High-Activity Classical and Alternative Complement Pathway Genotypes – Association with Donor-Specific Antibody-Triggered Injury and Renal Allograft Survival

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Supplementary Material

Table S1. Baseline characteristics – DSA-positive study patients versus DSA-negative matched control patients

Parameters	DSA+ (n=83)	DSA- (n=106)[a]	*P* value
Variables recorded at the time of transplantation			
Recipient age (years), median (IQR)	48 (36-54)	48 (38-56)	0.76
Female recipient sex, n (%)	37 (44.6)	45 (42.5)	0.88
Caucasian, n (%)	83 (100)	104 (98)[b]	0.51
Donor age (years), median (IQR)	46 (35-58)	48 (35-56)	0.95
Live donor, n (%)	13 (15.7)	18 (17.0)	0.85
ABO-incompatible allograft, n (%)	1 (1.2)	0 (0)	0.44
Recipient of a retransplant, n (%)	25 (30.1)	37 (34.9)	0.53
HLA mismatch in A, B and DR, median (IQR)	3 (2-4)	3 (2-4)	0.96
Current CDC panel reactivity ≥10%, n (%)[c]	14 (17.7)	16 (15.8)	0.84
Preformed anti-HLA DSA, n (%)[d]	24 (58.5)	19 (40.4)	0.13
Peritransplant immunoadsorption, n (%)[e]	25 (30.1)	19 (17.9)	0.05
Variables recorded at the time of ABMR screening			
Time to ABMR screening (years), median (IQR)	4.9 (1.7-13.1)	5.9 (2.9-10.6)	0.79
Recipient age (years), median (IQR)	55 (45-63)	55 (46-65)	0.84
Tacrolimus-based baseline immunosuppression	50 (60.2)	76 (71.7)	0.12
eGFR (ml/min/1.73 m^2), median (IQR)	54 (32-81)	63 (39-88)	0.088
Urinary protein/creatinine ratio (mg/g), median (IQR)	200 (79-488)	156 (83-453)	0.81

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; CDC, complement-dependent cytotoxicity; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

[a]The group of DSA-negative recipients was propensity score matched to DSA-positive study patients using female sex, recipient age at transplantation, urinary protein/creatinine ratio, prior transplantation, HLA mismatch and cytotoxic panel reactivity. For 1 (C4 genotyping) and 2 (complete evaluation of SNPs) cases, biological material was not sufficient for complete genotyping.

[b]Two recipients in the DSA-negative group were Asian.

[c]CDC panel reactivity was not recorded for 4 DSA-positive and 5 DSA-negative recipients.

[d]Pretransplant DSA data were available for 41 DSA-positive and 47 DSA-negative recipients (solid-phase HLA antibody screening on the waitlist was implemented at the Vienna transplant unit in July 2009).

[e]Sensitized patients (until 2009: ≥40% CDC-PRA; since 2009: preformed DSA) were subjected to peritransplant immunoadsorption.[34]

Table S2. Baseline demographics and patient characteristics – Vienna/Prague kidney transplant cohort[a]

Parameters	All patients (n=660)	High-activity C3/fB/fH complotype		P value
		yes (n=199)	no (n=461)	
Recipient age (years), median (IQR)	54 (42-62)	51 (41-60)	55 (44-63)	0.004
Female recipient sex, n (%)	249 (37.7)	66 (33.2)	183 (39.7)	0.11
Caucasian, n (%)	650 (98.5)	198 (99.5)	452 (98.0)	0.30
Glomerulonephritis as underlying renal disease, n (%)	230 (34.8)	80 (40.2)	150 (32.5)	0.06
Donor age (years), median (IQR)	53 (42-60)	54 (44-60)	52 (41-60)	0.27
Female donor sex, n (%)	289 (43.8)	92 (46.2)	197 (42.7)	0.41
Live donor, n (%)	94 (14.2)	35 (17.6)	59 (12.8)	0.11
Recipient of a retransplant, n (%)	75 (11.4)	29 (14.6)	46 (10)	0.09
Recipient presensitization[a]	244 (37)	80 (40.2)	164 (35.6)	0.26
HLA mismatch in A, B and DR, median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	0.99
Eplet mismatch, median (IQR)	42 (31-55)	43 (31-55)	41 (31-55)	0.76
Tacrolimus-based baseline immunosuppression	558 (84.5)	170 (85.4)	388 (84.2)	0.68
Induction with a depleting antibody	173 (26.2)	62 (31.2)	111 (24.1)	0.06

