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Research Paper

A non-coding signature in *SHROOM3* is associated with kidney disease progression in Fabry disease

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ABSTRACT

Fabry disease is a rare, X-linked lysosomal storage disorder that often leads to progressive kidney dysfunction. Despite carrying the same pathogenic *GLA* variant, patients exhibit considerable variability in the onset and progression of Fabry nephropathy, suggesting the involvement of additional genetic modifiers.

This study aimed to investigate the possible role of genetic polymorphisms in non-coding regions. A total of 284 patients with Fabry disease were included in the study and divided into two groups based on the progression of the kidney disease. Ten selected single nucleotide polymorphisms located in non-coding regions of podocyte-related genes were analyzed using quantitative PCR with TaqMan probes.

The analysis revealed significant associations between specific genotypes and an increased risk of rapid progression of Fabry nephropathy. In particular, the rs9992101 and rs17319721 polymorphisms in the *SHROOM3* gene were significantly associated with higher odds of accelerated kidney function decline. However, neither of these polymorphisms nor the polygenic risk scores were associated with conventional biomarkers of kidney disease.

Our results suggest that non-coding genetic variants in podocyte-related genes may contribute to the phenotypic variability observed in Fabry nephropathy. The integration of such genetic biomarkers into clinical practice could improve early risk stratification, support more individualized patient monitoring, and facilitate therapeutic decision-making.

1. Introduction

Fabry disease (FD, OMIM#301500) is a rare, X-linked lysosomal storage disorder caused by pathogenic variants in the *GLA* gene, leading to deficient activity of the enzyme α-galactosidase A [1,2]. The accumulation of globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) [2,3] in different cell types leads to progressive multiorgan involvement, with nephropathy being one of the most severe complications [4]. Fabry nephropathy is characterized by proteinuria and a decreased glomerular filtration rate (GFR), which could progress to end-stage kidney disease [5,6]. However, the clinical course and rate of kidney function decline vary considerably between patients, even within

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the same family [7], suggesting that additional genetic or environmental factors may contribute to disease progression.

In recent years, large-scale genome-wide association studies (GWAS) have led to significant advances in our understanding of the genetic basis of kidney diseases. These studies have identified numerous genetic loci associated with estimated GFR (eGFR) and other biomarkers of kidney function on a genome-wide scale [8,9]. The most comprehensive GWAS to date identified 264 loci associated with eGFR, 166 of which had not been previously reported. Notably, the majority of these loci are located in non-coding regions, suggesting a regulatory role of non-coding variants in modulating the expression of genes critical for podocyte function and other kidney cell types [10].

Considering that genetic polymorphisms have a significant impact on the course of other chronic kidney diseases, it is plausible that they could play a similar role in FD. However, there are few studies investigating such associations in the context of Fabry nephropathy, which represents a critical gap in our understanding of the pathogenesis and progression of the disease. With this in mind, whole-exome sequencing (WES) was performed in a recent multicenter study of 300 patients with FD. Bioinformatic analysis of a panel of 190 genes related to podocyte function and structure identified the *FAT1* variant rs749735949, which is associated with increased odds of rapid nephropathy progression [11].

The present study aimed to investigate the association of single nucleotide polymorphisms (SNPs) in non-coding regions of podocyte-related genes with the progression of nephropathy in patients with FD. By identifying genetic biomarkers associated with a higher risk of accelerated kidney function decline, we aimed to enhance the understanding of the mechanisms underlying phenotypic variability and explore the potential for early risk stratification and personalized management in Fabry nephropathy.

2. Materials and methods

2.1. Study participants

A total of 284 patients with FD were recruited from five specialized Fabry centers. The following institutions participated in the study: the Fabry Center in Slovenj Gradec, Slovenia ($n = 33$); Charles University in Prague, Czech Republic ($n = 125$); University of Zurich, Switzerland ($n = 68$); University of Porto, Portugal ($n = 47$); and Shaare Zedek Medical Center in Jerusalem, Israel ($n = 11$). The samples analyzed in this study were previously collected as part of an earlier investigation [11]. Participants had to fulfill the following inclusion criteria: they were at least 18 years old, had a confirmed diagnosis of FD, and had attended at least three follow-up visits in the previous five years. Exclusion criteria included uncontrolled hypertension, the presence of additional glomerular disease, and any physical or mental condition that could interfere with study conduct.

The patients with FD were divided into two cohorts. The first included patients with stable kidney function, characterized by a decrease in eGFR of less than 3 mL/min/1.73 m^2/year. The second cohort included patients with progressive nephropathy, characterized by a decrease in eGFR of more than 3 mL/min/1.73 m^2/year [12]. The eGFR was calculated using the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [13].

2.2. Genomic DNA extraction

Genomic DNA was extracted from EDTA-preserved blood samples using standard DNA isolation protocols specific to each institution. DNA quality and concentration were determined spectrophotometrically using NanoDrop One (Thermo Fisher Scientific, Waltham, MA, USA).

2.3. Selection of single nucleotide polymorphisms

The selection of polymorphisms analyzed in this study was based on previous research investigating the effects of genetic variations on kidney function. The present study focused on SNPs located in non-coding regions. Particular attention was paid to genetic variants that may contribute to the pathophysiology of Fabry nephropathy, especially those associated with podocyte damage. A total of ten SNPs were selected for analysis and were assessed in the DNA samples of all participants. Table 1 provides detailed information on the selected SNPs.

