

Genotype–phenotype correlations and clinical outcomes of genetic TRPC6 podocytopathies

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ABSTRACT

Background and hypothesis. Podocytopathy associated with likely pathogenic/pathogenic variants of Transient receptor potential cation channel subfamily C member 6 (TRPC6) (TRPC6-AP) has been recognized for about 20 years. As a result of its rarity however, the spectrum of clinical phenotypes and genotype–phenotype correlation of TRPC6-AP remains poorly understood. Here, we characterized clinical, histological and genetic correlates of familial and sporadic patients with TRPC6-AP.

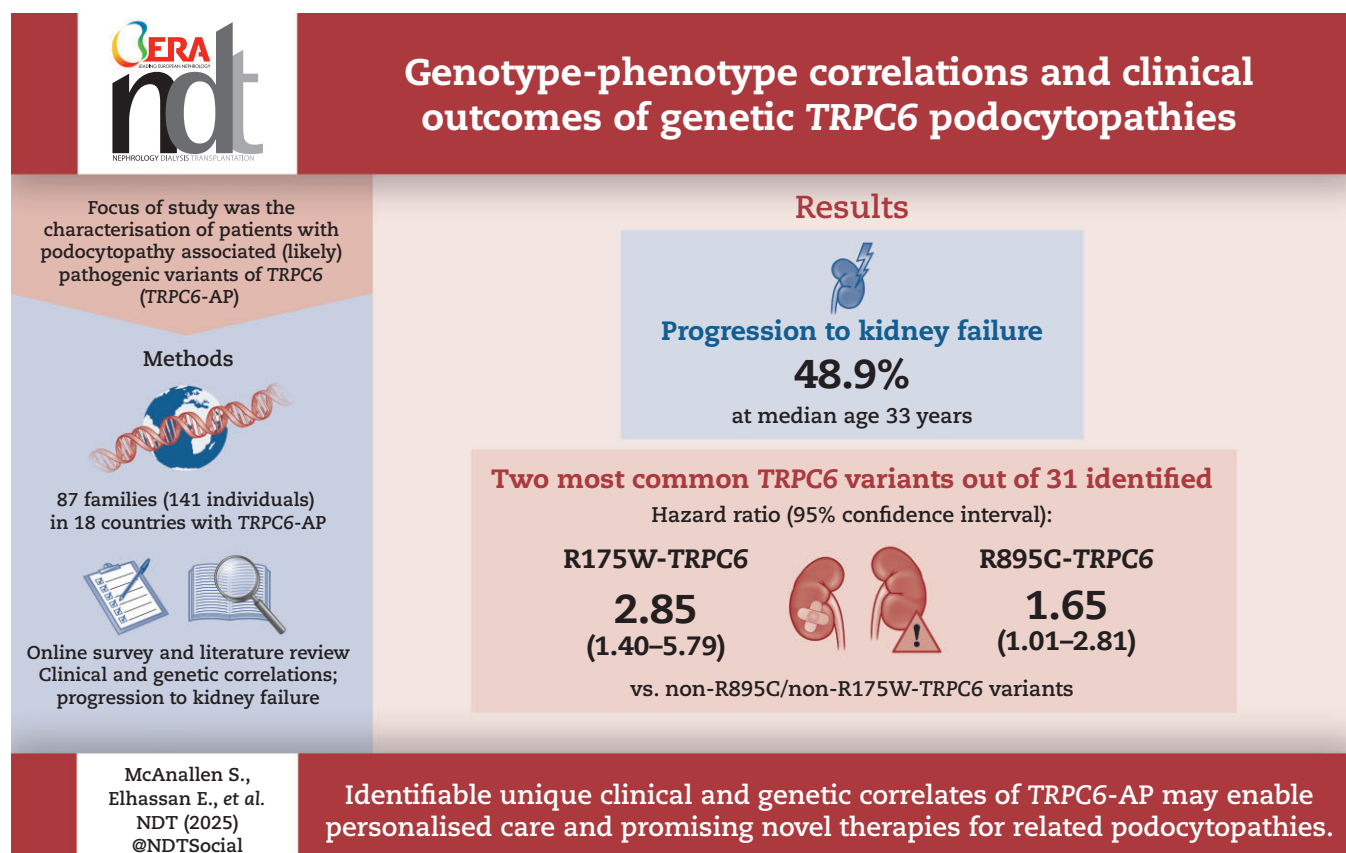
Methods. In this multicentre observational study, an online questionnaire followed by a systematic literature review was performed to create a cohort with comprehensive data on genetic and clinical outcomes [age of onset, clinical presentation, treatment response, kidney biopsy findings and progression to kidney failure (KF)]. Logistic regression, Cox proportional hazards model and Kaplan–Meier analyses investigated the associations between genetic variants and disease progression.

Results. Among 87 families (96 familial and 45 sporadic cases), 31 distinct missense TRPC6 variants (including 2 novel) were identified, with c.2683C>T p.(Arg895Cys) and c.523C>T p.(Arg175Trp) the commonest variants. Proteinuric kidney disease/nephrotic syndrome was the most common clinical presentation (83.7%), while focal segmental glomerulosclerosis was the most common histological finding (89.4%). By 33 (interquartile range 17–40) years, 48.9% (69/141) of patients had progressed to KF. Sporadic TRPC6-AP demonstrated an earlier progression to KF than familial cases ($P = .001$) and were more likely to present with nephrotic syndrome [odds ratio 4.34 (1.85–10.15); $P = .001$]. Gain-of-function TRPC6 variants were more frequent in familial than sporadic TRPC6-AP (70.8% vs 44.4%; $P = .004$). Compared with patients with other TRPC6 variants, patients with TRPC6 p.R175W and p.R895C variants progressed to KF earlier [median kidney survival of 21 years, hazard ratio 2.985 (95% confidence interval 1.40–5.79); and 38 years, hazard ratio 1.65 (95% confidence interval 1.01–2.81), respectively, log-rank $P = .005$].

Conclusion. Our study shows unique clinical and genetic correlations of TRPC6-AP, which may enable personalized care and promising novel therapies.

Keywords: CKD, FSGS, podocytopathy, steroid-resistant nephrotic syndrome, TRPC6

GRAPHICAL ABSTRACT



KEY LEARNING POINTS

What was known:

- Heterozygous Transient receptor potential cation channel subfamily C member 6 (TRPC6) variants are associated with proteinuric kidney disorders (TRPC6-AP), often leading to kidney failure, with variable phenotype penetrance and disease progression.
- The dysfunctional calcium influx into the podocyte leads to focal segmental glomerulosclerosis, with gain-of-function (GOF-TRPC6) variants causing excessive calcium influx.
- Poor genotype–phenotype correlations have been demonstrated.

This study adds:

- Eighty-seven families (141 cases) with 31 disease-causing missense TRPC6 variants, including 2 novel variants, were examined clinically and genetically.
- Sporadic TRPC6-AP were younger and present with nephrotic syndrome, while GOF-TRPC6 variants were more common in familial patients.
- Patients with TRPC6 p.(Arg175Trp) variant progressed earlier to kidney failure than other variants.

Potential impact:

- This investigation facilitates personalized care by allowing familial screening and informing kidney prognosis and treatment.
- This study highlights the importance of genetic diagnosis in reducing the burden of immunosuppression for individuals resistant to treatment.
- This study identifies families worldwide who may benefit from novel therapies targeting TRPC6 currently under development.

INTRODUCTION

Genetic testing has yielded significant insights into the physiology of the glomerular filtration barrier, facilitating the identification of likely pathogenic/pathogenic (LP/P) genetic variants causing dysfunction to the podocyte and its associated structural proteins in patients with steroid-resistant nephrotic syndrome (SRNS) or focal segmental glomerulosclerosis (FSGS), referred to as ‘podocytopathies’ [1–7]. Although patients with podocytopathies are often glucocorticoid-unresponsive and have limited options for targeted therapies [8, 9], establishing the underlying genetic basis of podocytopathies enables personalized management, allowing for immunosuppression-sparing approaches, prioritizing the protective effects of renin–angiotensin–aldosterone system inhibitors or using disease-specific therapies, such as coenzyme Q10 [9–11].

