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Relationship of gene polymorphisms for complement components C3 and factor H and kidney allograft function

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Abstract

Complement plays a central role in organ ischemia/reperfusion injury (IRI) and allograft rejection. A retrospective observational study included a cohort of 73 non-diabetic deceased donor kidney allograft recipients. We collected data on donor and recipient demographic, clinical and laboratory parameters. The main outcomes of our study were delayed graft function (DGF) and kidney allograft function during five years posttransplant. Gene single nucleotide polymorphisms (SNPs) for complement components C3 (rs2230199, G_C) and FH (rs800292, G_A) were determined. The genotyping results for FH polymorphism (184G > A) showed a distribution of GG (71.2%) and GA (28.8%) genotypes, with the AA genotype not detected in the cohort. The genotype frequencies of the C3 polymorphism (304 C > G) were CC (71.2%), CG (26.0%) and GG (2.8%).

Analysis of FH SNP demonstrated that patients with the GG genotype had a statistically higher frequency of DGF compared to those with the GA genotype (67.3% vs. 38.1%, $p = 0.022$). Univariate linear regression analysis confirmed that the FH GG genotype was the only significant determinant of DGF ($p = 0.025$). Analysis of C3 SNP showed that patients with the GC/GG genotype demonstrated significantly lower levels of creatinine clearance compared to those with the CC genotype at 1 year ($p = 0.002$), 3 years ($p = 0.001$) and 5 years ($p = 0.010$) posttransplant. These findings underscore the importance of genetic factors in influencing renal outcomes post-transplant.

Keywords Complement, Single nucleotid polymorphism, Transplantation

Introduction

Over the years, there have been a growing number of patients opting for one of the kidney replacement therapies (KRT), such as transplantation. Although the method is well established, the field of transplant immunology remains a constant challenge, and rejection and infection are the key reasons for the loss of a transplanted kidney. It has been shown that complement plays a central role not only in infections, but also in processes such as organ ischemia/reperfusion injury (IRI), antibody- and cell-mediated rejection and vascular injury in kidney recipients [1, 2]. The C3 component is critical to the

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activation of the complement cascade, acting as a junction point between the classical, lectin and alternative pathways. During kidney transplantation, IRI activates the complement system, causing C3 to be cleaved into active fragments C3a and C3b [3–5]. This initial activation primes the inflammatory response and sets the stage for further complement-mediated damage. C3 cleavage products, notably C3a, are strong inflammatory mediators that coordinate immune cell recruitment and activation at the injury site. Delayed graft function (DGF) is an IRI-related common, early complication and excessive complement activation in DGF causes an increased inflammatory cascade, which includes the release of pro-inflammatory cytokines and chemokines [6]. This persistent inflammatory environment exacerbates tissue damage and slows renal function recovery. Increasing evidence suggests that complement components serve as a link between the innate and adaptive immune responses and play a role in the pathogenesis of both T-cell-mediated rejection and antibody-mediated rejection following kidney transplantation [7]. Factor H (FH) is the key regulator of the alternative pathway of the complement system, playing a critical role in maintaining immune homeostasis and preventing excessive complement activation on host cells. In the context of DGF following kidney transplantation, FH's role is of particular interest due to its involvement in modulating complement-mediated injury and inflammation. The extent of complement activation, among other factors, may depend on the presence of functional single nucleotide polymorphisms (SNP's) that alter the activity, regulation, or expression of the key complement proteins [2]. It has been demonstrated that genetic variants 304 C > G in C3 (rs2230199) and 184G > A in FH (rs800292) lead to structural changes in respective proteins and may potentially cause dysregulation of the complement system [8, 9].

This study aimed to explore the possible relationship between SNP´s in complement components C3 (rs2230199) and FH (rs800292) and outcomes in kidney transplantation: DGF and allograft function within the five-year post-transplantation. The idea proposed in this study was that certain genetic polymorphisms in these complement components could influence the incidence of DGF and subsequent allograft function, providing insights into tailored transplantation strategies.

Materials and methods

Demographic and clinical features of the study group

A retrospective observational study was conducted at the Clinic for Nephrology, University Clinical Center of Serbia and the Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade. The study included a cohort of 73 non-diabetic kidney transplant recipients who received their first allograft from a deceased, brain-dead donor (DBD). The study included patients transplanted from 2008 to 2017. who were alive and had a functional allograft in 2019. Blood samples were taken on patients' regular follow-up visits.

