

ORIGINAL ARTICLE

C3 Polymorphisms and Allograft Outcome in Renal Transplantation

Mira Varaganam, Ph.D., Muhammad M. Yaqoob, M.D., Bernd Döhler, Ph.D.,
and Gerhard Opelz, M.D.

ABSTRACT

BACKGROUND

Complement activation plays a role in the development of chronic allograft nephropathy, a common cause of late allograft loss. The role of two complement component 3 (C3) allotypes, called C3F (fast) and C3S (slow) on the basis of their electrophoretic motility, in the long-term outcome of renal allografts remains controversial.

METHODS

We selected a random sample of 1147 donor and recipient pairs from the Collaborative Transplant Study DNA bank, and their DNA specimens were genotyped for the C3F and C3S alleles. The genotyping results were analyzed according to allograft outcome. Transplants were divided into four groups, according to the recipient and donor genotypes: SS recipient and FS or FF donor (the standard for comparison, since this combination has been reported to have the best outcome), SS recipient and donor, FS or FF recipient and SS donor, and FS or FF recipient and donor.

RESULTS

Baseline characteristics of the four transplant groups were similar. The hazard ratios for allograft survival in the SS recipient and FS or FF donor group as compared with the other three groups (SS recipient and donor, FS or FF recipient and SS donor, and FS or FF recipient and donor) were not significant: 0.90 (95% confidence interval [CI], 0.7 to 1.14; $P=0.33$), 0.87 (95% CI, 0.65 to 1.16; $P=0.33$), and 0.89 (95% CI, 0.65 to 1.23; $P=0.48$), respectively. The four groups had similar patient-survival rates and similar cumulative rates of acute rejection and allograft dysfunction, as assessed by means of serum creatinine levels.

CONCLUSIONS

Our results suggest that transplantation of FS or FF kidneys to SS recipients is not advantageous, possibly because chronic allograft nephropathy is a multifaceted disease involving the interplay of many biologic pathways.

From the Barts and the London School of Medicine and Dentistry and the National Health Service Trust, London (M.V., M.M.Y.); and the Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany (B.D., G.O.). Address reprint requests to Dr. Yaqoob at the Department of Renal Medicine and Transplantation, Royal London Hospital, Whitechapel, London E1 1BB, United Kingdom, or at m.m.yaqoob@qmul.ac.uk.

Drs. Varaganam and Yaqoob contributed equally to this article.

N Engl J Med 2009;360:874-80.
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KIDNEY TRANSPLANTATION IMPROVES the quality of life of patients with end-stage kidney failure, increasing their longevity and freeing them from the restrictions and complications of dialysis. Despite continued improvements in immunosuppressive drugs and medical care, the 10-year allograft survival rate remains disappointingly low, mainly because of chronic allograft nephropathy.¹

Activation of the complement cascade is inevitable in kidney transplantation, because of both the specific and nonspecific immunologic responses of the recipient. Complement component 3 (C3) is central to all three complement activation pathways.² Local production of C3 in the donor kidney is up-regulated in tubules, and the C3 is deposited on the tubule surface adjacent to the T-cell infiltrate in kidneys during allograft rejection.³

A single base substitution in C3 defines two allelic variants: S (slow) and F (fast), based on the differential mobility on gel electrophoresis of the resulting proteins in serum.⁴ The relative ease of use of polymerase-chain-reaction (PCR) technology and the central role of complement in many diseases have resulted in studies that have examined associations between C3F and C3S polymorphisms and disease states.

Generally, the presence of the F allele has a detrimental effect, as has been reported in IgA nephropathy,⁵ age-related macular degeneration,⁶ and systemic vasculitis.⁷ Such reports imply a functional variation in the phenotype associated with the genotype. The allelic frequency of C3F varies markedly among races: 20% among whites, 5% among blacks, and 1% among Asians.⁸ The presence of the C3F allele in either the donor or the recipient has been associated with allograft dysfunction.⁹ However, a recent study showed an improved long-term outcome with donor kidneys carrying the F allele transplanted into S recipients.¹⁰ If confirmed, this observation would mean that it would be important for transplantation laboratories to perform routine genotyping at the C3 locus. Given this finding, we genotyped DNA from 1147 pairs of donors and recipients to examine the importance of this gene in transplantation and its influence on long-term survival of allografts.

