Performance-enhancing strategies for deceased donor kidneys

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Functional EPO gene polymorphism rs1617640 affects graft survival after deceased donor kidney transplantation

Submitted

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Abstract

Introduction

High erythropoietin (EPO) levels following renal transplantation have been associated with increased mortality. However, EPO also has a cytoprotective effect. In this study, we investigated the role of the functional EPO single nucleotide polymorphism, rs1617640, in deceased donor kidney transplantation.

Materials & Methods

The genotypes of rs1617640 were determined in 986 deceased donors and 981 recipients of a deceased donor kidney. Subsequently, the effect of the different genotypes in recipients or donors on death censored graft survival, primary non-function, delayed graft function and acute rejection was analyzed. Furthermore, plasma EPO levels were determined in a part of our cohort.

Results

The TT genotype of EPO SNP rs1617640 in deceased donor kidneys was associated with reduced death censored graft survival and an increased incidence of delayed graft function compared to the GG genotype. Surprisingly, plasma EPO levels were significantly higher in recipients with the TT genotype compared to the GG genotype.

Discussion

This is the first study showing an association between functional promoter EPO SNP rs1617640 and outcome of deceased donor kidney transplantation. These findings give new insights in the role of EPO in renal transplantation and may inspire translation of EPO mediated cytoprotection to the transplantation clinic.

Introduction

Erythropoietin (EPO) mediated cytoprotection has been widely evidenced by experimental studies[1–3]. Based on these promising experimental studies, high dose EPO treatment of recipients of a deceased donor kidney has been tested in four clinical trials. The aim of these studies was to improve short-term outcome after transplantation. However, none of these studies showed improved short-term graft function or reduced incidence of delayed graft function (DGF)[4–7]. The used EPO doses were apparently not sufficient to attenuate short-term function following transplantation, while the risk of adverse events already increased in one clinical trial[4].

In experimental studies, it has been demonstrated that renal ischemia/reperfusion (I/R) injury can be reduced by EPO treatment pre- as well as post-reperfusion[1–3]. The pleiotropic function of EPO can be explained by its binding to different receptor complexes. Erythropoiesis is mediated by binding of EPO to a homodimeric receptor complex ($EPOR_2$) consisting of two EPO receptors (EPOR), while cytoprotection is induced by binding to a heteromeric complex ($EPOR_2$-βCR_2) consisting of two EPOR and two β common receptors (βCR). The binding affinity of the $EPOR_2$-βCR_2 is lower, indicating that higher doses of EPO are required to activate the protective receptor complex[3,8]. However, a combination of the vascular endothelial growth factor receptor-2 and the βCR (VEGFR-2-βCR) has also been proposed as an important receptor complex for EPO mediated cytoprotection[9].

Based on promising experimental studies, high dose EPO treatment has been tested in clinical kidney transplantation. The aim of these studies was to improve short-term outcome of deceased donor kidneys after transplantation by high dose EPO treatment of recipients. However, none of these studies showed improved short-term graft function or reduced incidence of delayed graft function (DGF)[6–9]. These EPO doses were apparently not sufficient to attenuate short-term function following transplantation, while stimulation of erythropoiesis already increased the risk of adverse events in one clinical trial[6].

Besides the local cytoprotective capacities of EPO, continuous high EPO levels are detrimental. For instance, recombinant erythropoietin (rhEPO) treatment of anemia (target hemoglobin level: 13.5 compared to 11.3 g/dl) was associated with an increased risk of cardiovascular adverse events in patients with chronic kidney disease[10]. In line with these findings, higher plasma EPO levels after transplantation are associated with increased mortality illustrating the contradictory effects of EPO[11].

Recently, a functional, single nucleotide polymorphism (SNP), rs1617640, was identified in the promoter region of the EPO gene[12]. Tong et al. showed that the TT genotype of rs1617640 increased EPO concentrations in the human vitreous body by 7.5 times compared to the GG genotype[12]. Several studies associated this SNP with development of diseases, like schizophrenia, myelodysplastic syndrome, diabetic retinopathy and diabetic end-stage renal disease[12–15]. The TT genotype was associated with an increased risk of diabetic retinopathy and diabetic end-stage renal disease[12,15], while the GG genotype was associated with an increased risk of myelodysplastic syndrome[13]. Furthermore, patients with the TT genotype more often required renal replacement therapy following cardiac surgery[16]. These findings show that this EPO SNP, rs1617640, is functional and plays a role in development of renal dysfunction.

