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ARTICLE

Shortened telomere length in white matter oligodendrocytes in major depression: potential role of oxidative stress

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Abstract

Telomere shortening is observed in peripheral mononuclear cells from patients with major depressive disorder (MDD). Whether this finding and its biological causes impact the health of the brain in MDD is unknown. Brain cells have differing vulnerabilities to biological mechanisms known to play a role in accelerating telomere shortening. Here, two glia cell populations (oligodendrocytes and astrocytes) known to have different vulnerabilities to a key mediator of telomere shortening, oxidative stress, were studied. The two cell populations were separately collected by laser capture micro-dissection of two white matter regions shown previously to demonstrate pathology in MDD patients. Cells were collected from brain donors with MDD at the time of death and age-matched psychiatrically normal control donors (N=12 donor pairs). Relative telomere lengths in white matter oligodendrocytes, but not astrocytes, from both brain regions were significantly shorter for MDD donors as compared to matched control donors. Gene expression levels of telomerase reverse transcriptase were significantly lower in white matter oligodendrocytes from MDD as compared to control donors. Likewise, the gene expression of oxidative defence enzymes superoxide dismutases (*SOD1* and *SOD2*), catalase (*CAT*) and glutathione peroxidase (*GPX1*) were significantly lower in oligodendrocytes from MDD as compared to control donors. No such gene expression changes were observed in astrocytes from MDD donors. These findings suggest that attenuated oxidative stress defence and deficient telomerase contribute to telomere shortening in oligodendrocytes in MDD, and suggest an aetiological link between telomere shortening and white matter abnormalities previously described in MDD.

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Introduction

The aetiology of depression remains theoretical and treatment advances have been few over the past 30 years. MDD is associated with a high rate of suicide and significantly increases the risk of numerous medical illnesses. Interestingly, MDD is highly comorbid with many diseases associated with advanced age such as cardiovascular disease, stroke, diabetes, osteoporosis, diabetes, immune impairments and dementia (Wolkowitz et al., 2011b). In fact, evidence is mounting that indicates MDD is associated with advanced cellular aging (Douillard-Guilloux et al., 2013; Kinser and Lyon, 2013). Numerous studies report shorter telomere lengths in peripheral blood mononuclear cells from MDD patients (Simon et al., 2006; Wolkowitz et al., 2010; Wikgren et al., 2012; Verhoeven et al., 2013). Telomeres are nucleoprotein complexes of guanine-rich DNA at the end of chromosomes that do not encode any gene product, but are essential for genome stability and the protection of chromosome ends from degradation or recombination (Blackburn, 2000). When telomeric DNA reaches a critically short length, as in cells undergoing repeated mitotic divisions, the cells become susceptible to senescence or apoptosis (Blackburn, 2000; Blackburn et al., 2006). Telomere shortening has been linked to a variety of stressors and stress-related factors such as metabolic stress (Epel, 2009; Epel et al., 2010), chronic life stress (Puterman et al., 2010), psychological stress (Malan et al., 2011), cumulative childhood stress (O'Donovan et al., 2011), inflammation (Kiecolt-Glaser et al., 2011) and glucocorticoids (Haussmann et al., 2012). Many, if not all of these stress factors are also strongly associated with MDD (Bonde, 2008; Maes et al., 2011; Wolkowitz et al., 2011b; Zunszain et al., 2011; Krishnadas and

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Cavanagh, 2012; Frodl and O'Keane, 2013). Hence, shortened telomeres in MDD have been proposed to be markers of accelerated cell aging as a result of exposure to life stressors (Simon et al., 2006; Wolkowitz et al., 2010).

The molecular mechanisms responsible for telomere shortening in MDD or as a result of psychological stress are unknown, although elevated levels of oxidative stress as a result of inflammation or stress hormones seems to be a likely mediator (Epel, 2009; Epel et al., 2010; Puterman et al., 2010). Due to its high level of oxidative metabolism, the brain is inherently vulnerable to oxidative stress and produces chemically reactive molecules containing oxygen (oxygen ions, peroxides, peroxynitrite), otherwise known as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are intracellular mediators of stress responses but with age increasingly contribute to cellular damage, reacting with various biological targets such as proteins, fatty acids, RNA and DNA (Radak et al., 2011; Aschbacher et al., 2013). Oxidative stress effects on DNA includes attack on oxidation-sensitive guanine nucleotides in guanine-rich telomere DNA, resulting in telomere shortening (Von Zglinicki and Martin-Ruiz, 2005).

