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Ermini L, Weale ME, Brown KM, Howell WM, Vaughan R, Rebollo Mesa I, Chowdhury P, Sacks SH, Sheerin NS. **Systematic assessment of the influence of complement gene polymorphisms on kidney transplant outcome.**
***Immunobiology* 2015. DOI: 10.1016/j.imbio.2015.12.006**

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DOI link to article:

http://dx.doi.org/10.1016/j.imbio.2015.12.006

Date deposited:

18/12/2015

Embargo release date:

12 December 2016

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Manuscript

Systematic assessment of the influence of complement gene polymorphisms on kidney transplant outcome

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Abstract

The importance of the innate immune system, including complement, in causing transplant injury and augmenting adaptive immune responses is increasingly recognised. Therefore variability in graft outcome may in part be due to genetic polymorphism in genes encoding proteins of the immune system. This study assessed the relationship between single nucleotide polymorphisms (SNPs) in complement genes and outcome after transplantation. Analysis was performed on two patient cohorts of 650 and 520 transplant recipients. 505 tagged SNPs in 47 genes were typed in both donor and recipient. The relationships between SNPs and graft survival, serum creatinine, delayed graft function and acute rejection were analysed. One recipient SNP in the gene encoding mannose binding lectin was associated with graft outcome after correction for analysis of multiple SNPs ($p=6.41 \times 10^{-5}$). When further correction was applied to account for analysis of the effect of SNPs in both donor and recipient this lost significance. Despite association p values of <0.001 no SNP was significantly associated with clinical phenotypes after Bonferroni correction. In conclusion, the variability seen in transplant outcome in this patient cohort cannot be explained by variation in complement genes. If causal genetic effects exist in these genes, they are too small to be detected by this study.

Key words: Complement, single nucleotide polymorphism, kidney transplantation, outcome

Abbreviations

APC; Antigen Presenting Cell
MBL; mannose binding lectin
QC; quality control
SNP; single nucleotide polymorphism.

Introduction

The complement system is a major element of the innate immune system. Its main physiological function is the killing and elimination of pathogens, apoptotic cell debris and immune complexes. It achieves this by formation of the pore forming membrane attack complex C5b-9, release of the potent inflammatory mediators C3a and C5a and by opsonisation, primarily with C3b. Complement is activated by three pathways that converge with the assembly of C3 and C5 convertases and subsequent amplification of the cascade. Excessive or inappropriate activation of complement can lead to tissue injury, so to prevent damage a series of serum and membrane bound inhibitors exist to limit activation directed at self. Despite these inhibitors, complement-mediated tissue injury is well documented.

In transplantation complement activation can occur at many stages and there is increasing evidence that it contributes to graft damage. Reperfusion of an ischemic graft leads to complement activation (Zhou, et al., 2000) and complement inhibition reduces injury (Pratt, et al., 2003, Thurman, et al., 2006). The presence of preformed or new donor specific antibodies leads to complement activation via the classical pathway. Hyperacute rejection is now rarely seen but acute and chronic antibody mediated rejection is increasingly recognised and complement is both a biomarker and probable mediator of graft injury (Stegall and Gloor).

The capacity of the transplanted graft to synthesise many complement proteins adds further complexity to complement's role in transplant injury. Complement gene expression is up-regulated by donor factors prior to transplantation and higher pre-transplant expression is associated with both early and late graft dysfunction (Naesens, et al., 2009, Hauser, et al., 2004). It is evident from studies in mice that the intra-renal synthesis of C3, the pivotal complement protein, contributes both ischemia reperfusion injury (Farrar, et al., 2006) and acute rejection (Pratt, et al., 2002). In patients, local expression of C3 in the transplanted kidney increases during acute rejection (Andrews, et al., 1994), spilling over into the circulation where it can contribute 10% of total C3 (Tang, et al., 1999).