Transient receptor potential cation channel subfamily C member 6 (TRPC6; MIM #603 652) encodes a widely expressed protein in the podocyte that maintains its slit diaphragm integrity by mediating calcium entrance and cellular signalling that interacts with podocin and nephrin [12]. Variants causing podocytopathy-associated TRPC6 (TRPC6-AP) have been linked to dominantly inherited monogenic FSGS [13, 14]. Functional studies that measure calcium influx into the podocyte demonstrate that gain-of-function (GOF-TRPC6) effect variants, often in the N-terminal ankyrin (ANK) repeat domain and C-terminal regions, increase calcium influx to the podocyte, precipitating cell damage and sclerosis [13–24]. Further studies showed that certain TRPC6 missense variants substantially reduce calcium influx activity into the podocyte, mediating loss-of-function (LOF-TRPC6) effects and usually associated with an earlier onset of SRNS/FSGS in affected individuals [24]. Notably, variants in the same motif can express differential functional effects, such as p.(Arg895Cys), characterized as GOF-TRPC6 [14], while p.(Arg895Lys) is LOF-TRPC6 [24]. TRPC6 protein-truncating variants have been described as having inactivating properties that completely disrupt calcium cellular entrance [25]. To date, genotype correlations have been shown to poorly influence disease progression [21].

In a recent multicentre case–control study, Wooden *et al.* utilized population-based next-generation sequencing data of 37 542 individuals to bridge the gap between the natural history and

phenotypic features associated with TRPC6-AP [21]. They demonstrated a similar burden of TRPC6 protein-truncating variants between cases and controls, suggesting a non-casual effect [21]. Furthermore, they described 39 families (64 cases) with 21 TRPC6 missense variants, 11 of which are novel, clustering in regions other than the transmembrane domain of TRPC6 [21]. Four variants exhibit the GOF-TRPC6 effect using protein structural modelling [21]. Exploration of the clinical data and histopathological findings has revealed poor genotype–phenotype correlations [21]. Nonetheless, this paper has included missense TRPC6 variants which are predicted to be benign/of uncertain significance, and further investigation is required.

To enhance our knowledge, we systematically reviewed 141 (114 published and 27 previously unreported) cases of podocytopathy secondary to LP/P TRPC6 missense variants to decipher associated clinical, histological and genetic correlates.

MATERIALS AND METHODS

Study design and ethics

To understand the phenotypic and genetic outcomes of LP/P TRPC6-AP, an invitation e-mail was sent to 260 potential respondents, including authors, nephrologists and collaborators involved in inherited kidney disease (Fig. 1). All member centres associated with the European Rare Kidney Disease Reference Network were invited to contribute. Anonymized clinical, histological and genetic data related to affected individuals was obtained via an 82-item cross-sectional survey from 1 February 2023 until 30 June 2023 and structured into nine sections, with the following as primary areas: clinical presentation, diagnostic evaluation, management, disease progression and genetic diagnosis (Supplementary data, Table S1). We aimed to include all clinically and genetically affected individuals and their relatives with LP/P TRPC6-AP, proteinuria, SRNS and/or FSGS (see sections on genetic variants assessment and definitions). Thirty-seven initial families (47 cases) from centres in Europe, Australia, Singapore and North America were evaluated (Fig. 1).

Ethical approval was obtained from the Research Ethics Committee in Beaumont Hospital, Dublin (REC 19/28), and all

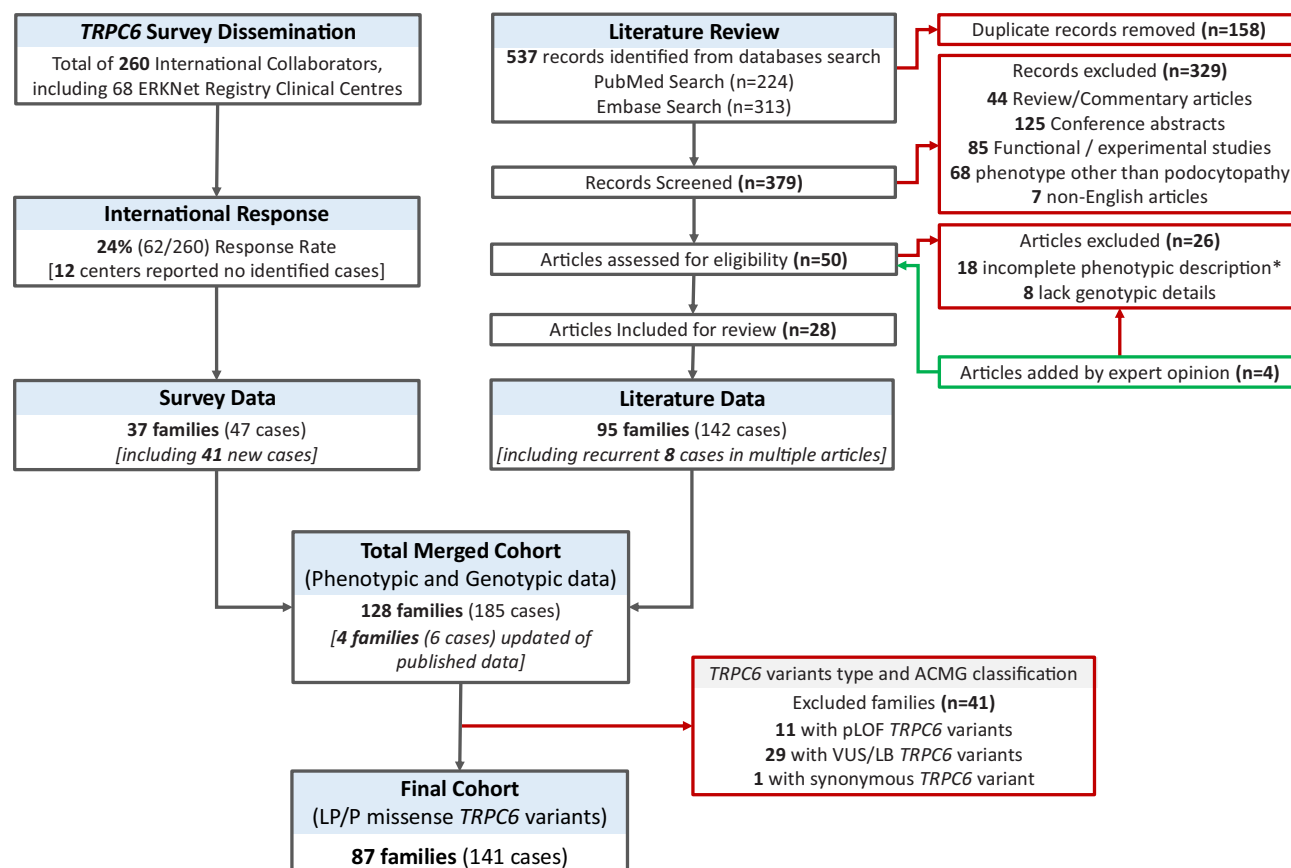


Figure 1: Flow diagram illustrating the survey distribution, literature review, exclusion criteria and final cohort of patients with LP/P non-synonymous TRPC6 variants. ERKNet, the European Rare Kidney Disease Reference Network; LB, likely benign; n, number; VUS, variants of uncertain significance; pLOF, protein loss-of-function variants.

centres participated voluntarily, following local legal and ethical guidelines.