One might assume that the deleterious effects of telomere shortening may contribute to brain pathology associated with stress-related disorders such as MDD. For example, accelerated telomere shortening as a result of life stresses could contribute to loss of susceptible cells in the brain, an intriguing possibility because numerous researchers have reported reduced numbers of cells in post-mortem brains from MDD subjects (Ongür et al., 1998; Duman et al., 2000; Rajkowska, 2000; Rajkowska and Miguel-Hidalgo, 2007). However, to our knowledge there are only two studies that have examined telomere lengths in post-mortem brain tissues from individuals with stress-related psychiatric disorders (Teyssier et al., 2010; Zhang et al., 2010). In both of these studies, telomere lengths were similar in psychiatric disorders including MDD and normal control subjects, suggesting that telomere shortening in MDD does not negatively impact cellular health of the brain. However, cells in the brain are likely to have differing susceptibilities to stress-induced telomere shortening. For example, astrocytes are more resistant to oxidative stress damage as compared to oligodendrocytes (Desagher et al., 1996; Juurlink, 1997; Wilson, 1997; Juurlink et al., 1998). Previous research reporting telomere lengths in post-mortem brain tissues utilized admixtures of cells from a gross dissection of brain and the lengths reported would be expected to be an average of telomere lengths among the multitude of cell types collected in the dissection.

In the present study, we used laser capture microdissection to collect two glia cell populations from post-mortem brain tissues to examine telomere lengths in MDD. White matter regions of the brain were chosen for study because of the ease of collecting glia cells from white matter. Two separate populations of glia, astrocytes and oligodendrocytes, were captured from white matter tracts in the frontal (Brodmann area 10, BA10) and temporal lobe (uncinate fasciculus; UF). These brain areas were chosen for study because structural magnetic resonance imaging studies have demonstrated white matter abnormalities in these regions in MDD subjects, presumably reflecting cellular pathology (Tham et al., 2011). In the same cells, we also measured the expression of five genes whose protein products maintain telomere length (telomerase reverse transcriptase, *TERT*) or protect cells and oxidation-sensitive telomeres from ROS (cytoplasmic superoxide dismutase, *SOD1*; mitochondrial superoxide dismutase, *SOD2*; catalase, *CAT*; intracellular glutathione peroxidase, *GPX1*). Findings here suggest that attenuated oxidative stress defence and deficient telomerase could contribute to telomere shortening in brain oligodendrocytes in MDD.

Method

Subjects and tissue acquisition

Human brain tissues were obtained at autopsy at the Medical Examiner's Office of Cuyahoga Country (USA) using an approved Institutional Review Board protocol. The details of the collection and psychiatric autopsy are found in previous publications (Ordway et al., 2009; Chandley et al., 2013). Briefly, Axis I diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV; APA, 1994) by a trained interviewer using the Structured Clinical Interview for DSM Axis I Disorders modified for third-person reporting (First et al., 1996). Demographic information of all brain donors is shown in Table 1; diagnoses were active at the time of death. Psychiatrically normal control and MDD donors were arranged into subject pairs, matched as closely as possible for age, gender, post-mortem interval, tissue pH and smoking history. Two MDD subjects had comorbid alcohol abuse disorder and one MDD subject had comorbid alcohol dependence. One of the comorbid alcohol abuse subjects was used only in the assay of BA10 cortex white matter cells. See Supplement Methods for further description.

