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hTERT $^{-1327}$T/C polymorphism is not associated with age-related telomere attrition in peripheral blood

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Abstract

Regulation of the telomerase catalytic subunit, hTERT, is a complex process accomplished on many levels. Transcription of the *hTERT* gene has been widely studied but less is known about the implication of genetic variations. Recently, a functional T to C transition polymorphism was indicated 1327 bp upstream the hTERT transcription starting site. The $^{-1327}$C/C genotype was associated with shorter telomere length compared to the alternative genotypes in healthy individuals and in coronary artery disease patients. We tested this observation and analysed telomere length and the $^{-1327}$T/C polymorphism in 226 myocardial infarction patients and 444 controls from southern Sweden. No significant difference in telomere length was found among the genotypes after age adjustments in the control group ($p = 0.794$) or in the MI group ($p = 0.339$). Moreover, no increased age-related attrition was observed for the $^{-1327}$C/C genotype as previously indicated, rather a telomere elongation in the control group ($p = 0.021$) not seen in the MI group ($p = 0.249$).

Keywords: Telomere length; hTERT; Polymorphism; Age; Myocardial infarction

The protection of our genome is to an essential part executed by telomeres, special structures consisting of 5–10 kb TTAGGG repeats at the chromosome ends. Without the telomeres, chromosomes would degrade and form end to end fusions [1]. Due to limitations of lagging strand synthesis telomeres abbreviate during the lifespan of a normal cell [2]. Numerous studies have shown that telomeres in human peripheral blood cells shorten with age [3–5] but with great interindividual variation, most likely regulated by hereditary and environmental factors. Telomeres are believed to have functional implications for the aging process since critically short telomeres induce irreversible replicative senescence [6].

Telomere length is partly inherited [4,7] but the mechanism is not elucidated and both an X-linked and a paternally linked inheritance have been observed [8,9]. Paternal age is positively associated to telomere length of the children, supporting a paternal inheritance [10]. Indications that various disorders such as cardiovascular diseases [11], depression [12], psychological stress [13], diabetes [14,15] and Alzheimer's disease [16] are associated with shorter telomeres compared to controls have been presented. Both coronary artery disease (CAD) and myocardial infarction (MI) patients seem to have abbreviated telomeres [17–20] but contradictory results have been reported [21,22]. The ongoing inflammatory process with enhanced cell replication in atherosclerosis is one plausible explanation to the reduced telomere length found in CAD patients [23]. Interestingly, genetically modified mice with short telomeres and apolipoprotein E (Apo-E)- deficiency were protected from diet induced atherosclerosis indicating that telomere exhaustion rather could restrict atheroma progression by senescence induction. Oxidative stress has implications for arteriosclerosis [24] and is a causative candidate for altering telomere length. Experiments on cell cultures have also shown that oxidative stress can induce enhanced telomere attrition and premature senescence [25].

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Telomerase has reverse transcriptase activity and ability to prolong telomeres, and its main components are the catalytic subunit (hTERT) and the RNA template (hTR). The enzyme is active during embryonic development but silenced in most somatic cells after birth [26]. Many malignant tumors show telomerase activity and thereby telomere lengthening capacity [27]. hTERT mRNA expression seems to be most important for telomerase activity, but also alternative splicing, posttranslational alterations and hTERT localization in the cell contribute [28]. Less is known about the impact of hereditary hTERT gene variations. Matsubara et al. [29] screened the promoter region of hTERT for functional polymorphisms in a Japanese healthy population, and a frequent T to C transition was found 1327 bp upstream the transcription starting site ($^{-1327}$T/C). Individuals homozygous for the $^{-1327}$C/C genotype showed shorter telomere length in their peripheral leucocytes compared to the $^{-1327}$T/T and $^{-1327}$T/C genotypes. Moreover, the only age-related attrition was found for the $^{-1327}$C/C genotype [30]. There was an overrepresentation of the $^{-1327}$C/C genotype in patients with CAD disease compared to controls and they had shorter telomeres compared to other CAD patients with alternative genotypes, indicating that a subgroup of CAD patients is more prone to telomere shortening [30].

In the present study, we tested the $^{-1327}$T/C hTERT polymorphism in relation to telomere length in patients with myocardial infarction and controls from southern Sweden. In these cohorts no difference in telomere length was found for the various hTERT genotypes.

Materials and methods

Study population. The study material was part of the Malmö cancer diet study and consisted of 226 cases of myocardial infarction (MI) (155 men and 71 women) and 444 controls (306 men and 138 women). The age span of both the controls and MI patients was 48–68 years (mean 61). Blood was drawn and DNA extracted from mononuclear cell preparations. The present study was approved by the Ethical committee in Malmö (# LU 51–90).

SNP analyses. DNA was extracted from frozen granulocyte samples using QIAamp-96 spin blood kits (QIAGEN, VWR Sweden). DNA concentration was determined by fluorescent measurement (FLOUstar Optima plate reader, BMG LABTECH GmbH) using the PicoGreen dsDNA Quantification Kit (Molecular Probes), and normalized working solutions, 2.5 ng DNA/μL, were prepared.

