Original Article

Kidney Res Clin Pract 2023;42(3):389-402
pISSN: 2211-9132 • eISSN: 2211-9140
https://doi.org/10.23876/j.krcp.22.061

KIDNEY RESEARCH AND CLINICAL PRACTICE

A non-muscle myosin heavy chain 9 genetic variant is associated with graft failure following kidney transplantation

Felix Poppelaars[1], Siawosh K. Eskandari[1], Jeffrey Damman[2], Marc A. Seelen[1], Bernardo Faria[1,3,*], Mariana Gaya da Costa[1,4,*]

[1]*Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands*
[2]*Department of Pathology, Erasmus University Medical Center, Rotterdam, the Netherlands*
[3]*Nephrology and Infectious Disease R&D Group, INEB, Institute of Investigation and Innovation in Health (i3S), University of Porto, Porto, Portugal*
[4]*Department of Anesthesiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands*

Background: Despite current matching efforts to identify optimal donor-recipient pairs for kidney transplantation, alloimmunity remains a major source of late transplant failure. Additional genetic parameters in donor-recipient matching could help improve long-term outcomes. Here, we studied the impact of a non-muscle myosin heavy chain 9 gene (*MYH9*) polymorphism on allograft failure.

Methods: We conducted an observational cohort study, analyzing the DNA of 1,271 kidney donor-recipient transplant pairs from a single academic hospital for the *MYH9* rs11089788 C>A polymorphism. The associations of the *MYH9* genotype with risk of graft failure, biopsy-proven acute rejection (BPAR), and delayed graft function (DGF) were estimated.

Results: A trend was seen in the association between the *MYH9* polymorphism in the recipient and graft failure (recessive model, p = 0.056), but not for the *MYH9* polymorphism in the donor. The AA-genotype *MYH9* polymorphism in recipients was associated with higher risk of DGF (p = 0.03) and BPAR (p = 0.021), although significance was lost after adjusting for covariates (p = 0.15 and p = 0.10, respectively). The combined presence of the *MYH9* polymorphism in donor-recipient pairs was associated with poor long-term kidney allograft survival (p = 0.04), in which recipients with an AA genotype receiving a graft with an AA genotype had the worst outcomes. After adjustment, this combined genotype remained significantly associated with 15-year death-censored kidney graft survival (hazard ratio, 1.68; 95% confidence interval, 1.05–2.70; p = 0.03).

Conclusion: Our results reveal that recipients with an AA-genotype *MYH9* polymorphism receiving a donor kidney with an AA genotype have significantly elevated risk of graft failure after kidney transplantation.

Keywords: Genetics, Kidney transplantation, Molecular motor proteins, Nephrology

Introduction

Despite the excellent short-term outcomes following solid organ transplantation, the long-term survival of kidney transplants has improved only negligibly in recent years [1]. Consequently, one out of five patients on the waitlist

Received: March 29, 2022; **Revised:** June 6, 2022; **Accepted:** July 7, 2022
Correspondence: Felix Poppelaars
Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, Postbus 196, 9700 AD Groningen, the Netherlands.
E-mail: f.poppelaars@umcg.nl
ORCID: https://orcid.org/0000-0002-5173-8078

*Bernardo Faria and Mariana Gaya da Costa contributed equally to this study.

for kidney transplantation are candidates whose previous grafts failed [2]. Maximizing the long-term outcomes of transplantation and preventing retransplantation is paramount—not only for improving transplant recipients' outcomes but also for reducing waitlist pressure. Alloimmunity, otherwise known as host anti-donor immune responses, remains the preeminent driver of late graft loss, despite strong efforts to optimally match donor-recipient pairs [3,4]. Recently, there are signs of a paradigm shift in the transplant field, with suggestions that allograft matching efforts should be updated to include novel genetic markers that better ensure long-term graft survival after kidney transplantation [5,6].

In this regard, non-muscle myosin heavy chain II-A (MHCII-A), encoded by the myosin heavy chain 9 gene (*MYH9*), is a target of particular interest (Fig. 1A). Non-muscle MHCII-A is a ubiquitously expressed contractile protein involved in myriad processes ranging from cell division and adhesion to providing cytoskeletal support [7]. Mutations in the *MYH9* cause a complex set of disorders, known as *MYH9*-related diseases, that can affect every system in the body but are characterized by congenital thrombocytopenia, giant platelets and leucocyte inclusions [7]. Although non-muscle MHCII-A is expressed by a variety of cell types, the podocyte lineage in particular expresses high levels of this protein [7]. Unsurprisingly, patients with *MYH9*-related disorders can clinically present with persistent proteinuria and a progressive decline in kidney function leading to end-stage kidney disease (ESKD) [7,8]. Subsequent studies linked common *MYH9* polymorphisms to an increased risk of developing focal segmental glomerulosclerosis and non-diabetic ESKD [9,10]. However, it is worth noting that these associations were later shown to be dependent on strong linkage disequilibrium of these *MYH9* polymorphisms with variants in the apolipoprotein L1 gene (*APOL1*) [7,11]. Still, there are studies that show an association between *MYH9* polymorphisms and chronic kidney disease (CKD) independently of linkage with *APOL1*, suggesting a potential role for *MYH9* polymorphisms in the pathogenesis of ESKD [12,13].

In a recent genome-wide linkage analysis, a significant association between the *MYH9* rs11089788 polymorphism and kidney function was identified in a meta-analysis of three European populations [14]. This *MYH9* polymorphism was additionally found to be significantly associated with progressive loss of kidney function in other cohorts [13,15]. Importantly, the associations between *MYH9* rs11089788 and kidney function could not be explained by linkage disequilibrium with *APOL1* [15].

Here, we investigated the impact of the recently discovered rs11089788 *MYH9* polymorphism on long-term graft survival in the context of kidney transplantation (Fig. 1B). As a secondary outcome, we also assessed the association of this polymorphism with biopsy-proven acute rejection (BPAR) and delayed graft function (DGF).

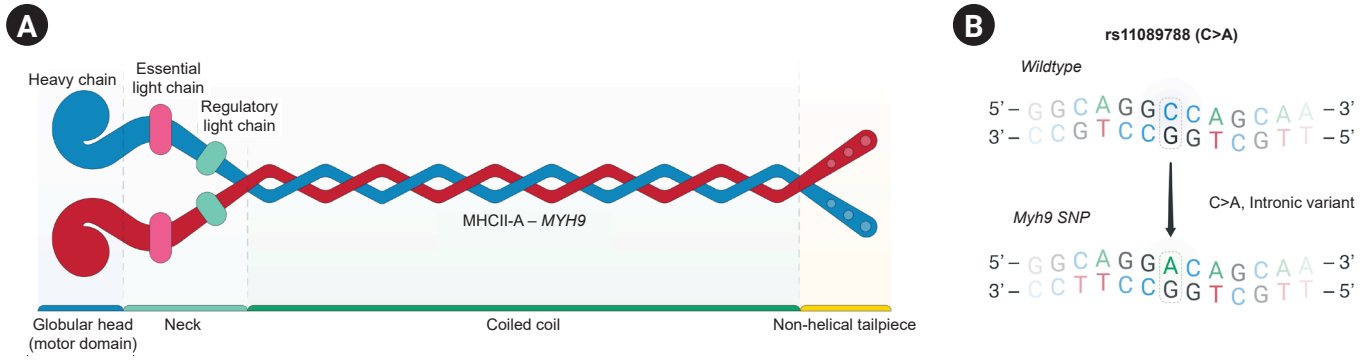

Figure 1. Illustration of the non-muscle MHCII-A and the examined *MYH9* polymorphisms. (A) Non-muscle MHCII-A is a contractile protein comprised of several domains: A globular motor head portion (heavy chain), a neck domain (essential light chain and regulatory light chain), coiled coil tail segment (MHCII-A), and non-helical tailpiece that can be phosphorylated. The coiled coil segment is notably encoded by the *MYH9*. (B) In this study, we assessed the association of rs11089788 (C>A) *MYH9* single-nucleotide polymorphism (SNPs) in kidney allograft donors and recipients with long-term graft survival outcomes.
MHCII-A, myosin heavy chain II-A; *MYH9*, myosin heavy chain 9 gene.

