



Original article

[Genetic study in young patients with chronic kidney disease stage G5 from unknown etiology. The GENSEN study design[☆]



Miquel Blasco^{a,b,*,1}, Borja Quiroga^{b,c,1}, José M. García-Aznar^d, Roser Torra^{b,e}, Alberto Ortiz^{b,f,g}, Patricia de Sequera^{b,h,i}, Grupo colaborativo GENSEN

^a Servicio de Nefrología y Trasplante Renal, Centro de Referencia en Enfermedad Glomerular Compleja del Sistema Nacional de Salud (CSUR), IDIBAPS, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

^b RICORS2040, Madrid, Spain

^c IIS-La Princesa, Servicio de Nefrología, Hospital Universitario de la Princesa, Madrid, Spain

^d Healthincode, Área Clínica de Diagnóstico Genético - Nefrología, A Coruña, Spain

^e Enfermedades renales hereditarias, Servicio de Nefrología, Fundació Puigvert, Institut d'Investigacions Biomèdiques (IIB-Sant Pau), Departament de Medicina, Universitat Autònoma de Barcelona (UAB). Spain

^f Servicio de Nefrología e Hipertensión, IIS-Fundación Jiménez Díaz UAM, Madrid, Spain

^g Departamento de Medicina, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain

^h Servicio de Nefrología, Hospital Universitario Infanta Leonor, Madrid, Spain

ⁱ Universidad Complutense de Madrid, Madrid, Spain

ARTICLE INFO

Article history:

Received 17 July 2023

Accepted 5 September 2023

Available online 25 July 2024

Keywords:

Etiological diagnosis
Chronic kidney disease
Hereditary diseases
Genetic study
Unknown etiology
Precision medicine

ABSTRACT

Introduction: Chronic kidney disease (CKD) of non-inherited etiology is one of the main causes of renal replacement therapy in our setting. Previous studies in other territories suggest that hereditary diseases could be one of the potential causes of this pathology, especially in younger patients. The GENSEN study will evaluate the presence of pathogenic genetic variants in subjects who have developed CKD category G5 before the age of 46 years, of non-inherited etiology.

Methods: Observational, prospective, multicenter study, which evaluates the diagnostic utility of massive high-throughput sequencing (HTS) directed to a set of genes, in the identification of the cause of CKD. Patients from all over Spain will be included, from whom a blood or saliva sample will be taken and a panel of 529 genes associated with hereditary kidney disease will be analyzed. This publication communicates the study protocol.

DOI of original article:

<https://doi.org/10.1016/j.nefro.2023.09.002>.

* The list of collaborators of the GENSEN study can be found in Appendix Annex 2. Likewise, the genes, OMIM phenotype, associated renal pathology and Pubmed can be found in Appendix Annex 1.

* Corresponding author.

¹ These authors have also contributed to this work.

2013-2514/© 2023 Sociedad Española de Nefrología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Conclusion: The GENSEN study will make it possible to evaluate the diagnostic performance of the gene panel study in young subjects in our setting with the development of CKD category G5 without a clear cause. An etiological diagnosis would offer potential benefits for patients and relatives (targeted therapies, clinical trials, detection of extrarenal manifestations, evaluation of relatives for live donation, estimation of the risk of recurrence in the renal graft, genetic counseling, among others) and would allow to apply this genetic study to the nephrology of our country.

© 2023 Sociedad Española de Nefrología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Estudio genético en pacientes jóvenes con enfermedad renal crónica avanzada de etiología no filiada – Diseño del estudio GENSEN

R E S U M E N

Palabras clave:

Diagnóstico etiológico
Enfermedad renal crónica
Enfermedades hereditarias
Estudio genético
Etiología no filiada
Medicina de precisión

Introducción: La enfermedad renal crónica (ERC) de etiología no filiada es una de las principales causas de tratamiento sustitutivo renal en nuestro medio. Estudios previos en otros territorios sugieren que las enfermedades hereditarias podrían ser una de las potenciales causas de esta patología, especialmente en los pacientes más jóvenes. El estudio GENSEN evaluará la presencia de variantes genéticas patogénicas en sujetos que hayan desarrollado ERC categoría G5 antes de los 46 años, de etiología no filiada.

Métodos: Estudio observacional, prospectivo y multicéntrico, que evalúa la utilidad diagnóstica de la secuenciación masiva de alto rendimiento (HTS) dirigida a un conjunto de genes, en la identificación de la causa de la ERC. Se incluirán pacientes de todo el territorio español, a los que se extraerá una muestra de sangre o saliva, analizando posteriormente un panel de 529 genes asociados con enfermedad renal hereditaria. Esta publicación comunica el protocolo del estudio.