There are a number of potential ways in which complement activation can contribute to graft rejection. Complement proteins can directly damage renal cells, a mechanism likely to contribute to acute antibody-mediated rejection. Sublytic deposition of membrane attack complex on cells can induce synthesis of cytokines and growth factors which, with the anaphylotoxins, can enhance graft inflammation and fibrosis. As well as its well documented influence on B cell immunity (Dempsey, et al., 1996), complement can also augment the T-cell allo-immune response (Heeger, et al., 2005). Complement receptor binding of opsonised cells increases interaction between lymphocytes and antigen presenting cells (APC) (Kerekes, et al., 1998) and C3a and C5a can activate and drive T cells into specific effector phenotypes (Lalli, et al., 2007, Liu, et al., 2008, Peng, et al., 2008, Peng, et al., 2009). Complement-mediated inflammatory injury may also provide a source of antigen and alter the local inflammatory environment (Lewis, et al., 2008). Whatever the mechanism, it is clear from animal studies that blocking complement effector functions can dramatically improve graft survival (Gueler, et al., 2008, Rother, et al., 2008, Pavlov, et al., 2008).

There is significant variation in transplantation outcome. Differences in outcome can in part be explained by factors such as donor age and ischemic time. However, this is insufficient to explain all the variation seen and the possible influence of both donor and recipient genetic risk factors has been proposed. The known contribution of genetic variation in major histocompatibility antigens does not fully explain differences in outcome (Su, et al., 2004).

As with many genes, those of the complement system contain many polymorphisms with putative functionality. The *C3* gene has many polymorphic variants, the best known being the F/S polymorphism (rs2230199). The F/S polymorphism has been associated with many inflammatory diseases and we reported an effect on transplant outcome (Brown, et al., 2006), although this was not replicated in a subsequent study (Varagunam, et al., 2009). Other studies have reported an effect of complement polymorphism following transplantation. For example polymorphisms in Mannose-binding lectin (MBL) have been implicated in the development of ischemic renal injury (de Vries, et al., 2004). The serum concentration of MBL is determined by promoter and exon 1 polymorphisms and these

SNPs influence graft survival after kidney (Berger, et al., 2005), simultaneous pancreas-kidney (Berger, et al., 2007), lung transplantation (Munster, et al., 2008) and have been associated with risk of infections post-transplantation (Verschuren, et al., 2008, Cervera, et al., 2009).

Genetic variation in the complement pathway could explain some of the variability in transplant outcome. In this study we have used a set of tagged single nucleotide polymorphisms (SNPs) across all complement genes to determine the effects of genetic polymorphic variation in complement genes on renal transplant outcome.

Materials and methods

Sample collection

The sample and data collection and analysis was approved by the local Research Ethics Committee (24932). Donor and recipient pairs were identified for which DNA was available from both parties. DNA was identified in donor and recipient pairs transplanted at Guy's and St Thomas' Hospital NHS Foundation Trust between 1993 and 2004 (KCL cohort, 650 pairs) and at Newcastle upon Tyne Hospitals NHS Foundation Trust between 1994 and 2005 (NCL cohort, 520 pairs). Only donor-recipient pairs who were aged over 18 years at the time of transplantation were included. To reduce the possibility of confounding due to population stratification, only Caucasian donors and recipients were included in the study.

Definition of phenotypes

Graft survival was defined as days from transplant date to a graft failure event: defined as a return to dialysis; a return to the transplant waiting list; or re-transplantation. Patients were defined as having acute rejection if they had one or more episodes of acute rejection that was biopsy proven (Banff (97) categories 2 and 4 (KCL patients only)). Delayed graft function was defined as the need for dialysis within the first week post-transplantation. Serum creatinine (Cr) measurements were supplied by the renal unit responsible for patient follow up. The serum Cr concentration at 1, 3 and 5 years (mean of the 3 measures closest

to each time point) after kidney transplant was used for analysis. Grafts that had failed (as defined above) were not included in the analysis.

SNP selection and genotyping

SNPs were selected to cover 47 genes considered by expert review to be the principal genes involved in the complement pathway (Supplementary Table 1 and 2). For tagging purposes, genes were clustered together into groups whenever inter-gene distances were less than 200kb. A hybrid tagging-plus-putative-function selection strategy was used, similar in design to Cavalleri *et al.* (Cavalleri, et al., 2007). First, common SNPs with putative functional effect were selected using the TAMAL v2 program (Hemminger, et al., 2006). SNPs were selected if there was evidence that the minor allele frequency (MAF) was greater than 5% in European populations and one of the following functional criteria were met: (1) non-synonymous coding SNP; (2) SNP within predicted promoter region; (3) SNP within evolutionarily conserved region; (4) SNP within predicted transcription factor binding site; (5) SNP within conserved miRNA target; or (6) SNP within a splice region. Some additional putative functional SNPs were also added via a manual literature search. 106 putative functional SNPs were selected in this way. Next, these SNPs were assessed for their linkage disequilibrium tagging ability using the Tagger program (de Bakker, et al., 2005), and additional SNPs were chosen to ensure an r^2 greater than 0.9 with all SNPs with MAF>5% in the HapMap Phase 2 European-ancestry sample and residing within the transcribed region of each gene. This resulted in 505 SNPs being selected for genotyping (Supplementary Table 2).

