Angiopoietins in renal replacement therapy

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CHAPTER 6

Angiopoietin-2 single nucleotide polymorphisms affect graft survival after renal transplantation

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ABSTRACT

Background

Elevated angiopoietin-2 (Ang2) levels in renal transplant recipients have been associated with cardiovascular events and mortality in dialysis patients. Upon renal transplantation, Ang2 levels have been associated with graft failure and mortality. In this study, we investigated the role of the functional Ang2 single nucleotide polymorphisms (SNPs) in deceased donor renal transplantation.

Methods

The genotypes of rs2442635 rs2442468, rs2515435 and rs2916702 were determined in deceased donors and recipients of a deceased donor kidney from a total of 1270 transplantations. Ang2 genotypic variations were associated in recipients or donors with death censored graft survival, primary non-function (PNF), delayed graft function (DGF), acute rejection and all-cause mortality.

Results

The homozygote genotype of rs2442468 in recipients was associated with death censored graft survival compared to the homozygote of the major allele. On the other hand, the heterozygote and homozygote genotypes of Ang2 SNPs rs2442635, rs2515435 and rs2916702 in recipients were associated with death censored graft survival. The homozygote genotype of rs2442468 in the donors was associated with a reduced incidence of PNF. The heterozygote and homozygote genotypes of rs2442635, rs2515435 and rs2916702 were associated with an increased risk of PNF compared to the homozygote of the major allele. Compared to the homozygote of the major allele, the heterozygote genotype of rs2916702 in recipients was significantly associated with increased mortality. The genetic Ang2 profile does not influence DGF.

Conclusion

This is the first study showing that the genetic Ang2 profile of the deceased donor and recipient is associated with transplantation outcome. This finding needs to be confirmed by a replication study.

INTRODUCTION

Both angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2), important regulators of angiogenesis, play an essential role in the regulation of vascular stability and are involved in the inflammatory balance provoked by renal ischemia/reperfusion injury (IRI)[1,2]. They bind to the extracellular domain of the tyrosine kinase Tie2 receptor, which is expressed by endothelial and hemopoietic stem cells[3-5]. Precursor pericytes and vascular smooth-muscle cells (SMCs) are responsible for the constant production and release of Ang1, while Ang2 is stored in Weibel Palade bodies (WPB) in the endothelial cells which is rapidly released upon endothelial activation[6-8]. In the healthy adult vasculature, a constant Ang1-mediated Tie2 phosphorylation seems to control and maintain vascular quiescence, damping inflammation and inhibiting endothelial apoptosis, while Ang2 boosts endothelial activation and dysfunction[9-11].

Endothelial dysfunction is an important mediator of IRI and brain death-induced inflammation[12,13]. In the development of allograft vasculopathy after renal transplantation, a critical role for angiopoietins and their Tie2 receptor is proposed given their known role in maintenance of the vascular integrity[14]. Intervention in this mechanism may ameliorate renal IRI as human renal IRI induces endothelial activation after reperfusion, reflected by Ang2 release from the kidney[1,2]. Thus, imbalance in favor of Ang2 causes endothelial activation and dysfunction[15,16]. However, the precise underlying mechanism inducing release of the endothelium stabilizing Ang1 and the destabilizing Ang2 remains unclear since a one on one antagonistic functioning of these angiopoietins is being criticized as Ang2 seems to be the more dynamic player responding rapidly to the internal environment[17,18]. After renal IRI an increased release of Ang2 by the kidney was demonstrated in grafts of living kidney donors and renal transplant recipients (RTR) while the release of Ang1 was not affected[2,19]. In addition, inhibiting Ang2 is likely to prevent transplant IRI in rats[20]. This indicates that increased levels of circulating Ang2 may play an important role in renal allograft vasculopathy. Therefore, it is important to increase understanding of the role of angiopoietin mediated signaling in renal transplantation.

Circulating Ang2 levels have been associated with poor outcome after trauma, sepsis, pancreatitis, chronic kidney disease (CKD) and transplantation[21-24]. Increased Ang2 levels were associated with increased mortality in a RTR case-cohort study[19]. Furthermore, a correlation of increased Ang2 levels with increased C-Reactive Protein levels (CRP), N-terminal pro-Brain Natriuretic Peptide (Nt-pro-BNP) and proteinuria was demonstrated. Ang2 levels correlated negatively with estimated Glomerular Filtration Rate (eGFR), hemoglobin levels and albumin concentration[14] which underlines the importance of Ang2 in (renal) inflammatory conditions.