Methods

Patient selection and study endpoint

Patients receiving a single kidney transplantation at the University Medical Center Groningen in the Netherlands were recruited between March 1993 and February 2008. A total of 1,271 of the 1,430 screened donor-recipient kidney transplant pairs were included in this study as previously reported [16–22]. Reasons for patient exclusion were technical complications during surgery, lack of DNA, loss of follow-up, retransplantation at recruitment, and simultaneous pancreas and kidney transplantation or combined liver and kidney transplantation. The primary endpoint of this study was long-term death-censored graft survival and the maximum follow-up period was 15 years. Graft failure was defined as the need for dialysis or retransplantation. Secondary endpoints included occurrence of DGF (described by the United Network for Organ Sharing as, "The need for at least one dialysis treatment in the first week after kidney transplantation.") and BPAR (based on the Banff '07 classification).

Ethical approval for this study and the study protocol was given by the Institutional Review Board of the University Medical Center Groningen in Groningen, the Netherlands (Medical Ethical Committee 2014/077). The study protocol adhered to the Declaration of Helsinki. All subjects provided written informed consent.

DNA extraction and *MYH9* genotyping

Peripheral blood mononuclear cells from blood or splenocytes were obtained from both the donor and recipient. DNA isolation was done with a commercial kit according to the manufacturer's instructions and stored at –80 °C. Genotyping of the single-nucleotide polymorphism (SNP) was performed using the Illumina VeraCode GoldenGate Assay kit as per the manufacturer's instructions (Illumina). We opted for the *MYH9* rs11089788 C>A SNP, which has previously been associated with kidney function in healthy individuals and with disease progression in patients with CKD [13–15]. Genotype clustering and calling were performed using BeadStudio Software (Illumina). The overall genotype success rate was 99.9%, and only two samples were excluded from subsequent analyses because of a missing call rate.

Statistical analyses

IBM SPSS version 25 (IBM Corp.) was used for statistical analyses. Data are presented as the total number of patients with percentage for nominal variables, mean ± standard deviation for parametric variables, and median (interquartile range) for nonparametric variables. Differences among groups were tested with the chi-square test for categorical variables or Student t test for normally distributed variables, and the Mann-Whitney U test for not-normally divided variables, respectively. The log-rank test was used to identify differences in kidney allograft survival or rejection-free survival among the different genotypes. Logistic regression was used to assess the association of the *MYH9* polymorphism with DGF. Univariable analyses were used to examine the associations of the *MYH9* polymorphism, recipient, donor, and transplant characteristics with BPAR and death-censored graft survival. Significant associations in univariable analyses were then assessed in a multivariable Cox regression. Two-tailed tests were regarded as significant at $p < 0.05$.

Results

Study population and determinants of graft failure

All patients who underwent a single kidney transplantation at the University Medical Center Groningen were recruited for this study (n = 1,271). Baseline patient characteristics are shown in Table 1. In our cohort, there was only one case of an ABO-incompatible kidney transplantation. During the mean study period of 6.2 ± 4.2 years, 215 of 1,271 kidney transplant recipients (16.9%) developed graft failure. The main reason for graft failure was rejection (n = 126; containing acute rejection, transplant glomerulopathy, and chronic antibody-mediated rejection). Other causes for graft loss were surgical complications (n = 33), relapse of original kidney disease (n = 16), other causes (n = 16), vascular disease (n = 12), and unknown causes (n = 12). In univariable analyses, DGF, recipient age, recipient blood type (AB vs. others), donor type (living vs. cadaveric), donor age, donor blood type (AB vs. others), cold ischemia time, warm ischemia time, use of cyclosporin, and use of corticoste-

Table 1. Baseline characteristics of donor-recipient pairs

Characteristic	All patients (n = 1,271)	Functioning graft (n = 1,056)	Graft loss (n = 215)	p-value[a]	Hazard ratio	p-value[b]
Donor						
MYH9 SNP	317 (24.0)	264 (25.0)	53 (24.8)	0.97		0.98
CC	687 (54.1)	572 (54.2)	115 (53.7)			
CA	265 (20.9)	219 (20.8)	46 (21.5)			
AA						
Age (yr)	44.4 ± 14.4	44.1 ± 14.6	46.1 ± 13.4	0.04*	1.02	<0.001*
Male sex	645 (50.7)	535 (50.7)	110 (51.2)	0.89		0.96
Blood group						
Type O	642 (50.5)	541 (51.3)	101 (47.2)	0.03*	0.39	0.004*
Type A	502 (39.5)	414 (39.3)	88 (41.1)		0.42	0.01*
Type B	97 (7.6)	82 (7.8)	15 (7.0)		0.36	0.01*
Type AB	27 (2.1)	17 (1.6)	10 (4.7)		Reference	0.04*
Donor type						
Living	282 (22.2)	257 (24.3)	25 (11.6)	<0.001*	Reference	0.002*
Brain death	787 (61.9)	642 (60.8)	145 (67.4)		1.94	
Circulatory death	202 (15.9)	157 (14.9)	45 (20.9)			
Recipient						
MYH9 SNP	326 (25.7)	270 (25.6)	56 (26.2)	0.15		0.31
CC	635 (50.0)	539 (51.1)	96 (44.9)			
CA	308 (24.3)	246 (23.3)	62 (29.0)			
AA						
Age (yr)	47.9 ± 13.5	48.5 ± 13.4	45.0 ± 13.2	<0.001*	0.99	0.03*
Male sex	739 (58.1)	607 (57.5)	132 (61.4)	0.29		0.21
Primary kidney disease						
Glomerulonephritis	340 (26.8)	271 (25.6)	69 (32.2)	0.28		0.45
Polycystic disease	208 (16.4)	188 (17.8)	20 (9.3)			
Vascular disease	145 (11.4)	123 (11.6)	22 (10.3)			
Pyelonephritis	148 (11.6)	120 (11.4)	28 (13.1)			
Diabetes	51 (4.0)	44 (4.2)	7 (3.3)			
Idiopathic	168 (13.2)	134 (12.7)	34 (15.9)			
Others	211 (16.6)	177 (16.7)	34 (15.9)			
Blood group						
Type O	567 (44.6)	474 (44.9)	93 (43.3)	0.004*	0.46	0.002*
Type A	536 (42.2)	448 (42.4)	88 (40.9)		0.46	0.002*
Type B	113 (8.9)	98 (9.3)	15 (7.0)		0.35	0.002*
Type AB	55 (4.3)	36 (3.4)	19 (8.8)		Reference	0.008*
Dialysis vintage (wk)	172 (91–263)	174 (87–261)	168 (109–270)	0.15		0.10
Highest PRA (%)	10.1 ± 23.6	10.0 ± 23.3	10.9 ± 25.0	0.60		0.75
Antihypertensives	1,131 (89.0)	945 (89.5)	186 (86.5)	0.20		0.36
Induction immunosuppression						
Anti-CD3 MoAb	19 (1.5)	14 (1.3)	5 (2.3)	0.27		0.51
ATG	103 (8.1)	79 (7.5)	24 (11.2)	0.07		0.14
Interleukin-2 RA	199 (15.7)	163 (15.4)	36 (16.7)	0.63		0.12

