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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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3 1 **Diagnostic yield of massively parallel sequencing in patients with chronic kidney disease**
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5 2 **of unknown etiology: Rationale and design of a national prospective cohort study**
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3 24 **ABSTRACT**
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6 25 *Introduction:* Chronic kidney disease (CKD) can be caused by a variety of systemic or
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9 26 primary renal diseases. The cause of CKD remains unexplained in approximately 20% of
10
11 27 patients. Retrospective studies indicate that massively parallel sequencing (MPS)-based gene
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13 28 panel testing may lead to a genetic diagnosis in 12-56% of patients with unexplained CKD,
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15 29 depending on patient profile. The diagnostic yield of MPS-based testing in a routine
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17 30 healthcare setting is unclear. Therefore, the primary aim of the VARIETY study is to
18
19 31 prospectively address the diagnostic yield of MPS-based gene panel testing in patients with
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21 32 unexplained CKD and an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m²
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23 33 before the age of 50 years in clinical practice.
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28 34 *Methods and analysis:* The VARIETY study is an ongoing, prospective, nation-wide
29
30 35 observational cohort study to investigate the diagnostic yield of MPS-based testing in patients
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32 36 with unexplained CKD in a routine healthcare setting in the Netherlands. Patients are
33
34 37 recruited from outpatient clinics in hospitals across the Netherlands. At least 282 patients will
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36 38 be included to meet the primary aim. Secondary analyses include subgroup analyses
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38 39 according to age and eGFR at first presentation, family history, and the presence of extra-
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40 40 renal symptoms.
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45 41 *Ethics and dissemination:* Ethical approval for the study has been obtained from the
46
47 42 institutional review board of the University Medical Center Groningen. Study findings should
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49 43 inform physicians and policymakers towards optimal implementation of MPS-based
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51 44 diagnostic testing in patients with unexplained CKD.
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3 45 **ARTICLE SUMMARY**
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6 46 **Strengths and limitations of this study**
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- 10 47 • First prospective study to examine the diagnostic yield of massively parallel
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12 48 sequencing in patients (age 18-50 at first presentation) with unexplained chronic
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14 49 kidney disease in a routine healthcare setting
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17 50 • Nation-wide study with relatively large sample size, allowing analyses of specific
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19 51 subgroups according to age and kidney function at first presentation
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22 52 • Study findings should inform physicians and policymakers in the implementation of
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24 53 gene panel testing in adults (age < 50) with unexplained CKD
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26 54 • A potential limitation is that the definition of ‘unexplained chronic kidney disease’ is
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28 55 not unequivocal
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56 INTRODUCTION

57 Chronic kidney disease (CKD) affects 11-16% of the population worldwide [1–3], is
58 associated with extensive co-morbidity and an increased risk of premature mortality, and may
59 ultimately result in end-stage kidney disease (ESKD) requiring dialysis or transplantation
60 [4,5]. CKD may be caused by a variety of systemic (e.g., diabetes, hypertension) or primary
61 renal diseases (e.g. IgA nephropathy, membranous nephropathy). Current diagnostic
62 approaches, including kidney biopsy, are often non-specific or inconclusive, contraindicated
63 or omitted due to lack of clinical consequences [6,7]. Therefore, the cause of CKD remains
64 unknown in approximately 20% of patients with ESKD [8–10]. However, knowledge of the
65 underlying kidney disease can be pivotal as it may influence prognosis and medical treatment.
66 In the setting of kidney transplantation, it may influence (living-related) donor selection and
67 post-transplant recurrence risk. Approximately 27-34% of patients with CKD report a positive
68 family history of kidney disease (first or second degree relative with CKD) [11,12] and a
69 genetic cause can be identified in at least 10% of adults with CKD [13,14], indicating that in
70 many cases a hereditary origin for the disease should be considered. Genetic testing could
71 therefore be a valuable tool in the diagnostic process of CKD of unknown etiology.

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

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3 81 MPS [20]. For this reason, it is difficult to define profiles of patients (e.g. based on age and
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5 82 severity at disease onset, extra-renal manifestations, positive family history) that should
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7 83 preferentially undergo genetic testing. A recent joint publication by the ERA Working Group
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9 84 on Inherited Kidney Disorders (WGIKD) and the Molecular Diagnostics Taskforce of the
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11 85 European Rare Kidney Disease Reference Network (ERKNet) called for further research to
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13 86 explore the diagnostic yield of genetic testing in CKD of unknown origin in a clinical setting
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15 87 [21].
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19 88 Therefore, the objective of this national prospective cohort study is to determine the
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21 89 diagnostic yield, i.e. the percentage of participants with a genetic diagnosis, using a large
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23 90 MPS-based multi-gene panel for kidney diseases in young patients (first presentation at age
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25 91 18-50) with unexplained CKD ($\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$) in a routine healthcare setting. In
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27 92 addition, we aim to identify specific patient profiles with a high diagnostic yield. These
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29 93 findings can guide physicians and policymakers in implementing MPS-based diagnostic
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31 94 testing in patients with unexplained CKD.
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95 **METHODS AND ANALYSIS**

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97 **Study design**

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

107

108 **Study population**

109 The targeted study population consist of all patients with unexplained CKD and an eGFR <60
110 ml/min/1.73m² before the age of 50 years. Unexplained CKD is defined as the absence of all
111 the following criteria: a biopsy-proven diagnosis (e.g. IgA nephropathy), a specific
112 morphological renal diagnosis (e.g. polycystic kidney disease suspected of
113 autosomal/recessive polycystic kidney disease), or a specific or plausible renal diagnosis (e.g.
114 history of long-term insulin-dependent diabetes mellitus before the onset of CKD, lithium-
115 induced nephropathy). Since hypertensive nephropathy is a non-specific diagnosis and
116 hypertension is also a very common consequence of CKD [22], patients with hypertensive
117 nephropathy in the absence of a clear underlying disorder such as renal artery stenosis are
118 considered to have unexplained CKD. Patients with renal hypoplasia, renal atrophy, and non-
119 specific histological conditions (such as secondary focal segmental glomerulosclerosis,

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3 120 glomerulonephritis of unknown cause, or interstitial nephritis) are also considered to have
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5 121 unexplained CKD.

