BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-057829
Article Type:	Protocol
Date Submitted by the Author:	29-Sep-2021
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Keywords:	NEPHROLOGY, End stage renal failure < NEPHROLOGY, Chronic renal failure < NEPHROLOGY, GENETICS

Diagnostic yield of massively parallel sequencing in patients with chronic kidney disease of unknown etiology: Rationale and design of a national prospective cohort study

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Word count: 2883

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ABSTRACT

Introduction: Chronic kidney disease (CKD) can be caused by a variety of systemic or primary renal diseases. The cause of CKD remains unexplained in approximately 20% of patients. Retrospective studies indicate that massively parallel sequencing (MPS)-based gene panel testing may lead to a genetic diagnosis in 12-56% of patients with unexplained CKD, depending on patient profile. The diagnostic yield of MPS-based testing in a routine healthcare setting is unclear. Therefore, the primary aim of the VARIETY study is to prospectively address the diagnostic yield of MPS-based gene panel testing in patients with unexplained CKD and an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 before the age of 50 years in clinical practice.

Methods and analysis: The VARIETY study is an ongoing, prospective, nation-wide observational cohort study to investigate the diagnostic yield of MPS-based testing in patients with unexplained CKD in a routine healthcare setting in the Netherlands. Patients are recruited from outpatient clinics in hospitals across the Netherlands. At least 282 patients will be included to meet the primary aim. Secondary analyses include subgroup analyses according to age and eGFR at first presentation, family history, and the presence of extra-renal symptoms.

Ethics and dissemination: Ethical approval for the study has been obtained from the institutional review board of the University Medical Center Groningen. Study findings should inform physicians and policymakers towards optimal implementation of MPS-based diagnostic testing in patients with unexplained CKD.

ARTICLE SUMMARY

Strengths and limitations of this study

- First prospective study to examine the diagnostic yield of massively parallel sequencing in patients (age 18-50 at first presentation) with unexplained chronic kidney disease in a routine healthcare setting
- Nation-wide study with relatively large sample size, allowing analyses of specific subgroups according to age and kidney function at first presentation
- Study findings should inform physicians and policymakers in the implementation of gene panel testing in adults (age < 50) with unexplained CKD
- A potential limitation is that the definition of 'unexplained chronic kidney disease' is not unequivocal

INTRODUCTION

Chronic kidney disease (CKD) affects 11-16% of the population worldwide [1–3], is associated with extensive co-morbidity and an increased risk of premature mortality, and may ultimately result in end-stage kidney disease (ESKD) requiring dialysis or transplantation [4,5]. CKD may be caused by a variety of systemic (e.g., diabetes, hypertension) or primary renal diseases (e.g. IgA nephropathy, membranous nephropathy). Current diagnostic approaches, including kidney biopsy, are often non-specific or inconclusive, contraindicated or omitted due to lack of clinical consequences [6,7]. Therefore, the cause of CKD remains unknown in approximately 20% of patients with ESKD [8–10]. However, knowledge of the underlying kidney disease can be pivotal as it may influence prognosis and medical treatment. In the setting of kidney transplantation, it may influence (living-related) donor selection and post-transplant recurrence risk. Approximately 27-34% of patients with CKD report a positive family history of kidney disease (first or second degree relative with CKD) [11,12] and a genetic cause can be identified in at least 10% of adults with CKD [13,14], indicating that in many cases a hereditary origin for the disease should be considered. Genetic testing could therefore be a valuable tool in the diagnostic process of CKD of unknown etiology.

Recent studies suggest that massively parallel sequencing (MPS) techniques (previously referred to as next-generation sequencing) [15] could be used as diagnostic tool in adults with unexplained CKD and should even be considered as first mode of diagnostics in patients with ESKD prior to the age of 50 years [16]. Depending on patient selection, MPS led to a genetic diagnosis in 12-56% of patients with unexplained CKD [14,17–19]. However, most of these studies have been performed in a research setting, and therefore little is known about the diagnostic utility of MPS for adults with unexplained CKD in a routine healthcare setting. Moreover, currently available studies have been commonly based on subgroups of larger retrospective cohorts, and are heterogeneous in design and selection of genes used in

MPS [20]. For this reason, it is difficult to define profiles of patients (e.g. based on age and severity at disease onset, extra-renal manifestations, positive family history) that should preferentially undergo genetic testing. A recent joint publication by the ERA Working Group on Inherited Kidney Disorders (WGIKD) and the Molecular Diagnostics Taskforce of the European Rare Kidney Disease Reference Network (ERKNet) called for further research to explore the diagnostic yield of genetic testing in CKD of unknown origin in a clinical setting [21].

Therefore, the objective of this national prospective cohort study is to determine the diagnostic yield, i.e. the percentage of participants with a genetic diagnosis, using a large MPS-based multi-gene panel for kidney diseases in young patients (first presentation at age 18-50) with unexplained CKD (eGFR <60 mL/min/1.73 m^2) in a routine healthcare setting. In addition, we aim to identify specific patient profiles with a high diagnostic yield. These findings can guide physicians and policymakers in implementing MPS-based diagnostic testing in patients with unexplained CKD.

METHODS AND ANALYSIS

Study design

The VARIETY (Validation of algoRithms and IdEnTification of genes in Young patients with unexplained chronic kidney disease) study is a prospective nation-wide observational cohort study designed to investigate the diagnostic yield of genetic testing in patients with unexplained CKD in a routine healthcare setting in the Netherlands. The study will collect and analyze data obtained during routine clinical practice and through a questionnaire. Participants will be included from both academic and non-academic hospitals throughout the Netherlands. The anonymized data are collected, stored and analyzed in the University Medical Center Groningen (UMCG). All participants will give written informed consent on enrolment.

Study population

The targeted study population consist of all patients with unexplained CKD and an eGFR <60 ml/min/1.73m^2 before the age of 50 years. Unexplained CKD is defined as the absence of all the following criteria: a biopsy-proven diagnosis (e.g. IgA nephropathy), a specific morphological renal diagnosis (e.g. polycystic kidney disease suspected of autosomal/recessive polycystic kidney disease), or a specific or plausible renal diagnosis (e.g. history of long-term insulin-dependent diabetes mellitus before the onset of CKD, lithium-induced nephropathy). Since hypertensive nephropathy is a non-specific diagnosis and hypertension is also a very common consequence of CKD [22], patients with hypertensive nephropathy in the absence of a clear underlying disorder such as renal artery stenosis are considered to have unexplained CKD. Patients with renal hypoplasia, renal atrophy, and non-specific histological conditions (such as secondary focal segmental glomerulosclerosis,

glomerulonephritis of unknown cause, or interstitial nephritis) are also considered to have unexplained CKD.

Patients with a current age >50 years, but who presented with an eGFR <60 ml/min/1.73m^2 before the age of 50 years, and renal transplant recipients who had a pre-transplant eGFR <60 ml/min/1.73m^2 before the age of 50 years are also eligible for inclusion. In addition, genetic testing with a specific MPS-based gene panel (see 'Genetic testing') is required for participation. Exclusion criteria for participation in the VARIETY study are: age <18 years at time of inclusion or patients who do not give or are unable to give informed consent for genetic testing or for the current study.

Recruitment

Patients are recruited from outpatient clinics in both academic and non-academic hospitals across the Netherlands. Patients will be screened by nephrologists or trained study investigators. In case of a study investigator, a list with potential participants will be sent to the treating nephrologist to confirm the diagnosis of unexplained CKD. Eligible participants will be informed about the study by the investigators or their treating nephrologists aware of the study protocol. A study investigator or treating nephrologist will ask for informed consent for this study. Information for patients has been made available in the form of a patient information folder and a website (in Dutch): www.onbegrepennierziekte.nl.

Data collection

Detailed clinical and demographic data are collected from patients' electronic health record (EHR) and through a questionnaire following informed consent. The data will subsequently be entered into a secure electronic case report form.

Electronic health record

The following information will be collected from the EHR: age at inclusion, sex, primary renal disease diagnosis, age at CKD onset/presentation, dialysis or kidney transplantation, age at start dialysis or kidney transplantation, presence of extra-renal features, medication use at inclusion, medical history, family history (including three-generation pedigree), blood pressure at CKD onset and at inclusion, presence of hematuria and/or nephrotic syndrome, laboratory results (serum creatinine, eGFR, total cholesterol, HDL, LDL, triglycerides, 24 hour urine albumin excretion, 24 hour urine creatinine excretion, 24 hour urine total protein excretion, hematuria) at CKD onset/presentation and inclusion, renal histopathology and imaging of the native kidneys, and results of genetic testing in relation to kidney disease. We will also collect information regarding the clinical consequences of a genetic diagnosis and if genetic counseling was performed by a nephrologist or clinical geneticist. If participants are referred to a clinical geneticist, we will also collect the results of any additional genetic testing.

Questionnaire

Data collected from the EHR will be expanded with a questionnaire to collect additional data on family history, medical history, current health complaints, and extra-renal manifestations (Supplemental Table 1).

Genetic testing

We will include patients who have undergone MPS-based multi-gene panel testing, initiated by a clinical geneticist or nephrologist following pre-test counseling as part of clinical care in patients with unexplained CKD, in accordance with guidelines in the Netherlands [23]. Figure 1 shows the suggested flowchart for genetic testing in the VARIETY study, based on these

recommendations. The criteria as shown in this flow chart are slightly more liberal than the published recommendations, which will help to define the optimal age and eGFR ranges where genetic testing is still of clinical benefit. To stimulate the implementation of the guideline, we made a website for the VARIETY study (www.onbegrepennierziekte.nl) where nephrologists can find information about genetic testing and pre-test genetic counseling.

In order to reduce heterogeneity in the diagnostic approach, we will assess the diagnostic yield of a specific MPS-based gene panel, namely the 'CKD-Y' ('Chronic Kidney Disease in Young patients') targeted exome sequencing (ES) panel available at the University Medical Center (UMC) Utrecht, The Netherlands. The older version of this panel (v18) contains 141 different genes associated with early-onset CKD, including *PKD1* and *PKD2* (Figure 2). On March 8 2021, the CKD-Y panel was updated (v21) and the number of genes changed from 141 to 256 (Figure 3). This panel was chosen as it is an ES-based panel and contains all the current genes associated with early-onset CKD. In addition, this panel can be ordered by nephrologists without referral to the clinical geneticist. Alternatively, the hereditary kidney disease panel from UMC Utrecht was allowed. This is another targeted ES panel, consisting of 379 genes in the v18 version and 495 genes in the updated v21 version (Supplementary Figure 1-2). Since this panel contains some kidney cancer oncogenes, it may only be ordered by a clinical geneticist. The hereditary kidney disease panel includes all genes of the CKD-Y panel, making it possible to determine if a variant could also have been identified with the CKD-Y panel. Potential findings from the hereditary kidney disease panel that do not overlap with the CKD-Y panel will not be included in the analyses. We will record which version of the CKD-Y and/or hereditary kidney disease panel was used.

Primary and secondary analyses

The primary analysis will address the diagnostic yield of the CKD-Y panel, defined as the percentage of positive test results (i.e. pathogenic variant(s) explaining the cause of the disease), in the overall cohort of patients with unexplained CKD and an eGFR <60 mL/min/1.73 m^2 between 18 and 50 years. The pathogenicity of variants will be determined according to the standards and guidelines from the American College of Medical Genetics and Genomics (ACMG) [24]. With this standard, variants are classified into five categories using several lines of evidence, such as available literature, patient databases and *in silico* prediction programs. Class 1 variants are clearly not pathogenic; class 5 variants are clearly pathogenic. Class 3 are variants of uncertain significance/pathogenicity (VUS), these variants do not confirm or exclude the diagnosis (Table 1) [24]. For the determination of the diagnostic yield, only class 4 and class 5 variants will be considered as a 'positive test result' to determine the diagnostic yield. In cases with two class 4/5 variants in an autosomal recessive gene, these will only be considered a 'positive test result' if testing in parents has confirmed the variants are positioned in trans.