Conclusión: El estudio GENSEN permitirá evaluar el rendimiento diagnóstico del estudio del panel de genes en sujetos jóvenes de nuestro medio con desarrollo de ERC categoría G5 sin causa clara. Un diagnóstico etiológico ofrecería potenciales beneficios para pacientes y familiares (terapias dirigidas, ensayos clínicos, detección manifestaciones extrarrenales, evaluación de familiares para donación de vivo, estimación del riesgo de recurrencia en el injerto renal, consejo genético, entre otros) y permitiría acercar el estudio genético a la nefrología de nuestro país.

© 2023 Sociedad Española de Nefrología. Publicado por Elsevier España, S.L.U. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Kidney disease is a public health problem that affects around 850 million people worldwide, with an estimated prevalence in Spain of approximately 15%.¹⁻³ The development of chronic kidney disease (CKD) is accompanied by a shortened duration of life especially due to impairment of cardiovascular health. Thus its prevention and early detection should be a priority.⁴ Among the strategies aimed at alleviating the consequences of CKD, certainly the etiological diagnosis of the renal disease is a need that currently is not adequately addressed. In fact, the Spanish Registry of Renal Patients (REER), in its 2021 report, places CKD of unknown origin as the second cause of kidney disease among incident patients on renal replacement therapy, accounting for 18% of cases, only after diabetes, which constitutes 25.5%.⁵

In recent years, the development of genetic medicine has made it possible to identify more than 600 genetic alterations

as a cause of renal disease of Mendelian inheritance. To date, the most relevant published study in this subject analyzed by exome sequencing 3,315 patients with CKD of whom 281 (8.5%) had no clear etiology. Among the subjects with CKD of undefined cause, the genetic study identified in 51 (18.1%) an underlying pathology of monogenic origin.⁶

The importance of a diagnosis of certainty of the cause of kidney disease offers benefits at different levels, including the possibility of specific treatment or participation in clinical trials or the development of new avenues of research with directed therapeutic targets; but also has prognostic value, especially at the time of renal transplantation, allowing a correct approach to a possible graft dysfunction, even being able to administer prophylactic measures to prevent recurrence.⁷ Beyond the patient's own future, the diagnosis of a hereditary disease would offer the possibility of screening offspring through genetic counseling with its inherent benefits or the option of diagnosing a hidden renal disease in potential donors.⁸

Although the recent *Kidney Disease: Improving Global Outcomes* (KDIGO) 2022 guidelines address the possible indications for ordering a genetic study and urge its generalization, the reality is that hereditary nephropathies still account for a significant percentage of kidney disease of unfiled etiology.⁹

In order to alleviate this need, the Spanish Society of Nephrology (S.E.N.) is promoting the GENSEN study entitled Detection of hereditary diseases in patients with advanced chronic renal failure of non-inherited etiology; the design of which is presented in this article.

Methods

Study population

Patients will be included if they have developed CKD category G5, estimated glomerular filtration rate (eGFR) less than 15 mL/min/1.73 m² and/or are on renal replacement therapy, before the age of 46 years, without a clear etiology. It is estimated that a total of 500 subjects will be recruited over a period of approximately 12 months. All participants must give written consent prior to inclusion. The study has been approved by the research ethics committee of the Hospital Universitario de La Princesa and has been conducted in compliance with the Declaration of Helsinki and the relevant legal requirements for Biomedical Research (Law 14/2007). Both personal data and biological samples will be obtained, treated and stored with the strict confidentiality in accordance with the provisions of the Organic Law 3/2018, of December 5 on the Protection of Personal Data and Guarantee of Digital Rights.

Study design

Observational, prospective, multicenter study to evaluate the diagnostic utility of massive high-throughput sequencing in young patients (<46 years) with advanced CKD of unidentified cause. Subjects of pediatric and adult age are currently being recruited in 30 centers throughout Spain.

Intervention

After an exhaustive review of compliance with the inclusion criteria and the signing of the informed consent form, patients will be included in the GENSEN study. Available demographic, clinical and histological data will be collected from a form. Blood samples (3–5 mL in ethylene diamine tetraacetic acid [EDTA] tubes) or saliva samples will be collected and sent within 24 h to the *Health in Code* logistics center (A Coruña, Spain) where they will be processed and analyzed. The samples will be collected using appointments for health care analysis and/or medical visits defined by the periodic clinical follow-up of the participants, thus avoiding the performance of other unnecessary procedures.

Methodology

DNA sequencing and variant analysis

The genetic study will be performed using high-throughput sequencing (HTS) technology applied to a customized library of genes associated with hereditary nephropathies. First, DNA will be extracted from blood or saliva samples by automatic genomic DNA purification (QIA Symphony SP[®], QIAGEN, Hilden, Germany). Libraries will be enriched using a SureSelectXT Low input specific hybridization probe kit (Agilent Technologies, Santa Clara, California, USA) for the Illumina multiplex paired-end sequencing method and the obtained fragments will be sequenced on the NovaSeq 6000 sequencing system platform (Illumina, San Diego, California, USA) following the manufacturer's instructions. Enrichment of the areas of interest allows capturing the coding regions and adjacent intronic areas of the genes selected for the personalized gene library, which includes 529 genes associated with hereditary renal pathologies (Table 1, Appendix Annex 1). The probes have been designed to adequately cover all coding exons and 50 base pairs (bp) of flanking intronic sequences, therefore, this test will not be able to identify genetic variants located in deeply intronic areas away from splice sites or UTR regions (*untranslated regions* of genes). The clusters were prepared using the cBot device (Illumina, San Diego, California, USA).

