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# Joint Assessment of Donor and Recipient *hTERT* Gene Polymorphism Provides Additional Information for Early Kidney Transplantation Outcomes

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**Background:** There are several genes and genetic *loci* affecting telomere length, including *hTERT* gene and *BICD1* gene as well as polymorphisms within chromosome 18. It has been demonstrated that the age of the donor is a negative factor associated with long-term kidney allograft function, and that post-transplant complications accelerate transplanted organ aging, thus contributing to estimated glomerular filtration rate (eGFR) decreases. The aim of this study was a joint assessment of donors' and recipients' *hTERT* and *BICD1* genes as well as chromosome 18 polymorphisms with regard to early kidney transplantation outcomes.





**Material/Methods:** The study enrolled 74 pairs of Polish Caucasian kidney allograft cadaveric donors (60% male, mean age 45.99±14.62) and recipients (50.0% male, mean age 48.89±13.50). The transplantation procedure (Tx) was performed between 2001 and 2012. All samples were genotyped in duplicate using Real-Time PCR.

**Results:** This study showed that rs2735940 *hTERT* CX-TT donor-recipient genotype pair was associated with almost five times higher odds (OR=4.82; 95% CI: 1.32–18; *p*=0.016) of delayed graft function (DGF), and that rs2735940 *hTERT*, rs2630578 *BICD1*, and rs7235755 chromosome 18 polymorphisms combined pairs were not associated with acute rejection (AR).

**Conclusions:** In conclusion, both the donor's and the recipient's rs2735940 *hTERT* gene polymorphism was associated with early graft function after transplantation. The odds of DGF were almost five times higher for a combination of CX (CT or CC) donor genotype and TT recipient genotype. Joint assessment of donor-recipient genotype pairs provides more information for prediction of early kidney transplantation outcomes.

**MeSH Keywords:** Cell Aging • Delayed Graft Function • Graft Rejection • Polymorphism, Genetic Telomere Homeostasis

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## Background

Prevalence of chronic kidney disease (CKD) is rising worldwide, including in Poland where diabetes, glomerulonephritis, and arterial hypertension are the main causes of kidney function deterioration and loss [1–3]. The importance of primary health care in the management of CKD has been emphasized [4]. Although Polish family physicians in everyday practice routinely assess both creatinine concentration values and estimated glomerular filtration rates (eGFRs), there is still a high need for renal replacement therapy (RRT) and its availability remains unsatisfactory [5]. It has been shown that promoting cardiovascular health is associated with lower risk of CKD [1]. In a recent Polish population-based study it was shown that supplementation of folic acid among individuals with atherosclerosis risk factors resulted in significant decreases in creatinine concentration, followed by increase in eGFR [6]. This observation links environmental factors and lifestyle with kidney function. However, no less important are genetic factors, which continue to be of great interest in the field of transplantation [7]. Among these genetic factors, apart from polymorphisms influencing the immune response after transplantation, the factors related directly to cell senescence are under investigation [8].

There are several genes of interest that affect organ aging. Primarily *hTERT* gene, *BICD1* gene, and polymorphisms within chromosome 18 alter telomere shortening [9]. These age-related findings are especially important in kidney transplantation, because it has been demonstrated that the age of the donor is a negative factor associated with long-term kidney allograft function, and post-transplant complications accelerate transplanted organ aging thus contributing to decreases in eGFR [10,11]. In our previous studies, we found a lack of association between recipient's sex, comorbid conditions, or post-transplant infections and telomere length [12]. Subsequently, we have focused on donor's gene polymorphisms evaluated in renal allograft tissue collected by biopsy. Nevertheless, little is

known with respect to combined analysis of both the donor- and the recipient-dependent genetic factors affecting cell senescence. Moreover, gene polymorphisms are still the subject of many studies in the transplantation area.

The aim of this study was a joint assessment of donors' and recipients' *hTERT* gene, *BICD1* gene, and chromosome 18 polymorphisms with regard to early kidney transplantation outcomes.

