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Original Paper

hTERT, BICD1 and Chromosome 18 Polymorphisms Associated with Telomere Length Affect Kidney Allograft Function After Transplantation

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Key Words
Allograft • DGF • Gene • Polymorphism • Telomere length

Abstract
Background/Aims: It has been confirmed that telomere length (TL) correlates with chronological donor age and that telomere shortening is accelerated in allografts. The aim of this study was to analyse the associations between graft rs2735940 *hTERT* and rs2630578 *BICD1* gene polymorphisms and rs7235755/rs2162440 chromosome 18 polymorphisms, relative TL and kidney function after transplantation. ***Methods:*** The study enrolled 119 Polish Caucasian kidney allograft recipients (64M/55F, mean age 47.3±14.0 years). The relative TL was assessed in biopsy specimens. To identify genotypes of the studied polymorphisms, real-time PCR was performed. ***Results:*** The graft rs2735940 *hTERT* gene polymorphism TT genotype was associated with a significantly lower risk of delayed graft function (DGF) (TT vs. TC+CC; OR=0, p=0.009) and significantly shorter TL in the '0' biopsy (TT vs. CC: 207±153 vs. 400±161, p=0.036). The graft rs2630578 *BICD1* gene polymorphism CC genotype was associated with lower creatinine concentrations in the first month (CC vs. GC: 1.11±0.06 vs. 2.0±1.25 mg/dL, p=0.03). The AA genotype of the graft rs7235755 chromosome 18 polymorphism was associated with longer relative TL in specimens collected 12 to 60 months after transplantation (AA vs.

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GG+GA p=0.04; AA vs. GG: 489±152 vs. 246±145, p=0.035) and the presence of A allele was associated with higher creatinine concentrations one month after transplantation (GA+AA vs. GG p=0.026; GA vs. GG: 2.18±1.59 vs. 1.76±0.88 mg/dL, p=0.02). It was found that shorter TL in the first six months was associated with higher creatinine concentrations 12 and 18 months after transplantation (Rs=-0.32; p=0.07 and Rs=-0.54; p=0.006, respectively). ***Conclusions:*** Graft rs2735940 *hTERT* and rs2630578 *BICD1* gene polymorphisms and rs7235755/rs2162440 chromosome 18 polymorphisms, apart from the association with TL, affect early kidney function after transplantation. Relative TL correlated negatively with creatinine concentrations, allowing the use of TL as a predictor of long-term kidney function.

Introduction

Telomere length (TL) has been analysed with regard to cell senescence and ageing of animals and humans, including age-related diseases [1-4]. There have been also attempts to link the accelerating transplanted organ ageing with TL and telomerase activity assessments [5, 6]. The context of kidney transplantation outcome is currently of particular interest, because TL has turned out to be a determinant of post-transplant organ function [7]. The common organ shortage has resulted in expanding kidney donor criteria to over 60 years of age [8]. The effect of the biological organ age of the donated allograft is still unknown. It has been confirmed that TL correlates with chronological donor age and that telomere shortening is accelerated in allografts [5-7]. Reaching the limit of proliferative capacity may lead to organ failure and subsequent loss. The possibility of allograft vitality prediction would allow the stratification of donors and recipients in order to improve transplantation outcomes [6].

Telomerase, which is also called telomere terminal transferase, is an enzyme responsible for telomere elongation through adding DNA sequence repeats. Human telomerase consists of the catalytic subunits telomerase reverse transcriptase (TERT), telomerase RNA (TR or TERC), and dyskerin (DKC1). Therefore, there are several genes encoding telomerase subunits, including *hTERT*, *TERC*, *DKC1* and *TEP1* [9]. The *hTERT* gene is located on chromosome 5 and determines telomerase activity. There are over 200 *hTERT* gene polymorphisms influencing cell cycle and promoting cancer development [10]. Moreover, changes among the *hTERT* gene may be correlated not only with cell immortality, but also with telomere shortening. Among patients with peripheral arterial disease, the loss-of-function haplotype CC of the rs2735940 and rs2853669 *hTERT* gene polymorphisms has been associated with shorter TL [9]. The bicaudal D homolog 1 (*BICD*1) rs2630778 gene polymorphism has also been linked with telomere shortening [11]. However, the analysis of the rs2630578 *BICD1* gene polymorphism in individuals with coronary heart disease and type 2 diabetes gave no significant results [12]. Other genetic loci (rs2162440 and rs7235755) found on chromosome 18 (18q12.2) in a two stage genome wide association (GWA) study on 2790 individuals from the UK were associated with shorter TL (C and G allele, respectively) [13]. All this evidence indicates that, apart from ageing, genetic factors significantly influence TL.

