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The functional VNTR MNS16A of the *TERT* gene is associated with human longevity in a population of Central Italy

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ABSTRACT

Background: Telomerase, encoded by *TERT*, is the ribonucleoprotein polymerase that maintains telomere ends and it plays a crucial role in cellular senescence. *TERT* single nucleotide polymorphisms (SNPs) have been associated both with various malignancies and telomere length (TL). The association of *TERT* SNPs with longevity remains uncertain and varies with ethnicity. The aim of this study was to investigate whether the functional variable number of tandem repeat (VNTR) MNS16A of *TERT* is associated with longevity.
Methods: MNS16A genotypes have been determined for 1072 unrelated healthy individuals from Central Italy (18–106 years old) divided into three gender-specific age classes defined according to demographic information and accounting for the different survivals between sexes: for men (women), the first class consists of individuals <66 years old (<73 years old), the second class of individuals 66–88 years old (73–91 years old), and the third class of individuals >88 years old (>91 years old). TL was assessed using genomic DNA from whole blood of 72 selected individuals by a multiplex real-time PCR assay.
Results: MNS16A appears associated to longevity, showing significant associations in Comparison 2 (Age Class 3 vs. Age Class 2) under both additive (odds ratio [O.R.] 0.749; $p = 0.019$) and dominant (O.R. 0.579; $p = 0.011$) models. The MNS16A*L allele is significantly underrepresented in Age Class 3 (O.R. 0.759; $p = 0.020$) compared to Age Class 2. A significant telomere attrition is reported along the three age classes ($p = 0.0001$), that remains significant only in L*/L* genotype carriers ($p = 0.002$) when the analysis was conducted according to MNS16A genotype.
Conclusions: The *TERT* MNS16A*L allele appears negatively associated with longevity. The concomitant significant telomere cross sectional attrition rate observed for L*/L* genotype suggests that this polymorphism could influence human longevity by affecting TL.

1. Introduction

Human longevity is a complex phenotype where environmental, genetic, epigenetic and stochastic factors are involved. Twin studies suggest that approximately 25% of the variation in adult life-span is attributable to inter-individual genetic differences (Herskind et al., 1996; Ljungquist et al., 1998). Many common gene variants have been associated with longevity, but only few of them have been consistently replicated (Novelli et al., 2008). Genes involved in cellular maintenance pathways and repair mechanisms have been widely studied in human longevity since aging (among other things) is due to a progressive accumulation of molecular damage and an attenuation of the cellular defense mechanisms.

Telomeres are specialized DNA structures, consisting of TTAGGG repeats, located at the terminal ends of chromosomes that progressively shorten every mitotic division, playing a critical role in maintaining the genome integrity (Blackburn, 2010; Chan and Blackburn, 2004). Human telomerase is the ribonucleoprotein polymerase that maintains telomere ends and consists of two molecules each of human telomerase reverse transcriptase (TERT), telomerase RNA (TR or TERC), and dyskerin (DKC1) (Cohen et al., 2007). Cells lacking telomerase inevitably undergo senescence. Telomere shortening is associated with organismal aging, and leukocyte telomere length (LTL) is inversely related to age (Lindsey et al., 1991; Slagboom et al., 1994). Indeed, increased telomere length (TL) shortening has been associated to several clinical conditions and to multiple aging-associated diseases (Armanios and

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Blackburn, 2012; Epel et al., 2004; Willeit et al., 2011). Moreover, several SNPs in *TERT* and *TERC* genes were associated to LTL (Codd et al., 2010; Levy et al., 2010; Melin et al., 2012; Shen et al., 2011). The collective evidence clearly suggests the telomere maintenance genes (*TERT* and *TERC*) as candidate genes for human longevity.