All patients were on hemodialysis prior to transplantation. The immunosuppressive treatment included induction with antithymocyte globulin (ATG) and methylprednisolone, and maintenance with mycophenolic acid preparations, tacrolimus and steroids. According to the Center's protocol tacrolimus target levels were obtained from 10 to 12ng/ml during the 1st posttransplant month, 8 to 10ng/ml from 2nd to 4th month, and 6 to 8ng/ml from the 5th posttransplant month. According to our center's protocol, all patients received mycophenolate mofetil 2 g per day or mycophenolate sodium 1.44 g per day during the first posttransplant year and half the dose onwards in cases with no acute rejection (AR) or additional immunological risk including donor-specific antibodies (DSA). Exclusion criteria were immunosuppressive maintenance protocols that did not include tacrolimus and multiorgan transplantation.

Before the study commenced, all patients were thoroughly informed about the goals of the study and signed informed consent. All procedures performed in the study were in accordance with the ethical standards of the Institutional Research Committee at which the study was conducted (IRB approval number 61206-328/2).

The following data were taken from the patient's medical records: demographic data, information on the underlying kidney disease, date and type of transplant, human leukocyte antigen (HLA) typing, data on AR, DGF and slow graft function (SGF), endogenous creatinine clearance and proteinuria, and biochemical parameters (C-reactive protein, blood cell count, glycemia, blood urea nitrogen, alkaline phosphatase, serum uric acid, serum albumin, trough tacrolimus level) on the 5th, 7th, 15th day, and during the first posttransplant year at months 1, 3, 6 and 12. We obtained data on the donor's age, hypertension, diabetes, body mass index (BMI) and kidney donor profile index (KDPI).

Long-term follow-up comprised medical record data at 1-, 3-, and 5-year post-transplantation intervals. Panel reactive antibodies (PRA) were measured by complement-dependent cytotoxicity (CDC) assay before transplantation and 30 days after transplantation. All patients were tested negative for hepatitis B, C, and HIV.

Acute rejection, slow and delayed graft function criteria

Diagnosis of AR was made by allograft biopsy, biopsy-proven AR (BPAR), or based on deterioration of allograft function that has improved after a high dose of corticosteroid therapy.

Slow graft function was defined as serum creatinine concentration ≥ 3.0 mg/dL on the 5th posttransplant day

without the need for hemodialysis (HD). Hemodialysis during the initial two weeks following transplantation met the criteria for DGF[10].

BKV, CMV and VZV monitoring

We recorded reactivations of BK virus (BKV) and cytomegalovirus (CMV) using the quantitative polymerase chain reaction (PCR) method. We checked the donor and the recipient for CMV IgG antibodies routinely before transplantation. CMV PCR was monitored weekly for a 3-month period after transplantation, while follow-up was extended for high-risk patients (symptoms and signs of infection/reactivation after treatment of AR) and in patients in whom antiviral therapy had been initiated. All recipients were checked for BKV PCR during the first posttransplant year (in months 1, 2, 3, 4, 6, 8 and 12) and yearly afterwards or by indication in all cases of deterioration of allograft function with or without inflammatory syndrome. Serology for varicella zoster (VZV) was checked in cases of symptoms and signs of virus reactivation.

Table 1 Demographic, clinical, and genotypic profiles of patients

Pretransplant variable	*N* = 73
Gender, male, n (%)	51 (69.9)
Age (years), mean ± SD	46.9 ± 10.9
Positive PRA (5–30%), n (%)	5 (6.8)
Genotype, n (%)	
FH (rs800292)	
GG, *n* (%)	52 (71.8)
GA, n (%)	21 (28.8)
AA, n (%)	0 (0.0)
C3 (rs2230199)	
CC, *n* (%)	52 (71.2)
GC, n (%)	19 (26.0)
GG, n (%)	2 (2.7)
Etiology of CKD, n (%)	
NSCL, n (%)	25 (34.2)
GN, n (%)	18 (24.7)
TIN, n (%)	4 (5.5)
PKD, n (%)	10 (13.7)
Other, n (%)	22 (30.2)
Comorbidities, n (%)	
HTN, n (%)	58 (79.5)
CMP, n (%)	1 (1.4)
Ischemic coronary heart disease, n (%)	1 (1.4)
CVI, n (%)	1 (1.4)