The aim of this study was to analyse the associations between graft rs2735940 *hTERT* and rs2630578 *BICD1* gene polymorphisms and rs7235755/rs2162440 chromosome 18 polymorphisms, relative TL and kidney function after transplantation.

Material and Methods

Participants

The study enrolled 119 Polish Caucasian kidney allograft recipients (64M/55F, mean age 47.32±14.03; transplantation performed between 2001 and 2012). Organs were harvested from cadaveric donors

(59.8%M/40.2%F, mean age 45.99±14.62 years). The population of recipients was dived into subgroup A – up to 2 years after transplantation (n=70) and subgroup B – more than 2 years after transplantation (n=49). The division was necessary to set the inclusion criteria. Subgroup A included first renal allograft recipients recruited consecutively immediately after transplantation and after giving their consent to participate in the study, while subgroup B included the first renal allograft recipients with a functioning organ, recruited from the Transplant Outpatient Clinic that delivers ongoing care for kidney transplant recipients following discharge, after giving their consent to participate in the study. Exclusion criteria were: more than one renal transplantation, lack of consent, or organ loss/return to dialysis. Kidney allograft recipients' detailed characteristics are presented in Table 1. The main causes of impaired kidney function before transplantation were glomerulonephritis, type 1 and 2 diabetes (T1DM and T2DM), arterial hypertension and autosomal dominant polycystic kidney disease (ADPKD). Arterial hypertension, post-transplant diabetes (PTDM) and atherosclerosis were the main comorbid conditions.

Table 1. Clinical characteristics of the studied group

Recipients	(N or n/N)	Mean±SD or %
Mean age [years]	119	47.32±14.03
Male Sex	64/119	53.8%
BMI [kg/m^2]	67	25.75±4.50
AH	91/115	79.1%
HD before Tx	66/84	78.6 %
PD before Tx	14/84	16.64%
Preemptive Tx	4/84	4.76%
Mean time of D before Tx [months]	80	28.02±19.65
Residual diuresis [mL/d]	78	1201.9±1267.7
CIT [hours]	93	17.7±6.65
PRA [%]	84	3.4±11.4
Diuresis 7th day after Tx [mL/d]	43	3348.8±1646.1
Cr 7th day after Tx [mg/dL]	98	3.35±2.62
Cr 1 month after Tx [mg/dL]	100	1.88±1.45
Cr 6 months after Tx [mg/dL]	72	1.50±0.52
Cr 12 months after Tx [mg/dL]	74	1.50±0.59
Cr 24 months after Tx [mg/dL]	56	1.62±1.11
DGF	36/119	30.25%
AR	34/119	28.57%
CAD	17/119	14.29%
Post Tx infection	39/119	32.8%
Post Tx UTI	25/39	64.1%
Post Tx CMV infection	6/39	15.38%
Post Tx BKV infection	3/39	7.69%
Post Tx TBC infection	1/39	2.56%
Post Tx Sepsis	1/39	2.56%
Mortality	3/119	2.52%

n – number of recipients with indicated feature, N – number of all recipients with available data, SD – standard deviation, BMI – body mass index, AH – arterial hypertension, HD - hemodialysis, Tx – transplantation, PD –peritoneal dialysis, D – dialysis, CIT – cold ischemia time, PRA – panel reactive antibody, Cr – creatinine, DGF – delayed graft function, AR – acute rejection, CAD – chronic allograft dysfunction, UTI – urinary tract infection, CMV – cytomegalovirus, BKV – BK virus, TBC - tuberculosis

