

Polymorphisms of genes coding for telomerase reverse transcriptase and telomerase RNA component and the need for target lesion revascularization after percutaneous coronary intervention

Kamil Bujak¹, Mateusz Lejawa^{2,3}, Martyna Fronczek^{2,3},
Andrzej Lekston¹, Mariusz Gąsior¹, Tadeusz Osadnik^{3,4}

¹ 3rd Department of Cardiology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland

² Kardio-Med Silesia, Zabrze, Poland

³ Department of Pharmacology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland

⁴ 2nd Department of Cardiology and Angiology, Silesian Center for Heart Diseases, Zabrze, Poland

Introduction Telomeres are regions located at the end of each chromosome that comprise tandem repeats of the DNA sequence (TTAGGG)_n. Telomeres are shortened during each cell division because DNA polymerase cannot fully replicate the 3' end of the DNA strand. They are necessary for the maintenance of genome stability and integrity.^{1,2} On the other hand, excessive shortening of telomeres resulting from mitoses and / or oxidative stress leads to the cessation of cell divisions in the majority of somatic cells (ie, cellular senescence) or cellular death via apoptosis.³ The average telomere length is mostly genetically determined and decreases with age.⁴ Extensive population studies showed that telomere length is linked with the risk of many age-related diseases, such as coronary artery disease (CAD), Alzheimer's disease, and malignant tumors.¹ Moreover, several single nucleotide polymorphisms (SNPs) affecting the average telomere length have been identified in genome-wide association studies.^{4,5} Some of these polymorphisms are associated with a risk for diseases mentioned above, which may indicate that telomere length is not only an epiphenomenon of other processes but also has a direct impact on the risk for those illnesses.^{1,4}

Significant differences in telomere length are also observed across different tissues.² In some tissues, telomere length can be maintained—despite numerous cell divisions—due to telomerase activity, which can be observed mainly in embryonic stem cells and undifferentiated cells. In most

adult somatic cells, telomerase expression and activity are low and decline further with age.^{1,2} Telomerase is a ribonucleoprotein that synthesizes primarily telomeric DNA. The enzyme consists of 2 main subunits: telomerase reverse transcriptase (TERT), responsible for catalyzing the addition of the TTAGGG sequences to the 3' end of the DNA strand, and telomerase RNA component (TERC), serving as a template for the newly synthesized telomeric DNA.^{2,6}

Despite the potential significance of telomere length and telomerase components in neointimal formation,⁷⁻⁹ polymorphisms in genes coding for TERT (rs2736100) and TERC (rs12696304) have not yet been studied in the context of in-stent restenosis. The study aimed to evaluate the relationship between *TERT* and *TERC* polymorphisms and the clinically-driven target lesion revascularization (TLR) for in-stent restenosis in patients who underwent percutaneous coronary intervention (PCI) with stent implantation for stable CAD.

Methods A total of 657 unrelated White patients, who underwent elective PCI with implantation of at least one bare-metal stent between 2007 and 2012 at our center and completed a 4-year follow-up for TLR were included in the analysis. Target lesion revascularization was defined as PCI of a lesion localized within the previously implanted stent or within 5 mm of the stent, or coronary artery bypass grafting of the target vessel for in-stent restenosis.

Correspondence to:

Kamil Bujak, MD,
Silesian Centre for Heart Diseases,
ul. Skłodowskiej-Curie 9,
41-800 Zabrze, Poland,
phone: +48 32 373 38 60,
email: k.bujak@scchd.pl

Received: November 28, 2020.

Revision accepted: February 5, 2021.

Published online: February 11, 2021.

Pol Arch Intern Med. 2021;

131 (3): 299-301

doi:10.20452/pamw.15810

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The study conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the Silesian Medical Chamber in Katowice, Poland. Written informed consent was obtained from included patients.