Table 1. Classification of variants according to ACMG guidelines [24]

Class	Description
1	Clearly not pathogenic, common polymorphism
2	Unlikely to be pathogenic, diagnosis not confirmed molecularly
3	Unknown significance/pathogenicity, does not exclude or confirm diagnosis
4	Likely to be pathogenic, consistent with the diagnosis
5	Clearly pathogenic, result confirms the diagnosis

Secondary analyses include subgroup analyses according to age and eGFR at first presentation, family history, and the presence of extra-renal symptoms. A positive family history for CKD is recorded if the participant either has a first (parent or child), second (siblings, grandparents, grandchildren), third (aunts, uncles, nephews, nieces) or fourth (cousins) degree relative with CKD. Family history will be obtained from combining information present in the EHR with information obtained from the questionnaire. Other secondary analysis aims to define the percentage of genetic tests with a clinical consequence. A genetic diagnosis is considered to have a clinical consequence if it: 1) negated the need for kidney biopsy, 2) triggered or negated the need for immunosuppressive therapy, 3) provides prognostic information, i.e. the risk of post-transplantation anti-GBM glomerulonephritis, 4) led to, or should lead to, referral to other specialties (e.g. ophthalmologist), 5) led to targeted work-up for associated symptoms or extra-renal manifestations, 6) affected surveillance frequency, 7) led to, or should lead to, genetic testing in potential living related kidney donors, 8) enabled more precise (preconception) genetic counseling for the patient or family members, or 9) led to more precise or extensive follow-up of potentially affected family members.

Tertiary outcomes will be the percentage of participants in which a VUS was identified and the number of incidental/secondary findings (results unrelated to the initial indication for genetic testing). In addition, if a molecular diagnosis is identified and a kidney biopsy from the native kidney is present, we will assess if the biopsy findings match the molecular diagnosis.

Participants without results from genetic testing will be excluded from all analyses. If information is missing from the EHR, we will ask the general practitioner to deliver the missing data within participants' consent. If the data cannot be retrieved, it will be regarded as

"unknown". In case eGFR at CKD onset is missing, the first available eGFR or serum creatinine measurement since the diagnosis of CKD will be used.

Statistical analysis

Statistical analysis will be performed with IBM SPPS statistics for Windows, version 23 (IBM Corporation, Armonk, NY, USA). An overall significance level of 0.05 will be handled. Continuous variables that are normally distributed will be presented as mean and standard deviation. Non-normally distributed variables will be expressed as median and interquartile range. Frequencies and percentages will be used to describe categorical variables such as gender, family history, renal replacement therapy, extra-renal manifestations, and diagnostic yield. The Chi-square or Fisher's exact test will be used to compare differences in categorical variables between the different subgroups of the secondary analysis. Logistic regression will be performed to identify characteristics associated with a genetic diagnosis.

Sample size calculation

The minimal sample size was calculated using the following formula [25], based on the study's primary endpoint:

$$n = \frac{Z^2 * P(1-P)}{d^2}$$

Based on the literature, the expected percentage of positive test results is 17% [14]. Assuming a level of confidence (z) of 1.96 and precision (d) of 0.05 [25], a minimum of 217 participants are required for a reliable assessment of the primary outcome. In order to be clinically and politically significant, we aim to increase the sample size of this prospective cohort study beyond the largest currently available retrospective study, i.e. to include at least 282 patients in the current study [20,25].

Data management

Study data will be recorded digitally using the secure REDCap electronic data capture tool (REDCap, Nashville, TN) hosted at the UMCG [26,27]. Data collection and entry is performed by trained investigators from the UMCG. To minimize differences and errors in data entry, investigators from the UMCG will travel to other participating centers for data collection and entry in REDCap. Data validation in REDCap will be performed according to a data validation plan, which has been made in collaboration with the UMCG Research Data Support and approved by the Institutional Review Board. Data analysis will take place on validated and anonymized data. Upon study closure, data will be extracted from REDCap and exported to SPSS for analysis.

Patient and public involvement

Patients and/or the public were not involved in the design of this study.

ETHICS AND DISSEMINATION

Ethical approval for the study has been obtained from the institutional review board of the UMCG (METc 2019/106). The study is conducted in accordance with the WMA Declaration of Helsinki. The results of the study will be presented at (inter)national congresses and submitted for open access publication in peer-reviewed journals. In addition to the primary results, related to the main research questions as defined above, case reports/series may be submitted for publication in case of unique or interesting findings and these will also be submitted for publication in peer-reviewed journals. In accordance with the information sheet for participants, the main results and any publications from the VARIETY study will also be made available on the study website. After completion of the study and publication of the main results, request for re-use of the data can be submitted to the corresponding author.

CONCLUSION AND STUDY STATUS

Genetic testing shows promising results as a diagnostic tool in adults with CKD and it has the potential to resolve CKD cases with an unknown etiology. However, further research is needed in a clinical setting to define the position of MPS-based diagnostics in clinical practice and to determine which subpopulations will have the highest diagnostic yield. Here, we outlined the design for a prospective cohort study that will determine the diagnostic yield of MPS-based renal gene panel testing in patients with unexplained CKD. The fact that unexplained CKD has not been uniformly defined by international (guideline) committees or institutions may slightly impact external validity of our findings. However, results from this study are likely a step forward in informing physicians and policymakers involved in implementation of genetic testing in patients with unexplained CKD. Inclusion started on 31 July 2019. As of September 2021, 248 patients have been included.

Acknowledgements

Several authors of this publication (AE and NK) are members of the European Reference Network for Rare Kidney Diseases (ERKNet) - Project ID no. 739532.

Author contributions

AH and MB wrote the first draft of the manuscript. ME, LV, BZ, AE and NK gave feedback and contributed to manuscript revision. All authors read and approved the submitted version.

Funding statement

This collaboration project is co-financed by Sanofi Genzyme and the Dutch Ministry of Economic Affairs and Climate Policy by means of the PPP Allowance made available by the

Top Sector Life Sciences & Health to stimulate public-private partnerships (grant numbers RVO/6320 and IMAGEN/LSHM20009).

Competing interest statement

Dr. De Borst and Dr. Vogt have received research support and lecture fees (all to institution) from Sanofi Genzyme related to the current study. Prof. Knoers has received reimbursement of travel expenses for lectures related to the current study from Sanofi Genzyme.

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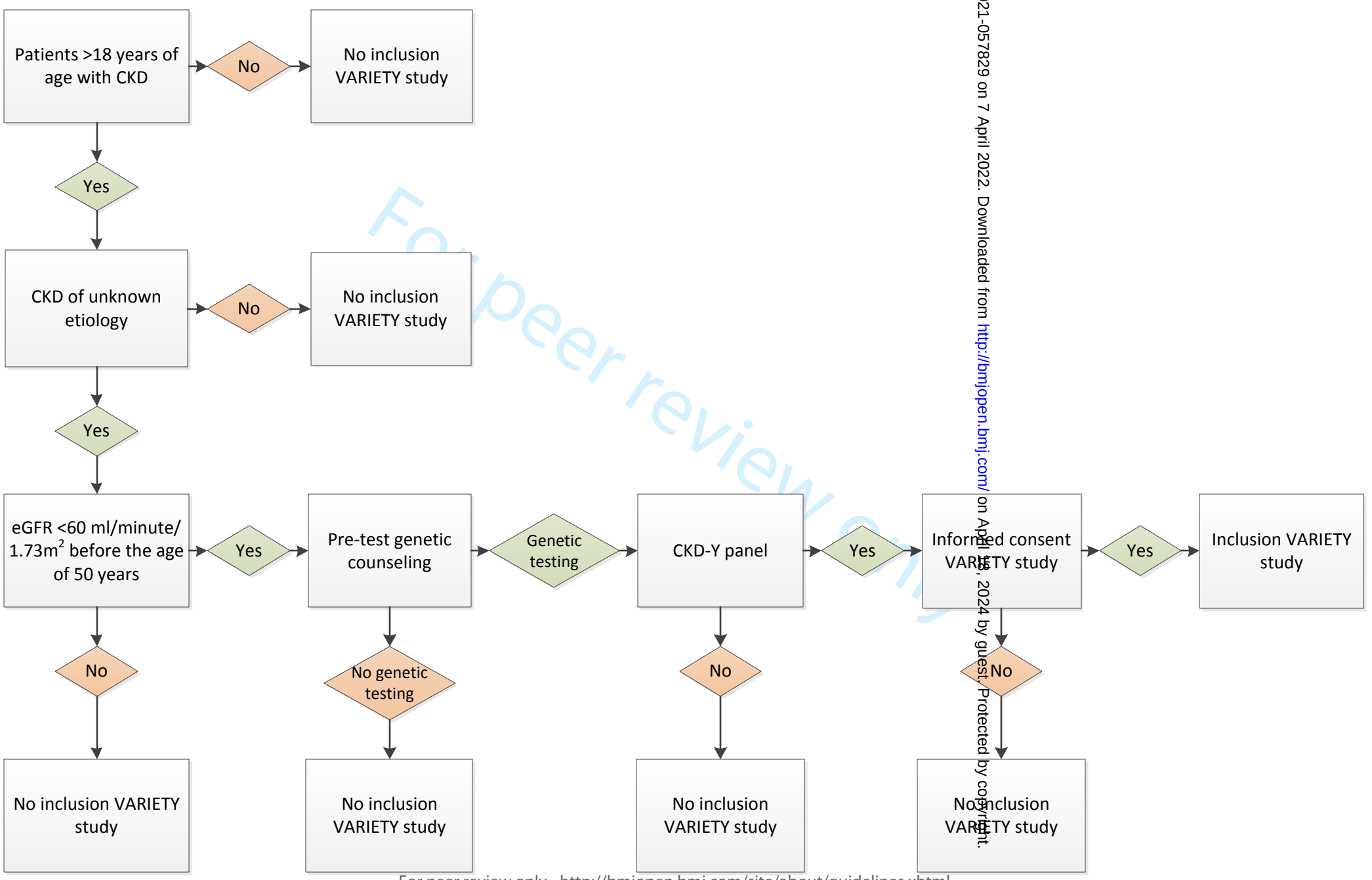
FIGURE LEGENDS

Figure 1. Flowchart for inclusion VARIETY study. CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate.

Figure 2. Overview of the 141 genes that are analyzed in the exome sequencing b Chronic Kidney Disease in Young patients (CKD-Y) panel version v18 at University Medical Center Utrecht

Figure 3. Overview of the 256 genes that are analyzed in the exome sequencing based Chronic Kidney Disease in Young patients (CKD-Y) panel version v21 at University Medical Center Utrecht. Bold genes are also on the CKD-Y panel v18.



141 genes in the young kidney failure (CKD-Y) panel v18						
ACE	CEP290	EMP2	INF2	MYH11	PKD1	TBX18
ACTN4	CFB	EYA1	INVS	MYH9	PKD2	TMEM67
ADCK4	CFH	FAN1	IQCB1	MYO1E	PKHD1	TNXB
AGT	CFHR5	FAT1	ITGA3	NEK8	PLCE1	TRAF3IP1
AGTR1	CFI	FGA	ITGA8	NOTCH2	PMM2	TRAP1
AGXT	CHD7	FN1	JAG1	NPHP1	PTPRO	TRPC6
ALG1	CLCN5	FOXC2	KANK1	NPHP3	REN	TTC21B
AMN	COL4A3	FRAS1	KANK2	NPHP4	RMND1	UMOD
ANKS6	COL4A4	FREM1	KANK4	NPHS1	ROBO2	VIPAS39
APOA1	COL4A5	FREM2	KIAA0556	NPHS2	RPGRIP1L	VPS33B
APOL1	COQ2	GATA3	KIAA0586	NUP107	RRM2B	WDR19
ARHGDIA	COQ6	GLA	LAMB2	NUP205	SALL1	WT1
ATXN10	CRB2	GLIS2	LMNA	NUP93	SCARB2	XPNPEP3
B2M	CTNS	GRHPR	LMX1B	NXF5	SDCCAG8	ZMPSTE24
BBIP1	CUBN	GRIP1	LRIG2	OCRL	SGPL1	ZNF423
BCS1L	CYP11B1	GSN	LYZ	OFD1	SIX5	
C3	CYP11B2	HNF1B	MAFB	OSGEP	SLC41A1	
CD151	DACT1	HOGA1	MAGI2	PAX2	SLC4A1	
CD2AP	DCDC2	HPSE2	MAP7D3	PBX1	SLC7A7	
CD46	DGKE	IFT27	MAPKBP1	PDSS1	SMARCAL1	
CEP164	DSTYK	IFT81	MUC1	PDSS2	SOX17	