fB, complement factor B; fH, complement factor H; IQR, interquartile range.

[a]Donor-specific antibodies at the time of transplantation or cytotoxic panel reactivity >10 %.

Table S3. Primer and probe sequences for analysis of C4A and C4B CNV.

Primer name	**Sequence 5'-3'**
C4_Forward	GCAGGAGACATCTAACTGGCTTCT
C4_Reverse	CCGGACCTGCATGCTCCT
Probe name[a]	
C4A	ACCCCTGTCCAGTGTTAG
C4B	ACCTCTCTCCAGTGATAC

CNV, copy number variation.
[a]Underlined letters show the differences of the probes that distinguish the C4A and C4B genes.

Table S4. Primer sequences and PCR conditions used to determine $C3_{R102G}$ and fB_{R32Q} SNPs.

Primer name	Sequence 5'-3'	PCR conditions
C3_Forward[a]	AGTTGCTGACGCTGGTTGGA	95°C-15 min 94°C-20 sec 57°C-10 sec 72°C-110 sec (35 cycles) 72°C-10 min
C3_Reverse[a]	GCTTGTGGTTGACGGTGAAGAT	
fB_Forward[a]	GGGAAAGTGATGTGGGTAGGAC	95°C-15 min 95°C-15 sec 60,9°C-30 sec 72°C-30 sec (35 cycles) 72°C-10 min
fB_Reverse[a]	GCACAGGGTACGGGTAGAAG	
fB_e1Forward[b]	TCACATGGAATTTCCCAGTTATG	95°C-5 min 95°C-15 sec 59°C-15 sec 72°C-150 sec (35 cycles) 72°C-10 min
fB_e3Reverse2[b]	CAGTGGTAGGTGACGCTGTCT	
fB_e2Reverse[b]	TGTCACCCTGCCTAGTCTCATC	96°C-1 min 96°C-10 sec 56°C-10 sec 60°C-4 min (25 cycles)

fB, complement factor B; SNP, single nucleotide polymorphism.
[a]Primers used for RFLP-PCR reaction.
[b]Primers used for PCR reaction and sequencing.

Table S5. Genotype distributions and allele frequencies in DSA-positive recipients and matched DSA-negative controls.

Gene; polymorphism	DSA+ study recipients (N=83)		DSA- control subjects (N=106)		*P* value (study vs. control subjects)
	Observed (%)	Expected (%)[a]	Observed (%)	Expected (%)[a]	
C3					
rs2230199 (c.304C>G; p.R102G; C3S and C3F)					
Genotype[b], n (%)					
G/**G** (**102G**/**102G**)	6 (7.2)	3.3 (4.0)	6 (5.8)	2.6 (2.5)	0.62
G/C (**102G**/102R)	21 (25.3)	26.4 (31.8)	21 (20.2)	27.8 (26.7)	
C/C (102R/102R)	56 (67.5)	53.3 (64.2)	77 (74.0)	73.6 (70.8)	
***P* value (observed vs. expected)**	0.061		0.013		
Allelic frequency[b], n (%)					
G (**102G**)	33 (19.9)		33 (15.9)		
C (102R)	133 (80.1)		175 (84.1)		
fB					
rs641153 (c.95G>A; FB R32Q)					
Genotype[b], n (%)					
G/**G** (**32R**/**32R**)	65 (78.3)	66.0 (79.5)	91 (87.5)	91.4 (87.9)	
G/A (**32R**/32Q)	18 (21.7)	16.0 (19.3)	13 (12.5)	12.2 (11.7)	0.093
A/A (32Q/32Q)	0 (0.0)	1.0 (1.2)	0 (0.0)	0.4 (0.4)	
***P* value (observed vs. expected)**	0.268		0.497		
Allelic frequency[b], n (%)					
G (**32R**)	148 (89.2)		195 (93.8)		
A (32Q)	18 (10.8)		13 (6.3)		
fH					
rs800292 (c.184G>A; FH V62I)					
Genotype[b], n (%)					
G/**G** (**62V**/**62V**)	50 (60.2)	50.1 (60.4)	69 (66.3)	70.3 (67.6)	
G/A (**62V**/62I)	29 (34.9)	28.8 (34.7)	33 (31.7)	30.4 (29.2)	
A/A (62I/62I)	4 (4.8)	4.1 (4.9)	2 (1.9)	3.3 (3.2)	0.45
***P* value (observed vs. expected)**	0.938		0.387		