The clinical effects of EPO are difficult to predict, because activation of the local protective pathways also results in an increased risk of cardiovascular adverse effects by enhanced platelet reactivity and stimulation of erythropoiesis[3,4,17]. Therefore, the aim of this study is to increase knowledge of the endogenous role of EPO in renal transplantation. We hypothesized that the genotype TT of rs1617640 is associated with reduced graft survival after deceased donor kidney transplantation. The effect of EPO SNP rs1617640 on post-transplant graft function has therefore been investigated in the REGaTTA cohort, a large single center renal transplant cohort.

Materials & Methods

Study population

Transplantations (n=1430) between 1993 and 2008 were retrospectively selected for our genetic study. Exclusion criteria were more than two re-transplantations (N=22), absence of DNA (N=65), simultaneous kidney/pancreas or kidney/liver transplantation (N=65), loss of follow-up (N=4), technical problems (N=3). 1271 donors and recipients were genotyped. Nine recipients and four donors were excluded as genotyping of rs1617640 was unsuccessful. Living donor kidney transplantations were excluded (N=282) as ischemia/reperfusion injury is limited in these transplantations. This resulted in inclusion of 981 recipients and 986 donors. 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. A cardiac event was defined as myocardial ischemia or sudden cardiac death. The study protocol was approved by the institutional review board of the University Medical Center Groningen. All recipients gave informed consent. This was not required for deceased donors. All procedures have been performed according to Dutch law and to the principles of the Declaration of Helsinki.

Plasma EPO levels after transplantation were used to investigate the functional role of the EPO SNP on plasma EPO levels after renal transplantation. Sinkeler et al. measured plasma EPO levels after transplantation 11. Blood samples of all recipients, who visited the outpatient clinic between August 2001 and July 2003 and who had a functioning graft for at least one year, were included. Recipients on erythropoiesis stimulating treatment or ferritin-depleted patients were excluded. EPO SNP analyses of 274 donor kidneys and 273 recipients were covered by Sinkeler's cohort and these recipients were used to investigate the functional effect of the EPO SNP on plasma EPO levels. For the adjusted univariate general linear model, 12 donor kidneys and 13 recipients were excluded because of missing at least one of the a priori defined covariates. The endogenous EPO resistance index (ERI) was calculated by the following formula: ERI = plasma EPO (IU/l) / plasma hemoglobin (g/dl).

Rs1617640, located in the promoter region of the EPO gene on chromosome 7, was selected as it is the only known functional EPO SNP. Tong et al. showed that EPO levels in the human vitreous body were 7.5 times higher in TT genotype subjects compared to GG genotype subjects[12]. As rs1617640 is located in the promoter region, the functional effect of the GT genotype is expected in between the effect of the TT- and GG genotype. Therefore, the outcome of the GT- and GG genotype was compared to the TT genotype (reference group).

Study endpoints

The primary end point of this study was death censored graft survival, defined as the need for dialysis or re-transplantation. Secondarily, the effects on primary non-function (PNF), delayed graft function (DGF) and acute rejection 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. Acute rejection was defined as biopsy proven rejection within the first year after transplantation. The Banff 2007 classification was used to re-evaluate all biopsies. 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[18–21]. 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).

Laboratory analysis

All general clinical parameters, like plasma creatinine and proteinuria, have been measured by the laboratory of the University Medical Center Groningen. EPO levels (IU/l) after renal transplantation have been measured by Immulite 2000 assay[11].

Statistics

Data were analyzed with SPSS 20.0 (SPSS Inc., Chicago, USA). Data were presented as mean±standard deviation 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-or χ^2 test depending on the type of data.

The effect of EPO SNP rs1617640 on graft survival 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, total human leucocyte antigen-A (HLA), -B and, -DR mismatch, cold ischemia time, the number of transplantation, use of corticosteroids, use of cyclosporine and use of mycophenolate mofetil.

The effect of the EPO SNP on PNF, DGF and acute rejection was analyzed by binary logistic regression, adjusting for the potentially influencing factors mentioned above.

EPO levels and ERI were log transformed to obtain normally distributed values. Mean values of the genotype groups were compared in a univariate general linear model, adjusting for factors potentially influencing plasma EPO levels as shown by Sinkeler et al.[11]: urinary protein excretion, creatinine clearance, plasma hemoglobin level, recipient age, recipient gender, use of azathioprine, use of cyclosporine, plasma C-reactive protein, mean corpuscular volume, plasma triglycerides level, total plasma cholesterol level, use of diuretics, renin-angiotensin-aldosterone inhibition and plasma ferritin level.