C_1839086_10, a TaqMan SNP genotyping assay was bought from Applied Biosystems (Foster City, CA, USA). Assay and PCR conditions were according to the included protocol except that PCR was run on 10 ng DNA in a 10 μl reaction volume. The reaction was performed on a GeneAmp 9700 system (Applied Biosystems), PCR-plates were read (allelic discrimination) on the ABI Prism 7900 HT instrument and genotypes were called with the SDS v2.1 software (Applied Biosystems). 7% of the samples were rerun to check for genotype call accuracy, showing 100% consistency.

Telomere length by real time PCR. Telomere length was investigated using real time PCR as described earlier [9,31] but with the following changes. Telomere and β-globin primer sequence written 5′–3′ and final concentration were: CGGTTTGTTTGGGTTTGGGTTTGGGTTTGG GTTTGGGTT, 100 nM (Tel 1b), GGCTTGCCTTACCCTTACCCTT ACCCTTACCCTTACCCT, 900 nM (Tel 2b), TGTGCTGGCCCATC ACTTTG, 400 nM (HBG3), ACCAGCCACCACTTTCTGATAGG, 400 nM (HBG4).

DNA from subjects and the reference cell line CCRF-CEM was diluted to 1.75 ng/μl in a TE buffer containing *Escherichia coli* DNA (Sigma–Aldrich). Final concentrations of both the telomere and β2-globin PCR mix was 50 mM KCl, 10 mM Tris–HCl (pH 8.0), 0.2 mM of each dNTP, 150 nM Rox (Molecular Probes), 0.2× Sybr Green (Roche Diagnostics GmBH), 1% DMSO and 1.25 U AmpliTaq Gold DNA Polymerase (Applied Biosystems). Final concentrations specific for the telomere and β2-globin mix was 1.7 mM MgCl, 2.5 mM DTT and 2 mM MgCl, 5 mM DTT, respectively. Cycling conditions for the telomere amplification comprised an initial denaturation step at 95 degrees for 10 min and 25 cycles at 95 degrees for 15 s and 54 degrees for 1 min. The same denaturation step initialised the β2-globin amplification but was followed by 35 cycles at 95 degrees for 15 s and 56 degrees for 1 min. Telomere/single copy gene (T/S) values were calculated by $2^{-\Delta Ct}$ and relative T/S values were determined by dividing sample T/S values with the T/S value of reference DNA.

Statistical methods. SPSS version 13.0 was used for all statistical calculations. Chi square test and ANCOVA adjusting for age was performed to compare the genotype groups and calculation of confidence intervals (CI). Age-related attrition was calculated using Spearman rank correlation coefficient.

Results and discussion

The genotype distribution among healthy individuals were: $^{-1327}$T/C $n = 235$ (52.9%), $^{-1327}$T/T $n = 101$

Table 1
$^{-1327}$hTERT genotype distribution

	Controls (%)	MI patients (%)	*p*-value
T/C	52.9	47.8	0.208
T/T	22.7	27.4	0.181
C/C	24.3	24.8	0.897

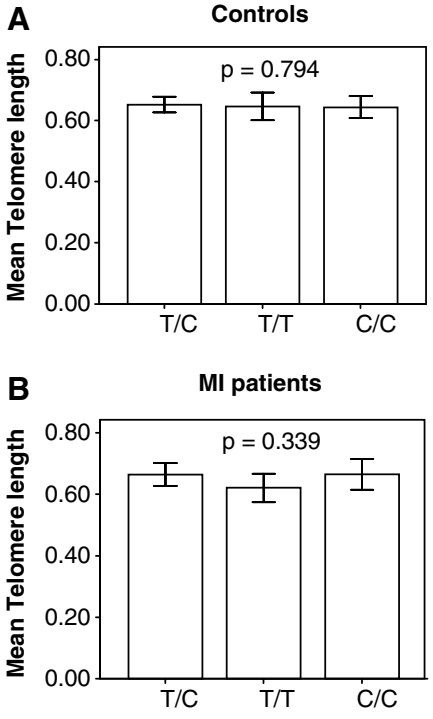

Fig. 1. Mean relative telomere length and hTERT $^{-1327}$T/C polymorphism group after age adjustments. (A) Controls; (B) MI Patients.