This set of SNPs was typed in our DNA samples by Sequenom using the iPlex platform. This utilises a multiplex PCR system followed by a single base pair extension step to produce primer extension products which are then analysed by MALDI-TOF mass spectrometry.

SNPs and DNA samples were subjected to the following quality control procedures. Sample were excluded if they could not be matched to the clinical database; if they had a reported gender in the clinical database incompatible with observed X-chromosome genotypes; if they had genotypes which completely matched another sample (with the exception of one known monozygotic twin pair); or if they had more than 20% missing genotype data in either the KCL or Newcastle datasets. SNPs were excluded if they had more than 10% missing data; if they had a sample MAF<0.002, or if they had an exact-test p-value for departure from Hardy-Weinberg equilibrium <0.001. QC resulted in final post-QC figures of 1295 samples, 621 donor-recipient pairs and 416 SNPs in the KCL dataset, and 1039 samples, 512 donor-recipient pairs and 448 SNPs in the Newcastle dataset. 409 SNPs passed QC in both datasets.

Selection of covariates

A model selection procedure on the clinical variables available to us was performed to identify which covariates should be entered into downstream analysis. We carried out a stepwise Cox proportional hazards model selection procedure on graft survival in the KCL cohort, using the AIC-based forward-backward model selection procedure implemented in the step() function of the R statistical package (http://www.r-project.org/), based on the following variables: date of transplant, follow-up unit code, donor and recipient gender, donor and recipient age, donor cause of death, number of previous grafts received by the recipient, graft origin (local / imported), donor type (cadaveric / living), transplant state (heartbeating / non-heartbeating), donor-recipient relatedness (e.g. 0 for unrelated, 0.5 for full sibs), and broad specificity mis-match status entered separately for HLA-A, -B and –DRB. The three variables emerging with the strongest statistical support for an effect on graft survival were donor type (cadaveric / living), recipient age and donor age. However, because of missing data values for donor age only donor type and recipient age were used as covariates in all downstream analyses. For comparison we also ran our graft survival analyses on the KCL dataset using the top 6 covariates (donor type, recipient age, donor age, HLA-DRB

mismatch, number of previous grafts, and recipient gender), and failed to find any significant hits for this analysis (data not shown).

Association analysis

Graft survival was tested via Cox proportional hazards modelling. Binary endpoints (acute rejection, delayed graft function, data from KCL cohort only) were tested via logistic regression. Creatinine levels after one, three and five years, as well as the difference between one and three and one and five years, were tested via standard linear regression. All association analyses employed the same covariates as previously described.

Graft survival analyses were performed on the combined KCL and Newcastle datasets, with centre origin (KCL / Newcastle) added as an additional covariate. Within cohort analyses were performed to establish whether association effects were consistent in direction between the two centres. Creatinine analyses were performed separately on KCL and Newcastle datasets, because creatinine measurements were undertaken using separate protocols at the two centres.

Unless otherwise stated, all analyses tested a linear effect of allele 'dose' on the phenotype when transformed on the relevant scale, with minor homozygotes / heterozygotes / major homozygotes coded as 0/1/2 respectively. Three genetic association effects were tested for each SNP on each phenotype: a donor genotype main effect, a recipient genotype main effect, and a donor x recipient linear interaction effect. Tests were performed using likelihood ratio tests that compared the model with the effect against the relevant simpler hierarchical model (interaction effects were tested with main effects present in both models). All statistical analyses were done with Plink software version 1.07 (http://pngu.mgh.harvard.edu/ ~ purcell/plink/) and the R statistical package version 2.14 (http://www.r-project.org/).