2.4. TaqMan genotyping

SNP genotyping was performed using Predesigned TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, MA, USA) in combination with the TaqMan® Universal PCR Master Mix (Applied Biosystems, Waltham, MA, USA), following the manufacturer's protocol. Reactions were performed in a 386-well format on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). Each reaction contained genomic DNA, assay-specific primers and probes, and master mix reagents. Allele discrimination was conducted by measuring endpoint fluorescence, and genotype calls were automatically assigned using QuantStudio™ Real-Time PCR Software v1.3 (Applied Biosystems, Waltham, MA, USA).

To assess the reliability of the genotyping results, 10% of the samples were randomly selected and re-analyzed by the same sequencing method. Repeated genotyping resulted in 100% concordance, confirming the reproducibility and accuracy of the method.

2.5. Statistical analysis

Statistical analyses were performed with RStudio version 4.3.0 (R Core Team, Vienna, Austria). Descriptive statistics were used to summarize the characteristics of the study population. The Shapiro–Wilk test was applied to assess the normality of continuous variables. Data are presented as medians with interquartile ranges (25th–75th percentiles) for continuous variables and as frequencies (percentages) for categorical variables. Group comparisons for continuous variables were carried out using the Mann–Whitney *U* test. Deviations from Hardy-Weinberg equilibrium (HWE) for all SNPs were assessed using the chi-square test. Univariate and multivariate logistic regression analyses were performed to assess associations with binary outcomes. Analyses of associations between genotypes and continuous variables were performed using the non-parametric Kruskal–Wallis test. Both additive and dominant genetic models were used. Linkage disequilibrium (LD) between SNP pairs was assessed using the genetics package in R. Additionally, a quadrant analysis was performed to examine the relationship among the urinary protein-to-creatinine ratio (UPCR) and the urinary albumin-to-creatinine ratio (UACR), and eGFR for the two *SHROOM3* genotypes. The thresholds used were 50 g/mol for UPCR, 30 g/mol for UACR, and 60 mL/min/1.73 m^2 for eGFR [14,15]. Associations between genotype distribution and quadrant categories were tested using Fisher's exact test. Finally, an unweighted polygenic risk score (PRS) was calculated by summing the number of risk alleles across the analyzed SNPs, considering the alternative allele as the risk allele. The PRS distribution was

Table 1
Selected single nucleotide polymorphisms.

Gene	rs number	Alele	Identification number
SHROOM3	rs9992101	G>A	C_29716172_10
SHROOM3	rs17319721	G>A	C_32612310_10
MYH9	rs3752462	T>C	C__2473811_1_
DACH1	rs626277	A>C	C__3146571_10
DAB2	rs11959928	T>A	C__3019605_10
CD2AP	rs9369717	T>G	C__2949640_10
PDSS2	rs2500574	C>T	C_11632891_10
SPATA5L1	rs2467853	T>G	C_15796031_10
MANBA	rs228611	G>A	C__804211_10
TGFB1	rs1800469	A>G	C__8708473_10

compared with the expected theoretical distribution using a Poisson–binomial model, and its association with clinical parameters was assessed using Spearman's correlation and visualized with linear regression analyses. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 284 patients with FD were included, of whom 203 (71.5%) had stable kidney function (146 women and 61 men) and 81 (28.5%) had progressive nephropathy (36 women and 45 men). A significant difference in laboratory biomarkers was found between patients with stable kidney function (FD-SKF) and those with progressive nephropathy (FD-PN). While the median age was comparable between groups, the FD-PN group exhibited significantly reduced kidney function, with significantly lower median eGFR values and a steeper decline in the eGFR slope. Increased urinary UPCRs and UACRs in the FD-PN group indicated the presence of proteinuria (Table 2).

A total of 180 patients (64%) were receiving disease-specific therapy, 100 patients (35%) were not receiving any treatment, and treatment information was unavailable for four patients (1%).

3.2. Genotype distribution patterns across selected single nucleotide polymorphisms

As shown in Table 3, the SNPs *SHROOM3* rs9992101, *SHROOM3* rs17319721, *DACH1* rs626277, *DAB2* rs11959928, *PDSS2* rs2500574, and *SPATA5L1* rs2467853 exhibited a common genotype distribution pattern: heterozygotes were the most frequent, followed by reference (wild-type) homozygotes, and lastly alternative allele homozygotes. For *CD2AP* rs9369717, reference allele homozygotes were the most common genotype, consistent with the relatively low minor allele frequency (MAF), followed by alternative allele heterozygotes and homozygotes. In the case of *MANBA* rs228611, heterozygotes were also the most common genotype; the frequency of both homozygous genotypes was relatively similar, consistent with the nearly 50% frequency of the alternative allele at the population level. For *MYH9* rs3752462 and *TGFB1* rs1800469, heterozygotes were again the most common, followed by homozygotes for the alternative allele and then homozygotes for the reference allele. This genotype distribution is consistent with data from the gnomAD database (v4.1.0), which also reports a higher frequency of the alternative allele in the European (non-Finnish) population.