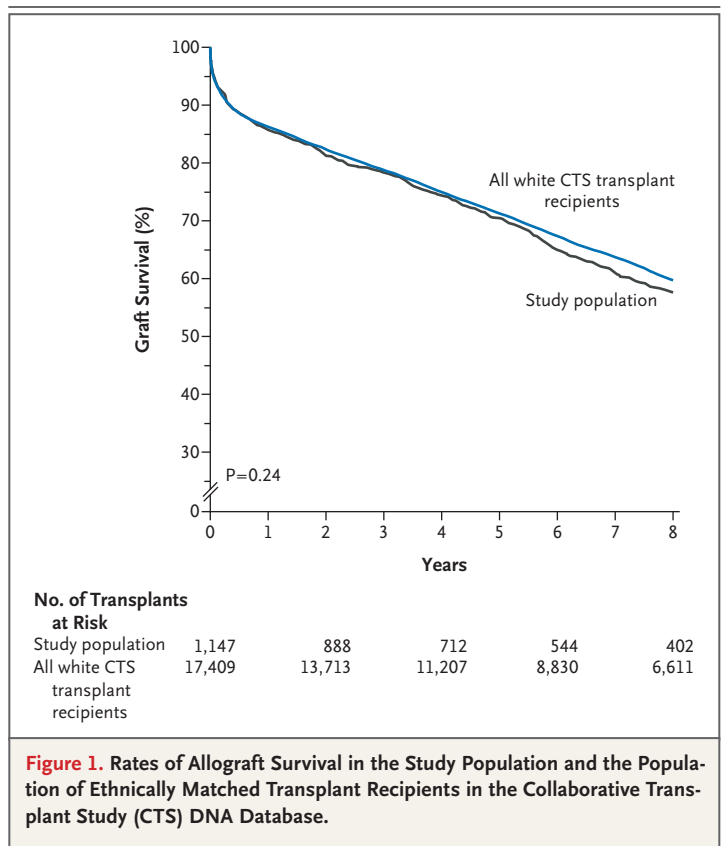


Figure 1. Rates of Allograft Survival in the Study Population and the Population of Ethnically Matched Transplant Recipients in the Collaborative Transplant Study (CTS) DNA Database.

METHODS

STUDY DESIGN

The DNA samples and data on patients were collected between 1986 and 2005. The Collaborative Transplant Study (CTS) collates information and samples from voluntarily participating transplantation centers around the world with the goal of expanding scientific knowledge in the area of transplantation. Written informed consent for the study was obtained from patients at the individual participating centers, and approval for the study was granted by the University of Heidelberg (application no. 083/2005). Samples were genotyped in a blinded fashion, and the results were analyzed according to clinical outcome.

STUDY POPULATION

The kidney-transplant recipients included in the study were 18 years of age or older at the time of transplantation. Transplantations were performed

Characteristic	SS Recipient		FS or FF Recipient		P Value
	SS Donor (N=452)	FS or FF Donor (N=289)	SS Donor (N=252)	FS or FF Donor (N=154)	
Geographic origin — no. (%)					0.68
Europe	406 (90)	256 (89)	219 (87)	138 (90)	
North America	46 (10)	33 (11)	33 (13)	16 (10)	
Time of transplantation					0.12
Year	1996	1995	1995	1995	
Mean time after start of year — mo	0.0±4.6	0.5±4.6	0.7±4.9	0.0±4.9	
Repeat transplant — no. (%)					0.41
First transplant	401 (89)	245 (85)	221 (88)	137 (89)	
Repeat transplant	51 (11)	44 (15)	31 (12)	17 (11)	
Recipient sex — no. (%)					0.55
Female	189 (42)	125 (43)	106 (42)	56 (36)	
Male	263 (58)	164 (57)	146 (58)	98 (64)	
Donor sex — no. (%)					0.80
Female	185 (41)	112 (39)	103 (41)	57 (37)	
Male	267 (59)	177 (61)	149 (59)	97 (63)	
Recipient age — yr	48.6±12.6	47.7±12.6	47.3±12.8	47.6±12.0	0.46
Donor age — yr	42.1±16.0	40.3±16.8	40.6±15.7	40.5±16.8	0.48
Cold-ischemia time — hr	19.9±7.5	20.5±7.8	20.3±7.7	19.0±7.6	0.63
HLA-A, B, and DR mismatches — no. (%)					0.68
0–1	56 (12)	42 (15)	32 (13)	21 (14)	
2–4	328 (73)	215 (74)	186 (74)	117 (76)	
5–6	68 (15)	32 (11)	34 (13)	16 (10)	
Panel-reactive antibody — no. (%)†					0.31
0–10%	307 (72)	188 (70)	175 (75)	100 (72)	
11–50%	78 (18)	38 (14)	32 (14)	20 (14)	
>50%	44 (10)	41 (15)	26 (11)	19 (14)	
Diabetes as disease first affecting renal function — no. (%)	36 (8)	21 (7)	19 (8)	16 (10)	0.69
Initial immunosuppression — no. (%)					0.26
With cyclosporine	375 (83)	253 (88)	209 (83)	127 (82)	
With tacrolimus	61 (13)	29 (10)	37 (15)	18 (12)	
With neither	16 (4)	7 (2)	6 (2)	9 (6)	
With azathioprine	207 (46)	139 (48)	109 (43)	73 (47)	0.68
With mycophenolic acid	153 (34)	86 (30)	83 (33)	43 (28)	
With neither	92 (20)	64 (22)	60 (24)	38 (25)	
Steroid use — no. (%)					0.36
Yes	444 (98)	281 (97)	250 (99)	150 (97)	
No	8 (2)	8 (3)	2 (1)	4 (3)	
Induction antibody therapy — no. (%)					0.55
Yes	152 (34)	102 (35)	77 (31)	46 (30)	
No	300 (66)	187 (65)	175 (69)	108 (70)	