PRA: panel reactive antibodies, CKD: chronic kidney disease, NSCL: nephroangiosclerosis, GN: glomerulonephritis, TIN: tubulointerstitial nephritis, PKD: polycystic kidney disease, HTN: hypertension, CMP: cardiomyopathy, CVI: cerebrovascular insult

Sample processing, genetic testing (gene and SNP selection, and genotyping)

Sample processing and deoxyribonucleic acid (DNA) isolation were conducted using a standard column method (Fermentas Thermo Fisher Scientific Inc, St. Leon-Rot, Germany) from the whole blood samples collected during regular check-ups between January 2019 and March 2019. The purity of the isolated DNA was assessed by measuring the absorbance at 260 and 280 nm. Additionally, gene SNPs for complement components C3 and FH were detected using the TaqMan PCR method (Thermo Fischer Scientific Inc, USA).

Statistical data analysis

Parametric data were analyzed using appropriate parametric tests, such as the Student's T-test for comparing two groups and analysis of variance (ANOVA) for comparing multiple groups. Non-parametric data were assessed using Chi-square, Fisher's exact probability test, Mann-Whitney U test, and Kruskal-Wallis test as deemed suitable. Correlation and regression analyses were conducted to explore relationships and predictive factors. Descriptive statistics was employed to summarize data, including measures of central tendency (mean, median) and variability (standard deviation, interquartile range).

Results

Baseline characteristics of the study cohort are provided in Table 1. A total of 51 (69.9%) patients were male and the mean age in the study group was 46.9 ± 10.9 years. The average follow-up period was 54 months. Underlying kidney diseases causing chronic kidney disease (CKD) were: nephroangiosclerosis in 34.2%, glomerulonephritis in 24.7%, polycystic kidney disease in 13.7% and tubulointerstitial nephritis in 5.5% of patients. The most often recorded comorbidity was hypertension in 79.5%.

All patients were treated with hemodialysis before transplantation from one to 20 years. All patients received ATG as induction therapy, with a dose depending on the total immunological risk, i.e. the number of HLA mismatch (HLA MM), PRA, the age of the donor and recipient, the length of the cold ischemia time (CIT) etc. A total of 21 (28.8%) patients received dose of a 9 mg/kg, and 52 (71.2%) patients received a cumulative dose of 12 mg/kg (9 mg/kg before arterial anastomosis, and 3 mg/kg on the first posttransplant day).

All patients received valganciclovir as prophylaxis for CMV infection for three months and trimethoprim/sulfamethoxazole prophylaxis for Pneumocystis jirovecii infection for six months. The genotype frequencies observed for both rs800292 (FH gene) and rs2230199 (C3 gene) in the study population were found to be in Hardy-Weinberg equilibrium. The genotyping results

for FH polymorphism (184G > A) showed a distribution of GG (71.2%) and GA (28.8%) genotypes, with the AA genotype not detected in the cohort. The genotype frequencies of the C3 polymorphism (304 C > G) were CC (71.2%), CG (26.0%) and GG (2.8%).

The mean donor age was 50.4 ± 13.8 years. A total of 43 (58.9%) of patients experienced DGF, which lasted on average 16 days (from 3 to 105 days). Delayed graft function demanded more than five HD sessions in the majority of patients. We did not find statistically significant differences between the patients with and without DGF regarding tacrolimus through levels on day 7, first and third month following transplantation (10.2 ± 4.4 vs. 9.9 ± 4.1, $p = 0.761$; 11.9 ± 2.5 vs. 12 ± 4.2, $p = 0.885$ and 9 ± 2.1 vs. 8.9 ± 2.3, $p = 0.867$, respectively).

The mean CIT was 18.8 ± 14 h, while the mean secondary warm ischemia time (WIT) was 30.5 ± 12.7 min. Slow graft function was recorded in 17 (23.3.%) of patients. The total number of HLA mismatches on A, B and DR loci was 1 in 1 (1.4%), 2 in 5 (6.8%), 3 in 27 (37%), 4 in 34 (46.6%) and 5 in 1 (1.4%) patient respectively. Biochemical variables at the end of the first posttransplant year are presented in Table 2.