3.3. Association between single nucleotide polymorphism genotypes and progressive nephropathy

Logistic regression analyses were performed for each polymorphism to evaluate the association between specific genotypes and the progression of nephropathy. For each SNP, the homozygous reference genotype served as the reference for calculating the odds of nephropathy progression for the heterozygous and homozygous alternative genotypes. The results are shown in Table 4.

In the case of the *SHROOM3* rs9992101 polymorphism, individuals homozygous for the alternative A allele were significantly more likely to develop progressive nephropathy compared with individuals with the reference genotype GG [odds ratio (OR) = 2.40; 95% confidence interval

Table 2
Patient characteristics.

Parameter	FD-SKF (n = 203)	FD-PN (n = 81)	p-value
Age (years)	47.9 (39.5–62.0)	51.2 (40.8–61.3)	0.410
eGFR (mL/min/1.73 m^2)	98.0 (87.3–111.8)	43.0 (19.0–78.0)	< **0.001**
eGFR slope (mL/min/1.73 m^2/year)	−0.82 (−1.50–-0.09)	−4.86 (−6.25–-3.86)	< **0.001**
UPCR (g/mol)	18.9 (10.0–25.3)	51.9 (14.8–100.3)	< **0.001**
UACR (g/mol)	2.2 (0.9–6.3)	10.0 (2.9–63.0)	< **0.001**

FD-SKF, Fabry patients with stable kidney function; FD-PN, Fabry patients with progressive nephropathy; eGFR, estimated glomerular filtration rate; UPCR, urinary protein-to-creatinine ratio; UACR, urinary albumin-to-creatinine ratio.

Table 3
Genotype distributions in study participants and allele frequencies of selected SNPs.

SNP	Genotype	FD-SKF n (%)	FD-PN n (%)	p-value (HWE)	MAF	MAF (gnomAD)
SHROOM3 rs9992101 (G>A)	GG	79 (38.9)	23 (28.4)	0.733	0.410	0.404
	AG	94 (46.3)	37 (45.7)			
	AA	30 (14.8)	21 (25.9)			
SHROOM3 rs17319721 (G>A)	GG	72 (35.5)	21 (25.9)	0.788	0.437	0.430
	GA	97 (47.8)	37 (45.7)			
	AA	34 (16.7)	23 (28.4)			
MYH9 rs3752462 (T>C)	CC	80 (39.4)	40 (49.4)	0.711	0.342	0.322
	CT	103 (50.7)	31 (38.3)			
	TT	20 (9.9)	10 (12.3)			
DACH1 rs626277 (A>C)	AA	78 (38.4)	32 (39.5)	0.856	0.371	0.398
	AC	95 (46.8)	42 (51.8)			
	CC	30 (14.8)	7 (8.7)			
DAB2 rs11959928 (T>A)	TT	64 (31.5)	27 (33.3)	0.829	0.442	0.438
	TA	97 (47.8)	38 (46.9)			
	AA	42 (20.7)	16 (19.8)			
CD2AP rs9369717 (T>G)	TT	106 (52.2)	48 (59.3)	0.881	0.268	0.266
	GT	79 (38.9)	29 (35.8)			
	TT	18 (8.9)	4 (4.9)			
PDSS2 rs2500574 (C>T)	CC	25 (12.3)	15 (18.5)	0.686	0.359	0.366
	CT	85 (44.9)	39 (48.2)			
	TT	93 (45.8)	27 (33.3)			
SPATA5L1 rs2467853 (T>G)	TT	76 (37.5)	27 (33.3)	0.836	0.405	0.377
	GT	91 (44.8)	41 (50.6)			
	GG	36 (17.7)	13 (16.1)			
MANBA rs228611 (G>A)	AA	58 (28.6)	24 (29.6)	0.257	0.486	0.474
	AG	89 (43.8)	39 (48.2)			
	GG	56 (27.6)	18 (22.2)			
TGFB1 rs1800469 (A>G)	GG	86 (42.6)	38 (46.9)	0.557	0.326	0.313
	AG	98 (48.5)	35 (43.2)			
	AA	18 (8.9)	8 (9.9)			

Table 4
Univariable and age-adjusted logistic regression analysis of the association between SNP genotypes and progressive nephropathy.