Bioinformatics analysis will be performed using an internal *end-to-end pipeline* developed by *Health in Code* (HIC-Mutations, Version 11.8.8326.1681), according to *Whole Exome Sequencing* (WES) analysis best practices. Sequencing data will be analyzed through a process that includes sample demultiplexing, alignment refinement and adjustment, variant calling, normalization, sequence quality control, generation of coverage statistics by region of interest, and quantification of copy number variation (CNV) along with evaluation of quality control points.¹⁰ This test can identify single nucleotide variants (SNVs) and insertions/deletions (INDELs) up to 50 bp. Genetic variants are identified using the GRCh37/hg19 reference genome and reported following the recommendations of the *Human Genome Variation Society* (HGVS) (www.hgvs.org).

The prioritization and interpretation of genetic variants is performed considering clinical, genetic and population scientific criteria, contrasting these data with different population, disease, gene and variant databases and using computational prediction tools. According to the guidelines of the American College of Medical Genetics (ACMG),¹¹ the classification of variants is performed based on five categories: pathogenic, probably pathogenic, variant of uncertain significance, probably benign and benign. The interpretation of the results is carried out according to current scientific knowledge, clinical evidence and information available in genomic databases, subject to change as new scientific evidence becomes available.

The genes included in this test have been selected on a clinical basis according to their relationship with a particular phenotype and classified based on the evidence supporting this association using different sources, including databases and scientific publications. The presence of variants that do

Table 1 – Panel of 529 genes associated with hereditary renal diseases.

