Complement modulation in renal replacement therapy

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Chapter 11

The Complotype: a major determinant of late renal transplantation outcome.

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Abstract

Background

There is a need for accurate prediction of graft loss after renal transplantation. Activation of the complement cascade during transplantation is involved in renal allograft injury. The inherited set of complement genes is called the Complotype and determines the ability to activate and regulate the complement system. We investigated whether the Complotype of donor-recipient pairs can improve prediction and thus risk stratification of renal allograft loss.

Methods

We enrolled patients who underwent a kidney transplantation in Groningen between March 1993 and February 2008. We designed a Complotype genetic risk score with a maximal area under the receiver-operating characteristic curve (AUC) by selecting risk-increasing and risk-decreasing alleles from 60 complement single nucleotide polymorphisms (SNPs) in the donor and recipient.

Results

The primary analysis included 1265 donor-recipient pairs. The Complotype risk score resulted in a dose-response relationship between this genetic score and the risk of graft loss. In fully adjusted models, higher Complotype risk scores were independently associated with increased allograft loss (hazard ratio, 1.75; 95% confidence interval, 1.48 to 2.08; P=0.001 per SD increase). In addition to donor, recipient and transplant characteristics, the Complotype risk score independently improved risk stratification and prediction for graft loss.

Conclusions

This is the first report showing that combined effects of complement polymorphisms significantly impact graft loss of renal transplant patients. Assessment of the Complotype in donor-recipient pairs forms a potential tool for may help to identify patients at high risk for graft loss. Additionally, future studies should determine if the Complotype could help to determine the ideal recipient for donor kidneys.

Introduction

Despite significant advances in renal transplantation, allograft loss remains a challenging issue.[1] Patients with graft failure are readmitted to dialysis treatment and relisted for a new transplant, leading to reduced quality of life, increased organ shortage, and high medical costs.[2,3] Preemptive therapeutic interventions in transplant recipients at high risk of graft loss are crucial to improve graft survival and achieve optimal results.[4] Risk prediction models have the potential to inform physicians and guide donor allocation, decision-making, and clinical care.[5] The demand for additional predictors of long-term outcome is ongoing since current risk factors are insufficient to explain the likelihood of graft failure.[6]

Activation of the complement cascade has been considered to be a major component of renal allograft injury.[7] The complement system is activated during different phases in renal transplantation; in deceased donors, during organ perfusion, at the time of reperfusion and in rejection.[8,9] In general, activation of the complement system occurs via the Classical-, Lectin-, or Alternative pathway. Activation of each pathway leads to cleavage of C3 and formation of C5b-9.[10] In experimental studies, complement inhibition in renal transplantation is a promising strategy to improve renal allograft outcome.[8,11,12] Furthermore, clinical trials with complement-targeting therapeutics are currently underway to test the effect of complement inhibition in renal transplantation.[8]

Complement polymorphisms have been shown to impact long-term allograft survival after kidney transplantation.[13–17] In the past, complement deficiencies were thought to be rare and of little clinical importance. However, recently various gene variants have been identified that result in either functional or quantitative differences.[18] Additionally, increasing numbers of clinical entities are now thought to arise from (partial) complement deficiencies or even certain polymorphisms.[18,19] However, the complement system is a complex network consisting of more than 40 proteins.[10] Ideally, one would, therefore, want to look at the total makeup of the complement genes. Moreover, the combination of complement polymorphisms is likely to have a larger impact on the long-term outcome than one particular SNP.[20]

The total makeup of the inherited set of complement genes is called the Complotype and is believed to determine one's individual ability to activate and regulate the complement system.[21,22] A Complotype leading to amplified complement activity will make an individual susceptible to inflammation, while a Complotype that dampens complement activity will increase the individual risks to infection.[22,23] We hypothesize that the Complotype impacts long-term outcome, forming a major determinant of late renal allograft loss and thereby providing valuable prognostic information. We conducted a study to explore the effect of multiple complement polymorphisms in the donor and recipient on long-term renal allograft survival in a large population-based study. Furthermore, we wanted to determine whether assessment of the multiple complement polymorphisms in donor-recipient pairs improves risk stratification and the prediction of renal allograft loss.

Materials and Methods

Subjects

We enrolled patients who underwent single kidney transplantation at the University Medical Center Groningen in the Netherlands between March 1993 until February 2008. From the 1430 renal transplantations, 1271 recipient and donor pairs were included in the cohort as previously described.[17,24] Subjects were excluded due to technical complications during surgery, lack of DNA, re-transplantation or loss of follow-up. This study is in accordance with the declaration of Helsinki and all patients provided written informed consent. The medical ethics committee of the University Medical Center Groningen approved the study under file n° METc 2014/077.

DNA isolation and genotyping

Peripheral blood mononuclear cells were isolated from blood or splenocytes collected from the donors and recipients. DNA was extracted with a commercial kit as instructed by the manufacturer and stored at -80°C. Genotyping of 60 complement single nucleotide polymorphisms (SNP) were determined via the Illumina VeraCode GoldenGate Assay kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions (Supplementary Table S1). Genotype clustering and calling were performed using BeadStudio Software (Illumina). The overall genotype success rate was 99.5% and 6 samples with a high missing call rate were excluded from subsequent analyses.

Genetic risk score

We created a Complotype risk score that assigns points for the presence of a risk-decreasing or a risk-increasing allele in the donor and recipient. However, to take into account the strength of the association of the SNPs with graft loss, the point for the presence of a complement SNP is multiplied by the regression coefficient (= logarithm of the hazard ratio) creating a weighted risk score.[25] A regression coefficient is negative when an SNP is protective and the regression coefficient is positive when an SNP is hazardous. The total sum of the protective and hazardous SNPs in both the donor and recipient creates the Complotype risk score. To determine which complement polymorphisms should be included in this model; SNPs were added via a step-forward approach, starting with the SNP with the highest hazard ratios to generate the genetic risk score. Next, we determined the area under the curve (AUC) after the addition of each SNP to assess whether adding the SNP improved to the risk score. SNPs were added to the genetic risk score until the highest value was reached for the AUC.[26]

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 and STATA version 13.1. Data are displayed as median [IQR] for non-parametric variables; mean ± standard deviation for parametric variables and total number of patients with percentage [n (%)] for nominal data. Differences between groups were examined with the Mann-Whitney-U test or the student t-test for not-normally and normally distributed variables, respectively, and categorical variables with the $\chi 2$ test. Log-rank tests

were performed between groups to assess the difference in allograft survival.