The following parameters were recorded: the recipient and donor ages and gender, the recipient's body mass index (BMI), the type of dialysis treatment and its duration before transplantation, the cause of impaired kidney function, residual diuresis, panel reactive antibodies (PRA), cold ischaemia time (CIT), date of the transplantation, comorbid conditions, and the occurrence and type of infection after transplantation. The frequencies of delayed graft function (DGF), AR (acute rejection) and chronic allograft dysfunction (CAD) were observed. The diagnosis of DGF was determined as the need for dialysis during the first 7 days after transplantation. Identification of AR was confirmed clinically (pain and/or swelling of the kidney graft, body temperature ≥38°C, elevated serum creatinine ≥25% in the absence of other pathology including infection, urinary tract obstruction, allograft artery stenosis, or cyclosporine and tacrolimus toxicity), and by biopsy review. The diagnosis of CAD was based on functional and morphological (biopsy confirmed) deterioration of a renal allograft at least 3-6 months after transplantation. Blood samples were collected for creatinine concentration evaluation during the first 7 days after transplantation, and 1, 3, 6, 12, 18, 24, 30, 36, 48 and 60 months after kidney transplantation. Creatinine concentration assessment was performed using a colorimetric method. Biopsy specimens were collected for analysis of the RTL and renal pathologist review (Banff working classification criteria were used) in the peri-transplant period (biopsy '0'), 3, 6, 12, 18, 24, 36, 48 and 60 months after transplantation and in the case of deteriorating renal transplant function. All patients received the standard

immunosuppressive protocol with triple drug therapy including a calcineurin inhibitor (tacrolimus), mycophenolate mofetil or mycophenolate sodium, and steroids. Some individuals in subgroup B received cyclosporine, azathioprine and steroids after transplantation, but were then converted to tacrolimus and mycophenolate mofetil at least 6 years prior to the study. If necessary, the immunosuppressive protocol was modified through everolimus or sirolimus initial administration/conversion. In some cases, induction immunosuppressive therapy was offered. Informed consent was obtained from all patients. The local ethics committee of the Pomeranian Medical University in Szczecin, Poland, approved protocol of the study.

Relative TL analysis

DNA was extracted using a mini column-based DNA isolation kit (A&A Biotechnology, Gdynia, Poland) and stored at –20°C. A high concentration genomic DNA sample was prepared in decimal concentrations to cover all possible measurements. According to standard procedure, telomere length was assessed using two pairs of primers, i.e. telomere-specific and a single copy gene-specific (albumin). The design of primers specific to a difficult sequence of telomeres is challenging and there are already numerous protocols for this application [14, 15]. We used the primers that had already been shown to work specifically [14], as we found them to be the most optimal of all of the primers tested in the context of specificity and to result in no primer-dimer or non-specific products.

The PCR conditions used to amplify the telomere fragment were as follows: initial denaturation and polymerase activation (hot start) was performed in 95˚C for 10min followed by two cycles of 94˚C/15 s and 49˚C/15 s without fluorescence acquisition. The signal was detected during another 40 cycles i.e. 94˚C/10 s, 66˚C/10 s and 72˚C/10 s. Melting analysis (65-95˚C range, 0.2˚C resolution) at the end of the reaction was performed to verify the specificity of the product and indicated Tm=81.7. The efficiency of the reaction was no lower than 97.8%. Importantly, this result was repeatable for all of the samples that were analysed (in serial dilutions). After the optimisation of the primer concentration, which was estimated to be 0.5 μM, we selected the optimal magnesium chloride concentration of 2.5 mM. Similarly, reaction conditions used to identify the optimal annealing temperature, magnesium chloride and primer concentrations for albumin were as follows: denaturation 95˚C/10 min – hot start; followed by 45 cycles 94˚C/10 s, 61˚C/10 s and 72/10 s. The Tm of the product (analysis performed as above) was 80.7˚C and the efficiency was 99.6%. The concentration of primers was 0.5 μM and magnesium chloride 2.5 mM. The telomere length was assessed using a qPCR system (Roche, LC 2.0) and SybrGreen kit (Roche, Manheim, Germany).

hTERT, BICD1 and chromosome 18 polymorphisms analysis

All samples were genotyped in duplicate using allelic discrimination assays with Taqman® probes (Applied Biosystems, Carlsbad, California, USA) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA). To discriminate *hTERT* rs2735940, *BICD1* rs2630578 as well as chromosome 18 rs7235755 and rs2162440 alleles, TaqMan® Pre-Designed SNP Genotyping Assays were used (assay IDs: C___2412786_10, C___7497299_10 and C__15966471_20, respectively), including appropriate primers and fluorescently labelled (FAM and VIC) MGB™ probes to detect the alleles. Genotypes were assigned using all of the data from the study simultaneously.