256 genes in the young kidney failure (CKD-Y) panel v21						
ACE	BICC1	**CUBN**	HYLS1	MOCOS	**PLCE1**	TMEM138
ACTG2	BMPR2	CUL3	**IFT27**	MTR	**PMM2**	TMEM216
ACTN4	**C3**	**CYP11B1**	IFT74	MTRR	POC1B	TMEM231
ADAMTS9	C8ORF37	**CYP11B2**	**IFT81**	MTX2	PODXL	TMEM237
AGT	CACNA1D	CYP17A1	IL1RAP	**MUC1**	**PTPRO**	**TMEM67**
AGTR1	CACNA1H	DAAM2	**INF2**	**MYH11**	**REN**	TMEM72
AGXT	CC2D2A	**DACT1**	INPP5E	**MYH9**	**RMND1**	TNS2
AHI1	**CD151**	**DCDC2**	**INVS**	**MYO1E**	**ROBO2**	**TNXB**
ALG1	**CD2AP**	**DGKE**	**IQCB1**	**NEK8**	**RPGRIP1L**	TOGARAM1
ALMS1	**CD46**	DLC1	**ITGA3**	NOS1AP	**RRM2B**	TP53RK
AMN	CDK20	DNAJB11	**ITGA8**	**NOTCH2**	**SALL1**	TPRKB
ANKS6	CEP104	**DSTYK**	ITGB4	**NPHP1**	SARS2	**TRAF3IP1**
ANLN	**CEP164**	E2F3	ITSN1	**NPHP3**	**SCARB2**	**TRAP1**
APOA1	**CEP290**	**EMP2**	ITSN2	**NPHP4**	SCNN1A	TRIM32
APOE	CEP41	**EYA1**	**JAG1**	**NPHS1**	SCNN1B	TRIM8
APOL1	CEP83	FAM149B1	**KANK1**	**NPHS2**	SCNN1G	**TRPC6**
APRT	**CFB**	**FAN1**	**KANK2**	NR3C1	**SDCCAG8**	**TTC21B**
ARHGAP24	**CFH**	**FAT1**	**KANK4**	NR3C2	SEC61A1	TTC8
ARHGDIA	CFHR1	**FGA**	KATNIP	**NUP107**	**SGPL1**	**UMOD**
ARL13B	CFHR2	**FN1**	KCNJ5	NUP133	SIX1	**VIPAS39**
ARL6	CFHR3	**FOXC2**	**KIAA0586**	NUP160	**SIX5**	**VPS33B**
ARMC9	CFHR4	**FRAS1**	KIF3B	**NUP205**	SLC22A12	WDPCP
ATXN10	**CFHR5**	**FREM1**	KIRREL1	NUP85	SLC2A9	**WDR19**
AVIL	**CFI**	**FREM2**	KLHL3	**NUP93**	SLC3A1	WDR35
B2M	**CHD7**	GANAB	**LAMB2**	**NXF5**	**SLC41A1**	WDR60
B9D1	CLCN2	GAPVD1	**LMNA**	**OCRL**	**SLC4A1**	WDR73
B9D2	**CLCN5**	**GATA3**	**LMX1B**	**OFD1**	**SLC7A7**	WNK1
BBIP1	**COL4A3**	GATM	**LRIG2**	**OSGEP**	SLC7A9	WNK4
BBS1	**COL4A4**	**GLA**	**LYZ**	**PAX2**	**SMARCAL1**	**WT1**
BBS10	**COL4A5**	**GLIS2**	LZTFL1	**PBX1**	**SOX17**	XDH
BBS12	**COQ2**	**GRHPR**	**MAFB**	PCM1	STX16	**XPNPEP3**
BBS2	**COQ6**	**GRIP1**	**MAGI2**	**PDSS1**	TBC1D8B	YRDC
BBS4	COQ8B	**GSN**	**MAP7D3**	**PDSS2**	**TBX18**	**ZMPSTE24**
BBS5	CPLANE1	**HNF1B**	**MAPKBP1**	PIBF1	TCTN1	**ZNF423**
BBS7	**CRB2**	**HOGA1**	MKKS	**PKD1**	TCTN2	
BBS9	CSPP1	**HPSE2**	MKS1	**PKD2**	TCTN3	
BCS1L	**CTNS**	HSD11B2	MMACHC	**PKHD1**	TMEM107	

SUPPLEMENTARY MATERIAL

379 genes in hereditary kidney disease panel v18						
ACE	CA2	DGAT1	**GSN**	MAGED2	**ROBO2**	TCTEX1D2
ACTG2	CACNA1H	**DGKE**	GUCY2C	**MAGI2**	RPGRIP1	TCTN1
ACTN4	CACNA1S	DMP1	HAAO	**MAP7D3**	**RPGRIP1L**	TCTN2
ADAMTS13	CASR	DNAJB11	**HNF1B**	**MAPKBP1**	**RRM2B**	TCTN3
ADCK3	CC2D2A	DST	HNF4A	MET	**SALL1**	THBD
ADCK4	CCDC114	**DSTYK**	**HOGA1**	MKKS	SALL4	TMEM104
AGT	**CD151**	DYNC2H1	HOXD13	MKS1	SARS2	TMEM107
AGTR1	**CD2AP**	DYNC2LI1	HPRT1	**MUC1**	**SCARB2**	TMEM138
AGXT	**CD46**	DZIP1L	**HPSE2**	**MYH11**	SCLT1	TMEM216
AHI1	CDKN1C	EGF	HSD11B2	**MYH9**	SCN11A	TMEM231
ALDOB	CEP120	EHHADH	IFT122	**MYO1E**	SCN4A	TMEM237
ALG1	**CEP164**	**EMP2**	IFT140	MYO5B	SCNN1A	**TMEM67**
ALG8	**CEP290**	ENPP1	IFT172	NEK1	SCNN1B	**TNXB**
ALMS1	CEP41	EPCAM	**IFT27**	**NEK8**	SCNN1G	TP53RK
AMN	CEP83	EVC	IFT43	NEUROG3	**SDCCAG8**	TPRKB
ANKS3	**CFB**	EVC2	IFT52	NGF	SDHB	**TRAF3IP1**
ANKS6	**CFH**	**EYA1**	IFT57	**NOTCH2**	SEC61A1	**TRAP1**
ANLN	CFHR1	FAH	IFT80	**NPHP1**	SEC61B	TRIM32
ANO1	CFHR2	FAHD2A	**IFT81**	**NPHP3**	SEC63	**TRPC6**
AP2S1	CFHR3	FAM134B	IKBKAP	**NPHP4**	**SGPL1**	TRPM6
APOA1	CFHR4	FAM20A	**INF2**	**NPHS1**	SIX1	TSC1
APOL1	**CFHR5**	FAM58A	INPP5E	**NPHS2**	SIX2	TSC2
APRT	**CFI**	**FAN1**	**INVS**	NR3C1	**SIX5**	**TTC21B**
AQP2	CHD1L	**FAT1**	**IQCB1**	NR3C2	SLC12A1	TTC8
ARHGAP24	**CHD7**	FBXL4	**ITGA3**	**NUP107**	SLC12A3	**UMOD**
ARHGDIA	CHRM3	**FGA**	**ITGA8**	**NUP205**	SLC16A12	UPK3A
ARL13B	**CLCN5**	FGF20	ITGB4	**NUP93**	SLC22A12	UQCC2
ARL6	CLCNKA	FGF23	**JAG1**	**NXF5**	SLC26A3	VDR
ARSA	CLCNKB	FGF8	KAL1	**OCRL**	SLC2A2	VHL
ATP6V0A4	CLDN16	FGFR1	**KANK1**	**OFD1**	SLC2A9	**VIPAS39**
ATP6V1B1	CLDN19	FH	**KANK2**	**OSGEP**	SLC34A1	**VPS33B**
ATP7B	CNNM2	FLCN	**KANK4**	**PAX2**	SLC34A3	WDPCP
ATXN10	COL4A1	**FN1**	KCNJ1	PAX8	SLC36A2	**WDR19**
AVP	**COL4A3**	**FOXC2**	KCNJ10	**PBX1**	SLC37A4	WDR34
AVPR2	**COL4A4**	FOXF1	KCNJ5	PCBD1	SLC3A1	WDR35
B2M	**COL4A5**	**FRAS1**	**KIAA0556**	PDE6D	**SLC41A1**	WDR60
B9D1	**COQ2**	**FREM1**	**KIAA0586**	**PDSS1**	**SLC4A1**	WDR73
B9D2	COQ4	**FREM2**	KIF14	**PDSS2**	SLC4A4	WNK1
BBIP1	**COQ6**	FXYD2	KIF7	PHEX	SLC5A2	WNK4
BBS1	COQ7	G6PC	KL	**PKD1**	SLC6A19	WNT4
BBS10	COQ9	GALNT3	KLHL3	**PKD2**	SLC6A20	**WT1**
BBS12	COX10	GALT	KYNU	**PKHD1**	**SLC7A7**	XDH

BBS2	CPT2	GANAB	LAGE3	**PLCE1**	SLC7A9	**XPNPEP3**
BBS4	**CRB2**	**GATA3**	**LAMB2**	**PMM2**	SLC9A3	XPO5
BBS5	CSPP1	GDNF	LCAT	PODXL	SLC9A3R1	YRDC
BBS7	**CTNS**	**GLA**	**LMNA**	PRDM12	SLIT2	ZEB2
BBS9	**CUBN**	GLI3	LMOD1	PRKCSH	**SMARCAL1**	ZIC3
BCS1L	CUL3	**GLIS2**	**LMX1B**	PSAP	**SOX17**	**ZMPSTE24**
BICC1	**CYP11B1**	GLIS3	LPP	PTEN	SPINT2	**ZNF423**
BMP4	**CYP11B2**	GNA11	**LRIG2**	PTH1R	SPTLC1	
BMPR2	CYP17A1	GPC3	LRP2	**PTPRO**	SPTLC2	
BSND	CYP24A1	GPC5	LRP4	PYGM	STRA6	
C2CD3	**DACT1**	GREB1L	**LYZ**	**REN**	STX16	
C3	**DCDC2**	**GRHPR**	LZTFL1	RET	TBC1D1	
C5orf42	DDX59	**GRIP1**	**MAFB**	**RMND1**	**TBX18**	

Supplementary Figure 1. The 379 genes that are on the hereditary kidney disease panel v18 at University Medical Center Utrecht. Bold genes are also on the CKD-Y panel v18.