(Continued to the next page)

Table 1. Continued

Characteristic	All patients (n = 1,271)	Functioning graft (n = 1,056)	Graft loss (n = 215)	p-value[a]	Hazard ratio	p-value[b]
Maintenance immunosuppression						
Azathioprine	72 (5.7)	53 (5.0)	19 (8.8)	0.03*		0.29
Corticosteroids	1,201 (94.5)	1,002 (94.9)	199 (92.6)	0.17	0.51	0.01*
Cyclosporin	1,085 (85.4)	911 (86.3)	174 (80.9)	0.04*	0.66	0.02*
Mycophenolic acid	907 (71.4)	775 (73.4)	132 (61.4)	<0.001*		0.06
Sirolimus	38 (3.0)	33 (3.1)	5 (2.3)	0.53		0.54
Tacrolimus	97 (7.6)	77 (7.3)	20 (9.3)	0.31		0.39
Transplantation						
CIT (hr)	17.7 (10.9–23.0)	17.0 (8.6–23.0)	20.0 (15.3–25.0)	<0.001*	1.03	0.001*
WIT (min)	37.0 (31–45)	37.0 (30–45)	38.0 (32–45)	0.12	1.02	0.003*
Total HLA mismatches	2 (1–3)	2 (1–3)	2 (1–3)	0.48		0.11
DGF	415 (32.7)	289 (27.4)	126 (58.6)	<0.001*	3.79	<0.001*

Data are expressed as number (%), mean ± standard deviation, or median (interquartile range).
ATG, anti-thymocyte globulin; CD3, cluster of differentiation 3; CIT, cold ischemia time; DGF, delayed graft function; *MYH9*, myosin heavy chain 9 gene; HLA, human leukocyte antigen; PRA, panel-reactive antibody; RA, receptor antagonist; SNP, single-nucleotide polymorphism; WIT, warm ischemia time.
[a]p-value for the differences in baseline characteristics between the groups, tested by Student t test or the Mann-Whitney U test for continuous variables, with the chi-square test for categorical variables; [b]p-value for univariable analysis with 15-year death-censored graft survival.
*p < 0.05, statistically significant.

roids were all associated with graft failure (p < 0.05).

Distribution of the *MYH9* polymorphism

The observed genotypic frequencies of the *MYH9* SNP (rs11089788 C>A) did not differ between donors (n = 1,269; CC, 25.0%; CA, 54.1%; AA, 20.9%) and recipients (n = 1,269; CC, 25.7%; CA, 50.0%; AA, 24.3%; p = 0.07). The distribution of the SNP was in Hardy-Weinberg equilibrium. Compared with the 1000 Genomes Project, the genotypic frequencies of the *MYH9* polymorphism in recipients and donors were significantly different (p < 0.001) [23]. In both recipients and donors, the A-allele of the *MYH9* SNP was more prevalent than the reported allele and genotype frequencies in the 1000 Genomes Project. The percentage of kidney allografts with DGF significantly differed based on the recipient *MYH9* genotype (33.7% in CC, 29.6% in CA, 37.7% in AA; p = 0.04), but not for the donor *MYH9* genotype (p = 0.93). For further analysis, heterozygotes (CA) and homozygotes (CC) genotypes were combined into one group (CA/CC). In logistic regression, recipients carrying the AA-genotype *MYH9* polymorphism had a significantly elevated risk of DGF (odds ratio [OR], 1.34) compared to CA/CC-genotype recipients (95% confidence interval [CI], 1.03–1.76; p = 0.03). In multivariable logistic regression, the AA genotype of the *MYH9* polymorphism in recipients was no longer significantly associated with DGF occurrence (OR, 1.26) compared with CA/CC-genotype recipients (95% CI, 0.92–1.72; p = 0.15) (Table 2). There was no difference in the overall BPAR frequency among the *MYH9* genotypes in the donors (34.7% in CC, 33.0% in CA, 35.8% in AA; p = 0.69). In contrast, the distribution of the *MYH9* polymorphism in recipients showed a trend toward higher risk of BPAR (31.6% in CC, 32.4% in CA, 39.3% in AA; p = 0.07) (Fig. 2A). A significant association was found with BPAR when the AA genotype of the *MYH9* polymorphism in the recipient was compared to CA and CC genotypes (39.3% in AA vs. 32.2% in CA/CC; p = 0.02) (Fig. 2B). In multivariable Cox regression, the AA genotype of the *MYH9* polymorphism in recipients was no longer significantly associated with BPAR occurrence (hazard ratio [HR], 1.22) compared with CA/CC-genotype recipients (95% CI, 0.97–1.54; p = 0.10) (Table 3). In summary, although the AA-genotype *MYH9* polymorphism in recipients was associated with DGF and BPAR, the significance was lost when correcting for potential confounders.

Table 2. Logistic regression analysis for the risk of delayed graft function

Variable	p-value	Odds ratio (95% CI)
MYH9 rs111089788 SNP in the recipient (AA vs. CA/CC)	0.15	1.26 (0.92–1.72)
Donor age (yr)	<0.001	1.02 (1.01–1.03)
Donor sex (male vs. female)	0.001	1.61 (1.22–2.13)
Donor type (deceased vs. living)	0.001	31.61 (4.14–214.57)
Total HLA mismatches	0.006	1.16 (1.04–1.30)
Dialysis vintage (wk)	0.007	1.08 (1.02–1.14)
Warm ischemia time (min)	0.02	1.01 (1.00–1.03)
Recipient age (yr)	0.44	1.00 (0.99–1.02)
Cold ischemia time (hr)	0.47	1.00 (1.00–1.00)

Multivariable logistic regression was performed for delayed graft function after kidney transplantation. Only variables with a p-value of <0.05 in the univariable analysis were included. Donor age, donor sex, donor type, total HLA mismatches, dialysis vintage, and warm ischemia time were significant, whereas the MYH9 SNP (rs11089788) in the recipient, recipient age, and cold ischemia time were not.
CI, confidence interval; HLA, human leukocyte antigen; *MYH9*, myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism.