Statistical analysis

The distribution of the genotypes and alleles was evaluated with use of the chi-square test or Fisher's exact test. Since distributions of most quantitative variables were significantly different from a normal distribution ($p<0.05$, Shapiro-Wilk test), we used non-parametric tests. Spearman's rank correlation coefficient was used to analyse correlations between variables and the Kruskal-Wallis test followed by Mann-Whitney U test were used to compare values between groups. Associations with a p-value <0.05 were considered statistically significant. Calculations were performed with Statistica 10 software.

Results

The frequency distribution of the rs2735940 *hTERT* gene, rs2630578 *BICD1* gene, as well as rs7235755 and rs2162440 within chromosome 18 polymorphisms genotypes among grafts are presented in Table 2. Because of full linkage disequilibrium between the

Table 2. The frequency distribution of studied polymorphisms genotypes among grafts

	Genotypes frequency (%)		
Polymorphism	CC n (%)	CT n (%)	TT n (%)
hTERT rs2735940	26 (30%)	48 (55%)	13 (15%)
Polymorphism	GG n (%)	GC n (%)	CC n (%)
BICD1 rs2630578	61 (70%)	23 (26.5%)	3 (3.5%)
Polymorphism	GG n (%)	GA n (%)	AA n (%)
C18 rs7235755 *	51 (58.5%)	31 (35.5%)	5 (6%)

* rs7235755 G and A alleles are equivalent to rs2162440 C and T alleles, respectively

Table 3. The distributions of the grafts' genotypes and alleles of the rs2735940 *hTERT* gene polymorphism in recipients with and without DGF

	DGF (n=27)		Without DGF (n=60)					
Genotypes and alleles; rs2735940	n	%	n	%	p^	Comparison	OR (95% CI) for DGF	p*
CC	7	27.0	19	73.0		TT vs TC+CC	0.00 (-)	**0.009**
TC	20	41.7	28	58.3	**0.01**	TT vs TC	0.00 (-)	**0.005**
TT	0	0.0	13	100.0		TT vs CC	0.00 (-)	**0.04**
C	34	34.0	66	66.0		T vs C	0.72 (0.37-1.39)	0.326
T	20	27.0	54	73.0				

^Chi-square test, * Fisher's exact test

chromosome 18 polymorphisms (rs7235755 G and A alleles are equivalent to rs2162440 C and T alleles, respectively), we present only rs7235755 genotypes. The distribution of all studied polymorphisms was consistent with Hardy-Weinberg equilibrium (p=0.28; p=0.7 and p=1.0 respectively).

Association between studied polymorphisms and DGF

Delayed graft function was diagnosed in 27.0% of recipients with the graft rs2735940 *hTERT* gene polymorphism CC genotype, in 41.7% of those with the TC graft genotype, and in 0.0% of those with TT. There were no TT graft genotype carriers with a diagnosis of DGF; therefore, it was impossible to assess the confidence intervals (TT vs TC+CC p=0.009) (Table 3). There were no statistically significant differences between recipients with DGF and without DGF in regard to the rs2630578 *BICD1* gene polymorphism, or rs7235755/rs2162440 within the graft chromosome 18 genotypes.

Association between studied polymorphisms and biopsy specimens relative TL

Table 4 presents the relative graft TL in kidney biopsy specimens stratified according to the rs2735940 *hTERT* gene, rs2630578 *BICD1* gene and rs7235755/rs2162440 chromosome 18 polymorphisms. The differences between relative TL in kidney biopsy specimens collected at month '0' with regard to all rs2735940 *hTERT* gene polymorphism genotypes were on the border of statistical significance (p=0.09). However, the comparison between two genotypes showed that relative TL was significantly shorter in the TT allograft specimens vs. CC genotype carriers (p=0.036). Similarly, allograft specimens with a T allele of the rs2735940 *hTERT* gene polymorphism collected from month '0' to the sixth month after transplantation were characterized with a shorter relative TL in comparison to CC homozygotes (p=0.047). Relative TL in biopsy specimens collected 12 to 60 months after transplantation was shorter in T allele carriers in comparison to CC homozygotes; however these differences were on the border of statistical significance (p=0.089). A comparison of all rs2630578 *BICD1* gene polymorphism genotypes with regard to relative TL in biopsy specimens collected at different time points revealed no statistically significant differences. The AA homozygotes of the graft rs7235755 chromosome 18 polymorphism in biopsy specimens collected from month '0' to the sixth month after transplantation were characterized with significantly shorter TL (GG+GA vs. AA p=0.027 and GG vs AA p=0.05). However, the comparison between genotypes