Ang2, consisting of 496 amino acids and compromising nine exons and eight introns, encodes the Ang2 protein growth factor[25] and is located on chromosome 8q23 which has been found to be highly polymorphic[26,27]. Single nucleotide polymorphisms (SNPs) have been identified in the Ang2 gene which may affect Ang2 gene expression or vascular angiogenesis[28]. So far, Ang2 gene polymorphism was studied in idiopathic recurrent miscarriage, unexplained intrauterine fetal death and gynecologic cancers[29-31]. In the literature, Ang2 SNPs are associated with the development of acute lung injury and increased risk of acute respiratory distress syndrome[32,33]. However, the functional effect of these Ang2 SNPs has not been demonstrated. The role of Ang2 SNPs in the development of renal disease or the effect on outcome after renal transplantation has never been investigated. In this study, we therefore study the effect of Ang2 SNPs on outcome after deceased renal transplantation since circulating Ang2 has been found to associate with graft failure and mortality in deceased donor-RTR. Secondarily, we tested the hypothesis that Ang2 SNPs are involved in the development of end-stage renal disease (ESRD).

MATERIALS & METHODS

Study population

Transplantations (n=1430) between 1993 and 2008 were retrospectively selected for our genetic study[34]. Exclusion criteria were absence of DNA, simultaneous kidney/pancreas or kidney/liver transplantation, loss of follow-up, technical problems or living donor renal transplantation. The inclusion and number of recipients and donors is shown in supplementary table 1, final analysis were performed with the deceased donor inclusions. Differences in numbers of patients are explained by technical problems in the SNP analysis. After transplantation time to graft failure was monitored and censored for death with a functioning graft. Graft failure was defined as return to dialysis or re-transplantation. Clinical parameters of donors and recipients were retrieved from medical files and documented. The study protocol was approved by the institutional review board of the University Medical Center Groningen. All recipients signed written informed consent. According to the Dutch Transplantation Law this was not required for deceased donors. The Institutional Review Board approved the study protocol, which was in adherence to the Declaration of Helsinki.

SNP selection

The following Ang2 tagging SNPs were selected: rs2442468, rs2442635, rs2515435 and rs2916702. This was based on recent publications selecting them on their genomic region, pairwise tagging of the HapMap population[27], minor allele frequency and the measured and calculated pairwise linkage disequilibrium[32,33]. The outcome of the mutated homozygote and heterozygote genotypes were compared to the homozygote of the major alle genotype (reference group).

Study endpoints

The primary end point of outcome after transplantation was death censored graft survival, defined as the need for dialysis or re-transplantation. Furthermore, the effects on primary non-function (PNF), delayed graft function (DGF), acute rejection and all-cause mortality were analyzed. PNF was defined as no graft function after transplantation and DGF was defined as need for dialysis within the first week after transplantation. For analysis of death censored graft survival, DGF and acute rejection, PNF kidney grafts were excluded as these kidneys never functioned.

DNA isolation and SNP analysis

DNA isolation and subsequent SNP analysis of the REGaTTA cohort has been described earlier[34-37]. Peripheral whole blood of recipients or lymphatic tissue of deceased donors was used for DNA extraction by a commercial kit following manufacturer's instructions. DNA concentration was calculated by the NanoDrop nucleic acid application. Isolation procedures were repeated if the concentration of DNA was too low. For SNP genotyping, the Illumina VeraCode GoldenGate assay kit (Illumina, San Diego, CA, USA) was used according to the terms of use. Genotype clustering and calling were performed using BeadStudio Software (Illumina).

Statistical analysis

Data were analyzed with SPSS 20.0 (SPSS Inc., Chicago, USA). Data were presented as mean ± standard deviation (SD) or median [interquartile range] depending on the distribution. The Hardy-Weinberg equilibrium was tested in donors and recipients. Patient characteristics were compared by Mann-Whitney U (continuous data) -or Chi-square test (binary data). The effect of Ang2 SNPs on death censored graft survival and all-cause mortality was initially analyzed and plotted by Kaplan-Meier analyses. The TT genotype was used as reference group meaning that the outcome of the GT- and GG genotype was compared to the TT genotype. Estimated survival was defined as the area under the survival curve. Cox regression analyses were performed to adjust for a priori defined factors, potentially influencing graft survival. These factors include donor age, donor gender, donor type, recipient age, recipient gender, cold ischemia time and the number of transplantations. The effect of the Ang2 SNPs on PNF, DGF and acute rejection was analyzed by binary logistic regression, adjusting for the potentially influencing factors mentioned above.

