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Published in:
BMJ Open

DOI:
[10.1136/bmjopen-2021-057829](https://doi.org/10.1136/bmjopen-2021-057829)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

de Haan, A., Eijgelsheim, M., Vogt, L., van der Zwaag, B., van Eerde, A. M., Knoers, N. V. A. M., & de Borst, M. H. (2022). Diagnostic yield of massively parallel sequencing in patients with chronic kidney disease of unknown etiology: rationale and design of a national prospective cohort study. *BMJ Open*, 12(4), [057829]. <https://doi.org/10.1136/bmjopen-2021-057829>

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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

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To cite: de Haan A, Eijgelsheim M, Vogt L, *et al*. Diagnostic yield of massively parallel sequencing in patients with chronic kidney disease of unknown etiology: rationale and design of a national prospective cohort study. *BMJ Open* 2022;**12**:e057829. doi:10.1136/bmjopen-2021-057829

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-057829>).

Received 29 September 2021
Accepted 16 March 2022



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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 CKD) 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 m² before the age of 50 years in clinical practice.

Methods and analysis The VARIETY study is an ongoing, prospective, nationwide 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 extrarenal 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.

INTRODUCTION

Chronic kidney disease (CKD) affects 11%–16% of the population worldwide,^{1–3} is associated with extensive comorbidity 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 (eg, diabetes, hypertension) or

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 (CKD) in a routine healthcare setting.
- Nationwide 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 at first presentation) with unexplained CKD.
- A potential limitation is that the definition of 'unexplained CKD' is not unequivocal.

primary renal diseases (eg, 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 aetiology.

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 (eg, based on age and severity at disease onset, extrarenal manifestations, positive family history) that should preferentially undergo genetic testing. A recent joint publication by the ERA Working Group on Inherited Kidney Disorders and the Molecular Diagnostics Taskforce of the European Rare Kidney Disease Reference Network 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, that is, the percentage of participants with a genetic diagnosis, using a large MPS-based multigene panel for kidney diseases in young patients (first presentation at age <50) with unexplained CKD (estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m²) 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 CKD) study is a prospective nationwide 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 analyse 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 anonymised data are collected, stored and analysed 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/1.73 m²

before the age of 50 years. Unexplained CKD is defined as the absence of all the following criteria: a biopsy-proven diagnosis (eg, IgA nephropathy), a specific morphological renal diagnosis (eg, polycystic kidney disease suspected of autosomal/recessive polycystic kidney disease), or a specific or plausible renal diagnosis (eg, history of long-term insulin-dependent diabetes mellitus before the onset of CKD, lithium-induced nephropathy). Since hypertensive nephropathy is a nonspecific 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 nonspecific 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/1.73 m² before the age of 50 years, and renal transplant recipients who had a pre-transplant eGFR <60 mL/min/1.73 m² 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 extrarenal features, medication use at inclusion, medical history, family history (including three-generation pedigree), blood pressure at CKD onset and at inclusion, presence of haematuria and/or nephrotic syndrome, laboratory results (serum creatinine, eGFR, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, 24-hour urine albumin excretion, 24-hour urine creatinine excretion, 24-hour urine total protein excretion, haematuria) 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 counselling 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 extrarenal manifestations (online supplemental table 1).

Genetic testing

We will include patients who have undergone MPS-based multigene panel testing, initiated by a clinical geneticist or nephrologist following pretest counselling 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 pretest genetic counselling.

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’ (‘CKD 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 8 March 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 (online supplemental figures 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