		Univariable analysis		Age-adjusted analysis	
SNP	Genotype	OR (95% CI)	*p*-value	OR_{adj} (95% CI_{adj})	p_{adj}-value
SHROOM3	GG	Reference		Reference	
rs9992101	AG	1.35 (0.75–2.49)	0.325	1.52 (0.82–2.89)	0.188
	AA	2.40 (1.16–4.99)	**0.018**	2.72 (1.30–5.77)	**0.008**
	AG + AA	1.61 (0.93–2.85)	0.097	1.83 (1.03–3.34)	**0.044**
SHROOM3	GG	Reference		Reference	
rs17319721	AG	1.31 (0.71–2.45)	0.393	1.53 (0.80–2.96)	0.199
	AA	2.32 (1.13–4.79)	**0.021**	2.69 (1.29–5.71)	**0.009**
	AG + AA	1.57 (0.89–2.83)	0.123	1.84 (1.02–3.44)	**0.047**
MYH9	TT	Reference		Reference	
rs3752462	CT	0.60 (0.26–1.46)	0.247	0.64 (0.27–1.61)	0.320
	CC	1.00 (0.44–2.41)	> 0.999	1.04 (0.44–2.61)	0.929
	CT + CC	0.78 (0.35–1.80)	0.538	0.81 (0.36–1.96)	0.622
DACH1	AA	Reference		Reference	
rs626277	AC	1.08 (0.62–1.87)	0.789	1.03 (0.59–1.81)	0.912
	CC	0.57 (0.21–1.37)	0.229	0.57 (0.21–1.38)	0.232
	AC + CC	0.96 (0.57–1.63)	0.866	0.92 (0.54–1.58)	0.763
DAB2	TT	Reference		Reference	
rs11959928	AT	0.93 (0.52–1.67)	0.804	0.94 (0.52–1.73)	0.851
	AA	0.90 (0.43–1.86)	0.784	0.96 (0.45–1.99)	0.907
	AT + AA	0.92 (0.53–1.61)	0.768	0.95 (0.54–1.68)	0.851
CD2AP	TT	Reference		Reference	
rs9369717	GT	0.81 (0.47–1.39)	0.451	0.84 (0.48–1.45)	0.528
	GG	0.49 (0.14–1.40)	0.219	0.38 (0.09–1.19)	0.134
	GT + GG	0.75 (0.44–1.26)	0.283	0.75 (0.44–1.27)	0.286
PDSS2	CC	Reference		Reference	
rs2500574	CT	0.76 (0.37–1.63)	0.480	0.80 (0.37–1.77)	0.580
	TT	0.48 (0.22–1.06)	0.065	0.52 (0.24–1.16)	0.105
	CT + TT	0.62 (0.31–1.27)	0.178	0.65 (0.32–1.38)	0.248
SPATA5L1	TT	Reference		Reference	
rs2467853	GT	1.27 (0.72–2.27)	0.417	1.24 (0.69–2.24)	0.478
	GG	1.02 (0.46–2.17)	0.967	1.07 (0.48–2.30)	0.867
	GT + GG	1.20 (0.70–2.09)	0.516	1.19 (0.69–2.08)	0.539
MANBA	GG	Reference		Reference	
rs228611	AG	1.36 (0.72–2.66)	0.351	1.33 (0.69–2.64)	0.400
	AA	1.29 (0.63–2.65)	0.487	1.32 (0.64–2.78)	0.452
	AG + AA	1.33 (0.74–2.50)	0.353	1.33 (0.72–2.54)	0.372
TGFB1	AA	Reference		Reference	
rs1800469	AG	0.89 (0.34–2.51)	0.817	0.81 (0.31–2.30)	0.676
	GG	0.97 (0.37–2.74)	0.952	0.94 (0.36–2.65)	0.898
	AG + GG	0.93 (0.38–2.52)	0.879	0.87 (0.35–2.37)	0.775

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

(CI): 1.16–4.99; $p = 0.018$]. This suggests that individuals carrying the AA genotype have more than twice the risk of accelerated kidney disease progression. The heterozygous AG genotype alone was not significantly associated with disease progression, nor was the combined group of A allele carriers (AG + AA).

Similarly, for *SHROOM3* rs17319721, significantly increased odds of progressive nephropathy were observed in individuals homozygous for the alternative A allele (OR = 2.32; 95% CI: 1.13–4.79; $p = 0.021$). Taken together, these results highlight a potentially important role of *SHROOM3* in disease progression, as two independent polymorphisms within this gene were significantly associated with progressive nephropathy.

LD between *SHROOM3* rs9992101 and rs17319721 was assessed to determine if they are co-inherited. The analysis revealed a very high LD ($D' = 0.96$), indicating almost complete linkage with minimal recombination between the SNPs in a cohort of 284 individuals. In addition, the correlation coefficient ($r^2 = 0.91$) indicates a strong correlation between the genotypes. This association was statistically significant ($p < 0.001$), suggesting that these loci are closely linked and their alleles are co-inherited more frequently than expected by chance.

No statistically significant correlations were found for the other polymorphisms. The ORs for these variants were close to 1, and all corresponding *p*-values were above 0.05, indicating no differences in nephropathy progression risk.

To better assess the independent effects of the genetic variants, we also performed multivariate logistic regression with age as an additional covariate. Since age is a known risk factor for worsening kidney function due to the physiological decline in GFR with increasing age, this covariate was included to determine whether the observed genetic associations were independent of this confounding factor.

The age-adjusted analysis confirmed the initial findings for both *SHROOM3* polymorphisms, with the AA genotype remaining significantly associated with a more than 2.5-fold increased odds of progressive nephropathy compared with GG homozygotes, regardless of age. In addition, the combined genotypes AG + AA also reached statistical significance in the age-adjusted model for the SNPs rs9992101 and rs17319721, with 1.8-fold increased odds. No significant associations were found for other SNPs after adjusting for age.

3.4. Association of SHROOM3 genotypes and kidney function biomarkers

Next, we investigated the potential association between *SHROOM3* genotypes and three conventional laboratory biomarkers of kidney function: UPCR, UACR, and eGFR, as measured at the final follow-up when DNA samples were also acquired. However, no significant differences were found between the *SHROOM3* genotypes and these biomarkers (Fig. 1).