Immunohistochemistry and laser capture micro-dissection

Frozen temporal lobe tissue (containing the UF, and part of the amygdala and hippocampus) or right BA10 were sectioned (10 μM) with a cryostat microtome at −20 °C (Leica CM 3050 S). GFAP immunostaining of tissue sections was conducted as previously described

Table 1. Subject demographic information

ID	Age	Gender	pH	RIN[a]	PMI[b]	Smoker	Toxicology	Tissue
Controls								
RR	37	M	6.47	7.3	17	No	NDD[c]	UF, BA10
KK	43	M	6.56	6.9	23	No, hx[d]	propoxyphene, oxycodone	UF, BA10
BB	52	M	6.28	6.4	17	No	NDD	UF, BA10
O	78	F	6.42	7.4	11	No	NDD	UF, BA10
KS82	47	M	6.10	7.1	25	No	Propoxyphene	UF, BA10
KS59	46	M	6.95	6.8	19	No	NDD	UF, BA10
ZZ	19	M	6.76	7.0	11	No	NDD	UF
A-1	82	M	6.72	6.7	16	No	NDD	UF
FF	27	M	6.88	8.4	17	Yes	NDD	UF, BA10
KS31	59	M	6.79	7.6	6	No, hx	Lidocaine	UF, BA10
KS43	67	M	6.95	7.2	24	Yes	NDD	UF, BA10
2A	47	M	6.80	7.1	17	Yes	NDD	UF
KS21	48	M	6.98	7.4	9	Yes	NDD	BA10
KS23	58	M	6.78	7.7	21	Yes	NDD	BA10
Mean	**51**		**6.67**	**7.21**	**16.6**			
S.E.M.	**5**		**0.07**	**0.13**	**1.5**			
MDD								
KS56	37	M	6.60	6.9	31	No	Ethanol	UF, BA10
KS58	42	M	6.50	6.8	27	?	CO	UF, BA10
DD	52	M	6.48	5.8	18	No	CO	UF, BA10
P	75	F	6.23	7.5	30	Yes	CO	UF, BA10
D	47	M	6.84	7.0	11	No	Ethanol	UF, BA10
6A	47	M	6.26	7.5	24	No	NDD	UF, BA10
KS64	20	M	6.70	6.7	20	No	Diphenhydramine	UF
1C	86	M	6.23	7.0	21	hx	NDD	UF
GG	30	M	6.91	8.0	18	Yes	NDD	UF, BA10
KS32	60	M	6.32	6.8	20	Yes	Ethanol	UF, BA10
WW	65	M	6.20	6.7	30	Yes	Codeine	UF, BA10
KS66	48	M	6.60	6.6	17	No	NDD	UF
KS12	41	M	6.24	6.7	19	Yes	Chlorpheniramine	BA10
KS24	64	M	6.80	7.2	26	Yes	Ethanol	BA10
Mean	**51.00**		**6.49**	**6.94**	**22.2**			
S.E.M.	**5**		**0.07**	**0.14**	**1.6**			
***p* value**[e]	**0.97**		**0.08**	**0.17**	**0.02**			

[a] RNA integrity number generated by the Agilent 2100e.
[b] Post-mortem interval.
[c] No drugs detectable.
[d] History.
[e] Results of an independent *t*-test comparing MDD group to control group.

(Ordway et al., 2009). CNP immunostaining was performed the same as for GFAP except all incubations occurred at 37 °C. LCM was performed on the Veritas Microdissection Instrument model 704 (Molecular Devices) with CapSure Macro caps (Molecular Devices) as reported previously (Ordway et al., 2009) with modification (see Supplement to Methods). For telomere length measurements, H_2O_2 was omitted from the immunostaining. Two captures were performed, one for DNA and one for RNA because the isolation methods for RNA and DNA differed. For both captures, 500 GFAP+ astrocytes and 500 CNP+ oligodendrocytes were collected by LCM in <2 h, separately from UF and BA10 white matter (Ordway et al., 2009; see Fig. 1). See Supplement to Methods for further description.