495 genes in hereditary kidney disease panel v21						
ACE	***CACNA1H***	**DGAT1**	**GREB1L**	***LRIG2***	**PRDM12**	**STRA6**
ACTA2	**CACNA1S**	***DGKE***	GREM1	LRP10	PRDX1	STRADA
ACTG2	**CASR**	DHCR7	***GRHPR***	**LRP2**	**PRKCSH**	***STX16***
ACTN4	CBWD1	DICER1	***GRIP1***	**LRP4**	**PSAP**	SYNPO
ADAMTS13	CBY1	***DLC1***	***GSN***	LRP5	**PTEN**	**TBC1D1**
ADAMTS9	***CC2D2A***	**DMP1**	**GUCY2C**	***LYZ***	**PTH1R**	***TBC1D8B***
ADCK3	**CCDC114**	***DNAJB11***	**HAAO**	***LZTFL1***	***PTPRO***	***TBX18***
ADCY10	CCDC28B	DOCK4	***HNF1B***	***MAFB***	**PYGM**	TBX6
AGK	***CD151***	**DST**	**HNF4A**	**MAGED2**	RBM8A	**TCTEX1D2**
AGT	***CD2AP***	***DSTYK***	***HOGA1***	***MAGI2***	***REN***	***TCTN1***
AGTR1	***CD46***	**DYNC2H1**	HOXA10	***MAP7D3***	RERE	***TCTN2***
AGXT	CDC73	**DYNC2LI1**	HOXA13	***MAPKBP1***	**RET**	***TCTN3***
AHI1	***CDK20***	**DZIP1L**	**HOXD13**	**MET**	RICTOR	**THBD**
ALDOB	**CDKN1C**	***E2F3***	**HPRT1**	***MKKS***	***RMND1***	**TMEM104**
ALG1	CENPF	**EGF**	***HPSE2***	***MKS1***	ROBO1	***TMEM107***
ALG5	***CEP104***	**EHHADH**	HRAS	***MMACHC***	***ROBO2***	***TMEM138***
ALG6	**CEP120**	ELP1	***HSD11B2***	***MOCOS***	**RPGRIP1**	***TMEM216***
ALG8	***CEP164***	***EMP2***	HSPA6	***MTR***	***RPGRIP1L***	***TMEM231***
ALG9	***CEP290***	**ENPP1**	***HYLS1***	***MTRR***	RRAGD	***TMEM237***
ALMS1	***CEP41***	**EPCAM**	ICK	***MTX2***	***RRM2B***	TMEM260
ALPL	CEP55	ERCC6	**IFT122**	***MUC1***	***SALL1***	***TMEM67***
AMN	***CEP83***	ERCC8	**IFT140**	***MYH11***	**SALL4**	***TMEM72***
ANKFY1	***CFB***	**EVC**	**IFT172**	***MYH9***	***SARS2***	***TNS2***
ANKS3	***CFH***	**EVC2**	***IFT27***	MYLK	***SCARB2***	***TNXB***
ANKS6	***CFHR1***	EVX1	**IFT43**	***MYO1E***	**SCLT1**	***TOGARAM1***
ANLN	***CFHR2***	EXOC8	**IFT52**	**MYO5B**	**SCN11A**	***TP53RK***
ANOS1	***CFHR3***	***EYA1***	**IFT57**	NAALADL2	**SCN4A**	TP63
AP2S1	***CFHR4***	**FAH**	***IFT74***	NCAPG2	***SCNN1A***	***TPRKB***
APOA1	***CFHR5***	**FAHD2A**	**IFT80**	**NEK1**	***SCNN1B***	***TRAF3IP1***
APOE	***CFI***	**FAM134B**	***IFT81***	***NEK8***	***SCNN1G***	***TRAP1***
APOL1	**CHD1L**	***FAM149B1***	***IL1RAP***	NEU1	***SDCCAG8***	***TRIM32***
APRT	***CHD7***	**FAM20A**	***INF2***	**NEUROG3**	**SDHB**	***TRIM8***
AQP2	**CHRM3**	FAM20C	***INPP5E***	**NGF**	***SEC61A1***	***TRPC6***
ARHGAP24	CHRNA3	**FAM58A**	INTU	***NOS1AP***	**SEC61B**	**TRPM6**
ARHGDIA	***CLCN2***	***FAN1***	***INVS***	***NOTCH2***	**SEC63**	TRPM7
ARL13B	***CLCN5***	***FAT1***	***IQCB1***	***NPHP1***	***SGPL1***	**TSC1**
ARL3	**CLCNKA**	**FBXL4**	ISL1	***NPHP3***	***SIX1***	**TSC2**
ARL6	**CLCNKB**	***FGA***	***ITGA3***	***NPHP4***	**SIX2**	TSHZ3
ARMC9	CLDN10	**FGF20**	***ITGA8***	***NPHS1***	***SIX5***	***TTC21B***
ARSA	**CLDN16**	**FGF23**	***ITGB4***	***NPHS2***	SKAP2	***TTC8***
ATP1A1	**CLDN19**	**FGF8**	***ITSN1***	NPNT	**SLC12A1**	TXNDC15
ATP6V0A4	**CNNM2**	**FGFR1**	***ITSN2***	***NR3C1***	**SLC12A3**	***UMOD***
ATP6V1B1	**COL4A1**	**FH**	***JAG1***	***NR3C2***	**SLC16A12**	**UPK3A**
ATP7B	***COL4A3***	**FLCN**	***KANK1***	NRAS	SLC19A2	**UQCC2**

ATXN10	***COL4A4***	***FN1***	***KANK2***	***NUP107***	***SLC22A12***	**VDR**
AVIL	***COL4A5***	***FOXC2***	***KANK4***	***NUP133***	SLC26A1	**VHL**
AVP	***COQ2***	**FOXF1**	***KATNIP***	***NUP160***	**SLC26A3**	***VIPAS39***
AVPR2	**COQ4**	FOXI1	**KCNJ1**	***NUP205***	**SLC2A2**	***VPS33B***
B2M	***COQ6***	***FRAS1***	**KCNJ10**	***NUP85***	***SLC2A9***	***WDPCP***
B9D1	**COQ7**	***FREM1***	***KCNJ5***	***NUP93***	**SLC34A1**	***WDR19***
B9D2	***COQ8B***	***FREM2***	KCTD1	***NXF5***	**SLC34A3**	**WDR34**
BBIP1	**COQ9**	**FXYD2**	KCTD3	***OCRL***	**SLC36A2**	***WDR35***
BBS1	**COX10**	**G6PC**	***KIAA0586***	***OFD1***	**SLC37A4**	***WDR60***
BBS10	***CPLANE1***	**GALNT3**	KIAA0753	***OSGEP***	***SLC3A1***	WDR72
BBS12	**CPT2**	**GALT**	**KIF14**	***PAX2***	***SLC41A1***	***WDR73***
BBS2	***CRB2***	***GANAB***	***KIF3B***	**PAX8**	***SLC4A1***	***WNK1***
BBS4	***CSPP1***	***GAPVD1***	**KIF7**	***PBX1***	**SLC4A4**	***WNK4***
BBS5	***CTNS***	***GATA3***	***KIRREL1***	**PCBD1**	**SLC5A2**	**WNT4**
BBS7	***CUBN***	***GATM***	**KL**	***PCM1***	**SLC6A19**	WNT9B
BBS9	***CUL3***	**GDNF**	***KLHL3***	**PDE6D**	**SLC6A20**	***WT1***
BCS1L	***CYP11B1***	GDF6	KRAS	***PDSS1***	***SLC7A7***	***XDH***
BICC1	***CYP11B2***	GFRA1	**KYNU**	***PDSS2***	***SLC7A9***	***XPNPEP3***
BMP4	***CYP17A1***	***GLA***	**LAGE3**	**PHEX**	**SLC9A3**	**XPO5**
BMPR2	**CYP24A1**	**GLI3**	LAMA5	***PIBF1***	**SLC9A3R1**	***YRDC***
BNC2	CYP27B1	***GLIS2***	***LAMB2***	***PKD1***	**SLIT2**	**ZEB2**
BSND	CYP2R1	**GLIS3**	**LCAT**	***PKD2***	SLIT3	**ZIC3**
C2CD3	CYP3A4	**GNA11**	LHX1	***PKHD1***	***SMARCAL1***	***ZMPSTE24***
C3	***DAAM2***	GNAS	***LMNA***	***PLCE1***	***SOX17***	ZNF365
C8ORF37	***DACT1***	GON7	**LMOD1**	***PMM2***	**SPINT2**	***ZNF423***
CA2	***DCDC2***	**GPC3**	***LMX1B***	***POC1B***	**SPTLC1**	
CACNA1D	**DDX59**	**GPC5**	**LPP**	***PODXL***	**SPTLC2**	

Supplementary Figure 2. The 495 genes that are on the hereditary kidney disease panel v21 at University Medical Center Utrecht. Bold genes are also hereditary kidney disease panel v18 and italic genes are also on the CKD-Y panel v21.

Supplementary Table 1. Questionnaire for participants (original version is in Dutch)

Number	Question	Answer possibilities
General questions		
1	What is your country of birth?	Open
2	What is the country of birth of your maternal grandmother?	Open
3	What is the country of birth of your maternal grandfather?	Open
4	What is the country of birth of your paternal grandmother?	Open
5	What is the country of birth of your paternal grandfather?	Open
Medical health and current health complaints		
6	At which age did you get the diagnosis chronic kidney disease?	Open
7	Did you undergo dialysis in the past or are you currently on dialysis?	Yes/no/unknown
8	Did you undergo a kidney transplantation in the past?	Yes/no/unknown
9	Do you have high blood pressure? If you are taking blood pressure-lowering medication and have a normal blood pressure thanks to the medication, you can also fill in "yes".	Yes/no/unknown
10	Have you ever been admitted to the emergency room for high blood pressure?	Yes/no/unknown
11	Are you unable or do you have trouble with sweating?	Yes/no/unknown

12	Do you suffer from heat- or cold intolerance? This means that you have trouble with handling heat or cold.	Yes/no/unknown
13	Have you experienced a burning pain or a feeling of tingling in the hands and/or feet now or in the past?	Yes/no/unknown
13a	If so, did this pain or tingling feeling arise or get worse with fever, exertion, stress, or if the hands or feet became very hot or cold?	Open
14	Do you have dark, red-purple spots in your skin? Especially between your belly button and knees?	Yes/no/unknown
15	Do you have any problems with seeing or any eye complaints?	Yes/no/unknown
15a	If so, what are your problems with seeing and/or eye complaints?	Open
16	Do you have any hearing problems of hearing disabilities?	Yes/no/unknown
16a	If so, what for hearing problems or disabilities do you have?	Open
17	Have you suffered from gout now or in the past?	Yes/no/unknown
18	Have you ever had a stroke (cerebral infraction, brain hemorrhage or TIA)?	Yes/no/unknown
19	Did you ever have a myocardial infarction?	Yes/no/unknown
20	Do you have a heart rhythm disorder?	Yes/no/unknown
20a	If so, which heart rhythm disorder do you have?	Open
21	Do you have a thickening of the heart muscle (hypertrophic cardiomyopathy)?	Yes/no/unknown

22	Do you have health complaints not mentioned in the previous questions?	Yes/no/unknown
22a	If so, which heath complaints do you experience?	Open
Family history		
23	How many biological children, alive or deceased, do you have?	Open
24	Do you (still) have any desire to have children?	Yes/no/unknown
25	How many siblings, alive or deceased, do you have?	Open
26	How many half-brothers and/or half-sisters, alive or deceased, do you have?	Open
27	How many siblings, alive or deceased, does your mother have?	Open
28	How many siblings, alive or deceased, does your father have?	Open
29	Are your grandparents still alive?	Yes/no/unknown
29a	Did one of your grandparents pass away before the age of 50 years?	Yes/no/unknown
30	Are your parents blood relatives (e.g. second cousins)?	Yes/no/unknown
30a	If so, how are your parents related to each other?	Open
31	Are you and your partner blood relatives (e.g. cousins, second cousins)?	Yes/no/I do not have a partner/unknown
31a	If so, how are you and your partner related to each other?	Open
32	Does gout run in your family?	Yes/no/unknown
33	Do you have family members with a high blood pressure at a young age?	Yes/no/unknown
34	Does anyone in your family have an intellectual disability?	Yes/no/unknown

35	Dou you have family members with kidney disease (children, parents, siblings, grandparents, uncles/aunts, cousins, nephews/nieces)?	Yes/no/unknown
35a	If so, how many family members have a kidney disease?	Open
35b	In how many family members if the cause for the kidney disease unknown?	Open
35c	If you know the cause of the kidney disease of other family members, please write down the cause of the kidney disease in this field. If you do not know the cause, you can leave this field empty.	Open
35d	How many family members with a kidney disease have had a kidney transplantation or dialysis?	Open
Final questions		
36	Have you visited a clinical geneticist or have you been referred to a clinical geneticist?	Yes/no/unknown
37	Do you already known the results from genetic testing at the time of completing this questionnaire?	Yes/no/unknown

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6-7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-12
Bias	9	Describe any efforts to address potential sources of bias	13
Study size	10	Explain how the study size was arrived at	12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12
		(*b*) Describe any methods used to examine subgroups and interactions	12
		(*c*) Explain how missing data were addressed	11
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

NA: not applicable

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Diagnostic yield of massively parallel sequencing in patients with chronic kidney disease of unknown etiology: Rationale and design of a national prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-057829.R1
Article Type:	Protocol
Date Submitted by the Author:	28-Jan-2022
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Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Diagnostics, Genetics and genomics
Keywords:	NEPHROLOGY, End stage renal failure < NEPHROLOGY, Chronic renal failure < NEPHROLOGY, GENETICS

Diagnostic yield of massively parallel sequencing in patients with chronic kidney disease of unknown etiology: Rationale and design of a national prospective cohort study

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Word count: 2918

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ABSTRACT

Introduction: Chronic kidney disease (CKD) can be caused by a variety of systemic or primary renal diseases. The cause of CKD remains unexplained in approximately 20% of patients. Retrospective studies indicate that massively parallel sequencing (MPS)-based gene panel testing may lead to a genetic diagnosis in 12-56% of patients with unexplained CKD, depending on patient profile. The diagnostic yield of MPS-based testing in a routine healthcare setting is unclear. Therefore, the primary aim of the VARIETY (Validation of algoRithms and IdEnTification of genes in Young patients with unexplained chronic kidney disease) study is to prospectively address the diagnostic yield of MPS-based gene panel testing in patients with unexplained CKD and an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 before the age of 50 years in clinical practice.