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7 122 Patients with a current age >50 years, but who presented with an eGFR <60 ml/min/1.73m²
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9 123 before the age of 50 years, and renal transplant recipients who had a pre-transplant eGFR <60
10 124 ml/min/1.73m² before the age of 50 years are also eligible for inclusion. In addition, genetic
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12 125 testing with a specific MPS-based gene panel (see 'Genetic testing') is required for
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14 126 participation. Exclusion criteria for participation in the VARIETY study are: age <18 years at
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16 127 time of inclusion or patients who do not give or are unable to give informed consent for
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18 128 genetic testing or for the current study.
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25 26 130 **Recruitment**

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28 131 Patients are recruited from outpatient clinics in both academic and non-academic hospitals
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30 132 across the Netherlands. Patients will be screened by nephrologists or trained study
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32 133 investigators. In case of a study investigator, a list with potential participants will be sent to
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34 134 the treating nephrologist to confirm the diagnosis of unexplained CKD. Eligible participants
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36 135 will be informed about the study by the investigators or their treating nephrologists aware of
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38 136 the study protocol. A study investigator or treating nephrologist will ask for informed consent
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40 137 for this study. Information for patients has been made available in the form of a patient
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42 138 information folder and a website (in Dutch): www.onbegrepennierziekte.nl.

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47 48 49 140 **Data collection**

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51 141 Detailed clinical and demographic data are collected from patients' electronic health record
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53 142 (EHR) and through a questionnaire following informed consent. The data will subsequently
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55 143 be entered into a secure electronic case report form.
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3 145 *Electronic health record*
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5 146 The following information will be collected from the EHR: age at inclusion, sex, primary
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7 147 renal disease diagnosis, age at CKD onset/presentation, dialysis or kidney transplantation, age
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10 148 at start dialysis or kidney transplantation, presence of extra-renal features, medication use at
11
12 149 inclusion, medical history, family history (including three-generation pedigree), blood
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14 150 pressure at CKD onset and at inclusion, presence of hematuria and/or nephrotic syndrome,
15
16 151 laboratory results (serum creatinine, eGFR, total cholesterol, HDL, LDL, triglycerides, 24
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18 152 hour urine albumin excretion, 24 hour urine creatinine excretion, 24 hour urine total protein
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20 153 excretion, hematuria) at CKD onset/presentation and inclusion, renal histopathology and
21
22 154 imaging of the native kidneys, and results of genetic testing in relation to kidney disease. We
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24 155 will also collect information regarding the clinical consequences of a genetic diagnosis and if
25
26 156 genetic counseling was performed by a nephrologist or clinical geneticist. If participants are
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28 157 referred to a clinical geneticist, we will also collect the results of any additional genetic
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30 158 testing.
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37 160 *Questionnaire*
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39 161 Data collected from the EHR will be expanded with a questionnaire to collect additional data
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41 162 on family history, medical history, current health complaints, and extra-renal manifestations
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43 163 (Supplemental Table 1).
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49 165 **Genetic testing**

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51 166 We will include patients who have undergone MPS-based multi-gene panel testing, initiated
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53 167 by a clinical geneticist or nephrologist following pre-test counseling as part of clinical care in
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55 168 patients with unexplained CKD, in accordance with guidelines in the Netherlands [23]. Figure
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57 169 1 shows the suggested flowchart for genetic testing in the VARIETY study, based on these
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3 170 recommendations. The criteria as shown in this flow chart are slightly more liberal than the
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5 171 published recommendations, which will help to define the optimal age and eGFR ranges
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7 172 where genetic testing is still of clinical benefit. To stimulate the implementation of the
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9 173 guideline, we made a website for the VARIETY study (www.onbegrepennierziekte.nl) where
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11 174 nephrologists can find information about genetic testing and pre-test genetic counseling.
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14 175 In order to reduce heterogeneity in the diagnostic approach, we will assess the
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16 176 diagnostic yield of a specific MPS-based gene panel, namely the ‘CKD-Y’ (‘Chronic Kidney
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18 177 Disease in Young patients’) targeted exome sequencing (ES) panel available at the University
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20 178 Medical Center (UMC) Utrecht, The Netherlands. The older version of this panel (v18)
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22 179 contains 141 different genes associated with early-onset CKD, including *PKD1* and *PKD2*
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24 180 (Figure 2). On March 8 2021, the CKD-Y panel was updated (v21) and the number of genes
25
26 181 changed from 141 to 256 (Figure 3). This panel was chosen as it is an ES-based panel and
27
28 182 contains all the current genes associated with early-onset CKD. In addition, this panel can be
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30 183 ordered by nephrologists without referral to the clinical geneticist. Alternatively, the
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32 184 hereditary kidney disease panel from UMC Utrecht was allowed. This is another targeted ES
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34 185 panel, consisting of 379 genes in the v18 version and 495 genes in the updated v21 version
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36 186 (Supplementary Figure 1-2). Since this panel contains some kidney cancer oncogenes, it may
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38 187 only be ordered by a clinical geneticist. The hereditary kidney disease panel includes all genes
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40 188 of the CKD-Y panel, making it possible to determine if a variant could also have been
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42 189 identified with the CKD-Y panel. Potential findings from the hereditary kidney disease panel
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44 190 that do not overlap with the CKD-Y panel will not be included in the analyses. We will record
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46 191 which version of the CKD-Y and/or hereditary kidney disease panel was used.
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56 193 **Primary and secondary analyses**

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194 The primary analysis will address the diagnostic yield of the CKD-Y panel, defined as the
 195 percentage of positive test results (i.e. pathogenic variant(s) explaining the cause of the
 196 disease), in the overall cohort of patients with unexplained CKD and an eGFR <60
 197 mL/min/1.73 m² between 18 and 50 years. The pathogenicity of variants will be determined
 198 according to the standards and guidelines from the American College of Medical Genetics and
 199 Genomics (ACMG) [24]. With this standard, variants are classified into five categories using
 200 several lines of evidence, such as available literature, patient databases and *in silico* prediction
 201 programs. Class 1 variants are clearly not pathogenic; class 5 variants are clearly pathogenic.
 202 Class 3 are variants of uncertain significance/pathogenicity (VUS), these variants do not
 203 confirm or exclude the diagnosis (Table 1) [24]. For the determination of the diagnostic yield,
 204 only class 4 and class 5 variants will be considered as a 'positive test result' to determine the
 205 diagnostic yield. In cases with two class 4/5 variants in an autosomal recessive gene, these
 206 will only be considered a 'positive test result' if testing in parents has confirmed the variants
 207 are positioned in trans.

209 **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

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212 Secondary analyses include subgroup analyses according to age and eGFR at first

213 presentation, family history, and the presence of extra-renal symptoms. A positive family

214 history for CKD is recorded if the participant either has a first (parent or child), second

215 (siblings, grandparents, grandchildren), third (aunts, uncles, nephews, nieces) or fourth

216 (cousins) degree relative with CKD. Family history will be obtained from combining

217 information present in the EHR with information obtained from the questionnaire. Other

218 secondary analysis aims to define the percentage of genetic tests with a clinical consequence.