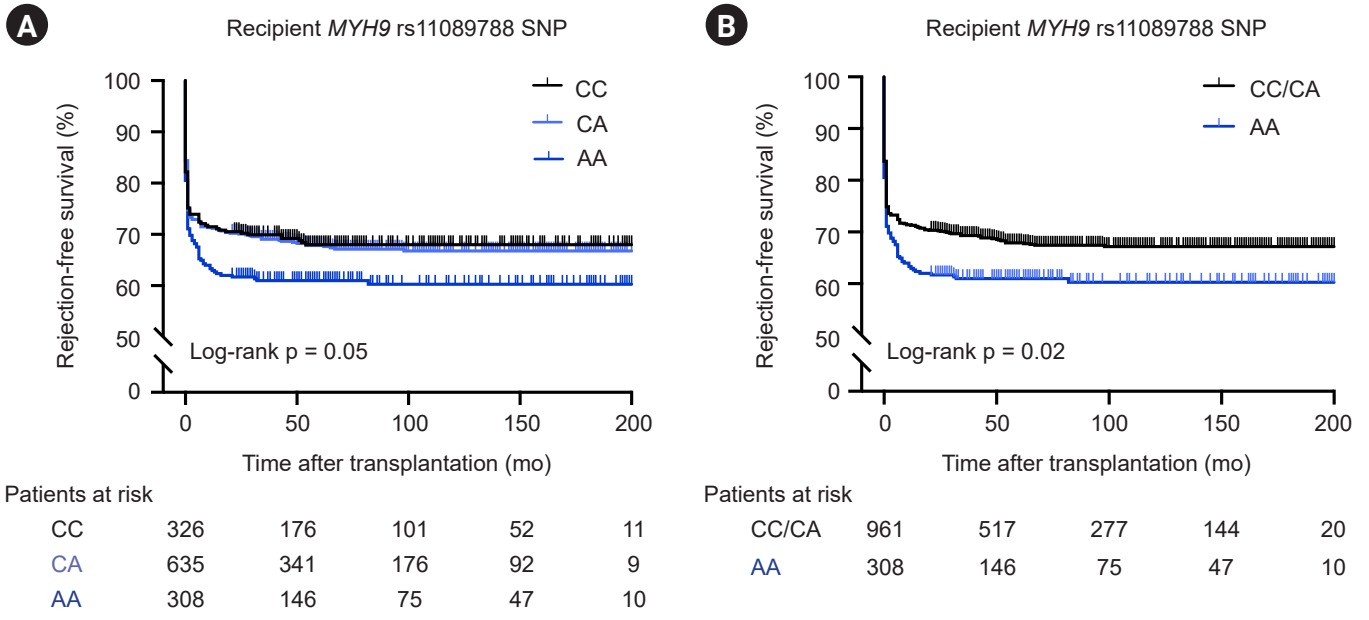

Figure 2. Kaplan-Meier curves for rejection-free survival of kidney allografts according to the presence of a non-muscle *MYH9* polymorphism in the recipient. (A) Cumulative rejection-free survival of kidney allografts according to the presence of the *MYH9* single-nucleotide polymorphism (SNP) rs11089788 in the recipient. (B) Cumulative rejection-free survival of kidney allografts in recipients with the AA genotype of the MYH9 SNP rs11089788 vs. the AC/CC genotype. Log-rank test was used to compare the incidence of biopsy-proven rejection between the groups.
MYH9, myosin heavy chain 9 gene.

Long-term kidney graft survival based on the *MYH9* genotypes

Kaplan-Meier survival analysis showed no association between the *MYH9* SNP in the recipient or the donor and death-censored kidney graft survival (Fig. 3). However, a trend was seen for a heightened rate of graft failure in recipients with an AA genotype of the *MYH9* polymorphism compared with CA- and CC-genotype recipients (graft loss, 33.2% in AA vs. 24.1% in CA/CC; p = 0.06) (Fig. 3B). Next, donor-recipient pairs were separated into four groups according to the presence or absence of the AA genotype

Table 3. Multivariable analysis for the risk of biopsy-proven acute rejection

Variable	p-value	Hazard ratio (95% CI)
MYH9 rs111089788 SNP in the recipient (AA vs. CA/CC)	0.10	1.22 (0.97–1.54)
Recipient age (yr)	<0.001	0.97 (0.97–0.98)
Total HLA mismatches	<0.001	1.20 (1.11–1.29)
Delayed graft function (yes vs. no)	0.02	1.31 (1.05–1.62)
Recipient sex (female vs. male)	0.04	1.25 (1.01–1.55)
Warm ischemia time (min)	0.08	0.99 (0.98–1.00)

Multivariable Cox regression was performed for biopsy-proven acute rejection after kidney transplantation. Only variables with a p < 0.05 in the univariable analysis were included. Recipient age, total HLA mismatches, delayed graft function, and recipient sex were significant, whereas the *MYH9* polymorphism (rs11089788) in the recipient and warm ischemia time were not.
CI, confidence interval; HLA, human leukocyte antigen; *MYH9*, myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism.

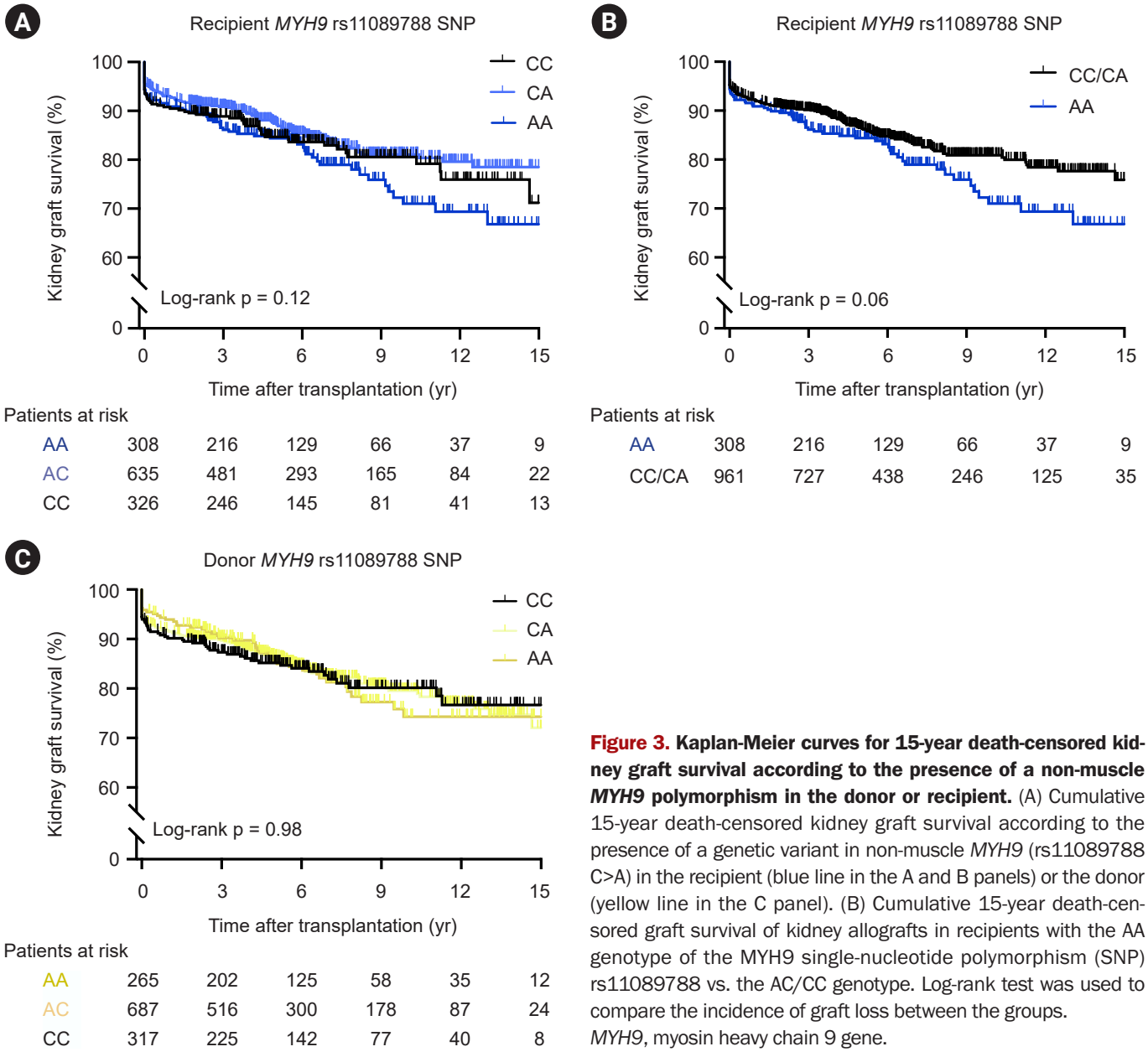

Figure 3. Kaplan-Meier curves for 15-year death-censored kidney graft survival according to the presence of a non-muscle *MYH9* polymorphism in the donor or recipient. (A) Cumulative 15-year death-censored kidney graft survival according to the presence of a genetic variant in non-muscle *MYH9* (rs11089788 C>A) in the recipient (blue line in the A and B panels) or the donor (yellow line in the C panel). (B) Cumulative 15-year death-censored graft survival of kidney allografts in recipients with the AA genotype of the MYH9 single-nucleotide polymorphism (SNP) rs11089788 vs. the AC/CC genotype. Log-rank test was used to compare the incidence of graft loss between the groups.
MYH9, myosin heavy chain 9 gene.