Methods and analysis: The VARIETY study is an ongoing, prospective, nation-wide observational cohort study to investigate the diagnostic yield of MPS-based testing in patients with unexplained CKD in a routine healthcare setting in the Netherlands. Patients are recruited from outpatient clinics in hospitals across the Netherlands. At least 282 patients will be included to meet the primary aim. Secondary analyses include subgroup analyses according to age and eGFR at first presentation, family history, and the presence of extra-renal symptoms.

Ethics and dissemination: Ethical approval for the study has been obtained from the institutional review board of the University Medical Center Groningen. Study findings should inform physicians and policymakers towards optimal implementation of MPS-based diagnostic testing in patients with unexplained CKD.

ARTICLE SUMMARY

Strengths and limitations of this study

- First prospective study to examine the diagnostic yield of massively parallel sequencing in patients (age <50 at first presentation) with unexplained chronic kidney disease in a routine healthcare setting
- Nation-wide study with relatively large sample size, allowing analyses of specific subgroups according to age and kidney function at first presentation
- Study findings should inform physicians and policymakers in the implementation of gene panel testing in adults (age < 50) with unexplained CKD
- A potential limitation is that the definition of 'unexplained chronic kidney disease' is not unequivocal

INTRODUCTION

Chronic kidney disease (CKD) affects 11-16% of the population worldwide [1–3], is associated with extensive co-morbidity and an increased risk of premature mortality, and may ultimately result in end-stage kidney disease (ESKD) requiring dialysis or transplantation [4,5]. CKD may be caused by a variety of systemic (e.g., diabetes, hypertension) or primary renal diseases (e.g. IgA nephropathy, membranous nephropathy). Current diagnostic approaches, including kidney biopsy, are often non-specific or inconclusive, contraindicated or omitted due to lack of clinical consequences [6,7]. Therefore, the cause of CKD remains unknown in approximately 20% of patients with ESKD [8–10]. However, knowledge of the underlying kidney disease can be pivotal as it may influence prognosis and medical treatment. In the setting of kidney transplantation, it may influence (living-related) donor selection and post-transplant recurrence risk. Approximately 27-34% of patients with CKD report a positive family history of kidney disease (first or second degree relative with CKD) [11,12] and a genetic cause can be identified in at least 10% of adults with CKD [13,14], indicating that in many cases a hereditary origin for the disease should be considered. Genetic testing could therefore be a valuable tool in the diagnostic process of CKD of unknown etiology.

Recent studies suggest that massively parallel sequencing (MPS) techniques (previously referred to as next-generation sequencing) [15] could be used as diagnostic tool in adults with unexplained CKD and should even be considered as first mode of diagnostics in patients with ESKD prior to the age of 50 years [16]. Depending on patient selection, MPS led to a genetic diagnosis in 12-56% of patients with unexplained CKD [14,17–19]. However, most of these studies have been performed in a research setting, and therefore little is known about the diagnostic utility of MPS for adults with unexplained CKD in a routine healthcare setting. Moreover, currently available studies have been commonly based on subgroups of larger retrospective cohorts, and are heterogeneous in design and selection of genes used in

MPS [20]. For this reason, it is difficult to define profiles of patients (e.g. based on age and severity at disease onset, extra-renal manifestations, positive family history) that should preferentially undergo genetic testing. A recent joint publication by the ERA Working Group on Inherited Kidney Disorders (WGIKD) and the Molecular Diagnostics Taskforce of the European Rare Kidney Disease Reference Network (ERKNet) called for further research to explore the diagnostic yield of genetic testing in CKD of unknown origin in a clinical setting [21].

Therefore, the objective of this national prospective cohort study is to determine the diagnostic yield, i.e. the percentage of participants with a genetic diagnosis, using a large MPS-based multi-gene panel for kidney diseases in young patients (first presentation at age <50) with unexplained CKD (eGFR <60 mL/min/1.73 m^2) in a routine healthcare setting. In addition, we aim to identify specific patient profiles with a high diagnostic yield. These findings can guide physicians and policymakers in implementing MPS-based diagnostic testing in patients with unexplained CKD.

METHODS AND ANALYSIS

Study design

The VARIETY (Validation of algoRithms and IdEnTification of genes in Young patients with unexplained chronic kidney disease) study is a prospective nation-wide observational cohort study designed to investigate the diagnostic yield of genetic testing in patients with unexplained CKD in a routine healthcare setting in the Netherlands. The study will collect and analyze data obtained during routine clinical practice and through a questionnaire. Participants will be included from both academic and non-academic hospitals throughout the Netherlands. The anonymized data are collected, stored and analyzed in the University Medical Center Groningen (UMCG). All participants will give written informed consent on enrolment.

Study population

The targeted study population consist of all patients with unexplained CKD and an eGFR <60 ml/min/1.73m^2 before the age of 50 years. Unexplained CKD is defined as the absence of all the following criteria: a biopsy-proven diagnosis (e.g. IgA nephropathy), a specific morphological renal diagnosis (e.g. polycystic kidney disease suspected of autosomal/recessive polycystic kidney disease), or a specific or plausible renal diagnosis (e.g. history of long-term insulin-dependent diabetes mellitus before the onset of CKD, lithium-induced nephropathy). Since hypertensive nephropathy is a non-specific diagnosis and hypertension is also a very common consequence of CKD [22], patients with hypertensive nephropathy in the absence of a clear underlying disorder such as renal artery stenosis are considered to have unexplained CKD. Patients with renal hypoplasia, renal atrophy, and non-specific histological conditions (such as secondary focal segmental glomerulosclerosis,

glomerulonephritis of unknown cause, or interstitial nephritis) are also considered to have unexplained CKD.

Patients with a current age >50 years, but who presented with an eGFR <60 ml/min/1.73m^2 before the age of 50 years, and renal transplant recipients who had a pre-transplant eGFR <60 ml/min/1.73m^2 before the age of 50 years are also eligible for inclusion. In addition, genetic testing with a specific MPS-based gene panel (see 'Genetic testing') is required for participation. Exclusion criteria for participation in the VARIETY study are: age <18 years at time of inclusion or patients who do not give or are unable to give informed consent for genetic testing or for the current study.

Recruitment

To ensure a representative sample of CKD patients, patients are recruited from outpatient clinics in both academic and non-academic hospitals across the Netherlands. Depending on the hospital, patients will be screened by the primary treating nephrologists or by trained study investigators. In case of a study investigator, a list with potential participants will be sent to the treating nephrologist to confirm the diagnosis of unexplained CKD. Eligible participants will be informed about the study by the investigators or their treating nephrologists aware of the study protocol. A study investigator or treating nephrologist will ask for informed consent for this study. Information for patients has been made available in the form of a patient information folder and a website (in Dutch): www.onbegrepennierziekte.nl.

Data collection

Detailed clinical and demographic data are collected from patients' electronic health record (EHR) and through a questionnaire following informed consent. The data will subsequently be entered into a secure electronic case report form.

Electronic health record

The following information will be collected from the EHR: age at inclusion, sex, primary renal disease diagnosis, age at CKD onset/presentation, dialysis or kidney transplantation, age at start dialysis or kidney transplantation, presence of extra-renal features, medication use at inclusion, medical history, family history (including three-generation pedigree), blood pressure at CKD onset and at inclusion, presence of hematuria and/or nephrotic syndrome, laboratory results (serum creatinine, eGFR, total cholesterol, HDL, LDL, triglycerides, 24 hour urine albumin excretion, 24 hour urine creatinine excretion, 24 hour urine total protein excretion, hematuria) at CKD onset/presentation and inclusion, renal histopathology and imaging of the native kidneys, and results of genetic testing in relation to kidney disease. We will also collect information regarding the clinical consequences of a genetic diagnosis and if genetic counseling was performed by a nephrologist or clinical geneticist. If participants are referred to a clinical geneticist, we will also collect the results of any additional genetic testing.

Questionnaire

Data collected from the EHR will be expanded with a questionnaire to collect additional data on family history, medical history, current health complaints, and extra-renal manifestations (Supplemental Table 1).

Genetic testing

We will include patients who have undergone MPS-based multi-gene panel testing, initiated by a clinical geneticist or nephrologist following pre-test counseling as part of clinical care in patients with unexplained CKD, in accordance with guidelines in the Netherlands [23]. Figure 1 shows the suggested flowchart for genetic testing in the VARIETY study, based on these recommendations. The criteria as shown in this flow chart are slightly more liberal than the published recommendations, which will help to define the optimal age and eGFR ranges where genetic testing is still of clinical benefit. To stimulate the implementation of the guideline, we made a website for the VARIETY study (www.onbegrepennierziekte.nl) where nephrologists can find information about genetic testing and pre-test genetic counseling.

In order to reduce heterogeneity in the diagnostic approach, we will assess the diagnostic yield of a specific MPS-based gene panel, namely the 'CKD-Y' ('Chronic Kidney Disease in Young patients') targeted exome sequencing (ES) panel available at the University Medical Center (UMC) Utrecht, The Netherlands. The older version of this panel (v18) contains 141 different genes associated with early-onset CKD, including *PKD1* and *PKD2* (Figure 2). On March 8 2021, the CKD-Y panel was updated (v21) and the number of genes changed from 141 to 256 (Figure 3). This panel was chosen as it is an ES-based panel and contains all the current genes associated with early-onset CKD. In addition, this panel can be ordered by nephrologists without referral to the clinical geneticist. Alternatively, the hereditary kidney disease panel from UMC Utrecht was allowed. This is another targeted ES panel, consisting of 379 genes in the v18 version and 495 genes in the updated v21 version (Supplementary Figure 1-2). Since this panel contains some kidney cancer oncogenes, it may only be ordered by a clinical geneticist. The hereditary kidney disease panel includes all genes of the CKD-Y panel, making it possible to determine if a variant could also have been identified with the CKD-Y panel. Potential findings from the hereditary kidney disease panel that do not overlap with the CKD-Y panel will not be included in the primary analyses.

Patients with older versions of the CKD-Y and hereditary kidney disease panels can be included. We will record which version of the CKD-Y and/or hereditary kidney disease panel was used. The procedures for ES and variant filtering have been described before [24]. Copy number variation (CNV) detection was performed using an in-house adapted, diagnostically validated, version of the ExomeDepth CNV detection tool [25].

Primary and secondary analyses

The primary analysis will address the diagnostic yield of the CKD-Y panel, defined as the percentage of positive test results (i.e. pathogenic variant(s) explaining the cause of the disease), in the overall cohort of patients with unexplained CKD and an eGFR <60 mL/min/1.73 m^2 before the age of 50 years. We will perform a sensitivity analysis in patients with onset eGFR <60 mL/min/1.73 m^2 between the age of 18 and 50 years. The pathogenicity of variants will be determined according to the standards and guidelines from the American College of Medical Genetics and Genomics (ACMG) [26]. With this standard, variants are classified into five categories using several lines of evidence, such as available literature, patient databases and *in silico* prediction programs. Class 1 variants are clearly not pathogenic; class 5 variants are clearly pathogenic. Class 3 are variants of uncertain significance/pathogenicity (VUS), these variants do not confirm or exclude the diagnosis (Table 1) [26]. For the determination of the diagnostic yield, only class 4 and class 5 variants will be considered as a 'positive test result' to determine the diagnostic yield. In cases with two class 4/5 variants in an autosomal recessive gene, these will only be considered a 'positive test result' if testing in parents has confirmed the variants are positioned in trans.