Search strategy and selection criteria

A systematic literature search was performed in MEDLINE (via PubMed) and Embase for published cases with TRPC6-AP since it was first published, 1 June 2005, to 30 December 2024. Utilizing the following terms ‘podocytopathy’, ‘FSGS’, ‘proteinuria’, ‘nephrotic syndrome’, ‘TRPC6’ and ‘Transient Receptor Potential Canonical 6’, 537 records were obtained. A complete search query is available in [Supplementary data, Table S2](#). After excluding duplicates, non-original records (reviews, editorials and conference abstracts) and *in vitro* studies were excluded, along with articles with incomplete phenotypic or genetic details of TRPC6 variants. Twenty-eight initial articles were identified and reviewed, yielding 95 families (142 cases), including information on 4 families (6 cases) updated from survey data (Fig. 1). Cases from the same cohort reported in multiple publications were identified and included once. Details of included and excluded articles are provided in [Supplementary data, Tables S3–S5](#). The analysis yielded 128 families (185 cases) with phenotypic and genotypic data readily available for TRPC6 variant classification by pooling data from the survey and literature review.

Genetic variants assessment

Using the merged data, all TRPC6 variants, identified using either exome sequencing or targeted gene panels related to monogenic

nephropathies, were re-evaluated and classified by expert genetics researchers (E.A.E.E., F.P.V. and K.A.B.), according to the American College of Medical Genetics (ACMG) guidelines [26]. ACMG LP/P TRPC6 variants were considered as ‘disease-causing’, based on previous reports, familial segregation and *de novo* data, *in silico* algorithms, and, when available, functional studies demonstrating TRPC6 variant deleterious effects. Nucleotide positions were numbered in the coding sequence of human TRPC6 mRNA (NM_004621.6) and referenced to the GRCh38/hg38 reference assembly. The pathogenicity of missense variants was predicted using Rare Exome Variant Ensemble Learner [27], Combined Annotation Dependent Depletion (version 1.7) [28] and AlphaMissense [29]. The minor allele frequency of TRPC6 variants was retrieved from the Genome Aggregation Database (gnomAD v4.1.0, <https://gnomad.broadinstitute.org/>). Existing ClinVar database entries were checked (last accessed on 30 December 2024).

Following the report of Wooden *et al.* [21], protein LOF-TRPC6 variants were excluded ($n = 11$ families). Variants that are likely benign or of uncertain significance with no evidence towards pathogenicity were excluded ($n = 29$ families). One survey case with a synonymous TRPC6 NM_004621.6: c.75C>T (rs201051533) variant was excluded from the analysis, as the variant was classified as likely benign because it is relatively frequent and predicted to have no impact on the splice sequence using SpliceAI [30] and Pangolin [31], and classified as a likely benign/benign in the ClinVar database. [Supplementary data, Table S6](#) provides all excluded TRPC6 variants ($n = 37$) and [Supplementary data, Table S7](#) summarizes clinical details of patients with TRPC6 missense

variants of uncertain significance. Eighty-seven families (141 cases) with LP/P TRPC6-AP comprised the final merged cohort.

Data extraction, definitions, and disease endpoints

At initial presentation, age at disease onset, pattern of clinical manifestation, family history of SRNS/proteinuria and/or kidney disease, estimated glomerular filtration rate (eGFR) and proteinuria quantification were collected. Childhood-onset TRPC6-AP was defined by disease manifestation at age <18 years. Individuals with *de novo* TRPC6 variants following parental genetic testing, or unaffected parents/relatives after a clinical evaluation, or no family history of kidney disease were considered sporadic TRPC6-AP, while familial TRPC6-AP were those with one or more clinically and/or genetically affected relatives. The follow-up duration is from the initial clinical presentation to the last follow-up.

Nephrotic syndrome was defined by urine protein:creatinine ratio (uPCR) >300 mg/mmol or equivalent, serum albumin <30 g/L and oedema, or as reported by the primary physician. Proteinuric chronic kidney disease was defined with a uPCR >15 mg/mmol $\pm$ eGFR <60 mL/min. Creatinine-based eGFR measurements were calculated utilizing the Chronic Kidney Disease Epidemiology Collaboration or modified Schwartz formula [32, 33], as applicable. If a native kidney biopsy was performed, histology was obtained, and the most prominent histopathological glomerular lesion was reported. Response to immunosuppressive treatment using corticosteroids and/or calcineurin inhibitors was evaluated against guidelines [34]. Post-transplant disease recurrence was defined as recurrence of nephrotic-range proteinuria (uPCR >300 mg/mmol) in patients who had undergone transplantation without an apparent alternative cause [35].

The primary outcome was the risk of kidney disease progression to kidney failure (KF) at last follow-up, defined by the need for kidney replacement therapy (chronic dialysis for ≥ 3 months or pre-emptive kidney transplantation). Also, we examined clinical and genetic determinants associated with other outcomes: initial presentation with SRNS/NS, sporadic TRPC6-AP and disease onset in childhood.

Statistical analysis

All statistical analyses were performed using STATA SE software version 18.0 (Stata Corp., College Station, TX, USA). Descriptive data was employed with median and interquartile range (IQR) used for continuous variables, while count and frequency were used for the categorical variables. The Wilcoxon signed rank and Fisher exact tests were employed to compare the differences between groups in continuous and categorical variables, respectively. As applicable, logistic regression analyses calculated odds ratios (ORs) and confidence intervals (CIs) to assess the associations between variables. Kaplan–Meier methods and Cox regression analyses of time-dependent covariates were performed to predict progression to KF. Log-rank testing and hazard ratios (HRs) were employed to analyse significant differences and were accompanied by 95% CI. The incidence rate of KF and person-time was determined by dividing the number of failures by the person-time. A P-value of <.05 was considered significant.

RESULTS

Cohort characteristics

The analysed cohort comprised 87 families, including 45 sporadic cases and 96 affected individuals from 42 families. Twenty-seven

cases were acquired from the online questionnaire and 114 from the literature review (Fig. 1). The median (IQR) age at presentation was 20.5 (8.4–31.5) years, with male and female patients almost equally represented (50.4% vs 48.2%). The majority of patients were Caucasian (58.9%), 19.1% were Asian and 2.1% were African. Clinical presentation varied across patients, predominantly presenting with proteinuric kidney disease (46.8%) and SRNS/NS (36.9%). Kidney biopsy was performed in 94/141 (66.7%), in which FSGS was the dominant histopathological diagnosis (89.4%). Over a median follow-up of 5 (IQR 1–11) years, 48.9% (69/141) progressed to KF at a median age of 33 (IQR 17–40) years. The median time from initial clinical presentation to KF was 4 (IQR 0.5–10) years, corresponding to an incidence rate of 16.8 (95% CI 13.2–21.3) per 1000 person-years (Supplementary data, Fig. S1). A summary of the phenotype characteristics and clinical outcomes per familial status is given in Table 1 and Supplementary data, Table S8 for full details.

Clinical presentation and disease progression at last follow-up according to familial status

Next, we compared clinical findings between the familial and sporadic cases. Compared with familial cases, sporadic cases were younger at disease-onset and more likely to present with SRNS/NS (Table 1). Immunosuppression use was more common among sporadic TRPC6-AP at 60% (12/20) compared with familial cases at 25% (10/40) ($P = .011$), with no significant difference in treatment response. Additionally, sporadic cases exhibited earlier progression to KF than familial cases (Table 1). None of the 42 individuals with KF and known kidney transplant status experienced recurrence post-transplant, regardless of their family status. Significant differences were not observed in terms of gender, ethnicity and histological findings based on familial status.