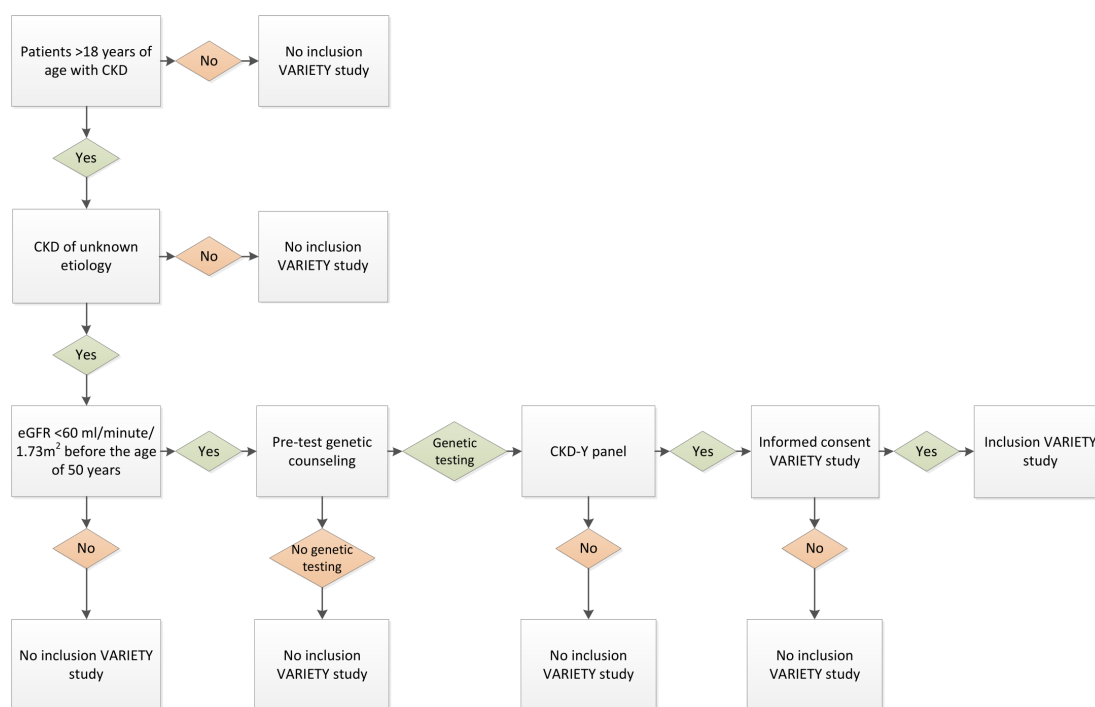


Figure 1 Flow chart for inclusion in the VARIETY study. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; VARIETY, Validation of algoRithms and IdEnTification of genes in Young patients with unexplained CKD.

141 genes in the Chronic Kidney Disease in Young patients (CKD-Y) panel						
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	RPM2B	WDR19
ARHGDI2	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	

Figure 2 Overview of the 141 genes that are analysed in the exome sequencing based Chronic Kidney Disease in young patients (CKD-Y) panel V.18 at University Medical Center Utrecht.

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,

256 genes in Chronic Kidney Disease in Young patients (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	ILIRAP	MUC1	PTPRO	TMEM67
AGTR1	CACNA1H	DAAM2	INF2	MYH11	REN	TMEM72
AGXT	CC2D2A	DACT1	INPP5E	MYH9	RMND1	TNS2
AH1	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
ARHGDI2	CFHR1	FGA	KATNIP	NUP107	SGPL1	UMOD
ARL13B	CFHR2	FN1	KCNJ5	NUP130	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	LZTF1	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	CSPPI	HPSE2	MKS1	PKD2	TCTN3	
BCS1L	CTNS	HSD11B2	MMACHC	PKHD1	TMEM107	

Figure 3 Overview of the 256 genes that are analysed in the exome sequencing based Chronic Kidney Disease in young patients (CKD-Y) panel V.21 at University Medical Center Utrecht. Bold genes are also on the CKD-Y panel V.18.

Table 1 Classification of variants according to ACMG guidelines²⁶

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

ACMG, American College of Medical Genetics.

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 (ie, 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 m² before the age of 50 years. We will perform a sensitivity analysis in patients with onset eGFR <60 mL/min/1.73 m² 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.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 programmes. Class one variants are clearly not pathogenic; class five variants are clearly pathogenic. Class three 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.