In additional sensitivity analyses, associations of the Complotype risk score with graft loss were tested by Cox proportional hazards regression analysis in subgroups. Univariate analysis was performed to determine the association of genetic, donor, recipient and transplant characteristics with long-term outcome. The factors identified in these analyses were thereafter tested in a multivariable cox regression. Additionally, multivariable cox regression with a stepwise forward selection was performed. The Harrell's C statistic was used to assess the predictive value of the Complotype risk score when added to the reference model.[27] We also determined the integrated discrimination improvement (IDI) and the net reclassification improvement (NRI) for the addition of the Complotype risk score to the reference model.[28] Tests were 2-tailed and regarded as statistically significant when $P<0.05$.

Results

Determinants of renal allograft loss

A total of 1265 kidney transplant donor-recipient pairs were included. The donor and recipient characteristics are described in Table 1. The mean follow-up after transplantation was 5.6 ± 3.3 years with a maximum follow-up period of 10 years. During median follow-up, 4.7% of the recipients developed primary non-function (PNF). In total, 16.1% of the recipients lost their renal graft during follow-up, whereas 15.0% of the recipients dieds with a functioning graft. Of all the tested characteristics, the following were significantly associated with renal allograft survival (Table 1); donor and recipient age, donor type, cold and warm ischemia time, dialysis vintage of the recipient prior to transplantation, the use of corticosteroids or cyclosporine after transplantation, the occurrence of rejection and delayed graft function (DGF).

Complement polymorphisms

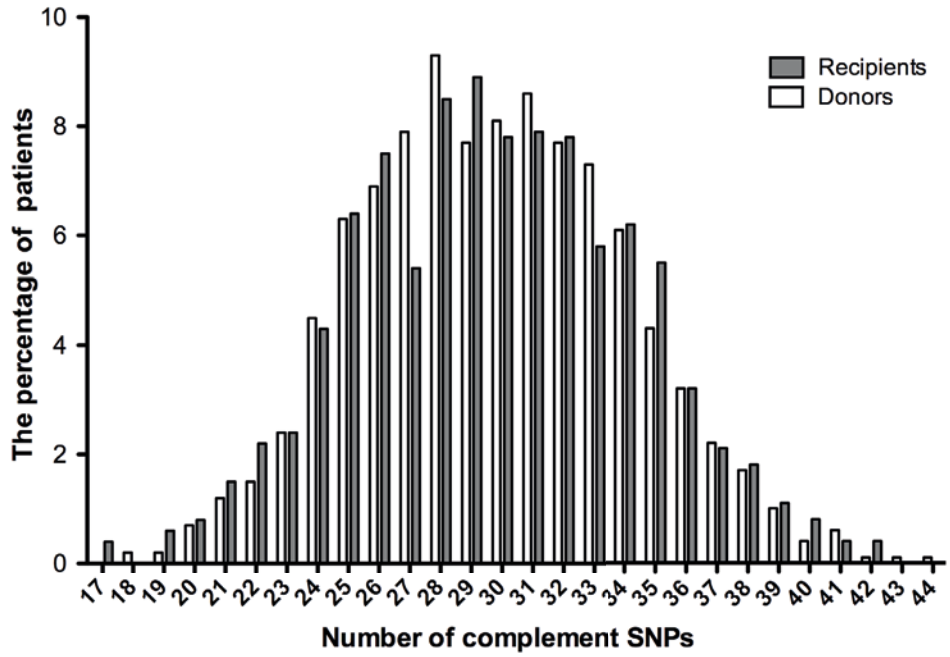
Next, we examined 60 SNPs (in 19 complement genes) that were previously reported to be associated with pathology. Four SNPs were excluded due to low call rates (minor allele frequency of less than 0.1%). We first investigated the frequency of complement polymorphisms and counted the number of minor alleles for each complement SNPs present in the donors and recipient (Figure 1). Among the donors, the numbers minor alleles of complement SNPs ranged from 18 to 44 with a median of 30. Similar results were seen in the recipients; there the number minor alleles of complement SNPs ranged from 17 to 42 with a median of 30. These results confirm the hypothesis that multiple complement SNPs are common.