TRPC6 variants distribution and functional characteristics

The distribution of LP/P missense variants in the coding sequence of TRPC6 and their functional impact on calcium channel function is illustrated in Fig. 2. A total of 31 unique substitutions in TRPC6, including 2 previously unreported novel variants, were described (Table 2 and Supplementary data, Fig. S2). The two most prevalent TRPC6 variants were c.2683C>T p.(Arg895Cys) (R895C-TRPC6) and c.523C>T p.(Arg175Trp) (R175W-TRPC6), identified in 18 and 13 families, respectively. Most variants were clustered in exon 2 (23 variants across 51 families) and exon 13 (5 variants across 29 families). TRPC6 variants predominantly clustered in neighbouring regions that form an intracellular interface serving as an inhibitory domain of the TRPC6 channel, particularly the N-terminal ANK domain (17 variants in 41 families) and the C-terminal region (8 variants in 36 families), with none located within the transmembrane domain of TRPC6, as shown in Fig. 2. Sixteen variants were identified as exhibiting GOF-TRPC6 effects, 4 exhibited LOF-TRPC6, while the other 11 TRPC6 variants had not been functionally assessed previously (Supplementary data, Table S9). Except for the initial description of a large kindred by Winn et al. [36], no founder effect was identified. Geographical distribution of variants causing TRPC6-AP in this cohort is outlined in Supplementary data, Fig. S3.

There were significantly higher GOF-TRPC6 variants in familial cases than in sporadic cases (70.8% vs 44.4%; $P = .004$) (Table 3). No statistically significant differences were noted regarding the exonic distribution of TRPC6 variants, the type of protein domains or the types of prevalent variants (all $P \geq .05$).

Table 1: Clinical characteristics of patients with TRPC6-AP.

Variables	Families/cases			P-value ⁱ
	Total (87/141)	Familial cases (42/96)	Sporadic cases (45 cases) ^h	
Gender, n (%)				
Male	71 (50.4)	50 (52.1)	21 (46.7)	.575
Female	68 (48.2)	44 (45.8)	24 (53.3)	
Unknown	2 (1.4)	2 (2.1)	0 (0)	
Ethnicity, n (%)				
Caucasian (White)	83 (58.9)	57 (59.4)	26 (57.8)	.151
East Asian (Asian)	27 (19.1)	20 (20.8)	7 (15.6)	
Hispanic	6 (4.3)	6 (6.2)	0 (0)	
African American (Black)	3 (2.1)	2 (2.1)	1 (2.2)	
Others ^a	22 (15.6)	11 (11.5)	11 (24.4)	
Age at initial presentation, years ^b	20.5 (8.4–31.5)	25 (15–35)	8.8 (5–25)	<.001
Age at last follow-up, years ^b	30 (17–42)	35 (21.5–46)	15 (8–34)	<.001
Duration of follow-up, years ^b	5 (1–11)	6 (1–13)	3 (0.4–9)	.053
Clinical presentation, n (%)				
Proteinuric CKD	66 (46.8)	52 (54.2)	14 (31.1)	<.001
SRNS/NS	52 (36.9)	24 (25)	28 (62.2)	
Pregnancy-related presentation	12 (8.5)	9 (9.4)	3 (6.7)	
Asymptomatic at time of genetic testing	6 (4.3)	6 (6.2)	0 (0)	
Others	5 (3.5)	5 (5.2)	0 (0)	
Proteinuria at initial presentation, mmol/mg (n = 33)	300 (150–770)	300 (145–770)	459 (190–795)	.783
eGFR at initial presentation, mL/min (n = 37)	86.7 (33–99)	91 (20.4–104)	69 (60–91)	.771
Disease onset in childhood, n (%) ^c	58 (41.1)	28 (29.2)	30 (66.7)	<.001
Initial manifestation with KF, n (%)	21 (14.9)	15 (15.6)	6 (13.3)	1
Initial manifestation with SRNS/NS, n (%)	62 (44)	29 (30.2)	33 (73.3)	<.001
Kidney biopsy, n (%) ^d	94 (66.7%)	54 (57.9)	40 (90.9)	<.001
Histopathological findings, n (%)				
FSGS	84 (89.4)	50 (92.6)	34 (85)	.163
MCD	1 (1.1)	1 (1.8)	0 (0)	
Other histological patterns ^e	9 (9.5)	3 (5.6)	6 (15)	
Immunosuppression treatment, n/N (%)	22/60 (37.3)	10/40 (25)	12/20 (60)	.011
Response to immunosuppression treatment, n (%) ^f				
Complete or partial response	7 (30.4)	2 (20)	5 (38.5)	.405
Resistant to immunosuppression	16 (69.6)	8 (80)	8 (61.5)	
Progression of kidney disease, n (%)				
CKD	72 (51.1)	48 (50)	24 (53.3)	.722
KF	69 (48.9)	48 (50)	21 (46.7)	
Age at KF, years	33 (17–40)	35 (22.9–43.5)	11 (7–36)	.001
Time from presentation to KF, years	4 (0.5–10)	5 (1–11)	2 (0.4–9)	.103
Kidney transplantation, n/N (%) ^g	42/50 (84)	30/36 (83.3)	12/14 (92.3)	1
Post-kidney Tx recurrence, n (%)	0 (0)	0 (0)	0 (0)	1

^aOthers comprise cases in which the self-reported ancestry is identified as Middle Eastern, Turkish, Iranian, Indian or mixed non-Caucasian.

^bMissing data in five familial cases.

^cDefined as disease-onset at age <18 years.

^dMissing data in six cases out of a total of 132 kidney biopsies.

^eOther histopathological patterns include C1q nephropathy (n = 1), immunoglobulin A (IgA) nephropathy and membranoproliferative glomerulonephritis (n = 1), IgA nephropathy and minor glomerular abnormality (n = 2), and diffuse mesangial sclerosis (n = 1).

^fResponse to immunosuppression was defined as per clinical published cases or the criteria applied from the online survey (see definitions in Materials and methods).

^gMissing data in 19 cases. n indicates the number of cases who underwent kidney transplants, while N indicates the total number of cases with KF and available data on kidney transplant status. Seventeen cases were excluded as transplant status was not reported.

^hSporadic cases were defined as individuals who did not report any family history of kidney disease, had no clinically affected relatives after a clinical evaluation, or presented with a *de novo* TRPC6 variant after parental genetic testing.

ⁱWilcoxon signed rank test was employed for continuous variables and Fisher exact test for categorical variables.

Continuous co-variants are presented as median (IQR).

CKD, chronic kidney disease; IS, immunosuppressive treatment; n, number of cases; N, total number of cases with available data; MCD, minimal change disease; Tx, transplantation.

Kidney survival and disease progression

Kidney failure-free survival estimates of several phenotypic and TRPC6 genotypic characteristics were examined (Fig. 3). Sixty-nine (48.9%) patients reached the primary kidney endpoint (KF—see definitions), with a median KF-free survival of 40 (95% CI 30–53) years for the entire cohort. Sporadic TRPC6-AP demonstrated an earlier progression to KF than familial cases (log-rank $P = .005$; Fig. 3A). Patients with disease-onset in childhood developed KF

more frequently and exhibited accelerated progression to KF than those with adulthood-onset disease (log-rank $P < .001$; Fig. 3B). Compared with patients who presented with SRNS/NS at the onset, a more prolonged median kidney survival was associated with non-SRNS/NS presenters (log-rank $P = .002$; Fig. 3C) and presentations other than SRNS/NS (log-rank $P = .005$; Fig. 3D).

