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Longer telomere length in patients with schizophrenia

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ABSTRACT

Previous studies have reported an association between shorter leukocyte telomere length and schizophrenia (SCZ). The aim of the present study was to replicate this finding in a large sample of SCZ patients (n = 539) and population-based controls (n = 519). In addition, the possible influence of SCZ severity on telomere length – as measured by age of onset, mode of onset, and course of the disorder – was investigated.
Telomere length was negatively associated with age in both patients and controls. This is a consistently reported phenomenon, related to the problem of DNA end-replication. However, in contrast to previous findings, SCZ patients displayed longer telomeres compared to controls (p = 0.015). No association was found with any SCZ-severity subphenotype. Interestingly, recent studies have reported associations between longer leukocyte telomere length and both smaller hippocampal volume, and poorer episodic memory performance. Both phenotypes are common in patients with SCZ. Further studies are warranted to investigate whether the present association between SCZ and increased telomere length was driven by such associations, or rather by association with the clinical disease per se or other associated phenotypes, endophenotypes or lifestyle factors.

1. Introduction

Telomeres form the ends of linear chromosomes, consist of nucleotide repeats and associated proteins (de Lange, 2002), and protect chromosomal termini from the loss of genetic material and from end-to-end recombination. Telomeres are therefore crucial for the maintenance of chromosomal integrity. However, the average telomere length of most proliferating cells (including blood leukocytes) declines substantially with age (Iwama et al., 1998; Blackburn, 2001). Whether short telomeres actually contribute to the aging process, or are merely a sign of aging, remains unknown.

Telomere length and attrition are also influenced by genetic (Slagboom et al., 1994; Nordfjall et al., 2010; Codd et al., 2013), and environmental factors. The latter include smoking (Saliques et al., 2010; Babizhayev and Yegorov, 2011) and psychological stress, both of which lead to a decrease in telomere length. Implicated psychological stress factors include childhood adversity, care-giving, work-related stress, ambivalent social relationships, experience of major negative life events, and chronic pain (von Zglinicki, 2002; Epel et al., 2004; Simon et al., 2006; Damjanovic et al., 2007; Houben et al., 2008; Kananen et al., 2010; Tyrka et al., 2010; Drury et al., 2011; Entringer et al., 2011; Kiecolt-Glaser et al., 2011; Malan et al., 2011; Ahola et al., 2012; Humphreys et al., 2012; Shalev et al., 2012; Sibille et al., 2012; Uchino et al., 2012).

In the case of psychiatric disorders, associations have been reported between decreased telomere length and anxiety disorders (Kananen et al., 2010; Hoen et al., 2012), posttraumatic stress disorder (Malan et al.,

Abbreviations: SCZ, schizophrenia; NGFN, National Genome Research Network; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; SCID, Structured Clinical Interview for DSM-IV axis I disorders; OPCRIT, Operational Criteria Checklist for Psychotic Illness; FNTD, Fagerstrom Test of Nicotine Dependence; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic; dsDNA, double strand; qPCR, quantitative Polymerase chain reaction; Hgb, β-hemoglobin; ng, nanograms; µl, microliter; nM, nanomolar; CV, inter-assay coefficient of variation.

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2011; O'Donovan et al., 2011), mood disorders (Simon et al., 2006), and major depression (Simon et al., 2006; Lung et al., 2007; Hartmann et al., 2010; Hoen et al., 2011; Wolkowitz et al., 2011).

An association with decreased telomere length has also been reported for schizophrenia (SCZ), although each individual study investigated relatively small samples (Kao et al., 2008; Yu et al., 2008; Fernandez-Egea et al., 2009; Mansour et al., 2011). Kao et al., investigated telomere length in a sample of 51 SCZ patients, 24 unaffected family members, and 53 controls (Kao et al., 2008). The authors found significantly shorter telomeres in the SCZ patients compared to family members and controls. In a study of 68 SCZ patients and 76 controls, Yu et al., found an association between telomere length and disease severity, with more severely affected patients displaying shorter telomere length compared to both controls and less severely affected patients (Yu et al., 2008). Fernandez-Egea et al., measured telomere DNA content, which is highly correlated with telomere length, in 41 individuals with nonaffective psychosis (including n = 27 with SCZ and 9 with schizophreniform disorder), and 41 controls (Fernandez-Egea et al., 2009). The authors found significantly decreased telomere DNA content in individuals with nonaffective psychosis compared to controls. In contrast, Mansour et al. (2011) compared 60 SCZ cases with 60 controls and found no association between SCZ and telomere length.

The aim of the present study was to replicate previous findings of shorter telomere length in SCZ in a large sample of SCZ patients and population-based control individuals.