A total of 14 (19.1%) patients had one episode AR, 9 episodes (64.2%) were BPAR, T-cell mediated AR. In other cases, it was not possible to perform a biopsy for technical reasons. Nevertheless, all included patients were treated with pulse doses of methylprednisolone, which led to the recovery of allograft function and made it possible to conclude that it was an AR episode. The fact that no repeated episodes of AR were recorded during the further follow-up, that the control PRA were 0% and that no de-novo DSA were detected; points to the fact that all of these assumed episodes were most likely T-cell-mediated episodes of AR, as well.

Table 2 Patient's and donor's characteristics in subgroups with and without DGF occurrence

Variable		All patients (*N* = 73)	DGF (*N* = 43, 58.9%)	Non-DGF (*N* = 30, 41.1%)	*p*
Transplant variables					
Donor's age (years), mean ± SD		50.4 ± 13.8	51.8 ± 11	47.9 ± 17.7	0.355
CIT (hours), mean ± SD		18.8 ± 14 (median 18 (14–21)	21.2 ± 16.6	16.6 ± 4.4	0.200
2°WIT (minutes), mean ± SD		30.5 ± 12.7	32.7 ± 13.1	26.3 ± 10.9	0.074
SGF, n (%)		17 (23.3)	0	17 (56.7)	< 0.001
AR, n (%)		14 (19.1)	5 (11.6)	9 (30)	0.050
HLA mismatch in A, B, and DR, n (%)	1	1 (1.4)	1 (2.3)	0	0.958
	2	5 (6.8)	4 (9.3)	1 (3.3)	0.643
	3	27 (37)	15 (34.9)	12 (40)	0.656
	4	34 (46.6)	23 (53.5)	11 (36.7)	0.156
	5	1 (1.4)	0	1 (3.3)	0.855
Biochemical parameters at the end of the first posttransplant year					
Hb (g/l), mean ± SD		129.7 ± 19.9	125.6 ± 16.6	137.2 ± 23.4	0.053
Leucocytes count, mean ± SD		6.8 ± 1.8	6.4 ± 1.5	7.5 ± 2	0.036
C-reactive protein (mg/l), median (IQR)		1.9 (0.6–5.8)	1.9 (0.55–8.55)	1 (0.7-4)	0.609
Glucose (mmol/l), mean ± SD		5.5 ± 0.9	5.4 ± 0.8	5.6 ± 1.1	0.387
Serum urea (mmol/l), mean ± SD		9.8 ± 4.9	10.9 ± 5.3	7.9 ± 3.5	0.028
Serum creatinine (μmol/l), mean ± SD		137.3 ± 50.4	146.1 ± 54.5	124.2 ± 41.2	0.087
Serum albumin (mmol/l), mean ± SD		44.1 ± 3.6	43.9 ± 3.9	44.4 ± 3.2	0.673
Serum uric acid (mmol/l), mean ± SD		399 ± 75.9	416.4 ± 82.1	368.5 ± 52.4	0.019
Alkaline phosphatase (IU/l), mean ± SD		81.8 ± 36.8	86.7 ± 39.8	71.5 ± 28	0.193
Proteinuria (g/24 h), mean ± SD		0.3 ± 0.22	0.3 ± 0.25	0.22 ± 0.21	0.260
Infections during the first posttransplant year					
Positive PCR BKV in blood, n (%)		15 (20.5)	9 (20.9)	6 (20)	0.923
PCR CMV in blood, n (%)		9 (12.3)	6 (14)	3 (10)	0.613
Positive IgM VZV, n (%)		5 (6.8)	4 (9.3)	1 (3.3)	0.643
Donor's characteristics					
KPDI (%)		53.8 ± 28.6	55 ± 27.3	49.9 ± 34.7	0.664
BMI (kg/m^2)		25.2 ± 2.6	25.4 ± 2.6	24.6 ± 2.8	0.477
Donor's HTN (*N* = 60)		60	13/38 (34.2%)	6/22 (27.3%)	0.578
Donor's DM		0	0	0	/

DGF: delayed graft function, SGF: slow graft function, CIT: cold ischaemia time, WIT: warm ischaemia time, SGF: slow graft function, AR: biopsy proven/clinically suspected acute rejection, HLA: human leukocyte antigen, Hb: hemoglobin, PCR: polymerase chain reaction test, BKV: BK virus, CMV: cytomegalovirus, IgM: immunoglobulin M, VZV: varicella zoster virus, CNS: central nervous system, KDPI: kidney donor profile index, BMI: body mass index, HTN: hypertension, DM: diabetes mellitus