In terms of the most frequent TRPC6 variants, patients with R175W-TRPC6 and R895C-TRPC6 variants progressed to KF earlier [Fig. 3F, HR 2.85 (95% CI 1.4–5.79) and HR

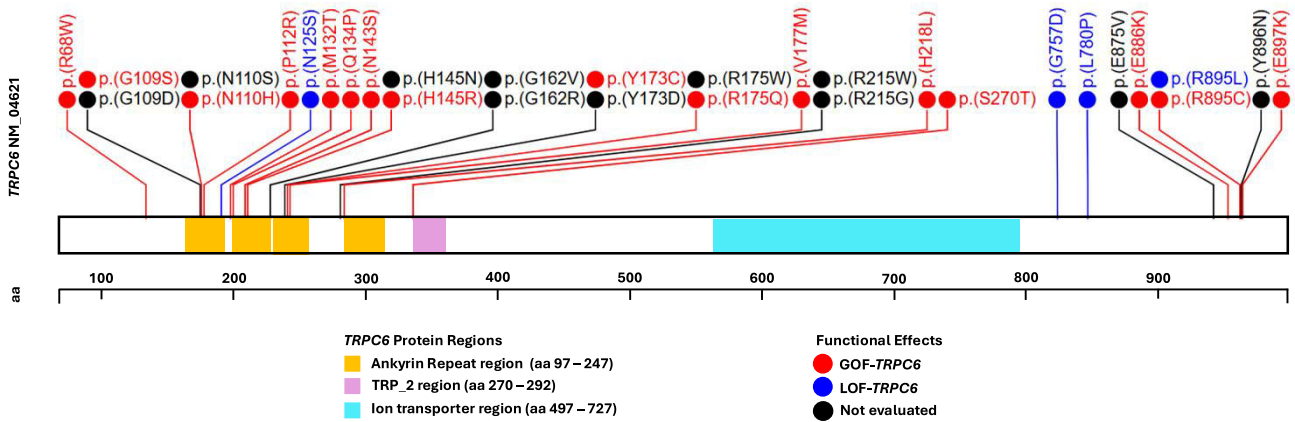


Figure 2: Distribution and functional effects of TRPC6 variants. Lollipop plot illustrating the position of non-synonymous TRPC6 variants in relation to a schematic representation of three protein regions: the ankyrin repeat region, transient receptor potential 2 region and ion transporter region (see key region below). Non-synonymous likely pathogenic/pathogenic TRPC6 variants are annotated according to their functional impact on calcium channel function and are categorised into three groups: GOF (red solid circles), LOF (blue solid circles) and variants that were not functionally evaluated (black solid circles). The lollipop was created utilizing plot template of proteinpaint.stjude.org. aa, amino acid; TRP, transient receptor potential.

1.65 (95% CI 1.01–2.81), respectively] compared with patients with non-R175W/non-R895C-TRPC6 variants (log-rank $P = .005$). In contrast, no significant kidney survival difference was identified based on exonic distribution, the type of protein domains, and the functional effects of TRPC6 variants (all $P \geq .05$) (Supplementarydata, Fig. S4).

Genomic and phenotypic correlations

Univariate and multivariable logistic regression analyses were performed to determine which clinical and genetic factors associated with TRPC6-AP were essential in the progression and severity of the disease. Regarding disease onset, in the univariate analysis, sporadic TRPC6-AP, the initial presentation with SRNS/NS and patients with LOF-TRPC6 were associated with childhood-onset TRPC6-AP (Table 4A). In multivariate analysis, the risk of childhood-onset TRPC6-AP was six times higher in R175W-TRPC6 patients compared with non-R895C/non-R175W-TRPC6 variants (Table 4A).

Regarding familial status, sporadic TRPC6-AP were more likely to present with SRNS/NS and to harbour LOF-TRPC6 variants, whereas in the multivariate analysis, none of the genetic covariates were statistically significant (Table 4B).

In the multivariate Cox hazard model, none of the genetic covariates predicted progression to KF except age at initial presentation in individuals with TRPC6-AP [HR 0.89 (0.86–0.93); $P < .001$] (Table 5).

DISCUSSION

Genotype–phenotype correlations enable the identification of critical observations regarding the pathophysiology of the affected gene and its impact on clinical outcomes. Here, we comprehensively examined the largest cohort of a rare podocytopathy caused by LP/P TRPC6 variants. We have gathered data of 141 cases (84 families) from major genomic medicine centres and reviewed published literature. We have identified several essential attributes of TRPC6-AP, such as its global distribution, the influence of various specific variants on kidney phenotype, including age of KF, and the distinction in phenotypes between sporadic and familial TRPC6-AP. We demonstrate that patients with TRPC6-AP exhibit heterogeneous clinical manifestations, typically present-

ing with nephrotic syndrome or subnephrotic-range proteinuria, indicating variation in the severity and type of symptoms despite the same underlying genetic cause. Additionally, a small percentage of familial cases displayed incomplete phenotypic penetrance (4.3%). Due to its prominent tissue expression, the TRPC6 channel is involved in numerous cellular functions, mainly enabling the cellular entrance of calcium ions, and interacts with glomerular cytoskeleton proteins [37, 38]. As a result, TRPC6 plays a role in a diverse array of genetic and non-genetic diseases [38]. In TRPC6-AP, this phenotypic variability is characteristic of different expressivity. It highlights how genetic variants can lead to a spectrum of clinical presentations, influenced by factors such as modifier genes, environmental influences or other regulatory mechanisms [38, 39].

TRPC6-AP also has an aggressive course, with nearly half of patients progressing to KF in young adulthood. Family sizes in our series were typically smaller than those in the initial description [36]. The prognosis was worse for sporadic cases, which were more likely to manifest in childhood and with nephrotic syndrome. Moreover, patients with the most prevalent variants in this cohort, R175W-TRPC6 and R895C-TRPC6, progressed to KF earlier compared with individuals with other variants. Disease did not recur post transplant, which is indicative of the healthy donor kidney's normal TRPC6 expression *in situ* and may indicate that the disease is not immunogenic, in contrast to primary FSGS [40].

A recent multicentre study has characterized the landscape of podocytopathy caused by missense TRPC6 variants [21]. Our results corroborate and expand upon their findings in multiple areas. Firstly, TRPC6-AP results in a variable disease presentation and progression to KF, regardless of sex and affected TRPC6 protein domains. All variants were predicted to be clustered across the encoded protein but not within the six transmembrane domains, with no particular variant hotspot region identified. Secondly, the two cohorts had a comparable period from clinical diagnosis to KF, 4 years versus 5 years. Thirdly, familial individuals displayed noticeable disease variability, characterized by different phenotypic expressivity, and family sizes were distinctively small. However, notably, Wooden et al. did not identify a difference in progression to KF between the two most prevalent TRPC6 variants identified in their cohort, R895C-TRPC6 and E897K-TRPC6—both have GOF-TRPC6 effects. Our larger cohort identified