Results

Transplant survival

Donor, recipient and interaction associations were assessed between SNPs in the complement system and transplant survival, censoring for death with a functioning graft (with transplant type and recipient age as covariates). The SNPs that had low p values in both the KCL and NCL cohorts are presented in Table 1. The SNP most closely associated with graft loss was in the gene encoding mannose binding lectin (rs4935047, donor main effect, $p=6.41 \times 10^{-5}$) (Figure 1). This is a significant association when the Bonferroni correction is applied to correct for analysis of multiple SNPs ($alpha=1.2 \times 10^{-4}$). However, each SNP was tested for donor, recipient and interaction effects. When a further correction is made to account for this, formal significance is lost ($alpha=4.1 \times 10^{-5}$). There are 4 SNPs in complement C7 with $P<0.01$. The presence of 4 SNPs in this list may be explained by linkage disequilibrium between the SNPs.

Additional survival analyses were performed treating death with a functioning graft as a failure event. The ten SNPs with the lowest p values and consistent in-group effect are shown in Table 2. No SNP demonstrated a significant association with graft survival after correction for multiple tests but again there are SNPs with low P values in genes within the lectin pathway and C7. Although this raises the possibility of a biological effect, further studies would be needed to determine whether an effect was present. No effect of the SNP (rs2230199) responsible for the F/S polymorphism in C3 was observed.

Serum Creatinine

Serum Cr was used as a biomarker to assess whether complement SNPs were associated with graft function. Associations with Cr at 1, 3 and 5 years following transplantation as well as the change in Cr between these time points were assessed. The ten SNPs with the lowest p value are shown in Table 3 for the KCL (A) and NCL (B) cohorts. The lowest p value achieved in either cohort was for a SNP in MBL (rs930507, recipient main effect in delta-Cr between 1 and 3 years post-transplant, $p=7.16 \times 10^{-5}$) (Figure 2). However, this did

not reach statistical significance after correction for multiple analyses (alpha=2.26 x 10^{-5}) and this was not replicated in the NCL cohort.

Delayed graft function and acute rejection

Associations between complement SNPs and two other transplant phenotypes, delayed graft function and biopsy proven acute rejection were also tested. Biopsy analysis was derived from clinical reports using the same Banff criteria. SNPs with the lowest p values are shown for these two phenotypes are shown in Tables 4A and 4B. Although SNPs were associated with both of these phenotypes with a significance level of $p<0.001$, this association was not significant after Bonferroni correction for multiple tests (alpha=1.11 x 10^{-4})

Discussion

This study represents the largest and most comprehensive study on the effect of complement genetics of both short-term and long-term renal transplant outcomes. Donor and recipient genotypes were tested along with their putative interaction effects. Despite possessing larger sample sizes than previous studies, we failed to find any significant effects after correcting for the multiple tests implicit in our study design.

The complement system has been implicated in the pathogenesis of renal allograft injury at several stages during the life of the graft. Even prior to implantation complement gene expression is increased in grafts and the magnitude of this increase correlates with long-term graft outcome (Naesens, et al., 2009). Complement is activated in a kidney at the time of reperfusion and there are many pre-clinical studies showing that preventing complement activation has a beneficial effect (Lewis, et al., 2008, Pratt, et al., 1996). Complement activation influences the development allo-immune responses in experimental models of kidney transplantation, altering T cell and dendritic cell function (Pratt, et al., 1997). Inflammatory damage to the graft caused by complement activation may provide a source of alloantigen and a stimulus to the adaptive immune response. (Peng, et al., 2008).

In addition to the known effects of complement in transplantation there are reports of polymorphic variation in complement genes affecting the outcome after kidney transplantation (Brown, et al., 2006, Gunesacar, et al., 2015, Jeong, et al., 2011). However, an independent study did not find an association with C3 polymorphisms and transplant outcome (Varagunam, et al., 2009), and although a more recent study has shown that donor C3F allotype protects from primary non-function there was no effect on long-term outcome (Damman, et al., 2012). These findings suggest that the original finding was a false positive.
Graft survival is a complex phenotype affected by many donor and recipient factors. Part of the variability in kidney transplant outcome is explained by polymorphic variation in the Major Histocompatibilty Complex (MHC) and other loci possibly associated with immune function (O'Brien, et al., 2013). Given this, and given the general observation that any complex trait, when examined in sufficient depth, yields multiple controlling genetic loci, and given that a role for the complement system in transplantation is becoming increasing more likely from molecular studies, we propose that the negative findings from our study are more likely to stem from a lack of power to detect genetic signals in the genes examined than from a complete absence of such signals. The lack of power can be attributed to any one, or a combination, of the following factors: (1) low effect sizes, implying insufficient sample sizes; (2) low linkage disequilibrium between causal variants and the SNPs typed in our study; (3) (for the survival and quantitative trait analyses) insufficient control for other major covariates, such that genetic signals are swamped by noise.
We acknowledge that all three of the above may have played a part in our negative findings. With regards to linkage disequilibrium, we note that at the time of the design of our SNP panel, the HapMap Phase II dataset was the best resource available for determining linkage disequilibrium among common SNPs. Since then, more extensive datasets such as the 1000 genomes resource have become available, and it has been noted, for example, that some common polymorphisms of potential functional significance in MBL2 are not well tagged by our panel because they were not present in the HapMap Phase II dataset. Furthermore, the fact that 12% of our SNPs failed QC due to technical issues (standard for