2. Materials and methods

2.1. Study participants

722 SCZ patients and 722 population-based control individuals of German descent were included. All patients and the majority of the controls (n = 491) were recruited between 1988 and 2005 within the German *National Genome Research Network* (NGFN) (Hoefgen et al., 2005). Additional control individuals (n = 231) were recruited with the cooperation of the residents' registration office in Mannheim and the Red Cross administration of Baden-Württemberg, as well as via newspaper and internet advertisements.

Lifetime best estimate diagnoses were assigned according to DSM-IV criteria by two experienced psychiatrists or psychologists on the basis of multiple sources of information, including interviews with the German version of the Structured Clinical Interview for DSM-IV axis I disorders (SCID) (First et al., 1998), the Operational Criteria Checklist for Psychotic Illness (OPCRIT) (McGuffin et al., 1991), medical records, and family history. Age of onset, mode of onset, course of disorder, and lifetime smoking were also assessed. Age at onset was defined as the age at which a patient showed the first symptoms of SCZ. Mode of the disorder and course of the disorder were assessed by the raters involved in performing best estimate diagnoses using OPCRIT (item numbers 5 and 90, OPCRIT version v3.32) as quantitative categories containing 5 levels each. In controls lifetime psychiatric symptoms were assessed using a structured self-report questionnaire (adapted from the German version of the International Diagnostic Interview (IDD); Zimmerman et al., 1986; Kühner, 1997). Life-time smoking behavior was assessed using the Fagerstrom Test of Nicotine Dependence (FNTD) (Fagerstrom et al., 1990).

Quality control criteria for telomere length measurement data as described in Section 2.2 were fulfilled in 584 of the 722 SCZ patients (326 male and 258 female) aged between 17 and 80 years (mean age 37.00 ± SD 11.65), and in 644 of the 722 control individuals (368 male and 276 female) aged between 18 and 80 years (mean age 37.07 ± SD 11.52). No significant difference in mean telomere length was observed between samples omitted from further analyses and samples that passed the quality control filters. In total, 77.7% of the patients and 44.3% of the control individuals had a lifetime history of smoking.

The study protocol was approved by the Ethics Committees of the Medical Faculties of the Universities of Bonn and Heidelberg, and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to participation.

2.2. Telomere length assessment

For telomere measurement, a qPCR-based method was used (Cawthon, 2002), as described elsewhere (Kao et al., 2008; Eerola et al., 2010; Kananen et al., 2010) (for details see Supplementary material). Leukocyte telomere length was measured using DNA extracted from peripheral blood samples. Extraction of genomic DNA was either performed manually (Miller et al., 1988), or using an automated system (PerkinElmer Chemagen Technologie GmbH; Rodgau; Germany). DNA concentration and integrity were assessed using fluorescent measurement of dsDNA (Quant-iT™ PicoGreen®; Invitrogen; life technologies GmbH; Darmstadt, Germany).

2.3. Statistical analyses

Sex and age of the participants, as well as the batch used for telomere length measurement, were included as covariates in all models in order to rule out confounding effects. Monotonic relationships between clinical status, age, lifetime smoking, severity of SCZ measures (age of onset, mode of onset, and course of the disorder) and telomere length were assessed by linear regression. All statistical analyses were performed with IBM SPSS 20.0 (Chicago, Illinois, USA).

3. Results

3.1. DNA extraction method affects telomere length

The DNA extraction method had a significant influence on telomere length measurement, with longer telomeres being observed in DNA samples extracted automatically ($p < 0.00001$, data not shown) compared to manually extracted DNA samples. This finding is in accordance with our earlier observation in the Finrisk cohort (n = 4300), in which DNA was extracted using five different methods. In a linear regression analysis of the Finrisk sample, the effect of DNA extraction method on telomere length was highly significant ($p = 3.1E\text{-}81$; adjusted for age and sex), with the automated extraction method producing the longest measurement values (J.L. and I.H., unpublished). For the majority of patients and controls, DNA was extracted manually (Miller et al., 1988). Therefore, the analyses were restricted to manually extracted DNA samples (n = 562 patients and n = 523 controls). To exclude potential bias secondary to outliers, individuals with relative telomere length more than 3 standard deviations different from the mean (n = 23 patients and n = 4 controls) were excluded from further analysis (for sample characteristics see Supplemental Table in Appendix A).

3.2. Longer telomeres in SCZ patients compared to controls

Telomere length was significantly longer in patients with SCZ (n = 539) compared to controls (n = 519) ($B = 0.073$, $p = 0.015$; adjusted for age, batch, and sex) (Fig. 1). Smoking rates were significantly higher in SCZ patients than in controls (SCZ: 79.5%; controls: 44.4%; $x^2 = 92.12$, $p < 0.001$). However, the finding of longer telomere length in SCZ patients remained significant after adjustment for smoking ($B = 0.073$, $p = 0.034$).