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

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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 immunoabsorption, 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 m²), 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.

aThe 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.

bTwo recipients in the DSA-negative group were Asian.

cCDC panel reactivity was not recorded for 4 DSA-positive and 5 DSA-negative recipients.

dPretransplant 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).

eSensitized patients (until 2009: ≥40% CDC-PRA; since 2009: preformed DSA) were subjected to peritransplant immunoabsorption.34
Table S2. Baseline demographics and patient characteristics – Vienna/Prague kidney transplant cohort\textsuperscript{a}

| Parameters                                           | All patients (n=660) | High-activity C3/fB/fH comploypey (n=199) | no (n=461) | P value |
|------------------------------------------------------|----------------------|------------------------------------------|-------------|---------|
| 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\textsuperscript{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    |

\textsuperscript{a}Donor-specific antibodies at the time of transplantation or cytotoxic panel reactivity >10 %.

fB, complement factor B; fH, complement factor H; IQR, interquartile range.
| Primer name | Sequence 5'-3' |
|-------------|---------------|
| C4_Forward  | GCAGGAGACATCTAACTGGCTTCT |
| C4_Reverse  | CCGGACCTGCATGCTCCT |

| Probe name^a |
|-------------|
| C4A         | ACCCTCTGTCAGTGTAAG |
| C4B         | ACCTCTCTCCAGTGATAC |

CNV, copy number variation.
^aUnderlined letters show the differences of the probes that distinguish the C4A and C4B genes.
Table S4. Primer sequences and PCR conditions used to determine C3R102G and fBR32Q SNPs.

| Primer name       | Sequence 5'-3' | PCR conditions |
|-------------------|---------------|----------------|
| C3_Forward<sup>a</sup> | AGTTGCTGACGCTGGTTGGA | 95°C-15 min, 94°C-20 sec, 57°C-10 sec, 72°C-10 min, 35 cycles |
| C3_Reverse<sup>a</sup> | GCTTGTGGTTGACGGTGAAGAT | 94°C-20 sec, 57°C-10 sec, 72°C-110 sec, 35 cycles |
| fB_Forward<sup>a</sup> | GGGAAAGTGATGTGGGTAGGAC | 95°C-15 min, 95°C-15 sec, 60.9°C-30 sec, 35 cycles |
| fB_Reverse<sup>a</sup> | GCACAGGGTACGGGTAGAAG | 95°C-15 min, 95°C-15 sec, 72°C-30 sec, 35 cycles |
| fB_e1Forward<sup>b</sup> | TCACATGGAATTTCAGTTATG | 95°C-5 min, 95°C-15 sec, 59°C-15 sec, 35 cycles |
| fB_e3Reverse2<sup>b</sup> | CAGTGGTAGGTGACGCTGTCT | 96°C-1 min, 96°C-10 sec, 56°C-10 sec, 25 cycles |
| fB_e2Reverse<sup>b</sup> | TGTCCACCTGCCTAGTCTCATC | 96°C-1 min, 96°C-10 sec, 56°C-10 sec, 25 cycles |

<sup>a</sup>Primers used for RFLP-PCR reaction.  
<sup>b</sup>Primers used for PCR reaction and sequencing.

fB, complement factor B; SNP, single nucleotide polymorphism.
| Gene; polymorphism | DSA+ study recipients (N=83) | DSA- control subjects (N=106) | $P$ value (study vs. control subjects) |
|-------------------|-----------------------------|-------------------------------|-------------------------------------|
|                   | Observed (%)                | Expected (%)                 | Observed (%)                       | Expected (%)                 |                                |
| **C3**            |                             |                               |                                     |                               |                                |
| rs2230199(c.304C>G; p.R102G; C3S and C3F) |                             |                               |                                     |                               |                                |
| Genotype, 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, 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, n (%)   |                             |                               |                                     |                               |                                |
| G/G (32R/32R)     | 65 (78.3)                   | 66.0 (79.5)                   | 91 (87.5)                          | 91.4 (87.9)                   | 0.093                          |
| G/A (32R/32Q)     | 18 (21.7)                   | 16.0 (19.3)                   | 13 (12.5)                          | 12.2 (11.7)                   |                                |
| 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, 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, 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<sup>b</sup>, n (%) | Study | Control |
|-------------------------------------|-------|---------|
| 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.

<sup>a</sup>Expected genotype frequencies at Hardy Weinberg equilibrium were calculated from allele frequencies in study and control subjects.

<sup>b</sup>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.

| Gene    | rs   | Variant                  | Blood complement profile, median (IQR) | Biopsy results, n (%) | 5-year survival rates, % | P value |
|---------|------|--------------------------|----------------------------------------|------------------------|--------------------------|---------|
|         |      |                          | C3, g/L (102G/102G) (n=6) | (102G/102R) (n=21) | (102R/102R) (n=56) | P value | C4, g/L (0.29-0.41) (0.25-0.37) (0.26-0.42) | 0.25 | 0.33 | 0.35 | C4d+ABMR | 0.797 | 2 (33.3) | 6 (28.6) | 13 (23.2) | 0.499 | 13 (26.0) | 8 (27.6) | 0 (0) | 0.49 |
|         |      |                          | CH50, U/mL (51-55) | (50-70) | (57-70) | 0.035 | 61 (52-67) | 63 (53-82) | 75 (63-86) | 0.042 |         |        |        |       |       |       |       |       |
|         |      |                          | %AP activity (114) | (92-110) | (102-115) | 0.014 | 110 | 109 | - | 0.711 | 110 | (100-115) | 109 | (100-114) | 118 | (102-130) | 0.27 |
|         |      |                          | 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 | 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 |
|         |      |                          | 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.
| Gene; polymorphism | DSA+ study recipients (N=83) | Vienna/Prague cohort (N=660) | P value (DSA-positive vs. Vienna/Prague cohort) |
|--------------------|-----------------------------|-----------------------------|---------------------------------------------|
|                    | Observed (%) | Expected (%)<sup>a</sup> | Observed (%) | Expected (%)<sup>a</sup> | |
| 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) | 0.048 |
| G/C (102R/102G) | 21 (25.3) | 26.4 (31.8) | 189 (28.6) | 184 (27.9) | |
| 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) | 0.19 |
| G/A (32R/32Q) | 18 (21.7) | 16.0 (19.3) | 92 (13.9) | 94.1 (14.3) | |
| 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.

*aExpected 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.