Table 1
Baseline characteristics of the donors and recipients

	All Patients (n = 1271)	Patients without graft loss (n = 1066)	Patients with graft loss (n = 205)	P-value*	HR	P-value#
Donor						
Age, years	44.4 ± 14.4	44.0 ± 14.7	46.7 ± 12.9	0.008	1.02	<0.001
Male gender, n (%)	645 (50.7)	541 (50.8)	101 (49.3)	0.5		0.9
Blood group						
Type O, n (%)	642 (50.5)	546 (51.2)	96 (46.8)			
Type A, n (%)	502 (39.5)	418 (39.2)	84 (41.0)	0.02		0.1
Type B, n (%)	97 (7.6)	83 (7.8)	14 (6.8)			
Type AB, n (%)	27 (2.1)	17 (1.6)	10 (4.9)			
Donor type						
Living, n (%)	282 (22.2)	258 (24.2)	24 (11.7)		1.00	
Brain death, n (%)	787 (61.9)	650 (61.0)	137 (66.8)	<0.001	1.78	<0.001
Non-heart beating, n (%)	202 (15.9)	158 (14.8)	44 (21.5)		2.92	
Cause of death						
Trauma, n (%)	305 (30.8)	266 (32.9)	39 (21.5)			
CVA, n (%)	549 (55.5)	436 (54.0)	113 (62.4)	0.01		0.2
Other, n (%)	135 (13.7)	106 (13.1)	29 (16.0)			
Recipient						
Age, years	47.9 ± 13.5	48.4 ± 13.4	45.3 ± 13.3	<0.001	0.99	0.048
Male gender, n (%)	739 (58.1)	453 (42.5)	80 (39.0)	0.3		0.2
Blood group						
Type A, n (%)	536 (42.2)	452 (42.4)	84 (41.0)			
Type B, n (%)	113 (8.9)	99 (9.3)	14 (6.8)	0.002		0.1
Type O, n (%)	567 (44.6)	479 (44.9)	88 (42.9)			
Type AB, n (%)	55 (4.3)	36 (3.4)	19 (9.3)			
Dialysis vintage, weeks	189 ± 136	186 ± 135	205 ± 135	0.1	1.00	0.046
PRA level >5%, n (%)						
Transplantation						
CIT, in hours	16.7 ± 9.4	16.1 ± 9.5	19.7 ± 8.6	<0.001	1.03	<0.001
WIT, in minutes	39.0 ± 11.3	38.72 ± 10.9	40.7 ± 13.2	0.03	1.02	0.005
Total HLA mismatches	2 [1 – 3]	2 [1 – 3]	2 [1 – 3]	0.4		0.1
Immunosuppression						
Anti-CD3 Moab, n (%)	19 (1.5)	14 (1.3)	5 (2.4)	0.2		0.4
Azathioprine, n (%)	72 (5.7)	55 (5.2)	17 (8.3)	0.1		0.3
ATG, n (%)	103 (8.1)	80 (7.5)	23 (11.2)	0.1		0.2
Corticosteroids, n (%)	1201 (94.5)	1012 (94.9)	189 (92.2)	0.1	0.50	0.009
Cyclosporin, n (%)	1085 (85.4)	919 (86.2)	166 (81.0)	0.1	0.68	0.03
Interleukin-2 RA, n (%)	199 (15.7)	163 (15.3)	36 (17.6)	0.4		0.1
Mycophenolic acid, n (%)	907 (71.4)	778 (73.0)	129 (62.9)	0.004		0.1
Sirolimus, n (%)	38 (3.0)	33 (3.1)	5 (2.4)	0.8		0.5
Tacromilus, n (%)	97 (7.6)	78 (7.3)	19 (9.3)	0.3		0.5
Transplant outcome						
DGF, n (%)	415 (32.7)	293 (27.5)	122 (54.6)	<0.001	3.85	<0.001
Acute rejection, n (%)	392 (30.8)	299 (28.0)	93 (45.4)	<0.001	1.80	<0.001

Data are presented as mean ± SD or median [IQR]. P-value indicates P-value for the difference in baseline characteristics between the patients with and without graft loss, tested by Student's t-Test or Mann–Whitney U test for continuous variables and with $\chi 2$ test for categorical variables. The hazard ratios (HR) plus 95 % confidence interval (CI) are shown if the univariate analysis for graft loss was significant. P-value# shows the corresponding P-value.*

Figure 1
The frequency and distribution of complement polymorphisms in the donor and recipient.



The number of risk alleles of complement polymorphisms was counted for each donor and recipient.

Complotype risk score

To explore to what extent combinations of complement SNPs can be used as predictors of long-term renal graft survival, we performed multiple SNPs testing in the recipient and the donor. Complement SNPs had protective or hazardous effects on long-term graft survival and the effect per SNP differed between the donor and recipient, creating four groups (protective SNPs in the recipient, hazardous SNPs in the recipient, protective SNPs in the donor and hazardous SNPs in the donor). We first ranked the risk alleles according to the hazard ratio (Table 2 – 4). Next, we created a genetic score and subsequently calculated the area under a curve as seen in Figure 2. The genetic score for protective SNPs in the donor started with the SNP with the highest HR: CFB-rs641153 that had an AUC of 0.52. The accuracy increased promptly with the addition of each SNP until the strongest 20 SNPs were included in the model and the AUC was 0.60, 95% CI: 0.56 – 0.64 (Fig. 2, upper left panel). Furthermore, the genetic score for hazardous SNPs in the donor started with SNP C3-rs1047286, but the highest AUC (0.64, 95% CI: 0.60 – 0.68) was seen when the strongest 22 SNPs were included (Fig. 2, upper right panel). The genetic scores for protective and hazardous SNPs in the recipient were constructed using a similar approach (Fig. 2, lower panel). In the recipient, the discriminative accuracy of the genetic scores was

the highest with the strongest 18 protective SNPs (AUC 0.59, 95% CI: 0.55 – 0.63) and the genetic score for the hazardous SNPs with the highest 22 SNPs (AUC 0.60, 95% CI, 0.56 – 0.64), respectively. We finally combined the four genetic scores, thereby creating the Complotype risk score.