custom-designed panels of this nature) means that gaps in the coverage for some genes exist.

With regards to the effect of covariates on survival and quantitative trait analyses (note binary trait analyses should not be affected because these covariates are likely to be uncorrelated with our genetic traits), we carried out a model selection process to include any covariates present in our clinical database that had a demonstrably large effect on graft survival in our cohorts. We accept, however, that our clinical database did not include a number of variables of potential relevance, including underlying renal disease, cold ischemic time, donor baseline pathology, HLA immunization, drug regimen and compliance. Missing data presented a consequent problem for downstream analysis.

Candidate gene association studies, even those that have examined many genes across a single pathway, have had a mixed record in identifying genetic variants for complex traits (Ioannidis, 2005, Lohmueller, et al., 2003). However, the well-known effects at the HLA locus, and the fact that renal transplants between identical twins have the best graft survival outcomes, are proof that genetic effects are certainly at work in renal transplant outcomes. Elucidation of these await the implementation of still larger studies.

Acknowledgements

The work was funded by the UK Medical Research Council (grant reference G0701320).

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Table legends

Table 1. SNPs with lowest p-values for association with graft survival (combined analysis across both KCL and NCL cohorts) and consistent within-cohort effects. Patient death was treated as a censoring event. SNPs are ordered by overall p-value regardless of the genetic effect tested. SNP – rs Identifier (effect; S – synonymous, NS – non-synonymous, NC – non-coding); Gene - nearest gene; MAF - minor allele frequency; Log hazard ratio (all) - natural log of hazard ratio for heterozygotes versus major homozygote (all data); Log hazard ratio (KCL) - natural log of hazard ratio for KCL data only; Log hazard ratio (NCL) - natural log of hazard ratio for NCL data only; SE (all) / p-value (all) / N (all) = standard error/p-value/non-missing-data-size for log hazard ratio (all); Effect = D if donor main effect, R if recipient main effect, I if DxR interaction. Bonferroni corrected alpha level = 4.1E-05.

Table 2. SNPs with lowest p-values for association with graft survival (combined analysis across both KCL and NCL cohorts) and consistent within-cohort effects.. Patient death treated as a failure event failure. SNPs are ordered by overall p-value regardless of the genetic effect tested. SNP – rs Identifier (effect; S – synonymous, NS – non-synonymous, NC – non-coding); Gene - nearest gene; MAF - minor allele frequency; Log hazard ratio (all) - natural log of hazard ratio for heterozygotes versus major homozygote (all data); Log hazard ratio (KCL) - natural log of hazard ratio for KCL data only; Log hazard ratio (NCL) - natural log of hazard ratio for NCL data only; SE (all) / p-value (all) / N (all) = standard error/p-value/non-missing-data-size for log hazard ratio (all); Effect = D if donor main effect, R if recipient main effect, I if DxR interaction. Bonferroni corrected alpha level = 2.26E-05.

Table 3. SNP associations with serum creatinine. The top ten SNPs associated with serum creatinine in the KCL patient cohort (A) and the NCL patient cohort (B). SNP – rs Identifier (effect; S – synonymous, NS – non-synonymous, NC – non-coding); Gene - nearest gene; MAF - minor allele frequency; SE/p-value/N - standard error/p-value/non-missing-data-size

for Slope; CRT1Y - Creatinine at 1 year; CRT3Y - Creatinine at 3 years; CRT5Y - Creatinine at 5 years; CRT1_3Y - Change in creatinine between 1 and 3 years; CRT1_5Y - change in creatinine between 1 and 5 years; Effect - D if donor main effect, R if recipient main effect, I if DxR interaction. Bonferroni corrected alpha level = 2.26E-05.