We recorded only one case of D+/R- CMV transplantation, without any episodes of CMV reactivation during the follow-up. The number of patients with BK viremia was 15 (20.5%) during the first posttransplant year. Nine (12.3%) patients had PCR confirmation of CMV replication in blood. Five patients had positive IgM on ELISA testing for VZV (6.8%) (Table 2). As expected patients with DGF had no SGF ($p<0.01$), had significantly higher serum urea nitrogen concentration (10.9 ± 5.3 vs. 7.9 ± 3.5, $p=0.028$), serum uric acid concentrations (416.4 ± 82.1 vs. 368.5 ± 52.4, $p=0.019$) and leucocyte count at the end of the first posttransplant year (6.4 ± 1.5 vs. 7.5 ± 2, $p=0.036$).

Table 2. also shows the donor's characteristics (body mass index, hypertension, diabetes) and KDPI and their distribution in the DGF and non-DGF groups. A body mass index higher than 25 was found in 15 patients with DGF and in 4 patients without DGF, $p=0.858$. None of the donors were diabetic. No statistical significance was found between the DGF and non-DGF groups regarding hypertension and KDPI ($p=0.578$ and 0.664, respectively).

As shown in Table 3. analysis of FH SNP (184G > A, rs800292) demonstrated that patients with the GG genotype (67.3%) had a statistically higher frequency of DGF compared to those with the GA genotype (38.1%). Interestingly, patients with the GG genotype exhibited significantly lower levels of proteinuria in comparison to those with the GA genetic profile. The analysis of other clinical and demographic parameters did not reveal statistically significant differences among the observed FH genotypes. Further investigation into clinical and demographic parameters among transplant patients has uncovered significant findings with regard to genetic polymorphism C3 (304 C > G, rs2230199). During the first year after transplantation, the incidence of infections was higher in the GC/GG genotype group (33.3%) compared to the CC genotype group (15.4%), although this difference did not reach statistical significance ($p=0.086$). Furthermore, notable differences were found in creatinine clearance between C3 genotype groups. Patients with the GC/GG genotype demonstrated significantly lower levels of creatinine clearance compared to those with the CC genotype at 1 year ($p=0.002$), 3 years ($p=0.001$) and 5 years ($p=0.010$) post-transplantation. After making adjustments for the history of AR, donor's age and DGF we confirmed the independent effect of C3 GC/GG genotype on creatinine clearance (Ccr) in the first, third and fifth year following transplantation: Ccr at year 1– B coefficient 0.040, $p=0.024$, OR 1.040 [95% CI (1.005–1.077)], Ccr at year 3 - B coefficient 0.057, $p=0.009$, OR 1.058 [95% CI (1.014–1.105)], Ccr at year 5 posttransplant - B coefficient 0.043, $p=0.007$, OR 1.044 [95% CI (1.012–1.076)]. No other significant associations were observed between C3 genotype groups and additional clinical or demographic parameters in this study.

Table 3 Evaluation of FH and C3 polymorphism in relation to clinical and demographic characteristics