* Plus–minus values are means ±SD. P values are given for comparisons among all four groups.

† Data on panel-reactive antibody level were missing for some patients because of incomplete reporting. The data are shown for 429 allografts with recipient and donor genotypes of SS, 267 allografts with recipient genotype SS and donor genotype FS or FF, 233 allografts with recipient genotype FS or FF and donor genotype SS, and 139 with recipient and donor genotypes of FS or FF.

from 1986 through 2005 in Europe and North America, and all transplants were from deceased donors. All the recipients and donors were white.

The DNA used in the study was randomly chosen from a bank at the Collaborative Transplant Study facility in Heidelberg, Germany, containing DNA from transplant recipients and their donors. In the DNA bank, each donor or recipient is assigned a patient identification number, and his or her DNA sample is given a separate DNA identification number. Both identification numbers are assigned for processing purposes and are not known to anyone outside the computing department.

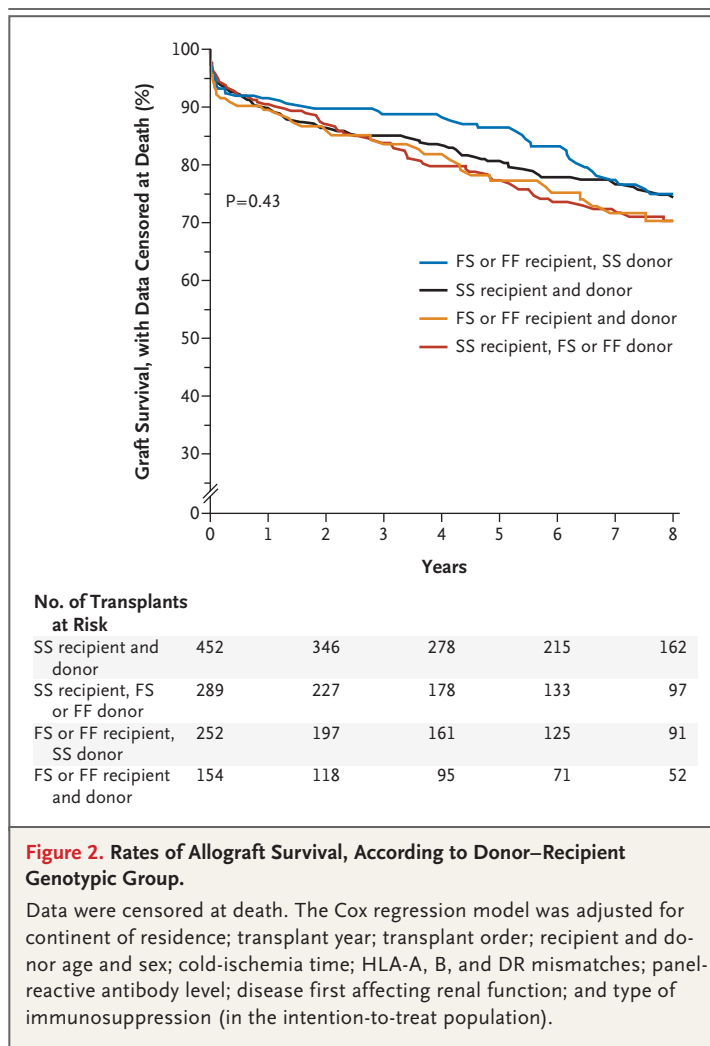
The DNA used in this study was systematically, randomly sampled on the basis of identification numbers. All patients with a patient identification number with a last digit of 3, 6, or 9 were chosen. Since the number of these chosen patients exceeded the 1000 to 1500 needed for genotyping, we then selected, among those patients, only those who had a DNA identification number with a last digit of 2, 4, 6, or 8.