## Material and Methods

The study enrolled 74 pairs of Polish Caucasian kidney allograft cadaveric donors (60% male, mean age 45.99±14.62 years) and matched recipients (50.0% male, mean age 48.89±13.50 years). The transplantation procedure (Tx) was performed between 2001 and 2012. The inclusion criteria for the recipients were as follows: consecutively recruitment after the transplantation procedure and after giving consent to participate in the study, or recruitment from the Transplant Outpatient Clinic, which was delivering ongoing care for kidney transplant recipients with a functioning organ, and after receiving the patient's consent. The exclusion criteria were as follows: >1 kidney transplantation, refusing to participate in the study, loss of transplanted organ, or return to dialysis. The necessity of Tx was caused mainly by glomerulonephritis, diabetes mellitus type 1 or type 2, arterial hypertension, or autosomal dominant polycystic kidney disease (ADPKD).

The following parameters were recorded for each case: age and gender of both the donor and the recipient, cause of the impaired kidney function, and date of the transplantation. Moreover, the transplanted kidney function was monitored by creatinine measurement and observation of delayed graft function (DGF) and acute rejection (AR) occurrence (Table 1). The diagnosis of DGF was based on a common definition: the need for dialysis during the first week after transplantation procedure. In the case of AR, clinical confirmation (pain and/or

**Table 1.** Clinical characteristics of the kidney allograft recipients.

	Recipients (N or n/N)	Mean ±SD or%
Mean age [years]	74	48.89±13.50
Males	37/74	50.0%
DGF	22/74	29.7%
AR	14/74	18.9%
CAD	8/74	10.8%
Post-Tx infection	19/48	39.6%

n – number of recipients with indicated feature; N – number of all recipients with available data; SD – standard deviation; DGF – delayed graft function; AR – acute rejection; CAD – chronic allograft dysfunction; Post-Tx – post transplant.

swelling of the allograft, elevated temperature  $\geq 38^{\circ}\text{C}$  and serum creatinine  $\geq 25\%$  in the absence of other pathology), and morphological confirmation (biopsy review) were performed. The colorimetric method was used for serum creatinine concentration assessment. All patients received typical immunosuppressant agents as triple drug therapy including calcineurin inhibitor (tacrolimus), mycophenolate mofetil or mycophenolate sodium, and steroids. Informed consent was obtained from all study participants. The Pomeranian Medical University in Szczecin Ethics Committee approved the protocol for the study (approval No. KB-0080/100/09).

### The genotyping of the *hTERT*, *BICD1*, and chromosome 18 polymorphisms

All samples were analyzed twice, with use of allelic discrimination assays and Taqman<sup>®</sup> probes (Applied Biosystems, Carlsbad, CA, USA). To perform the reactions, CFX96 Touch<sup>™</sup> Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) was used. The identification of relevant polymorphisms alleles rs2735940 *hTERT*, rs2630578 *BICD1*, and rs7235755 as well as rs2162440 within chromosome 18 was based on TaqMan<sup>®</sup> Pre-Designed SNP Genotyping Assays (IDs: C\_\_2412786\_10, C\_\_7497299\_10 and C\_\_15966471\_20, respectively) using chosen primers and fluorescently labeled probes to detect the alleles.

### Statistical analysis

The genotypes and alleles distribution was analysed with chi-square test. Analysis of donor and recipient genotypes as independent variables associated with DGF or AR as dependent variables was performed using multivariate logistic regression. We considered  $p$ -value  $< 0.05$  as statistically significant. Statistica 10 software was used.

The power of the study to detect an association of the analysed polymorphisms in donor-recipient pairs with kidney transplantation outcomes was estimated using the PS program ver. 3.0.43. The study sample size ( $n=74$ ) was sufficient to detect with 80% probability the true effect size of differences in rs2735940 *hTERT* CX-TT donor-recipient genotype combination frequencies between groups measured as odds ratio (OR) equal to 4.6 for DGF and 5.5 for AR.

## Results

We observed a full linkage disequilibrium between the studied polymorphisms within chromosome 18 (rs2162440 C and T alleles correspond to rs7235755 G and A alleles, respectively) and thus we analyzed rs7235755 polymorphism genotypes only. The frequencies of rs2735940 *hTERT* gene and rs2630578

*BICD1* gene polymorphisms, as well as rs7235755 within chromosome 18 polymorphisms, among the recipients and donors were consistent with the Hardy-Weinberg equilibrium. These results have been described elsewhere [9,12].