ACE, ACTB, ACTG1, ACTN4, ADAMTS13, ADAMTS9, ADCY10, AGT, AGTR1, AGXT, AHI1, ALG1, ALG8, ALG9, ALMS1, ALPL, AMER1, ANKFY1, ANKS6, ANLN, ANOS1, AP2S1, APOA1, APOA4, APOE, APOL1, APRT, AQP2, ARHGAP24, ARHGDI1, ARL13B, ARL3, ARL6, ARMC9, ATN1, ATP6V0A4, ATP6V1B1, ATP6V1C2, AVIL, AVP, AVPR2, B2 M, B3GLCT, B9D1, B9D2, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BICC1, BMP2, BMP4, BMPER, BNC2, BSLC2, BSND, C1QA, C1QB, C1QC, C3, C4A, C4B, C5orf42, C8orf37, CA1, CA2, CASP10, CASR, CC2D2A, CCB1, CCDC28B, CCL2, CCNQ, CD151, CD2AP, CD46, CD81, CD96, CDC42, CDC5L, CDC73, CDK10, CDK20, CDKN1C, CENPF, CEP104, CEP120, CEP164, CEP290, CEP41, CEP55, CEP83, CFB, CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CFI, CFP, CHD1L, CHD7, CHRM3, CISD2, CIT, CLCN5, CLCN7, CLCNKA, CLCNKB, CLDN10, CLDN16, CLDN19, CNNM2, COL4A1, COL4A3, COL4A4, COL4A5, COL4A6, COPA, COQ2, COQ4, COQ6, COQ7, COQ8A, COQ8B, COQ9, CPT1A, CRB2, CRKL, CSPP1, CTH, CTLA4, CTNS, CTU2, CUBN, CUL3, CYP24A1, DACH1, DACT1, DCDC2, DCHS1, DDX59, DGKE, DHCR7, DLC1, DMP1, DNAJB11, DNASE1, DSTYK, DVL1, DVL3, DYNC2H1, DYNC2L1, DZIP1L, EGF, EHHADH, EMP2, ENPP1, EP300, ESCO2, ETFA, ETFB, ETFDH, ETV4, EXOC8, EYA1, FAH, FAM20A, FAN1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCI, FAS, FASLG, FAT1, FAT4, FCGR2A, FCGR3A, FGA, FGF10, FGF20, FGF23, FGFR2, FGFR3, FLCN, FLNA, FMN1, FN1, FOXI1, FOXP1, FRAS1, FREM1, FREM2, FUZ, FXYD2, G6PD, GANAB, GAPVD1, GATA3, GCM2, GDF11, GEMIN4, GLA, GLI3, GLIS2, GLIS3, GNA11, GPC3, GPHN, GREB1L, GRHRP, GRIP1, H19, HAAO, HAS2, HES7, HGD, HNF1A, HNF1B, HNF4A, HOGA1, HPRT1, HPSE2, HSD11B2, HSD17B4, HSPA9, IFT122, IFT140, IFT172, IFT27, IFT43, IFT46, IFT52, IFT74, IFT80, IFT81, INF2, INPP5E, INTU, INVS, IQCB1, IRF5, ITGA3, ITGA8, ITGAM, ITGB4, ITS1N1, ITS2N2, JAG1, JAM3, KANK1, KANK2, KANK4, KAT6B, KCNA1, KCNJ1, KCNJ10, KCNJ15, KCNJ16, KCTD1, KIAA0556, KIAA0586, KIAA0753, KIF14, KIF7, KLHL3, KMT2D, KYNU, LAGE3, LAMA5, LAMB2, LCAT, LMNA, LMX1B, LRIG2, LRP2, LRP4, LRP5, LYZ, LZTFL1, MAD2L2, MAFB, MAGED2, MAGI2, MAPKBP1, MBTPS2, MCM5, MKKS, MKS1, MMACHC, MNX1, MOCOS, MOCS1, MUC1, MYCN, MYH9, MYO1E, NAA10, NCPAG2, NDUFAF3, NDUFB8, NDUFS2, NEK1, NEK8, NEU1, NFIA, NIPBL, NOS1AP, NOTCH2, NPHP1, NPHP3, NPHP4, NPHS1, NPHS2, NR3C2, NR1P1, NSDHL, NUP107, NUP133, NUP160, NUP205, NUP85, NUP93, NXF5, NXN, OCRL, OFD1, OPLAH, OSGEF, PAX2, PAX8, PBX1, PCBD1, PCSK5, PDE6D, PDS1, PDS2, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PGM3, PHEX, PHGDH, PIBF1, PIEZO2, PIGN, PIGT, PKD1, PKD2, PKHD1, PLA2R1, PLCE1, PMM2, PODXL, PORCN, PRKCD, PRKCSH, PRPS1, PTEN, PTPN22, PTPRO, PUF60, RAD21, RAI1, RARRES1, REN, RERE, RET, RFWD3, RMND1, RNU4ATAC, ROBO2, ROR2, RFGRI1, RPL26, SALL1, SALL4, SARS2, SCARB2, SCNN1A, SCNN1B, SCNN1G, SDCCAG8, SEC61A1, SEC61B, SEC63, SEMA3E, SF3B4, SGPL1, SI, SIX1, SIX2, SIX5, SLC12A1, SLC12A2, SLC12A3, SLC12A7, SLC22A12, SLC26A1, SLC26A7, SLC2A2, SLC2A9, SLC34A1, SLC34A3, SLC36A2, SLC3A1, SLC41A1, SLC4A1, SLC4A2, SLC4A4, SLC5A1, SLC5A2, SLC6A19, SLC6A20, SLC7A7, SLC7A9, SLC9A3R1, SLIT2, SMARCA1, SNRPB, SON, SOX11, SOX17, SPRY2, SRGAP1, STAT1, STAT4, STK11, STRA6, STS, SUFU, SYNPO, TBC1D24, TBC1D8B, TBX18, TBX4, TBXT, TCTN1, TCTN2, TCTN3, TFAP2A, THBD, THOC6, TMEM107, TMEM138, TMEM216, TMEM231, TMEM237, TMEM260, TMEM67, TNFSF4, TNIP1, TNS2, TNXB, TP53RK, TP63, TPRKB, TRAF3IP1, TRAP1, TREX1, TRIM32, TRIP11, TRPC6, TRPM6, TRPS1, TRRAP, TSC1, TSC2, TTC21B, TTC37, TTC8, TXNDC15, TXNL4A, UMOD, UPK3A, USP8, VANGL1, VANGL2, VDR, VHL, VIPAS39, VPS33B, VTN, WDPCC, WDR19, WDR34, WDR35, WDR72, WDR73, WFS1, WNK1, WNK4, WNT3, WNT4, WNT5A, WNT7A, WT1, XDH, XPNPEP3, XPO5, XRCC2, XRCC4, ZAP70, ZIC3, ZMPSTE24, ZNF148, ZNF365, ZNF423

not meet the optimal quality parameters, either because they have low coverage, because they are located in regions of high homology or other technical peculiarities will be confirmed by complementary tests such as Sanger sequencing or *Multiplex Ligation dependent Probe Amplification* (MLPA).

In particular, this design enriches intergenic regions in the *CFH* and *CD46* genes, allowing us to infer the risk haplotypes of factor H and MCP (*membrane cofactor protein*, complement regulator gene) in relation to the development of atypical hemolytic uremic syndrome (aHUS).^{12,13}

In addition, capture probes have been introduced in the *Variable Number of Tandem Repeats* (VNTR) region of *MUC1*, susceptible to present the most prevalent pathogenic variant in this gene linked to the development of autosomal dominant tubulointerstitial nephropathy (NTAD).¹⁴ The horizontal coverage of this highly repetitive region, together with a high depth inreading, allows the detection of changes in the nucleotide sequence that must be confirmed by complementary techniques such as Sanger sequencing or, in cases suggestive of being caused by defects in *MUC1*, the SNaPShot technique.¹⁵

Despite the high sensitivity and specificity of this test for the 529 selected genes, there are some limitations of the test when the following situations occur: presence of mosaicism and somatic variants whose low frequencies may not be identified, presence of complex chromosomal rearrangements, balanced translocations and inversions that alter the zygosity of variants, genetic variants that produce allelic dropouts, presence of pseudogenes or homologous regions, incorrect identification of variants in homopolymer or areas with high guanine and cytosine (GC) content, and errors in the reference sequence.

