Clinica Chimica Acta 454 (2016) 62–65

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

# Genetic variants within telomere-associated genes, leukocyte telomere length and the risk of acute coronary syndrome in Czech women

Dana Dlouha [a,*], Jan Pitha [a], Jana Mesanyova [a], Jolana Mrazkova [a], Adela Fellnerova [a], Vladimir Stanek [b], Vera Lanska [c], Jaroslav A. Hubacek [a]

[a] Laboratory for Atherosclerosis Research, Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[b] Department of Cardiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[c] Statistical Unit, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

ARTICLE INFO

*Article history:*
Received 22 July 2015
Received in revised form 28 December 2015
Accepted 31 December 2015
Available online 4 January 2016

*Keywords:*
Leukocyte telomere length
Polymorphism
Acute coronary syndrome
Women
Risk factors

ABSTRACT

The association between leukocyte telomere length (LTL) and cardiovascular disease (CVD) has been published in many reports, although almost exclusively in men. In our study we analysed the association between LTL and five selected variants within three candidate genes (*TERC* rs12696304; *TERF2IP* rs3784929 and rs8053257; *UCP2* rs659366 and rs622064), which are not only involved in telomere-length maintenance but also potentially associated with higher risk of acute coronary syndrome (ACS) in Czech women (505 cases and 642 controls). We detected significantly shorter LTL in women with ACS ($P < 0.001$), but the difference disappeared after multiple adjustments. We did not find any significant associations between analysed variants and LTL, except for rs622064 within the *UCP2* gene, in which case AA homozygotes had a higher LTL ($P < 0.04$). Genotype frequencies of the analysed SNPs did not differ between controls and women with ACS. Variants within *UCP2* (rs622064; CC vs. A allele carriers OR = 1.61; 95% CI: 1.21–2.15, $P < 0.002$) and within *TERF2IP* (rs8053257; A allele carriers vs. GG, OR = 1.78; 95% CI: 1.07–3.18, $P < 0.03$) were associated with increased risk of type 2 diabetes mellitus (T2DM). Analysed polymorphisms were not major determinants of telomere length or ACS risk in Czech females.



## 1. Introduction

Acute coronary syndrome is one of the major causes of mortality in females in industrially developed countries. Despite years of research and the fact that major risk factors were defined decades ago, we are still not able to predict future cases in time or with reasonable accuracy to allow for focused individual intervention.

Genetic markers (not only common polymorphisms or rare mutations) and epigenetic markers, such as microRNA, DNA methylation and telomere length could potentially assist in predicting the risk of future disease.

Telomeres in eukaryotic cells are located at the end of nuclear chromosomes and are involved in maintaining the integrity and stability of the genome during replication. Telomere attrition is probably involved in vascular and endothelial cell senescence and apoptosis and may contribute to atherogenesis [1]. Initial telomere length at birth is widely variable and is determined by both genetic and environmental factors [2,3]. Gender differences in LTL and onset of cardiovascular disease (CVD) have previously been detected. In general, women have a longer LTL and they have a lower risk of CVD and atherosclerosis development during the pre-menopausal period [4–7]. The main risk factors for CVD in women generally include: age, BMI, hypertension, diabetes, cigarette smoking, dyslipidaemia, family history and physical inactivity. A number of studies have focused on the association between LTL and the risk of CVD, suggesting that short LTL is associated with hypertension, type 2 diabetes mellitus, carotid atherosclerosis and stroke [8–13]. *Vice versa*, CVD risk factors, such as cigarette smoking, obesity and inflammation could affect telomere shortening [14,15].

Single nucleotide polymorphisms in telomere-associated pathway genes could alter chromosomal integrity by influencing gene expression and inducing structural aberrations of telomere maintenance proteins. This alteration could lead to telomere dysfunction and shortening [16]. Mutations in the *TERC* gene (OMIM ID 602322) result in the reduction of telomerase activity leading to premature telomere shortening [17]. An association between LTL and *TERC* SNPs has been detected [18,19]

*Abbreviations:* CVD, cardiovascular diseases; BMI, Body Mass Index; 3PMFs, the Prague Pre- and Post-Menopausal Females study; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; SCG, single-copy gene; T/S, telomere repeats/single-copy gene copy ratio; LTL, leukocyte telomere length; ACS, acute coronary syndrome; T2DM, type 2 diabetes mellitus; SNPs, single nucleotide polymorphisms; OMIM, Online Mendelian Inheritance in Man; LDL, low density lipoprotein; HDL, high density lipoprotein; SD, standard deviation; CRP, C-reactive protein; IHD, ischaemic heart disease; AP, angina pectoris; MI, myocardial infarction; CV, cardiovascular; STEMI, ST elevation myocardial infarction; non-STEMI, Non-ST elevation myocardial infarction; WHO, World Health Organisation; MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease.