Genomic DNA purification from LCM sample

Genomic DNA was isolated from captured cells using a silica-gel membrane based QIAamp DNA Micro Kit (Qiagen, USA) as described previously (Shammas et al., 2008) with modification. To prevent generation of abasic sites on the DNA and to minimize oxidative damage, 50 μM phenyl-tert-butyl nitron (SIGMA, USA) was supplemented (O'Callaghan et al., 2008). For the lysis of cells on LCM caps, the original (56 °C for 10 min) high

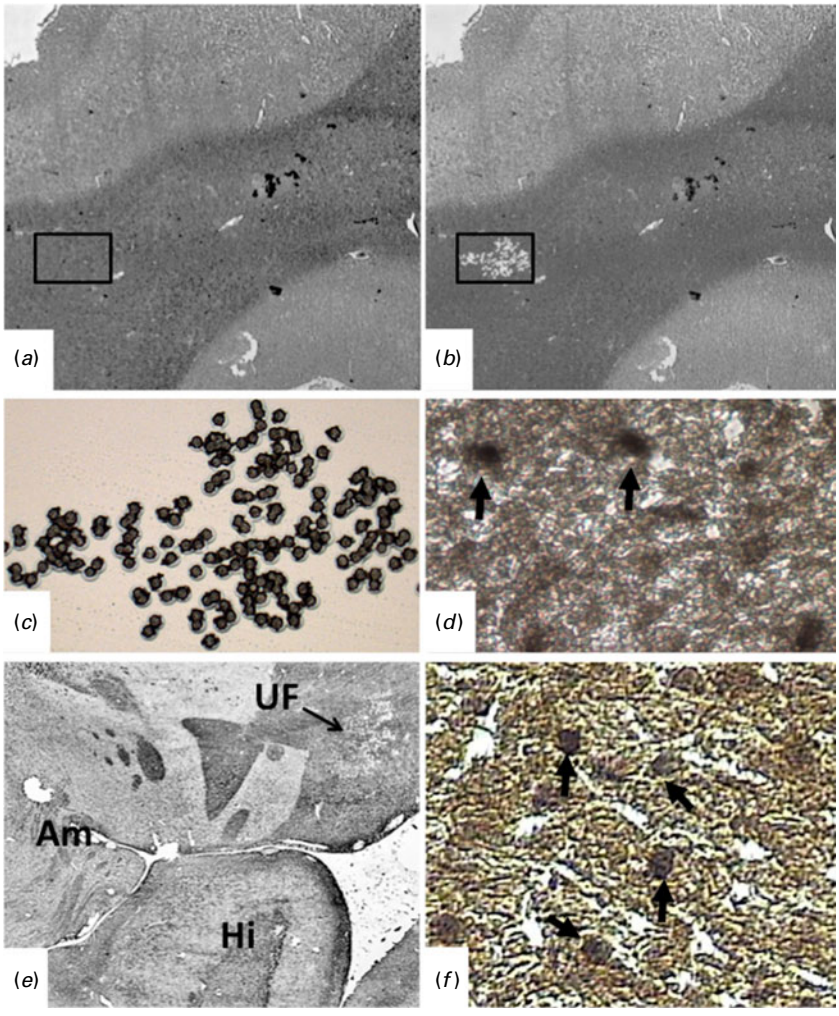

Fig. 1. Laser capture micro-dissection of astrocytes and oligodendrocytes. Panels A (before LCM) and B (after LCM) show the region of BA10 white matter where cells were captured (magnification 2×). Panel C shows (10×) captured astrocytes on the LCM cap from the area of the rectangle in Panel A. Panel D is an image (60×) of GFAP immunostained astrocytes (arrows pointing to astrocytes). Panel E is an image (approximately 2×) of a CNP immunostained section showing the location of the uncinate fasciculus (UF), where oligodendrocytes were captured (Am, amygdala; Hi, anterior hippocampus). Panel E is an image (60x) of CNP immunostained oligodendrocytes (arrows pointing to oligodendrocytes). Note that slides were not coverslipped and appear as they do to the user during cell capture.

temperature of the kit protocol was reduced to 37 °C for 3 h. DNA samples were stored at −80 °C until required.