Allelic frequency[b], n (%)		
G (**62V**)	129 (77.7)	171 (82.2)
A (62I)	37 (22.3)	37 (17.8)

DSA, donor-specific antibody; fB, complement factor B; fH, complement factor H.
[a]Expected genotype frequencies at Hardy Weinberg equilibrium were calculated from allele frequencies in study and control subjects.
[b]Risk variants are marked with bold font.

Table S6. Complement gene variants in DSA-positive recipients in relation to complement profile, biopsy results and survival.

	C3 rs2230199 (c.304C>G; p.R102G)				**fB** rs641153 (c.95G>A; p.R32Q)				**fH** rs800292 (c.184G>A; p.V62I)			
	G/G (**102G/102G**) (n=6)	**G**/C (**102G**/102R) (n=21)	C/C (102R/102R) (n=56)	***P* value**	**G/G** (**32R/32R**) (n=65)	**G**/A (**32R**/32Q) (n=18)	A/A (32Q/32Q) (n=0)	***P* value**	**G/G** (**62V/62V**) (n=50)	**G**/A (**62V**/62I) (n=29)	A/A (62V/62I) (n=4)	***P* value**
Blood complement profile, median (IQR)												
C3, g/L	1.24 (1.09-1.40)	1.20 (1.08-1.46)	1.43 (1.21-1.61)	0.02	1.38 (1.20-1.54)	1.39 (1.09-1.65)	-	0.847	1.38 (1.20-1.55)	1.38 (1.19-1.58)	1.43 (1.12-1.75)	0.78
C4, g/L	0.33 (0.29-0.41)	0.30 (0.25-0.37)	0.35 (0.26-0.42)	0.25	0.33 (0.26-0.39)	0.38 (0.30-0.45)	-	0.093	0.35 (0.26-0.41)	0.31 (0.26-0.41)	0.34 (0.29-0.53)	0.73
CH50, U/mL	51 (49-55)	59 (50-70)	62 (57-70)	0.035	62 (51-72)	62 (59-67)		0.934	61 (52-67)	63 (53-82)	75 (63-86)	0.042
%AP activity	114 (112-117)	103 (92-110)	110 (102-115)	0.014	110 (102-114)	109 (100-115)	-	0.711	110 (100-115)	109 (100-114)	118 (102-130)	0.27
Biopsy results, n (%)												
ABMR	4 (66.7)	15 (71.4)	28 (50.0)	0.21	37 (56.9)	10 (55.6)	-	0.563	26 (52.0)	18 (62.1)	3 (75.0)	0.51
C4d+ABMR	2 (33.3)	6 (28.6)	13 (23.2)	0.797	17 (26.2)	4 (22.2)	-	0.499	13 (26.0)	8 (27.6)	0 (0)	0.49
5-year survival rates, %												
Death-censored	65	80	84	0.493	79	89	-	0.468	87	71	100	0.18
Patient survival	100	85	82	0.518	82	88	-	0.482	85	83	75	0.87

ABMR, antibody-mediated rejection; fB, complement factor B; fH, complement factor H.