Table 4. SNP associations for acute rejection and delayed graft function. The top ten SNPs associated with biopsy proven acute rejection in the KCL patient cohort (A). The top ten SNPs associated with biopsy proven acute rejection in the KCL patient cohort (B). SNP – rs Identifier (effect; S – synonymous, NS – non-synonymous, NC – non-coding, MIS - Missense); Gene - nearest gene; MAF - minor allele frequency; SE/p-value/N - standard error/p-value/non-missing-data-size for Slope; OR – Odds ratio. Effect - D if donor main effect, R if recipient main effect, I if DxR interaction. Bonferroni corrected alpha level = 1.113E-04.

Table 1

SNP (effect)	Gene	MAF	Log hazard ratio (all)	Log hazard ratio (KCL)	Log hazard ratio (NCL)	SE (all)	p-value (all)	N (all)	Effect
rs4935047 (NC)	MBL2	0.441	-0.402	-0.410	-0.393	0.169	6.41E-05	1120	D
rs874603 (NC)	MASP1	0.076	-1.882	-3.260	-0.849	1.001	2.72E-04	1115	R
rs1910016 (NC)	C7	0.488	-0.383	-0.101	-0.772	0.204	1.18E-03	1041	D
rs12658133 (NC)	C7	0.189	0.690	0.610	0.771	0.228	1.89E-03	1098	I
rs419137 (NC)	CFH	0.125	0.877	0.968	0.566	0.629	2.69E-03	1096	R
rs3792640 (NC)	C7	0.192	0.607	0.542	0.665	0.222	5.24E-03	1115	I
rs2455308 (NC)	C7	0.192	0.643	0.631	1.322	0.238	5.44E-03	1126	I
rs2842704 (NC)	C4BPA	0.141	-0.832	-0.968	-0.837	0.289	5.50E-03	1091	I
rs1048971 (S)	CR2	0.360	0.086	0.062	0.191	0.206	5.54E-03	1107	R
rs7499077 (NC)	ITGAM	0.278	0.500	0.466	0.581	0.188	6.90E-03	1096	I

Table 2

SNP (effect)	Gene	MAF	Log hazard ratio (all)	Log hazard ratio (KCL)	Log hazard ratio (NCL)	SE (all)	p-value (all)	N (all)	Effect
rs1078375 (NC)	C7	0.417	-0.442	-0.337	-0.534	0.119	2.16E-04	1047	I
rs4935047 (NC)	MBL2	0.441	-0.236	-0.233	-0.233	0.139	3.28E-04	1120	D
rs294185 (NC)	C1QG	0.390	0.383	0.237	0.575	0.119	1.21E-03	1108	I
rs4151657 (NC)	BF	0.331	0.397	0.561	0.104	0.134	2.83E-03	1115	I
rs2675982 (NC)	C7	0.069	-0.627	-0.613	-0.376	0.683	4.25E-03	1124	R
rs6772329 (NC)	MASP1	0.081	-1.870	-2.873	-1.393	0.832	4.32E-03	1059	D
rs2443040 (NC)	C7	0.375	0.373	0.365	0.404	0.132	4.57E-03	1033	I
rs7545126 (NC)	MCP	0.110	-0.646	-0.202	-1.168	0.594	5.81E-03	1109	R
rs6772329 (NC)	MASP1	0.081	1.045	1.450	0.878	0.438	6.96E-03	1059	I
rs1143664 (S)	C1S	0.075	-0.999	-1.142	-0.108	0.368	8.08E-03	1118	I

Table 3

A

SNP (effect)	CHR	Gene	MAF	Coefficient	SE	p-value	N	Effect	Creatinine
rs930507 (S)	10	MBL2	0.161	-15.989	3.972	7.16E-05	317	R	CRT1_3Y
rs6602392 (NC)	10	IL2RA	0.107	-16.017	4.334	2.53E-04	376	D	CRT1Y
rs11569450 (NC)	19	C3	0.105	18.765	5.350	5.31E-04	269	R	CRT1_5Y
rs2300955 (NC)	1	C8A	0.182	-12.948	3.724	5.67E-04	380	D	CRT1Y
rs5964488 (NC)	23	VSIG4	0.155	-11.102	3.224	6.40E-04	372	D	CRT1Y
rs835209 (NC)	5	C9	0.035	-26.550	7.764	7.00E-04	362	D	CRT1Y
rs11569523 (NC)	19	C3	0.056	21.800	6.437	8.17E-04	268	R	CRT1_5Y
rs2300955 (NC)	1	C8A	0.182	-32.434	9.868	1.11E-03	385	I	CRT3Y
rs11569523 (NC)	19	C3	0.056	18.952	5.794	1.19E-03	328	R	CRT1_3Y
rs619545 (NC)	1	C8A	0.267	10.173	3.158	1.39E-03	374	D	CRT1Y