Additionally, we performed a quadrant analysis of kidney function biomarkers based on *SHROOM3* genotypes and found no statistically significant differences among the groups (Fig. 2).

Finally, we calculated an unweighted PRS by summing the number of alternative (risk) alleles across the analyzed SNPs. The PRS distribution was consistent with the expected Poisson–binomial distribution, and no significant associations with kidney function—UACR and UPCR—were observed (Fig. 3).

4. Discussion

The role of genetic modifiers in the pathophysiology of FD is still poorly understood. Nevertheless, previous studies have demonstrated associations between specific genetic variants and cerebrovascular complications [16,17], nephropathy progression [11], overall FD phenotype [18], and disease progression [19]. Further research in this area is therefore essential as it may contribute to a more detailed understanding of the variability and progression of the disease in individual patients.

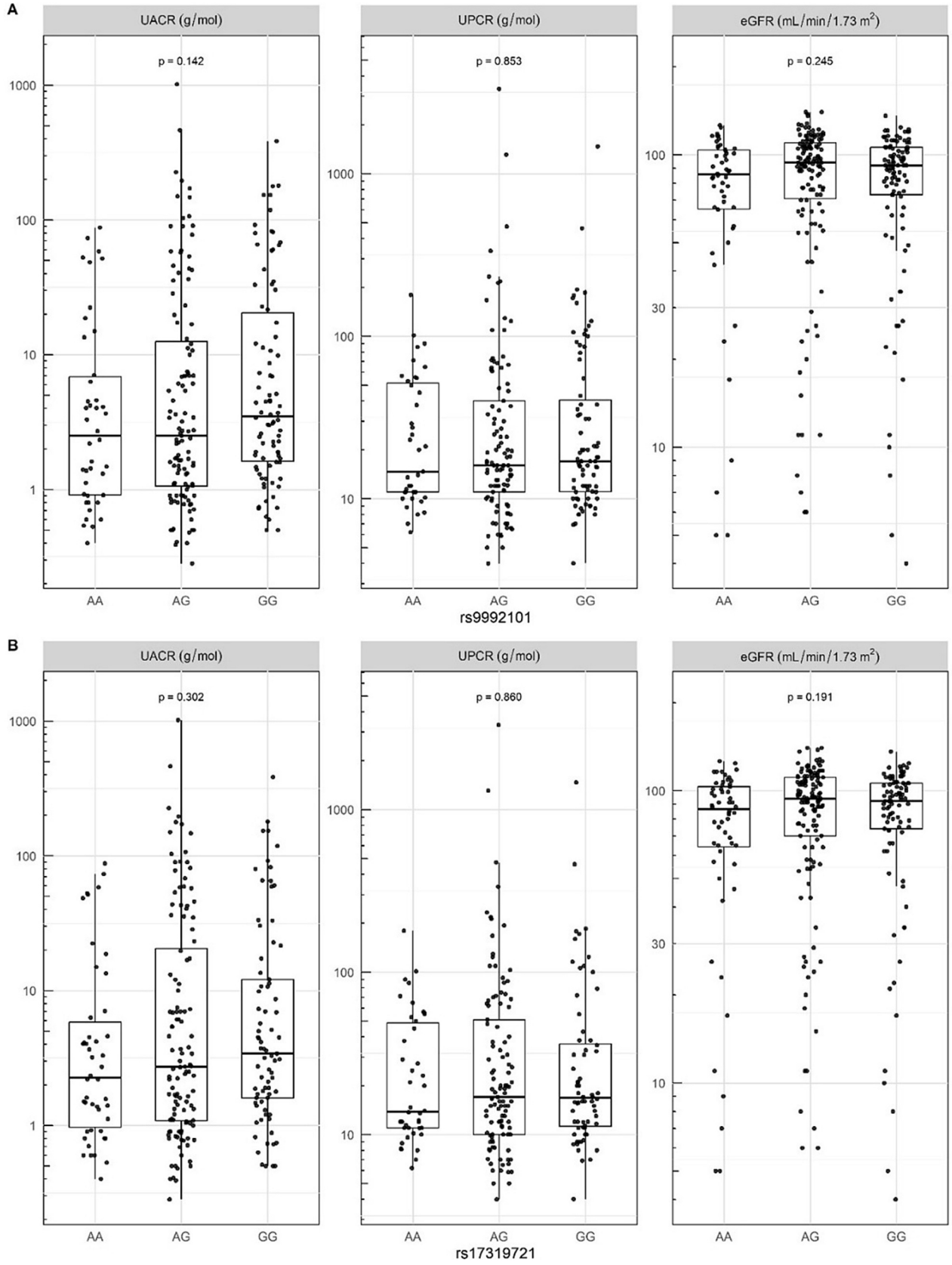

Fig. 1. Association between A) rs9992101 and B) rs17319721 genotypes and biomarkers of kidney function plotted on a logarithmic scale. UACR, urinary albumin-to-creatinine ratio; UPCR, urinary protein-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