Frequencies of combined genotypes in recipients with DGF and without DGF as well as in recipients with AR and without AR are shown in Table 2. Distribution of *hTERT* rs2735940, *BICD1* rs2630578, as well as chromosome 18 rs7235755 polymorphisms genotype pairs did not differ significantly in individuals with DGF in comparison to those without DGF ( $p=0.11$ ,  $p=0.55$ ,  $p=0.23$  respectively). However, in recipients with AR compared to those without AR, the distribution of rs2735940 *hTERT* polymorphism genotype pairs differed significantly ( $p=0.04$ ) and in the case of rs7235755 chromosome 18 polymorphisms genotype pairs distribution, a borderline significant trend was observed ( $p=0.09$ ). Despite these differences, multivariate regression analysis revealed that all studied recipient and donor polymorphism genotypes were not independent risk factors for AR (data not shown). On the other hand, though distribution of analyzed genotype pairs revealed no significant differences with regard to DGF, the multiple logistic regression analysis adjusted for donor's and recipient's age and gender showed that rs2735940 *hTERT* TT donor's genotype was an independent factor decreasing the risk for DGF on the borderline of significance (OR=0.41; 95% CI: 0.16–1.07;  $p=0.06$ ) and that rs2735940 *hTERT* TT recipient's genotype was an independent factor significantly increasing the risk for DGF (OR=2.47; 95% CI: 1.06–5.79;  $p=0.03$ ) (Table 3).

Due to limited study sample size, we combined the genotype pairs to assess the risk for DGF and AR more accurately in renal transplant recipients. Thus, for the rs2735940 *hTERT* gene polymorphism we defined the CX genotype, which contains at least one C allele (i.e., CT or CC). For example, the CX-TT genotype combination means that C-containing donor's genotype (i.e., CT or CC) coexists with TT recipient's genotype as a pair. Distribution of rs2735940 *hTERT* gene combined genotype pairs differed significantly in individuals with DGF, compared to those without DGF ( $p=0.02$ ) and in the case of AR no significant differences were found ( $p=0.16$ ) (Table 4). Multivariate regression analysis including *hTERT* gene polymorphism genotype pairs as independent variable with regard to DGF as an dependent variable revealed that *hTERT* rs2735940 CX-TT donor-recipient genotype combination was an independent risk factor for DGF (OR=4.82; 95% CI: 1.32–18;  $p=0.016$ ) (Table 5).

## Discussion

Taking into consideration our previous studies and reports from other authors, we decided to perform a joint assessment of donor's and recipient's genotype pairs. This approach was

**Table 2.** The distributions of the donors' and recipients' genotypes pairs of the rs2735940 *hTERT*, rs2630578 *BICD1* genes and rs7235755 chromosome 18 polymorphisms in recipients with and without DGF and in recipients with and without AR.

Genotypes donor-recipient	DGF (n=22) without DGF (n=52)					AR (n=14) without AR (n=60)				
	n	%	n	%	p	n	%	n	%	p
rs2735940										
CC-CC	3	33.3	6	66.7		1	11.1	8	88.9	
CC-CT	2	22.2	7	77.8		0	0.0	9	100.0	
CC-TT	2	50.0	2	50.0		2	50.0	2	50.0	
CT-CC	0	0.0	5	100.0		3	60.0	2	40.0	
CT-CT	9	36.0	16	64.0	0.11	3	12.0	22	88.0	0.04
CT-TT	6	60.0	4	40.0		1	10.0	9	90.0	
TT-CC	0	0.0	4	100.0		1	25.0	3	75.0	
TT-CT	0	0.0	5	100.0		1	20.0	4	80.0	
TT-TT	0	0.0	3	100.0		2	66.7	1	33.3	
rs2630578										
GG-GG	11	31.4	24	68.6		4	11.4	31	88.6	
GG-GC	3	21.4	11	78.6		4	28.6	10	71.4	
GG-CC	1	50.0	1	50.0		0	0.0	2	100.0	
GC-GG	3	23.1	10	76.9	0.55	3	23.1	10	76.9	0.42
GC-GC	3	42.9	4	57.1		3	42.9	4	57.1	
CC-GG	0	0.0	2	100.0		0	0.0	2	100.0	
CC-GC	1	100.0	0	0.0		0	0.0	1	100.0	
rs7235755										
GG-GG	5	18.5	22	81.5		5	18.5	22	81.5	
GG-GA	7	50.0	7	50.0		2	14.3	12	85.7	
GG-AA	0	0.0	2	100.0		1	50.0	1	50.0	
GA-GG	4	23.5	13	76.5	0.23	1	5.9	16	94.1	0.09
GA-GA	5	55.6	4	44.4		4	44.4	5	55.6	
AA-GG	0	0.0	1	100.0		1	100.0	0	0.00	
AA-GA	0	0.0	1	100.0		0	0.0	1	100.0	
AA-AA	1	33.3	2	66.7		0	0.0	3	100.0	

