

The R506Q (G1691A) Polymorphism in the Factor V Leiden Gene in Diabetic Nephropathy and Type 2 Diabetes Mellitus

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Abstract

Diabetic nephropathy is a leading microvascular complication of type 2 diabetes mellitus and a major cause of chronic kidney disease worldwide (Thomas *et al.*, 2015; Alicic *et al.*, 2017). In addition to metabolic and hemodynamic factors, genetic determinants may influence individual susceptibility and disease progression. The present study investigated the distribution of the R506Q (G1691A) polymorphism in the Factor V Leiden gene and its association with microalbuminuria dynamics in patients with type 2 diabetes mellitus and early diabetic nephropathy. Sixty-seven patients and thirty-eight healthy controls were included. Genomic DNA was extracted from peripheral blood leukocytes, and genotyping was performed using real-time PCR with allelic discrimination. Twenty-four-hour urinary albumin excretion was assessed at baseline and followed for 24 months. Heterozygous carriers were detected in 8.96% of patients. Carriers of the A allele demonstrated significantly slower progression of microalbuminuria compared with wild-type individuals ($p < 0.05$). The findings suggest that the R506Q polymorphism may act as a genetic modifier in the progression of diabetic nephropathy.

Keywords: Factor V Leiden, R506Q polymorphism, microalbuminuria, type 2 diabetes mellitus, diabetic nephropathy, real-time PCR

Резюме

Диабетната нефропатия е водещо микросъдово усложнение на захарен диабет тип 2 и основна причина за хронично бъбречно заболяване в световен мащаб (Thomas *et al.*, 2015; Alicic *et al.*, 2017). В допълнение към метаболитните и хемодинамичните фактори, генетичните детерминанти могат да повлияят на индивидуалната податливост и прогресията на заболяването. Настоящото проучване изследва разпределението на полиморфизма R506Q (G1691A) в гена на фактор V Leiden и неговата връзка с динамиката на микроалбуминурията при пациенти с диабет тип 2 и ранен диабетна нефропатия. В проучването са включени 67 пациенти и 38 здрави контроли. Геномната ДНК е екстрахирана от левкоцити от периферна кръв, а генотипирането е извършено чрез PCR в реално време с алелна дискриминация. 24-часовото отделяне на албумин в урината е оценено в началото на проучването и проследено в продължение на 24 месеца. Хетерозиготни носители са открити при 8,96% от пациентите. Носителите на алел А демонстрираха значително по-бавно прогресиране на микроалбуминурията в сравнение с индивидите от див тип ($p < 0,05$). Резултатите предполагат, че полиморфизмът R506Q може да действа като генетичен модификатор в прогресирането на диабетната нефропатия.

Introduction

Diabetic nephropathy (DN) is one of the most common and clinically significant microvascular complications of type 2 diabetes mellitus

(T2DM) and a leading cause of chronic kidney disease worldwide (Thomas *et al.*, 2015; Alicic *et al.*, 2017). Its pathogenesis involves complex in-

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teractions between metabolic, hemodynamic, and inflammatory mechanisms leading to glomerular hyperfiltration, podocyte damage, and progressive albuminuria (Doke and Susztak, 2021).

Factor V is a key cofactor in the coagulation cascade. Activated Factor Va participates in the composition of the prothrombin complex and catalyzes the conversion of prothrombin to thrombin. Factor V Leiden polymorphism (R506Q, G1691A) causes resistance to activated protein C and leads to increased thrombin generation (Kujovich, 2011). Although traditionally considered a risk factor for venous thrombosis, recent experimental data suggest that low-grade and prolonged coagulation activation may modulate cellular signaling and affect renal homeostasis (Bae *et al.*, 2010).

Evidence is accumulating that thrombin-dependent signaling may regulate podocyte apoptosis and nephrin phosphorylation, thereby participating in the maintenance of the glomerular barrier (Wang *et al.*, 2011). This is particularly relevant in DN, where podocyte damage is a central pathogenetic mechanism (Doke and Susztak, 2021). Clinical observations suggest that carriage of the Factor V Leiden mutation may be associated with lower albuminuria and slower progression of renal damage in patients with diabetes mellitus (Isermann *et al.*, 2007). Recent analyses further emphasize the role of coagulation factors as potential modifiers of inflammation and renal fibrosis (Navarro-González and Mora-Fernández, 2020). These data raise the question of whether the R506Q polymorphism may act as a genetic modifier of the clinical course of diabetic nephropathy.