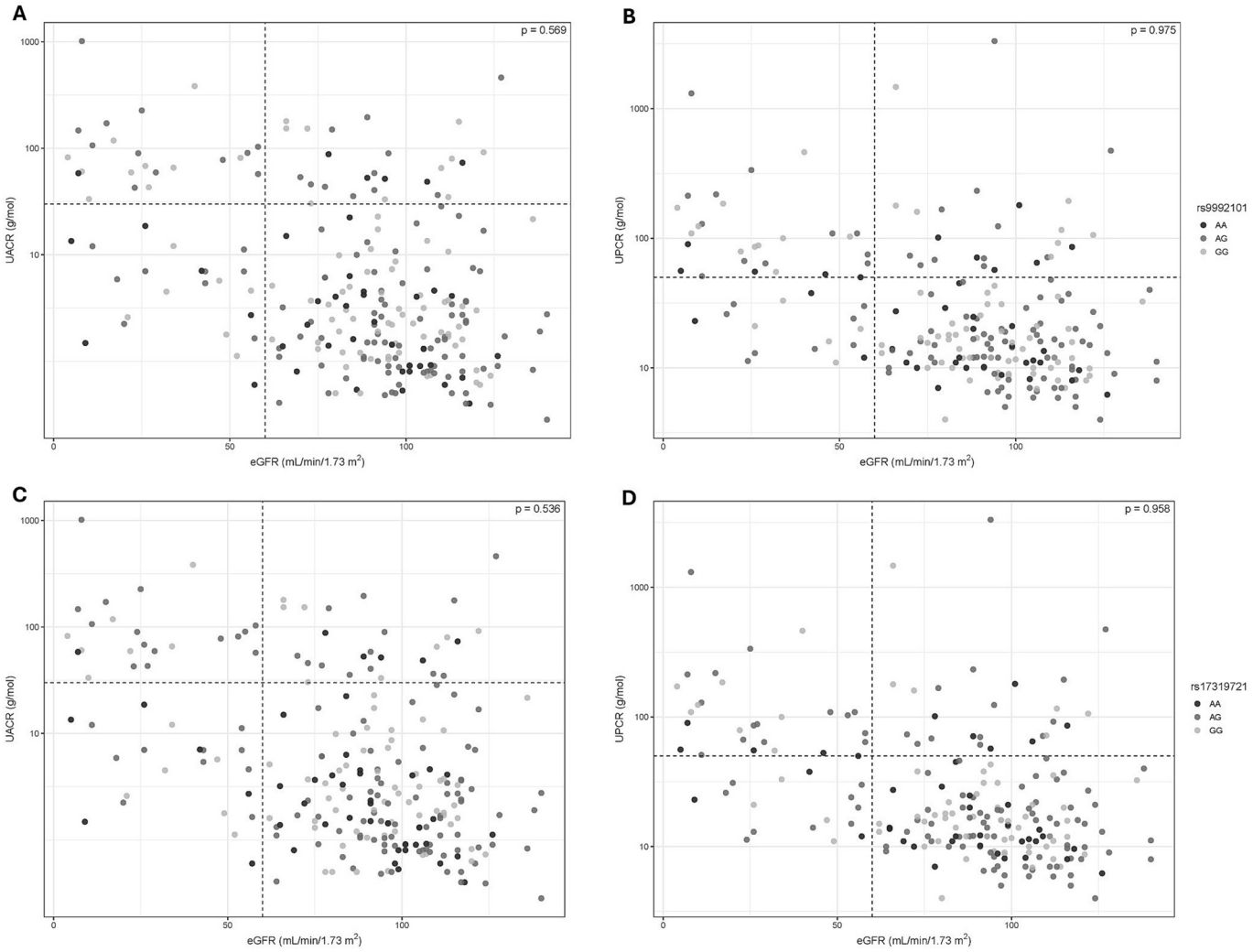

Fig. 2. Quadrant analysis of kidney function biomarkers for *SHROOM3* genotypes. For rs9992101, A) UACR vs. eGFR, B) UPCR vs. eGFR; for rs17319721, C) UACR vs. eGFR, D) UPCR vs. eGFR. UACR and UPCR are plotted on a logarithmic scale. UACR, urinary albumin-to-creatinine ratio; UPCR, urinary protein-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

In a previous study, we focused on variants in coding regions associated with podocyte function and structure [11]. In the current study, however, we selected ten SNPs located in non-coding regions of genes also associated with podocyte homeostasis. Non-coding regions do not encode proteins; nevertheless, they play a crucial role in gene regulation and disease mechanisms. Such variants can affect gene expression levels, impair alternative splicing, alter mRNA stability, induce epigenetic changes, or impair the function of regulatory RNAs [20,21]. These mechanisms highlight the potential importance of non-coding variants in modulating disease phenotype and progression in FD. In the present study, we discovered two intronic variants in the *SHOOM3* gene that are associated with a more rapid decline in eGFR in patients with FD.