Brown et al.¹⁰ reported a hazard ratio of 2.21 (95% confidence interval [CI], 1.04 to 4.72) for graft loss among SS donor kidneys as compared with FF or FS kidneys. We calculated the number of samples that would yield a hazard ratio of 1.2, assuming an alpha of 0.05 and a statistical power of 95%, using the PS program (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>).

DNA was isolated from buffy-coat or spleen specimens with the use of a salting-out method.¹¹ Approximately 80% of the DNA samples were extracted by staff of the Collaborative Transplant Study; the remainder of the samples were donated by the participating centers after isolation.

CLINICAL DATA OF PATIENTS

Details concerning the donors and recipients are stored in the Collaborative Transplant Study database. This information includes age; sex; continent of residence; transplant year; number of previous transplants; cold-ischemia time; presence of HLA-A, B, and DR mismatches; panel-reactive antibody level; disease leading to renal failure; means of initial immunosuppression; serum creatinine level; number of rejection episodes; timing and cause of death; and details about allograft loss and transplantation outcome.



C3 ALLOTypING

C3 allotyping was done with the use of an amplification refractory mutation system according to the method of Brown et al.,¹⁰ and quality control was performed by validating our primers using genotyped samples from their laboratory. Each reaction mixture contained control primers, which functioned as internal controls. Briefly, the common antisense primer sequence was 5'-CGTCCG-GCCACGGGTA-3', and the sense primer sequence was 5'-AGTTCAAGTCAGAAAAGGTGG-3' for C3F and 5'-CGTCCGGCCACGGGTA-3' for C3S, which gave an amplified product of 278 bp. Control primers were 5'-TGCCAAGTGGAGCACCCAA-3' and 5'-GCATCTTGCTCTGTGCAGAT-3', which yielded a 750-bp product. Cycling conditions were initial

Table 2. Hazard Ratios from Multivariate Cox Regression Analysis among Homozygous Recipients and Donors.*

C3 Allotypes of Recipient and Donor	No. of Recipient–Donor Pairs	Survival of Grafts		Survival of Patients		Survival of Grafts, Data Censored at Death	
		Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
SS recipient and donor	452	1.00		1.00		1.00	
SS recipient and FF donor	31	0.83 (0.45–1.55)	0.56	0.78 (0.31–1.94)	0.60	1.06 (0.51–2.20)	0.88
FF recipient and SS donor	28	0.84 (0.42–1.65)	0.61	1.77 (0.81–3.87)	0.15	0.53 (0.17–1.67)	0.28
FF recipient and donor	0						

* C3 denotes complement component 3.