Table 4. Grafts' relative telomere length in kidney biopsies specimens stratified according to rs2735940 *hTERT* gene, rs2630578 *BICD1* gene and rs7235755/rs2162440 chromosome 18 polymorphisms genotypes

GRTL	Mean±SD	Mean±SD	Mean±SD	p^	p*	p*
			rs2735940 *hTERT*			
	CC	TC	TT		CC vs CT	TT vs CC
Biopsy 0 m	400±161	293±153	207±153	0.09	0.14	**0.036**
Biopsy 0-6 m	330±117	263±136	267±195	0.13	**0.047**	0.24
Biopsy 12-60 m	343±133	241±182	278±236	0.23	0.089	0.66
			rs2630578 *BICD1*			
	GG	GC	CC		GG vs GC	GG vs CC
Biopsy 0 m	325±180	305±126	215±106	0.61	0.44	0.39
Biopsy 0-6 m	290±147	285±135	202±79	0.49	0.26	0.95
Biopsy 12-60 m	270±166	308±187	-#	-	0.59	-
			rs7235755 chromosome 18			
	GG	GA	AA		GG + GA vs AA	GG vs AA
Biopsy 0 m	300±178	338±139	-#	-	-	-
Biopsy 0-6 m	285±151	304±118	90±26	0.09	**0.027**	**0.05**
Biopsy 12-60 m	246±145	286±193	489±152	0.11	**0.04**	**0.035**

^Kruskal-Wallis (K-W) test for differences between 3 genotypes, *Mann-Whitney test, #no biopsies with indicated graft genotype were available, SD – standard deviation, GRTL – grafts' relative telomere length, m - month

in specimens collected 12 to 60 months transplantation showed significantly longer TL among AA homozygotes (GG+GA vs. AA p=0.04 and GG vs AA p=0.035).

Association between studied polymorphisms and creatinine concentrations

Table 5 presents the creatinine serum concentrations of recipients stratified according to the graft rs2735940 *hTERT* gene, rs2630578 *BICD1* gene and rs7235755/rs2162440 chromosome 18 polymorphisms. A comparison of creatinine concentrations assessed at different time points (1, 6, 12 and 24 months after transplantation) with regard to the graft rs2735940 *hTERT* gene polymorphism showed no statistically significant differences. Creatinine concentrations 1 and 6 months after transplantation differed among the graft rs2630578 *BICD1* gene polymorphism genotypes (p=0.067 and 0.049, respectively) and were lower among the CC homozygotes (GC vs. CC p=0.03 and GC vs. CC p=0.067, respectively). Differences in creatinine concentrations 12 and 24 months after transplantation with regard to rs2630578 *BICD1* gene polymorphism genotypes were not statistically significant. Creatinine concentrations one month after transplantation differed among the graft rs7235755 chromosome 18 polymorphism genotypes (p=0.08) and were higher among the A allele carriers (GA+AA vs. GG p=0.026 and GG vs GA p=0.02). A comparison of creatinine concentrations assessed 6, 12 and 24 months after transplantation with regard to graft rs7235755 chromosome 18 polymorphism genotypes showed no statistically significant differences.

Correlations between creatinine serum concentrations and biopsy specimen relative TL

The analysis of relative TL in kidney allograft biopsy specimens collected at different time points and creatinine concentrations revealed negative correlations (presented in detail in Table 6). The higher the concentration of creatinine assessed in the first week after transplantation (third and seventh day), the shorter the TL in biopsies performed between 12 and 24 months after transplantation (borderline statistical significance, p<0.1). Moreover, the shorter TL in the early period after transplantation (0-6 months), the higher the creatinine concentrations 12 and 18 months after transplantation (p=0.07 and p=0.006 respectively). The correlation between TL at 0-6 months and creatinine concentration at 24 months after transplantation was not statistically significant.

