ORIGINAL RESEARCH

Cerebellar Telomere Length and Psychiatric Disorders

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Abstract We tested whether telomere length is altered in
the brains of patients diagnosed with major depression
(MD), bipolar disorder (BD) and schizophrenia (SZ) by
measuring mean telomere length (mTL) with real-time
PCR. The samples are cerebellar gray matter from 46 SZ,
46 BP, and 15 MD patients, and 48 healthy controls. We
found no difference in mTL between SZ and controls, BD
and controls, MD and controls, or all cases and controls; no
correlation between mTL and age was observed, either.
This suggests that brain gray matter is unlikely to be related
to the telomere length shortening reported in blood of
psychiatric patients. White matter deserves further inves-
tigation as it has been reported to have a different mTL
dynamic from gray matter. Since mTL has been reported to
be a heritable quantitative trait, we also carried out gen-
ome-wide mapping of genetic factors for mTL, treating
mTL as a quantitative trait. No association survived cor-
rection of multiple testing for the number of SNPs studied.
The previously reported rs2630578 (*BICD1*) association
was not replicated. This suggests that telomere length of
cerebellar gray matter is determined by multiple loci with
"weak effects."

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Introduction

Telomeres are DNA–protein complexes located at the ends
of chromosomes involved in preventing chromosome
fusion and maintaining genome stability (Blackburn 2000).
Telomeres comprise a variable number of repeats of the
sequence TTAGGG (Blackburn 1991; Zakian 1995) and
their lengths decline with cell division (Lansdorp 2008).
Oxidative stress has been proposed to be an important
modulator of telomere length shortening (von Zglinicki
2002).

Telomere shortening in psychiatric diseases

Telomere shortening has been reported in psychological
stress and psychiatric disorders, including schizophrenia
(SZ), bipolar disorder (BD) and major depression (MD)
(Epel et al. 2004; Kao et al. 2008; Lung et al. 2007; Simon
et al. 2006). However, the previous telomere studies of
psychiatric diseases all measured mean telomere length
(mTL) of DNA from leukocytes in blood, not from the
more relevant brain tissue.

Prefrontal and limbic system dysfunction are known to
be strongly involved in the development of SZ, BD, and
MD (Barch 2005; Maletic et al. 2007; McIntosh et al. 2008;
Miklowitz and Johnson 2006). Also, telomere length has
been shown to vary among tissues (Prowse and Greider
1995; Wang et al. 2005). Though cerebellum is not thought
to be the most relevant brain region for psychiatric disease,
studies have shown that the cerebellum may play a role in

BD, SZ, and MD (Andreasen and Pierson 2008; Konarski et al. 2005; Schmahmann et al. 2007). Most importantly, cerebellum has very similar cellular composition, development and aging dynamics as other brain regions, in contrast to blood.

Therefore, we have tested whether telomere length is altered in the cerebellum of psychiatric patients with MD, BD, and SZ. The facts that oxidative status in the cerebellum, cerebral cortex, hippocampus, and peripheral leucocytes is associated with anxiety levels in mice (Rammal et al. 2008), and that oxidative status is also thought to be related to telomere length (von Zglinicki 2002), support a prediction that psychiatric disease status and mTL are associated.

Genetics of telomere length

BD, SZ, and MD all have underlying genetic factors (Alaerts and Del-Favero 2009; Shyn et al. 2009). If telomere shortening is related to psychiatric disease, discovery of genetic factors that determine telomere length or shortening would be important for understanding the genetics of these diseases.

The mTL of most somatic cells is negatively correlated with age, although it varies greatly among individuals, even those in the same age group (Andrew et al. 2006; Okuda et al. 2000; Vasa-Nicotera et al. 2005; Vaziri et al. 1994; Vaziri et al. 1993). Estimates of the heritability of mTL in leucocytes vary: past twin and family studies have placed it around 78–84% (Jeanclos et al. 2000; Slagboom et al. 1994), while a more recent, large, female twin study found a heritability of 36% after adjusting for batch effects, with a large common environmental effect of 49% (Andrew et al. 2006).

Data from yeast suggest that telomere length variation is associated with polymorphisms at a large number of loci (Gatbonton et al. 2006). In humans, although a genetic association mapping study of two candidate genes failed to generate a positive result (Zhang et al. 2007), linkage analysis has mapped telomere regulatory regions to two different chromosomal regions (chromosome 14q23.2 and chromosome 12p11.2) in two leukocyte studies (Andrew et al. 2006; Vasa-Nicotera et al. 2005). The latter group further found that single nucleotide polymorphism (SNP) rs2630578 in *BICD1* was associated with telomere length (Mangino et al. 2008).