of the *MYH9* polymorphism in the donor and recipient. Kaplan-Meier survival analyses showed a significant difference in graft failure rates among the four groups (p = 0.04) (Fig. 4A). Intriguingly, the AA genotype of the *MYH9* polymorphism in the donor seemed to have a marginal positive impact on graft survival, whereas the AA genotype in the recipient had a modest detrimental impact compared with donor-recipient pairs with the combined CC/CA genotype. Recipients with an AA genotype receiving a graft with an AA genotype had the worst outcomes. This combined genotype was identified in 6.3% of the donor-recipient pairs. Moreover, the significant association with graft failure increased when the combined AA genotype of the *MYH9* polymorphism in donor-recipient pairs was compared with other groups (p = 0.01) (Fig. 4B). The cumulative 15-year death-censored kidney allograft survival was 50.4% in this combined AA-genotype group and 74.9% in the reference group. The association of the combined *MYH9* AA-genotype group with long-term graft survival was maintained when primary non-function cases were excluded (p = 0.001) (Supplementary Fig. 1, available online), demonstrating that the association between the *MYH9* rs11089788 polymorphism and graft failure is independent of early graft failure. These data suggest that matching donor-recipient pairs on the *MYH9* polymorphism may impact long-term graft survival in kidney transplantation.

Kaplan-Meier survival analyses for the combined AA genotype of the *MYH9* polymorphism in donor-recipient pairs were reestimated for patients transplanted in the 1990s and 2000s because immunosuppression has improved through time, and this could influence the risk of graft loss. In these subgroups, the significance was maintained in patients transplanted after 2000 (p = 0.04) (Supplementary Fig. 2, available online), while a trend

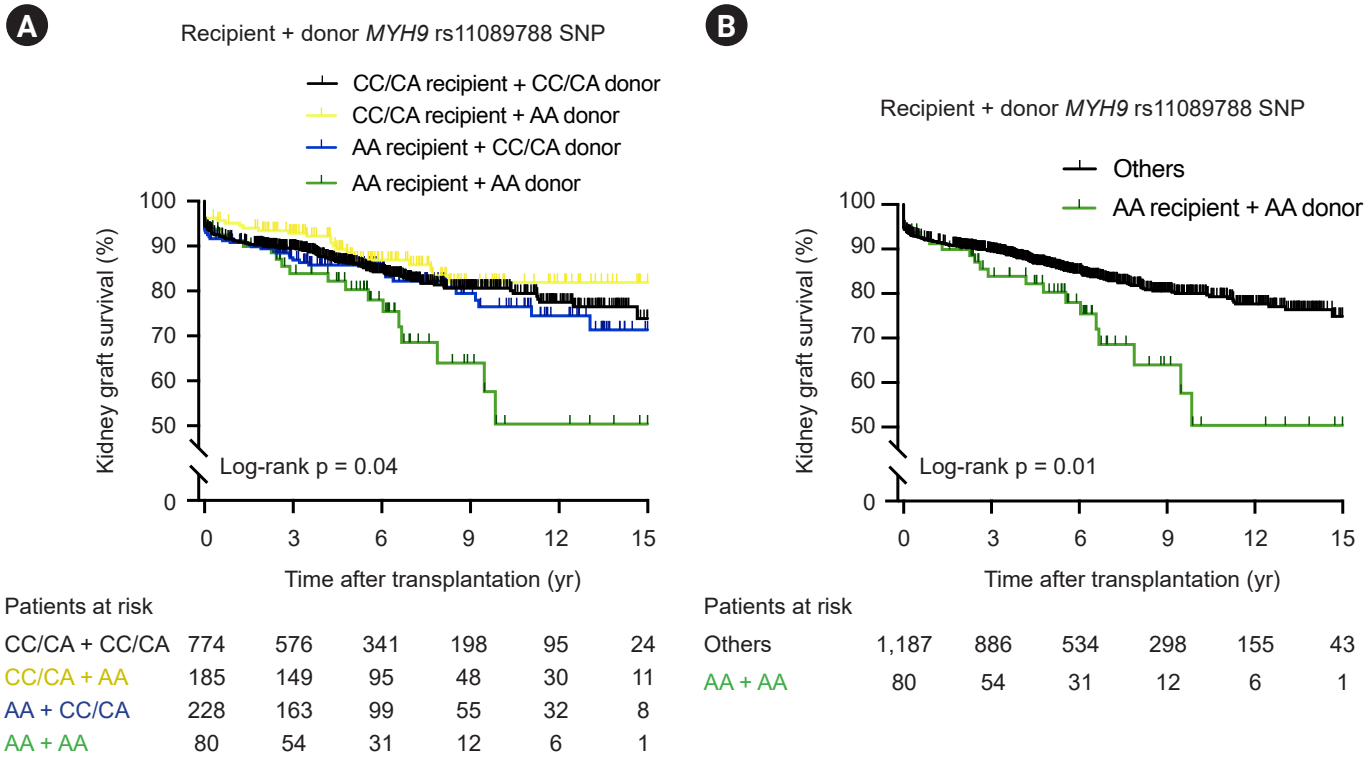

Figure 4. Kaplan-Meier curves for 15-year death-censored kidney graft survival according to the presence of a non-muscle *MYH9* polymorphism in donor-recipient pairs. Cumulative 15-year death-censored kidney graft survival is shown according to the presence of the *MYH9* polymorphism in donor-recipient pairs. (A) Pairs were divided into four groups according to the absence (black line) or presence of the AA genotype in the recipient (blue line), donor (yellow line), or both (green line). (B) The presence of the AA genotype in both the recipient and donor (green line) was compared to the rest (black line). Log-rank test was used to compare the incidence of graft loss between the groups.
MYH9, myosin heavy chain 9 gene; SNP single-nucleotide polymorphism.

was seen in patients transplanted before 2001 (p = 0.10) (Supplementary Fig. 2, available online). Nevertheless, in accordance with our previous results, the combined AA genotype of the *MYH9* polymorphism in donor-recipient pairs remained harmful for long-term graft survival.

Regression analysis for the *MYH9* polymorphism in donor-recipient pairs and graft failure

Finally, we investigated whether the *MYH9* variant in donor-recipient pairs is an independent risk factor for graft failure. In univariable analysis, the combined AA genotype of the *MYH9* SNP in donor-recipient pairs was associated with a hazard ratio of 1.78 (95% CI, 1.13–2.79; p = 0.01) for graft failure after complete follow-up. We then determined whether the baseline characteristics differed between the donor-recipient pairs with the combined AA genotype of the *MYH9* SNP and those with other *MYH9* genotypes (Table 4). The proportion of living donor kidney transplants was significantly higher in the combined AA-genotype group (p = 0.001), and linked to this finding, the median cold ischemia time was significantly lower for donor-recipient pairs with the combined AA-genotype group (p = 0.002). Furthermore, the total number of human leukocyte antigen (HLA) mismatches was significantly higher in the combined AA-genotype group (p = 0.004). However, the total number of HLA mismatches was not significantly associated with graft loss in univariable analysis (p = 0.11) (Table 1). Furthermore, when we adjusted for HLA mismatches, the hazard ratio and significance increased for the association between the combined AA genotype of the *MYH9* SNP in donor-recipient pairs and graft loss (HR, 2.02; 95% CI, 1.26–3.26; p = 0.004). Also, the total number of HLA mismatches was not statistically different between patients who experienced graft failure in the combined AA-genotype group compared with those with graft failure in the other group (p = 0.37) (Supplementary Table 1, available online). Hence, although a difference was detected in the total number of HLA mismatches between the combined AA-genotype group and the other genotypes group, it seems unlikely that this is a confounder given the association between the *MYH9* genotype and allograft outcome.