B

SNP (effect)	CHR	Gene	MAF	Coefficient	SE	p-value	N	Effect	Creatinine
rs7519492 (NC)	1	intergenic	0.130	-8.612	21.027	1.28E-04	361	R	CRT3Y
rs16840422 (NC)	1	CFH	0.140	-40.170	15.342	1.71E-04	337	R	CRT1_3Y
rs7519492 (NC)	1	intergenic	0.130	-36.495	14.713	1.80E-04	341	R	CRT1_3Y
rs1738548 (NC)	11	CD59	0.278	18.767	17.722	1.98E-04	184	I	CRT5Y
rs16840422 (NC)	1	CFH	0.140	-13.132	21.896	2.74E-04	357	R	CRT3Y
rs1887632 (NC)	1	CR1	0.091	-34.981	14.773	3.06E-04	333	I	CRT1_3Y
rs6674522 (NC)	1	CFHR2	0.112	-37.659	15.288	5.65E-04	335	R	CRT1_3Y
rs710459 (NC)	3	MASP1	0.427	-1.832	11.911	6.05E-04	188	D	CRT1_5Y
rs11569450 (NC)	19	C3	0.111	-11.408	21.787	6.50E-04	357	R	CRT3Y
rs6697228 (NC)	1	C8A	0.142	-11.599	11.018	6.93E-04	187	D	CRT1_5Y

Table 4

A

SNP (effect)	Chr	Gene	MAF	Coefficient	SE	OR	p-value	N	effect
rs696764 (NC)	5	C9	0.135	1.671	0.557	5.317	1.72E-04	323	I
rs3886100 (NC)	1	CR1	0.421	1.064	0.299	2.898	2.88E-04	362	R
rs11672613 (NC)	19	C3	0.432	0.549	0.164	1.732	6.07E-04	370	D
rs831627 (NC)	11	CD59	0.035	1.555	0.566	4.735	1.67E-03	359	R
rs6661764 (NC)	1	CR1	0.268	0.579	0.198	1.784	2.72E-03	362	R
rs13157656 (MIS)	5	C7	0.221	1.047	0.376	2.849	3.37E-03	311	I
rs696760 (NC)	5	C9	0.102	1.966	0.713	7.142	3.95E-03	323	I
rs3795341 (NC)	1	CFHR4	0.169	-0.528	0.196	0.590	6.42E-03	363	R
rs1048118 (S)	23	CFP	0.243	-0.391	0.144	0.676	6.47E-03	359	R
rs17020983 (NC)	1	C4BPA	0.064	-1.811	0.679	0.163	6.88E-03	321	I

B

SNP (effect)	Chr	Gene	MAF	Coefficient	SE	OR	p-value	N	effect
rs7732104 (NC)	5	C7	0.166	-0.705	0.210	0.494	8.17E-04	536	R
rs7040603 (NC)	9	C5	0.194	-1.137	0.372	0.321	1.56E-03	506	I
rs3181274 (NC)	11	CD59	0.351	-0.514	0.165	0.598	1.70E-03	539	D
rs1444903 (NC)	5	C6	0.123	-0.741	0.240	0.477	1.80E-03	433	R
rs17514136 (NC)	9	Ficolin2	0.270	-0.534	0.175	0.586	2.16E-03	538	R
rs7951 (S)	19	C3	0.074	2.624	0.872	13.791	2.36E-03	523	I
rs4926 (MIS)	11	Serp1	0.261	0.798	0.278	2.221	3.50E-03	529	I
rs3886100 (NC)	1	CR1	0.421	0.816	0.28	2.261	3.87E-03	538	D
rs12722588 (NC)	10	IL2RA	0.174	-0.599	0.209	0.549	4.27E-03	539	D
rs11569450 (NC)	19	C3	0.105	0.841	0.314	2.319	4.48E-03	539	D

Figure legend

Figure 1. Kaplan-Meier plots of graft survival stratified by donor rs4935047 genotype. Top line (solid, straight ticks) is for the major homozygote; middle line (dotted, cross ticks) is for the heterozygote; bottom line (dashed, star ticks) is for the minor homozygote.