To date, two association studies have been conducted in Ashkenazi and Danish populations, with conflicting results. First, Atzmon et al. (2010), using a cohort of Ashkenazi Jewish centenarians, their offspring, and offspring-matched controls, found an overrepresentation of synonymous and intronic mutations in *TERT* and *TERC* genes among centenarians compared to controls. Moreover, they reported a significant association of a common *TERT* haplotype (defined by rs2853669, rs2736098, rs33954691, and rs2853691 SNPs) with both exceptional longevity and longer telomere length. In the second study, Soerensen et al. (2012) investigated two *TERC* (rs3772190 and rs12696304) and four *TERT* (the same defining the haplotype associated with longevity by Atzmon et al. (2010)) SNPs in middle-aged, old, and oldest-old Danes (58–100 years) and their association with LTL and longevity. They failed to replicate the association of *TERT* SNPs reported by Atzmon et al. (2010), but they found the association of *TERC* SNPs with both LTL and longevity. They also investigated 11 tag-SNPs, covering the common variation in *TERT* in Caucasians, on 1089 members of the "1905 cohort" and 736 middle-aged controls, finding no significant associations (Soerensen et al., 2012).

In the present study, we attempt to add knowledge on the role of the *TERT* gene in longevity by analyzing the functional variable number of tandem repeat (VNTR) MNS16A (Wang et al., 2003). The VNTR MNS16A is located down-stream of exon 16 of the *TERT* gene and up-stream the putative promoter region of an antisense *TERT* transcript (Hofer et al., 2011; Wang et al., 2003). MNS16A was found to have two repeat elements forming a 23 bp core sequence or a 26 bp core sequence with a CAT insertion representing a transcription factor binding site for GATA-1. To date, six different VNTR alleles have been described. The two most common alleles are the VNTR-302 and VNTR-243, followed by rare VNTR-333 and VNTR-272. Recently, two very rare alleles, namely VNTR-364 and VNTR-213, were found in just one subject from Austria (Hofer et al., 2011) and one subject from Taiwan (Chang et al., 2011), respectively. Promoter activity was demonstrated to depend on the number of tandem repeats implicating functionality of MNS16A genotype (Wang et al., 2003). It was proposed that this minisatellite might act as a repressor for the promoter of the *TERT* antisense transcript (Wang et al., 2003).

Herein, we tested the association of VNTR MNS16A of *TERT* with human longevity by performing a cross-sectional study on a population from Central Italy divided into three gender-specific age classes defined according to demographic information and accounting for the different survivals between sexes. We also determined LTL in a selected group of subjects (age-, sex- and genotype- matched) to assess the association of VNTR MNS16A with telomere length.

2. Material and methods

2.1. Demographics

Peripheral blood was obtained from 1072 (569 females and 503 males) unrelated individuals, 18–106 years old, recruited from the same geographical area of Central Italy (Marche region) on the eastern side of the Apennines Mountains. The whole population studied is composed of Caucasian individuals. The same donors provided information concerning their health condition so as to ascertain that no pathological condition existed (e.g. cancer, diabetes, heart diseases, hypertension, obesity, and chronic inflammatory diseases). Subject's age was self-reported and was corroborated with official documentation. Almost all subjects were recruited in blood donor centers and through family physicians. The population under study was divided into three sex-specific age classes: for men [women], the first class consists of individuals <66 years old [<73 years old], the second class of individuals 66–88 years old [73–91 years old], and the third class of individuals >88 years old [>91 years old]. These gender-specific age classes were defined according to demographic information and accounting for the different survival rates of men and women in the Italian population (Passarino et al., 2006). Briefly, the age classes ranging from 18 to 106 years were obtained on the basis of a synthetic survival curve constructed using historic mortality data taken from the Italian population from 1890 onward (computed using death counts per calendar year and per year of birth as reported for the Italian population in the Human Mortality Database, available online at www.mortality.org) (Passarino et al., 2006). The study protocol was approved by the Joint Ethical Committee (JEC) University of Camerino-Azienda ASUR Marche ZT-10 Camerino, in accordance with the Declaration of Helsinki in its revised edition and with international and local regulatory requirements.

2.2. TERT MNS16A genotyping

Genomic DNA extraction was carried out from peripheral blood through standardized salting-out method and DNA was stored at −20 °C until gene analysis.

