history of KF before age 50 years.
[b]Fisher's exact test, two-tailed P-value.
[c]Two sample Wilcoxon rank sum test.
IQR, interquartile range; KF, kidney failure; uCKD; chronic kidney disease of undetermined aetiology.

associated with KF in the UK Biobank Study comprising >380 000 individuals [20]. The microduplications are low-penetrant with variable phenotypes mainly associated with CAKUT [7], possibly explaining their similar frequencies in controls without CKD [2, 7]. Additionally, specific kidney disease-associated genes in these microduplications have not been established. Using whole-genome sequencing, we identified a causative *MYH9* variant that was unrelated to the 16p13.11 duplication initially identified in the same patient. Similarly, we identified a pathogenic *COL4A3* variant in a woman also carrying an Xp21.1 deletion. These examples highlight that the genomic disorders identified in patients with CKD are not necessarily causal. It is possible that some genomic disorders accumulated in CKD are not the primary cause of kidney disease but may be risk factors contributing to the development of CKD or simply incidental. Large-scale health database initiatives like Genomic England, UK Biobank and All Of Us—containing genomic and clinical data—will be great resources in clarifying these associations in future studies.

We further identified a causative 16p11.2 microdeletion in patient F67 along with a hypomorphic *TBX6* allele in the remaining gene copy. *TBX6* has been identified as the underlying CAKUT-associated gene in 16p11.2 deletions, displaying a complex compound heterozygous recessive inheritance in both humans and mice [7, 16, 17]. This illustrates another dimension of complexity in deciphering the relationship between CNVs and the observed phenotypes, as common genetic variants on the other chromosome may modify the disease phenotype or penetrance.

Our study was limited by its small sample size. Additionally, there is a risk of enrolment bias, as patients with genomic disorders that impair social and neurocognitive skills [2, 21] in addition to causing CKD, may have lower participation rates. The study is strengthened by the combination of SNP-array with whole-genome sequencing in patients with non-causative CNVs, detecting both CNVs and other types of genetic variants. This allows for the identification of alternative genetic explanations and uncovers the complexity of interpreting genomic disorders in CKD.

To conclude, genomic disorders were frequent in this cohort with uKF $\leq$50 years, but the identified CNVs only explained the kidney disease in 5/123 (4%) patients, with *NPHP1* deletions being the most common. Chromosomal microarray has methodological shortcomings as a stand-alone screening method compared with whole-genome sequencing, and this study highlights the importance of critically evaluating the causality assessment of identified CNVs in patients with kidney disease as causative sequence variants may otherwise be missed in patients carrying prevalent CNVs. Further studies are also needed to clarify the accumulation of genomic disorders in patients with uKF.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

AUTHORS' CONTRIBUTIONS

J.G., H.B., D.L.L. and M.R. conceived and designed the study. J.G., K.V.P. and M.R. recruited patients and acquired data. J.G., J.A.G., M.A.A. and M.R. conducted genetic analyses and analysed genetic data. J.G. drafted the article with critical input from co-authors. All authors participated in analysis and interpretation of the data and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Complete SNP-array and whole-genome sequencing data cannot be shared publicly to safeguard the anonymity of the study participants, but genomic data will be deposited at the Danish National Genome Center according to Danish law. The remaining data will be shared upon reasonable request to the corresponding author.

Table 4: Diagnostic sequence variants identified by whole-genome sequencing in patients with CNVs.

Family ID	Gene	Disease	Genomic loci	Variant (HGVS)	Zygosity[a]	REVEL Score	SpliceAI Score	Functional studies	ACMG criteria[b]	Classification[c]	Previosly reported	Causality
F257	NPHP1	NPHP1-nephronophthisis	2q13	NM_001128178.3:c.1362_1363dupGC p.Leu455Argfs*4	Hem				PVS1, PM2, PM3	P	No	Causative
F441	NPHP1	NPHP1-nephronophthisis	2q13	NM_000272.5: c.1309T>G p.Trp437Gly	Hem	0.87			PM2, PM3, PP3 (moderate)	VUS	No	Uncertain
F429	COL4A3	Autosomal dominant Alport syndrome	2q36.3	NM_000091.5:c. 765G>A p.?	Het		0.90	Skipped exon 13 in minigene assay [22]	PVS1, PS3, PM2, PP3 (moderate),	P	[22, 23]	Likely causative
F520	MYH9	MYH9-related disease	22q12.3	NM_002473.6:c. 287C>T p.Ser96Leu	Het	0.935			PS2, PM1 (moderate), PM2, PP3 (strong)	P	[24]	Causative
F67	TBX6	TBX6-associated CAKUT	16p11.2	NM_004608.4: c.1227G>A p.Pro490= NM_0.004608.4:c.-48-240A>G NM_004608.4:c.-49+34G>T	Hem			Haplotype increases risk of CAKUT phenotype in mice [7, 16]			[7, 16]	Causative

Genomic coordinates are based on UCSC genome build hg38. Causality is an assessment of whether the identified CNV and corresponding genomic disorder provides an explanation for the kidney phenotype. The heterozygous 2q13 deletions involved NPHP1 and the heterozygous deletion of 16p11.2 involving TBX6 were re-identified using whole-genome sequencing. Representative examples of deletions called by SNP-array vs. whole-genome sequencing are provided in Supplementary data, Figs S1 and S2.

[a]Variants were reported as hemizygous when other gene copy was located within a genomic deletion.

[b]Full-filled ACMG criteria[15], PM2 is a supporting criterion, REVEL score and SpliceAI were used for PP3 criteria:

REVEL score ≥0.932 , PP3_Strong

REVEL score (0.773, 0.932) , PP3_Moderate

REVEL score (0.644, 0.773) , PP3_Supporting

REVEL score <0.15 , BP4_Supporting

SpliceAI score >0.9 PP3_strong

SpliceAI score >0.8 PP3_moderate

SpliceAI score >0.5 PP3_Supporting

SpliceAI score <0.1 , BP4_Supporting

[c]Classification according to ACMG/AMP guidelines for sequence variants [15].

ACMG/AMP, American College of Medical Genomics and Association for Molecular Pathology; Hem, hemizygous; Het, heterozygous; HGVS, Human Genome Society Variation nomenclature; LP, likely pathogenic; P, pathogenic; VUS, variant of uncertain significance.

CONFLICT OF INTEREST STATEMENT

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