Table 2: NS genetic variations in TRPC6 (NM_004621.6) reported in this study

Inheritance ^a	Variant c.change; p.(change) (exon) ^b	Global AF ^c (gnomAD)	In silico prediction tools			Effects on Ca ²⁺ channel function ^e	ACMG classification (evidence)	Families (n)
			CADD ^d	AlphaMissense	REVEL			
AD (also de novo)	c.2683C>T; p.(Arg895Cys) (Ex. 13)	0.000001859	32	0.986	0.91	GOF	Pathogenic (PS3, PM2_supp, PM5, PP3_mod)	18
AD	c.523G>T; p.(Arg175Trp) (Ex. 2)	0	32	0.826	0.55	NE	Pathogenic (PS4, PS2_mod, PM2_supp, PM5, PP1)	13
AD	c.643C>T; p.(Arg215Trp) (Ex. 2)	0.000003718	27.3	0.869	0.75	NE	Likely Pathogenic (PS4, PM2_supp, PM5, PP3)	5
AD	c.2689G>A; p.(Glu897Lys) (Ex. 13)	0	32	0.981	0.9	GOF	Pathogenic (PS4, PS3, PM2_supp, PP3_mod)	4
AD	c.2656G>A; p.(Glu886Lys) (Ex. 13)	0.000000062	31	0.989	0.85	GOF	Likely Pathogenic (PS4, PS3, PM2_supp, PP1, PP3_mod)	4
AD	c.335C>G; p.(Pro112Arg) (Ex. 2)	0	28.6	0.966	0.64	GOF	Pathogenic (PS4, PS3, PM2_supp, PM5, PS2_mod)	3
AD	c.374A>G; p.(Asn125Ser) (Ex. 2)	0.0003414	24.5	0.16	0.59	LOF	Likely Pathogenic (PS3, PP1, BS2)	3
AD	c.434A>G; p.(His145Arg) (Ex. 2)	0	23.9	0.979	0.29	GOF	Likely Pathogenic (PS4, PS3, PM2_supp, PP1)	3
AD	c.428A>G; p.(Asn143Ser) (Ex. 2)	0.00003841	24.9	0.206	0.46	GOF	Pathogenic (PS4, PS3, PM2_supp, PP1, PP3_mod)	3
AD	c.2270G>A; p.(Gly175Asp) (Ex. 9)	0.0000006196	25.3	0.949	0.85	LOF	Likely Pathogenic (PS4, PM2_supp, PP1, PP3_mod)	3
AD	c.2624A>T; p.(Glu875Val) (Ex. 12)	0	35	0.558	0.78	NE	Likely Pathogenic (PS4, PM2_supp, PM5, PP3_mod)	2
AD (also de novo)	c.326G>A; p.(Gly109Asp) (Ex. 2)	0	28.2	0.999	0.82	NE	Likely Pathogenic (PS2_mod, PM2_supp, PM5, PP1, PP3_mod)	2
AD	c.325G>A; p.(Gly109Ser) (Ex. 2)	0	28.2	0.983	0.78	GOF	Likely Pathogenic (PS3, PM2_supp, PM5, PP3)	2
AD	c.329A>G; p.(Asn110Ser) (Ex. 2)	0	25.7	0.44	0.47	NE	Likely Pathogenic (PS4, PM2_supp, PM5)	2
AD	c.524G>A; p.(Arg175Gln) (Ex. 2)	0.000004957	32	0.583	0.49	GOF	Likely Pathogenic (PS4, PS3, PM2_supp, PM5)	2
AD	c.2684G>T; p.(Arg895Leu) (Ex. 13)	0	32	0.992	0.87	LOF	Pathogenic (PS4, PS3, PM2_mod, PM2_supp, PM5, PP3_mod)	2
AD	c.202C>T; p.(Arg68Trp) (Ex. 2)	0.00002861	31	0.306	0.53	GOF	Likely Pathogenic (PS3, PM2_supp, PM5_supp, PP1)	1
AD	c.328A>C; p.(Asn110His) (Ex. 2)	0	26.5	0.728	0.51	GOF	Likely Pathogenic (PS3, PM2_supp, PM5_supp, PP1)	1
AD	c.395T>C; p.(Met132Thr) (Ex. 2)	0	25.5	0.97	0.7	GOF	Likely Pathogenic (PS3, PM2_supp, PP3)	1
AD	c.401A>C; p.(Gln134Pro) (Ex. 2)	0	27.2	0.996	0.7	GOF	Likely Pathogenic (PS3, PM2_supp, PP1, PP3)	1
AD	c.433G>A; p.(His145Asn) (Ex. 2)	0	26.8	0.912	0.37	NE	Likely Pathogenic (PM2_supp, PM5, PP1, PP3)	1
AD (also de novo)	c.484G>C; p.(Gly162Arg) (Ex. 2)	0	28.7	0.992	0.69	NE	Likely Pathogenic (PM2_supp, PM5_supp, PS2_mod, PP3_mod)	1
de Novo	c.485G>T; p.(Gly162Val) (Ex. 2)	0	28.4	0.987	0.73	NE	Likely Pathogenic (PS2_mod, PM2_supp, PP3_mod, PM5_supp)	1
de Novo	c.517T>G; p.(Tyr173Asp) (Ex. 2)	0	28.8	0.957	0.72	NE	Likely Pathogenic (PS2_mod, PM2_supp, PM5_supp, PP3_mod)	1
Singleton	c.518A>G; p.(Tyr173Cys) (Ex. 2)	0	28.2	0.699	0.76	GOF	Likely Pathogenic (PS2_mod, PM2_supp, PM5_supp, PP3_mod)	1
Singleton	c.529G>A; p.(Val177Met) (Ex. 2)	0.000001239	28.2	0.689	0.73	GOF	Likely Pathogenic (PS3, PM2_supp, PP3_mod)	1
AD	c.643C>G; p.(Arg215Gly) (Ex. 2)	0	28.2	0.958	0.71	NE	Likely Pathogenic (PS3, PM2_supp, PP3_mod)	1
AD	c.653A>T; p.(His218Leu) (Ex. 2)	0.000007436	22.6	0.204	0.57	GOF	Likely Pathogenic (PS3, PM2_supp)	1
AD	c.808T>A; p.(Ser270Thr) (Ex. 2)	0	25.8	0.933	0.9	GOF	Likely Pathogenic (PS3, PM2_supp, PP1, PP3_mod)	1
AD	c.2339T>C; p.(Leu780Pro) (Ex. 9)	0.000004957	27.4	0.567	0.68	LOF	Likely Pathogenic (PS3, PM2_supp, PP3)	1
AD	c.2686 T>A; p.(Tyr896Asn) (Ex. 13)	0	29.5	0.976	0.8	NE	Likely Pathogenic (PS4, PM2_supp, PP3_mod)	1

^aFamily history of parent-offspring inheritance is referred to as autosomal dominant 'AD'. De novo indicates that parental genetic testing has confirmed their genetically unaffected state.
^bGenomic alterations relative to Genome Reference Consortium Human Build 38.
^cThe Genome Aggregation Database version 4.1.0 accessed 30 December 2024.
^dCADD score version 1.7.
^eFunctional effects of variant effect on calcium channel function were obtained from the following references [13–24].
AD, autosomal dominant; AF, allelic frequency; Ca²⁺, calcium; CADD, Combined Annotation Dependent Depletion; Ex., exon; gnomAD, the Genome Aggregation Database; GOF, gain of function of calcium channel function with increased calcium influx; LOF, loss of function of calcium channel function with decreased calcium influx; Mod, moderate; n, number of families; NE, not evaluated; REVEL, Rare Exome Variant Ensemble Learner Score; S, strong; Supp, supportive; VUS, variant of uncertain significance.

Table 3: Genetic characteristics of patients with LP/P TRPC6 variants.

Variables	Families/cases			P-value ^b
	Total (87/141)	Familial cases (42/96)	Sporadic cases (45 cases) ^a	
Exonic distribution, n (%)				
Exon 2	83 (58.9)	56 (58.3)	27 (60)	.873
Exon 13	50 (35.5)	35 (36.5)	15 (33.3)	
Non-Exon 2/non-Exon 13	8 (5.6)	5 (5.2)	3 (6.7)	
Protein domains, n (%)				
N-terminal ANK domain region	65 (46.1)	42 (43.8)	23 (51.1)	.612
C-terminal region	58 (41.1)	40 (41.7)	18 (40)	
Non-N-terminal/non-C-terminal regions	18 (12.8)	14 (14.5)	4 (8.9)	
Functional effects of TRPC6 variants on Ca ²⁺ influx, n (%)				
GOF-TRPC6	88 (62.4)	68 (70.8)	20 (44.4)	.004
LOF-TRPC6	11 (7.8)	4 (4.2)	7 (15.6)	
not evaluated variants	42 (29.8)	24 (25)	18 (40)	
TRPC6 variants prevalence, n (%)				
R895C-TRPC6	30 (21.3)	20 (20.8)	10 (22.2)	.261
R175W-TRPC6	19 (13.5)	10 (10.4)	9 (20)	
Non-R895C/non-R175W-TRPC6	92 (65.2)	66 (68.8)	26 (57.8)	

^aSporadic cases were defined as individuals who did not report any family history of kidney disease, had no clinically affected relatives after a clinical evaluation, or presented with a de novo TRPC6 variant after parental genetic testing.