TERT VNTR MNS16A was genotyped according to the allele-specific PCR method as previously reported (Wang et al., 2003). PCR was performed using PCR Master Mix (2×) (Fermentas), with a primer set, 5′-AGGATTCTGATCTCTGAAGGGTG-3′ and 5′-TCTGCCTGAGGAAGGAC GTATG-3′ (Sigma-Aldrich, MO, USA). The amplification procedure consisted of an initial denaturing step for 5 min at 95 °C followed by 35 cycles for 30 s at 95 °C, 45 s at 60 °C, and 1 min at 72 °C, as well as a final extension step for 10 min at 72 °C. Each sample was genotyped in duplicate. The PCR products were visualized on a 2% agarose gel containing 0.25 mg/ml of ethidium bromide and genotypes were assigned as previously reported (Wang et al., 2003): the 243 bp and 272 bp bands were classified as the short (S*) allele, and the 333 bp and 302 bp bands were classified as the long (L*) allele, thus defining the MNS16A genotypes as L*/L*, S*/L* and S*/S*.

2.3. LTL measurement

A total of 72 samples were selected from our population to check a possible genotype-dependent telomere length variability along the three age classes studied. Eight samples (4 males and 4 females) were chosen for each genotype (L*/L*, S*/L*, S*/S*) from each age class. The total amount of telomeric DNA was measured by monochrome multiplex Real-Time Quantitative PCR, by measuring for each sample the relative amount of telomeric DNA (T) as compared to the amount of a single copy gene DNA (S) in the same sample (T/S ratio) (Cawthon, 2009). This assay allows the measurement of both telomere and single copy gene (albumin) signals in the same well by using 2 specifically designed couples of primers: telg (5′-ACACTAAGGTTTGGGTTTGGGTTT GGGTTTGGGTTAGTGT-3′), telc (5′-TGTTAGGTATCCCTATCCCTATCCCTA TCCCTATCCCTAACA-3′), albugcr2 (5′-CGGCGGCGGGCGGCGCGGGCTG GGCGGCCATGCTTTTCAGCTCTGCAAGTC-3′), and albdgcr2 (5′-GCCCGG CCCGCCGCGCCCGTCCCGCCGAGCATTAAGCTCTTTGGCAACGTAGGTTTC-3′). PCR reactions were conducted on a BioRad MyiQ single color thermal cycler using iQ5 2.0 Standard Edition Optical System Software to collect raw data. A total 25 μl of PCR reaction mix containing 20 ng genomic DNA, SYBR Green JumpStart Taq ReadyMix 2× (Sigma), 1 M betaine, 1 mM DTT, 100 nM telg, 900 nM telc, 700 nM albugcr2, and 500 nM albdgcr2 was prepared for each sample. Each sample as well as calibrator sample and standard curve points were run in triplicate. During the thermal cycle (95 °C for 15 min; 34 cycles at 98 °C for 2 s, 48 °C for 60 s, 74 °C for 15 s with signal acquisition, 84 °C for 20 s, 85 °C for 15 s with signal acquisition; melting curve step) telomere amplification signal is collected at 74 °C in the early cycles when albumin signal is under baseline, while acquisition at 85 °C provided the albumin Ct values. To check for PCR reaction specificity, a melting analysis

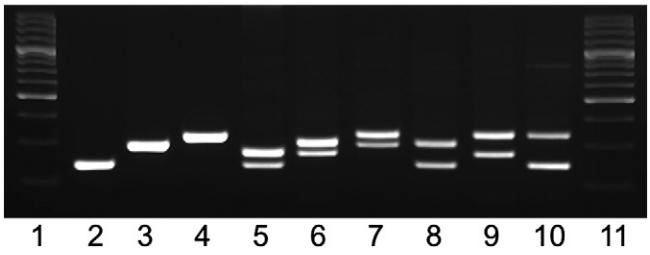

Fig. 1. Representative electrophoresis gel displaying the nine MNS16A genotypes found in our population. Lanes 1 and 11: 100 bp ladders (Fermentas, 100 bp plus DNA ladder 100–3000 bp), lane 2: 243/243 (S*/S*); lane 3: 302/302 (L*/L*); lane 4: 333/333 (L*/L*); lane 5: 243/272 (S*/S*); lane 6: 272/302 (S*/L*); lane 7: 302/333 (L*/L*); lane 8: 243/302 (S*/L*); lane 9: 272/333 (S*/L*); lane 10: 243/333 (S*/L*).