Secondary analyses include subgroup analyses according to age and eGFR at first presentation, family history and the presence of extrarenal 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, that is, the risk of post-transplantation anti-glomerular basement membrane (GBM) nephritis, (4) led to, or should lead to, referral to other specialties (eg, 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 counselling 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 on 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, V.23 (IBM). 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, extrarenal manifestations, and diagnostic yield. The χ^2 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, that is, to include at least 282 patients in the current study.^{20 27}

Data management

Study data will be recorded digitally using the secure REDCap (Research Electronic Data Capture) web application (REDCap, Nashville, Tennessee, USA) hosted at the UMCG.^{28 29} Data collection and entry is performed by trained investigators from the UMCG. To minimise differences and errors in data entry, investigators from the UMCG will travel to other participating centres 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 anonymised data. On 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 aetiology. 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.

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Acknowledgements Several authors of this publication (AMvE, NVAMK and MHdB) are members of the European Reference Network for Rare Kidney Diseases (ERKNet) - Project ID no. 739 532.

Contributors AdH and MHdB wrote the first draft of the manuscript. ME, LV, BvdZ, AMvE and NVAMK gave feedback and contributed to manuscript revision. All authors read and approved the submitted version.

Funding This collaboration project is cofinanced 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 interests MHdB and LV have received research support and lecture fees (all to institution) from Sanofi Genzyme related to the current study. NVAMK has received reimbursement of travel expenses for lectures related to the current study from Sanofi Genzyme.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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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
ARHGDI1A	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	FXYP2	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