Next, multivariable analysis was performed to adjust for other potential confounders, including donor and patient characteristics as well as transplant variables (Table 5). In these Cox regression analyses, the combined AA genotype of the *MYH9* SNP in donor-recipient pairs remained significantly associated with graft failure. We also performed a multivariable Cox regression analysis using all variables that were significantly associated with graft failure in univariable analysis (Table 6). In this model, the *MYH9* SNP (rs11089788) in donor-recipient pairs, DGF occurrence, recipient age, and donor age were all significantly associated with graft loss. After adjustment, the hazard ratio for graft failure of the combined AA genotype for the *MYH9* SNP in donor-recipient pairs was 1.68 (95% CI, 1.05–2.70; p = 0.03). Our results reveal that recipients with an AA genotype of the *MYH9* SNP receiving a kidney allograft with an AA genotype have a significantly elevated risk of graft failure after kidney transplantation.

Finally, we analyzed the causes of allograft failure among the different groups to uncover the potential mechanism by which the combined *MYH9* AA genotype lowers long-term allograft survival. We did not, however, find any major differences in the causes of graft loss between the donor-recipient with the combined AA genotype and those with other *MYH9* genotypes (Supplementary Table 2, available online). Additionally, there was no significant difference in the percentage of rejection-related graft loss between the two groups (71.4% in the combined AA genotype vs. 60.1% in the other genotypes; p = 0.31).

Discussion

A multitude of strategies can be pursued to improve long-term outcomes after kidney transplantation, ranging from the development of novel drugs that can halt alloimmune cascades, to the refinement of donor-recipient matching systems to minimize the severity of allograft recognition. Regarding allograft matching, HLA-centric systems remain the cornerstone of allocating kidney allografts, although a paradigm shift in the approach to donor-recipient matching is urgently needed [24]. Genetic analyses in transplantation provides a particularly unique opportunity for the development of innovative strategies that can improve donor-recipient pairing and drive personalized medicine, in part by enabling individualized risk stratification [25,26]. Presently, we report the impact of a recently discovered polymorphism in *MYH9* on long-term kidney allograft survival. The key finding of our study is that recipients with an

Table 4. Baseline characteristics of donor-recipient pairs based on their *MYH9* genotype

Characteristic	All patients (n = 1,271)	AA–AA pair (n = 80)	Other pairs (n = 1,187)	p-value[a]
Donor				
Age (yr)	44.4 ± 14.4	46.8 ± 12.9	44.2 ± 14.5	0.12
Male sex	645 (50.7)	43 (53.8)	601 (50.6)	0.59
Blood group				
Type O	642 (50.5)	40 (50.0)	600 (50.7)	0.93
Type A	502 (39.5)	32 (40.0)	469 (39.6)	
Type B	97 (7.6)	7 (8.8)	89 (7.5)	
Type AB	27 (2.1)	1 (1.3)	26 (2.2)	
Donor type				
Living	282 (22.2)	30 (37.5)	251 (21.1)	0.001*
Brain death	787 (61.9)	35 (43.8)	749 (63.1)	
Circulatory death	202 (15.9)	15 (18.8)	187 (15.8)	
Recipient				
Age (yr)	47.9 ± 13.5	48.1 ± 13.1	47.9 ± 13.5	0.91
Male sex	739 (58.1)	43 (53.8)	694 (58.5)	0.41
Blood group				
Type O	567 (44.6)	31 (38.8)	534 (45.0)	0.38
Type A	536 (42.2)	34 (42.5)	501 (42.2)	
Type B	113 (8.9)	11 (13.8)	101 (8.5)	
Type AB	55 (4.3)	4 (5.0)	51 (4.3)	
Dialysis vintage (wk)	172 (91–263)	169 (74–267)	173 (91–262)	0.67
Highest PRA (%)	10.1 ± 23.6	9.3 ± 23.2	10.2 ± 23.6	0.78
Induction immunosuppression				
Anti-CD3 MoAb	19 (1.5)	1 (1.3)	18 (1.5)	0.85
ATG	103 (8.1)	6 (7.5)	97 (8.2)	0.83
Interleukin-2 RA	199 (15.7)	15 (18.8)	184 (15.5)	0.44
Maintenance immunosuppression				
Azathioprine	72 (5.7)	5 (6.3)	67 (5.6)	0.82
Corticosteroids	1,201 (94.5)	78 (97.5)	1,119 (94.3)	0.22
Cyclosporin	1,085 (85.4)	69 (86.3)	1,012 (85.3)	0.81
Mycophenolic acid	907 (71.4)	56 (70.0)	847 (71.4)	0.80
Sirolimus	38 (3.0)	3 (3.8)	35 (2.9)	0.68
Tacrolimus	97 (7.6)	8 (10.0)	89 (7.5)	0.42
Transplantation				
CIT (hr)	17.7 (10.9–23.0)	15.5 (2.8–20.0)	18.0 (11.5–23.0)	0.002*
WIT (min)	37.0 (31.0–45.0)	36.5 (30.3–45.0)	37.0 (31.0–45.0)	0.90
Total HLA mismatches	2 (1–3)	3 (1–3)	2 (1–3)	0.004*
DGF	415 (32.7)	33 (41.3)	380 (32.0)	0.09

Data are expressed as number (%), mean ± standard deviation, or median (interquartile range).
ATG, anti-thymocyte globulin; CD3, cluster of differentiation 3; CIT, cold ischemia time; DGF, delayed graft function; HLA, human leukocyte antigen; MoAb, monoclonal antibody; *MYH9*, myosin heavy chain 9 gene; PRA, panel-reactive antibody; RA, receptor antagonist; WIT, warm ischemia time.
[a]p-value for the differences in baseline characteristics between the groups, tested by Student t test or the Mann-Whitney U test for continuous variables, with the chi-square test for categorical variables.
*$p < 0.05$, statistically significant.

AA genotype of the *MYH9* rs11089788 variant receiving a kidney allograft with an AA genotype of the same variant, have a significantly elevated risk of developing graft loss. In contrast, no association for the *MYH9* polymorphism with long-term allograft survival was found in either the recipient or donor when assessed individually. Hence, our study provides evidence that matching recipients with donor kidneys based on the *MYH9* polymorphism may well impact the risk of graft loss.

To our knowledge, our study is the first to show an association between this *MYH9* variant and long-term graft survival after kidney transplantation. Specifically, we found that the combined AA genotype in donor-recipient pairs nearly doubled the risk of graft failure. Genome-wide linkage analysis recently highlighted the *MYH9* rs11089788 polymorphism as a top variant for kidney function in a meta-analysis of three European populations [14]. In accordance with our results, the C-allele of the *MYH9* rs11089788 polymorphism was consistently associated with better kidney function in healthy Europeans [14]. Furthermore, in a Chinese cohort of immunoglobulin A nephropathy patients, the A-allele of this variant was associated with hastened progression to kidney failure [13]. Other groups, however, did not recapitulate an association between this *MYH9* variant and kidney outcomes [27,28]. In particular, Franceschini et al. [28] found no relationship between the *MYH9* rs11089788 polymorphism and kidney function or CKD in native Americans. Importantly, we also found no relationship between this *MYH9* variant in the recipient or the donor alone with death-censored kidney graft survival. Our findings, thus, suggest that only donor-recipient interactions in *MYH9* may lead to kidney function decline after renal transplantation.

