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Telomere quantification in frontal and temporal brain tissue of patients with schizophrenia

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ABSTRACT

Recent imaging studies have suggested that accelerated aging occurs in schizophrenia. However, the exact cause of these findings is still unclear. In this study we measured telomere length, a marker for cell senescence, in gray and white matter brain tissue from the medial frontal gyrus (MFG) and superior temporal gyrus (STG) of 9 patients with schizophrenia and 11 controls. No alterations in telomere length were found in MFG gray and white matter and in STG gray matter. A significant reduction in telomere length was observed in STG white matter of patients with schizophrenia as compared to controls (fold change of −0.42, U = 5, P = 0.008). Our results support previous findings that telomere length in gray matter is not affected, whereas they suggest that increased cell senescence may affect white matter temporal brain tissue.

1. Introduction

Recent longitudinal imaging studies have suggested that progressive brain volume decrease during the course of schizophrenia reflects a process of accelerated aging (Cropley et al., 2016; Kochunov et al., 2013; Koutsouleris et al., 2014; Schnack et al., 2016). This hypothesis is supported by circumstantial evidence such as cognitive decline and lower life expectancy in schizophrenia and shared risk factors with other aging-related disorders (Kirkpatrick et al., 2008). However, the exact cause of this process of accelerated aging found in schizophrenia remains uncertain.

Telomere length is a marker frequently measured to assess cell senescence and biological age (Bojesen, 2013). Telomeres consist of a repetitive set of nucleotides at the end of the chromosome bound by specific proteins and during each successive cell division telomere length usually decreases (Levy et al., 1992). The telomerase enzyme is capable of extending telomeres again (Bojesen, 2013). If chromosomes are no longer capped by telomeres due to extensive telomere decrease, this could result in chromosomal instability, cellular senescence and cell apoptosis (d'Adda di Fagagna et al., 2003). Higher chronological age is associated with shorter telomere length but other factors such as cell division rate, genetic background and environmental circumstances also influence telomere length (Müezzinler et al., 2013).

Shortened telomere length, most commonly measured in leukocytes, has been found in various disorders (Bojesen, 2013). Telomere length has also been extensively measured in leukocytes of patients with schizophrenia, with contradicting results thus far (Polho et al., 2015). Only two studies have assessed telomere length in brain tissue of patients with schizophrenia, using a relative qPCR quantification method (Mamdani et al., 2015; Zhang et al., 2010). No differences in telomere length were found between patients and controls in tissue from the cerebellum, amygdala, dorsolateral prefrontal cortex, hippocampus, nucleus accumbens and substantia nigra. These studies however, focused on cortical and subcortical tissue, while white matter may be more susceptible to cell senescence (Nakamura et al., 2007).

In this study we set out to measure absolute telomere length in gray and white matter brain tissue from the medial frontal gyrus (MFG) and superior temporal gyrus (STG) of patients with schizophrenia and controls, as previous imaging studies have found both gray and white matter alterations in these brain regions in

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schizophrenia (Kikinis et al., 2010; Lee et al., 2009; Ohi et al., 2016; Ohtani et al., 2014; Olabi et al., 2011; Sun et al., 2009).

2. Material and methods

2.1. Study sample

For this study human post-mortem brain tissue was acquired from the Netherlands Brain Bank. We included tissue from 9 patients with schizophrenia and 11 controls from the MFG and STG. Controls had to be non-demented and had no known history of a psychiatric disorder. All included patients were diagnosed with schizophrenia during life. Not every tissue type was available from each donor; an overview is depicted in Table 1. Clinical data were collected from an extensive post-mortem psychological autopsy report, which was critically reviewed by an experienced psychiatrist to confirm the diagnosis of schizophrenia. Informed consent was obtained for brain autopsy and the use of tissue and clinical information for research purposes.

2.2. Telomere length assay

After visual inspection of the acquired tissue blocks, gray and white matter was separated using tweezers and a scalpel knife. Genomic DNA was extracted from these tissues using QIAmp DNA Mini Kits (QIAGEN, Hilden, Germany). DNA concentration and purity was determined with the NanoDrop 2000 (Thermo scientific, MA), all DNA samples had a 260/280 nm ratio > 1.8.

Telomere length was determined using the q-PCR method described by Cawthon (2002), modified with synthetic standards as described by O'Callaghan (O'Callaghan and Fenech, 2011) to measure absolute telomere length. Assays were performed in duplo using a QuantStudio 12 K Flex Real-Time PCR system. The difference between duplicates was <0.5 Ct for each included sample. 36B4 was used as single copy gene (S) to normalize for DNA input. Primer sequences and conditions have been published elsewhere (Boks et al., 2015). A standard curve was established on each 96-well plate by serial dilution of a known amount of oligonucleotide for either 36b4 or telomere. The R^2 of each standard curve was >0.95. In total 2 plates for 36b4 and 2 plates for telomere were run, patient and controls were equally distributed across these plates.

2.3. Statistical analysis

Differences in baseline characteristics and telomere length between the two diagnostic groups and were examined using Fisher's Exact test and Mann–Whitney *U* test when appropriate, as data were not normally distributed and consisted of a small sample size. The difference between gray and white matter telomere length of the same donor was examined using the Wilcoxon Signed Ranks Test. As we examined gray and white matter telomere length in two different brain regions between patients and controls, results with a p-value of <0.0125 (0.05/4) were considered significant. Spearman's rank correlation coefficients were calculated to investigate the relation between telomere length and age.

3. Results

3.1. Characteristics of the cohort

Characteristics of the cohort are shown in Table 1. In the whole group there was a significant correlation between telomere length of gray and white matter tissue (rs = 0.572, P = 0.001) and telomere length was significantly longer in gray matter than white matter in both brain regions (MFG Z = −3.027 P = 0.002, STG Z = −3.408 P = 0.001). We found no significant differences in telomere length between males and females in the examined brain regions (data not shown).

3.2. Differences between patients and controls

We found a significant decrease in telomere length in STG white matter tissue in patients as compared to controls (fold change of −0.42, U = 5, P = 0.008). An overview of the individual cases is shown in Fig. 1A. For the other brain regions no significant differences were found between patients and controls (Table 1).

3.3. Correlation with age

Telomere length for the whole group was not significantly correlated with age (data not shown). When data were stratified for diagnostic group we found a significant correlation (albeit not significant after correction for multiple testing) between telomere length in STG white matter tissue in controls and age (rs = −0.753,

Table 1
Characteristics of patients and controls.

	Patient (N = 9)	Control (N = 11)	P-value
Gender M/F	3/6	5/6	0.670
Age	68.2 (±11.1)	78.1 (±14.7)	0.112
Post mortem interval (H)	10:20 (±4:26)	7:47 (±3:38)	0.131
pH	6.7 (±0.6)	6.6 (±0.3)	0.842
Used antipsychotics before death	8/9	0/9	
Cause of death	3 Pneumonia 2 Cancer 1 Cardiac arrest 1 Heart failure 1 Medication 1 Pulmonary embolism	3 Heart failure 1 Cardiac arrest 1 Cardiac not specified 1 Ventricular fibrillation 1 Renal insufficiency 1 Intestinal ischemia 1 Starvation 2 Ischemia not specified	
Telomere length (kb telomere/genome)			
MFG gray (Patient N = 9/Control N = 10)	552 (±223)	583 (±165)	0.720
MFG white (Patient N = 9/Control N = 9)	333 (±91)	342 (±121)	1.000
STG gray (Patient N = 8/Control N = 11)	350 (±199)	251 (±86)	0.351
STG white (Patient N = 6/Control N = 9)	85 (±26)	146 (±45)	0.008