RESULTS

The characteristics of deceased donor renal transplantations are shown in table 1. In both donors and recipients, the SNP distribution was tested according to the Hardy Weinberg equilibrium. The distribution of rs2442635 in donor kidneys and the distribution of rs2916702 in recipients was skewed (supplementary data – table 2).

Table 1. Transplantation characteristics

Parameter	Deceased donor kidney Tx (989)	Living donor kidney Tx (282)
Gender recipients: male, n (%)	573 (58)	166 (59)
Age recipients, years[a]	51 [40-60]	43 [31-55]
Gender donors: male, n (%)	519 (53)	126 (45)
Age donors, years[a]	46 [32-55]	50 [43-57]
Donor type DBD, n(%) DCD, n(%)	 787 (80) 202 (20)	
Cold ischemia time[a]	1200 [960-1440]	152 [120-182]
Tx without HLA mismatch, n (%)	231 (23)	
Previous transplant, n (%)	107 (11)	21 (7)
PNF, n (%)		
DGF, n (%)	400 (40)	15 (5.3)
Acute rejection, n (%)		
Death censored graft failure	181 (18)	24 (9)
All-cause mortality, n (%)	175 (18)	17 (6)

[a]Median [interquartile range]. Abbreviations: DBD: deceased brain death; DCD: deceased cardiac death; Tx: transplantation; PNF: primary non-function; DGF: delayed graft function.

The effect of Ang2 SNPs on development of end-stage renal disease

The distribution of the Ang2 SNPs between recipients and donors has been compared to determine the role of these SNPs in the development of ESRD (figure 1). No difference in the distribution of rs2442468 was observed. However, rs2442635 and rs2515435 tended to be aberrantly distributed. This skewed distribution between donors and recipients was significant for rs2916702.

The effect of Ang2 SNPs on death censored graft survival

The homozygote genotype of rs2442468 in recipients was associated with reduced death censored graft survival compared to the homozygote of the major allele (table 2). On the other hand, the heterozygote and homozygote genotypes of Ang2 SNPs rs2442635, rs2515435 and rs2916702 in recipients were associated with improved death censored graft survival compared to the homozygote of the major allele (table 2). This association was significant for all heterozygote genotypes and the homozygote genotypes of rs2442635 and rs2515435. A tendency was observed between the homozygote genotype of rs2916702 and the reference group. No differences in death censored graft survival were observed between genotypes of the Ang2 SNPs in the donor kidneys (table 2). The effect on death censored graft survival is illustrated in figure 2.

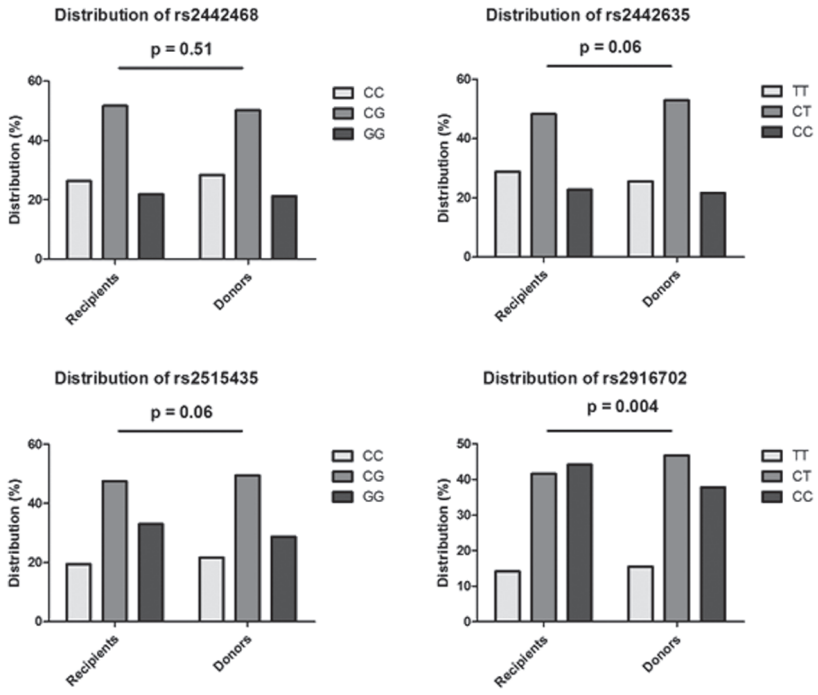

Figure 1. Distribution of four Ang2 SNPs in kidney donors and recipients. No differences in the distribution of the Ang2 SNPs rs2442468, rs2442635 and rs2515435 were observed in kidney donors and recipients. The distribution of the Ang2 SNP rs2916702 did differ between kidney donors and recipients (p=0.004).