219 A genetic diagnosis is considered to have a clinical consequence if it: 1) negated the need for

220 kidney biopsy, 2) triggered or negated the need for immunosuppressive therapy, 3) provides

221 prognostic information, i.e. the risk of post-transplantation anti-GBM glomerulonephritis, 4)

222 led to, or should lead to, referral to other specialties (e.g. ophthalmologist), 5) led to targeted

223 work-up for associated symptoms or extra-renal manifestations, 6) affected surveillance

224 frequency, 7) led to, or should lead to, genetic testing in potential living related kidney

225 donors, 8) enabled more precise (preconception) genetic counseling for the patient or family

226 members, or 9) led to more precise or extensive follow-up of potentially affected family

227 members.

228 Tertiary outcomes will be the percentage of participants in which a VUS was

229 identified and the number of incidental/secondary findings (results unrelated to the initial

230 indication for genetic testing). In addition, if a molecular diagnosis is identified and a kidney

231 biopsy from the native kidney is present, we will assess if the biopsy findings match the

232 molecular diagnosis.

233 Participants without results from genetic testing will be excluded from all analyses. If

234 information is missing from the EHR, we will ask the general practitioner to deliver the

235 missing data within participants' consent. If the data cannot be retrieved, it will be regarded as

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3 236 “unknown”. In case eGFR at CKD onset is missing, the first available eGFR or serum
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5 237 creatinine measurement since the diagnosis of CKD will be used.
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9 239 **Statistical analysis**

10 240 Statistical analysis will be performed with IBM SPSS statistics for Windows, version 23
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12 241 (IBM Corporation, Armonk, NY, USA). An overall significance level of 0.05 will be handled.
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14 242 Continuous variables that are normally distributed will be presented as mean and standard
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16 243 deviation. Non-normally distributed variables will be expressed as median and interquartile
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18 244 range. Frequencies and percentages will be used to describe categorical variables such as
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20 245 gender, family history, renal replacement therapy, extra-renal manifestations, and diagnostic
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22 246 yield. The Chi-square or Fisher’s exact test will be used to compare differences in categorical
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24 247 variables between the different subgroups of the secondary analysis. Logistic regression will
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26 248 be performed to identify characteristics associated with a genetic diagnosis.
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34 250 **Sample size calculation**

35 251 The minimal sample size was calculated using the following formula [25], based on the
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37 252 study’s primary endpoint:
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$$n = \frac{Z^2 * P(1 - P)}{d^2}$$

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46 254 Based on the literature, the expected percentage of positive test results is 17% [14]. Assuming
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48 255 a level of confidence (z) of 1.96 and precision (d) of 0.05 [25], a minimum of 217 participants
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50 256 are required for a reliable assessment of the primary outcome. In order to be clinically and
51
52 257 politically significant, we aim to increase the sample size of this prospective cohort study
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54 258 beyond the largest currently available retrospective study, i.e. to include at least 282 patients
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56 259 in the current study [20,25].
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261 **Data management**

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

272 **Patient and public involvement**

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

275 **ETHICS AND DISSEMINATION**

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

286 **CONCLUSION AND STUDY STATUS**

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

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303 **Author contributions**

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

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4
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10 313 **Competing interest statement**