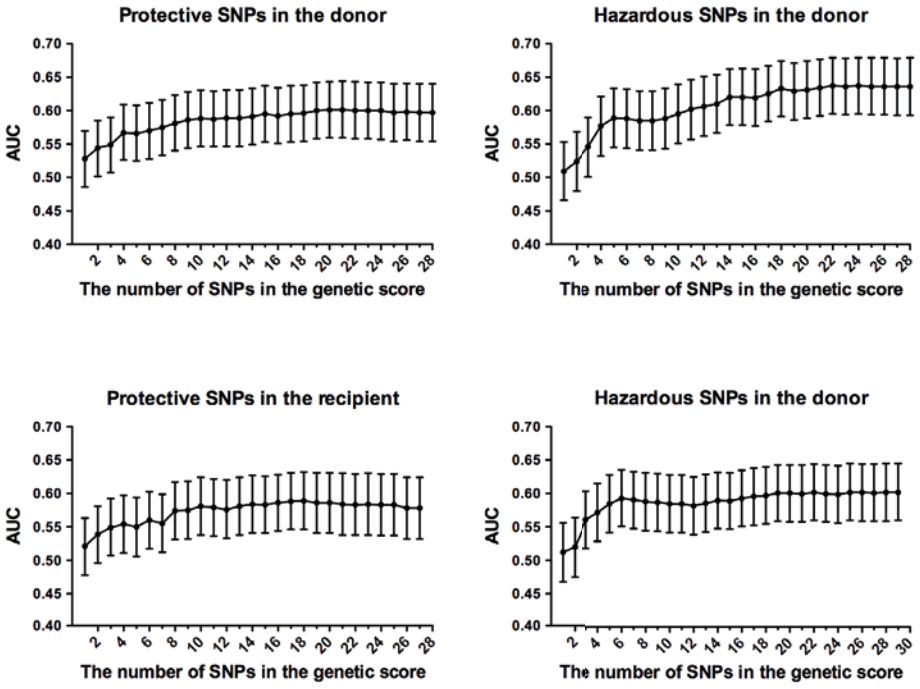
Table 2
Overview of the protective complement single nuclear polymorphisms in the donor

Gene	SNP	Allele frequency (%)		HR	95% CI
		Risk	Reference		
CFB	rs641153	AA+AG (14.0)	GG (86.0)	0.60	0.38 – 0.97
THBD	rs6076016	AA (11.5)	AT+TT (88.5)	0.66	0.40 – 1.08
MASP2	rs72550870	AG (4.9)	AA (95.1)	0.73	0.34 – 1.55
C1QC	rs294179	GG (20.1)	AA+AG (79.9)	0.74	0.51 – 1.08
C1QC	rs294183	AA (11.3)	AG+GG (88.7)	0.74	0.45 – 1.21
CLU	rs9331888	CC (7.7)	CG+GG (92.3)	0.75	0.42 – 1.35
CLU	rs2279590	CC+CT (14.6)	TT (85.4)	0.77	0.51 – 1.18
CD35	rs6656401	CC+CT (36.1)	TT (63.9)	0.82	0.61 – 1.09
THBD	rs1042580	GG+GA (59.6)	AA (40.4)	0.85	0.64 – 1.12
CFH	rs529825	CC+CT (47.2)	TT (52.8)	0.86	0.65 – 1.13
CFH	rs800292	GG+GA (46.0)	AA (54.0)	0.86	0.65 – 1.14
MBL2	rs7096206	CG+GG (38.7)	CC (61.3)	0.86	0.65 – 1.15
C1QA	rs292001	CC (16.2)	CT+TT (83.8)	0.87	0.59 – 1.28
THBD	rs3176123	CC+CA (34.6)	AA (65.4)	0.90	0.67 – 1.20
CFH	rs380390	GG (14.7)	CC+CG (85.3)	0.90	0.60 – 1.34
CFH	rs1329428	CC+CT (67.6)	TT (32.4)	0.90	0.68 – 1.20
FCN2	rs7851696	AA+AC (22.9)	CC (77.1)	0.90	0.65 – 1.27
CLU	rs11136000	AA+AG (63.0)	GG (37.0)	0.91	0.69 – 1.21
CFI	rs10033900	CC (19.0)	CT+TT (81.0)	0.91	0.64 – 1.31
C2	rs9332739	CC+CG (10.5)	GG (89.5)	0.94	0.59 – 1.48
CD55	rs10746463	AG+GG (54.7)	AA (45.3)	0.95	0.72 – 1.25
THBD	rs2424505	AA+AG (16.4)	GG (83.6)	0.96	0.66 – 1.39
FCN2	rs3124953	CC+CT (36.8)	TT (63.2)	0.97	0.73 – 1.29
CFHR1	rs436719	AA (41.3)	AC+CC (58.7)	0.97	0.73 – 1.29
C1QB	rs631090	AG+GG (13.7)	AA (86.3)	0.98	0.65 – 1.48
CFHR5	rs9427661	AG+GG (20.0)	AA (80.0)	0.98	0.70 – 1.39
THBD	rs1040585	AA+AC (14.5)	AA (85.5)	0.98	0.67 – 1.45
C1QA	rs587585	AG+GG (27.0)	AA (73.0)	0.99	0.72 – 1.35

All the complement single nuclear polymorphisms (SNPs) in the donor, with rs ID number, that were associated with a lower risk of graft loss are displayed. Data are presented as hazard ratio (HR) plus 95% confidence interval (CI) according to the univariate analysis for graft loss after renal transplantation.

In conclusion, the Complotype risk score looks at the presence of the 20 protective SNPs and 22 hazardous SNPs in the donor and in the recipient at 18 protective SNPs and 22 hazardous SNPs. Weight is added to SNPs according to their hazard ratio, creating a negative score for protective polymorphisms and a positive score for hazardous ones. The greater the effect of the SNP on allograft survival, the bigger the score is. Overall, Complotype risk scores above zero indicate the presence of more hazardous SNPs in a donor-recipient pair and Complotype risk scores below zero indicates the presence of more protective SNPs in a donor-recipient pair.

Figure 2
Area under the ROC of Complotypes based on increasing numbers of SNPs.



Complement SNPs in the donor and recipient were analyzed to see if they had a hazardous or protective effect on graft loss. Next, genetic scores were created for protective complement SNPs in the donor (upper left panel), hazardous complement SNPs in the donor (upper right panel), protective complement SNPs in the recipient (lower left panel) and hazardous complement SNPs in the recipient (lower right panel). SNPs were next added in order of the hazard ratio to each genetic score, until the model reached the maximal value of the area under the curve.