p value calculated with Chi-square test for comparison between all donor-recipient genotype combinations. DGF – delayed graft function; AR – acute rejection.

not novel, but studies of donor gene variation are scarcer than those of transplant recipient variation [13,14]. According to Chand et al. this is an unfavorable situation, because such reports are highly informative [7]. Especially given that not only are the drug metabolism genes relevant for donor-recipient association, but also for genes related with aging [13]. The decline of kidney function that is dependent on increase in age

varies among individuals. Thus, accelerated kidney aging resulting from post-transplant stressors may limit allograft survival [13,15]. This study showed that donor-recipient combinations of rs2735940 *hTERT* CX-TT genotypes was associated with almost five times higher odds of DGF, and that rs2735940 *hTERT* and rs2630578 *BICD1*, as well as rs7235755 chromosome 18 polymorphisms combined pairs were not associated with AR.

**Table 3.** Multiple logistic regression analysis including rs2735940 *hTERT* gene polymorphism genotype in donors and recipients as independent variables in regard to DGF as dependent variable.

Independent variables	DGF			
	OR	-95% CI	+95% CI	p
Donor's age [years]	1.02	0.97	1.06	0.48
Recipient's age [years]	1.55	0.47	5.15	0.47
Donor's male gender	0.86	0.25	2.97	0.81
Recipient's male gender	1.01	0.97	1.06	0.54
<i>rs2735940 hTERT</i> TT donor's genotype	<b>0.41</b>	0.16	1.07	<b>0.06</b>
<i>rs2735940 hTERT</i> TT recipient's genotype	<b>2.47</b>	1.06	5.79	<b>0.03</b>

p value calculated with logistic regression analysis; OR – odds ratio; DGF – delayed graft function.

**Table 4.** The distributions of the donors' and recipients' genotypes combined pairs of the rs2735940 *hTERT* in recipients with and without DGF and in recipients with and without AR.

Genotypes donor-recipient	DGF (n=22) without DGF (n=52)				p	AR (n=14) without AR (n=60)				p
	n	%	n	%		n	%	n	%	
<i>rs2735940</i>										
CX-CX*	14	29.2	34	70.8		7	14.6	41	85.4	
CX-TT	8	57.1	6	42.9	<b>0.02</b>	3	21.4	11	78.6	0.16
TT-CX	0	0.0	9	100.0		2	22.2	7	77.8	
TT-TT	0	0.0	3	100.0		2	66.7	1	33.3	

p value calculated with Chi-square test for comparison between 4 donor-recipient genotype combinations. \* CX is a genotype containing C allele (i.e. CC or CT).

**Table 5.** Multiple logistic regression analysis including rs2735940 *hTERT* gene polymorphism genotypes in donor-recipient pairs as independent variable in regard to DGF as dependent variable.

Independent variables	DGF			
	OR	-95% CI	+95% CI	p
Donor's age [years]	1.01	0.97	1.05	0.71
Recipient's age [years]	1.02	0.98	1.07	0.32
Donor's male gender	1.00	0.63	1.60	1.00
Recipient's male gender	1.28	0.43	3.80	0.66
<i>rs2735940 hTERT</i> CX-TT donor-recipient genotype combination*	<b>4.82</b>	1.32	18.0	<b>0.016</b>

p value calculated with logistic regression analysis; OR – odds ratio; DGF – delayed graft function. \* CX is a genotype containing C allele (i.e. CC or CT).