Table 2. The effect of donor kidney and recipient genotypes of angiopoietin-2 SNPs on death censored graft survival after deceased donor renal transplantation

Recipient SNP	Homozygote (Reference)	Heterozygote	p	Homozygote (SNP)	p
rs2442468 (n)	CC (249)	CG (482)		GG (200)	
Est. survival (years, 95% CI) [a]	14.2 (13.5-14.9)	14.1 (13.6-14.7)	0.95	**13.0 (12.0-13.9)**	0.10
Hazard ratio (95% CI) [b]	1.00	0.99 (0.65-1.51)		1.47 (0.93-2.30)	
rs2442635 (n)	TT (268)	**CT (446)**		**CC (215)**	
Est. survival (years, 95% CI) [a]	12.8 (12.0-13.6)	**14.3 (13.8-14.9)**	**0.00**	**14.4 (13.6-15.1)**	**0.03**
Hazard ratio (95% CI) [b]	1.00	**0.54 (0.37-0.78)**		**0.60 (0.38-0.94)**	
rs2515435 (n)	CC (307)	**CG (439)**		**GG (183)**	
Est. survival (years, 95% CI) [a]	13.1 (12.3-13.8)	**14.2 (13.7-14.8)**	**0.01**	**14.5 (13.7-15.3)**	**0.03**
Hazard ratio (95% CI) [b]	1.00	**0.62 (0.43-0.89)**		**0.59 (0.36-0.95)**	
rs2916702 (n)	TT (413)	**CT (376)**		CC (137)	
Est. survival (years, 95% CI) [a]	13.3 (12.6-13.9)	**14.5 (13.9-15.0)**	**0.02**	14.1 (13.2-15.0)	0.17
Hazard ratio (95% CI) [b]	1.00	**0.69 (0.43-0.92)**		0.69 (0.41-1.16)	

Table 2. The effect of donor kidney and recipient genotypes of angiopoietin-2 SNPs on death censored graft survival after deceased donor renal transplantation (*Continued*)

Donor kidney SNP	Homozygote (Reference)	Heterozygote	p	Homozygote (SNP)	p
rs2442468 (n)	CC (261)	CG (464)		GG (204)	
Est. survival (years, 95% CI) [a]	13.7 (12.9-14.4)	13.9 (13.3-14.5)	0.91	14.2 (13.3-15.0)	0.65
Hazard ratio (95% CI) [b]	1.00	0.98 (0.66-1.44)		0.90 (0.56-1.44)	
rs2442635 (n)	TT (244)	CT (487)		CC (201)	
Est. survival (years, 95% CI) [a]	14.1 (13.3-14.9)	13.9 (13.4-14.5)	0.92	13.5 (12.6-14.4)	0.54
Hazard ratio (95% CI) [b]	1.00	1.02 (0.68-1.55)		1.16 (0.72-1.87)	
rs2515435 (n)	CC (272)	CG (452)		GG (204)	
Est. survival (years, 95% CI) [a]	14.1 (13.3-14.8)	13.9 (13.4-14.5)	0.95	13.5 (12.6-14.4)	0.56
Hazard ratio (95% CI) [b]	1.00	0.99 (0.66-1.47)		1.15 (0.72-1.82)	
rs2916702 (n)	TT (360)	CT (426)		CC (143)	
Est. survival (years, 95% CI) [a]	14.1 (13.5-14.8)	13.7 (13.1-14.3)	0.83	13.9 (12.8-14.9)	0.99
Hazard ratio (95% CI) [b]	1.00	1.04 (0.72-1.51)		1.00 (0.61-1.67)	

Abbreviations: Est.: estimated; CI: confidence interval.
[a]Kaplan-meier survival analysis with log-rank test. Primary non-function grafts were excluded.
[b]Cox regression analysis adjusted for donor age, donor gender, donor type, recipient age, recipient gender, HLA mismatch, cold ischemia time, number of transplantation. Primary non-function grafts were excluded.