Table 3
Overview of the hazardous complement single nuclear polymorphisms in the donor

Gene	SNP	Allele frequency (%)		HR	95% CI
		Risk	Reference		
C3	rs1047286	CC (3.3)	CG+GG (96.7)	1.68	0.89 – 3.17
CFH	rs3753394	TT (19.0)	CT+CC (81.0)	1.65	1.02 – 2.68
THBD	rs1962	CC (7.2)	CT+TT (92.8)	1.60	1.03 – 2.49
FCN1	rs2989727	GG (12.7)	AA+AG (87.3)	1.53	1.07 – 2.18
FCN2	rs17549193	CC (7.6)	CT+TT (92.4)	1.44	0.92 – 2.27
FCN1	rs1071583	CC (12.2)	CT+CC (87.8)	1.44	1.00 – 2.08
FCN2	rs17514136	TT (5.8)	CC+CT (94.2)	1.36	0.80 – 2.29
CFH	rs1065489	GG (2.5)	GT+TT (97.5)	1.32	0.62 – 2.81
MBL2	rs11003125	CC (13.4)	CG+GG (86.6)	1.22	0.84 – 1.78
CFP	rs1048118	CC (13.3)	CT+TT (86.7)	1.22	0.83 – 1.78
FCN2	rs3124952	TT (23.1)	CC+CT (76.9)	1.22	0.89 – 1.67
C5	rs2900180	AA+AG (59.7)	GG (40.3)	1.20	0.90 – 1.59
CFHR5	rs3748557	AA+AT (40.5)	TT (59.5)	1.20	0.91 – 1.57
MBL2	rs7095891	CC+CT (44.5)	TT (55.5)	1.18	0.90 – 1.56
C5	rs3761847	AG+GG (70.6)	AA (29.4)	1.18	0.86 – 1.61
C3	rs2230199	CC (3.9)	CG+GG (96.1)	1.16	0.59 – 2.26
CFH	rs1061170	TT (37.9)	CT+CC (62.1)	1.15	0.86 – 1.54
C5	rs17611	AA (17.9)	AG+GG (82.1)	1.15	0.81 – 1.63
C5	rs10818488	AA+AG (76.5)	GG (23.5)	1.15	0.82 – 1.62
SERPING1	rs2511989	CC+CT (67.2)	TT (32.8)	1.11	0.82 – 1.49
CFH	rs3766404	CT+CC (28.6)	TT (71.4)	1.08	0.80 – 1.46
CFH	rs1061147	AA+AG (64.1)	GG (35.9)	1.08	0.81 – 1.45
CFH	rs10801554	AG+GG (61.2)	AA (38.8)	1.07	0.81 – 1.42
CFB	rs4151667	AA+AT (9.7)	TT (90.3)	1.03	0.65 – 1.64
CFHR5	rs12092294	CT+TT (20.0)	CC (80.0)	1.03	0.73 – 1.44
CFHR5	rs6694672	AC+CC (20.1)	AA (79.9)	1.01	0.72 – 1.42
CFHR5	rs9427662	CT+TT (19.7)	CC (80.3)	1.01	0.72 – 1.42
C5	rs2300929	AG+GG (18.4)	AA (81.6)	1.01	0.71 – 1.43

All the complement single nuclear polymorphisms (SNPs) in the donor, with rs ID number, that were associated with a higher risk for graft loss are displayed. Data are presented as hazard ratio (HR) plus 95 % confidence interval (CI) according to the univariate analysis for graft loss after renal transplantation.

Table 4
Overview of the protective complement single nuclear polymorphisms in the recipient

Gene	SNP	Allele frequency (%)		HR	95% CI
		Risk	Reference		
CLU	rs2279590	CC (14.3)	CT+TT (85.7)	0.70	0.45 – 1.10
CFH	rs380390	CC (32.4)	CG+GG (67.6)	0.74	0.56 – 0.99
FCN1	rs1071583	CC (13.0)	CT+TT (87.0)	0.76	0.48 – 1.19
FCN1	rs2989727	GG (14.6)	AA+AG (85.4)	0.76	0.50 – 1.17
CFHR1	rs436719	CC (11.3)	AC+AA (88.7)	0.76	0.48 – 1.22
CFH	rs1061170	CT+CC (61.1)	TT (38.9)	0.76	0.58 – 1.01
CFH	rs1061147	CC (39.0)	AC+AA (61.0)	0.77	0.59 – 1.02
THBD	rs6076016	AA+AT (59.8)	TT (40.2)	0.77	0.59 – 1.02
MASP2	rs72550870	AG (3.9)	AA (96.1)	0.78	0.35 – 1.76
MBL2	rs11003125	CC (12.2)	CG+GG (87.8)	0.78	0.50 – 1.23
CLU	rs11136000	AA (13.6)	AG+GG (86.4)	0.78	0.51 – 1.21
CFH	rs10801554	AG+GG (55.6)	AA (44.4)	0.80	0.61 – 1.05
FCN2	rs3124953	CC+CT (35.9)	TT (64.1)	0.80	0.60 – 1.08
CFB	rs641153	AA+AG (13.1)	GG (86.9)	0.81	0.52 – 1.25
THBD	rs1040585	AA+AC (n=216)	CC (n=1052)	0.81	0.54 – 1.21
THBD	rs3176123	CC (5.1)	CA+AA (94.9)	0.82	0.56 – 1.19
C1QC	rs294183	AA (16.4)	AG+GG (83.6)	0.83	0.56 – 1.23
FCN2	rs7851696	AA+AC (26.1)	CC (73.9)	0.84	0.60 – 1.16
FCN2	rs3124952	CC (26.0)	CT+TT (74.0)	0.85	0.60 - 1.21
THBD	rs2424505	AA+AG (n=217)	GG (n=1054)	0.88	0.50 – 0.88
C3	rs1047286	CC+CG (44.2)	GG (55.8)	0.89	0.68 – 1.18
CFP	rs1048118	CC+CT (40.5)	TT (59.5)	0.90	0.68 – 1.20
C1QA	rs292001	CC (18.5)	CT+TT (81.5)	0.93	0.65 – 1.33
C3	rs2230199	CC+CG (5.6)	GG (94.4)	0.94	0.51 – 1.72
C1QC	rs294179	GG (22.1)	AA+AG (77.9)	0.94	0.67 – 1.31
MBL2	rs7096206	CG+GG (37.9)	CC (62.1)	0.95	0.71 – 1.26
FCN2	rs17514136	TT (8.2)	CT+CC (91.8)	0.99	0.61 – 1.60

All the complement single nuclear polymorphisms (SNPs) in the recipient, with rs ID number, that were associated with a lower risk for graft loss are displayed. Data are presented as hazard ratio (HR) plus 95 % confidence interval (CI) according to the univariate analysis for graft loss after renal transplantation.