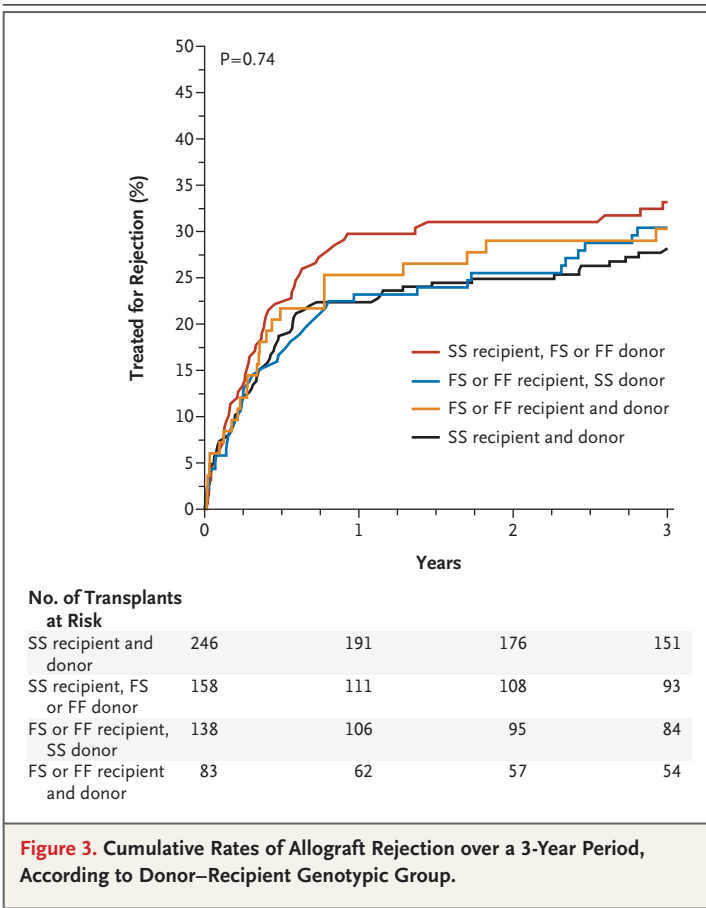


Figure 3. Cumulative Rates of Allograft Rejection over a 3-Year Period, According to Donor–Recipient Genotypic Group.

did not deviate from the Hardy–Weinberg equilibrium.

STATISTICAL ANALYSIS

Statistical analysis was performed with the use of SPSS software, version 15, and R software, version 2.5. Categorical variables were compared by means of chi-square analysis and continuous variables by means of the Kruskal–Wallis test. The Kaplan–Meier algorithm was used to compare survival among genotype groups. Survival results were adjusted for potential confounders using stepwise backward Cox regression.

RESULTS

To rule out selection bias, allograft survival in our study population was compared with that of the entire population of white transplant recipients in the DNA bank of the CTS. Allograft survival did not differ significantly among the 17,409 white donor–recipient pairs in the DNA bank and our 1147 donor–recipient pairs (P=0.24) (Fig. 1). There were no significant differences in the baseline characteristics among the four genotypic groups (Table 1). With regard to transplant outcome, we found that allograft survival was similar in all four groups after censoring of data at death (Fig. 2). Since the F allele in donor kidneys transplanted into SS recipients has been reported as beneficial with regard to long-term allograft survival, this transplantation was of greatest interest. There was no significant difference in the rate of allograft survival between this SS recipient and FS or FF donor group and each of the other three groups (SS recipient and donor, FS or FF recipient and SS donor, FS or FF recipient and donor), with associ-

denaturation at 94°C for 1 minute; then 5 cycles at 94°C for 25 seconds, 70°C for 45 seconds, and 72°C for 30 seconds; followed by 20 cycles of 94°C for 25 seconds, 63°C for 45 seconds, and 72°C for 30 seconds; and finally, 5 cycles at 94°C for 25 seconds, 55°C for 1 minute, and 72°C for 2 minutes. The frequency of C3 genotypes in our study

ated hazard ratios and 95% confidence intervals of 0.90 (0.7 to 1.14, $P=0.33$), 0.87 (0.65 to 1.16, $P=0.33$), and 0.89 (0.65 to 1.23, $P=0.48$), respectively.

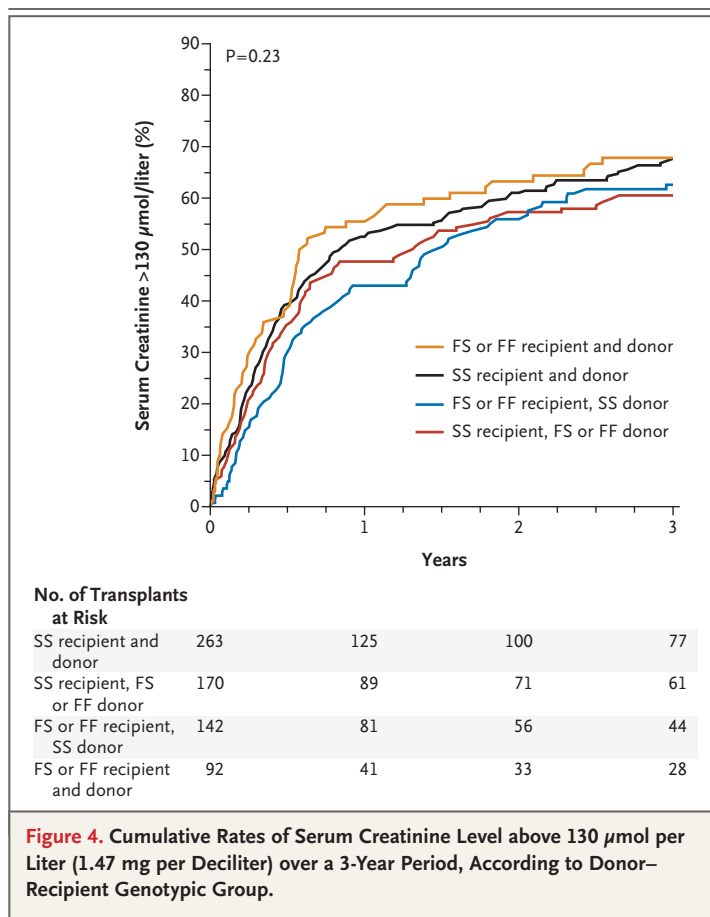
Because of the low frequency of the FF allotype among both the recipients and donors, we combined the FF and FS groups for statistical analysis. In the analysis of the FF allotype separately (Table 2 and the Supplementary Appendix, available with the full text of this article at NEJM.org), there was still no significant difference in allograft survival (after censoring of data at death) in the FF recipient and SS donor group (28 grafts) and the SS recipient and FF donor group (31 grafts) as compared with the SS recipient and donor group (452 grafts). There were no FF recipient and donor pairs (0 grafts) for analysis.