Table S7. Genotype distributions and allele frequencies in DSA-positive recipients and the Vienna/Prague transplant cohort.

Gene; polymorphism	DSA+ study recipients (N=83)		Vienna/Prague cohort (N=660)		*P* value (DSA-positive vs. Vienna/Prague cohort)
	Observed (%)	Expected (%)[a]	Observed (%)	Expected (%)[a]	
C3					
rs2230199 (c.304C>G; p.R102G)					
Genotype, n (%)					
G/**G** (102G/102G)	6 (7.2)	3.3 (4.0)	16 (2.4)	18.5 (2.8)	
G/C (102R/102G)	21 (25.3)	26.4 (31.8)	189 (28.6)	184 (27.9)	0.048
C/C (102R/102R)	56 (67.5)	53.3 (64.2)	455 (68.9)	457.5 (69.3)	
***P* value (observed vs. expected)**	0.061		0.485		
Allelic frequency, n (%)					
G (102G)	33 (19.9)		221 (16.7)		
C (102R)	133 (80.1)		1099 (83.3)		
fB					
rs641153 (c.95G>A; FB R32Q)					
Genotype, n (%)					
G/**G** (32R/32R)	65 (78.3)	66.0 (79.5)	563 (85.3)	561.9 (85.1)	
G/A (32R/32Q)	18 (21.7)	16.0 (19.3)	92 (13.9)	94.1 (14.3)	0.19
A/A (32Q/32Q)	0 (0.0)	1.0 (1.2)	5 (0.8)	3.9 (0.6)	
***P* value (observed vs. expected)**	0.268		0.563		
Allelic frequency, n (%)					
G (32R)	148 (89.2)		1218 (92.3)		
A (32Q)	18 (10.8)		102 (7.7)		
fH					
rs800292 (c.184G>A; FH V62I)					
Genotype, n (%)					
G/**G** (62V/62V)	50 (60.2)	50.1 (60.4)	415 (62.9)	409.7 (62.1)	
G/A (62V/62I)	29 (34.9)	28.8 (34.7)	210 (31.8)	220.6 (33.4)	0.84
A/A (62I/62I)	4 (4.8)	4.1 (4.9)	35 (5.3)	29.7 (4.5)	
***P* value (observed vs. expected)**	0.938		0.217		

Allelic frequency, n (%)		
G (62V)	129 (77.7)	1040 (78.8)
A (62I)	37 (22.3)	280 (21.2)

DSA, donor-specific antibody; fB, complement factor B; fH, complement factor H.
[a]Expected genotype frequencies at Hardy Weinberg equilibrium were calculated from allele frequencies.

Table S8. High-activity C3/fB/fH complotype and death-censored graft survival in the Vienna/Prague kidney transplant cohort - multivariate Cox regression analysis.

Variable	Hazard ratio	95% confidence interval	p-value
High-activity C3/fB/fH complotype, yes vs. no	1.55	1.04 – 2.32	0.031
Recipient age >65 years, yes vs. no	1.46	0.85 – 2.51	0.17
Female recipient sex, yes vs. no	1.17	0.79 – 1.75	0.44
Glomerulonephritis as primary renal disease, yes vs. no	0.70	0.45 – 1.08	0.11
Donor age, per year	1.02	1.01 – 1.04	0.004
Female donor sex, yes vs. no	1.66	1.11 – 2.49	0.015
Live donor, yes vs. no	1.02	0.60 – 1.74	0.94
Retransplant, yes vs. no	3.17	1.90 – 5.28	<0.001
HLA eplet mismatch, per increase in mismatch score	1.01	1.00 – 1.02	0.013
Tacrolimus-based baseline immunosuppression, yes vs. no	1.10	0.65 – 1.89	0.72
Induction with a depleting antibody, yes vs. no	0.76	0.47 – 1.23	0.26

fB, complement factor B; fH, complement factor H.