We selected 2 common variants of *TERT* and *TERC* (rs2736100 and rs12696304, respectively), which were previously found to be associated with leukocyte telomere length (LTL) in genome-wide association studies.^{4,5} Genomic DNA was extracted from whole blood samples. Genotyping for SNPs of the *TERT* and *TERC* genes was performed by the TaqMan method (Thermo Fisher Scientific, Waltham, Massachusetts, United States; Assay IDs, C___1844009_10 and C___407063_10, respectively; catalog #4351379) on the Cobas z480 Real-Time PCR System (Roche, Basel, Switzerland) following manufacturer's instruction. Positive controls were established through sequencing by the Sanger method. For quality control, genotyping of 10% of samples was randomly repeated, which led to obtaining completely consistent results. The genotypes of the *TERT* and *TERC* polymorphisms were successfully determined for 654 (99.5%) and 656 (99.8%) patients, respectively.

Statistical analysis The χ^2 test was used to determine whether the observed genotype frequencies were consistent with the Hardy–Weinberg equilibrium. In a per-lesion analysis, both univariable and multivariable Cox regression models (adjusted for clinical, angiographic, and procedural covariates) were performed to evaluate the association between the genotypes and TLR (in the dominant, recessive, and codominant models). A *P* value of less than 0.05 was considered significant. Statistical analysis was performed using the R language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria; <http://www.R-project.org/>) as well as Statistica, version 13.3 (TIBCO Software, Palo Alto, California, United States).

Results The study group comprised 657 patients with 781 coronary lesions treated with PCI. The median (interquartile range [IQR]) age of patients was 63 (56–70) years. Among them, 465 (70.8%) were male, 173 (26.3%) had diabetes, 272 (41.4%) had a history of previous PCI, and 64 (9.7%) had previous coronary artery bypass grafting. In 112 patients (17%), there was more than one lesion treated with PCI. The most frequently treated artery was the right coronary artery (295 lesions, 37.8% of all stented lesions). The median (IQR) total stent length was 18 (13–23) mm, and the median (IQR) stent diameter was 3 (2.5–3.5) mm. Of all included individuals, 72 patients (with 79 lesions) underwent clinically-driven TLR for in-stent restenosis during a 4-year follow-up. The observed genotype frequencies were consistent with the Hardy–Weinberg equilibrium (TABLE 1). There were no differences in the TLR rate between the

genotypes of *TERT* and *TERC* polymorphisms (TABLE 1). There was no relationship between *TERT* and *TERC* genotypes and the risk of TLR in univariable Cox regression analysis, even after adjustment for clinical, angiographic, and procedural covariates (TABLE 1).

Discussion Telomerase plays an essential role in regulating gene expression, cell differentiation, proliferation, and apoptosis, all of which seem to be independent of the enzyme's primary function of maintaining telomere length.² All of the above functions of telomerase might be involved in vascular smooth muscle cells (VSMCs) proliferation and extracellular matrix synthesis, that is, main processes contributing to neointimal formation after stent implantation.¹⁰ In vitro studies proved that telomerase activity in VSMCs correlates with cell proliferation, and inhibition of telomerase decreases cellular growth.¹¹ Torella et al⁷ showed in a rat model of angioplasty-induced arterial injury that telomerase activity and neointimal growth in old rats were lower than in adult rats. The inhibition of neointimal formation by everolimus in drug-eluting stents is linked to an antiproliferative effect of this drug on VSMCs. Recent studies demonstrated that this effect results partly from the ability of everolimus to inhibit the TERT-dependent activation of promoters of genes associated with the cell S-phase entry.¹² Telomerase also regulates the expression of other genes potentially linked with the formation of neointima, such as those encoding vascular endothelial growth factor and epidermal growth factor receptor.^{2,10} Endorf et al⁸ found that TERT affects neointimal formation through epigenetic regulation of proliferative gene expression in smooth muscle cells. A study by Armstrong et al⁹ showed that in patients with implanted stents, the average LTL correlates positively with neointimal stent strut coverage assessed by optical coherence tomography 6 months after PCI.