Over the past two decades, the human microbiome has established itself as a functional system involved in the regulation of metabolic, immune, and vascular processes. Dysbiosis of the intestinal microbiome is associated with type 2 diabetes mellitus and its microvascular complications, including diabetic nephropathy. In parallel, the microbiome of the urinary tract (urobiome), which has long been considered sterile, is also of interest. Modern molecular methods show that the urinary tract has its own microbial population with low biomass but with potential functional significance (Brubaker *et al.*, 2021; Roth *et al.*, 2022). Its role in renal pathol-

ogy and in the context of diabetic nephropathy is still being elucidated.

Possible interactions between genetic factors, coagulation mechanisms, and microbiome changes outline a new direction in understanding the progression of diabetic nephropathy. In this context, the study of the R506Q (G1691A) polymorphism in the Factor V Leiden gene as a potential modifier of albuminuria in patients with T2DM and early DN represents a clinically and pathophysiologically relevant scientific question.

Materials and Methods

Study design

An observational, comparative clinical study with a molecular genetic component was conducted to assess the relationship between the R506Q (G1691A) polymorphism in the Factor V Leiden gene and the progression of microalbuminuria in patients with type 2 diabetes mellitus and early diabetic nephropathy.

Study population

A total of 105 individuals were included in the study, divided into two groups:

- Group 1: 67 patients with type 2 diabetes mellitus and diabetic nephropathy, stage I–II chronic kidney disease (CKD).

Average values:

- Serum creatinine: $96.22 \pm 18.61 \mu\text{mol/L}$
- eGFR (MDRD): $103.26 \pm 16.12 \text{ mL/min/1.73 m}^2$
- Group 2 (control): 38 clinically healthy individuals with no history of diabetes mellitus, kidney disease, or other chronic conditions.

The diagnosis of type 2 diabetes mellitus was made according to the criteria of the World Health Organization (Table 1).

DNA analysis and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA concentration and purity were assessed spectrophotometrically. Genotyping of the R506Q (G1691A) polymorphism in the Factor V Leiden gene was performed by real-time PCR with allelic discrimination (Allelic Discrimination assay) on a Montania 4896 device (Anatolia Geneworks, Turkey). CE-IVD certified diagnostic

Table 1. Clinical characteristics of the study groups

Group	n	Age (years, mean $\pm$ SD)	Female, n (%)	Male, n (%)
T2DM + DN (CKD stages I–II)	67	64.14 ± 10.96	24 (35.8%)	43 (64.2%)
Control	38	35.80 ± 9.47	32 (84.2%)	6 (15.8%)

kits (Generi Biotech, Czech Republic) were used, allowing differentiation between the wild type (G1691G), heterozygous variant (G1691A), and homozygous mutant genotype (A1691A). A negative control without DNA template was included in each series.

The PCR reaction was performed in a final volume according to the manufacturer's recommendations under the following conditions: initial denaturation and enzyme activation at 95°C for 3 min, followed by 50 cycles including denaturation at 95°C for 10 s and annealing/extension at 60°C for 20 s. The final phase included cooling to 4°C. The results were analyzed using the device software based on fluorescent signals and allele discrimination.

Monitoring of microalbuminuria

Microalbuminuria was determined by measuring albumin excretion in 24-hour urine (mg/24 h). The study was performed at baseline and followed up for a period of 24 months. The dynamics of albumin excretion were analyzed depending on the Factor V Leiden genotype.

Statistical analysis

Statistical analysis was performed using descriptive statistics to determine mean values and standard deviations. The normality of the distribution was checked before applying parametric tests. Comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by post hoc multiple comparison tests when necessary. Correlation analysis was used to assess the relationship between microalbuminuria dynamics and the Factor V Leiden genotype. The level of statistical significance was set at $p < 0.05$.