Results

Main patient and transplantation characteristics of the donors and recipients are shown in table 1. The EPO SNP in donor kidneys or recipients did not affect the incidence of cardiac events in recipients. In both donors and recipients EPO SNP rs1617640 was distributed according to the Hardy-Weinberg equilibrium (recipients: p=0.45; donors: p=0.06). The genotype frequencies of TT, GT and GG are shown in figure 1. No difference in distribution of rs1617640 between donors and recipients was observed (p=0.11).

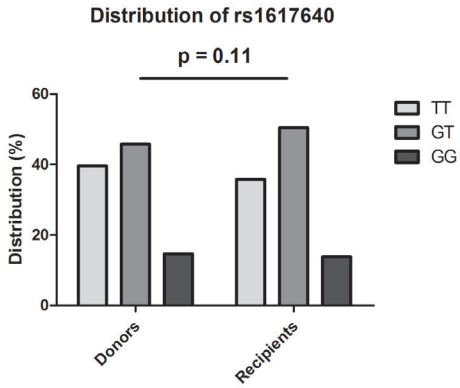

Figure 1 – Distribution of rs1617640 in donors and recipients. No differences were observed in distribution of this EPO SNP between donors and recipients.

Table 1 – Transplantation characteristics of donor- and recipient genotypes

Donor kidney SNP	TT (n=353)	GT (n=497)	GG (n=136)	p-value[a]
Gender recipients: male, n (%)	205 (58)	291 (59)	76 (56)	0.86
Age recipients, years[a]	51 [39-59]	51 [40-60]	51 [42-60]	0.97
Gender donors: male, n (%)	183 (52)	261 (53)	74 (54)	0.88
Age donors, years[a]	46 [32-54]	45 [31-55]	48 [34-55]	0.43
Donortype				
DBD, n (%)	276 (78)	403 (81)	105 (77)	0.45
DCD, n (%)	77 (22)	94 (19)	31 (23)	
Cold ischemia time[a]	1260 [960-1440]	1220 [960-1490]	1080 [910-1380]	**0.026**
Tx with no HLA mismatch, n (%)	67 (19)	133 (27)	31 (23)	0.096
Previous transplant, n (%)	46 (13)	50 (10)	11 (8)	0.21
Long-term immunosuppression				
Corticosteroids, n (%)	332 (94)	468 (94)	133 (98)	0.21
Cyclosporin, n (%)	300 (85)	426 (86)	127 (93)	**0.04**
Mycophenolate mofetil, n (%)	231 (65)	332 (67)	103 (67)	0.08
Recipient SNP	**TT (n=388)**	**GT (n=449)**	**GG (n=144)**	**p-value[a]**
Gender recipients: male, n (%)	228 (58.8)	258 (57.5)	83 (57.6)	0.93
Age recipients, years[a]	51 [40-60]	51 [41-60]	50 [39-60]	0.44
Gender donors: male, n (%)	193 (49,7)	239 (53.2)	81 (56.2)	0.36
Age donors, years[a]	46 [32-55]	46 [31-56]	44 [31-52]	0.58
Donortype				
DBD, n (%)	296 (76.3)	361 (80.4)	122 (84.7)	0.079
DCD, n (%)	92 (23.7)	88 (19.6)	22 (15.3)	
Cold ischemia time[a]	1220 [960-1440]	1200 [960-1440]	1200 [930-1470]	0.81
Tx with no HLA mismatch, n (%)	88 (22.7)	110 (24.6)	29 (20.1)	**0.026**
Previous transplant, n (%)	44 (11.4)	44 (9.8)	19 (13.2)	0.49
Long-term immunosuppression				
Corticosteroids, n (%)	364 (94)	426 (95)	138 (96)	0.62
Cyclosporin, n (%)	336 (87)	393 (87)	120 (83)	0.48
Mycophenolate mofetil, n (%)	269 (69)	294 (66)	101 (70)	0.39

DBD – deceased brain death; DCD – deceased circulatory death.

Donor and recipient characteristics were analyzed by Kruskal-Wallis or χ^2 test depending on type of data.