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12 314 Dr. De Borst and Dr. Vogt have received research support and lecture fees (all to institution)
13
14 315 from Sanofi Genzyme related to the current study. Prof. Knoers has received reimbursement
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17 316 of travel expenses for lectures related to the current study from Sanofi Genzyme.
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3 386 **FIGURE LEGENDS**
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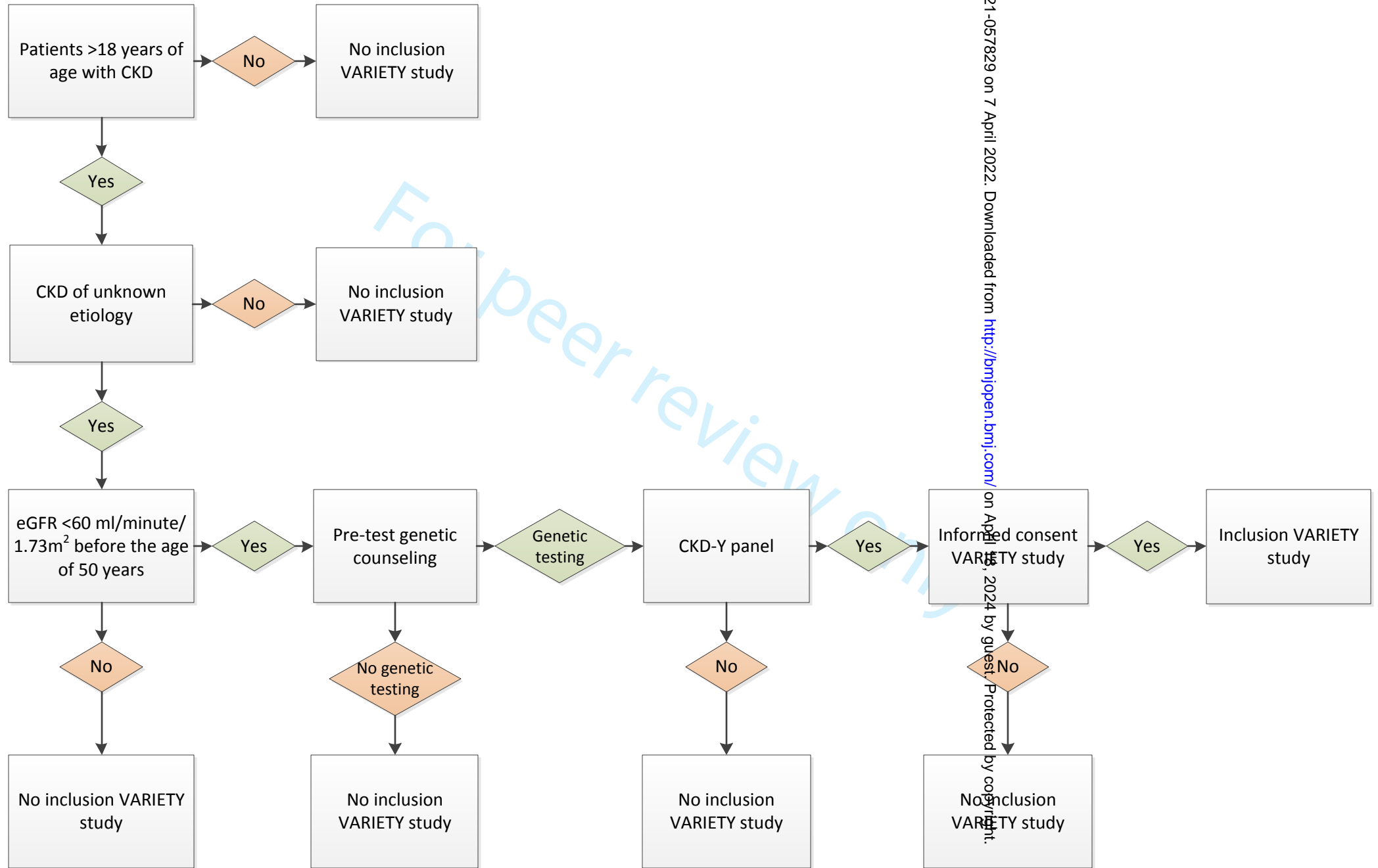
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6 387 **Figure 1. Flowchart for inclusion VARIETY study.** CKD: chronic kidney disease; eGFR:
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8 388 estimated glomerular filtration rate.
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13 390 **Figure 2. Overview of the 141 genes that are analyzed in the exome sequencing b**
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16 391 **Chronic Kidney Disease in Young patients (CKD-Y) panel version v18 at University**
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18 392 **Medical Center Utrecht**
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23 394 **Figure 3. Overview of the 256 genes that are analyzed in the exome sequencing based**
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26 395 **Chronic Kidney Disease in Young patients (CKD-Y) panel version v21 at University**
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28 396 **Medical Center Utrecht.** Bold genes are also on the CKD-Y panel v18.
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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
ARHGDI1	COQ6	GLA	LAMB2	NUP205	SALL1	WT1
ATXN10	CRB2	GLIS2	LMNA	NUP93	SCARB2	XPNPEP3
B2M	CTNS	GRHR	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
AH11	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
ARHGDI1	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	GRHRP	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
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	FXSD2	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>TOGARAM1</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>SCNN1A</i>	<i>TPRKB</i>
<i>APOA1</i>	<i>CFHR5</i>	<i>FAHD2A</i>	<i>IFT80</i>	<i>NEK1</i>	<i>SCNN1B</i>	<i>TRAF3IP1</i>
<i>APOE</i>	<i>CFI</i>	<i>FAM134B</i>	<i>IFT81</i>	<i>NEK8</i>	<i>SCNN1G</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>ARHGDI1A</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>	VDR
<i>AVIL</i>	<i>COL4A5</i>	<i>FOXC2</i>	<i>KANK4</i>	<i>NUP133</i>	<i>SLC26A1</i>	VHL
<i>AVP</i>	<i>COQ2</i>	FOXF1	<i>KATNIP</i>	<i>NUP160</i>	<i>SLC26A3</i>	VIPAS39
AVPR2	<i>COQ4</i>	FOXI1	KCNJ1	<i>NUP205</i>	<i>SLC2A2</i>	VPS33B
<i>B2M</i>	<i>COQ6</i>	<i>FRAS1</i>	KCNJ10	<i>NUP85</i>	<i>SLC2A9</i>	WDPCP
<i>B9D1</i>	<i>COQ7</i>	<i>FREM1</i>	<i>KCNJ5</i>	<i>NUP93</i>	<i>SLC34A1</i>	WDR19
<i>B9D2</i>	<i>COQ8B</i>	<i>FREM2</i>	KCTD1	<i>NXF5</i>	<i>SLC34A3</i>	WDR34
<i>BBIP1</i>	<i>COQ9</i>	FXYD2	KCTD3	<i>OCRL</i>	<i>SLC36A2</i>	WDR35
<i>BBS1</i>	COX10	G6PC	<i>KIAA0586</i>	<i>OFD1</i>	<i>SLC37A4</i>	WDR60
<i>BBS10</i>	<i>CPLANE1</i>	GALNT3	KIAA0753	<i>OSGEP</i>	<i>SLC3A1</i>	WDR72
<i>BBS12</i>	CPT2	GALT	KIF14	<i>PAX2</i>	<i>SLC41A1</i>	WDR73
<i>BBS2</i>	<i>CRB2</i>	<i>GANAB</i>	<i>KIF3B</i>	PAX8	<i>SLC4A1</i>	WNK1
<i>BBS4</i>	<i>CSPP1</i>	<i>GAPVD1</i>	KIF7	<i>PBX1</i>	<i>SLC4A4</i>	WNK4
<i>BBS5</i>	<i>CTNS</i>	<i>GATA3</i>	<i>KIRRELI</i>	PCBD1	<i>SLC5A2</i>	WNT4
<i>BBS7</i>	<i>CUBN</i>	<i>GATM</i>	KL	<i>PCM1</i>	<i>SLC6A19</i>	WNT9B
<i>BBS9</i>	<i>CUL3</i>	GDNF	<i>KLHL3</i>	PDE6D	<i>SLC6A20</i>	WT1
<i>BCS1L</i>	<i>CYP11B1</i>	GDF6	KRAS	<i>PDSS1</i>	<i>SLC7A7</i>	XDH
<i>BICC1</i>	<i>CYP11B2</i>	GFRA1	KYNU	<i>PDSS2</i>	<i>SLC7A9</i>	XPNPEP3
BMP4	<i>CYP17A1</i>	<i>GLA</i>	LAGE3	PHEX	<i>SLC9A3</i>	XPO5
BMPR2	CYP24A1	GLI3	LAMA5	<i>PIBF1</i>	<i>SLC9A3R1</i>	YRDC
BNC2	<i>CYP27B1</i>	<i>GLIS2</i>	<i>LAMB2</i>	<i>PKD1</i>	SLIT2	ZEB2
BSND	<i>CYP2R1</i>	GLIS3	LCAT	<i>PKD2</i>	SLIT3	ZIC3
C2CD3	<i>CYP3A4</i>	GNA11	LHX1	<i>PKHD1</i>	<i>SMARCAL1</i>	ZMPSTE24
C3	<i>DAAM2</i>	GNAS	<i>LMNA</i>	<i>PLCE1</i>	<i>SOX17</i>	ZNF365
C8ORF37	<i>DACT1</i>	GON7	LMOD1	<i>PMM2</i>	SPINT2	ZNF423
CA2	<i>DCDC2</i>	GPC3	<i>LMX1B</i>	<i>POC1B</i>	SPTLC1	
CACNA1D	DDX59	GPC5	LPP	<i>PODXL</i>	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 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