Objectives

Following the KDIGO guidelines for CKD, which indicate that the cause of CKD should be identified and the risk categorized by determining eGFR and albuminuria, the main objective of the GENSEN study is to offer a potentially etiological diagnosis to young patients with CKD category G5 with no previous etiological affiliation. A correct diagnosis should allow a better understanding of their disease (personal and family prognosis) and personalized management (targeted treatments, clinical trials).

This project also opens the door to know the phenotype-genotype correlations of the studied population. In the case of evidencing a clear association between the clinical and histological presentation and/or family history with the different pathogenic variants found, it would allow a more targeted diagnostic approach in the future, with the possibility of early diagnosis and individualized treatment of the different pathologies.

In addition, if a large number of patients throughout the country are analyzed, the study will provide valuable information regarding the percentage of people with hereditary kidney diseases in our environment (underdiagnosed to date), the most frequent hereditary pathologies and gene variants, as well as the possible inter-territorial differences in these findings.

Finally, evaluating the performance of this diagnostic approach is the first step to introduce DNA sequencing techniques for the etiological diagnosis of CKD in all nephrology units in the country, especially when suggested by the clinical and/or family history, but also for patients without a clear etiological diagnosis.

Discussion

The GENSEN project is a milestone in Spanish Nephrology, it is framed within the strategic lines of the S.E.N. and evidences a necessity that is already incorporated in the clinical practice guidelines. Among the objectives defined by the project, the possibility of reaching an etiological diagnosis of certainty in patients with previously unaffiliated renal diseases will make it possible to achieve various benefits.

Firstly, the identification of the etiology of a renal disease can modify the natural history of some pathologies that have a specific treatment (e.g., Fabry disease, primary hyperoxaluria or cystinosis), as well as avoid overtreatment in some of the cases, which are often erroneously labeled (such as Alport disease, which currently has no specific management but is frequently diagnosed as focal and segmental glomerulosclerosis, leading to a useless treatment with immunosuppressants). In fact, nephroangiosclerosis or hypertensive nephropathy is at the top of the etiological diagnoses in CKD. We now know that quite a few cases of nephroangiosclerosis are misdiagnosed,¹⁶ attributing the deterioration of renal function to this pathology, but that in reality it is due to hereditary causes (undiagnosed) such as alterations in the collagen genes (Alport disease)¹⁷ or uromodulin (autosomal dominant tubulointerstitial nephropathy).¹⁸ Recently, inaxaplin, a drug that inhibits the intracellular membrane channel activity of proteins encoded by high-risk *APOL1* variants, decreased proteinuria in patients with these variants, putting the pathogenic focus on the podocyte.¹⁹ The presence of high-risk *APOL1* variants explains the high prevalence of the misnamed hypertensive nephropathy in African Americans.

Secondly, reaching an etiological diagnosis prevents recurrences at the time of renal transplantation of pathologies such as atypical hemolytic uremic syndrome or primary hyperoxaluria, which currently benefit from specific treatments such as eculizumab or ravulizumab for the former^{20,21} or lumasiran for the latter.²²

Thirdly, etiological diagnosis allows screening for extrarenal manifestations such as in autosomal dominant polycystic kidney disease (intracranial aneurysms), in PX2 nephropathy (ocular involvement), in recessive and X-linked forms of Alport (ocular and hearing involvement), etc.

Finally, the diagnosis of hereditary kidney disease makes it possible to offer genetic counseling to patients, with the consequent reproductive recommendation.²³

Previous studies performed in cohorts with unaffiliated nephropathy have demonstrated a variable diagnostic range between 10 and 40%.^{24,25} In addition, identification and classification of variants may allow reclassification of patients who had previously received a diagnosis of variant of uncertain significance.