[a]Median [interquartile range]

The effect of the EPO gene polymorphism rs1617640 on outcome after renal transplantation

The general, uncorrected effect of EPO SNP rs161740 on graft survival in donors and recipients is shown in figures 2A and 2B. The EPO SNP in recipients did not affect death censored graft survival, while figure 2A shows that death censored graft survival of the TT genotype was inferior compared to the GG genotype. The estimated mean death censored graft survival of a TT donor kidney was 13.7 years, while this was 15.1 years for a GG donor kidney (p=0.031). The estimated mean graft survival of GT genotype kidneys was 14.0 years and not significantly increased compared to TT genotype kidneys.

The adjusted effect for a priori defined potential confounders is shown in table 2. Cox regression analysis revealed that GG genotype donor kidneys were independently associated with improved graft survival compared to the TT genotype (Table 2; hazard ratio: 0.52, 95% CI: 0.27-0.99, p = 0.046).

PNF of deceased donor kidneys was not affected by rs1617640 (Table 2). In line with the effect on graft survival, the incidence of DGF was higher in TT genotype donor kidneys compared to the GG genotype (Table 2; 39.2% compared to 28.7%, respectively). The GG genotype was independently associated with a reduced incidence of DGF (Table 2; odds ratio: 0.52, 95% CI: 0.31-0.87, p = 0.016). No differences were observed in incidence of acute rejection between donor genotypes GG or GT compared to TT (Table 2; 27.1%, 29.2% and 35.9% respectively).

The GT- or GG genotype in recipients did not affect death censored graft survival, incidence of PNF, incidence of DGF or acute rejection compared to the TT genotype (Table 2).