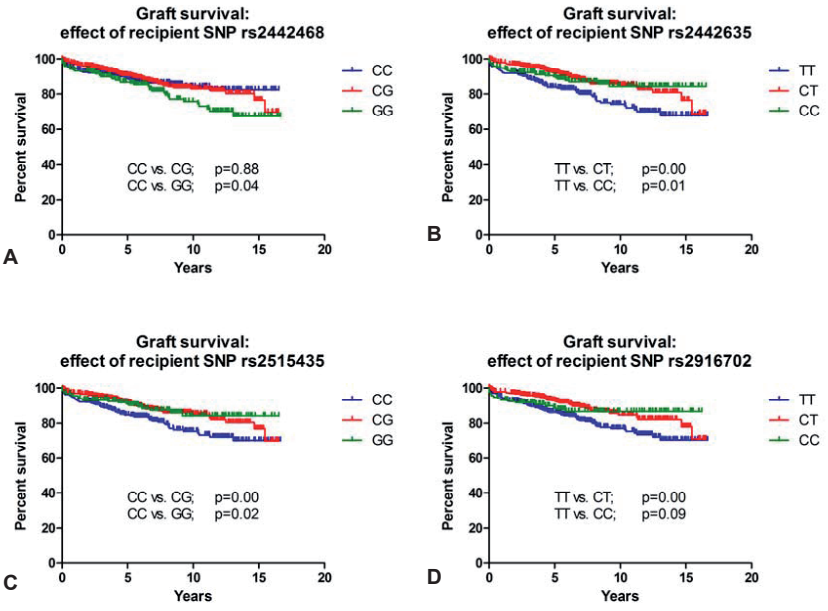

Figure 2. Effect of Ang2 SNPs in renal transplant recipients on death censored graft survival. A) Estimated mean graft survival of CC recipient genotype is superior to the GG genotype (p=0.04). B) Estimated mean graft survival of CC recipient genotype is superior to the TT genotype (p=0.01) and the estimated mean graft survival of CT recipient genotype is superior to the TT genotype (p<0.01). C) Estimated mean graft survival of GG recipient genotype is superior to the CC genotype (p=0.02) and the estimated mean graft survival of CG recipient genotype is superior to the CC genotype (p<0.01). D) Estimated mean graft survival of CT recipient genotype is superior to the TT genotype (p<0.01).

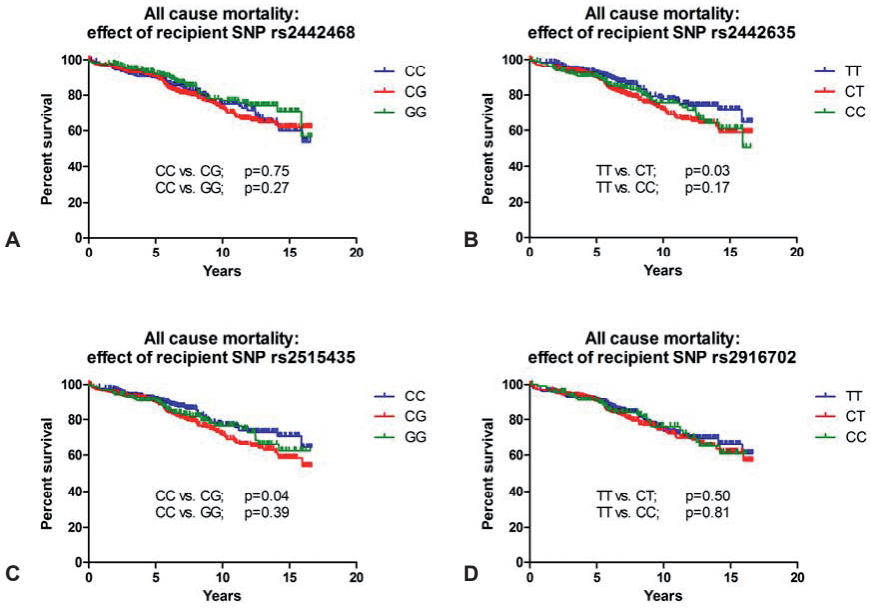

Figure 3. Effect of Ang2 SNPs on all-cause mortality in renal transplant recipients. A) No differences in patient survival were observed between recipient genotypes. B) Estimated mean patient survival of TT recipient genotype is superior to the CT genotype (p=0.03). C) Estimated mean patient survival of CC recipient genotype is superior to the CG genotype (p=0.04). D) No differences in patient survival were observed between recipient genotypes.

The effect of Ang2 SNPs on primary non-function and delayed graft function

In recipients, the Ang2 SNPs were not associated with PNF, while in donor kidneys significant associations were observed. The homozygote genotype of rs2442468 in the donors was associated with a reduced incidence of PNF. However, the heterozygote and homozygote genotypes of rs2442635, rs2515435 and rs2916702 were associated with an increased risk of PNF compared to the homozygote of the major allele. For delayed graft function no relevant differences between the genotypes of the Ang2 SNPs in recipients or donor kidneys were observed, as only the heterozygote genotype of rs2442468 in recipients was significantly associated with reduced DGF (table 4).