step was included at the end of each PCR run: no non-specific PCR product was detected. In our experimental conditions the standard curves' correlation coefficients were all $R^2 > 0.99$.

2.4. Statistical analysis

Allele and genotype frequencies were calculated by genotype counting method. Hardy–Weinberg Equilibrium (HWE) was assessed by comparing the genotype frequencies with the expected values using a contingency table χ^2 statistics. Genotype association was tested under additive, dominant and recessive models. Cochran–Armitage test of trend was employed for the additive model. Pearson's χ^2 or Fisher's exact test (when expected is <5) was used to evaluate dominant model (L*/L* + L*/S* vs. S*/S*), recessive model (L*/L* vs. L*/S* + S*/S*), and allelic association. The strength of association was expressed as odds ratios (O.R.s) with 95% confidence intervals. For LTL measures, the normality of distribution was assessed by Kolmogorov–Smirnov test, while the differences between study groups were analyzed using one-way ANOVA. Data are expressed as mean ± S.D. Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). Two-tailed probability values of less than 0.05 were regarded as statistically significant. Bonferroni's correction for multiple testing was not performed, since we are assessing specific questions on a gene previously tested for association with human longevity, and we are not searching for associations without a priori hypotheses (Pernerger, 1998).

3. Results

3.1. MNS16A genotype and allele distributions

The allele and genotype frequency distributions of the MNS16A were compared among the age classes, males and females together because no significant differences were noticed between the sexes (data not shown). No significant deviation from HWE expectation was detected among age classes (Supplementary Table 1). The two very rare alleles VNTR-364 and VNTR-213 were not present in our population. In Fig. 1 we report the electrophoretic patterns of the nine genotypes found in the present study. In accordance with a previous report (Wang et al., 2003), the 243 bp and 272 bp bands were classified as the short (S*) allele, and the 333 bp and 302 bp bands were classified as the long (L*) allele. The MNS16A genotypes were therefore defined as L*/L*, S*/L* and S*/S* and their distribution along the three age classes is reported in Table 1. While both genotype and allele distributions did not change significantly along the three age classes (Table 1; genotype: $\chi^2 = 6.91$ d.f. 4 p = 0.141; allele: $\chi^2 = 5.42$ d.f. 2 p = 0.067), significant associations of *TERT* VNTR MNS16A were found in Comparison 2 (Age Class 3 vs. Age Class 2) under both additive (odds ratio [O.R.] 0.749; p = 0.019) and dominant (O.R. 0.579; p = 0.011) models (Table 1). The MNS16A*L allele was significantly underrepresented in Age Class 3 (O.R. 0.759; p = 0.020) compared to Age Class 2 (Table 1).

3.2. Average LTL and MNS16A

Age-associated telomere shortening is a well documented feature of leukocyte cells from human blood (Lindsey et al., 1991; Slagboom et al., 1994). LTL was measured on 72 samples (age-, sex- and genotype-matched), selected from the whole population studied and expressed as T/S ratio, to detect the effect of MNS16A genotype on LTL. The mean value of T/S ratio was 1.111 ± 0.290, ranging from 0.683 to 1.833, and it followed a normal distribution in the study population (Kolmogorov–Smirnov test p = 0.153). No significant difference was noticed between the sexes (females, 1.099 ± 0.283; males 1.124 ± 0.301; p = 0.721). As expected, LTL showed a significant age-dependent cross sectional decrease (Age Class 1, mean T/S = 1.315 ± 0.298; Age Class 2, mean T/S = 1.029 ± 0.256; Age Class 3, mean T/S = 1.011 ± 0.223; p = 0.0001) (Fig. 2). LTL did not differ among the three MNS16A genotype groups (Table 2). However, the LTL attrition along the three age classes was significant only for MNS16A L*/L* genotype carriers (p = 0.002) (Table 2).