Table 5
Overview of the hazardous complement single nuclear polymorphisms in the recipient

Gene	SNP	Allele frequency (%)		HR	95% CI
		Risk	Reference		
CFH	rs1065489	GG (3.9)	GT+TT (96.1)	1.78	1.01 – 3.20
CFHR5	rs3748557	AA (5.0)	AT+TT (95.0)	1.55	0.92 – 2.62
CLU	rs9331888	CC+CG (52.6)	GG (47.4)	1.39	1.05 – 1.84
SERPING1	rs2511989	CC (18.7)	CT+TT (81.3)	1.37	0.99 – 1.90
C1QA	rs587585	AG+GG (25.8)	AA (74.2)	1.31	0.97 – 1.77
CFHR5	rs9427662	AG+GG (17.2)	AA (82.8)	1.31	0.93 – 1.83
CFHR5	rs9427661	CT+TT (17.4)	CC (82.6)	1.30	0.92 – 1.82
C1QB	rs631090	AG+GG (13.5)	AA (86.5)	1.28	0.89 – 1.85
CFHR5	rs12092294	CT+TT (17.0)	CC (83.0)	1.28	0.91 – 1.80
CFHR5	rs6694672	AC+CC (16.9)	AA (83.1)	1.28	0.91 – 1.80
C5	rs3761847	GG (16.6)	AA+AG (83.4)	1.25	0.89 – 1.77
CFH	rs3766404	AG+GG (25.0)	AA (75.0)	1.22	0.90 – 1.66
C5	rs17611	AA (18.5)	AG+GG (81.5)	1.20	0.86 – 1.67
THBD	rs1042580	GG+GA (57.0)	AA (43.0)	1.19	0.90 – 1.58
C5	rs10818488	AA (18.3)	AG+GG (81.7)	1.19	0.85 – 1.67
CFI	rs10033900	CT+CC (74.2)	TT (25.8)	1.18	0.87 – 1.60
CFH	rs529825	CC+CT (47.3)	TT (52.7)	1.17	0.89 – 1.54
FCN2	rs17549193	CC+CT (49.2)	TT (50.8)	1.16	0.88 – 1.52
THBD	rs1962	CC+CT (41.6)	TT (58.4)	1.15	0.88 – 1.52
CFH	rs800292	CC+CT (47.2)	TT (52.8)	1.15	0.87 – 1.51
CFH	rs1329428	CC (19.2)	CT+TT (80.8)	1.15	0.82 – 1.60
CD55	rs10746463	AG+GG (53.3)	AA (46.7)	1.14	0.87 – 1.51
MBL2	rs7095891	CC+CT (44.3)	TT (55.7)	1.12	0.85 – 1.48
C5	rs2900180	AA+AG (57.0)	GG (43.0)	1.11	0.84 – 1.46
C2	rs9332739	CG (7.8)	GG (92.2)	1.10	0.67 – 1.81
CD35	rs6656401	CC+CT (36.0)	TT (64.0)	1.07	0.80 – 1.42
C5	rs2300929	AG+GG (18.2)	AA (81.8)	1.06	0.74 – 1.50
CFB	rs4151667	AT+TT (7.3)	TT (92.7)	1.05	0.81 – 1.36
CFH	rs3753394	TT+CT (50.7)	CC (49.3)	1.04	0.79 – 1.36

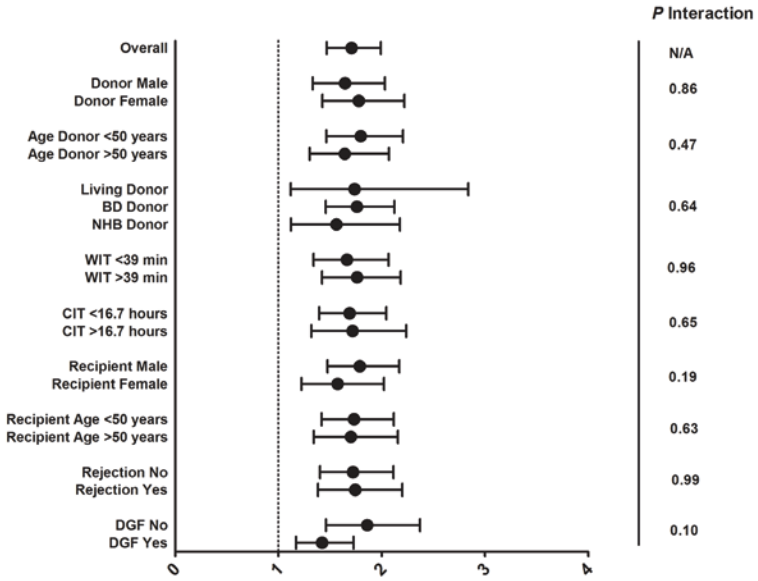
All the complement single nuclear polymorphisms (SNPs) in the recipient, with rs ID number, that were associated with a higher risk for graft loss are displayed. Data are presented as hazard ratio (HR) plus 95 % confidence interval (CI) according to the univariate analysis for graft loss after renal transplantation.