SHROOM3 encodes an actin-binding protein that plays a central role in maintaining epithelial cell integrity and cytoskeleton organization [22]. Its interaction with Rho-kinase and non-muscle myosin II facilitates the remodeling of the actin cytoskeleton, a process crucial for the maintenance of podocyte structure and function [23]. As podocytes are a pivotal component of the glomerular filtration barrier, disruptions in their architecture can result in impaired filtration and proteinuria [24]. As demonstrated in previous studies, reduced expression of *Shroom3* in animal models leads to albuminuria, foot process effacement, and altered podocyte morphology. *In vitro* experiments have further demonstrated that suppression of *Shroom3* results in the disruption of lamellipodia formation and the destabilization of slit diaphragm components. Conversely, restoration of *SHROOM3* expression has been shown to partially rescue glomerular structure and function, highlighting its protective role in maintaining kidney health [23,25].

Genetic studies have identified several polymorphisms in the *SHROOM3* gene associated with reduced kidney function. In a large GWAS conducted in the Japanese population, 21 of 50 variants associated with eGFR mapped to *SHROOM3*, highlighting its importance in kidney physiology [26]. Two of these intronic SNPs—rs17319721 and rs9992101—have been consistently associated with kidney function indicators, such as eGFR, serum creatinine, and cystatin C in several independent populations [26–28]. In particular, rs17319721 is one of the most strongly associated polymorphisms in GWAS of kidney traits [27]. Functional analyses have shown that the A allele of rs17319721 increases *SHROOM3* expression by enhancing binding of the β-catenin transcription factor in the presence of TGF-β1. This promotes profibrotic signaling pathways and accelerates interstitial fibrosis, which is a key contributor to the progression of chronic kidney disease [23].

In our study, we found a significant association between the rs17319721 and rs9992101 polymorphisms and the risk of developing progressive nephropathy in patients with FD. The AA genotypes of both rs9992101 and rs17319721 were associated with 2.4- and 2.3-fold higher odds of advanced nephropathy, respectively, compared with GG carriers. After adjustment for age, these associations retained their statistical significance, suggesting that the observed effects are