The importance of the *MYH9* for the kidney has been investigated by several groups but remains controversial. Initial reports linked certain variants in the *MYH9* to a greater risk of CKD [9,10]. Later studies uncovered that this association was based on strong linkage disequilibrium between *MYH9* variants and variants in *APOL1* [7,11].

Table 5. Associations of *MYH9* polymorphism with graft loss

Model	*MYH9* SNP (rs1800472) in donor-recipient pairs	
	Hazard ratio[a] (95% CI)	p-value
1	1.78 (1.13–2.79)	0.01
2	1.90 (1.19–3.02)	0.007
3	1.95 (1.24–3.08)	0.004
4	1.91 (1.16–3.12)	0.01

Model 1, crude model; model 2, adjusted for model 1 plus recipient characteristics (recipient age, recipient sex, recipient blood type, and dialysis vintage); model 3, adjusted for model 1 plus donor characteristics (donor age, donor sex, donor blood type, and donor origin); model 4: adjusted for model 1 plus transplant characteristics (cold and warm ischemia time, and the number of human leukocyte antigen-mismatches).
CI, confidence interval; *MYH9*, myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism.
[a]AA + AA vs. others.

Table 6. Multivariable analysis for the risk of graft loss

Variable	p-value	Hazard ratio (95% CI)
rs111089788 in donor-recipient pairs (AA + AA vs. others)	0.03	1.68 (1.05–2.70)
Delayed graft function (yes vs. no)	<0.001	3.47 (2.56–4.72)
Recipient age (yr)	<0.001	0.98 (0.97–0.99)
Donor age (yr)	0.001	1.02 (1.01–1.03)
Recipient blood type (AB vs. others)	0.06	NA
Warm ischemia time (min)	0.12	1.01 (1.00–1.02)
Corticosteroids	0.20	1.53 (0.80–2.95)
Cold ischemia time (hr)	0.32	1.00 (1.00–1.00)
Donor type (living vs. deceased)	0.41	0.76 (0.39–1.46)
Cyclosporin A	0.71	1.04 (0.71–1.66)
Donor blood type (AB vs. others)	0.90	NA

Multivariable Cox regression was performed for kidney graft survival. Only variables with a $p < 0.05$ in the univariable analysis were included. In the final model, the *MYH9* SNP (rs11089788) in donor-recipient pairs, the occurrence of delayed graft function, recipient age, and donor age were significant, whereas recipient blood type, warm ischemia time, use of corticosteroids, cold ischemia time, donor type, use of cyclosporin A, and donor blood type were not.
CI, confidence interval; *MYH9*, non-muscle myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism; NA, not available.

Nonetheless, patients with rare mutations in *MYH9* leading to *MYH9*-related diseases often present with signs of CKD and can develop ESKD [7,8]. Consistent with these results, heterozygous mice with mutations in *Myh9* manifest similar pathological kidney phenotypes as humans with *MYH9*-related diseases, including proteinuria, focal segmental glomerulosclerosis, and CKD [29]. Intriguingly, *Myh9* knockdown in zebrafish lead to the malformation and dysfunction of their glomeruli [30]. More specifically, these zebrafish failed to correctly develop the glomerular capillary structure, lacking fenestration in the endothelial cells and having an absence or reduced number of mesangial cells together with irregular thickening of the glomerular basement membrane [30]. Although kidney clearance experiments showed that the glomerular barrier function remained unaltered, glomerular filtration in these zebrafish was significantly reduced [30]. Altogether, these findings demonstrate a key role for *MYH9* and non-muscle MHCII-A in kidney development and physiology.

In humans, non-muscle myosin II-A, whose heavy chains are encoded by *MYH9*, is expressed in the podocytes, tubular cells, endothelial cells of the peritubular capillaries, interlobular arteries, and arterioles [31]. A potential mechanism underpinning the association between *MYH9* polymorphism and graft failure would likely be dependent on kidney-expressed non-muscle MHCII-A. On the basis of our findings, however, alternative mechanisms may be more probable. Firstly, in the recipients, a trend was found for the association between the *MYH9* polymorphism and graft loss, while there was no association in the donor genotypes. Secondly, the AA genotype of the *MYH9* variant in the recipient, but not the donor, was associated with BPAR and DGF, although significance was lost after adjusting for potential confounders. Lastly, in the genotypic analysis of the donor-recipient pairs, the isolated donor AA genotype was marginally protective while the isolated AA genotype in the recipient had a modest detrimental effect on graft survival. Additional evidence supporting a systemic role of the *MYH9* variant in determining kidney allograft outcomes is provided by a case report of a patient with focal segmental glomerulosclerosis where proteinuria rapidly recurred following a deceased donor kidney transplantation that therapeutically responded to plasmapheresis [32]. Moreover, the fact that donor-recipient pairs with the combined AA genotype of the *MYH9* variant had the highest risk of graft loss in our population suggests both donor-recipient interactions in *MYH9* with perhaps a leading role for extra-renal expressed non-muscle MHCII-A. A case report of two kidney transplants in pediatric patients suggested a similar donor-recipient *MYH9* interaction [33].

There is ongoing debate about whether DGF affects long-term allograft outcomes in kidney transplantation. Recently, Phillips et al. [34] demonstrated that DGF duration, rather than DGF occurrence itself, negatively impacted graft and patient survival after kidney transplantation. In accordance with our results, Phillips et al. [34] found that DGF occurrence was associated with long-term graft survival in univariable analysis. However, after adjustment for other characteristics, the significance was lost, whereas in our study DGF occurrence remained significant in multivariable analysis. There are several differences between our study and Phillips et al. [34] that need to be considered. Firstly, Phillips et al. [34] only focused on renal allografts from donation after circulatory death donors, whereas our study also included renal allografts from living donors and brain-dead donors. Secondly, there is a gap in the transplantation era between the two studies. Our study includes kidney transplantation between 1993 and 2008, whereas Phillips et al. [34] include kidney transplantation between 2006 and 2016. Thirdly, there are important differences in how the multivariable models were constructed. Due to their larger sample size, Phillips et al. [34] were able to adjust for more covariates, however, we corrected for covariates that they did not. They also used different methods of multivariable analysis than we did. Additionally, their follow-up was shorter than ours. Altogether, these differences most likely explain the different results, nevertheless, we do not doubt that DGF duration, rather than occurrence, is a better outcome predictor.

Our study has several limitations that warrant consideration. First, our study design is observational in nature and thus cannot determine whether associations are based on causality. Therefore, we cannot exclude the possibility that the *MYH9* rs11089788 variant is a tag SNP in the neighboring *APOL1*-to-*APOL6* region, justifying further investigation in this regard. Second, we investigated a single polymorphism in *MYH9* and did not examine the impact of *MYH9* haplotypes. Third, we could not investigate whether the association between the *MYH9* variant and BPAR differed for T-cell mediated rejection or antibody-mediated

rejection, due to the lack of a standardized assay over the years for donor-specific antibodies determination. Forth, we cannot exclude ethnic differences in the associations between the *MYH9* variant and graft outcomes, because we studied donor-recipient pairs from a single center in the Netherlands. Fifth, information on certain comorbidities such as cardiovascular disease was lacking. Nevertheless, crucial strengths of our study were the analysis of the recently described *MYH9* polymorphism in both donors and recipients, our large patient population, the long and complete follow-up, and the hard clinical endpoints.