Although the most relevant limitation of genetic testing for etiological diagnosis is the cost (Fig. 1), it is also an opportunity for multidisciplinary work, since it requires an arrangement between geneticists and nephrologists. The new applications of HTS technologies make it possible to unify different analyses in a single test that reduces the cost associated with different techniques applied and resources used. In addition,

HTS offers a wide range of improved strategies that can be implemented on an ongoing basis, for example, by including new regions of interest for genetic analysis. Among the most important indications for requesting it, the KDIGO 2022 guidelines recommend its performance in case of family history, development of early CKD, consanguinity, manifestations or syndromic phenotype, in the living donor, as an alternative to a renal biopsy when this cannot be performed, as a guide for certain treatments, at risk of recurrence in transplantation or as prognostic information in some pathologies. In France, genetic diagnosis is offered free of charge to patients nationwide with CKD of uncertain cause under 46 years of age.²⁶

In any case, taking into account the costs derived from the initiation of renal replacement therapy (inherent to the technique and derived complications), and from a cost-efficient point of view, the etiological diagnosis of nephropathy with potential treatment also represents a benefit superior to the mere progression of renal disease without therapy. Just the fact in of diagnosing CKD (screening for albuminuria) has recently been shown to be cost-effective, regardless of the etiology of the nephropathy, proving that only general measures to prevent progression of CKD results in a direct economic benefit.²⁷

Our study is not free of some methodological limitations such as, for example, its observational design, being ethically impossible to propose a clinical trial. Furthermore, it is a voluntary study in which each participating center recruits patients according to inclusion criteria. One of the inclusion criteria is that the subject presents a non-filiated etiology of CKD, which may present certain heterogeneity depending on the techniques available in the different centers (biomarkers, genetic studies, renal biopsy, staining). In an attempt to alleviate this limitation, it was decided to include individuals with progression to CKD category G5 at an early age (< 46 years), being the cohort in which there is the highest prevalence of hereditary diseases. The strengths of this study include the repercussion of the results in increasing knowledge on hereditary renal diseases and promoting genetic diagnosis through collaboration with specialists in this discipline, with clear prognostic benefit for patients, overcoming the aforementioned limitations.

In conclusion, the GENSEN project is a pioneering multicenter study whose primary objective is to determine the etiology of CKD in young patients in whom an etiological diagnosis of certainty is not available.

Financing

The GENSEN study is promoted by the Spanish Society of Nephrology (S.E.N.) and funded by AstraZeneca, Alexion, Chiesi and Alnylam, with unconditional grants.

MB's research is funded by Instituto de Salud Carlos III (ISCIII) RICORS program to RICORS2040 (RD21/0005/0001) funded by European Union - NextGenerationEU, Mechanism for Recovery and Resilience (MRR) and PI PI PI22/00240, Fundació la Marató de TV3 202026-10.

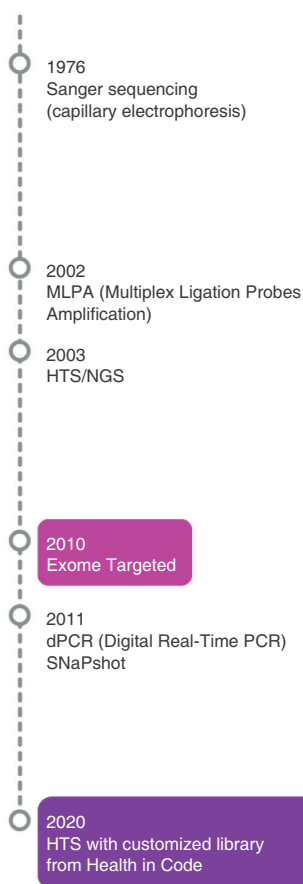
AO research is funded by Instituto de Salud Carlos III (ISCIII) RICORS program to RICORS2040 (RD21/0005/0001) funded by

GENSEN study

A differential technological approach to precision medicine



EVOLUTION OF THE TECHNIQUES



COMPARISONS OF TECHNIQUES

Over the years, we have observed how technological advances have helped us to unify the different techniques in order to be able to evaluate everything under a single sequencing. Below, we present a comparison of the technology used for the GENSEN study (HTS with custom library) versus targeted exome, as well as the limitations found in each.

TARGETED EXOME	HTS WITH CUSTOMIZED LIBRARY
The exome captures genes not associated with the patient's pathology that require IC adaptation.	Targeting a set of genes related to the patient's clinical indication.
Capture of intronic regions within +/- 20 bp of coding regions	Enrichment of intronic regions within +/- 50 bp of coding regions.
Average read depth: 100x	Increased horizontal coverage and read depth (average coverage >500x + horizontal coverage at 30x >99%).
Does not capture deeply intronic or intergenic regions.	Enrichment of deeply intronic or intergenic target regions of interest.
Allows analysis of CNVs	Analysis of CNVs with proprietary software.
Low sensitivity for detecting mosaicism.	More sensitivity to detect mosaicisms.
Captures unselected genes that could be associated with renal disease at a later stage.	Limitations Does not allow capturing unselected genes that could later be associated with kidney disease.
Reanalysis of the data does not require re-sequencing of the sample.	Reanalysis of data requires re-sequencing of the sample.

Fig. 1 – Sequencing techniques and GENSEN study.

Left column: evolution of DNA sequencing techniques. Right column: comparison of the most commonly used sequencing techniques. HTS: High Throughput Sequencing (HTS: High Throughput Sequencing).