Table 5. Recipients' creatinine serum concentrations stratified according to grafts' rs2735940 *hTERT* gene, rs2630578 *BICD1* gene and rs7235755/ rs2162440 chromosome 18 polymorphisms genotypes

Cr [mg/dl]	Mean±SD	Mean±SD	Mean±SD	p^	p*	p*
			rs2735940 *hTERT*			
	CC	CT	TT		CC vs CT	CC vs TT
Creatinine 1 m	1.60±0.57	2.04±1.76	1.43±0.44	0.71	0.88	0.43
Creatinine 6 m	1.31±0.32	1.46±0.51	1.55±0.44	0.49	0.41	0.27
Creatinine 12 m	1.38±0.63	1.48±0.57	1.44±0.45	0.62	0.42	0.41
Creatinine 24 m	1.34±0.40	1.80±1.59	1.42±0.46	0.62	0.38	0.59
			rs2630578 *BICD1*			
	GG	GC	CC		CC vs GC	CC vs GG
Creatinine 1 m	1.81±1.44	2.0±1.25	1.11±0.06	0.067	**0.03**	0.089
Creatinine 6 m	1.40±0.46	1.59±0.41	1.02±0.04	**0.049**	0.067	0.16
Creatinine 12 m	1.37±0.56	1.60±0.59	1.54 (-)	0.20	0.10	0.10
Creatinine 24 m	1.61±1.43	1.58±0.60	1.46 (-)	0.35	0.35	0.35
			rs7235755 chromosome 18			
	GG	GA	AA		GA + AA vs GG	GG vs GA
Creatinine 1 m	1.61±1.23	2.18±1.59	1.76±0.88	0.08	**0.026**	**0.02**
Creatinine 6 m	1.46±0.47	1.40±0.47	1.33±0.24	0.94	0.84	0.92
Creatinine 12 m	1.48±0.60	1.34±0.56	1.48±0.42	0.51	0.49	0.31
Creatinine 24 m	1.76±1.46	1.24±0.36	1.59±0.46	0.15	0.71	0.29

^Kruskal-Wallis (K-W) test for differences between 3 genotypes, *Mann-Whitney test, SD – standard deviation, Cr - creatinine, m – month

Table 6. Correlations between creatinine serum concentrations assessed at different time points and relative telomere biopsy specimens length

Correlated parameters	N	Rs	p value
3rd day creatinine & 12 months biopsy	19	-0.41	0.08
3rd day creatinine & 12-24 months biopsy	29	-0.36	0.06
7th day creatinine & 12 months biopsy	23	-0.37	0.08
7th day creatinine & 12-24 months biopsy	36	-0.29	0.08
12th month creatinine & 0-6 months biopsy	33	-0.32	0.07
18th month creatinine & 0-6 months biopsy	24	-0.54	**0.006**
24th month creatinine & 0-6 months biopsy	25	-0.15	0.48

p value calculated for Spearman's rank correlation coefficient (Rs)

Discussion

Our main findings suggest that the TT genotype in the graft rs2735940 *hTERT* gene polymorphism is associated with a lower risk of DGF and the T allele is associated with shorter relative TL in the early period after transplantation. The G allele of the graft rs2630578 *BICD1* gene polymorphism is associated with higher creatinine concentrations in the early period after transplantation. The A allele in the graft rs7235755 chromosome 18 polymorphism is associated with longer relative TL in biopsy specimens collected 12 to 60 months after transplantation and higher creatinine concentrations in the early period after transplantation. Moreover, with a shorter TL in the early period after transplantation, higher creatinine concentrations were observed 12 and 18 months after transplantation. Moreover, a higher concentration of creatinine assessed in the first week after transplantation was associated with shorter TL in biopsies performed between 12 and 24 months after transplantation.

We observed associations between polymorphisms in genes implicated in the immune response and renal allograft function in our earlier studies [16-18]. The current study assessed the linkage between inflammation and accelerated ageing of the transplanted kidney. Results described elsewhere indicate that the age of the donor, the duration of