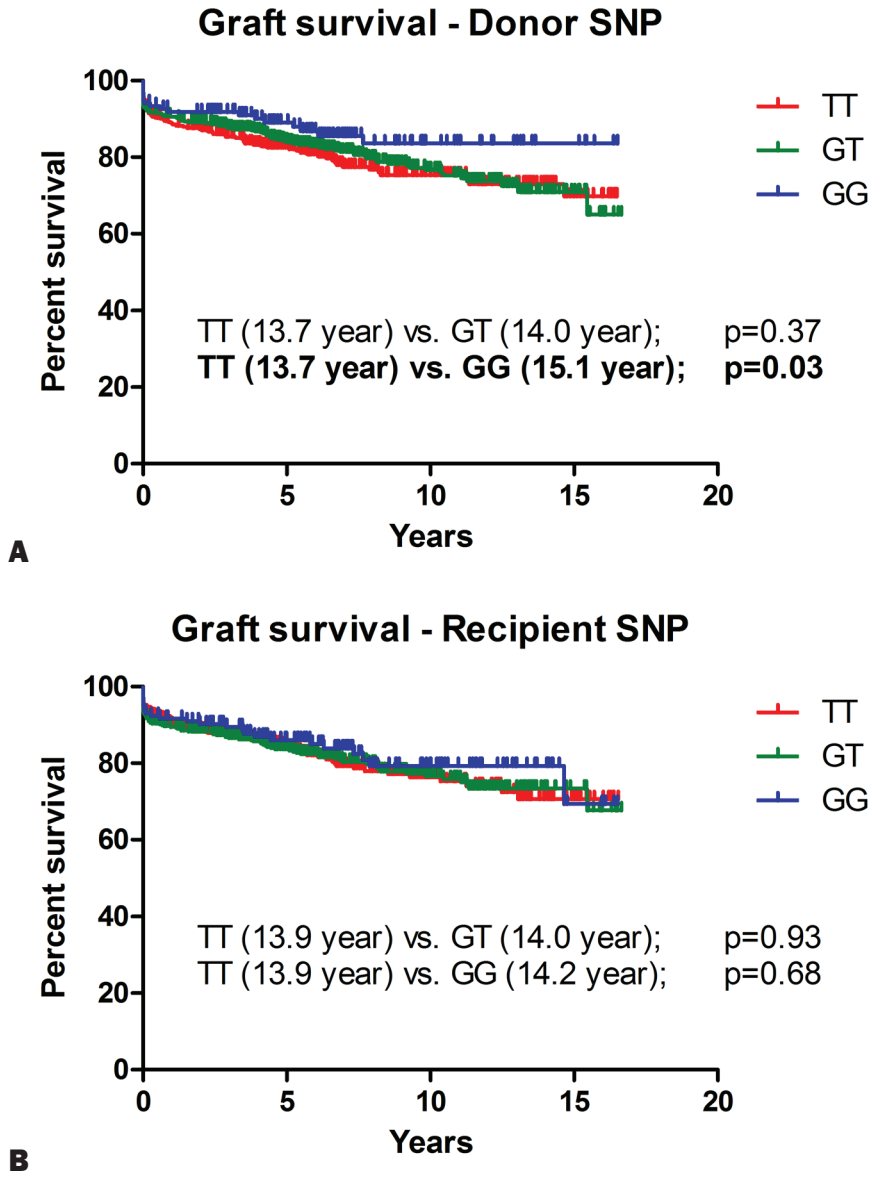

Figure 2 – Effect of rs1617640 in recipients and donor kidneys on death censored graft survival. Estimated mean graft survival of GG genotype donor kidneys is superior to the TT genotype (A). No differences in graft survival were observed between recipient genotypes (B). The Kaplan-Meier survival curves were compared using a log-rank test.

Table 2 – The effect of donor kidney and recipient genotypes of SNP rs1617640 on outcome of renal transplantation

Donor kidney SNP	Reference: TT (n=353)	GT (n=496)	p-value	GG (n=136)	p-value
Death censored graft survival					
Est. mean GS, yr (95% CI)[a]	13.7 (13.0-14.3)	14.0 (13.5-14.6)	0.37	**15.1 (14.2-15.9)**	**0.031**
Hazard ratio (95% CI)					
Crude[b]	1.00	0.85 (0.60-1.21)	0.37	**0.50 (0.26-0.96)**	**0.037**
Adjusted[c]	1.00	0.92 (0.64-1.32)	0.65	**0.52 (0.27-0.99)**	**0.046**
PNF					
Incidence	5.4 %	6.0 %		5.1 %	
Odds ratio (95% CI)					
Crude[d]	1.00	1.13 (0.63-2.04)	0.69	0.95 (0.39-2.32)	0.92
Adjusted[e]	1.00	1.12 (0.61-2.07)	0.71	1.02 (0.41-2.55)	0.96
DGF					
Incidence	39.2 %	37.7 %		28.7 %	
Odds ratio (95% CI)					
Crude[d]	1.00	0.94 (0.70-1.26)	0.66	**0.62 (0.40-0.97)**	**0.035**
Adjusted[f]	1.00	1.05 (0.75-1.47)	0.79	**0.52 (0.31-0.87)**	**0.016**
One year acute rejection					
Incidence	35.9 %	29.2 %		27.1 %	
Hazard ratio (95% CI)					
Crude[d]	1.00	0.76 (0.56-1.02)	0.07	0.66 (0.42-1.04)	0.07
Adjusted[f]	1.00	0.78 (0.57-1.07)	0.12	0.68 (0.43-1.10)	0.11
Recipient SNP	**Reference: TT (n=388)**	**GT (n=449)**	**p-value**	**GG (n=144)**	**p-value**
Death censored graft survival					
Est. mean GS, yr (95% CI)[a]	13.9 (13.3-14.6)	14.0 (13.5-14.6)	0.93	14.2 (13.2-15.1)	0.68
Hazard ratio (95% CI)					
Crude[b]	1.00	1.02 (0.71-1.47)	0.92	0.90 (0.53-1.52)	0.68
Adjusted[c]	1.00	1.07 (0.74-1.55)	0.73	0.95 (0.56-1.62)	0.86
PNF					
Incidence	6.2 %	5.8 %		4.2 %	
Odds ratio (95% CI)					
Crude[d]	1.00	0.93 (0.53-1.65)	0.81	0.66 (0.26-1.65)	0.37
Adjusted[e]	1.00	1.00 (0.55-1.82)	0.99	0.74 (0.29-1.91)	0.54
DGF					
Incidence	39 %	36.9 %		33.3 %	
Odds ratio (95% CI)					
Crude[d]	1.00	0.91 (0.68-1.22)	0.54	0.78 (0.52-1.18)	0.24
Adjusted[f]	1.00	1.07 (0.77-1.51)	0.68	0.96 (0.60-1.56)	0.88
One year acute rejection					
Incidence	31.6 %	32.9 %		28.3 %	
Hazard ratio (95% CI)					
Crude[d]	1.00	1.06 (0.79-1.43)	0.71	0.85 (0.55-1.31)	0.71
Adjusted[f]	1.00	1.00 (0.73-1.37)	0.99	0.80 (0.51-1.26)	0.33

Est. – estimated; GS – graft survival; CI – confidence interval; PNF – primary non-function; DGF – delayed graft function

[a]Kaplan-meier survival analysis with log-rank test. Primary non-function grafts were excluded.