To test whether there is a genetic variant(s) with strong effect that determines telomere length in brain, we have carried out a genome-wide SNP-based association mapping of regulatory regions of mTL in human brain. Previous studies have only examined mTL in leukocytes; we have used brain tissue since telomere length and its determinants could differ between brain and blood, and brain is biologically more relevant to psychiatric diseases.

Materials and methods

We studied genomic DNA extracted from 155 brain samples (gray matter of cerebellums) from individuals of European ancestry from the Stanley Medical Research Institute (SMRI). The samples are from persons with SZ ($n = 46$), BD ($n = 46$), MD ($n = 15$), and from healthy controls ($n = 48$). We measured mTL by quantitative PCR (Brouilette et al. 2007; Cawthon 2002) with some modifications (see supplementary methods). The amplification data were analyzed using SDS software (version 2.1, Applied Biosystems). The relative T/S ratio was calculated as previously described (Cawthon 2002). We genotyped subjects using the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Santa Clara, CA); the SNP genotyping was performed by David W. Craig and Margot Redman at the Translational Genomic Research Institute (TGEN). There were 240,576 common SNPs of sufficiently high quality (MAF > 0.1, HWE p-value > 0.001) to include in the subsequent mapping analysis. Average call rate was 99.9%.

Analysis of covariance (ANCOVA) was employed to test the significance of telomere length differences between SZ (or BD or MD or all cases) and controls. Telomere length (T/S ratio) was the dependent variable, and disease status (case or control), age, and sex were covariates.

For genome-wide association analysis of SNP genotypes with mTL, linear regression was carried out using PLINK (Purcell et al. 2007) with mTL as the dependent variable. Since the ANCOVA showed that age and disease status did not have an effect on mTL in this study, these factors were excluded in the linear regression. Data on lifetime antipsychotic use were analyzed as quantitative covariates. Smoking and alcohol use were analyzed as binary covariates.

Results

We observed no difference in mTL between SZ and controls, BD and controls, MD and controls, or all cases and controls (all p-values > 0.1; see Fig. 1; Table 1). Also, age, gender, medication and drug use had no effect on mTL (all p-values greater than 0.05, data not shown).

In the mTL quantitative trait locus (QTL) genome-wide mapping analysis, SNP rs12519633 on chromosome 5 showed the most significant association, with a p-value of 4.31E-06. The top three most significant associations are

shown in Table 2, but the permutated *p*-values are not significant after correcting for the number of SNPs tested, since all are greater than 0.05.
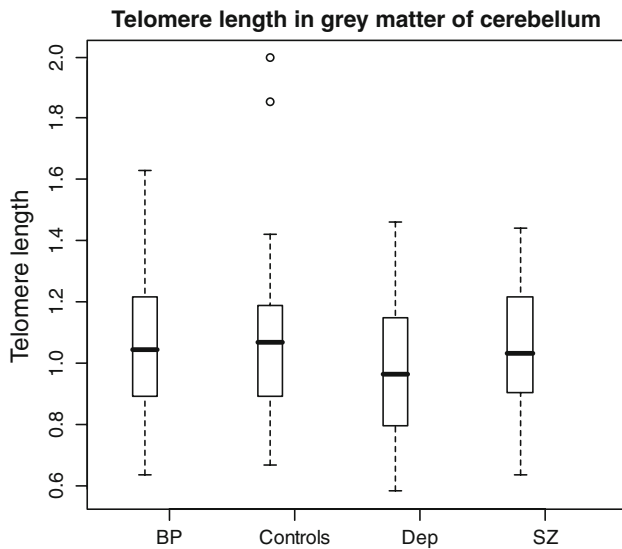

Fig. 1 Telomere length in cerebellar gray matter. The x-axis shows four groups of individuals. BP represents bipolar patients, SZ represents schizophrenia patients, controls represent healthy individuals and Dep represents patients with depression. The y-axis shows telomere length. For each *box plot*, the *top bar* is the maximum observation, the *lower bar* is the minimum observation, the top of the box is the upper or third quartile, the bottom of box is the lower or first quartile, the *middle bar* is the median value and circles are possible outliers

Discussion

In the present study, we investigated the correlation of mTL in brains with disease status for SZ, BD, or MD for the first time. No difference of mTL in the cerebellar gray matter was found in SZ, BD, MD, or all cases compared to controls. The mTL in MD was nonsignificantly shorter than in BD, SZ, and controls.