Table 1. Classification of variants according to ACMG guidelines [26]

Class	Description
1	Clearly not pathogenic, common polymorphism
2	Unlikely to be pathogenic, diagnosis not confirmed molecularly
3	Unknown significance/pathogenicity, does not exclude or confirm diagnosis
4	Likely to be pathogenic, consistent with the diagnosis
5	Clearly pathogenic, result confirms the diagnosis

Secondary analyses include subgroup analyses according to age and eGFR at first presentation, family history, and the presence of extra-renal symptoms. A positive family history for CKD is recorded if the participant either has a first (parent or child), second (siblings, grandparents, grandchildren), third (aunts, uncles, nephews, nieces) or fourth (cousins) degree relative with CKD. Family history will be obtained from combining information present in the EHR with information obtained from the questionnaire. Other secondary analysis aims to define the percentage of genetic tests with a clinical consequence. A genetic diagnosis is considered to have a clinical consequence if it: 1) negated the need for kidney biopsy, 2) triggered or negated the need for immunosuppressive therapy, 3) provides prognostic information, i.e. the risk of post-transplantation anti-GBM glomerulonephritis, 4) led to, or should lead to, referral to other specialties (e.g. ophthalmologist), 5) led to targeted work-up for associated symptoms or extra-renal manifestations, 6) affected surveillance frequency, 7) led to, or should lead to, genetic testing in potential living related kidney donors, 8) enabled more precise (preconception) genetic counseling for the patient or family members, or 9) led to more precise or extensive follow-up of potentially affected family members.

Tertiary outcomes will be the percentage of participants in which a VUS was identified and the number of incidental/secondary findings (results unrelated to the initial indication for genetic testing). In addition, if a molecular diagnosis is identified and a kidney biopsy from the native kidney is present, we will assess if the biopsy findings match the molecular diagnosis. Finally, we will perform health economic analyses to determine if MPS in patients with unexplained CKD is cost-effective.

We will report the number of participants who withdraw from participating in the VARIETY study after initial inclusion and the number of participants who have initially been included, but upon further analysis by the study team did not match the inclusion criteria. Participants without results from genetic testing will be excluded from all analyses. If information is missing from the EHR, we will ask the general practitioner to deliver the missing data within participants' consent. If the data cannot be retrieved, it will be regarded as "unknown". In case eGFR at CKD onset is missing, the first available eGFR or serum creatinine measurement since the diagnosis of CKD will be used.

Statistical analysis

Statistical analysis will be performed with IBM SPSS statistics for Windows, version 23 (IBM Corporation, Armonk, NY, USA). An overall significance level of 0.05 will be handled. Continuous variables that are normally distributed will be presented as mean and standard deviation. Non-normally distributed variables will be expressed as median and interquartile range. Frequencies and percentages will be used to describe categorical variables such as gender, family history, renal replacement therapy, extra-renal manifestations, and diagnostic yield. The Chi-square or Fisher's exact test will be used to compare differences in categorical variables between the different subgroups of the secondary analysis. Logistic regression will be performed to identify characteristics associated with a genetic diagnosis.

Sample size calculation

The minimal sample size was calculated using the following formula [27], based on the study's primary endpoint:

$$n = \frac{Z^2 * P(1-P)}{d^2}$$

Based on the literature, the expected percentage of positive test results is 17% [14]. Assuming a level of confidence (z) of 1.96 and precision (d) of 0.05 [27], a minimum of 217 participants are required for a reliable assessment of the primary outcome. In order to be clinically and politically significant, we aim to increase the sample size of this prospective cohort study beyond the largest currently available retrospective study, i.e. to include at least 282 patients in the current study [20,27].

Data management

Study data will be recorded digitally using the secure REDCap electronic data capture tool (REDCap, Nashville, TN) hosted at the UMCG [28,29]. Data collection and entry is performed by trained investigators from the UMCG. To minimize differences and errors in data entry, investigators from the UMCG will travel to other participating centers for data collection and entry in REDCap. Data validation in REDCap will be performed according to a data validation plan, which has been made in collaboration with the UMCG Research Data Support and approved by the Institutional Review Board. Data analysis will take place on validated and anonymized data. Upon study closure, data will be extracted from REDCap and exported to SPSS for analysis.

Patient and public involvement

Patients and/or the public were not involved in the design of this study.

ETHICS AND DISSEMINATION

Ethical approval for the study has been obtained from the institutional review board of the UMCG (METc 2019/106). The study is conducted in accordance with the WMA Declaration of Helsinki. The results of the study will be presented at (inter)national congresses and submitted for open access publication in peer-reviewed journals. In addition to the primary results, related to the main research questions as defined above, case reports/series may be submitted for publication in case of unique or interesting findings and these will also be submitted for publication in peer-reviewed journals. In accordance with the information sheet for participants, the main results and any publications from the VARIETY study will also be made available on the study website. After completion of the study and publication of the main results, request for re-use of the data can be submitted to the corresponding author.

CONCLUSION AND STUDY STATUS

Genetic testing shows promising results as a diagnostic tool in adults with CKD and it has the potential to resolve CKD cases with an unknown etiology. However, further research is needed in a clinical setting to define the position of MPS-based diagnostics in clinical practice and to determine which subpopulations will have the highest diagnostic yield. Here, we outlined the design for a prospective cohort study that will determine the diagnostic yield of MPS-based renal gene panel testing in patients with unexplained CKD. The fact that unexplained CKD has not been uniformly defined by international (guideline) committees or institutions may slightly impact external validity of our findings. However, results from this study are likely a step forward in informing physicians and policymakers involved in implementation of genetic testing in patients with unexplained CKD. Inclusion started on 31 July 2019. As of September 2021, 248 patients have been included.

Acknowledgements

Several authors of this publication (AE and NK) are members of the European Reference Network for Rare Kidney Diseases (ERKNet) - Project ID no. 739532.

Author contributions

AH and MB wrote the first draft of the manuscript. ME, LV, BZ, AE and NK gave feedback and contributed to manuscript revision. All authors read and approved the submitted version.

Funding statement

This collaboration project is co-financed by Sanofi Genzyme and the Dutch Ministry of Economic Affairs and Climate Policy by means of the PPP Allowance made available by the

Top Sector Life Sciences & Health to stimulate public-private partnerships (grant numbers RVO/6320 and IMAGEN/LSHM20009).

Competing interest statement

Dr. De Borst and Dr. Vogt have received research support and lecture fees (all to institution) from Sanofi Genzyme related to the current study. Prof. Knoers has received reimbursement of travel expenses for lectures related to the current study from Sanofi Genzyme.

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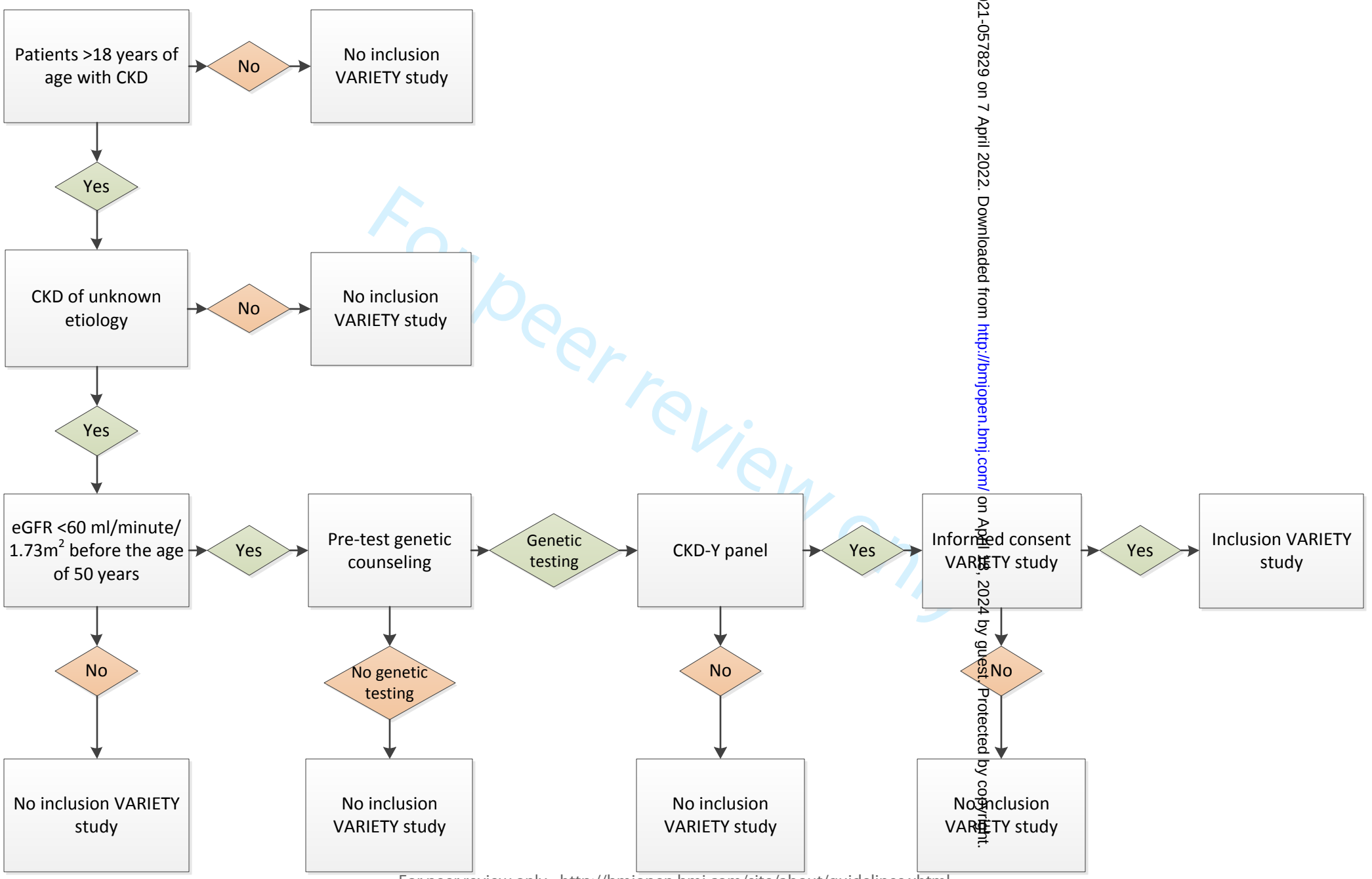
FIGURE LEGENDS

Figure 1. Flowchart for inclusion VARIETY study. CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate.

Figure 2. Overview of the 141 genes that are analyzed in the exome sequencing based Chronic Kidney Disease in Young patients (CKD-Y) panel version v18 at University Medical Center Utrecht

Figure 3. Overview of the 256 genes that are analyzed in the exome sequencing based Chronic Kidney Disease in Young patients (CKD-Y) panel version v21 at University Medical Center Utrecht. Bold genes are also on the CKD-Y panel v18.