Parameter	FH (184G > A, rs800292)		p	C3 (304 C > G, rs2230199)		p
Genotype (n, %)	GG (52, 71.2%)	GA (21, 28.8%)		CC (52, 71.2%)	GC/GG (21, 28.8%)	
DGF	35 (67.3%)	8 (38.1%)	0.022	30 (57.7%)	13 (61.9%)	0.741
AR	7 (13.5%)	7 (33.3%)	0.051	9 (17.3%)	5 (23.8%)	0.523
Recipient's age (years)	47.7±10.5	44.9±11.9	0.335	47.3±9.6	45.9±13.9	0.661
Donor's age (years)	50.2±12.8	50.8±16.6	0.875	51.1 ± 12.9	48.5±16.1	0.507
CIT (hours)	median 18 (14.25-21)	median 19 (14-21.5)	0.863	median 18.75 (14–21)	median 18 (15–21)	0.789
2°WIT (min)	31.4±13.2	28.2±11.6	0.368	29.3±10.5	33±16.6	0.324
Infections during the first year following transplantation						
BK viremia	11 (21.2%)	4 (19%)	0.840	8 (15.4%)	7 (33.3%)	0.086
CMV viremia	8 (15.4%)	1 (4.8%)	0.211	6 (11.5%)	3 (14.3%)	0.747
VZV IgM	3 (5.8%)	2 (9.5%)	0.565	3 (5.8%)	2 (9.5%)	0.621
Allograft function and proteinuria						
sCr 1st year, (μmol/l)	138.3±54.5	135.1±40.2	0.794	135.5±50.8	142.3±50.5	0.636
sCr 3rd year, (μmol/l)	137.3±51.1	130.4±35.5	0.546	130.6±43.1	147.6±55.5	0.196
sCr 5th year, (μmol/l)	129±51.5	126.1±39.7	0.829	119.1±38.1	147.9±62.1	0.082
Ccr 1st year, (ml/min)	67.8±27.7	72.8±35.7	0.613	74.8±32.1	54.1±16.4	0.002
Ccr 3rd year, (ml/min)	70.1±29.5	70.5±29.9	0.965	76.7±30.5	54.2±18.6	0.001
Ccr 5th year, (ml/min)	69.9±31.5	72.3±38.8	0.835	78.2±34.4	53.5±23.7	0.010
prt 1st year, (g/day)	median 0.15 (0.1–0.24)	median 0.22 (0.17–0.53)	0.045	median 0.178 (0.11–0.30)	median 0.195 (0.1–0.39)	0.848
prt 3rd year, (g/day)	median 0.16 (0.1–0.31)	median 0.15 (0.11–0.22)	0.877	median 0.15 (0.1-0.275)	median 0.17 (0.12–0.31)	0.484
prt 5th year, (g/day)	median 0.18 (0.1–0.29)	median 0.16 (0.12–0.27)	0.992	median 0.19 (0.14–0.30)	median 0.13 (0.04–0.21)	0.157

DGF: delayed graft function; CIT: cold ischemia time, 2° WIT: secondary warm ischemia time; AR: biopsy proven/clinically suspected acute rejection; BKV: BK virus; CMV: cytomegalovirus, VZV: varicella zoster virus, sCr: serum creatinine, Ccr: endogenous creatinine clearance, Prt: proteinuria

Table 4 Influence of demographic, clinical, biochemical parameters and KDPI on the DGF by univariate linear regression analysis

Variable	Unstandardized Coefficients		*p*	OR	95% Confidence Interval for OR	
	B	SE			Lower	Upper
Donor's age	-0.510	1.008	0.292	1.021	0.982	1.061
KDPI	0.006	0.014	0.654	1.006	0.979	1.034
Recipient's age	0.024	0.022	0.275	1.024	0.981	1.070
CIT	0.099	0.060	0.095	1.014	0.983	1.241
2°WIT	0.049	0.028	0.084	1.050	0.994	1.109
HLA MM total	-0.169	0.359	0.638	0.844	0.418	1.708
AR	-0.783	0.533	0.142	0.457	0.161	1.300
CMV	0.378	0.751	0.615	1.459	0.335	6.360
BK viremia	0.057	0.591	0.923	1.059	0.333	3.370
FH (rs800292, GG)	-1.208	0.538	0.025*	0.299	0.104	0.858
C3 (rs2230199, GG + GC)	0.175	0.530	0.741	1.192	0.422	3.366

* $p<0.05$, KDPI: kidney donor profile index, CIT: cold ischemia time, 2° WIT: secondary warm ischemia time, HLAMM: HLA mismatch, AR: biopsy proven/clinically suspected acute rejection; CMV: cytomegalovirus, BKV: BK virus

Univariate linear regression analysis confirmed that the FH GG genotype was the only significant determinant of DGF (Table 4).

Discussion

In this study, we investigated the association of SNP polymorphisms for complement components C3 (rs2230199) and FH (rs800292) with allograft function. Our findings revealed that carriers of the C allele for C3 (rs2230199) were associated with predictive changes in allograft function, specifically in terms of creatinine clearance. Additionally, the GG genotype of FH rs800292 emerged as a significant predictor for DGF.