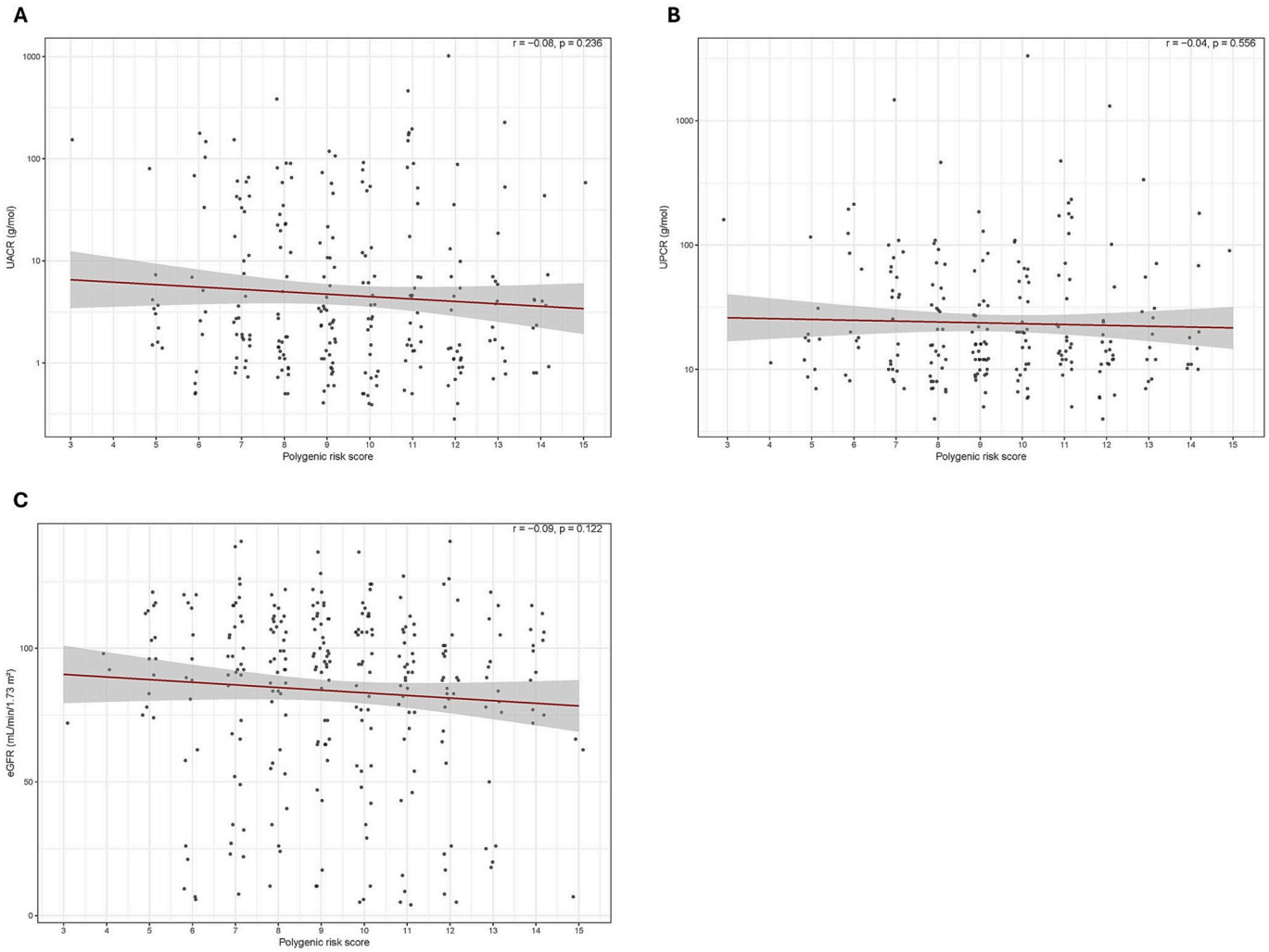

Fig. 3. Scatter plots showing the relationship between polygenic risk score and A) UACR, B) UPCR, and C) eGFR. A linear regression line with a 95% confidence interval is shown for visualization purposes, while statistical significance was tested using Spearman's rank correlation. UACR, urinary albumin-to-creatinine ratio; UPCR, urinary protein-to-creatinine ratio; eGFR, estimated glomerular filtration rate; r, Spearman's rank correlation coefficient.

independent of this confounding factor. Of particular significance was the observation that the A allele, in both heterozygous and homozygous forms, was associated with a statistically significant increase in the risk of progressive nephropathy. This result supports the hypothesis of an additive or dose-dependent genetic effect. Moreover, LD analysis suggests that these two variants, located less than 10 kb apart, are likely co-inherited, which may indicate a shared underlying biological mechanism driving the observed associations.

We also evaluated the association of rs17319721 and rs9992101 with conventional kidney function biomarkers, namely UACR, UPCR, and eGFR. Contrary to expectations and previous population-based reports [26–28], our analysis revealed no statistically significant differences between genotypes for either polymorphism. Likewise, there was no significant association between PRS and these markers. Several factors may explain this lack of association. First, the relatively small size of our cohort likely limited the statistical power to detect modest genotype-phenotype correlations. Second, the clinical course of Fabry nephropathy is highly heterogeneous, shaped by genetic, environmental, and treatment-related influences, which may have obscured the contribution of individual genetic variants to conventional kidney biomarkers. Finally, the use of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) by some participants may have reduced the variability in UACR and UPCR between genotypic groups, as these therapies are known to reduce proteinuria and slow kidney disease progression regardless of genetic background.

This study has several strengths. To our knowledge, it is the first study to investigate the association between non-coding polymorphisms and the progression of Fabry nephropathy. Our approach was hypothesis-driven and biologically plausible, as we focused on SNPs previously associated with podocyte biology and kidney function. In addition, we used a clearly defined clinical endpoint: a reduced eGFR slope assessed over a 5- to 10-year period, providing a comprehensive assessment of kidney involvement. Adjustment for age in multivariate logistic regression models increased the robustness of our results and reduced the likelihood of confounding factors.

However, this study also has its limitations. The relatively small sample size, typical of rare disease research, may have limited our statistical power to detect associations with continuous biomarkers of kidney function. In view of the limited cohort size, our models were adjusted only for age; additional genetic or environmental confounders could not be reliably included and may contribute to residual confounding. In addition, our analysis focused on only ten SNPs, all of which have been previously associated with kidney diseases. However, we cannot exclude the potential influence of other genetic variants or regulatory mechanisms in these genes or nearby loci. The study population consisted of Caucasians, which increases the internal validity but may limit the generalizability of the results to other populations. Future studies with larger cohorts with more diverse populations will be

important to confirm and extend these results.

To conclude, variants in *SHROOM3* have been implicated in impaired epithelial organization, dysregulated actin dynamics, and altered TGF-β–driven fibrotic signaling [22,23], all of which are relevant pathways in chronic kidney injury. In the context of FD, where glycolipid accumulation induces podocyte stress, lysosomal dysfunction, and pro-fibrotic remodeling [4], additional genetic modifiers that influence cytoskeletal integrity or tubular repair capacity may exacerbate susceptibility to kidney damage. A biologically plausible interaction therefore exists in which *SHROOM3* polymorphisms could modulate tubular resilience or fibrosis progression, potentially affecting the severity or trajectory of Fabry-associated nephropathy. The inclusion of such biomarkers in future predictive models has the potential to facilitate earlier risk stratification and lead to personalized monitoring and treatment strategies. This could be particularly important in FD, as early therapeutic intervention can have a significant impact on long-term kidney outcomes.

CRediT authorship contribution statement

Tina Levstek: Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Nika Breznik:** Writing – review & editing, Visualization, Formal analysis. **Kaja Balant Marin:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Tisa Podkrajšek:** Writing – review & editing, Investigation. **Bojan Vujkovac:** Writing – review & editing, Resources, Data curation. **Albina Nowak:** Writing – review & editing, Resources, Data curation. **João-Paulo Oliveira:** Writing – review & editing, Resources, Data curation. **Gabriela Dostálová:** Writing – review & editing, Resources, Data curation. **Aleš Linhart:** Writing – review & editing, Resources, Data curation. **Markéta Šafaříková:** Writing – review & editing, Resources, Data curation. **Gheona Altarescu:** Writing – review & editing, Resources, Data curation. **Katarina Trebušak Podkrajšek:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Slovenian Ethics Committee for Research in Medicine (0120–260/2020/6, 0120–521/2020/3).

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Declaration of competing interest

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Data availability

Data will be made available on request.

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