European Union - NextGenerationEU, Mechanism for Recovery and Resilience (MRR) and SPACKDc PMP21/00109, FEDER funds and COST Action PERMEDIK CA21165, supported by COST (European Cooperation in Science and Technology). PREVENTCKD Consortium. Project ID: 101101220 Programme: EU4H. DG/Agency: HADEA: 2023-2024.

RT research is funded by Instituto de Salud Carlos III (ISCIII) RICORS program to RICORS2040 (RD21/0005/0001) funded by European Union - NextGenerationEU, Mechanism for Recovery

and Resilience (MRR) and PI22/00361, Fundació la Marató de TV3 202036-30.

Conflict of interest

M. Blasco is a member of the current S.E.N. board and has received speaking fees, advisory boards and funding to attend courses and congresses from Otsuka, Chiesi, Novartis and Alexion in the last 36 months.

B. Quiroga is the current secretary of the S.E.N. and has received payments for lectures and funding to attend courses and congresses from Vifor-Pharma, Astellas, Amgen, Bial, Ferrer, Novartis, AstraZeneca, Sandoz, Laboratorios Bial, Esteve, Sanofi-Genzyme, Otsuka in the last 36 months.

A. Ortiz is coordinator of the ERA Registry. A. Ortiz has received grants from Sanofi and payments as a consultant or for presentations or travel funding from Adviccene, Alexion, Astellas, Astrazeneca, Amicus, Amgen, Boehringer Ingelheim, Fresenius Medical Care, GSK, Bayer, Sanofi-Genzyme, Menarini, Mundipharma, Kyowa Kirin, Lilly, Freeline, Idorsia, Chiesi, Otsuka, Novo-Nordisk, Sysmex and Vifor Fresenius Medical Care Renal Pharma. He is the Director of the UAM - AstraZeneca Chair in Chronic Kidney Disease and Water Electrolyte Disorders. He holds shares in Telara Farma.

P. de Sequera is the president of S.E.N. and has received payments for lectures and funding to attend courses and congresses from Vifor Pharma, Amgen, Fresenius, Nipro, AstraZeneca, Braun, Baxter, GSK and Astellas.

R. Torra is the president-elect of the European Renal Association and has received speaking fees and funding to attend courses and congresses from Sanofi-Genzyme, Takeda, Amicus, Chiesi, Kyowa-Kirin, Astra-Zeneca, Alnylam, Alexion, Otsuka.

J.M. García-Aznar is an employee of Healthincode.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.nefro.2023.09.002>.