The effect of Ang2 SNPs on all-cause mortality

No relevant differences in association with mortality after transplantation between the genotypes of the Ang2 SNPs in recipients or donor kidneys were observed, as only the heterozygote genotype of rs2916702 in recipients was significantly associated with increased mortality (table 5).

Table 3. The effect of donor kidney and recipient genotypes of angiopoietin-2 SNPs on primary non-function after deceased donor renal transplantation

Recipient SNP	Homozygote (Reference)	Heterozygote	p	Homozygote (SNP)	p
rs2442468 (n)	CC (264)	CG (514)		GG (210)	
Incidence of PNF	5.7 %	6.2 %	0.74	4.8 %	0.45
Odds ratio (95% CI) [a]	1.00	1.12 (0.59-2.13)		0.71 (0.29-1.73)	
rs2442635 (n)	TT (286)	CT (471)		CC (229)	
Incidence of PNF	6.3 %	5.3 %	0.85	6.1 %	0.96
Odds ratio (95% CI) [a]	1.00	0.94 (0.49-1.82)		1.02 (0.48-2.18)	
rs2515435 (n)	CC (329)	CG (463)		GG (194)	
Incidence of PNF	6.7 %	5.2 %	0.54	5.7 %	0.69
Odds ratio (95% CI) [a]	1.00	0.82 (0.44-1.54)		0.85 (0.39-1.86)	
rs2916702 (n)	TT (439)	CT (401)		CC (143)	
Incidence of PNF	5.9 %	6.2 %	0.61	4.2 %	0.52
Odds ratio (95% CI) [a]	1.00	1.17 (0.65-2.11)		0.74 (0.29-1.87)	
Donor kidney SNP	**Homozygote (Reference)**	**Heterozygote**	**p**	**Homozygote (SNP)**	**p**
rs2442468 (n)	CC (283)	CG (496)		**GG (209)**	
Incidence of PNF	7.8 %	6.0 %	0.53	**2.4 %**	**0.02**
Odds ratio (95% CI) [a]	1.00	0.83 (0.46-1.50)		**0.27 (0.09-0.79)**	
rs2442635 (n)	TT (249)	**CT (520)**		**CC (220)**	
Incidence of PNF	1.5 %	**5.4 %**	**0.01**	**6.9 %**	**0.01**
Odds ratio (95% CI) [a]	1.00	**3.9 (1.36-11.3)**		**4.83 (1.59-14.6)**	
rs2515435 (n)	CC (278)	**CG (484)**		**GG (223)**	
Incidence of PNF	2.2 %	**6.6 %**	**0.01**	**8.5 %**	**0.01**
Odds ratio (95% CI) [a]	1.00	**3.56 (1.36-9.32)**		**4.10 (1.48-11.3)**	
rs2916702 (n)	TT (370)	**CT (458)**		**CC (158)**	
Incidence of PNF	2.7 %	**7.0 %**	**0.00**	**9.5 %**	**0.00**
Odds ratio (95% CI) [a]	1.00	**3.22 (1.46-7.15)**		**4.06 (1.66-9.95)**	

Abbreviations: PNF: primary non-function; CI: confidence interval.
[a]Binary logistic regression adjusted for donor age, donor gender, donor type, recipient age, recipient gender, HLA mismatch, cold ischemia time, number of transplantation.

Table 4. The effect of donor kidney and recipient genotypes of angiopoietin-2 SNPs on delayed graft function after deceased donor renal transplantation