There was no significant difference among the four study groups in rejection rates (Fig. 3) or rates of allograft dysfunction as measured by the percentage of patients with a serum creatinine level above 130 μmol per liter (1.47 mg per deciliter) (Fig. 4). Hazard ratios for the SS recipient and FS or FF donor group with respect to allograft survival, survival of patients, and allograft survival after censoring of data at death were not significantly different among the study groups.

DISCUSSION

The activation of complement associated with transplantation due to innate and adaptive immune mechanisms has led to the investigation of the complement cascade in the context of allograft survival. The importance of deposition of C4d, a component of the complement cascade associated with antibody-mediated alloreactions, in acute and chronic rejection in the allograft capillaries has been acknowledged by virtue of its incorporation in the Banff classification.¹² Proximal tubular cells produce C3 in the transplanted kidney, and in the case of allograft rejection, up to 16% of the circulating complement pool in the recipient is contributed by the donor.^{13,14}

Obtaining a profile of genetic information from a single tube of blood is methodologically simple and relatively noninvasive for the patient. In this context, the result reported by Brown et al.¹⁰ indicating enhanced allograft survival of C3F kidneys transplanted into C3S recipients in their study of 478 donor–recipient pairs would have far-reaching implications, if confirmed. Indeed, the study



by Brown et al. has opened a debate concerning the functional as well as clinical importance of the C3 fast–slow polymorphism.¹⁵ The extent to which the substitution of glycine for arginine¹⁶ at position 80 leads to a functional difference between the C3 allotypes is unclear. The results of functional studies to date have provided conflicting results. Arvilommi¹⁷ found a difference between the allotypes in binding to receptors. However, in another study, Bartók and Walport¹⁸ were not able to reproduce these findings.

In the present study, we used the DNA bank at the CTS as a source to confirm the importance of the C3 alleles in a larger study sample (1147 donor–recipient pairs) than that of Brown et al.¹⁰ We detected no significant differences in long-term allograft survival on the basis of the distribution of C3 alleles among recipients and donors. Our sample size was larger, and the post-transplantation follow-up of our patients was more complete, than that of Brown et al. Whereas the study by Brown et al. involved 113 SS recipients

of an FS or FF kidney and 179 SS recipients of an SS kidney, our study involved 289 and 452 of such transplants, respectively. Furthermore, after 8 years, the study by Brown et al. had follow-up data for only 35 of 478 patients (7.3%), whereas the 8-year follow-up in our study was of 402 of 1147 patients (35%). Although disappointingly negative, our study used the methods of Brown et al. in a larger cohort, and the results question the suitability of the C3F/S polymorphism as a candidate for predicting the compatibility of donor grafts and recipients for transplantation.

The ultimate clinical importance of variants in the complement C3 gene will be determined only

through randomized clinical studies. The complications experienced by transplantation patients are considered the culmination of multifactorial pathways involving many genes, many of which have polymorphic alleles. A key question is whether polymorphisms contribute to the risk of graft loss over and above already identified, traditional risk factors. In the light of our present findings, we suggest that the quest for improving long-term allograft outcomes continue.

Supported by grants from the Renal Unit, Barts and the London National Health Service Trust, and the Department of Transplantation Immunology, University of Heidelberg.

No potential conflict of interest relevant to this article was reported.

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