141 genes in the young kidney failure (CKD-Y) panel v18						
ACE	CEP290	EMP2	INF2	MYH11	PKD1	TBX18
ACTN4	CFB	EYA1	INVS	MYH9	PKD2	TMEM67
ADCK4	CFH	FAN1	IQCB1	MYO1E	PKHD1	TNXB
AGT	CFHR5	FAT1	ITGA3	NEK8	PLCE1	TRAF3IP1
AGTR1	CFI	FGA	ITGA8	NOTCH2	PMM2	TRAP1
AGXT	CHD7	FN1	JAG1	NPHP1	PTPRO	TRPC6
ALG1	CLCN5	FOXC2	KANK1	NPHP3	REN	TTC21B
AMN	COL4A3	FRAS1	KANK2	NPHP4	RMND1	UMOD
ANKS6	COL4A4	FREM1	KANK4	NPHS1	ROBO2	VIPAS39
APOA1	COL4A5	FREM2	KIAA0556	NPHS2	RPGRIP1L	VPS33B
APOL1	COQ2	GATA3	KIAA0586	NUP107	RRM2B	WDR19
ARHGDIA	COQ6	GLA	LAMB2	NUP205	SALL1	WT1
ATXN10	CRB2	GLIS2	LMNA	NUP93	SCARB2	XPNPEP3
B2M	CTNS	GRHPR	LMX1B	NXF5	SDCCAG8	ZMPSTE24
BBIP1	CUBN	GRIP1	LRIG2	OCRL	SGPL1	ZNF423
BCS1L	CYP11B1	GSN	LYZ	OFD1	SIX5	
C3	CYP11B2	HNF1B	MAFB	OSGEP	SLC41A1	
CD151	DACT1	HOGA1	MAGI2	PAX2	SLC4A1	
CD2AP	DCDC2	HPSE2	MAP7D3	PBX1	SLC7A7	
CD46	DGKE	IFT27	MAPKBP1	PDSS1	SMARCAL1	
CEP164	DSTYK	IFT81	MUC1	PDSS2	SOX17	

256 genes in the young kidney failure (CKD-Y) panel v21						
ACE	BICC1	**CUBN**	HYLS1	MOCOS	**PLCE1**	TMEM138
ACTG2	BMPR2	CUL3	**IFT27**	MTR	**PMM2**	TMEM216
ACTN4	**C3**	**CYP11B1**	IFT74	MTRR	POC1B	TMEM231
ADAMTS9	C8ORF37	**CYP11B2**	**IFT81**	MTX2	PODXL	TMEM237
AGT	CACNA1D	CYP17A1	IL1RAP	**MUC1**	**PTPRO**	**TMEM67**
AGTR1	CACNA1H	DAAM2	**INF2**	**MYH11**	**REN**	TMEM72
AGXT	CC2D2A	**DACT1**	INPP5E	**MYH9**	**RMND1**	TNS2
AHI1	**CD151**	**DCDC2**	**INVS**	**MYO1E**	**ROBO2**	**TNXB**
ALG1	**CD2AP**	**DGKE**	**IQCB1**	**NEK8**	**RPGRIP1L**	TOGARAM1
ALMS1	**CD46**	DLC1	**ITGA3**	NOS1AP	**RRM2B**	TP53RK
AMN	CDK20	DNAJB11	**ITGA8**	**NOTCH2**	**SALL1**	TPRKB
ANKS6	CEP104	**DSTYK**	ITGB4	**NPHP1**	SARS2	**TRAF3IP1**
ANLN	**CEP164**	E2F3	ITSN1	**NPHP3**	**SCARB2**	**TRAP1**
APOA1	**CEP290**	**EMP2**	ITSN2	**NPHP4**	SCNN1A	TRIM32
APOE	CEP41	**EYA1**	**JAG1**	**NPHS1**	SCNN1B	TRIM8
APOL1	CEP83	FAM149B1	**KANK1**	**NPHS2**	SCNN1G	**TRPC6**
APRT	**CFB**	**FAN1**	**KANK2**	NR3C1	**SDCCAG8**	**TTC21B**
ARHGAP24	**CFH**	**FAT1**	**KANK4**	NR3C2	SEC61A1	TTC8
ARHGDIA	CFHR1	**FGA**	KATNIP	**NUP107**	**SGPL1**	**UMOD**
ARL13B	CFHR2	**FN1**	KCNJ5	NUP133	SIX1	**VIPAS39**
ARL6	CFHR3	**FOXC2**	**KIAA0586**	NUP160	**SIX5**	**VPS33B**
ARMC9	CFHR4	**FRAS1**	KIF3B	**NUP205**	SLC22A12	WDPCP
ATXN10	**CFHR5**	**FREM1**	KIRREL1	NUP85	SLC2A9	**WDR19**
AVIL	**CFI**	**FREM2**	KLHL3	**NUP93**	SLC3A1	WDR35
B2M	**CHD7**	GANAB	**LAMB2**	**NXF5**	**SLC41A1**	WDR60
B9D1	CLCN2	GAPVD1	**LMNA**	**OCRL**	**SLC4A1**	WDR73
B9D2	**CLCN5**	**GATA3**	**LMX1B**	**OFD1**	**SLC7A7**	WNK1
BBIP1	**COL4A3**	GATM	**LRIG2**	**OSGEP**	SLC7A9	WNK4
BBS1	**COL4A4**	**GLA**	**LYZ**	**PAX2**	**SMARCAL1**	**WT1**
BBS10	**COL4A5**	**GLIS2**	LZTFL1	**PBX1**	**SOX17**	XDH
BBS12	**COQ2**	**GRHPR**	**MAFB**	PCM1	STX16	**XPNPEP3**
BBS2	**COQ6**	**GRIP1**	**MAGI2**	**PDSS1**	TBC1D8B	YRDC
BBS4	COQ8B	**GSN**	**MAP7D3**	**PDSS2**	**TBX18**	**ZMPSTE24**
BBS5	CPLANE1	**HNF1B**	**MAPKBP1**	PIBF1	TCTN1	**ZNF423**
BBS7	**CRB2**	**HOGA1**	MKKS	**PKD1**	TCTN2	
BBS9	CSPP1	**HPSE2**	MKS1	**PKD2**	TCTN3	
BCS1L	**CTNS**	HSD11B2	MMACHC	**PKHD1**	TMEM107	

SUPPLEMENTARY MATERIAL

379 genes in hereditary kidney disease panel v18						
ACE	CA2	DGAT1	**GSN**	MAGED2	**ROBO2**	TCTEX1D2
ACTG2	CACNA1H	**DGKE**	GUCY2C	**MAGI2**	RPGRIP1	TCTN1
ACTN4	CACNA1S	DMP1	HAAO	**MAP7D3**	**RPGRIP1L**	TCTN2
ADAMTS13	CASR	DNAJB11	**HNF1B**	**MAPKBP1**	**RRM2B**	TCTN3
ADCK3	CC2D2A	DST	HNF4A	MET	**SALL1**	THBD
ADCK4	CCDC114	**DSTYK**	**HOGA1**	MKKS	SALL4	TMEM104
AGT	**CD151**	DYNC2H1	HOXD13	MKS1	SARS2	TMEM107
AGTR1	**CD2AP**	DYNC2LI1	HPRT1	**MUC1**	**SCARB2**	TMEM138
AGXT	**CD46**	DZIP1L	**HPSE2**	**MYH11**	SCLT1	TMEM216
AHI1	CDKN1C	EGF	HSD11B2	**MYH9**	SCN11A	TMEM231
ALDOB	CEP120	EHHADH	IFT122	**MYO1E**	SCN4A	TMEM237
ALG1	**CEP164**	**EMP2**	IFT140	MYO5B	SCNN1A	**TMEM67**
ALG8	**CEP290**	ENPP1	IFT172	NEK1	SCNN1B	**TNXB**
ALMS1	CEP41	EPCAM	**IFT27**	**NEK8**	SCNN1G	TP53RK
AMN	CEP83	EVC	IFT43	NEUROG3	**SDCCAG8**	TPRKB
ANKS3	**CFB**	EVC2	IFT52	NGF	SDHB	**TRAF3IP1**
ANKS6	**CFH**	**EYA1**	IFT57	**NOTCH2**	SEC61A1	**TRAP1**
ANLN	CFHR1	FAH	IFT80	**NPHP1**	SEC61B	TRIM32
ANO1	CFHR2	FAHD2A	**IFT81**	**NPHP3**	SEC63	**TRPC6**
AP2S1	CFHR3	FAM134B	IKBKAP	**NPHP4**	**SGPL1**	TRPM6
APOA1	CFHR4	FAM20A	**INF2**	**NPHS1**	SIX1	TSC1
APOL1	**CFHR5**	FAM58A	INPP5E	**NPHS2**	SIX2	TSC2
APRT	**CFI**	**FAN1**	**INVS**	NR3C1	**SIX5**	**TTC21B**
AQP2	CHD1L	**FAT1**	**IQCB1**	NR3C2	SLC12A1	TTC8
ARHGAP24	**CHD7**	FBXL4	**ITGA3**	**NUP107**	SLC12A3	**UMOD**
ARHGDIA	CHRM3	**FGA**	**ITGA8**	**NUP205**	SLC16A12	UPK3A
ARL13B	**CLCN5**	FGF20	ITGB4	**NUP93**	SLC22A12	UQCC2
ARL6	CLCNKA	FGF23	**JAG1**	**NXF5**	SLC26A3	VDR
ARSA	CLCNKB	FGF8	KAL1	**OCRL**	SLC2A2	VHL
ATP6V0A4	CLDN16	FGFR1	**KANK1**	**OFD1**	SLC2A9	**VIPAS39**
ATP6V1B1	CLDN19	FH	**KANK2**	**OSGEP**	SLC34A1	**VPS33B**
ATP7B	CNNM2	FLCN	**KANK4**	**PAX2**	SLC34A3	WDPCP
ATXN10	COL4A1	**FN1**	KCNJ1	PAX8	SLC36A2	**WDR19**
AVP	**COL4A3**	**FOXC2**	KCNJ10	**PBX1**	SLC37A4	WDR34
AVPR2	**COL4A4**	FOXF1	KCNJ5	PCBD1	SLC3A1	WDR35
B2M	**COL4A5**	**FRAS1**	**KIAA0556**	PDE6D	**SLC41A1**	WDR60
B9D1	**COQ2**	**FREM1**	**KIAA0586**	**PDSS1**	**SLC4A1**	WDR73
B9D2	COQ4	**FREM2**	KIF14	**PDSS2**	SLC4A4	WNK1
BBIP1	**COQ6**	FXYD2	KIF7	PHEX	SLC5A2	WNK4
BBS1	COQ7	G6PC	KL	**PKD1**	SLC6A19	WNT4
BBS10	COQ9	GALNT3	KLHL3	**PKD2**	SLC6A20	**WT1**
BBS12	COX10	GALT	KYNU	**PKHD1**	**SLC7A7**	XDH

BBS2	CPT2	GANAB	LAGE3	**PLCE1**	SLC7A9	**XPNPEP3**
BBS4	**CRB2**	**GATA3**	**LAMB2**	**PMM2**	SLC9A3	XPO5
BBS5	CSPP1	GDNF	LCAT	PODXL	SLC9A3R1	YRDC
BBS7	**CTNS**	**GLA**	**LMNA**	PRDM12	SLIT2	ZEB2
BBS9	**CUBN**	GLI3	LMOD1	PRKCSH	**SMARCAL1**	ZIC3
BCS1L	CUL3	**GLIS2**	**LMX1B**	PSAP	**SOX17**	**ZMPSTE24**
BICC1	**CYP11B1**	GLIS3	LPP	PTEN	SPINT2	**ZNF423**
BMP4	**CYP11B2**	GNA11	**LRIG2**	PTH1R	SPTLC1	
BMPR2	CYP17A1	GPC3	LRP2	**PTPRO**	SPTLC2	
BSND	CYP24A1	GPC5	LRP4	PYGM	STRA6	
C2CD3	**DACT1**	GREB1L	**LYZ**	**REN**	STX16	
C3	**DCDC2**	**GRHPR**	LZTFL1	RET	TBC1D1	
C5orf42	DDX59	**GRIP1**	**MAFB**	**RMND1**	**TBX18**	

Supplementary Figure 1. The 379 genes that are on the hereditary kidney disease panel v18 at University Medical Center Utrecht. Bold genes are also on the CKD-Y panel v18.