Renal allograft survival

To assess the clinical applications of the Complotype, we studied the predictive value of this genetic profiling in more detail. The Complotype risk score was significantly associated with long-term graft survival. Moreover, the hazard ratio of the Complotype was consistent in subgroups analysis (Figure 3). The confidence intervals of all subgroups showed substantial overlap with the overall hazard ratio at the top, which demonstrates the consistency of findings across subgroups. When analyzed according to quartiles, Kaplan–Meier curves showed decreased graft survival in donor-recipient pairs with a high Complotype risk score (Figure 4; log-rank P<0.001); 10 year graft survival was 90.3% in the first quartiles, 81.9% in the second quartiles, 78.3% in the third quartiles and 63.6% in the fourth quartiles, respectively. Multivariate regression analysis was performed to adjust for other determinants of renal graft survival. In the crude model, the Complotype risk score was associated with a hazard ratio of 1.71 per SD increase (95% CI: 1.47–1.99, P<0.001). We next performed a multivariate analysis with

pre-transplant variables only (Table 6, model 3). After adjustment, the Complotype risk score was associated with a hazard ratio of 1.75 per SD increase (95% CI: 1.51–2.08, P<0.001). In addition, we performed a multivariate analysis with pre- and post-transplant predictors and after adjustment, the Complotype risk score remained significantly associated (Table 6, model 4). Finally, we performed a multivariate analysis with a stepwise forward selection procedure. In the final model, Complotype, donor and recipient age, donor type, DGF, and rejection were included (Table 7).

Figure 3
Hazard ratios for the Complotype in different subgroups



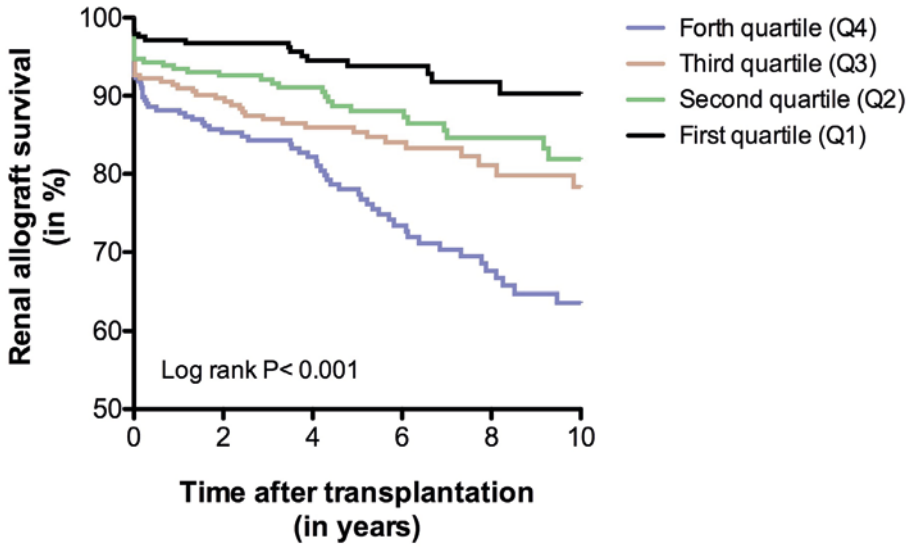
Forest plot of sub-analyses of the Complotype demonstrating that the hazard ratios for graft loss were consistent in different subgroups.

Table 6
Associations of Complotypes with renal allograft survival in donor recipient pairs.

	Total Complotype score		
	Hazard ratio (per SD)	**95% CI**	**P-value**
Model 1	1.71	1.47 – 1.99	P<0.001
Model 2	1.78	1.51 – 2.09	P<0.001
Model 3	1.75	1.48 – 2.08	P<0.001
Model 4	1.69	1.43 – 1.99	P<0.001

Data are presented as hazard ratio with 95% confidence interval (CI) and P-value.
Model 1: *crude model*
Model 2: *adjusted for model 1 plus donor and recipient age, donor origin and dialysis vintage.*
Model 3: *adjusted for model 2 plus donor and recipient gender, donor and recipient blood type, HLA-mismatches, PRA prior to transplantation.*
Model 4: *adjusted for model 2 plus cold and warm ischemia time, the use of corticosteroids and cyclosporine after transplantation, DGF and rejection.*

Figure 4
Kaplan–Meier curves of renal allograft survival according to the total Complotype score



The Kaplan–Meier curve for graft survival among renal transplant recipients according to quartiles of the Complotype score. Log rank tested showed that the graft survival was significantly different among the different patients from the quartiles.

Table 7
Competitive analysis of the associations of clinical factors with renal allograft survival.

Variables not in the equation		Variables in the equation		
Variables	**P-value**	**Variables**	**P-value**	**Hazard Ratio**
Donor type	0.11	DGF	<0.001	3.10 (2.19 – 4.38)
Dialysis vintage	0.98	Complotype	<0.001	1.65 (1.40 – 1.95)
Corticosteroids	0.26	Rejection	0.017	1.51 (1.08 – 2.11)
Cyclosporin	0.93	Donor age	0.001	1.02 (1.01 – 1.03)
Cold ischemia time	0.81	Recipient age	0.030	0.99 (0.97 – 1.00)
Warm ischemia time	0.17			

A multivariate cox regression was performed with a stepwise forward selection. Only variables that were significantly associated in the univariate analysis were included. Data are presented as hazard ratio with 95% confidence interval (CI) and P-value.

Prediction of renal allograft loss

The performance of the Complotype risk score for the prediction of graft loss was also assessed (Table 8). The Complotype risk score alone had a Harrell's C of 0.67 (95% CI: 0.63 – 0.72, P<0.001). Next, additional pre-transplant variables were included and the discriminative accuracy to predict graft loss of the model improved (Table 8, model 3). The highest discriminative accuracy was reached when only significant determinants of graft survival were added (Table 8, model 4), including pre- and post-transplant variables. The Harrell's C of the models significantly improved with the addition of the Complotype risk score. In addition, the Complotype risk score significantly improved the predictive

value of the models according to the integrated discrimination improvement index (IDI). Including the Complotype risk score to fully adjusted models leads to an IDI value of 7.7%, demonstrating that the Complotype risk score substantially enhances the prediction for graft loss. Similarly, the addition of the Complotype risk score to the pre-transplant model adequately reclassified patients at lower risk for graft loss and those at higher risk, as shown by a continuous net reclassification improvement of 0.68 (95% CI, 0.54 to 0.82).