* Corresponding author at: IKEM-CEM-LMG, Videnska 1958/9, 140 21 Prague 4, Czech Republic.
*E-mail address:* dadl@ikem.cz (D. Dlouha).

http://dx.doi.org/10.1016/j.cca.2015.12.041
0009-8981/

as well as an association between *TERC* variants and longevity in humans [20]. Two SNPs (rs3784929; rs8053257) within the *TERF2IP* locus (OMIM ID 605061) have been reported to be associated with greater risk of ischaemic stroke in obese women [16,21]. In addition, common variants (rs659366, rs622064) within the *UCP2* gene (OMIM ID 601693) have been shown to be associated with T2DM, ischaemic stroke and LTL in European Caucasians [22–25].

The aim of our study was to analyse whether LTL could reflect the risk of acute coronary syndrome in Czech women and to evaluate the potential association between LTL and five selected gene variants potentially connected with telomere-length maintenance (*TERC* C>G rs12696304; *TERF2IP* A>G rs3784929 and G>A rs8053257; *UCP2* G>A rs659366 and C>A rs622064). Another purpose of our study was to determine the link between analysed variants and the main risk factors for CVD.

## 2. Materials and methods

### 2.1. Subjects

Our case group comprised of 505 women with acute coronary syndrome (ACS; mean age 61 ± 9.3 years) hospitalised in the Institute for Clinical and Experimental Medicine for ACS (STEMI and non-STEMI acute myocardial infarction, minimal myocardial lesion and unstable angina pectoris) between the years 2006 and 2014 [26–28]. During 2–7 years of follow up (FU), 89 patients died. The control group consisted of 642 women from the Prague Pre- and Post-Menopausal Females study (3PMFs). This group was recruited from a 5% representative random sample of the population, consisting of 29 440 women aged between 45 and 54 years living in Prague and selected from the registers of health insurance companies. From a random sample of 1472 women, 908 women gave their informed consent to participate in the study and were primarily examined between 2003 and 2005 [6,29]. After excluding women who did not satisfy the reliable criteria for definition of reproductive status and those who provided incomplete data, 642 subjects were included. Out of all the examined women in our study, 305 were healthy, 518 suffered from CVD and 324 belonged to a heterogeneous group of subjects suffering from different diseases, such as diabetes, kidney or liver disease, hypo- or hyper-function of the thyroid gland and tumours. The protocol of this study was carried out according to the principles of the Declaration of Helsinki. All examined individuals were of Caucasian ethnicity and all signed informed consent forms, which, together with the protocol of the study, were approved by the institute's Ethics Committee.

Risk factors for CVD were defined as follows: (i) self-reported current smoking; (ii) dyslipidaemia as plasma total cholesterol over 5.2 mmol/L, and/or triglycerides over 2 mmol/L or self-reported lipid-lowering treatment; (iii) body mass index (BMI) equal to, or higher than, 30 kg/m$^2$; (iv) hypertension (self-reported antihypertensive treatment or seated systolic/diastolic blood pressure higher than 139/89 mm Hg); (v) diabetes as self-reported diabetes and/or fasting glucose over 7 mmol/L and/or antidiabetic medication; (vi) age ≥55 years; (vii) family history of ACS or stroke and finally (viii) CRP levels greater than 10 mg/L.

### 2.2. DNA extraction and SNPs analyses

Genomic DNA was extracted from EDTA blood using a standard method [30]. Genetic variants within *TERC* (C>G, rs12696304, ID assay C_407063_ 10), *TERF2IP* (A>G, rs3784929, ID assay C_25800757_10; G>A, rs8053257, ID assay C_31423806_10) and *UCP2* (G>A, rs659366, ID assay C_8760350_10; C>A, rs622064, ID assay C_8760411_10) were analysed using TaqMan technology (quantitative PCR-based method) on an AB 7300 RT PCR instrument.