[b]Cox regression analysis. Primary non-function grafts were excluded.

[c]Cox regression analysis adjusted for donor age, donor gender, donor type, recipient age, recipient gender, HLA mismatch, cold ischemia time, number of transplantation, use of corticosteroids, use of cyclosporine, use of mycophenolate mofetil. Primary non-function grafts were excluded.

[d]Logistic regression.

[e]Logistic regression adjusted for donor age, donor gender, donor type, recipient age, recipient gender, HLA mismatch, cold ischemia time, number of transplantation, use of corticosteroids, use of cyclosporine, use of mycophenolate mofetil.

[f]Logistic regression adjusted for donor age, donor gender, donor type, recipient age, recipient gender, HLA mismatch, cold ischemia time, number of transplantation, use of corticosteroids, use of cyclosporine, use of mycophenolate mofetil. Primary non-function grafts were excluded.

The effect of the EPO gene polymorphism rs1617640 on plasma EPO levels after renal transplantation

Plasma EPO (IU/l) and hemoglobin (Hb, g/dl) levels after transplantation were determined in recipients of the REGaTTA cohort (Table 3). No differences were observed in plasma EPO levels after transplantation between donor kidney GG- or GT- and TT genotypes. However, plasma EPO levels were significantly higher in recipients with the TT genotype compared to the GG genotype (Table 3; log EPO: 1.28 vs. 1.16; p = 0.015). The plasma EPO level in GT recipients was not significantly lower than in TT recipients (Table 3; log EPO: 1.28 vs. 1.23; p = 0.3). In figure 3, homozygote donor – recipient combinations were defined to illustrate that the plasma EPO level is depending on the genotype of the recipient.

Table 3 – The effect of rs1617640 on plasma EPO levels after transplantation

Donor kidney SNP	Reference: TT (n=89)	GT (n=141)	p-value	GG (n=44)	p-value
Hb (g/dl)	13.9 ± 1.6	13.9 ± 1.6		14.0 ± 1.5	
EPO (IU/l)	15.6 [10.2-23.7]	17.1 [12.0-23.3]		18.1 [10.6-27.6]	
ERI (EPO / Hb)	1.16 [0.74-1.89]	1.22 [0.84-1.76]		1.19 [0.71-2.06]	
Log EPO					
Crude[a]	1.21 ± 0.26	1.25 ± 0.25	0.34	1.25 ± 0.27	0.42
Adjusted[b]	1.21 ± 0.26	1.25 ± 0.25	0.41	1.26 ± 0.27	0.47
Log ERI					
Crude[a]	0.07 ± 0.27	0.11 ± 0.27	0.37	0.11 ± 0.29	0.51
Adjusted[c]	0.07 ± 0.27	0.11 ± 0.27	0.74	0.11 ± 0.29	0.38
Recipient SNP	**Reference: TT (n=94)**	**GT (n=139)**	**p-value**	**GG (n=40)**	**p-value**
Hb (g/dl)	13.9 ± 1.6	13.9 ± 1.6		13.9 ± 1.8	
EPO (IU/l)	17.9 [13.6-28.5]	16.6 [11.8-23.7]		13.1 [8.8-20.5]	
ERI (EPO / Hb)	1.35 [0.88-2.16]	1.20 [0.81-1.85]		0.97 [0.64-1.56]	
Log EPO					
Crude[a]	1.28 ± 0.27	1.23 ± 0.22	0.14	**1.15 ± 0.29**	**0.009**
Adjusted[b]	1.28 ± 0.27	1.23 ± 0.23	0.30	**1.16 ± 0.29**	**0.015**
Log ERI					
Crude[a]	0.14 ± 0.30	0.09 ± 0.24	0.14	**0.01 ± 0.30**	**0.011**
Adjusted[c]	0.14 ± 0.30	0.08 ± 0.24	0.20	**0.02 ± 0.30**	**0.028**

Hb – hemoglobin; EPO – erythropoietin; ERI – Erythropoietin resistance index (plasma EPO / plasma Hb)

[a]Univariate general linear model

[b]Univariate general linear model adjusting for recipient age and gender, urinary protein excretion, creatinine clearance, plasma hemoglobin level, use of azathioprine, use of cyclosporine, plasma C-reactive protein, mean corpuscular volume, plasma triglycerides level, total plasma cholesterol level, use of diuretics, renin-angiotensin-aldosterone inhibition and plasma ferritin level.