Significant telomere reductions have been reported in leukocytes and lymphocytes of MD, BD, and SZ. Decreased telomere length in MD (measured by Southern blot) has been reported in white blood cells when comparing 253 patients to 411 controls (Lung et al. 2007) (age MD = 44.48 ± 15.18, controls = 45.32 ± 13.85). A significant difference in mTL (measured by Southern blot) was also reported in a lymphocyte study of 44 combined mood disorders (15 MD and 15 BD with anxiety and 14 BD without anxiety) and 44 controls (*p*-value = 0.002) (Simon et al. 2006). Mean telomere length (measured by RT–PCR) was shortened in lymphocytes of 31 SZ (age 38.77 ± 10.2) compared to 41 controls (25.85 ± 7.43) (*p* = 0.002) (Kao et al. 2008). Kao et al. also replicated their finding in another age-matched male SZ-control study (26 controls and 33 SZ, age 33.19 ± 8.81, 36.24 ± 8.99, respectively).

Our findings should be most comparable to those of Kao et al., since our mTL RT–PCR measurement methods were very similar. We did not detect a correlation between affection status and mTL, while Kao et al. did. The likely explanation for the discrepancy is that we used brain tissue

Table 1 Sample characteristics and mean telomere length

	Male	Female	Age			Mean TL		*p*-Value*
			Range	Mean	SD	Mean	SD	
All cases	65	41	20–65	43.962	10.390	1.051	0.222	0.412
SZ	33	12	20–60	42.444	9.572	1.058	0.203	0.498
BP	23	23	20–65	44.783	11.400	1.071	0.231	0.883
MD	9	6	30–65	46.000	9.487	0.970	0.250	0.107
Controls	33	15	30–70	45.208	9.048	1.077	0.251	

* *p*-value of mean TL comparing cases (all cases, BP, SZ or MD) and controls

Table 2 Top significant association of QTL mapping of mean telomere length (with *p*-value < 1E-4)

rs#	Chr.	Positions	*p*-value (correlation between SNP genotype and mTL)	Permutation corrected *p*-value
rs12519633	5	121967053	4.31E-06	0.47
rs7245298	18	3840002	7.71E-06	0.72
rs2979684	8	24873358	1.11E-05	0.86

Annotations: rs#, rs number; Chr, the chromosome where the SNP is located; positions, physical positions in the chromosome; *p*-value, Wald test asymptotic *p*-value; permutation corrected *p*-value, empirical *p*-value based on 10^3 permutations, corrected for the number of SNPs tested for associations

instead of blood. Previous studies have shown that telomere length varies among tissues (Prowse and Greider 1995; Wang et al. 2005). Thomas et al. observed a significant age effect on mTL in white blood cells but not in buccal cells (Thomas et al. 2008).

Due to the lack of mitosis of neurons and slow replacement of glial cells, telomere length is considered to be stable in adult brain. However, Nakamura and colleagues found that telomeres in the cerebral gray matter were longer than those in the cerebral white matter, and observed no difference in telomere length between infants and individuals aged 60–69 years (Nakamura et al. 2007). We also found that telomere length is not significantly correlated with aging in the cerebellar gray matter of individuals under 70 years of age.

Though we were not able to study prefrontal cortex or limbic system regions due to sample availability problems, the findings of Nakamura et al. suggest those and other brain regions, particularly cerebral or cerebellar white matter of the brain, should be explored further. Studies of cerebellar and cerebral white matter of psychiatric disease patients for age- and disease-status-related telomere erosion and the correlation of telomere length between brain white matter and blood cells may also be important.

Although telomere length in cerebellar gray matter was age-independent and stable, it still showed inter-individual variability (mean $\pm$ SD (standard deviation) in our control samples (1.055 $\pm$ 0.251)). The SD in our samples was comparable to control group A in the study by Kao et al. (2008), despite the difference in mean (control group A, 1.51 $\pm$ 0.33; control group B, 1.51 $\pm$ 0.62).

SNP rs2630578 in *BICD1* was previously reported to be associated with telomere length in leukocytes (Mangino et al. 2008). We did not observe any association from this region, including rs2630578 ($p = 0.245$).

The genetic factor underlying mTL is evident, but our negative genome-wide QTL mapping results were negative, suggesting that multiple "weak effect" genes may exist that contribute to telomere length variation in human, similar to many other complex phenotypes. This is consistent with the yeast genome-wide linkage findings (Gatbonton et al. 2006). A large sample study would be needed to detect those "weak effect" genes controlling telomere length, including candidate genes such as *DDX11* and *Rtel* that have been suggested by previous linkage studies in human or other animal models (Ding et al. 2004; Vasa-Nicotera et al. 2005).

The mTLs measured in most studies, including ours, are actually the outcomes of the combined effects of two factors: at-birth initial telomere length and telomere erosion or restoration rate. Both factors could have independent genetic and environmental influences. As we only measured mTL at one point in time, it is impossible to differentiate between these two factors. A longitudinal study would be ideal for dissecting these two factors for studies of the genetics of telomere length. Brain is not a feasible target of such a study in humans, but an appropriate animal model would be an excellent alternative.

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