4. Discussion

During the last decade several studies have demonstrated a strong link between telomere length and human lifespan (Barrett and Richardson, 2011; Gomes et al., 2011). Telomere length shortens with age. Progressive shortening of telomeres leads to senescence, apoptosis, or oncogenic transformation of somatic cells, affecting the health and lifespan of an individual. Shorter telomeres have also been associated with increased incidence of diseases and poor survival (Shammas, 2011). Thus, recent studies attempted to uncover the role of the telomere maintenance genes (*TERT* and *TERC*) in human longevity (Atzmon et al., 2010; Soerensen et al., 2012).

Here, we provide novel evidence for *TERT* in longevity; in the Central Italy population we studied, that the L*allele at VNTR MNS16A of the *TERT* gene influences negatively life expectancy. This result was

Table 1
TERT MNS16A genotype and allele distributions in age classes studied. O.R. values showing significant differences are shown in bold.

	Genotype (N, %)			Allele (N, %)		Comparison 1 (Age Class 2 vs. Age Class 1)	Comparison 2 (Age Class 3 vs. Age Class 2)
Group	L*/L*	S*/L*	S*/S*	L*	S*	Additive (L*/L* vs. L*/S* vs. S*/S*) Common O.R. 1.099 p = 0.415	Additive (L*/L* vs. L*/S* vs. S*/S*) **Common O.R. 0.749 p = 0.019**
Age Class 1 (N = 333, females 189, males 144)	125 (37.5%)	156 (46.9%)	52 (15.6%)	406 (61.0%)	260 (39.0%)	Dominant (L*/L* + L*/S* vs. S*/S*) O.R. 1.240 (0.841–1.828) p = 0.277	Dominant (L*/L* + L*/S* vs. S*/S*) **O.R. 0.579 (0.378–0.886) p = 0.011**
Age Class 2 (N = 539, females 280, males 259)	209 (38.8%)	260 (48.2%)	70 (13.0%)	678 (62.9%)	400 (37.1%)	Recessive (L*/L* vs. L*/S* + S*/S*) O.R. 1.054 (0.795–1.397) p = 0.718	Recessive (L*/L* vs. L*/S* + S*/S*) O.R. 0.778 (0.553–1.095) p = 0.149
Age Class 3 (N = 200, females 100, males 100)	66 (33.0%)	93 (46.5%)	41 (20.5%)	225 (56.3%)	175 (43.7%)	Allele (L* vs. S*) O.R. 1.085 (0.890–1.324) p = 0.419	Allele (L* vs. S*) **O.R. 0.759 (0.601–0.958) p = 0.020**
Comparison along the three Age Classes	$\chi^2 = 6.91$ d.f. 4 p = 0.141			$\chi^2 = 5.42$ d.f. 2 p = 0.067			

Women, Age Class 1: <73 years old; Class 2: 73–91 years old; Class 3: >91 years old subjects.
Men, Age Class 1: <66 years old; Class 2: 66–88 years old; Class 3: >88 years old subjects.