Transition G > A at position 184 in exon 2 of the FH gene has been extensively studied and found to be associated with several inflammatory diseases [11–13]. In these studies, the G allele has consistently been identified as the "risk" allele and it has sparked significant interest due to its potential role in predisposing individuals to these complex diseases. The alteration from G to A in rs800292 results in the production of isoleucine rather than valine, causing structural changes that affect the protein's activity [8]. Pechti et al. demonstrated that both FH variants accelerate the decay of the C3bBb complex, with the variant carrying Ile at position 62 showing slightly superior cofactor activity [8]. As for kidney tissue, mutation and variants in the complement FH gene have been found to be associated with a broad range of renal pathological conditions, from atypical hemolytic uremic syndrome to C3 glomerulopathy that comprises several rare types of glomerulonephritis, including dense deposit disease, C3 glomerulonephritis and CFHR5 nephropathy [14, 15]. Authors suggest that FH most likely prevents C3 accumulation along the GBM primarily by regulating the AP in the circulation and secondly by attaching to the GBM. These actions represent the response to the various exogenous triggers of C3 activation such as infection and presumably transplantation. In patients with abnormal FH, the physiological mechanism of 'C3 tick-over', whereby spontaneous C3 activation occurs via the AP in the circulation, proceeds without inhibition. This results in severe secondary consumption of plasma C3 and the AP activation protein, factor B, even in the absence of any exogenous trigger of C3 activation [16]. These subtle differences may also potentially contribute to variations in delayed graft function within the kidney transplantation environment.

Our study has also demonstrated that patients with the GG genotype exhibit significantly lower levels of proteinuria compared to those with the GA genotype at 1-year post-transplant. Additionally, the GG genotype group exhibited lower creatinine clearance and higher serum creatinine levels at the same time point, indicating a reduced kidney capacity to filter various substances.

Classical pathway (CP) of complement has a triggering effect on DSA-mediated inflammation in the microvasculature and its genetic background could determine the severity of rejection [17]. The extent of DSA triggered complement activation depends on the presence or absence of functional SNP's determining the strength of the alternative pathway (AP) amplification loop, which is critical for full CP activation [17]. Experimental models support this by showing that specific variants of C3 (C3102G, confers resistance toward regulation), factor B (fB32R forms AP convertase more efficiently), and factor H (fH62V binds C3 less strongly and is a worse cofactor for factor I) increased activity of the AP convertase, and yielding 6-fold higher hemolytic activity compared with "protective" variants C3102R, fB32Q, and fH62I [2, 18]. Therefore we can assume that any change in the C3

control could ultimately condition the creation of DSA, with clinically manifested or subclinical microvascular injury and, in the long term, worse allograft function.

Numerous authors suggested that the DGF, being a marker of more profound IRI, in the long-term could have detrimental effects on the allograft function [19]. We assume that the DGF was related to more pronounced hemodynamic instability of donors, and consequent IRI, and with various factors regarding recipient, including posttransplant infections. This could have been the challenge, as patients with the GG genotype may react differentially to complement activation in the DGF scenario. The incidence of DGF observed in our study cohort was 58.9%. This finding can be attributed, at least in part, to the extended ischemia times encountered during our study, specifically characterized by a mean CIT of 18.8 h and a mean secondary WIT of 30.5 min. These prolonged ischemic periods likely contributed to the incidence of DGF observed in our analysis. To our knowledge, this study represents the first investigation into the relationship between FH 184G > A SNP and the occurrence of DGF in kidney allograft recipients.

The cohort was additionally assessed based on AR rates, infections during the initial transplant year, kidney function biochemical indicators, and their possible link with FH SNP 184G > A. Our group had an AR percentage of 19.1%. In certain cases, this could be attributed to the absence of positive histological confirmation in some patients, which might have offered clearer diagnostic imputations thus changing for that matter the demonstrated frequency of AR. Nonetheless, there was no connection established between FH genetic variations and acute transplant rejection nor with other examined clinical criteria.