Figure 2. Box plots of KCL 1- vs 3-year serum creatinine stratified by recipient rs930507 genotype. Numbers in each group are also indicated.

Figures

Figure 1

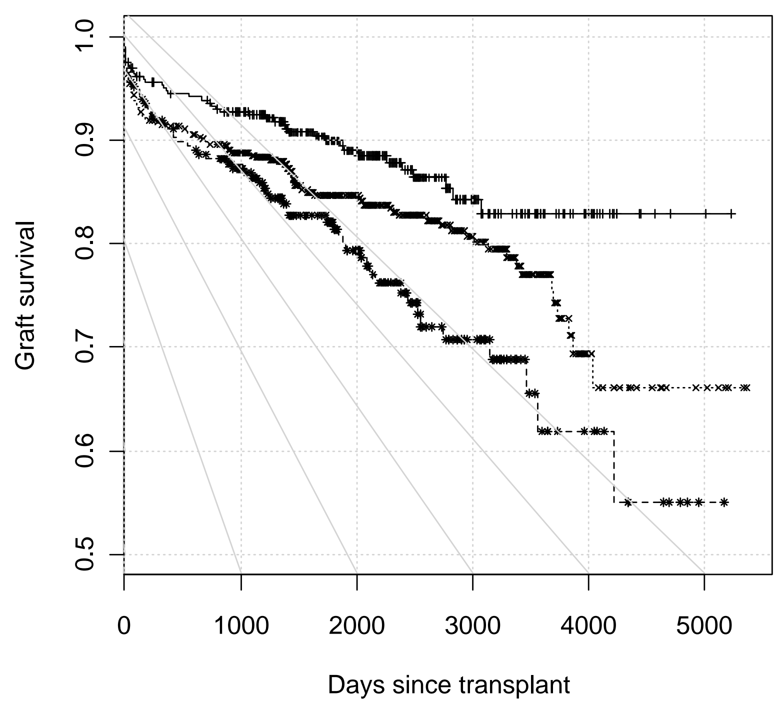
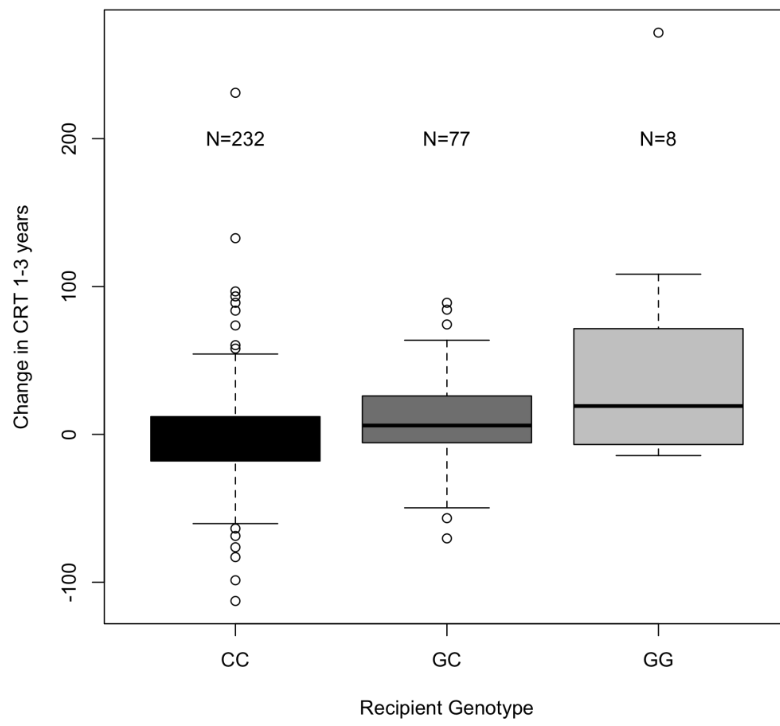


Figure 2



***Conflict of Interest**

The authors have no conflict of interest to declare.

Professor Neil Sheerin
On behalf of the authors

Supplementary tables

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