<i>ACE</i>	<i>CACNA1H</i>	<i>DGAT1</i>	<i>GREB1L</i>	<i>LRIG2</i>	<i>PRDM12</i>	<i>STRA6</i>
<i>ACTA2</i>	<i>CACNA1S</i>	<i>DGKE</i>	<i>GREM1</i>	<i>LRP10</i>	<i>PRDX1</i>	<i>STRADA</i>
<i>ACTG2</i>	<i>CASR</i>	<i>DHCR7</i>	<i>GRHPR</i>	<i>LRP2</i>	<i>PRKCSH</i>	<i>STX16</i>
<i>ACTN4</i>	<i>CBWD1</i>	<i>DICER1</i>	<i>GRIP1</i>	<i>LRP4</i>	<i>PSAP</i>	<i>SYNPO</i>
<i>ADAMTS13</i>	<i>CBY1</i>	<i>DLC1</i>	<i>GSN</i>	<i>LRP5</i>	<i>PTEN</i>	<i>TBC1D1</i>
<i>ADAMTS9</i>	<i>CC2D2A</i>	<i>DMP1</i>	<i>GUCY2C</i>	<i>LYZ</i>	<i>PTH1R</i>	<i>TBC1D8B</i>
<i>ADCK3</i>	<i>CCDC114</i>	<i>DNAJB11</i>	<i>HAAO</i>	<i>LZTFL1</i>	<i>PTPRO</i>	<i>TBX18</i>
<i>ADCY10</i>	<i>CCDC28B</i>	<i>DOCK4</i>	<i>HNF1B</i>	<i>MAFB</i>	<i>PYGM</i>	<i>TBX6</i>
<i>AGK</i>	<i>CD151</i>	<i>DST</i>	<i>HNF4A</i>	<i>MAGED2</i>	<i>RBM8A</i>	<i>TCTEX1D2</i>
<i>AGT</i>	<i>CD2AP</i>	<i>DSTYK</i>	<i>HOGA1</i>	<i>MAGI2</i>	<i>REN</i>	<i>TCTN1</i>
<i>AGTR1</i>	<i>CD46</i>	<i>DYNC2H1</i>	<i>HOXA10</i>	<i>MAP7D3</i>	<i>RERE</i>	<i>TCTN2</i>
<i>AGXT</i>	<i>CDC73</i>	<i>DYNC2LI1</i>	<i>HOXA13</i>	<i>MAPKBP1</i>	<i>RET</i>	<i>TCTN3</i>
<i>AHI1</i>	<i>CDK20</i>	<i>DZIP1L</i>	<i>HOXD13</i>	<i>MET</i>	<i>RICTOR</i>	<i>THBD</i>
<i>ALDOB</i>	<i>CDKN1C</i>	<i>E2F3</i>	<i>HPRT1</i>	<i>MKKS</i>	<i>RMND1</i>	<i>TMEM104</i>
<i>ALG1</i>	<i>CENPF</i>	<i>EGF</i>	<i>HPSE2</i>	<i>MKS1</i>	<i>ROBO1</i>	<i>TMEM107</i>
<i>ALG5</i>	<i>CEP104</i>	<i>EHHADH</i>	<i>HRAS</i>	<i>MMACHC</i>	<i>ROBO2</i>	<i>TMEM138</i>
<i>ALG6</i>	<i>CEP120</i>	<i>ELP1</i>	<i>HSD11B2</i>	<i>MOCOS</i>	<i>RPGRIP1</i>	<i>TMEM216</i>
<i>ALG8</i>	<i>CEP164</i>	<i>EMP2</i>	<i>HSPA6</i>	<i>MTR</i>	<i>RPGRIP1L</i>	<i>TMEM231</i>
<i>ALG9</i>	<i>CEP290</i>	<i>ENPP1</i>	<i>HYLS1</i>	<i>MTRR</i>	<i>RRAGD</i>	<i>TMEM237</i>
<i>ALMS1</i>	<i>CEP41</i>	<i>EPCAM</i>	<i>ICK</i>	<i>MTX2</i>	<i>RRM2B</i>	<i>TMEM260</i>
<i>ALPL</i>	<i>CEP55</i>	<i>ERCC6</i>	<i>IFT122</i>	<i>MUC1</i>	<i>SALL1</i>	<i>TMEM67</i>
<i>AMN</i>	<i>CEP83</i>	<i>ERCC8</i>	<i>IFT140</i>	<i>MYH11</i>	<i>SALL4</i>	<i>TMEM72</i>
