RESEARCH NOTE

Relative telomere length is associated with a functional polymorphism in the monoamine oxidase A gene in a South American sample

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Introduction

Human telomeres are nucleoprotein structures at both ends of linear chromosomes and are fundamental for the maintenance of genomic stability and cellular ageing, and for the adequate functioning of cells in the entire human organism (Castro-Vega *et al.* 2013; Eitan *et al.* 2014). Telomere length (TL) is increasingly being used as a genomic marker associated with several neuropsychiatric disorders, such as major depression, bipolar disorder, schizophrenia and dementia, among others (Eitan *et al.* 2014). Regulation of TL is the result of the complex interplay of multiple environmental and genetic factors (Castro-Vega *et al.* 2013; Eitan *et al.* 2014).

A recent meta-analysis in 19,713 subjects has revealed that heritability of TL in blood cells is close to 0.70 (Broer *et al.* 2013) and it highlights the importance of studying genetic variants related to telomere dynamics. On the other hand, chronic exposure to several types of increased psychological stress, in early and adult life, has been associated with shorter telomeres in white blood cells of healthy individuals (Starkweather *et al.* 2014). Recently, a systematic review identified a possible association between shorter TL and high levels of chronically perceived stress (Starkweather *et al.* 2014). In this regard, a meta-analysis has found that variants in monoamine oxidase A (*MAOA*) gene could play an important role in the molecular mechanisms of response to behavioural stress and development of psychopathology. Subjects of the MAOA low activity group that were exposed to child maltreatment showed a higher risk of developing antisocial disorder (Kim-Cohen *et al.* 2006).

MAOA protein plays an important role in the regulation of levels of norepinephrine, dopamine and serotonin neurotransmitters (Duncan *et al.* 2012). *MAOA* gene is located at Xp.11.4–Xp11.3, has a size of 90,660 bp and is expressed in several regions of brain (Duncan *et al.* 2012). A functional variable-number tandem repeat (VNTR) located in the promoter region of *MAOA* gene (*MAOA*-uVNTR) consists of a 30 bp repeat sequence (Sabol *et al.* 1998). Functional studies have shown that this VNTR regulates the transcription of *MAOA*, with four repeat alleles leading to higher expression (Duncan *et al.* 2012); it has been explored in a large number of genetic studies of neuropsychiatric disorders (Duncan *et al.* 2012). As the main objective of this study was to test the hypothesis of a possible association between TL and *MAOA*-uVNTR (Lung *et al.* 2005), we analysed it in a sample of Colombian healthy and young individuals, to rule out the possible confounding effects of neuropsychiatric diseases or ageing.

Materials and methods

Subjects

One hundred and ninety-nine healthy young subjects living in Bogotá, the capital city of Colombia, were included in this study. There were 137 women and 62 men aged between 18 and 30 years (mean, 20.8; SD, 2.7). The population living in Bogotá is the result of a historical admixture of Spaniards and Amerindians (Ojeda *et al.* 2013). Analysis of several ancestry informative markers showed no evidence of major population stratification in these samples (Ojeda *et al.* 2013). We excluded participants with self-report of personal history of neuropsychiatric diseases. This study was approved by the Institutional Ethics Committee and all the subjects have provided written informed consent (Morales *et al.* 2009).

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Table 1. Primers used for qPCR-based analysis of TL and *MAOA*-uVNTR genotyping.

Primer	Sequence
TELG	ACA CTA AGG TTT GGG TTT GGG TTT GGG TTT GGG TTA GTG T
TELC	TGT TAG GTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA ACA
ALBU	CGG CGGCGG GCG GCG CGG GCT GGG CGG AAA TGC TGC ACA GAA TCC TTG
ALBD	GCC CGG CCC GCC GCG CCC GTC CCG CCG GAA AAG CAT GGT CG CCT GTT
MAOA-F	ACA GCC TGA CCG TGG AGA AG
MAOA-R	GAA CGG ACG CTC CAT TCG GA

Underlined bases represent GC-rich tails.

DNA samples

DNA was extracted from peripheral blood samples using the salting out method as previously described (Morales *et al.* 2009). DNA concentrations were quantified using a Qubit ds-DNA HS Assay and measured in a Qbitfluorometer (Invitrogen, Carlsbad, USA) and all the samples were normalized to 10 ng/μL in TE-4 buffer and stored at 4°C until use.