Table 8
Additive value of the Complotype for the prediction of graft loss after renal transplantation.

	Harrell's C (95% CI)		Change (95% CI)*	P-value	IDI (%)	P-value
	without the Complotype	with the Complotype				
Model 1	0.50	0.67 (0.63 – 0.72)	0.174 (0.130 – 0.218)	<0.001	1.91	<0.001
Model 2	0.66 (0.61 – 0.70)	0.73 (0.69 – 0.78)	0.078 (0.072 – 0.08a5)	<0.001	4.30	<0.001
Model 3	0.66 (0.61 – 0.71)	0.73 (0.69 – 0.77)	0.075 (0.070 – 0.080)	<0.001	4.95	<0.001
Model 4	0.73 (0.69 – 0.77)	0.78 (0.74 – 0.81)	0.046 (0.041 – 0.050)	0.003	7.71	<0.001

*Data are presented as Harrell's concordance statistic (Harrell's C) with 95% confidence interval (CI) and integrated discrimination improvement (IDI) with P-value. *Change in C-statistics compared to the model without the Complotype.*
***Model 1:** crude model*
***Model 2:** adjusted for model 1 plus donor and recipient age, donor origin and dialysis vintage.*
***Model 3:** adjusted for model 2 plus donor and recipient gender, donor and recipient blood type, HLA-mismatches, PRA prior to transplantation.*
***Model 4:** adjusted for model 2 plus cold and warm ischemia time, the use of corticosteroids and cyclosporine after transplantation, DGF and rejection.*

Discussion

To optimize clinical care, risk prediction models are useful tools to identify recipients at high risk of graft loss.[5] In this study, we constructed a Complotype genetic score based on multiple complement SNPs in the donor and recipient. The main findings of this study are that the Complotype is significantly associated with long-term graft survival and we subsequently demonstrated a "dose-response" relationship between this genetic score and the risk of graft loss. Extending these findings, this association was observed after a maximum follow-up of 10 years, was present in all subgroups and independent of other determinants of renal allograft survival. Moreover, the Complotype risk score significantly improved risk prediction for graft loss beyond currently used clinical risk factors. Next to the additive effect of SNPs in complement proteins, we also showed that protective and hazardous SNPs could compensate for each other's effect. We are the first to show that combined effects of complement polymorphisms significantly impact graft loss of renal transplant patients. The present study provides clear evidence that rather than single polymorphisms, the risk for graft loss is determined by the combination of complement SNPs in both the donor and recipient.

The concept of the Complotype was first proposed by *Harris et al.* and was suggested to impact the individual susceptibility to inflammatory and infectious diseases.[22] Evaluating the Complotype of an individual could help prediction of disease and may guide decisions on preventive interventions in high-

risk groups. Several in vitro studies have shown that having multiple hazardous SNPs in complement genes leads to higher complement activation.[21,23] In accordance, the study by *Paun et al*. showed that a Complotype composed of out of three common hazardous complement SNPs was associated with higher complement activation levels *in vivo* and the occurrence of age-related macular degeneration (AMD). Since complement is vital in the pathogenesis of transplant renal injury, it is likely that the Complotype affects long-term allograft survival.[20] In accordance, complement SNPs have been shown to impact outcome after kidney transplantation in multiple studies in different populations.[13–16] However, previous studies have only investigated solitary SNPs or haplotypes of a single complement gene.[13,17,29] The complement system forms a tightly regulated network of proteins, therefore an accurate genetic view of this system is only observed with the total repertoire of polymorphisms in complement genes.[22] Our study demonstrates the fundamental principles of the Complotype. Firstly, multiple complement polymorphisms in one individual are common. Secondly, the combination of multiple polymorphisms has a greater impact on outcome than a single one. Thirdly, we demonstrate the possibility of genetic compensation by complement SNPs with opposite effects.

From a prognostic perspective, the Complotype risk score could be used for the prediction of patients at high risk for graft loss. However, the Complotype could also be used to determine compatibility between the donor kidney and the recipient. In current practice, HLA-typing and ABO-typing are done to assess compatibility.[30] Our study shows that the Complotype risk score and the HLA-typing do not provide equivalent prognostic information. The Complotype risk score could, therefore, help to identify the best-suited recipient for each donor kidney. Assessment of the Complotype could offer an indication of the potential complement-mediated renal allograft injury. However, the findings in this study do not rule out the possibility of complement-independent mechanisms of the tested SNPs. Furthermore, the Complotype risk score may be used to identify renal patients that could benefit from treatment with complement inhibitors. Based on our analysis of the Complotype risk score and previous studies, interventions targeting complement in renal transplantation will most likely improve long-term allograft survival. Our results can also have implications for other forms of transplantation such as the heart, lungs, and liver since evidence from other studies support the effect of complement polymorphisms on post-transplant outcome of these organs.[31–33]

Our study has some limitations. The association found in this study is expected to be causal, however since our study is prospective, but observational in nature, it cannot be proven by our results. Furthermore, measurements of complement activation were not performed in our cohort due to the lack of fresh serum samples. We can therefore not determine if individuals with a high Complotype risk score are genetically predisposed to having the highest complement activation during transplantation. In addition, we do not yet know the effects of all the SNPs on the subsequent protein; however, the majority has previously been described. Finally, we only looked at the presence of 60 SNPs in 19 complement proteins. We think that adding other complement SNPs to the model could further improve discrimination. On the other hand, strengths of our study include the high cohort size, the long follow-up and the hard and clinically relevant end point (graft loss).

In conclusion, we systematically evaluated the determinants of long-term renal allograft survival in a cohort of donor-recipient pairs. We demonstrated that the Complotype risk score is strongly

associated with graft loss and that the addition of this score significantly improved risk stratification for graft loss.

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