<i>ANKFY1</i>	<i>CFB</i>	<i>EVC</i>	<i>IFT172</i>	<i>MYH9</i>	<i>SARS2</i>	<i>TNS2</i>
<i>ANKS3</i>	<i>CFH</i>	<i>EVC2</i>	<i>IFT27</i>	<i>MYLK</i>	<i>SCARB2</i>	<i>TNXB</i>
<i>ANKS6</i>	<i>CFHR1</i>	<i>EVX1</i>	<i>IFT43</i>	<i>MYO1E</i>	<i>SCLT1</i>	<i>TOGARAMI</i>
<i>ANLN</i>	<i>CFHR2</i>	<i>EXOC8</i>	<i>IFT52</i>	<i>MYO5B</i>	<i>SCN11A</i>	<i>TP53RK</i>
<i>ANOS1</i>	<i>CFHR3</i>	<i>EYA1</i>	<i>IFT57</i>	<i>NAALADL2</i>	<i>SCN4A</i>	<i>TP63</i>
<i>AP2S1</i>	<i>CFHR4</i>	<i>FAH</i>	<i>IFT74</i>	<i>NCAPG2</i>	<i>SCNNIA</i>	<i>TPRKB</i>
<i>APOA1</i>	<i>CFHR5</i>	<i>FAHD2A</i>	<i>IFT80</i>	<i>NEK1</i>	<i>SCNNIB</i>	<i>TRAF3IP1</i>
<i>APOE</i>	<i>CFI</i>	<i>FAM134B</i>	<i>IFT81</i>	<i>NEK8</i>	<i>SCNNIG</i>	<i>TRAP1</i>
<i>APOL1</i>	<i>CHD1L</i>	<i>FAM149B1</i>	<i>IL1RAP</i>	<i>NEU1</i>	<i>SDCCAG8</i>	<i>TRIM32</i>
<i>APRT</i>	<i>CHD7</i>	<i>FAM20A</i>	<i>INF2</i>	<i>NEUROG3</i>	<i>SDHB</i>	<i>TRIM8</i>
<i>AQP2</i>	<i>CHRM3</i>	<i>FAM20C</i>	<i>INPP5E</i>	<i>NGF</i>	<i>SEC61A1</i>	<i>TRPC6</i>
<i>ARHGAP24</i>	<i>CHRNA3</i>	<i>FAM58A</i>	<i>INTU</i>	<i>NOS1AP</i>	<i>SEC61B</i>	<i>TRPM6</i>
<i>ARHGDI1</i>	<i>CLCN2</i>	<i>FAN1</i>	<i>INVS</i>	<i>NOTCH2</i>	<i>SEC63</i>	<i>TRPM7</i>
<i>ARL13B</i>	<i>CLCN5</i>	<i>FAT1</i>	<i>IQCB1</i>	<i>NPHP1</i>	<i>SGPL1</i>	<i>TSC1</i>
<i>ARL3</i>	<i>CLCNKA</i>	<i>FBXL4</i>	<i>ISL1</i>	<i>NPHP3</i>	<i>SIX1</i>	<i>TSC2</i>
<i>ARL6</i>	<i>CLCNKB</i>	<i>FGA</i>	<i>ITGA3</i>	<i>NPHP4</i>	<i>SIX2</i>	<i>TSHZ3</i>
<i>ARMC9</i>	<i>CLDN10</i>	<i>FGF20</i>	<i>ITGA8</i>	<i>NPHS1</i>	<i>SIX5</i>	<i>TTC21B</i>
<i>ARSA</i>	<i>CLDN16</i>	<i>FGF23</i>	<i>ITGB4</i>	<i>NPHS2</i>	<i>SKAP2</i>	<i>TTC8</i>
<i>ATP1A1</i>	<i>CLDN19</i>	<i>FGF8</i>	<i>ITSN1</i>	<i>NPNT</i>	<i>SLC12A1</i>	<i>TXNDC15</i>
<i>ATP6V0A4</i>	<i>CNNM2</i>	<i>FGFR1</i>	<i>ITSN2</i>	<i>NR3C1</i>	<i>SLC12A3</i>	<i>UMOD</i>
<i>ATP6V1B1</i>	<i>COL4A1</i>	<i>FH</i>	<i>JAG1</i>	<i>NR3C2</i>	<i>SLC16A12</i>	<i>UPK3A</i>
<i>ATP7B</i>	<i>COL4A3</i>	<i>FLCN</i>	<i>KANK1</i>	<i>NRAS</i>	<i>SLC19A2</i>	<i>UQC2</i>