Measurement of relative telomere length

Relative telomere length was measured using a monochrome multiplex quantitative PCR (MMQPCR) technique as described by Cawthon *et al.* in 2009, with slight modifications. It used four primers (designed by Cawthon), two targeting the telomere sequence (TELG and TELC) and two designed to bind a single-copy gene ALB (ALBU and ALBD) (table 1). Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, USA).

Final volume of MMQPCR was 15 μL, consisted of 7.5 μL of 2× SensiMix HRM (Bioline, London, UK), 0.6 μL of EvaGreen dye, 1.2 μL of a 10 μM working solution of each primer (for a final concentration of 0.8 μM) and 20 ng of genomic DNA. All assays were carried out in a CFX96 touch real-time PCR detection system (BioRad, Hercules, USA). CFX96 manager software (BioRad) was used for recording and analysing the relative fluorescence from the sample reactions and to estimate the Cq values. Specificity of the qPCR products was verified by visualization of expected amplification band sizes on agarose gel electrophoresis and the melting curve analysis (BioRad Precision Melt Analysis Software).

Negative controls (water) and a calibrator DNA sample were run in each plate. Assays were carried out in duplicate for each DNA sample. The relative TL for each subject was quantified by the mean T/S ratio (Cawthon 2009), where T corresponds to the Ct value at 74°C read (telomere signal) and S corresponds to the Ct value at 88°C read (albumin signal). The T/S ratio for each sample was normalized (to reduce variation across runs), taking the T/S ratio of the calibrator DNA as 1.0. Analysis with the LinRegPCR programme (Ramakers *et al.* 2003) showed that the efficiency of qPCR reactions were >0.99. Coefficient of variation for interassay reproducibility of MMQPCR was 3.3% and a standard curve with different DNA concentrations confirmed the linearity of the assay.

MAOA genotyping

MAOA-uVNTR genotyping was carried out as described by Sabol *et al.* (1998), using two primers (MAOA-F and MAOA-R, table 1). For this VNTR, five alleles have been reported: two (294 bp), three (324 bp), four (354 bp), five (384 bp) and 3.5 (340 bp) (Sabol *et al.* 1998). Fragment sizes were determined by comparison with molecular weight markers (HyperLadder V, Bioline). A random subsample (10% of subjects) was reanalysed for the MAOA polymorphism to assure consistency in the genotyping results. Two different investigators checked all genotypes to confirm and validate the results.

Statistical analysis

Normality of the distribution of T/S ratios was analysed with Shapiro–Wilk test. A χ^2 test was used to evaluate Hardy–Weinberg equilibrium (HWE) for *MAOA* genotype frequencies in females. Pearson's correlation r and unpaired t tests were used for analysis of correlation between age and relative TL, and for differences between males and females for mean TL. SNPStats and PLINK (ver. 1.07) programs (Sole *et al*. 2006; Purcell *et al*. 2007) were used for the analysis of the association between relative TL and *MAOA*-uVNTR, using a linear regression model, corrected by sex and age. T/S ratios and *MAOA* genotypes were the dependent and independent variables, respectively (male hemizygous subjects were combined with female homozygous subjects). In addition, 3/3 and 4/4 subjects were defined as low and high activity groups, respectively (3/4 heterozygous female subjects were excluded) (Kim-Cohen *et al.* 2006) and an unpaired t-test was used to compare relative TL between those two categories. P value <0.05 was considered as statistically significant.

Results

Relative TLs, defined as T/S ratios, had a normal distribution in the present sample ($P = 0.1$, for a Shapiro–Wilk test). In the current study, there was neither significant correlation between age and relative TL (Pearson's correlation $r = -0.09$, $P = 0.166$) nor significant differences between males and females (P=0.05) in relative TL.

Table 2. Association analysis of relative TL and *MAOA*-uVNTR.

MAOA group	*n* (%)	TL mean T/S (S.E.)	MAOA group	*n* (%)	TL mean T/S (S.E.)
3/3	39 (19.6)	1.03 (0.01)	Low	39 (28.7)	1.03 (0.01)
3/4–4/4	160 (80.4)	0.99 (0.01)	High	97 (71.3)	0.99 (0.01)
Total	199 (100)	$P = 0.007^a$	Total	136 (100)	$P = 0.002^b$

[a]*P* value from a linear regression model corrected by sex and age. [b]*P* value from an unpaired *t*-test.