Genetic variants like SNP in the C3 gene (rs2230199), known for their implications in conditions such as age-related macular degeneration (AMD), highlight the broader relevance of genetic research in healthcare, including kidney transplantation. Similarly to rs800292, the G allele of rs2230199 has been identified as a genetic risk factor for advanced AMD in Caucasian populations and AR in lung transplant recipients [20, 21]. The transversion of the common C allele to G leads to an amino acid substitution at position 102 (Arg > Gly), which defines two prevalent allotypic forms of C3 distinguished by their mobility in gel electrophoresis: S (Slow) and F (Fast) [9]. Results from our study demonstrated a statistically significant association between genotypes encoding C3F/F (GG) and C3F/S (GC) and creatinine clearance at 1 year, 3 years, and 5 years post-transplant. Individuals with GG and GC genotypes exhibited lower creatinine clearance at the respective time points, suggesting a sustained reduction in kidney filtration capacity over time. There is limited literature exploring the relationship between rs2230199 and clinical outcomes in kidney allograft recipients. Andrews et al. reported that the presence of the C3F allele predicted an increased risk of graft dysfunction [22]. Brown et al. investigated the C3F/S polymorphism in 513 pairs of white kidney donors and recipients and analyzed its relationship with demographic and clinical outcomes. They identified recipients with different C3 genotypes (C3S/S, C3S/F, C3F/F) and found that graft survival and function were significantly better in recipients of kidneys from C3F/F or C3F/S donors compared to C3S/S donors. They concluded that the C3F and C3S alleles likely have distinct functional effects on transplant outcomes [23]. In contrast, Varagunam et al. reported no significant differences in the long-term survival of transplanted organs based on the distribution of C3 alleles among donors and recipients [24]. Finally, a cohort from the United States comprising 1265 donor-recipient pairs, along with a smaller cohort from Iran, demonstrated no significant association between C3 allotypes and AR [25, 26]. Randomized clinical studies are the only means through which the real implications of variants in the complement C3 gene can be determined scientifically. Patient complications that occur within the context of transplantation arise from intricate pathways that are affected by multiple genes, many of which have various polymorphic variants. We can say that our study showed that allele C (C3) and allele G (FH) are considered to be the wild types, hence, more investigations using randomized clinical tests are required to clarify how exactly the C3 304 C > G and FH 184G > A gene variants affects transplanting results.

We did not find that the SNP rs2230199 for C3 was associated with a higher percentage of CMV, BKV, and VZV reactivations during the first year after kidney transplantation. Still, it is known that infections can trigger episodes of AR, complement activation and deterioration of allograft function [27].

Donor-related factors can influence the posttransplant course and DGF occurrence [28]. Still, we did not find that donor age, hypertension or obesity were related to DGF or that KDPI predicted DGF.

This study has several limitations. Firstly, we included patients who were transplanted from 2008 to 2017. and were alive with a functional kidney allograft in 2019. Patients who were transplanted between 2008 and 2017, and who did not survive or who experienced permanent loss of allograft function, were not included in the research. Secondly, AR was not confirmed by biopsy in all cases, potentially impacting the precision of our findings. Despite higher doses of corticosteroids improving clinical presentation, the absence of histological confirmation leaves room for ambiguity in terms of the nature and severity of the acute rejection that occurred within our group. While acknowledging the study's limitations,

including a small participant cohort and potential single-centre selection bias, our findings provide significant insights into the association of FH and C3 SNPs with long-term renal dysfunction post-kidney transplantation. This information can be used in future meta-analyses to gain a better understanding of genetic elements that affect post-transplantation outcomes. Our research also has a uniform group because all individuals got their first recipient kidneys from diseased donors and were on the same immunosuppressive drugs.

In summary, FH SNP (rs800292, GG) was found to be related to increased occurrence of DGF, without any significant impact on AR or viral infection after transplantation. Our study provides potential evidence that there is a link between C3F (GG) and C3FS (GC) genotypes and creatinine clearance rates at different times after kidney transplant surgery. Genetic variants play a crucial role in determining renal outcomes post-transplantation; hence, it might lead to tailored treatments for these patients.

We consider the degree of non-homogenous immunological risk and non-similar HLA matchings as well as KDPI to be the limits of our study. Our results though should be confirmed by studies involving a larger cohort and investigating the underlying mechanisms involved.

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Author contributions
MM, VP and ML analyzed and interpreted the patient data regarding the kidney transplantation, allograft function and immunological data. MM, VP, ML, MK, VB, SM, IV, IAM and MR participated in data collection and processing. VP performed genotyping assay, MM, VP, ML, MK, VB, SM, IV, IAM and MR participated in writing, reviewing and editing of the manuscript.All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Ethics approval and consent
The study was conducted in accordance with the Declaration of Helsinki, and approved by The Ethic Committee of the Faculty of Medicine University of Belgrade, Serbia, which granted approval to collect the medical data and carry out the study (IRB approval number 61206-328/2). All patients provided informed consent.

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