RNA purification and end-point PCR

Total RNA was isolated from laser-captured cells using the RNAqueous Micro Kit (includes DNase treatment; Ambion, USA) and kept at −80 °C. Reverse transcription was performed as published earlier (Ordway et al., 2009). End-point PCR was performed as previously described using three reference genes (glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*; 18S ribosomal 1 RNA, *RNA18S1*; ubiquitin C, *UBC*) to normalize target gene expression data (Ordway et al., 2009; Chandley et al., 2013). PCR amplicons were collected during the linear range of amplification as determined for each primer set in optimization experiments, and were quantified on an Agilent 2100e Bioanalyser (Agilent Technologies, USA). DNA 1000 Chips (Agilent Technologies) with a quantitative range of 0.1–50 ng/μl were used with the Bioanalyser. All gene primers were designed using GenBank accession numbers as shown in Table S1. See Supplement Methods for further details.

Telomere length analysis with end-point PCR

Genomic DNA from LCM samples was dried to 6.5 μl in a SpeedVac centrifuge and 3×1 μl was used for telomere length measurement and the other 3×1 μl was used for single copy gene (albumin, *ALB*) measurement (Cawthon, 2002). Because small amounts of DNA are available from LCM, end-point PCR was used to quantify relative telomere length as described previously (Ordway et al., 2009). Average relative telomere length as

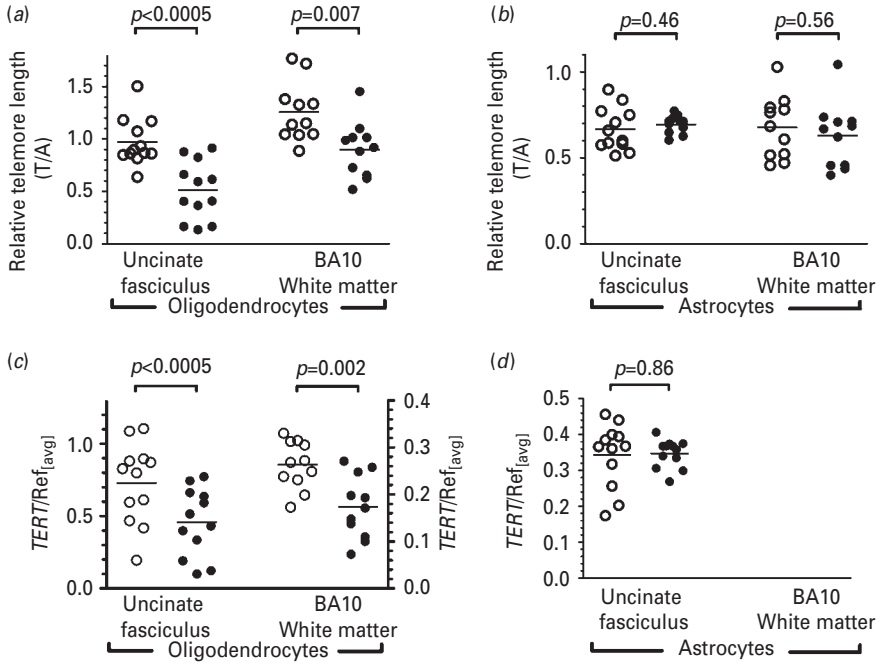

Fig. 2. Relative telomere lengths (A and B) and *TERT* expression (C and D) in laser captured oligodendrocytes and astrocytes from uncinate fasciculus (UF) and BA10 white matter from matched pairs of psychiatrically normal control donors (open symbols) and MDD donors (closed symbols). *TERT* expression was normalized using the averaged expression of stable reference genes (*GAPDH, RNA18S1* and *UBC*; see Supplemental Fig. 1). *p* values from the analysis of covariance with age as the covariate are shown.

represented by the telomere repeat copy number to single copy gene (*ALB*) copy number (T/A) ratio was determined using a modified version of a previously described real-time PCR assay (Cawthon, 2002).