Recipient SNP	Homozygote (Reference)	Heterozygote	p	Homozygote (SNP)	p
rs2442468 (n)	CC (249)	**CG (428)**		GG (200)	
Incidence of DGF	42.6 %	**33.0 %**	**0.03**	41.5 %	0.94
Odds ratio (95% CI) [a]	1.00	**0.66 (0.46-0.96)**		0.96 (0.61-1.49)	
rs2442635 (n)	TT (268)	CT (446)		CC (215)	
Incidence of DGF	40.7 %	32.5 %	0.08	42.8 %	0.72
Odds ratio (95% CI) [a]	1.00	0.72 (0.50-1.04)		1.08 (0.71-1.66)	
rs2515435 (n)	CC (307)	CG (439)		GG (183)	
Incidence of DGF	39.1 %	34.2 %	0.38	42.1 %	0.52
Odds ratio (95% CI) [a]	1.00	0.85 (0.60-1.21)		1.15 (0.75-1.79)	
rs2916702 (n)	TT (413)	CT (376)		CC (137)	
Incidence of DGF	38.5 %	35.6 %	0.64	39.4 %	0.87
Odds ratio (95% CI) [a]	1.00	0.92 (0.66-1.29)		1.04 (0.65-1.66)	
Donor kidney SNP	**Homozygote (Reference)**	**Heterozygote**	**p**	**Homozygote (SNP)**	**p**
rs2442468 (n)	CC (261)	CG (466)		GG (204)	
Incidence of DGF	34.1 %	38.4 %	0.12	39.2 %	0.45
Odds ratio (95% CI) [a]	1.00	1.35 (0.93-1.95)		1.19 (0.76-1.85)	
rs2442635 (n)	TT (244)	CT (487)		CC (201)	
Incidence of DGF	38.9 %	37.2 %	0.66	35.8 %	0.43
Odds ratio (95% CI) [a]	1.00	1.09 (0.75-1.58)		0.83 (0.53-1.31)	
rs2515435 (n)	CC (272)	CG (452)		GG (204)	
Incidence of DGF	39.0 %	38.7 %	0.79	32.8 %	0.08
Odds ratio (95% CI) [a]	1.00	1.05 (0.73-1.50)		0.67 (0.43-1.04)	
rs2916702 (n)	TT (360)	CT (426)		CC (143)	
Incidence of DGF	37.8 %	38.5 %	0.99	31.5 %	0.18
Odds ratio (95% CI) [a]	1.00	1.10 (0.79-1.55)		0.72 (0.45-1.16)	

Abbreviations: DGF: delayed graft function; CI: confidence interval.
[a]Binary logistic regression adjusted for donor age, donor gender, donor type, recipient age, recipient gender, HLA mismatch, cold ischemia time, number of transplantation. Primary non-function grafts were excluded.

Table 5. The effect of donor kidney and recipient genotypes of angiopoietin-2 polymorphisms on mortality after deceased donor renal transplantation

Recipient SNP	Homozygote (Reference)	Heterozygote	p	Homozygote (SNP)	p
rs2442468 (n)	CC (264)	CG (514)		GG (210)	
Est. survival (years, 95% CI) [a]	12.2 (11.4-13.0)	12.2 (11.6-12.7)	0.80	12.2 (11.4-13.1)	0.61
Hazard ratio (95% CI) [b]	1.00	1.04 (0.78-1.37)		1.09 (0.78-1.54)	
rs2442635 (n)	TT (286)	CT (471)		CC (229)	
Est. survival (years, 95% CI) [a]	12.2 (11.5-13.0)	12.2 (11.6-12.8)	0.51	12.2 (11.4-13.0)	0.40
Hazard ratio (95% CI) [b]	1.00	0.91 (0.69-1.20)		0.87 (0.63-1.20)	
rs2515435 (n)	CC (329)	CG (463)		GG (194)	
Est. survival (years, 95% CI) [a]	12.3 (11.6-13.0)	12.1 (11.5-12.7)	0.50	12.2 (11.3-13.2)	0.33
Hazard ratio (95% CI) [b]	1.00	0.91 (0.70-1.19)		0.84 (0.60-1.18)	
rs2916702 (n)	TT (439)	**CT (401)**		CC (143)	
Est. survival (years, 95% CI) [a]	12.0 (11.4-12.6)	**12.5 (11.8-13.1)**	**0.01**	12.3 (11.3-13.3)	0.24
Hazard ratio (95% CI) [b]	1.00	**0.72 (0.55-0.93)**		0.81 (0.57-1.15)	
Donor kidney SNP	**Homozygote (Reference)**	**Heterozygote**	**p**	**Homozygote (SNP)**	**p**
rs2442468 (n)	CC (238)	CG (496)		GG (209)	
Est. survival (years, 95% CI) [a]	12.5 (11.7-13.2)	11.9 (11.3-12.4)	0.50	12.4 (11.6-13.3)	0.74
Hazard ratio (95% CI) [b]	1.00	(0.83-1.45)		(0.67-1.33)	
rs2442635 (n)	TT (249)	CT (520)		CC (220)	
Est. survival (years, 95% CI) [a]	12.1 (11.2-12.9)	12.0 (11.5-12.6)	0.58	12.6 (11.7-13.4)	0.85
Hazard ratio (95% CI) [b]	1.00	(0.82-1.44)		(0.68-1.38)	
rs2515435 (n)	CC (278)	CG (484)		GG (223)	
Est. survival (years, 95% CI) [a]	12.0 (11.2-12.8)	12.2 (11.6-12.8)	0.69	12.2 (11.3-13.0)	0.89
Hazard ratio (95% CI) [b]	1.00	1.06 (0.80-1.40)		1.02 (0.73-1.43)	
rs2916702 (n)	TT (370)	CT (458)		CC (158)	
Est. survival (years, 95% CI) [a]	12.0 (11.3-12.6)	12.3 (11.7-12.9)	0.98	12.4 (11.4-13.4)	0.79
Hazard ratio (95% CI) [b]	1.00	(0.77-1.30)		(0.67-1.36)	

