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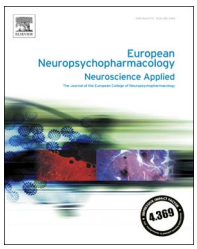

SHORT COMMUNICATION

Shorter leukocyte telomere length in patients at ultra high risk for psychosis

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Abstract
Telomere length attrition has been demonstrated in schizophrenia but not in individuals in ultra high risk (UHR) for psychosis. The present study aimed to compare the leukocyte telomere length (TL) between patients at UHR for psychosis and healthy controls (HC). Twenty-two participants with UHR and 88 HC were enrolled in this study. Telomere lengths were determined using a multiplex qPCR assay. After adjustment for age, sex, ethnicity, and education, patients

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in UHR, compared with HC groups, had shorter telomere length (RR: 0.929, $p=0.031$). Shorter leukocyte telomere length in UHR could represent early signs of accelerated aging in this population.

1. Introduction

Schizophrenia (SCZ), has been conceptualized as a neuro-developmental disease, wherein the onset of psychotic symptoms are a late manifestation of underlying illness processes (Lewis, 2002). As a result, studies have focused on populations in pre- and/or sub-clinical stages (Brietzke et al., 2011). Accumulating evidence indicates that 15-40% of individuals that fulfil criteria for ultra-high risk (UHR) develop a psychotic episode over a 2-year period (Correll, 2007).

Telomeres are tandem repeats of non-coding DNA sequences (TTAGGG/CCCTAA) at the end of each chromosome that protect them from loss of genetic material and end-to-end recombination (Moyzis et al., 1988). Telomeres progressively shorten with age, as a result of each cell division; therefore, telomeres are considered a sign of cellular aging. Telomere length (TL) has increasingly been studied in SCZ patients, with studies reporting shorter TL (Czepielewski et al., 2016; Kao et al., 2008; Yu et al., 2008), specially on patients with a poor response to treatment (Kota et al., 2015; Yu et al., 2008). However, there are conflicting reports, with separate studies reporting no differences in TL in cerebellum (Malaspina et al., 2014; Zhang et al., 2010), and the largest study to date actually documenting longer telomeres in this population (Nieratschker et al., 2013).

There is no data about early stages of psychosis and this may be particularly interesting since there is no effect of acute severe psychosis or medication. In this study, we aim to investigate the difference in TL between UHR and healthy controls.

2. Experimental procedures

2.1. Sample and clinical evaluation

Twenty two UHR subjects within an age range from 14 to 26 years were recruited from the Recognition Program and Intervention in Risk Mental States (PRISMA Early Intervention Program), a specialized ambulatory in São Paulo, Brazil, whose main objective is to assess, treat and follow patients at ultra-high risk to develop psychosis (Brietzke et al., 2011). We used the criteria for ultra-high risk for psychosis proposed and validated by Yung et al., according to the classification of the Comprehensive Assessment of At-risk Mental State (CAARMS) Scale (Yung et al., 2005). The inclusion criteria adopted for considering UHR include (i) Attenuated positive symptoms (APS) i.e. presence of positive symptoms in moderate severity but not reaching clearly the psychotic band; present more than one time per month for >1 h per week; (ii) Brief intermittent psychotic symptoms (BLIPS) which includes presence of brief episode of a full psychotic illness which might involve all of the symptoms of psychosis; (iii) Trait and state risk (TS) factors includes vulnerable family history of psychosis in a 1st degree relative or a diagnosis of schizotypal personality disorder associated with a decline in social and occupational functioning. The exclusion criteria for UHR group was (i) recent use of anti-inflammatories; (ii) long term treatment with drugs; (iii) acute and chronic general medical conditions; and (iv) subjects with autism spectrum disorders.

For the control group ($n=88$), healthy individuals with no current or past history of mental disorders according to the Structured Clinical Interview for DSM-IV Axis 1 (SCID-1) disorders (Castro et al., 2015) were selected. In addition, we included only subjects with no history of use of psychotropic medications and no family history of a major psychiatric disorder (defined as unipolar depression, bipolar disorder (BD), suicide or psychosis in any first-degree relative).

Clinical mood status was evaluated by the following scales: Montgomery-Äsberg Depression Rating Scale (MADRS), Young Mania Rating Scale (YMRS). The MADRS, YMRS, and CAARMS were not administered to the HC group.The research protocol was approved by the Ethics in Research Committee and all participants above 18 years of age provided written informed consent prior to their enrolment in this study. For participants under 18 years old, the written informed consent was provided by the legal responder.

A total volume of 10 mL of peripheral blood was collected via arm venepuncture in BD SST II Advanced tubes. The collection of blood samples were carried out between 8 and 10 am after a minimum 12-h fast. After collection, genomic DNA was isolated and stored at −80 °C for a maximum time of 2 years.

2.2. Measurement of leukocyte telomere length

Genomic DNA was isolated from whole blood using Gentra Puregene Blood Kit (Qiagen) following the manufacturer's instructions. After isolation, the DNA samples were quantified and diluted to 50ng/ul. Telomere length measurement was performed by multiplex real time PCR, as described by Cawthon with some modification (Cawthon, 2009). Measurement consists of determining the relative ratio (*T*/*S* ratio) of ng of telomeres (*T*) to ng of albumin (single-copy gene, *S*) in experimental samples using a standard curve. The *T*/*S* ratio is proportional to the average telomere length.

The primers for telomeres and albumin were 5′-ACACTAAGGTTT-GGGTTTGGGTTTGGGTTTGGGTTAGTGT-3′ (telg), 5′-TGTTAGGTATC-CCTATCCCTATCCCTATCCCTATCCCTAACA-3′ (telc), 5′-CGGCGGCGGG-CGGCGCGGGCTGGGCGGAAATGCTGCACAGAATCCTTG-3′ (albu) and 5′-GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAAG-CATGGTCGCCTGTT-3′ (albd). The final volume in the reaction wells was 25 μL and contained: 12.5 μL of 2x SYBR® Select Master Mix (Life-Thermo-Fischer Scientific); 1 μL sample DNA; 0.9 μL of 25 μM telg (final concentration 900 nM); 0.6 μL of 25 μM telc (600 nM); 0.9 μL of 25 μM albu (900 nM); 0.9 μL of 25 μM albd (900 nM) and 8.2 μL of UltraPure™ DNase/RNase-Free Distilled Water (Life-Thermo-Fischer Scientific). The standard curve was constructed based on a five-point 1:3 serial dilution DNA from a single person (range from 150 to 1.85 ng). The samples and standard curve were all run in triplicates in a ViiA™ 7 Real-Time PCR System with fast 96-Well Block (Life-Thermo-Fischer Scientific).

The thermal cycling profile was Stage 1: 15 min at 95 °C; Stage 2: 2 cycles of 15 s at 94 °C, 15 s at 49 °C; and Stage 3: 35 cycles of 15 s at 94 °C, 10 s at 68 °C, 19 s at 74 °C with signal acquisition (telomeres), 10 s at 85 °C, 19 s at 88 °C with signal acquisition (albumin). After amplification, a melt curve was used to confirm the specificity of the reaction.

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