dialysis before transplantation and PRA are negatively significantly correlated with relative TL. Moreover, we observed significant shortening of the TL in patients with DGF, AR and CAD. The strong association between the age of the donor and TL have been reported by other authors. Oetting et al. confirmed that ageing as a controlled biological process affects the transplanted kidney, leading to shortening of the TL [6]. In a study by Melk et al., somatic cell senescence mechanisms were found to influence kidney transplant outcomes through not only the age of the donor, but also through transplantation complications resulting in accelerated kidney allograft ageing [19]. Analysis of the polymorphisms associated with TL gives additional information on the predicted transplanted organ function. A significant decrease in TL and accelerated tissue senescence in primate kidneys exposed to the ischemia-reperfusion injury has been reported [20]. Independently of ischemia-reperfusion damage leading to telomere shortening, we observed that the TT genotype of the graft rs2735940 *hTERT* gene polymorphism is associated with a lower risk of DGF. The possible mechanism underlying this finding is not clear. We assume that the TT genotype of the rs2735940 polymorphism may play a protective role in the pathogenesis of DGF by altering telomerase transcription activity. Iizuka et al. found that the TT genotype of the rs2735940 *hTERT* gene polymorphism is associated with a significantly lower risk of epithelial malignancy [21]. Unfortunately, the authors made no attempt to explain this observation. Since neoplasm promotion results from uncontrolled cell division, the TT genotype may be implicated in telomerase activity control and thereby affect the cell cycle. Such an assumption is in agreement with a study by Zhang et al., where the TT genotype was associated with longer TL in comparison with the CC genotype, which had 43% lower transcription activity of the *hTERT* promoter [9]. Contrary to this evidence, in our study, the T allele of the rs2735940 *hTERT* gene polymorphism was associated with shorter relative TL in the biopsy specimens collected between 0 and 6 months after transplantation. However, this could be explained by the frequency of DGF occurrence. There were no grafts with the TT genotype among patients with DGF and therefore no specimens were collected in the first 7 days after transplantation. Obviously, TT genotype graft biopsies performed in month '0' were mostly from the pre-implantation period (during cold ischemia time). It is possible that the ischemic state affects rs2735940 *hTERT* gene polymorphism expression resulting in alterations in TL.

The rs2630578 *BICD1* gene polymorphism C allele has been linked to shorter TL and 44% lower BICD1 mRNA levels in comparison to the G allele [11]. Analysis of this polymorphism among individuals with psychiatric disorders gave no significant results [22]. However, a study on patients with hypertension and cardiac organ damage revealed that the rs2630578 *BICD1* gene polymorphism is associated with impaired function of heart muscle and lower ejection fraction. The G allele of this polymorphism showed a stronger association than the C allele [23]. In our study, higher creatinine concentrations in the early period after transplantation were observed among grafts with the G allele of the rs2630578 *BICD1* gene polymorphism. These results cannot be compared because of a lack of similar studies.

Studies concerning markers of cellular senescence, including TL as a predictor of kidney function, have focused mainly on the first year after transplantation. The authors of these reports claim that the cell cycle inhibitor CDKN2A rather than TL is the best single allograft outcome prognostic factor [24, 25]. There have also been attempts to link long-term kidney function with TL. It has been shown that shorter TL is associated with impaired kidney function five years, but this observation could be explained by older age. Moreover, this study regarded individuals with stable coronary heart disease and not transplanted kidneys [26]. We found that the A allele of the graft rs7235755 chromosome 18 polymorphisms was associated with longer relative TL in biopsy specimens collected 12 to 60 months after transplantation. Despite the fact that there are no studies on kidney allografts to compare with our results, Mangino et al. confirmed that the G allele of the rs7235755 polymorphism is correlated with shorter TL [13]. Surprisingly, biopsy specimens with the A allele collected 0-6 months after transplantation had shorter telomeres. However, this sample was very small and therefore not representative. The A allele of the rs7235755 polymorphisms was associated with higher creatinine concentrations in the early period after transplantation in

our study. We cannot explain the possible underlying mechanism of this observation. It may be due to the influence of ischemia-reperfusion damage and DGF occurrence. Maubaret et al. found no association between the rs2162440 chromosome 18 polymorphism and mean leukocyte TL and the risk of coronary artery disease [12]. It is possible that the impact of rs7235755/rs2162440 on the relative TL and kidney allografts is small and a much larger sample is needed to obtain reliable results.

The main limitation of our study is the small sample size. We observed many results on the border of statistical significance. Increasing the power of the study would allow us to obtain more reliable results. Unfortunately, the unavailability of funding made it impossible to recruit other transplantation centres. However, our findings give new insight into the association between TL-affecting polymorphisms and kidney allograft function.

Conclusions

Graft rs2735940 *hTERT* and rs2630578 *BICD1* gene polymorphisms and rs7235755/rs2162440 chromosome 18 polymorphisms, apart from an association with TL, affect early kidney function after transplantation. The results of the assessment of relative TL correlate negatively with creatinine concentrations, allowing us to use TL as a predictor of long-term kidney function.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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