Data analysis

Telomere data was analysed using a one-way between groups analysis of covariance. The independent variable was the diagnostic group; the dependent variables were T/A ratios and TERT gene expression levels. Age was used as a covariate based on the effects of age on telomere length (Blackburn et al., 2006) and *TERT* expression (see below). Gene expression levels of oxidative stress defence enzymes (SOD1, SOD2, GPX and CAT) were analysed using a multivariate analysis of variance. Appropriate preliminary checks were conducted to examine the potential violation of assumptions of analysis of variance. Analysis of variance was performed using IBM SPSS Statistics (version 21.0M). Pearson correlations were performed using GraphPad Prism (version 5.00; GraphPad Software, Inc.).

Results

Telomere lengths

Adjusting for age, T/A values (representing relative telomere lengths) were significantly lower in UF oligodendrocytes in MDD donors as compared to control donors ($F=25.6$, $p<0.0005$; Fig. 2*a*). Likewise, T/A values were significantly lower in oligodendrocytes captured from BA10 white matter comparing MDD to control donors ($F=9.02$, $p=0.007$; Fig. 2*a*). There was a statistically significant relationship between the covariate age and T/A values in UF oligodendrocytes ($F=8.28$, $p=0.009$), but not BA10 white matter oligodendrocytes ($F=0.377$; $p=0.55$). In contrast, T/A values in astrocytes were not significantly different comparing MDD to control donors in either the UF ($F=0.578$, $p=0.46$) or in BA10 white matter ($F=0.354$; $p=0.56$; Fig. 2*b*). Likewise, there were no significant relationships between age and T/A values in astrocytes (UF, $F=2.52$, $p=0.13$; BA10 white matter, $F=0.377$, $p=0.55$).

Telomerase reverse transcriptase

Telomerase reverse transcriptase (*TERT*) expression was measured using cells captured from the same donors as above. Adjusting for age (see below), *TERT* expression in oligodendrocytes was significantly lower in MDD as compared to control donors (UF: $F=23.9$, $p<0.0005$, Fig. 2*c*; BA10 white matter: $F=12.5$, $p=0.002$, Fig. 2*c*). A robust correlation was observed between age and *TERT* expression in UF oligodendrocytes ($F=59.8$, $p<0.0005$), but not in BA10 white matter oligodendrocytes ($F=0.90$, $p=0.36$). *TERT* expression was lower in astrocytes compared to oligodendrocytes. There was no significant difference between donor groups in *TERT* expression in UF astrocytes (Fig. 2*d*; $F=0.03$, $p=0.86$); *TERT* expression was undetectable in BA10 white matter astrocytes. Age significantly correlated with *TERT* expression in UF astrocytes ($F=4.65$, $p<0.05$).

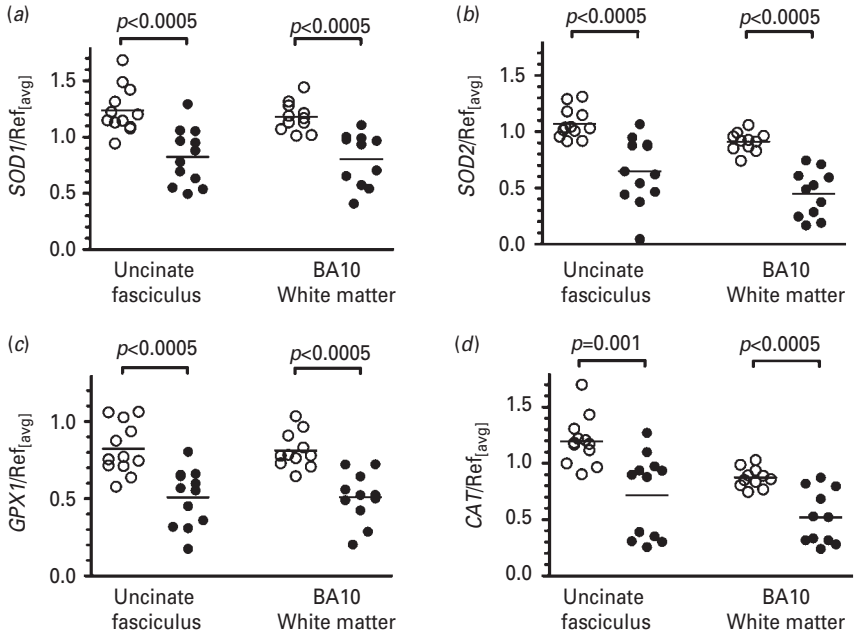

Fig. 3. Gene expression of oxidative stress defence enzymes in oligodendrocytes laser captured from uncinate fasciculus (UF) and BA10 white matter of psychiatrically normal control donors (open symbols) and MDD donors (closed symbols). Gene expression levels are normalized to the average of stable reference genes as noted in Fig. 1. p values from the multivariate analysis of variance are shown.