Results

Among the 67 patients with type 2 diabetes mellitus and diabetic nephropathy studied, heterozygous carriers of the R506Q (G1691A) polymorphism were found in 6 patients (8.96%) Table 2). In the remaining 61 patients (91.04%), a wild-type genotype (G1691G) was identified. A homozygous mutant genotype (A1691A) was not found in the studied cohort. The frequency of heterozygous carriage in the present study is comparable to data for the European population.

Heterozygous carriers of the R506Q (G1691A) polymorphism showed a slower increase in microalbuminuria over 24 months compared to patients with the wild-type genotype (G1691G) ($p < 0.05$) (Fig. 1).

Patients with the G1691G genotype showed

an increase in albumin excretion from 207 mg/24 h to 428 mg/24 h, while heterozygous carriers (G1691A) showed an increase from 178.03 mg/24 h to 395.21 mg/24 h. The difference in the dynamics of microalbuminuria between the two groups is statistically significant.

Table 2. Distribution of Factor V Leiden genotypes in patients with T2DM and diabetic nephropathy

Genotype	n	%
G1691G (wild type)	61	91.0
G1691A (heterozygous)	6	9.0
A1691A (homozygous mutant)	0	0.0

No homozygous mutant genotype was detected

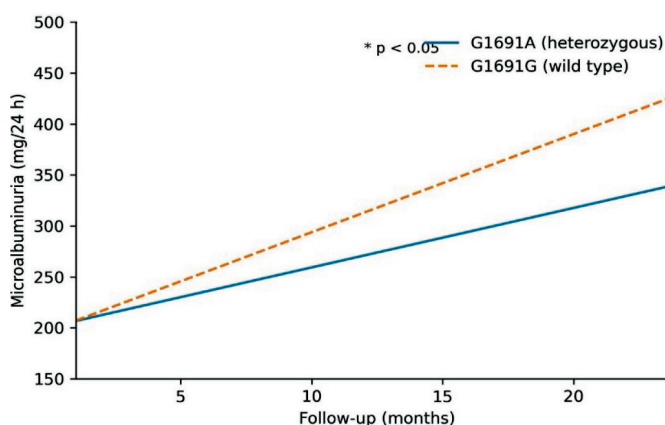


Fig. 1. Longitudinal changes in microalbuminuria according to G1691A genotype during follow-up

Discussion

The results obtained support the concept that Factor V Leiden polymorphism (R506Q, G1691A) is not only a thrombotic risk factor, but may also have a modifying role in diabetic nephropathy. The slower increase in microalbuminuria observed in A-allele carriers suggests a possible involvement of coagulation-related mechanisms in the regulation of the glomerular barrier.

Experimental data show that low-grade and prolonged thrombin activation may reduce podocyte apoptosis and preserve nephrin signaling (Wang *et al.*, 2011; Bae and Rezaie, 2020). These mechanisms are particularly important in diabetic nephropathy, where podocyte damage plays a central role in the progression of albuminuria (Doke and Susztak, 2021).

Our results are consistent with the observations of Peter *et al.* (2014), who report lower albumin excretion in carriers of the Factor V Leiden mutation. Data from Isermann *et al.* (2007) further suggest that activated protein C may exert nephroprotective effects by inhibiting endothelial and podocyte apoptosis. Recent analyses highlight the role of coagulation factors as potential modulators

of inflammation and renal fibrosis in diabetic nephropathy (Navarro-González and Mora-Fernández, 2020).

The combination of genetic factors, coagulation mechanisms, and potential microbiome influences outlines a complex pathophysiological framework in which individual genetic variability may determine different rates of disease progression.

A major limitation of the present study is the relatively small number of heterozygous carriers, which limits the statistical power of the analysis. Larger, prospective, multicenter studies are needed to confirm the observed associations.

Conclusion

The R506Q (G1691A) polymorphism in the Factor V Leiden gene is associated with differences in the dynamics of microalbuminuria in patients with type 2 diabetes mellitus and diabetic nephropathy. Carriage of the A allele is associated with a slower increase in albumin excretion during the follow-up period. The data suggest a possible modifying role of this genetic variant in the course of diabetic nephropathy, which requires confirmation in larger clinical cohorts.

Ethical approval

The study was approved by the Local Ethics Committee (2017). All participants signed informed consent forms.

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