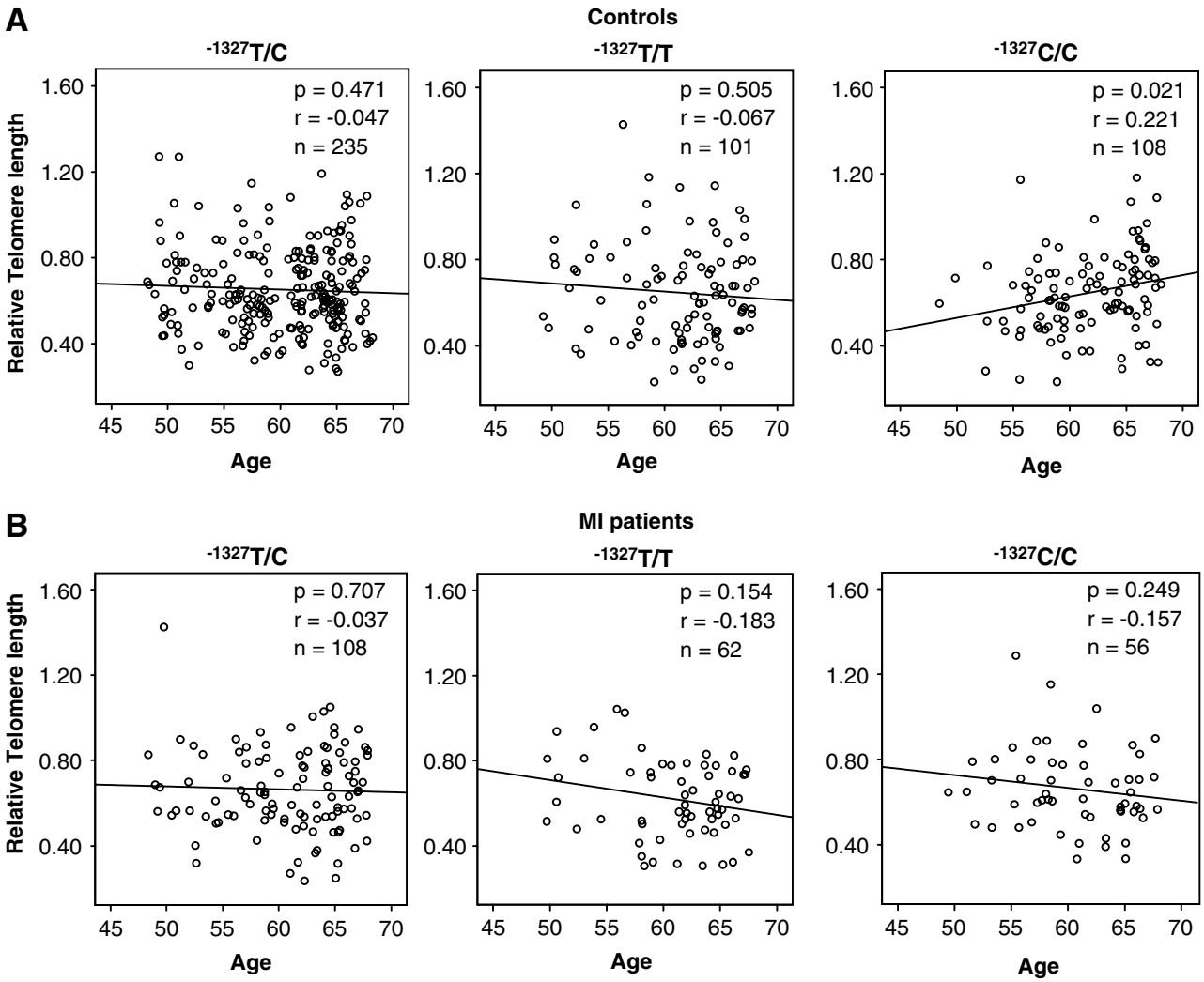

Fig. 2. Age-related telomere attrition. (A) Controls; (B) MI patients.

(22.7%) $^{-1327}$C/C $n = 108$ (24.3%) and this distribution did not differ from the MI patients: $^{-1327}$T/C $n = 108$ (47.8%), $^{-1327}$T/T $n = 62$ (27.4%) and $^{-1327}$C/C $n = 56$ (24.8%) ($p = 0.208$, $p = 0.181$ and $p = 0.897$, respectively). Thus, the $^{-1327}$C/C genotype previously found to be overrepresented in CAD patients (30), showed no difference in frequency between our MI patients and controls. A summary of genotype frequencies are given in Table 1.

When evaluating mean telomere length in controls we could not observe any significant difference among the genotypes after age adjustments ($p = 0.794$) (Fig. 1A). This was also true for MI patients ($p = 0.339$) (Fig. 1B). After merging the $^{-1327}$T/C and $^{-1327}$T/T genotypes and in comparison to the $^{-1327}$C/C group still no differences could be detected after age adjustments ($p = 0.551$) (not shown in figure).

The telomere loss per year in control samples was nonsignificant in both the $^{-1327}$T/C and the $^{-1327}$T/T group ($r = -0.067$, $p = 0.505$ and $r = -0.047$, $p = 0.471$, respectively). The $^{-1327}$C/C genotype actually showed an unexpected increase in telomere length with age ($r = 0.221$, $p = 0.021$) in the controls. No age-related changes in telomere length was observed for MI patients in any genotype group (Fig. 2A and B).

Thus, neither regarding $^{-1327}$hTERT genotype distribution nor telomere length in the various genotype groups we could demonstrate any differences between controls or MI patients, in contrast to previously published data on Japanese individuals [29,30]. Obviously, different ethnic groups (Japanese and Swedish) were analyzed and it was recently shown that gene expression patterns can differ depending on ethnicity, which partly can explain variation in disease incidence in diverse ethnic groups [32]. It can not be ruled out that the various $^{-1327}$hTERT genotypes cosegregate with a gene (or genes) with effects on telomere length regulation and which has ethnicity group coupled activity.

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