1 2 3 4 5 6 7 8 9	35	Dou you have family members with kidney disease (children, parents, siblings, grandparents, uncles/aunts, cousins, nephews/nieces)?	Yes/no/unknown
10 11	35a	If so, how many family members have a kidney disease?	Open
12 13 14 15 16	35b	In how many family members if the cause for the kidney disease unknown?	Open
17 18 19 20 21 22 23 24 25	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
26 27 28 29 30	35d	How many family members with a kidney disease have had a kidney transplantation or dialysis?	Open
31 32	Final questions		
33 34 35 36 37	36	Have you visited a clinical geneticist or have you been referred to a clinical geneticist?	Yes/no/unknown
38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	37	Do you already known the results from genetic testing at the time of completing this questionnaire?	Yes/no/unknown

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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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	6-7
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	NA
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	NA
		<i>Cross-sectional study</i> —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

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SCHOLARONE™
Manuscripts

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3 1 **Diagnostic yield of massively parallel sequencing in patients with chronic kidney disease**
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5 2 **of unknown etiology: Rationale and design of a national prospective cohort study**
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24 **ABSTRACT**

25 *Introduction:* Chronic kidney disease (CKD) can be caused by a variety of systemic or
26 primary renal diseases. The cause of CKD remains unexplained in approximately 20% of
27 patients. Retrospective studies indicate that massively parallel sequencing (MPS)-based gene
28 panel testing may lead to a genetic diagnosis in 12-56% of patients with unexplained CKD,
29 depending on patient profile. The diagnostic yield of MPS-based testing in a routine
30 healthcare setting is unclear. Therefore, the primary aim of the VARIETY (Validation of
31 algoRithms and IdEnTification of genes in Young patients with unexplained chronic kidney
32 disease) study is to prospectively address the diagnostic yield of MPS-based gene panel
33 testing in patients with unexplained CKD and an estimated glomerular filtration rate (eGFR)
34 <60 mL/min/1.73 m² before the age of 50 years in clinical practice.

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

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

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3 46 **ARTICLE SUMMARY**
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6 47 **Strengths and limitations of this study**
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- 10 48 • First prospective study to examine the diagnostic yield of massively parallel
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12 49 sequencing in patients (age <50 at first presentation) with unexplained chronic kidney
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14 50 disease in a routine healthcare setting
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17 51 • Nation-wide study with relatively large sample size, allowing analyses of specific
18
19 52 subgroups according to age and kidney function at first presentation
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22 53 • Study findings should inform physicians and policymakers in the implementation of
23
24 54 gene panel testing in adults (age < 50) with unexplained CKD
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26 55 • A potential limitation is that the definition of ‘unexplained chronic kidney disease’ is
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28 56 not unequivocal
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57 INTRODUCTION

58 Chronic kidney disease (CKD) affects 11-16% of the population worldwide [1–3], is
59 associated with extensive co-morbidity and an increased risk of premature mortality, and may
60 ultimately result in end-stage kidney disease (ESKD) requiring dialysis or transplantation
61 [4,5]. CKD may be caused by a variety of systemic (e.g., diabetes, hypertension) or primary
62 renal diseases (e.g. IgA nephropathy, membranous nephropathy). Current diagnostic
63 approaches, including kidney biopsy, are often non-specific or inconclusive, contraindicated
64 or omitted due to lack of clinical consequences [6,7]. Therefore, the cause of CKD remains
65 unknown in approximately 20% of patients with ESKD [8–10]. However, knowledge of the
66 underlying kidney disease can be pivotal as it may influence prognosis and medical treatment.
67 In the setting of kidney transplantation, it may influence (living-related) donor selection and
68 post-transplant recurrence risk. Approximately 27-34% of patients with CKD report a positive
69 family history of kidney disease (first or second degree relative with CKD) [11,12] and a
70 genetic cause can be identified in at least 10% of adults with CKD [13,14], indicating that in
71 many cases a hereditary origin for the disease should be considered. Genetic testing could
72 therefore be a valuable tool in the diagnostic process of CKD of unknown etiology.

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

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3 82 MPS [20]. For this reason, it is difficult to define profiles of patients (e.g. based on age and
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5 83 severity at disease onset, extra-renal manifestations, positive family history) that should
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7 84 preferentially undergo genetic testing. A recent joint publication by the ERA Working Group
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9 85 on Inherited Kidney Disorders (WGIKD) and the Molecular Diagnostics Taskforce of the
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11 86 European Rare Kidney Disease Reference Network (ERKNet) called for further research to
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13 87 explore the diagnostic yield of genetic testing in CKD of unknown origin in a clinical setting
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15 88 [21].
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19 89 Therefore, the objective of this national prospective cohort study is to determine the
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21 90 diagnostic yield, i.e. the percentage of participants with a genetic diagnosis, using a large
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23 91 MPS-based multi-gene panel for kidney diseases in young patients (first presentation at age
24
25 92 <50) with unexplained CKD (eGFR <60 mL/min/1.73 m²) in a routine healthcare setting. In
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27 93 addition, we aim to identify specific patient profiles with a high diagnostic yield. These
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29 94 findings can guide physicians and policymakers in implementing MPS-based diagnostic
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31 95 testing in patients with unexplained CKD.
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96 **METHODS AND ANALYSIS**

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98 **Study design**

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

108

109 **Study population**

110 The targeted study population consist of all patients with unexplained CKD and an eGFR <60
111 ml/min/1.73m² before the age of 50 years. Unexplained CKD is defined as the absence of all
112 the following criteria: a biopsy-proven diagnosis (e.g. IgA nephropathy), a specific
113 morphological renal diagnosis (e.g. polycystic kidney disease suspected of
114 autosomal/recessive polycystic kidney disease), or a specific or plausible renal diagnosis (e.g.
115 history of long-term insulin-dependent diabetes mellitus before the onset of CKD, lithium-
116 induced nephropathy). Since hypertensive nephropathy is a non-specific diagnosis and
117 hypertension is also a very common consequence of CKD [22], patients with hypertensive
118 nephropathy in the absence of a clear underlying disorder such as renal artery stenosis are
119 considered to have unexplained CKD. Patients with renal hypoplasia, renal atrophy, and non-
120 specific histological conditions (such as secondary focal segmental glomerulosclerosis,

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3 121 glomerulonephritis of unknown cause, or interstitial nephritis) are also considered to have
4
5 122 unexplained CKD.