[c]Univariate general linear model adjusting for recipient age and gender, urinary protein excretion, creatinine clearance, use of azathioprine, use of cyclosporine, plasma C-reactive protein, mean corpuscular volume, plasma triglycerides level, total plasma cholesterol level, use of diuretics, renin-angiotensin-aldosterone inhibition and plasma ferritin level.

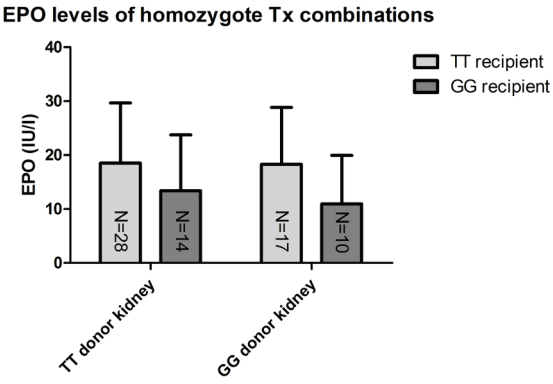

Figure 3 – Plasma EPO levels after transplantation of homozygote combinations of donors and recipients. This figure illustrates that the plasma EPO levels after transplantation depend on the genotype of the recipient and not the genotype of the donor as significantly shown in table 3. A GG donor kidney transplanted to a TT recipient tends to increase EPO production to the need of a TT recipient, while a TT kidney transplanted to a GG recipient tends to reduce EPO production.

Furthermore, the endogenous erythropoietin resistance (ERI) index was calculated, to define the effectiveness of EPO in stimulation of erythropoiesis. In TT recipients, the ERI was significantly higher compared to GG genotype recipients (Table 3; log ERI: 0.14 vs. 0.02; $p = 0.028$). The EPO SNP in donor kidneys did not affect the ERI (Table 3; log ERI: 0.07 vs. 0.11; $p = 0.38$).

Discussion

In this study, we showed that the functional EPO SNP, rs1617640, affected outcome of deceased donor kidneys. The TT genotype of deceased donor kidneys was associated with impaired death censored graft survival and a higher incidence of delayed graft function after transplantation. The EPO SNP in recipients did not affect outcome of renal transplantation. These results implicate renal EPO signaling as an important mediator of both short-term outcome and long-term graft survival.

Functionally, plasma EPO levels after transplantation were higher in recipients with the TT genotype compared to the GG genotype. Thus, plasma EPO levels were dependent on the genotype of the recipient instead of the genotype of the donor kidney. This is surprising as the transplanted kidney most likely produces these plasma EPO levels. However, a functional effect on expression level of EPO was expected as rs1617640 is located in the promoter region of the EPO gene. Higher EPO levels in the TT genotype confirms the functionality of rs1617640 as demonstrated by Tong et al.[12].

Strikingly, hemoglobin levels of GG recipients were normal despite lower plasma EPO levels, suggesting that the EPO variant of the GG genotype is more active or erythropoietic receptors are more efficiently activated. A more active EPO variant in GG genotype subjects is unlikely as rs1617640 is located in the promoter region. Besides, the GG genotype is strongly associated with myelodysplastic syndromes (MDS) as shown by Ma et al.[13]. This association makes a more active EPO variant of GG genotype subjects even more unlikely, because MDS is characterized by ineffective erythropoiesis and stimulation of erythropoiesis by rhEPO is one of the treatment options for MDS[22].

Thus, erythropoietic receptor complexes are presumably more efficiently activated in GG recipients. To investigate this effect, the endogenous EPO resistance index was calculated. The EPO resistance was significantly higher in TT recipients the compared to the GG genotype. This indicates that higher systemic EPO levels were required to regulate erythropoiesis. Thus, a GG donor kidney is able to increase the production of EPO to the need of a TT recipient for adequate erythropoiesis. This explains that plasma EPO levels after transplantation were dependent on the genotype of the recipient instead of the donor kidney.

In literature, increased EPO resistance is associated with increased mortality in patients with end-stage renal disease[23]. The mechanisms of increased EPO resistance are not fully elucidated, but include inflammation, receptor interactions and intracellular signaling[24]. In this study increased EPO levels, based on genetic background, were associated with increased EPO resistance. Clinically, these findings imply that TT genotype patients with end-stage renal disease might require higher doses of rhEPO to treat anemia compared to GG genotype patients. The precise mechanism of increased EPO resistance of the TT genotype has to be further investigated.