Abbreviations: Est.: estimated; CI: confidence interval.
[a]Kaplan-meier survival analysis with log-rank test.
[b]Cox regression analysis adjusted for donor age, donor gender, donor type, recipient age, recipient gender, HLA mismatch, cold ischemia time, number of transplantation.

DISCUSSION

Angiopoietin-2 has been shown to play a substantial role in IRI and outcome after renal transplantation. There is growing evidence that Ang2 is involved in allograft vasculopathy in kidney donors and RTR. This is the first study that demonstrates the role of the Ang2 gene in renal transplantation. Next, Ang2 SNPs were involved in the development of end-stage renal disease. After deceased donor transplantation, these SNPs were associated with improved death censored graft survival. However, the same Ang2 SNPs in the donor kidneys were associated with an increased risk of PNF. This implicates that the effect of the Ang2 gene in renal IRI is different from its role in development of ESRD.

The results of this study would be strengthened by confirmation in an independent cohort. Nevertheless, in this cohort the effect of the Ang2 SNPs on development of end-stage renal disease is evident. The distribution of the Ang2 SNPs is skewed towards the homozygote of the major allele. This means that this homozygote of the major allele increases the risk of end-stage renal disease. Subsequently, the homozygote of the major allele of these SNPs in recipients is associated with impaired graft survival. Thus, both before and after transplantation, recipients with these SNPs are predisposed to develop end-stage renal disease.

In contrast to the role of Ang2 SNPs in the long-term outcome, the heterozygote and homozygote genotypes were associated with an increased risk of PNF compared to the homozygote of the major allele. No effect on the incidence of DGF was observed. However, the effect on PNF indicates that the role in short-term outcome after transplantation is contrary to the long-term outcome. Presumably, the function of Ang2 in renal IRI is opposite to the development of end-stage renal disease.

Circulating Ang2 levels have been reported to predict mortality in renal transplant recipients[19]. Unfortunately, only serum Ang2 levels were determined in this particular study. Testing of the association between Ang2 genotype and mortality or graft failure was not performed. In our cohort, the Odd's ratio for mortality of the heterozygote genotype of rs2916702 and for DGF of the heterozygote genotype of rs2442468 in recipients were significantly reduced compared to the homozygote of the major allele. However, no differences in the incidence of DGF between the two homozygote genotypes were observed. These data are inconsequent and this significant difference is likely the result of a bias. We therefore conclude that the Ang2 SNPs do not affect the incidence of delayed graft function or mortality.

We acknowledge some limitations of our study. First, the single center retrospective design which makes generalization to the community setting difficult. Second, we did not examine the functions of Ang2 genotypes and Ang2 levels, thus the functional significance of Ang2 in deceased donors remains to be defined. Furthermore, we emphasize the need for replication of our findings given the large number of false positive generated in genetic association studies[38]. So to fully understand the disparity between our findings and the role of Ang2, the functionality of these Ang2 SNPs has to be confirmed in other transplantation cohorts, including both genotyping and functional Ang2 expression. However, the association of common Ang2 genetic variation with transplant outcome is biological plausible since an *in vitro* study has suggested that Ang2 gene variations alter gene expression[28].

To conclude, this study shows that the Ang2 genotype of RTR is associated with death censored graft survival after deceased donation. These SNPs were involved in the development of end stage renal disease and PNF. Subsequently, the homozygote of the major allele of these SNPs in RTR is associated with impaired graft survival. Hence, both before and after transplantation, recipients with these SNPs are predisposed to develop ESRD. The genetic Ang2 profile does not influence DGF or mortality after renal transplantation.

Supplementary data - table 1. Included number of patients for each SNP

SNP	Recipient SNP	Donor kidney SNP
rs2442468	1270	1270
rs2442635	1268	1271
rs2515435	1268	1264
rs2916702	1265	1266

Supplementary data - table 2. Hardy Weinberg equilibrium

SNP	Recipients	Donor kidneys
rs2442468	0.19	0.73
rs2442635	0.25	0.03
rs2515435	0.27	0.87
rs2916702	0.003	0.57

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