495 genes in hereditary kidney disease panel v21						
ACE	***CACNA1H***	**DGAT1**	**GREB1L**	***LRIG2***	**PRDM12**	**STRA6**
ACTA2	**CACNA1S**	***DGKE***	GREM1	LRP10	PRDX1	STRADA
ACTG2	**CASR**	DHCR7	***GRHPR***	**LRP2**	**PRKCSH**	***STX16***
ACTN4	CBWD1	DICER1	***GRIP1***	**LRP4**	**PSAP**	SYNPO
ADAMTS13	CBY1	***DLC1***	***GSN***	LRP5	**PTEN**	**TBC1D1**
ADAMTS9	***CC2D2A***	**DMP1**	**GUCY2C**	***LYZ***	**PTH1R**	***TBC1D8B***
ADCK3	**CCDC114**	***DNAJB11***	**HAAO**	***LZTFL1***	***PTPRO***	***TBX18***
ADCY10	CCDC28B	DOCK4	***HNF1B***	***MAFB***	**PYGM**	TBX6
AGK	***CD151***	**DST**	**HNF4A**	**MAGED2**	RBM8A	**TCTEX1D2**
AGT	***CD2AP***	***DSTYK***	***HOGA1***	***MAGI2***	***REN***	***TCTN1***
AGTR1	***CD46***	**DYNC2H1**	HOXA10	***MAP7D3***	RERE	***TCTN2***
AGXT	CDC73	**DYNC2LI1**	HOXA13	***MAPKBP1***	**RET**	***TCTN3***
AHI1	***CDK20***	**DZIP1L**	**HOXD13**	**MET**	RICTOR	**THBD**
ALDOB	**CDKN1C**	***E2F3***	**HPRT1**	***MKKS***	***RMND1***	**TMEM104**
ALG1	CENPF	**EGF**	***HPSE2***	***MKS1***	ROBO1	***TMEM107***
ALG5	***CEP104***	**EHHADH**	HRAS	***MMACHC***	***ROBO2***	***TMEM138***
ALG6	**CEP120**	ELP1	***HSD11B2***	***MOCOS***	**RPGRIP1**	***TMEM216***
ALG8	***CEP164***	***EMP2***	HSPA6	***MTR***	***RPGRIP1L***	***TMEM231***
ALG9	***CEP290***	**ENPP1**	***HYLS1***	***MTRR***	RRAGD	***TMEM237***
ALMS1	***CEP41***	**EPCAM**	ICK	***MTX2***	***RRM2B***	TMEM260
ALPL	CEP55	ERCC6	**IFT122**	***MUC1***	***SALL1***	***TMEM67***
AMN	***CEP83***	ERCC8	**IFT140**	***MYH11***	**SALL4**	***TMEM72***
ANKFY1	***CFB***	**EVC**	**IFT172**	***MYH9***	***SARS2***	***TNS2***
ANKS3	***CFH***	**EVC2**	***IFT27***	MYLK	***SCARB2***	***TNXB***
ANKS6	***CFHR1***	EVX1	**IFT43**	***MYO1E***	**SCLT1**	***TOGARAM1***
ANLN	***CFHR2***	EXOC8	**IFT52**	**MYO5B**	**SCN11A**	***TP53RK***
ANOS1	***CFHR3***	***EYA1***	**IFT57**	NAALADL2	**SCN4A**	TP63
AP2S1	***CFHR4***	**FAH**	***IFT74***	NCAPG2	***SCNN1A***	***TPRKB***
APOA1	***CFHR5***	**FAHD2A**	**IFT80**	**NEK1**	***SCNN1B***	***TRAF3IP1***
APOE	***CFI***	**FAM134B**	***IFT81***	***NEK8***	***SCNN1G***	***TRAP1***
APOL1	**CHD1L**	***FAM149B1***	***IL1RAP***	NEU1	***SDCCAG8***	***TRIM32***
APRT	***CHD7***	**FAM20A**	***INF2***	**NEUROG3**	**SDHB**	***TRIM8***
AQP2	**CHRM3**	FAM20C	***INPP5E***	**NGF**	***SEC61A1***	***TRPC6***
ARHGAP24	CHRNA3	**FAM58A**	INTU	***NOS1AP***	**SEC61B**	**TRPM6**
ARHGDIA	***CLCN2***	***FAN1***	***INVS***	***NOTCH2***	**SEC63**	TRPM7
ARL13B	***CLCN5***	***FAT1***	***IQCB1***	***NPHP1***	***SGPL1***	**TSC1**
ARL3	**CLCNKA**	**FBXL4**	ISL1	***NPHP3***	***SIX1***	**TSC2**
ARL6	**CLCNKB**	***FGA***	***ITGA3***	***NPHP4***	**SIX2**	TSHZ3
ARMC9	CLDN10	**FGF20**	***ITGA8***	***NPHS1***	***SIX5***	***TTC21B***
ARSA	**CLDN16**	**FGF23**	***ITGB4***	***NPHS2***	SKAP2	***TTC8***
ATP1A1	**CLDN19**	**FGF8**	***ITSN1***	NPNT	**SLC12A1**	TXNDC15
ATP6V0A4	**CNNM2**	**FGFR1**	***ITSN2***	***NR3C1***	**SLC12A3**	***UMOD***
ATP6V1B1	**COL4A1**	**FH**	***JAG1***	***NR3C2***	**SLC16A12**	**UPK3A**
ATP7B	***COL4A3***	**FLCN**	***KANK1***	NRAS	SLC19A2	**UQCC2**

ATXN10	***COL4A4***	***FN1***	***KANK2***	***NUP107***	***SLC22A12***	**VDR**
AVIL	***COL4A5***	***FOXC2***	***KANK4***	***NUP133***	SLC26A1	**VHL**
AVP	***COQ2***	**FOXF1**	***KATNIP***	***NUP160***	**SLC26A3**	***VIPAS39***
AVPR2	**COQ4**	FOXI1	**KCNJ1**	***NUP205***	**SLC2A2**	***VPS33B***
B2M	***COQ6***	***FRAS1***	**KCNJ10**	***NUP85***	***SLC2A9***	***WDPCP***
B9D1	**COQ7**	***FREM1***	***KCNJ5***	***NUP93***	**SLC34A1**	***WDR19***
B9D2	***COQ8B***	***FREM2***	KCTD1	***NXF5***	**SLC34A3**	**WDR34**
BBIP1	**COQ9**	**FXYD2**	KCTD3	***OCRL***	**SLC36A2**	***WDR35***
BBS1	**COX10**	**G6PC**	***KIAA0586***	***OFD1***	**SLC37A4**	***WDR60***
BBS10	***CPLANE1***	**GALNT3**	KIAA0753	***OSGEP***	***SLC3A1***	WDR72
BBS12	**CPT2**	**GALT**	**KIF14**	***PAX2***	***SLC41A1***	***WDR73***
BBS2	***CRB2***	***GANAB***	***KIF3B***	**PAX8**	***SLC4A1***	***WNK1***
BBS4	***CSPP1***	***GAPVD1***	**KIF7**	***PBX1***	**SLC4A4**	***WNK4***
BBS5	***CTNS***	***GATA3***	***KIRREL1***	**PCBD1**	**SLC5A2**	**WNT4**
BBS7	***CUBN***	***GATM***	**KL**	***PCM1***	**SLC6A19**	WNT9B
BBS9	***CUL3***	**GDNF**	***KLHL3***	**PDE6D**	**SLC6A20**	***WT1***
BCS1L	***CYP11B1***	GDF6	KRAS	***PDSS1***	***SLC7A7***	***XDH***
BICC1	***CYP11B2***	GFRA1	**KYNU**	***PDSS2***	***SLC7A9***	***XPNPEP3***
BMP4	***CYP17A1***	***GLA***	**LAGE3**	**PHEX**	**SLC9A3**	**XPO5**
BMPR2	**CYP24A1**	**GLI3**	LAMA5	***PIBF1***	**SLC9A3R1**	***YRDC***
BNC2	CYP27B1	***GLIS2***	***LAMB2***	***PKD1***	**SLIT2**	**ZEB2**
BSND	CYP2R1	**GLIS3**	**LCAT**	***PKD2***	SLIT3	**ZIC3**
C2CD3	CYP3A4	**GNA11**	LHX1	***PKHD1***	***SMARCAL1***	***ZMPSTE24***
C3	***DAAM2***	GNAS	***LMNA***	***PLCE1***	***SOX17***	ZNF365
C8ORF37	***DACT1***	GON7	**LMOD1**	***PMM2***	**SPINT2**	***ZNF423***
CA2	***DCDC2***	**GPC3**	***LMX1B***	***POC1B***	**SPTLC1**	
CACNA1D	**DDX59**	**GPC5**	**LPP**	***PODXL***	**SPTLC2**	

Supplementary Figure 2. The 495 genes that are on the hereditary kidney disease panel v21 at University Medical Center Utrecht. Bold genes are also hereditary kidney disease panel v18 and italic genes are also on the CKD-Y panel v21.

Supplementary Table 1. Questionnaire for participants (original version is in Dutch)

Number	Question	Answer possibilities
General questions		
1	What is your country of birth?	Open
2	What is the country of birth of your maternal grandmother?	Open
3	What is the country of birth of your maternal grandfather?	Open
4	What is the country of birth of your paternal grandmother?	Open
5	What is the country of birth of your paternal grandfather?	Open
Medical health and current health complaints		
6	At which age did you get the diagnosis chronic kidney disease?	Open
7	Did you undergo dialysis in the past or are you currently on dialysis?	Yes/no/unknown
8	Did you undergo a kidney transplantation in the past?	Yes/no/unknown
9	Do you have high blood pressure? If you are taking blood pressure-lowering medication and have a normal blood pressure thanks to the medication, you can also fill in "yes".	Yes/no/unknown
10	Have you ever been admitted to the emergency room for high blood pressure?	Yes/no/unknown
11	Are you unable or do you have trouble with sweating?	Yes/no/unknown

12	Do you suffer from heat- or cold intolerance? This means that you have trouble with handling heat or cold.	Yes/no/unknown
13	Have you experienced a burning pain or a feeling of tingling in the hands and/or feet now or in the past?	Yes/no/unknown
13a	If so, did this pain or tingling feeling arise or get worse with fever, exertion, stress, or if the hands or feet became very hot or cold?	Open
14	Do you have dark, red-purple spots in your skin? Especially between your belly button and knees?	Yes/no/unknown
15	Do you have any problems with seeing or any eye complaints?	Yes/no/unknown
15a	If so, what are your problems with seeing and/or eye complaints?	Open
16	Do you have any hearing problems of hearing disabilities?	Yes/no/unknown
16a	If so, what for hearing problems or disabilities do you have?	Open
17	Have you suffered from gout now or in the past?	Yes/no/unknown
18	Have you ever had a stroke (cerebral infraction, brain hemorrhage or TIA)?	Yes/no/unknown
19	Did you ever have a myocardial infarction?	Yes/no/unknown
20	Do you have a heart rhythm disorder?	Yes/no/unknown
20a	If so, which heart rhythm disorder do you have?	Open
21	Do you have a thickening of the heart muscle (hypertrophic cardiomyopathy)?	Yes/no/unknown

22	Do you have health complaints not mentioned in the previous questions?	Yes/no/unknown
22a	If so, which heath complaints do you experience?	Open
Family history		
23	How many biological children, alive or deceased, do you have?	Open
24	Do you (still) have any desire to have children?	Yes/no/unknown
25	How many siblings, alive or deceased, do you have?	Open
26	How many half-brothers and/or half-sisters, alive or deceased, do you have?	Open
27	How many siblings, alive or deceased, does your mother have?	Open
28	How many siblings, alive or deceased, does your father have?	Open
29	Are your grandparents still alive?	Yes/no/unknown
29a	Did one of your grandparents pass away before the age of 50 years?	Yes/no/unknown
30	Are your parents blood relatives (e.g. second cousins)?	Yes/no/unknown
30a	If so, how are your parents related to each other?	Open
31	Are you and your partner blood relatives (e.g. cousins, second cousins)?	Yes/no/I do not have a partner/unknown
31a	If so, how are you and your partner related to each other?	Open
32	Does gout run in your family?	Yes/no/unknown
33	Do you have family members with a high blood pressure at a young age?	Yes/no/unknown
34	Does anyone in your family have an intellectual disability?	Yes/no/unknown

35	Dou you have family members with kidney disease (children, parents, siblings, grandparents, uncles/aunts, cousins, nephews/nieces)?	Yes/no/unknown
35a	If so, how many family members have a kidney disease?	Open
35b	In how many family members if the cause for the kidney disease unknown?	Open
35c	If you know the cause of the kidney disease of other family members, please write down the cause of the kidney disease in this field. If you do not know the cause, you can leave this field empty.	Open
35d	How many family members with a kidney disease have had a kidney transplantation or dialysis?	Open
Final questions		
36	Have you visited a clinical geneticist or have you been referred to a clinical geneticist?	Yes/no/unknown
37	Do you already known the results from genetic testing at the time of completing this questionnaire?	Yes/no/unknown

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6-7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-12
Bias	9	Describe any efforts to address potential sources of bias	13
Study size	10	Explain how the study size was arrived at	12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12
		(*b*) Describe any methods used to examine subgroups and interactions	12
		(*c*) Explain how missing data were addressed	11
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

NA: not applicable

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.