Telomere length is regulated by telomerase activity and significantly correlated with chronological age. However, individual differences in telomere shortening dynamics are so strongly marked that heritability perforce has to be an important factor [16,17]. Thus, variation of telomere length is dependent on several polymorphisms including those analyzed in this study [18,19]. It is only a matter of time before genome wide

association studies (GWAS) will answer the question regarding other genetic *loci* that have significance for telomere length [7]. The functional meaning of rs2735940 *hTERT* gene polymorphism, which is known also as -1327C>T, has already been demonstrated. The T/C transition alters the *hTERT* transcriptional activity and results in change of telomere length [20,21]. Moreover, the T allele and TT genotype have been specifically

linked with longer telomeres. Zhang et al. found that rs2735940 *hTERT* gene polymorphism C allele was associated with 43% lower transcriptional activity of the *hTERT* promoter and decreased telomere length. Additionally, these authors defined the C-C haplotype (C allele of the rs2735940 and C allele of the rs2853669 *hTERT* polymorphisms) as telomerase “loss-of-function” haplotype [22]. However, besides our previous studies, these observations have yet not been transferred to the solid organ transplantation setting.

The occurrence of DGF results from ischemia-reperfusion injury (IRI) manifested as tissue damage and is associated with various immunological and non-immunological factors. It is broadly known that among them, genetic factors are of great importance [9,12,23]. There are also many promising studies focused on linkage between immune response mediators and cell status. High mobility group box 1 protein (HMGB1), which organizes DNA and regulates transcription, is secreted through activated macrophages and monocytes as a cytokine mediator of inflammation [24]. It was shown that xenon treatment attenuates DGF and enhances graft survival through diminished cytoplasmic translocation of HMGB [25]. The process of post-damage immune response is complex and according to the danger theory, it involves not only the innate and adaptive pathways, but also DNA repair mechanisms. Thus, IRI contributes to damage through reactive oxidative species (ROS), triggering the immune response and accelerating aging process [26].

We have demonstrated that rs2735940 *hTERT* gene polymorphism was associated with DGF. Interestingly, the donor's TT genotype was an independent factor decreasing the risk of DGF and the recipient's TT genotype was an independent factor increasing this risk. The possible mechanism underlying these associations is unclear. In the case of the donor, the impact of TT genotype might result from increasing the kidney cells telomerase transcription activity, leading to telomere elongation or limitation of telomere shortening, thus playing a protective role

with regard to DGF development. This is a plausible explanation, because a significant decrease in telomere length and accelerated tissue senescence in primate kidneys undergoing ischemia-reperfusion injury has been reported [27]. Taking into account that rs2735940 *hTERT* C allele is part of the “loss-of-function” haplotype, the protective effect of TT genotype is convincing [22]. However, in the case of the recipient, the pathogenesis of the possible association between the rs2735940 polymorphism and DGF is more complex. This hypothesis is challenging, but we suspect that the recipient's rs2735940 *hTERT* TT genotype is a significant factor for the severity of immune response after transplantation. This genotype is associated with longer telomeres and thus with greater cell vitality, which also includes leukocytes. Lower immune response has been linked with shorter telomeres [28,29]. Moreover, it has been shown that intense cardiorespiratory exercise was sufficient to differentially regulate key telomeric genes and miRNAs (including *TERT*) in white blood cells leading to their function improvement [30]. Intensive immune reaction to ischemia-reperfusion damage results in expansion of injury and higher risk of DGF. Such an explanation is in agreement with our observations, but this hypothesis needs further research.

## Conclusions

Both the donor's and the recipient's rs2735940 *hTERT* gene polymorphism was associated with early graft function after transplantation. The odds of DGF development was almost five times higher for a combination of CX (CT or CC) donor genotype and TT recipient genotype. Joint assessment of donor-recipient genotype pairs provides more information for prediction of early kidney transplantation outcomes.

## Conflict of interests

All authors declare no conflict of interest.

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