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7 123 Patients with a current age >50 years, but who presented with an eGFR <60 ml/min/1.73m²
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9 124 before the age of 50 years, and renal transplant recipients who had a pre-transplant eGFR <60
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11 125 ml/min/1.73m² before the age of 50 years are also eligible for inclusion. In addition, genetic
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13 126 testing with a specific MPS-based gene panel (see 'Genetic testing') is required for
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15 127 participation. Exclusion criteria for participation in the VARIETY study are: age <18 years at
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17 128 time of inclusion or patients who do not give or are unable to give informed consent for
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19 129 genetic testing or for the current study.
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25 26 131 **Recruitment**

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28 132 To ensure a representative sample of CKD patients, patients are recruited from outpatient
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30 133 clinics in both academic and non-academic hospitals across the Netherlands. Depending on
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32 134 the hospital, patients will be screened by the primary treating nephrologists or by trained
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34 135 study investigators. In case of a study investigator, a list with potential participants will be
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36 136 sent to the treating nephrologist to confirm the diagnosis of unexplained CKD. Eligible
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38 137 participants will be informed about the study by the investigators or their treating
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40 138 nephrologists aware of the study protocol. A study investigator or treating nephrologist will
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42 139 ask for informed consent for this study. Information for patients has been made available in
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44 140 the form of a patient information folder and a website (in Dutch):
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46 141 www.onbegrepennierziekte.nl.

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52 53 143 **Data collection**

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3 144 Detailed clinical and demographic data are collected from patients' electronic health record
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5 145 (EHR) and through a questionnaire following informed consent. The data will subsequently
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7 146 be entered into a secure electronic case report form.
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10 147

11 148 *Electronic health record*

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14 149 The following information will be collected from the EHR: age at inclusion, sex, primary
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16 150 renal disease diagnosis, age at CKD onset/presentation, dialysis or kidney transplantation, age
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18 151 at start dialysis or kidney transplantation, presence of extra-renal features, medication use at
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20 152 inclusion, medical history, family history (including three-generation pedigree), blood
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22 153 pressure at CKD onset and at inclusion, presence of hematuria and/or nephrotic syndrome,
23
24 154 laboratory results (serum creatinine, eGFR, total cholesterol, HDL, LDL, triglycerides, 24
25
26 155 hour urine albumin excretion, 24 hour urine creatinine excretion, 24 hour urine total protein
27
28 156 excretion, hematuria) at CKD onset/presentation and inclusion, renal histopathology and
29
30 157 imaging of the native kidneys, and results of genetic testing in relation to kidney disease. We
31
32 158 will also collect information regarding the clinical consequences of a genetic diagnosis and if
33
34 159 genetic counseling was performed by a nephrologist or clinical geneticist. If participants are
35
36 160 referred to a clinical geneticist, we will also collect the results of any additional genetic
37
38 161 testing.
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41 163 *Questionnaire*

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44 164 Data collected from the EHR will be expanded with a questionnaire to collect additional data
45
46 165 on family history, medical history, current health complaints, and extra-renal manifestations
47
48 166 (Supplemental Table 1).
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50 167

51 168 **Genetic testing**

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3 169 We will include patients who have undergone MPS-based multi-gene panel testing, initiated
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5 170 by a clinical geneticist or nephrologist following pre-test counseling as part of clinical care in
6
7 171 patients with unexplained CKD, in accordance with guidelines in the Netherlands [23]. Figure
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10 172 1 shows the suggested flowchart for genetic testing in the VARIETY study, based on these
11
12 173 recommendations. The criteria as shown in this flow chart are slightly more liberal than the
13
14 174 published recommendations, which will help to define the optimal age and eGFR ranges
15
16 175 where genetic testing is still of clinical benefit. To stimulate the implementation of the
17
18 176 guideline, we made a website for the VARIETY study (www.onbegrepennierziekte.nl) where
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20
21 177 nephrologists can find information about genetic testing and pre-test genetic counseling.
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24 178 In order to reduce heterogeneity in the diagnostic approach, we will assess the
25
26 179 diagnostic yield of a specific MPS-based gene panel, namely the ‘CKD-Y’ (‘Chronic Kidney
27
28 180 Disease in Young patients’) targeted exome sequencing (ES) panel available at the University
29
30 181 Medical Center (UMC) Utrecht, The Netherlands. The older version of this panel (v18)
31
32 182 contains 141 different genes associated with early-onset CKD, including *PKD1* and *PKD2*
33
34 183 (Figure 2). On March 8 2021, the CKD-Y panel was updated (v21) and the number of genes
35
36 184 changed from 141 to 256 (Figure 3). This panel was chosen as it is an ES-based panel and
37
38 185 contains all the current genes associated with early-onset CKD. In addition, this panel can be
39
40 186 ordered by nephrologists without referral to the clinical geneticist. Alternatively, the
41
42 187 hereditary kidney disease panel from UMC Utrecht was allowed. This is another targeted ES
43
44 188 panel, consisting of 379 genes in the v18 version and 495 genes in the updated v21 version
45
46 189 (Supplementary Figure 1-2). Since this panel contains some kidney cancer oncogenes, it may
47
48 190 only be ordered by a clinical geneticist. The hereditary kidney disease panel includes all genes
49
50 191 of the CKD-Y panel, making it possible to determine if a variant could also have been
51
52 192 identified with the CKD-Y panel. Potential findings from the hereditary kidney disease panel
53
54 193 that do not overlap with the CKD-Y panel will not be included in the primary analyses.
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3 194 Patients with older versions of the CKD-Y and hereditary kidney disease panels can be
4
5 195 included. We will record which version of the CKD-Y and/or hereditary kidney disease panel
6
7
8 196 was used. The procedures for ES and variant filtering have been described before [24]. Copy
9
10 197 number variation (CNV) detection was performed using an in-house adapted, diagnostically
11
12 198 validated, version of the ExomeDepth CNV detection tool [25].
13
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200 **Primary and secondary analyses**

201 The primary analysis will address the diagnostic yield of the CKD-Y panel, defined as the
202 percentage of positive test results (i.e. pathogenic variant(s) explaining the cause of the
203 disease), in the overall cohort of patients with unexplained CKD and an eGFR <60
204 mL/min/1.73 m² before the age of 50 years. We will perform a sensitivity analysis in patients
205 with onset eGFR <60 mL/min/1.73 m² between the age of 18 and 50 years. The pathogenicity
206 of variants will be determined according to the standards and guidelines from the American
207 College of Medical Genetics and Genomics (ACMG) [26]. With this standard, variants are
208 classified into five categories using several lines of evidence, such as available literature,
209 patient databases and *in silico* prediction programs. Class 1 variants are clearly not
210 pathogenic; class 5 variants are clearly pathogenic. Class 3 are variants of uncertain
211 significance/pathogenicity (VUS), these variants do not confirm or exclude the diagnosis
212 (Table 1) [26]. For the determination of the diagnostic yield, only class 4 and class 5 variants
213 will be considered as a 'positive test result' to determine the diagnostic yield. In cases with
214 two class 4/5 variants in an autosomal recessive gene, these will only be considered a
215 'positive test result' if testing in parents has confirmed the variants are positioned in trans.