In our Colombian sample, we only found two *MAOA* alleles: three and four repeats. The genotype frequencies for *MAOA*-uVNTR were in HWE in females ($P = 1.0$). A linear model, corrected by sex and age, showed a statistically significant association of *MAOA*-uVNTR with relative TL ($P = 0.007$), with 3/3 subjects having longer telomeres (higher T/S ratios) (table 2). These results were confirmed with an analysis of differences in relative TL between *MAOA* high and low activity categories ($P = 0.002$), with *MAOA* low activity subjects having longer telomeres (table 2).

Discussion

An increasing number of studies showed the growing importance of the analysis of TL as a possible genomic marker for several neuropsychiatric disorders and endophenotypes. Several workers have found changes in mean relative TL in DNA, extracted from peripheral blood cells, from patients with Alzheimer's disease, Parkinson's disease, schizophrenia, bipolar disorder and major depression (Eitan *et al.* 2014).

Given the large known heritability of TL (around 0.70) (Broer *et al.* 2013), it is important to study common genetic factors that could explain, in interaction with environmental factors, a part of the interindividual variability in TL. A meta-analysis of five studies found convincing evidence that the variants in *MAOA* gene could have a significant role to play in signalling response to psychological stress and development of mental disorders (Kim-Cohen *et al*. 2006), whereas chronic exposure to different types of abnormal behavioural stress, in both early and adult life, has been associated with shorter telomeres in leucocytes of healthy subjects (Starkweather *et al.* 2014). *MAOA*-uVNTR has been studied as a candidate marker for several neuropsychiatric disorders, such as attention deficit hyperactivity disorder, major depressive disorder, bipolar disorder, suicide attempt and panic disorder (Duncan *et al.* 2012).

In a sample of 199 Colombian healthy subjects, there was a significant association of *MAOA*-uVNTR and relative TL, with *MAOA* 3/3 carriers having longer telomeres. A comparison of mean TL between *MAOA* high and low activity groups confirmed these observations. In our study, we did not find a significant correlation between age and TL, which could be related to the age range (mean age of 20.8 years) of our subjects; Frenck *et al.* (1998) found that a strong linear correlation between age and telomere length in adult subjects appear mainly after middle of the second decade of life.

Our findings in young healthy subjects suggest that *MAOA* allele-specific might impact telomere dynamics and, therefore, lead to widespread changes in genome integrity (Castro-Vega *et al.* 2013), to ultimately alter brain functions. Different studies have demonstrated that depressed patients show accelerated cellular ageing according to a 'dose–response' gradient: those with the most severe and chronic major depressive disorder showed the shortest telomeres (Starkweather *et al.* 2014). These mechanisms could also be present in healthy young carriers of genotypes that are associated with higher risk for psychopathology (Ojeda *et al.* 2013). A possible functional link between telomere shortening and neural dysfunction could be oxidative stress (Eitan *et al.* 2014). In this context, mice that are knock-out for telomerase have brain alterations, such as anxiety-like behaviours and spatial memory defects (Lee *et al.* 2010; Rolyan *et al.* 2011).

We used a recent methodological approach for a cost-effective analysis of TL, monochrome multiplex quantitative PCR technique (Cawthon 2009). Further studies in other populations and in animal models are needed to confirm and understand the relationship between MAOA and TL in healthy subjects, including measures of stress sensitivity and history of stressful events.

Our work is one of the first studies of telomere length associated with candidate genes of neuropsychiatric relevance in Latin American samples. It is also one of the first studies of MAOA and TL in young subjects (mean age: 20.8 years). Our current results are concordant with previous findings in older healthy subjects from an Asian population (Taiwanese individuals with a mean age of 45.3 years) (Lung *et al*. 2005, 2007). However, given the fact that *MAOA*-uVNTR contributes only to a small amount of variance in risk for neuropsychiatric disorders (Duncan *et al.* 2012), further analysis of other candidate genes are necessary (Alaerts *et al.* 2009) in larger samples, and including patients with neuropsychiatric disorders and additional age ranges.

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