In conclusion, we found that patients with an AA genotype of the *MYH9* rs11089788 variant receiving a donor kidney with the AA genotype had an elevated risk of late graft loss. Considering the impact of this combined genotype, our findings suggest that donor-recipient interactions in *MYH9* negatively influence the long-term allograft survival of kidney allografts.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

The authors thank the members of the REGaTTA cohort (REnal GeneTics TrAnsplantation; University Medical Center Groningen, University of Groningen, Groningen, the Netherlands): Bakker SJL, van den Born J, de Borst MD, van Goor H, Hillebrands JL, Hepkema BG, Navis GJ, and Snieder H.

Data sharing statement

The data presented in this study are available on request from the corresponding author.

Authors' contributions

Conceptualization: JD, MAS
Data curation, Formal analysis: FP, BF, MGC
Investigation: FP, JD
Visualization: SKE
Writing–original draft: FP, SKE, BF, MGC
Writing–review & editing: All authors

All authors read and approved the final manuscript.

ORCID

Felix Poppelaars, https://orcid.org/0000-0002-5173-8078
Siawosh K. Eskandari, https://orcid.org/0000-0003-4867-4170
Jeffrey Damman, https://orcid.org/0000-0001-5997-7551
Marc A. Seelen, https://orcid.org/0000-0003-2240-4682
Bernardo Faria, https://orcid.org/0000-0001-9112-8920
Mariana Gaya da Costa, https://orcid.org/0000-0002-9201-5108

References

1. Ibrahim HN, Murad DN, Knoll GA. Thinking outside the box: novel kidney protective strategies in kidney transplantation. *Clin J Am Soc Nephrol* 2021;16:1890–1897.
2. Fiorentino M, Gallo P, Giliberti M, et al. Management of patients with a failed kidney transplant: what should we do? *Clin Kidney J* 2020;14:98–106.
3. Langewisch E, Mannon RB. Chronic allograft injury. *Clin J Am Soc Nephrol* 2021;16:1723–1729.
4. Mayrdorfer M, Liefeldt L, Wu K, et al. Exploring the complexity of death-censored kidney allograft failure. *J Am Soc Nephrol* 2021;32:1513–1526.
5. Farouk S, Zhang Z, Menon MC. Non-HLA donor-recipient mismatches in kidney transplantation: a stone left unturned. *Am J Transplant* 2020;20:19–24.
6. Otten HG, Joosten I, Allebes WA, et al. The PROCARE consortium: toward an improved allocation strategy for kidney allografts. *Transpl Immunol* 2014;31:184–190.
7. Pecci A, Ma X, Savoia A, Adelstein RS. MYH9: structure, functions and role of non-muscle myosin IIA in human disease. *Gene* 2018;664:152–167.
8. Tabibzadeh N, Fleury D, Labatut D, et al. MYH9-related disorders display heterogeneous kidney involvement and outcome. *Clin Kidney J* 2018;12:494–502.
9. Kao WH, Klag MJ, Meoni LA, et al. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. *Nat Genet* 2008;40:1185–1192.
10. Kopp JB, Smith MW, Nelson GW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet* 2008;40:1175–1184.
11. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 2010;329:841–845.

12. O'Seaghdha CM, Parekh RS, Hwang SJ, et al. The MYH9/APOL1 region and chronic kidney disease in European-Americans. *Hum Mol Genet* 2011;20:2450–2456.
13. Cheng W, Zhou X, Zhu L, et al. Polymorphisms in the nonmuscle myosin heavy chain 9 gene (MYH9) are associated with the progression of IgA nephropathy in Chinese. *Nephrol Dial Transplant* 2011;26:2544–2549.
14. Pattaro C, Aulchenko YS, Isaacs A, et al. Genome-wide linkage analysis of serum creatinine in three isolated European populations. *Kidney Int* 2009;76:297–306.
15. Voskarides K, Demosthenous P, Papazachariou L, et al. Epistatic role of the MYH9/APOL1 region on familial hematuria genes. *PLoS One* 2013;8:e57925.
16. Damman J, Kok JL, Snieder H, et al. Lectin complement pathway gene profile of the donor and recipient does not influence graft outcome after kidney transplantation. *Mol Immunol* 2012;50:1–8.
17. Damman J, Nijboer WN, Schuurs TA, et al. Local renal complement C3 induction by donor brain death is associated with reduced renal allograft function after transplantation. *Nephrol Dial Transplant* 2011;26:2345–2354.
18. Dessing MC, Kers J, Damman J, Navis GJ, Florquin S, Leemans JC. Donor and recipient genetic variants in NLRP3 associate with early acute rejection following kidney transplantation. *Sci Rep* 2016;6:36315.
19. Poppelaars F, Gaya da Costa M, Eskandari SK, Damman J, Seelen MA. Donor genetic variants in interleukin-6 and interleukin-6 receptor associate with biopsy-proven rejection following kidney transplantation. *Sci Rep* 2021;11:16483.
20. Poppelaars F, Gaya da Costa M, Faria B, Eskandari SK, Damman J, Seelen MA. A functional TGFB1 polymorphism in the donor associates with long-term graft survival after kidney transplantation. *Clin Kidney J* 2021;15:278–286.
21. Poppelaars F, Gaya da Costa M, Faria B, Eskandari SK, Seelen MA, Damman J. Tumor necrosis factor-α gene polymorphism is associated with short- and long-term kidney allograft outcomes. *J Inflamm Res* 2022;15:2243–2254.
22. Eskandari SK, Gaya da Costa M, Faria B, et al. An interleukin 6-based genetic risk score strengthened with interleukin 10 polymorphisms associated with long-term kidney allograft outcomes. *Am J Transplant* 2022;22 Suppl 4:45–57.
23. 1000 Genomes Project Consortium; Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature* 2015;526:68–74.
24. Montgomery RA, Tatapudi VS, Leffell MS, Zachary AA. HLA in transplantation. *Nat Rev Nephrol* 2018;14:558–570.
25. Zanoni F, Kiryluk K. Genetic background and transplantation outcomes: insights from genome-wide association studies. *Curr Opin Organ Transplant* 2020;25:35–41.
26. Yang JY, Sarwal MM. Transplant genetics and genomics. *Nat Rev Genet* 2017;18:309–326.
27. Pazik J, Lewandowski Z, Oldak M, et al. Association of MYH9 rs3752462 and rs5756168 polymorphisms with transplanted kidney artery stenosis. *Transplant Proc* 2016;48:1561–1565.
28. Franceschini N, Voruganti VS, Haack K, et al. The association of the MYH9 gene and kidney outcomes in American Indians: the Strong Heart Family Study. *Hum Genet* 2010;127:295–301.
29. Zhang Y, Conti MA, Malide D, et al. Mouse models of MYH9-related disease: mutations in nonmuscle myosin II-A. *Blood* 2012;119:238–250.
30. Müller T, Rumpel E, Hradetzky S, et al. Non-muscle myosin IIA is required for the development of the zebrafish glomerulus. *Kidney Int* 2011;80:1055–1063.
31. Noris M, Remuzzi G. Non-muscle myosins and the podocyte. *Clin Kidney J* 2012;5:94–101.
32. Babayeva S, Miller M, Zilber Y, et al. Plasma from a case of recurrent idiopathic FSGS perturbs non-muscle myosin IIA (MYH9 protein) in human podocytes. *Pediatr Nephrol* 2011;26:1071–1081.
33. Freedman BI, Nagaraj SK, Lin JJ, et al. Potential donor-recipient MYH9 genotype interactions in posttransplant nephrotic syndrome after pediatric kidney transplantation. *Am J Transplant* 2009;9:2435–2440.
34. Phillips BL, Ibrahim M, Greenhall GH, Mumford L, Dorling A, Callaghan CJ. Effect of delayed graft function on longer-term outcomes after kidney transplantation from donation after circulatory death donors in the United Kingdom: a national cohort study. *Am J Transplant* 2021;21:3346–3355.