216

217 **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

218

219

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

1
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3 236 Tertiary outcomes will be the percentage of participants in which a VUS was
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5 237 identified and the number of incidental/secondary findings (results unrelated to the initial
6
7 238 indication for genetic testing). In addition, if a molecular diagnosis is identified and a kidney
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9 239 biopsy from the native kidney is present, we will assess if the biopsy findings match the
10
11 240 molecular diagnosis. Finally, we will perform health economic analyses to determine if MPS
12
13 241 in patients with unexplained CKD is cost-effective.
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16
17 242 We will report the number of participants who withdraw from participating in the
18
19 243 VARIETY study after initial inclusion and the number of participants who have initially been
20
21 244 included, but upon further analysis by the study team did not match the inclusion criteria.
22
23 245 Participants without results from genetic testing will be excluded from all analyses. If
24
25 246 information is missing from the EHR, we will ask the general practitioner to deliver the
26
27 247 missing data within participants' consent. If the data cannot be retrieved, it will be regarded as
28
29 248 "unknown". In case eGFR at CKD onset is missing, the first available eGFR or serum
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31 249 creatinine measurement since the diagnosis of CKD will be used.
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37 251 **Statistical analysis**

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39 252 Statistical analysis will be performed with IBM SPSS statistics for Windows, version 23
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41 253 (IBM Corporation, Armonk, NY, USA). An overall significance level of 0.05 will be handled.
42
43 254 Continuous variables that are normally distributed will be presented as mean and standard
44
45 255 deviation. Non-normally distributed variables will be expressed as median and interquartile
46
47 256 range. Frequencies and percentages will be used to describe categorical variables such as
48
49 257 gender, family history, renal replacement therapy, extra-renal manifestations, and diagnostic
50
51 258 yield. The Chi-square or Fisher's exact test will be used to compare differences in categorical
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53 259 variables between the different subgroups of the secondary analysis. Logistic regression will
54
55 260 be performed to identify characteristics associated with a genetic diagnosis.
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262 Sample size calculation

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

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

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

272

273 Data management

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

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284 Patient and public involvement

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

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45 287 **ETHICS AND DISSEMINATION**
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7 288 Ethical approval for the study has been obtained from the institutional review board of the
8
9 289 UMCG (METc 2019/106). The study is conducted in accordance with the WMA Declaration
10
11 290 of Helsinki. The results of the study will be presented at (inter)national congresses and
12
13 291 submitted for open access publication in peer-reviewed journals. In addition to the primary
14
15 292 results, related to the main research questions as defined above, case reports/series may be
16
17 293 submitted for publication in case of unique or interesting findings and these will also be
18
19 294 submitted for publication in peer-reviewed journals. In accordance with the information sheet
20
21 295 for participants, the main results and any publications from the VARIETY study will also be
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23 296 made available on the study website. After completion of the study and publication of the
24
25 297 main results, request for re-use of the data can be submitted to the corresponding author.
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298 **CONCLUSION AND STUDY STATUS**

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

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315 **Author contributions**

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

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4
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10 325 **Competing interest statement**
11

12 326 Dr. De Borst and Dr. Vogt have received research support and lecture fees (all to institution)
13
14 327 from Sanofi Genzyme related to the current study. Prof. Knoers has received reimbursement
15
16 328 of travel expenses for lectures related to the current study from Sanofi Genzyme.
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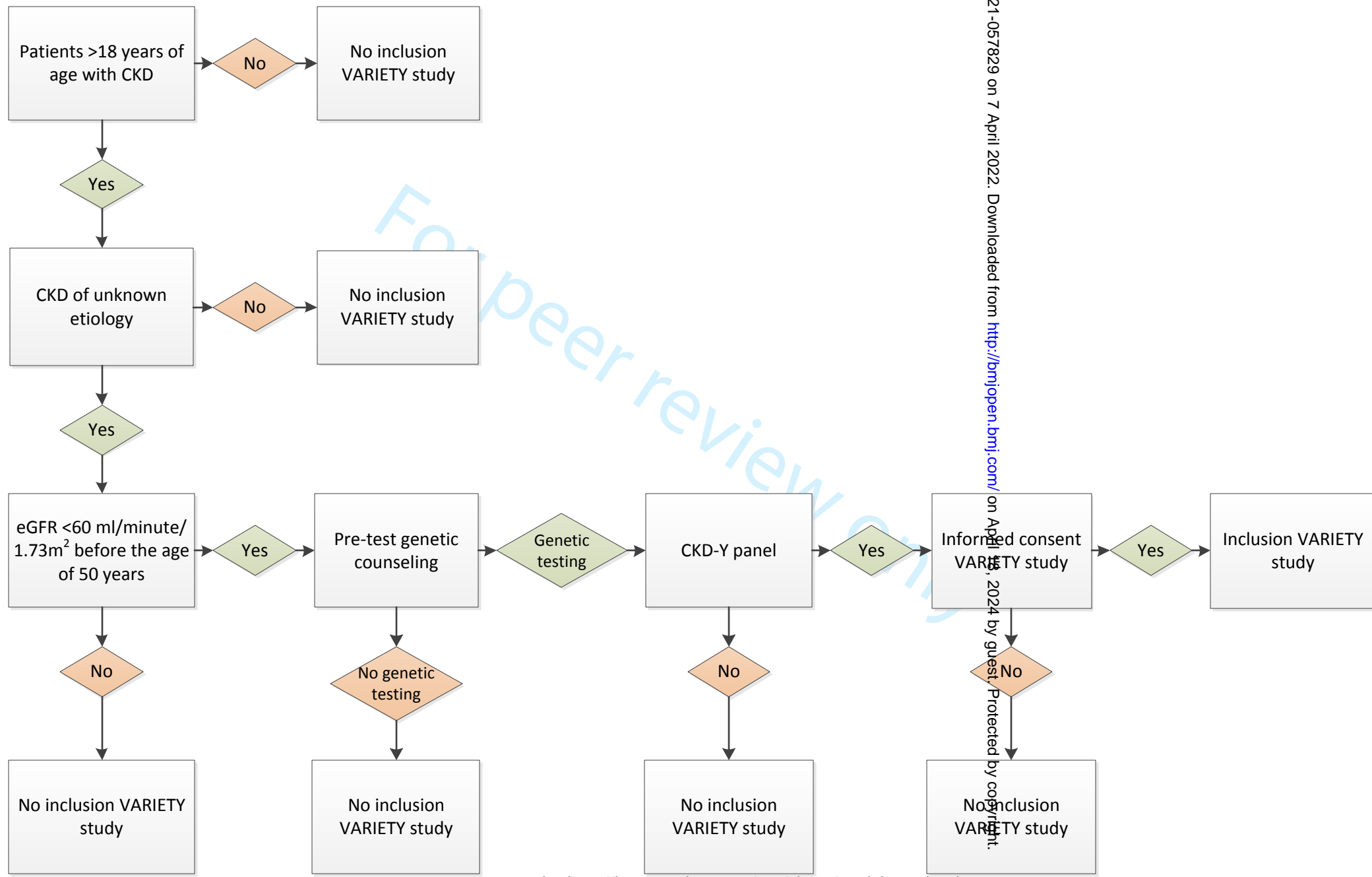
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3 404 **FIGURE LEGENDS**
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6 405 **Figure 1. Flowchart for inclusion VARIETY study.** CKD: chronic kidney disease; eGFR:
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8 406 estimated glomerular filtration rate.
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13 408 **Figure 2. Overview of the 141 genes that are analyzed in the exome sequencing based**
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16 409 **Chronic Kidney Disease in Young patients (CKD-Y) panel version v18 at University**
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18 410 **Medical Center Utrecht**
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23 412 **Figure 3. Overview of the 256 genes that are analyzed in the exome sequencing based**
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26 413 **Chronic Kidney Disease in Young patients (CKD-Y) panel version v21 at University**
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28 414 **Medical Center Utrecht.** Bold genes are also on the CKD-Y panel v18.
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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
ARHGDI1	COQ6	GLA	LAMB2	NUP205	SALL1	WT1
ATXN10	CRB2	GLIS2	LMNA	NUP93	SCARB2	XPNPEP3
B2M	CTNS	GRHR	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
ARHGDI1	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
ARHGDI1	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