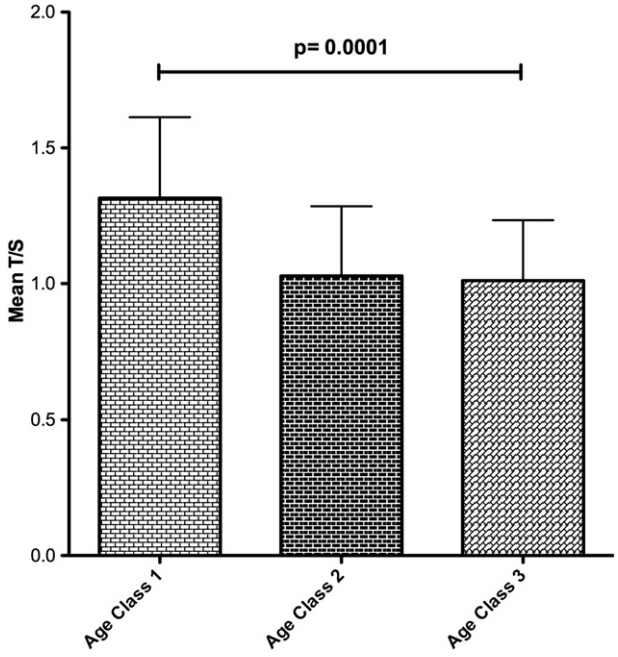

Fig. 2. Mean LTL expressed as T/S ratio among the three age classes studied. Values are expressed as mean ± S.D. Twelve females and twelve males were studied for each age class. Age Class 1, mean age = 39.7 ± 3.7 years; Age Class 2, mean age = 80.3 ± 1.0 years; Age Class 3, mean age = 97.9 ± 2.5 years.

further supported by the significant LTL attrition that we found along the three age classes, for L*/L* genotype carriers only.

Contrary to the previous studies (Atzmon et al., 2010; Soerensen et al., 2012) which employed SNPs to assess the association of *TERT* locus with longevity, we used a functional VNTR located downstream of the *TERT* gene. VNTRs constitute a relatively under-examined class of genomic variants in the context of complex disease because of their sequence complexity and the challenges in assaying them (El-Sayed Moustafa et al., 2012). It has been suggested that complex multiallelic loci such as VNTRs are among the most likely structural variants to be enriched for functional impact (Conrad et al., 2010; El-Sayed Moustafa et al., 2012). The involvement of VNTRs in complex traits has been previously demonstrated by the association of VNTR in the *INS* gene with type-1 diabetes (Julier et al., 1991), the VNTRs located in the *SLC6A3* and *SLC6A4* genes with several psychiatric conditions (Ueno, 2003) and the VNTRs in *SIRT3* and *APOB* genes with human longevity (Bellizzi et al., 2005; De Benedictis et al., 1998).

It has been reported that VNTR MNS16A affects the putative promoter region of the antisense transcript of *TERT* giving rise to a potential allele-dependent regulation of telomerase expression (Wang et al., 2003). Indeed, longer alleles at MNS16A exhibit stronger promoter activity compared to the shorter alleles (Wang et al., 2003). In turn, this lead to an increased expression of antisense *TERT* mRNA with a conceivable, at least partial, silencing of sense telomerase transcript (Wang et al., 2003). Accordingly, carriers of the MNS16A*S allele display higher telomerase activity than the L*/L* genotype carriers (Wang et al., 2003). This evidence clearly supports the results we obtained herein, where L*/L* subjects are less likely to attain longer life-span probably because their telomerase may be less effective in preserving telomere length. Even if this proof of principle remains to be tested, a further clue corroborating our hypothesis comes from our results on LTL attrition along the three age classes, that resulted significant only for L*/L* genotype carriers.

Several association studies of MNS16A in cancer can further help us to address its role in human longevity. A survival analysis on 299 non-Hispanic white patients with glioblastoma multiforme demonstrated that L* carriers have a worse survival duration compared to S*/S* genotype carriers (Wang et al., 2006). L*/L* genotype carriers were also found to display a worse overall survival in surgically resected non-small cell lung cancer (Jin et al., 2011). On the other side, the S* allele seems to confer an increased risk for glioma (Carpentier et al., 2007), breast cancer (Wang et al., 2008), colorectal cancer (Hofer et al., 2011) and lung cancer (Jin et al., 2011). Thus, these findings may suggest that the MNS16A*S allele is a sort of "double-edged sword" with conflicting roles of increasing the risk of cancer development but, at the same time, improving survival in some cancer types. This intricate tangle can be unraveled by assuming that the stronger telomerase activity of S* carriers can not only promote cancer cell immortalization and tumor development, but also expands normal organismal and cellular life-span.