To ensure the accuracy of genotyping, one plate (containing 94 DNA samples) was analysed twice within one week with 100% conformity. The call rate for genotyping was 99–100% for all SNPs.

Lipoprotein parameters were measured by the WHO Regional Lipid Reference Centre, IKEM, Prague on a Roche COBAS MIRA autoanalyser using reagents from Boehringer Mannheim Diagnostics (Mannheim, Germany) and Hoffmann-La Roche (Basel, Switzerland). Body height and weight were measured according to the standardised WHO MONICA Project protocol.

### 2.3. Measurement of telomere length

Telomere length was analysed as described previously [31,32]. Analysis was performed using a quantitative polymerase chain reaction (qPCR)-based method with slight modifications [33]. Relative telomere length was calculated as the ratio of telomere repeats to a single-copy gene (SCG) (T/S ratio). All qPCRs were performed in triplicate on a Rotor-Gene 3000 (Corbett Research Ltd). In order to examine the measurement stability of telomere length by qPCR analysis, both intra-assay (1.9–6.9%) and inter-assay reproducibility were evaluated (3.4–14.8%).

### 2.4. Statistical analysis

Analyses were performed using JMP 10 statistical software. Data are presented as percentages for categorical variables and means ± standard deviation (SD) for continuous variables, unless otherwise indicated. The Hardy–Weinberg test was used to confirm independent segregation of the alleles of individual genotypes (www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls). Comparisons between the two groups were performed using the Student's t-test. Non-parametric analysis (Wilcoxon/Kruskal–Wallis tests) was performed where appropriate. We used Cox proportional hazards models (adjusted for age) to examine the association between survivors and LTL. P values less than 0.05 were considered to be significant.

## 3. Results

Baseline characteristics of the study cohort are described in Table 1. In comparison to the control group, women with ACS were older and exhibited higher prevalence for T2DM and hypertension. Higher BMI and increased levels of biochemical parameters, such as CRP and serum glucose, were detected in the group of patients.

LTL was inversely associated with age ($P < 0.001$). Higher BMI and C-reactive protein levels correlated with shorter LTL ($P < 0.04$; resp. $P < 0.03$); for more details, see Table 2. Other contributors to CVD risk, such as dyslipidaemia, diabetes, hypertension and smoking status,

**Table 1**
Baseline characteristics of study participants divided into groups of women with ACS and healthy women — controls from 3PMFs. Values are given as means ± SD, numbers (%). Current and occasional smokers are included according to smoking prevalence. Telomere length is expressed as a relative telomere/single copy gene (T/S) ratio. P values were obtained using the two-sample Student's t-test for comparison of continuous variables, the $\chi^2$ test for categorical variables and the Mann–Whitney *U* test for the relative T/S ratio.

| Characteristics | Controls | ACS | P value |
|---|---|---|---|
| N | 642 | 505 | |
| Age (years) | 50 ± 2.7 | 61 ± 9.3 | <0.0001 |
| Smoking prevalence (N/%) | 211/33 | 167/35 | ns |
| Diabetes mellitus (N/%) | 89/14 | 118/23 | <0.0001 |
| Hypertension (N/%) | 178/28 | 287/57 | <0.0001 |
| BMI (kg/m$^2$) | 25.9 ± 4.8 | 28.7 ± 5.6 | <0.0001 |
| Plasma lipids | | | |
| Total Cholesterol (mmol/L) | 5.59 ± 0.95 | 5.24 ± 1.27 | <0.0001 |
| HDL (mmol/L) | 1.64 ± 0.40 | 1.26 ± 0.36 | <0.0001 |
| LDL (mmol/L) | 3.42 ± 0.86 | 3.36 ± 1.17 | ns |
| Ln Triglycerides (mmol/L) | 0.19 ± 0.46 | 0.26 ± 0.57 | ns |
| Serum glucose (mmol/L) | 5.17 ± 0.84 | 8.52 ± 4.17 | <0.0001 |
| Ln CRP (mg/L) | 0.03 ± 1.08 | 1.93 ± 1.15 | <0.0001 |
| Unadjusted LTL | 0.93 ± 0.38 | 0.86 ± 0.32 | <0.0003 |