<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>TOGARAM1</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>ARHGDI1A</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>	VDR
<i>AVIL</i>	<i>COL4A5</i>	<i>FOXC2</i>	<i>KANK4</i>	<i>NUP133</i>	<i>SLC26A1</i>	VHL
<i>AVP</i>	<i>COQ2</i>	FOXF1	<i>KATNIP</i>	<i>NUP160</i>	SLC26A3	VIPAS39
AVPR2	COQ4	FOXI1	KCNJ1	<i>NUP205</i>	<i>SLC2A2</i>	VPS33B
<i>B2M</i>	<i>COQ6</i>	<i>FRAS1</i>	KCNJ10	<i>NUP85</i>	<i>SLC2A9</i>	WDPCP
<i>B9D1</i>	<i>COQ7</i>	<i>FREM1</i>	<i>KCNJ5</i>	<i>NUP93</i>	<i>SLC34A1</i>	WDR19
<i>B9D2</i>	<i>COQ8B</i>	<i>FREM2</i>	KCTD1	<i>NXF5</i>	<i>SLC34A3</i>	WDR34
<i>BBIP1</i>	<i>COQ9</i>	FXYD2	KCTD3	<i>OCRL</i>	<i>SLC36A2</i>	WDR35
<i>BBS1</i>	COX10	G6PC	<i>KIAA0586</i>	<i>OFD1</i>	<i>SLC37A4</i>	WDR60
<i>BBS10</i>	<i>CPLANE1</i>	GALNT3	KIAA0753	<i>OSGEP</i>	<i>SLC3A1</i>	WDR72
<i>BBS12</i>	CPT2	GALT	KIF14	<i>PAX2</i>	<i>SLC41A1</i>	WDR73
<i>BBS2</i>	<i>CRB2</i>	<i>GANAB</i>	<i>KIF3B</i>	PAX8	<i>SLC4A1</i>	WNK1
<i>BBS4</i>	<i>CSPP1</i>	<i>GAPVD1</i>	KIF7	<i>PBX1</i>	<i>SLC4A4</i>	WNK4
<i>BBS5</i>	<i>CTNS</i>	<i>GATA3</i>	<i>KIRRELI</i>	PCBD1	<i>SLC5A2</i>	WNT4
<i>BBS7</i>	<i>CUBN</i>	<i>GATM</i>	KL	<i>PCM1</i>	<i>SLC6A19</i>	WNT9B
<i>BBS9</i>	<i>CUL3</i>	GDNF	<i>KLHL3</i>	PDE6D	<i>SLC6A20</i>	WT1
<i>BCS1L</i>	<i>CYP11B1</i>	GDF6	KRAS	<i>PDSS1</i>	<i>SLC7A7</i>	XDH
<i>BICC1</i>	<i>CYP11B2</i>	GFRA1	KYNU	<i>PDSS2</i>	<i>SLC7A9</i>	XPNPEP3
BMP4	<i>CYP17A1</i>	<i>GLA</i>	LAGE3	PHEX	<i>SLC9A3</i>	XPO5
BMPR2	CYP24A1	GLI3	LAMA5	<i>PIBF1</i>	<i>SLC9A3R1</i>	YRDC
BNC2	<i>CYP27B1</i>	<i>GLIS2</i>	<i>LAMB2</i>	<i>PKD1</i>	SLIT2	ZEB2
BSND	<i>CYP2R1</i>	GLIS3	LCAT	<i>PKD2</i>	SLIT3	ZIC3
C2CD3	<i>CYP3A4</i>	GNA11	LHX1	<i>PKHD1</i>	<i>SMARCAL1</i>	ZMPSTE24
C3	<i>DAAM2</i>	GNAS	<i>LMNA</i>	<i>PLCE1</i>	<i>SOX17</i>	ZNF365
C8ORF37	<i>DACT1</i>	GON7	LMOD1	<i>PMM2</i>	SPINT2	ZNF423
CA2	<i>DCDC2</i>	GPC3	<i>LMX1B</i>	<i>POC1B</i>	SPTLC1	
CACNA1D	DDX59	GPC5	LPP	<i>PODXL</i>	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 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 infarction, 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	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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	6-7
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	NA
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	NA
		<i>Cross-sectional study</i> —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.