<i>ATXN10</i>	<i>COL4A4</i>	<i>FNI</i>	<i>KANK2</i>	<i>NUP107</i>	<i>SLC22A12</i>	<i>VDR</i>
<i>AVIL</i>	<i>COL4A5</i>	<i>FOXC2</i>	<i>KANK4</i>	<i>NUP133</i>	<i>SLC26A1</i>	<i>VHL</i>
<i>AVP</i>	<i>COQ2</i>	<i>FOXF1</i>	<i>KATNIP</i>	<i>NUP160</i>	<i>SLC26A3</i>	<i>VIPAS39</i>
<i>AVPR2</i>	<i>COQ4</i>	<i>FOXJ1</i>	<i>KCNJ1</i>	<i>NUP205</i>	<i>SLC2A2</i>	<i>VPS33B</i>
<i>B2M</i>	<i>COQ6</i>	<i>FRAS1</i>	<i>KCNJ10</i>	<i>NUP85</i>	<i>SLC2A9</i>	<i>WDPCP</i>
<i>B9D1</i>	<i>COQ7</i>	<i>FREMI</i>	<i>KCNJ5</i>	<i>NUP93</i>	<i>SLC34A1</i>	<i>WDR19</i>
<i>B9D2</i>	<i>COQ8B</i>	<i>FREM2</i>	<i>KCTD1</i>	<i>NXF5</i>	<i>SLC34A3</i>	<i>WDR34</i>
<i>BBIP1</i>	<i>COQ9</i>	<i>FXD2</i>	<i>KCTD3</i>	<i>OCRL</i>	<i>SLC36A2</i>	<i>WDR35</i>
<i>BBS1</i>	<i>COX10</i>	<i>G6PC</i>	<i>KIAA0586</i>	<i>OFD1</i>	<i>SLC37A4</i>	<i>WDR60</i>
<i>BBS10</i>	<i>CPLANE1</i>	<i>GALNT3</i>	<i>KIAA0753</i>	<i>OSGEP</i>	<i>SLC3A1</i>	<i>WDR72</i>
<i>BBS12</i>	<i>CPT2</i>	<i>GALT</i>	<i>KIF14</i>	<i>PAX2</i>	<i>SLC41A1</i>	<i>WDR73</i>
<i>BBS2</i>	<i>CRB2</i>	<i>GANAB</i>	<i>KIF3B</i>	<i>PAX8</i>	<i>SLC4A1</i>	<i>WNK1</i>
<i>BBS4</i>	<i>CSPP1</i>	<i>GAPVD1</i>	<i>KIF7</i>	<i>PBX1</i>	<i>SLC4A4</i>	<i>WNK4</i>
<i>BBS5</i>	<i>CTNS</i>	<i>GATA3</i>	<i>KIRRELI</i>	<i>PCBD1</i>	<i>SLC5A2</i>	<i>WNT4</i>
<i>BBS7</i>	<i>CUBN</i>	<i>GATM</i>	<i>KL</i>	<i>PCM1</i>	<i>SLC6A19</i>	<i>WNT9B</i>
<i>BBS9</i>	<i>CUL3</i>	<i>GDNF</i>	<i>KLHL3</i>	<i>PDE6D</i>	<i>SLC6A20</i>	<i>WT1</i>
<i>BCSIL</i>	<i>CYP11B1</i>	<i>GDF6</i>	<i>KRAS</i>	<i>PDSS1</i>	<i>SLC7A7</i>	<i>XDH</i>
<i>BICCI</i>	<i>CYP11B2</i>	<i>GFRA1</i>	<i>KYNU</i>	<i>PDSS2</i>	<i>SLC7A9</i>	<i>XPNEP3</i>
<i>BMP4</i>	<i>CYP17A1</i>	<i>GLA</i>	<i>LAGE3</i>	<i>PHOX</i>	<i>SLC9A3</i>	<i>XPO5</i>
<i>BMPR2</i>	<i>CYP24A1</i>	<i>GLI3</i>	<i>LAMA5</i>	<i>PIBF1</i>	<i>SLC9A3R1</i>	<i>YRDC</i>
<i>BNC2</i>	<i>CYP27B1</i>	<i>GLIS2</i>	<i>LAMB2</i>	<i>PKD1</i>	<i>SLIT2</i>	<i>ZEB2</i>
<i>BSND</i>	<i>CYP2R1</i>	<i>GLIS3</i>	<i>LCAT</i>	<i>PKD2</i>	<i>SLIT3</i>	<i>ZIC3</i>
<i>C2CD3</i>	<i>CYP3A4</i>	<i>GNA11</i>	<i>LHX1</i>	<i>PKHD1</i>	<i>SMARCAL1</i>	<i>ZMPSTE24</i>
<i>C3</i>	<i>DAAM2</i>	<i>GNAS</i>	<i>LMNA</i>	<i>PLCE1</i>	<i>SOX17</i>	<i>ZNF365</i>
<i>C8ORF37</i>	<i>DACT1</i>	<i>GON7</i>	<i>LMOD1</i>	<i>PMM2</i>	<i>SPINT2</i>	<i>ZNF423</i>
<i>CA2</i>	<i>DCDC2</i>	<i>GPC3</i>	<i>LMX1B</i>	<i>POC1B</i>	<i>SPTLC1</i>	
<i>CACNA1D</i>	<i>DDX59</i>	<i>GPC5</i>	<i>LPP</i>	<i>PODXL</i>	<i>SPTLC2</i>	

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 or 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 health 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	Do 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 know the results from genetic testing at the time of completing this questionnaire?	Yes/no/unknown