In this cohort, no differences in distribution of rs1617640 were observed between donors and recipients. This implicates that the EPO SNP did not play a role in the development of chronic kidney disease. However, the TT genotype of rs1617640 is associated with the development of diabetic end-stage renal disease and an increased need of renal replacement therapy following cardiac surgery[12,16]. In the pathophysiology of both diabetic nephropathy and renal ischemia/reperfusion, activation of endothelial nitric oxide synthase (eNOS) is compromised indicating endothelial dysfunction[25,26]. Since EPO is able to increase eNOS activity, the effect of the EPO SNP on outcome of renal transplantation might be explained by endothelial dysfunction. This is supported by experimental studies indicating the pivotal role of eNOS for EPO mediated renoprotection[9,27,28].

Experimental studies evidently showed the protective capacities of EPO in renal ischemia/reperfusion injury[1,2,29–31]. EPO mediated cytoprotection is based on the binding of EPO to receptor complexes as $EPOR_2$-βCR_2 or βCR-VEGFR-2[8,9]. Binding affinity of these complexes is lower compared to the erythropoietic EPOR2 complex. Thus, in response to injury, a local peak of EPO levels induces cytoprotection, while continuous high plasma levels of EPO cause cardiovascular adverse events. As mentioned above, increased phosphorylation of eNOS is an important mediator of EPO to reduce endothelial dysfunction and improve renal function[9,27,28,32]. Only the genotype of the donor kidney affected outcome after transplantation, indicating that a local effect of EPO is responsible for these differences. In line with the erythropoietic receptor complex, we speculate that also resistance of the cytoprotective receptor complexes might be increased by continuous higher levels of EPO in TT subjects. The peak in local EPO concentration in response to injury in TT donor kidneys might be less effective to induce cytoprotection. Thus, increased EPO resistance of TT donor kidneys might explain the inferior outcome after transplantation compared to the GG genotype.

Recently, immunosuppressive capacities of EPO have been demonstrated by Cravedi et al. EPO inhibited proliferation of T-cells in vitro[33]. This effect was mediated by the EPOR, because ARA290, a non-erythropoietic EPO derivative which is derived from the binding site to the $EPOR_2$-βCR_2 complex[34–37], did not modulate T-cell immunity. Cravedi et al.[33] speculate that the role of EPO in T-cell immunity might be responsible for the observed protective effects of EPO in kidney transplant recipients[33]. However, in this study, the genotype of EPO in donor kidneys or recipients did not affect the incidence of acute rejection. Besides, only the genotype of donor kidneys affected outcome of renal transplantation. Therefore, it is more likely that reduced activation of local, protective pathways against endothelial dysfunction and ischemia/reperfusion injury in the TT genotype donor kidneys was responsible for the observed effect of the EPO SNP.

The single center, retrospective design of this study is the main limitation. However, the independent effects on graft survival and plasma EPO levels validate the functionality of the EPO SNP in this study design. Furthermore, EPO plasma levels were measured in ~28 % of all recipients at a single time after transplantation and the time between transplantation and blood samples varied. It would have been interesting to investigate the effect of the EPO SNP on plasma EPO levels at standardized times after transplantation.

This is the first clinical study showing the important role of local EPO signaling in deceased donor kidney transplantation. These findings in combination with experimental studies suggest an endogenous cytoprotective function of EPO. Intervention in EPO mediated cytoprotection may therefore improve short- and long-term graft function following deceased donor kidney transplantation. To date, clinical trials failed to translate EPO mediated cytoprotection to the clinic, while the risk of cardiovascular events increased[4–7]. Therefore, non-erythropoietic EPO derivatives have been developed, which can be titrated safely to protective levels. In experimental studies, these non-erythropoietic EPO derivatives showed great potential to improve renal function without increasing risk of cardiovascular adverse events[34–40].

In conclusion, the TT genotype of promoter EPO SNP rs1617640 in deceased donor kidneys reduced death censored graft survival and increased incidence of DGF. This effect is probably mediated by altered EPO signaling in the kidney, as the EPO SNP in recipients did not affect outcome after transplantation. The findings of this study give new insight in the role of EPO in renal transplantation and may therefore help to translate EPO mediated cytoprotection by non-erythropoietic EPO derivatives to the transplantation clinic.

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