Owing to its ability to confer with unlimited proliferative potential, *TERT* over-expression is a common feature of human cancers and can increase cancer incidence in the context of classical mouse *TERT* transgenesis (Bernardes de Jesus et al., 2012). However, at the same time, telomerase-transfected normal human cell types exhibit elongated telomeres, vigorous cell divisions, reduced senescence biomarkers and elongated life-span by at least 20 doublings (Bodnar et al., 1998). Thus, it is highly conceivable that strong telomerase activity may promote both cancer and longevity.

Although we found a significant LTL attrition along the three age classes ($p = 0.0001$), that remains significant only in L*/L* genotype carriers ($p = 0.002$) when the analysis was conducted according to MNS16A genotype, this result should be taken with caution due to limited sample size. Moreover, it should be noted that the difference between the average LTL among the two elderly groups (Age Class 2 and Age Class 3) (Table 2 and Fig. 2) seems to be insignificant, indeed appearing unchanged. This fact is supported by several studies that reported the leveling off of telomere length in advanced age groups (Ishikawa et al., 2012), the dependence of LTL on the health status of centenarians (Terry et al., 2008), and the lack of association of LTL with the overall survival or life span in elderly persons (Njajou et al., 2009), respectively.

It is noteworthy that some *TERT* polymorphisms correlated to telomere length but did not affect longevity or the parameters of healthy aging (Soerensen et al., 2012). This is probably due to the great environmental component affecting telomere length; oxidative stress, depression and uncertainty for the future have been found to significantly correlate with shorter telomeres (Cherkas et al., 2006; Epel et al., 2004; Lung et al., 2007; Yen and Lung, 2013). Thus, environmental factors

Table 2
Mean LTL expressed as T/S ratio among the MNS16A genotypes divided by age classes. Values are expressed as mean ± S.D. Values showing significant differences are shown in bold. p-Values were obtained by ANOVA.

Genotype	Age Class 1 (N = 24)	Age Class 2 (N = 24)	Age Class 3 (N = 24)	p-Value*
L*/L* (N = 8 for each Age Class)	1.455 ± 0.274	1.019 ± 0.198	1.003 ± 0.257	**0.002**
S*/L* (N = 8 for each Age Class)	1.285 ± 0.331	1.035 ± 0.262	1.073 ± 0.280	0.207
S*/S* (N = 8 for each Age Class)	1.140 ± 0.205	1.033 ± 0.328	0.966 ± 0.129	0.345
p-Value**	0.097	0.992	0.649	

* p-Value obtained by comparing the mean T/S ratio along the three age classes.
** p-Value obtained by comparing the mean T/S ratio along the three MNS16A genotypes.

may affect telomere length and longevity more than the genetic background in populations (such as the Italian population), where a great social and economic variability is present (De Rango et al., 2011). In certain restricted groups (such as Ashkenazi Jews from New York Atzmon et al., 2010), where environmental variability is lower, the genetic variation of telomerase may have a greater impact on aging and longevity. Further studies using larger sample size and detailed environmental variables (e.g. smoking, working and educational status) are warranted to clearly address the genetic contribution of *TERT* polymorphisms in determining LTL and human longevity. In particular, it should be noted that the significance of our data on MNS16A genotype and allele frequencies would not survive to a multiple comparison correction if applied. This fact, together with the lack of association of LTL across MNS16A genotypes (within age classes), further highlights the need for a replication study using another population and a bigger sample size.

In conclusion, this is the first study reporting the association between the functional VNTR MNS16A of *TERT* and human longevity. Our study provides interesting findings that should be investigated in different populations and in larger samples (for LTL). The crucial role played by *TERT* and telomere dynamics in aging process is warranted by many established functions of this enzyme and its involvement in several age-related diseases, so we further underscore the need of further investigations to definitively address the role of *TERT* variants in the regulation of human life-span.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2013.03.009.

Conflict of interest

The authors have no conflict to declare.

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