Previous studies found that SNPs of *TERT* (rs2736100) and *TERC* (rs12696304) are associated with both LTL and the risk of many age-related diseases.^{1,4,5} Despite that and the potential role of telomerase in neointima formation, we did not find any association between polymorphisms of genes coding for TERT and TERC and clinically-driven TLR in patients who underwent PCI for stable CAD.

There are several limitations to our study that must be considered. First, our study cohort was limited to the Central European, White patients. Therefore our results should not be generalized to other populations or ethnicities. Second, considering that some of the patients had more than one lesion stented, we could not exclude the confounding effect of common (patient-related) factors on clustered target lesions within a single patient.

In conclusion, this study showed no association between the polymorphisms of *TERT* (rs2736100) and *TERC* (rs12696304) and the risk for TLR after PCI.

TABLE 1 The allelic distribution, target lesion revascularization rate at 4 years, and the results of univariable and multivariable analyses of the relationship between *TERT* and *TERC* genotypes and the risk of target lesion revascularization (in the dominant, recessive, and codominant models)

Genotypes	<i>TERC</i> (rs12696304)				<i>TERT</i> (rs2736100)			
	Homozygous major (CC)	Heterozygous (CG)	Homozygous minor (GG)	<i>P</i> value	Homozygous major (AA)	Heterozygous (AC)	Homozygous minor (CC)	<i>P</i> value
Genotype distribution, n (%)	334 (50.8)	275 (41.9)	47 (7.2)	0.34 ^a	202 (30.7)	309 (47)	143 (21.8)	0.23 ^a
TLR rate, n (%)	41 (12.3)	28 (10.2)	3 (6.4)	0.41	17 (8.4)	36 (11.7)	19 (13.3)	0.32
	Dominant model (CC _{ref} vs CG + GG)				Dominant model (AA _{ref} vs AC + CC)			
HR (95% CI) unadjusted	0.79 (0.51–1.24)				1.46 (0.87–2.44)			
HR (95% CI) adjusted for clinical, angiographic, and procedural variables ^b	0.8 (0.51–1.25)				1.51 (0.9–2.54)			
	Recessive model ([CC + CG] _{ref} vs GG)				Recessive model ([AA + AC] _{ref} vs CC)			
HR (95% CI) unadjusted	0.65 (0.24–1.77)				1.37 (0.83–2.26)			
HR (95% CI) adjusted for clinical, angiographic, and procedural variables ^b	0.65 (0.24–1.8)				1.35 (0.81–2.24)			
	Codominant model 1 (CC _{ref} vs CG)				Codominant model 1 (AA _{ref} vs AC)			
HR (95% CI) unadjusted	0.83 (0.52–1.32)				1.36 (0.79–2.36)			
HR (95% CI) adjusted for clinical, angiographic, and procedural variables ^b	0.83 (0.53–1.33)				1.43 (0.82–2.49)			
	Codominant model 2 (CC _{ref} vs GG)				Codominant model 2 (AA _{ref} vs CC)			
HR (95% CI) unadjusted	0.60 (0.21–1.66)				1.67 (0.9–3.11)			
HR (95% CI) adjusted for clinical, angiographic, and procedural variables ^b	0.61 (0.22–1.69)				1.68 (0.9–3.14)			

a *P* value for the Hardy–Weinberg equilibrium

b Adjusted for diabetes, aorto-ostial lesion, bifurcation lesion, stent underexpansion, edge dissection, and lesion treated with more than one stent

Abbreviations: HR, hazard ratio; *TERC*, telomerase RNA component; *TERT*, telomerase reverse transcriptase; TLR, target lesion revascularization

ARTICLE INFORMATION

ACKNOWLEDGMENTS This work was supported by the National Science Centre, Poland (grant no. 2016/21/N/NZ5/01449; to KB).

CONFLICT OF INTEREST None declared.

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HOW TO CITE Bujak K, Lejawa M, Fronczek M, et al. Polymorphisms of genes coding for telomerase reverse transcriptase and telomerase RNA component and the need for target lesion revascularization after percutaneous coronary intervention. *Pol Arch Intern Med.* 2021; 131: 299–301. doi:10.20452/pamw.15810

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