^bFisher exact test was employed for categorical variables.

Ca²⁺, calcium.

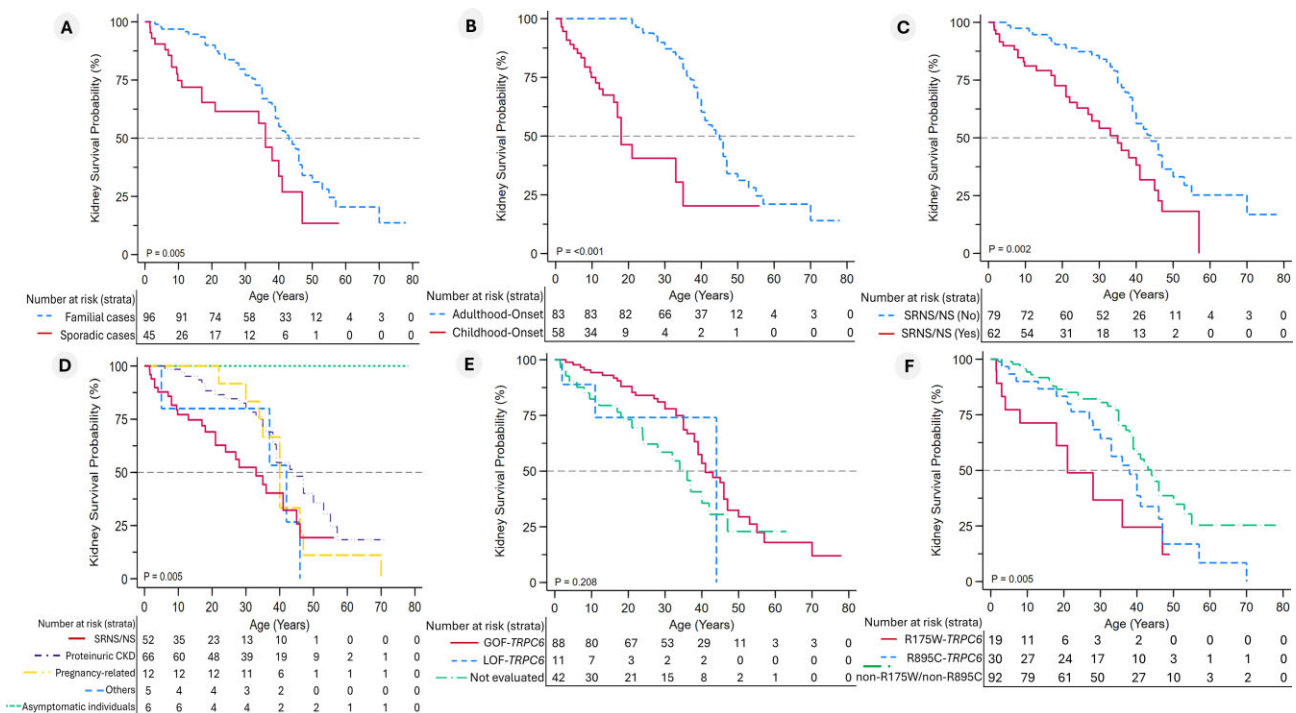


Figure 3: Kidney survival estimates of patients with LP/P non-synonymous TRPC6 variants. The Kaplan-Meier survival curves show survival probability according to: (A) family history; (B) age at disease onset (adulthood vs childhood); (C) initial presentation with SRNS/NS; (D) pattern of clinical presentations; (E) functional impact on TRPC6 variants on calcium channel function; and (F) TRPC6 prevalent variants [c.523C>T p.(Arg175Trp) (R175W-TRPC6) variant, c.2683C>T p.(Arg895Cys) (R895C-TRPC6) compared with other TRPC6 variants]. P-value from the log-rank test is given to compare the survival distributions among subgroups.

statistically significant rank-like differences in kidney survival between individuals with R175W-TRPC6 and R895C-TRPC6 compared with the other variants. The R175W-TRPC6 variant, which has not been evaluated functionally so far, has been reported in 13 families (19 cases) who, on average, reached KF at a median age of

21 years, compared with 38 and 44 years to R895C-TRPC6 and non-R175W/non-R895C-TRPC6 variants, respectively. This finding suggests that R175W-TRPC6 variant might exhibit loss of calcium ion influx into the podocyte TRPC6, leading to earlier progression to KF.

Table 4: Logistic regression models outlining genetic associations with clinical presentation in childhood and sporadic TRPC6-AP.

Variables ^a	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
(A) Childhood presentation (<18 years)				
Distribution of TRPC6 variants				
Protein domains—ANK region (vs non-ANK/non-C-terminal regions)	2.52 (0.8–7.88)	.112	0.8 (0.16–3.97)	.786
Protein domains—C-terminal region (vs non-ANK/non-C-terminal regions)	1.47 (0.46–4.71)	.512	1.44 (0.39–5.24)	.576
Exonic distribution—exon 2 (vs other exons)	1.41 (0.71–2.82)	.321	0.35 (0.03–3.63)	.381
Exonic distribution—exon 13 (vs other exons)	0.55 (0.26–1.13)	.104	0.17 (0.02–1.91)	.154
Functional (effects on Ca ²⁺ channel) status of TRPC6 variant ^b				
LOF-TRPC6 (vs GOF-TRPC6)	5.15 (1.27–20.87)	.022	1.16 (0.13–10.4)	.889
TRPC6 variant prevalence ^c				
R895C-TRPC6 (vs non-R895C/non-R175W-TRPC6)	0.98 (0.42–2.32)	.977	1.02 (0.2–5.11)	.979
R175W-TRPC6 (vs non-R895C/non-R175W-TRPC6)	3.69 (1.28–10.62)	.015	6.69 (1.05–42.59)	.044
(B) Sporadic TRPC6-associated podocytopathy				
Distribution of TRPC6 variants				
Protein domains—ANK region (vs non-ANK/non-C-terminal regions)	1.91 (0.56–6.5)	.297	1.79 (0.27–11.8)	.547
Protein domains—C-terminal region (vs non-ANK/non-C-terminal regions)	1.57 (0.45–5.45)	.474	1	1
Exonic distribution—exon 2 (vs other exons)	1.07 (0.52–2.2)	.851	1.99 (0.21–19.01)	.549
Exonic distribution—exon 13 (vs other exons)	0.87 (0.41–1.83)	.718	2.71 (0.32–22.95)	.361
Functional (effects on Ca ²⁺ channel) status of TRPC6 variant				
LOF-TRPC6 (vs GOF-TRPC6)	5.95 (1.58–22.40)	.008	3.08 (0.82–34.8)	.985
TRPC6 variant prevalence				
R895C-TRPC6 (vs non-R895C/non-R175W-TRPC6)	1.21 (0.52–3.07)	.597	3.12 (0.73–13.31)	.124
R175W-TRPC6 (vs non-R895C/non-R175W-TRPC6)	2.28 (0.83–6.26)	.108	0.80 (0.19–3.44)	.773

^aThe following clinical co-variables were adjusted to genetic factors in the multivariate model: sex, familial status (familial vs sporadic), age of initial presentation (years), initial presentation with SRNS/NS and ethnicity. To avoid the collinearity, models of childhood presentation and sporadic disease were only depended on familial status and age of initial presentation, respectively.

^bForty-five individuals with TRPC6 variants that were 'not evaluated' functionally were excluded from the univariate analysis.

^cComparing the two most prevalent TRPC6 variants—p.(Arg895Cys) (R895C-TRPC6) and p.(Arg175Trp) (R175W-TRPC6)—vs the remaining variants (non-R895C/non-R175W-TRPC6).