REFERENCES

- GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2020;395:709-33 [http://10.1016/S0140-6736\(20\)30045-3](http://10.1016/S0140-6736(20)30045-3)
- Asociación Información Enfermedades Renales Genéticas (AIRG-E), European Kidney Patients' Federation (EKPF), Federación Nacional de Asociaciones para la Lucha Contra las Enfermedades del Riñón (ALCER), Fundación Renal Íñigo Álvarez de Toledo (FRIAT), Red de Investigación Renal (REDINREN) Ortiz A. RICORS2040: the need for collaborative research in chronic kidney disease. *Clin Kidney J*. 2021;15:372-87 <http://10.1093/ckj/sfab170>
- Jager KJ, Kovesdy C, Langham R, Rosenberg M, Jha V, Zoccali C. A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases. *Nephrol Dial Transplant*. 2019;34:1803-5.
- Alberta Kidney Disease Network Thompson S, James M, Wiebe N, Hemmelgarn B, Manns B, Klarenbach S, et al. Cause of death in patients with reduced kidney function. *J Am Soc Nephrol*. 2015;26(10):2504-11 <http://10.1681/ASN.2014070714>
- <https://www.ont.es/wp-content/uploads/2023/01/MEMORIA-REER-2021-PRELIMINAR.pdf> [accessed el 9 de julio de 2023].
- Groopman EE, Marasa M, Cameron-Christie S, Petrovski S, Aggarwal VS, Milo-Rasouly H, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med*. 2019;380:142-51 <http://10.1056/NEJMoa1806891>
- Gansevoort RT, Arici M, Benzing T, Birn H, Capasso G, Covic A, et al. Recommendations for the use of tolvaptan in autosomal dominant polycystic kidney disease: a position statement on behalf of the ERA-EDTA Working Groups on Inherited Kidney Disorders and European Renal Best Practice. *Nephrol Dial Transplant*. 2016;31:337-48 <http://10.1093/ndt/gfv456>
- Snoek R, Stokman MF, Lichtenbelt KD, van Tilborg TC, Simcox CE, Paulussen ADC, et al. Preimplantation genetic testing for monogenic kidney disease. *Clin J Am Soc Nephrol*. 2020;15:1279-86 <http://10.2215/CJN.03550320>
- KDIGO Conference Participants. Genetics in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int*. 2022;101:1126-41 <http://10.1016/j.kint.2022.03.019>
- de Una D. System and Method to Detect Structural Genetic Variants. Madrid, Spain: OEPM (Oficina Española de Patentes y Marcas); 2019. p. 201731242.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405-24 <http://10.1038/gim.2015.30>
- Goodship TH, Cook HT, Fakhouri F, Fervenza FC, Frémeaux-Bacchi V, Kavanagh D, et al. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a Kidney Disease: Improving Global Outcomes(KDIGO) Controversies Conference. *Kidney Int*. 2017;91:539-51 <http://10.1016/j.kint.2016.10.005>
- España-Gordillo J, Jorge EG, Garrido CA, Carreras L, Lopez-Trascasa M, Sanchez-Corral P, et al. Insights into hemolytic uremic syndrome: segregation of three independent predisposition factors in a large, multiple affected pedigree. *Mol Immunol*. 2006;43:1769-75 <http://10.1016/j.molimm.2005.11.008>
- Asyrah N, Bullich G, Miquel R, Furlano M, Ruiz P, Lorente L, et al. Autosomal dominant tubulointerstitial kidney disease: clinical presentation of patients with ADTKD-UMOD and ADTKD-MUC1. *Am J Kidney Dis*. 2018;72:411-8 <http://10.1053/j.ajkd.2018.03.019>
- Knaup KX, Hackenbeck T, Popp B, Stoeckert J, Wenzel A, Büttner-Herold M, et al. Biallelic expression of Mucin-1 in autosomal dominant tubulointerstitial kidney disease: implications for nongenetic disease recognition. *J Am Soc Nephrol*. 2018;29:2298-309 <http://10.1681/ASN.2018030245>
- Carriazo S, Vanessa Perez-Gomez M, Ortiz A. Hypertensive nephropathy: a major roadblock hindering the advance of precision nephrology. *Clin Kidney J*. 2020;13:504-9 <http://10.1093/ckj/sfaa162>
- Savigne J, Harraka P. Pathogenic variants in the genes affected in alport syndrome (COL4A3-COL4A5) and their association with other kidney conditions: a review. *Am J Kidney Dis*. 2021;78:857-64 <http://10.1053/j.ajkd.2021.04.017>
- Olinger E, Hofmann P, Kidd K, Dufour I, Belge H, Schaeffer C, et al. Clinical and genetic spectra of autosomal dominant tubulointerstitial kidney disease due to mutations in UMOD and MUC1. *Kidney Int*. 2020;98:717-31 <http://10.1016/j.kint.2020.04.038>
- Egbuna O, Zimmerman B, Manos G, Fortier A, Chirieac MC, Dakin LA, et al. Inaxaplin for proteinuric kidney disease in persons with two APOL1 variants. *N Engl J Med*. 2023;388:969-79 <http://10.1056/NEJMoa2202396>
- Duineveld C, Bouwmeester RN, Wijnsma KL, Bemelman FJ, van der Heijden JW, Berger SP, et al. Eculizumab rescue therapy in patients with recurrent atypical hemolytic uremic syndrome after kidney transplantation. *Kidney Int Rep*. 2023;8:715-26 <http://10.1016/j.ekir.2023.01.016>

21. Begum F, Khan N, Boisclair S, Malieckal DA, Chitty D. Complement inhibitors in the management of complement-mediated hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria. *Am J Ther*. 2023;30:e209–19 <http://10.1097/MJT.0000000000001609>
22. Lombardi Y, Isnard P, Chavarot N, Chauvet S, Martinez F, Thervet É, et al. Stiripentol and lumasiran as a rescue therapy for oxalate nephropathy recurrence after kidney transplantation in an adult patient with primary hyperoxaluria type 1. *Am J Kidney Dis*. 2023;S0272-6386(23):00041 <http://10.1053/j.ajkd.2022.12.005>
23. Snoek R, van der Graaf R, Meinderts JR, van Reekum F, Bloemenkamp KWM, Knoers NVAM, et al. Pregnancy in advanced kidney disease: clinical practice considerations on a challenging combination. *Nephron*. 2020;144:185–9 <http://10.1159/000505781>
24. Snoek R, van Jaarsveld RH, Nguyen TQ, et al. Genetics-first approach improves diagnostics of ESKD patients <50 years old. *Nephrol Dial Transplant*. 2022;37:349–57 <http://10.1093/ndt/gfaa363>
25. Connaughton DM, Kennedy C, Shril S, Mann N, Murray SL, Williams PA, et al. Monogenic causes of chronic kidney disease in adults. *Kidney Int*. 2019;95:914–28 <http://10.1016/j.kint.2018.10.031>
26. Doreille A, Villié P, Mesnard L. National survey on genetic test prescription in French adult nephrologists: a call for simplification and education. *Clin Kidney J*. 2022;15:1213–5 <http://10.1093/ckj/sfac041>
27. Cusick MM, Tisdale RL, Chertow GM, Owens DK, Goldhaber-Fiebert JD. Population-wide screening for chronic kidney disease: a cost-effectiveness analysis. *Ann Intern Med*. 2023;176:788–97 <http://10.7326/M22-3228>