^dRegarding disease onset, in the univariate analysis, sporadic TRPC6-AP [OR 4.86 (2.27–10.38); $P < .001$], the initial presentation with SRNS/NS [OR 3.59 (1.77–7.25); $P < .001$] and patients with LOF-TRPC6 [OR 5.15 (1.27–20.87); $P = .022$] were associated with childhood-onset of TRPC6-AP.

^eRegarding familial status, in univariate analysis, sporadic TRPC6-AP were more likely to present with SRNS/NS [OR 6.35 (2.88–14.01); $P < .001$], and harbour LOF-TRPC6 variants [OR 5.95 (1.58–22.4); $P = .008$]. Each year of age upon presentation decreased the risk of sporadic TRPC6-AP by 7% [OR 0.93 (0.91–0.96); $P < .001$]. SRNS/NS at initial presentation was the only clinical co-variate in multivariate analysis that predicted sporadic TRPC6-AP [OR 4.34 (1.85–10.15); $P = .001$]. Ca²⁺, calcium; GOF, gain of function of calcium channel function with increased calcium influx; LOF, loss of function of calcium channel function with decreased calcium influx.

Table 5: Time-dependent Cox proportional hazards regression models of genetic determinants associated with the development of KF in LP/P TRPC6-AP.

Variables ^a	Median age of kidney survival (95% CI), years	Univariate analysis, HR (95% CI)	P-value	Multivariate analysis, HR (95% CI)	P-value
Distribution of TRPC6 variants					
TRPC6 protein domains—ANK region (vs non-ANK/non-C-terminal regions)	41 (28–50) vs 40 (35–NE)	1.82 (0.78–4.21)	.161	1.82 (0.52–6.36)	.350
TRPC6 protein domains—C-terminal region (vs non-ANK/non-C-terminal regions)	40 (27–47) vs 40 (35–NE)	1.74 (0.77–3.96)	.181	1	1
Exonic distribution—exon 2 (vs other exons)	40 (30–55) vs 40 (28–47)	0.89 (0.56–1.43)	.643	1.62 (0.26–10.04)	.602
Exonic distribution—exon 13 (vs other exons)	40 (28–47) vs 40 (28–53)	1.03 (0.64–1.67)	.875	2.29 (0.35–14.82)	.385
Functional (effects on Ca ²⁺ channel) status of TRPC6 variant					
LOF-TRPC6 (vs GOF-TRPC6)	44 (11–44) vs 41 (33–55)	1.54 (0.47–5.04)	.475	1.72 (0.31–9.53)	.530
TRPC6 variant prevalence					
R895C-TRPC6 (vs non-R895C/non-R175W-TRPC6)	38 (27–47) vs 44 (35–NE)	1.65 (1.01–2.81)	.045	0.61 (0.20–1.77)	.360
R175W-TRPC6 (vs non-R895C/non-R175W-TRPC6)	21 (8–36) vs 44 (35–NE)	2.85 (1.40–5.79)	.004	1.39 (0.41–4.67)	.590

^aThe following as co-variables were adjusted to genetic factors in the multivariate model to investigate the association between predictor variables and the kidney survival time: sex, initial presentation with SRNS/NS (yes vs no), presentation in childhood (yes vs no), ethnicity (Caucasian vs non-Caucasians), familial status (familial vs sporadic) and age of initial presentation. In univariate Cox proportional hazard model, patients with childhood-onset TRPC6-AP [HR 4.61 (2.67–7.96); $P < .001$], sporadic TRPC6-AP [HR 2.04 (1.21–3.45); $P = .007$] and presenting with SRNS/NS [HR 2.06 (1.27–3.34); $P = .003$] were more likely to progress to KF. However, in the multivariate analysis none of the genetic factors was statistically significant, the age at initial presentation was the most accurate determinant of progression to KF in individuals with TRPC6-AP [HR 0.88 (0.85–0.93); $P < .001$].

Ca²⁺, calcium; CKD, chronic kidney disease; GOF, gain of function of calcium channel function with increased calcium influx; LOF, loss of function of calcium channel function with decreased calcium influx; NA, not applicable; NE, not estimable.

The strengths of this study are several. The collaborative distribution of our survey and the incorporation of a comprehensive literature review of previously published cases improved the characterization of TRPC6-AP disease evolution. Only LP/P variants of TRPC6-AP were included, representing patients from 18 countries worldwide to ensure diversity and inclusivity of the largest population to date. To demonstrate its translational impact on patients with TRPC6-AP, we studied a series of clinical endpoints relevant to daily practice, encompassing progression to KF and phenotypic traits, which revealed novel indicators suggesting TRPC6 variant-specific characteristics on disease progression. Yet, several limitations should be noted. The retrospective design may introduce inherent biases, such as incomplete or missing data and the selection of severely impacted individuals. Detailed characteristics of meaningful phenotypic features at baseline, such as eGFR decline, proteinuria quantification and histological details, remain lacking. Additionally, the potential ramifications of specific TRPC6 variants, notably excluded some mis-sense variants of uncertain significance, are constrained by the dependence on ACMG-based classification, which may potentially over- or under-call these variants. Although we have used all available evidence to apply the selection criteria of submitted variants, the pathogenicity remains uncertain for some variants, particularly in light of the lack of functional studies or additional affected probands. This offers a promising opportunity to functionally characterize these variants and better clarify their cellular effects.

Overall, our study advances the understanding of genotype-phenotype correlations of podocytopathy caused by LP/P mis-sense TRPC6 variants. Future research endeavors are anticipated to examine potential targeted therapies for patients with this rare disease.

SUPPLEMENTARY DATA

Supplementary data are available at [Nephrology Dialysis Transplantation](#) online.

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AUTHORS' CONTRIBUTIONS

S.M.M., E.A.E.E. and P.J.C. conceptualized the study and wrote the original manuscript draft. S.M.M., E.A.E.E., S.S., F.P.V., M.C.H., J.H., M.C., P.M., S.M.T., S.J., J. Calado, S.D., E.C., S.T., T.F., A.S., J.C., J.F., C.D., R.F., A.R., A.M.P., S.D., L.L., M.F., L.F.Q., F.B., G.N., D.A., K.T., N.D., B.A.A., C.S., M.R., M.S., A.A., N.K.H., H.M., E.B., A.J.M., T.H., K.K., S.H., A.B., A.J.M., J.A.S., F.S. and P.J.C. contributed to patient data collection. E.A.E.E. analysed the data, including a literature review search and statistical analysis. E.A.E.E., F.P.V. and K.A.B. classified the genetic variants. S.H. and F.S. facilitated the dissemination of our survey via the European Rare Kidney Disease Reference Network. All authors reviewed the results and approved the final version of the manuscript. The results presented in this article have not been published previously in whole or part, except in abstract form.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding and named senior author.

CONFLICT OF INTEREST STATEMENT

S.M.M., E.A.E.E., P.J.C., S.H. and F.S. are members of the European Rare Kidney Disease Reference Network. E.A.E.E. reports funds from the Royal College of Surgeons in Ireland StAR PhD. P.J.C. is co-founder of the Irish Kidney Gene Project. S.M.M. reports funding from the Royal College of Physicians of Ireland post-CSCST-ASPIRE Fellowship programme in Transitional Nephrology. J.A.S. is on the Genes and Kidney Board for the European Renal Association and is the Academic Vice President of the UK Kidney Association. A.J.M. is supported by a Queensland Health Advancing Clinical Research Fellowship and is on board of ANZSN. A.J.M. has reported association with research bodies NHMRC, MRFF and Aus Gov, QLD Gov. F.B. reports association with Alnylam and Chiesi. S.J. reports association with Alnylam for lumasiran use and from Dioscope for an educational course on nephrogenetics. J.C. is a coordinator of the nephrogenetics working group, Portuguese Society of Nephrology. The remaining authors have no conflicts of interest to declare.

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