Oxidative stress defence enzymes

The expression of four antioxidant enzyme genes, *SOD1, SOD2, GPX1* and *CAT* were measured using the same RNA samples as above for *TERT* expression. Gene expression levels of all four antioxidant enzymes were significantly lower in UF oligodendrocytes of MDD donors as compare to control donors (Fig. 3*a–d; SOD1,* $F=19.7$, $p<0.0005$; *SOD2,* $F=20.5$, $p<0.0005$; *GPX1,* $F=19.2$, $p<0.0005$; *CAT,* $F=15.4$, $p=0.001$). Similar MDD-associated reductions in antioxidant gene expressions were observed in oligodendrocytes captured from BA10 white matter (Fig. 3*a–d; SOD1,* $F=21.2$, $p<0.0005$; *SOD2,* $F=46.8$, $p<0.0005$; *GPX1,* $F=25.4$, $p<0.0005$; *CAT,* $F=21.3$, $p<0.0005$). In contrast, gene expression levels of antioxidant enzymes in UF astrocytes were similar in control and MDD donors (Fig. 4*a–d; SOD1,* $F=2.23$, $p=0.09$; *SOD2,* $F=0.00$, $p=0.97$; *GPX1,* $F=0.63$, $p=0.44$; *CAT,* $F=1.43$, $p=0.24$). Likewise, expression levels of these genes in BA10 white matter astrocytes were not significantly different comparing control to MDD donors (Fig. 4*a–d; SOD1,* $F=0.01$, $p=0.91$; *SOD2,* $F=0.76$, $p=0.40$; *GPX1,* $F=0.27$, $p=0.61$; *CAT,* $F=0.36$, $p=0.56$).

Reference genes and analysis of potential confounds and correlates

There were no significant differences between control and MDD groups with regard to age, RNA quality as assessed by RIN, and brain tissue pH (Table 1). PMI was significantly longer for MDD subjects ($p<0.05$; Table 1). No differences in expression levels of reference genes were observed comparing normal control and MDD donor groups (Table S2, Fig S1). Possible influences of age, PMI, pH and RIN values were evaluated on target and reference gene expression levels (Table S3). Given the number (128) of correlations examined, a $p<0.01$ was considered as a potential confound. There were no consistent effects of any of these factors on gene expression levels that spanned across cell types or brain regions. Age significantly correlated with *TERT* expression levels in oligodendrocytes and was used as a covariate in the analyses of these data as noted above. RIN significantly correlated with the expression of *SOD2* only in UF oligodendrocytes ($r^2=0.31$, $p=0.005$). Using RIN as a covariate in the ANOVA for *SOD2* expression levels in UF oligodendrocytes did not alter the overall outcome of the statistical comparison of control and MDD groups (F14.8, $p=0.001$). pH values correlated significantly with *GPX1* expression levels only in BA10 white matter oligodendrocytes ($r^2=0.32$, $p=0.006$), and using pH as a covariate in the ANOVA did not alter the overall outcome of the analysis of control and MDD groups ($F=15.1$, $p=0.001$). As an additional check, pH, PMI, and RIN were used as covariates in the ANOVA (regardless of the results of Pearson correlation analysis) of all other gene expressions as well as the T/A values from oligodendrocytes and astrocytes and statistical conclusions were unaltered by these analyses.

For MDD cases where known (seven cases for BA10 white matter; ten cases for UF), the duration of illness did not correlate with the T/A ratios in both cell types in both brain regions (Table S4). There was no significant

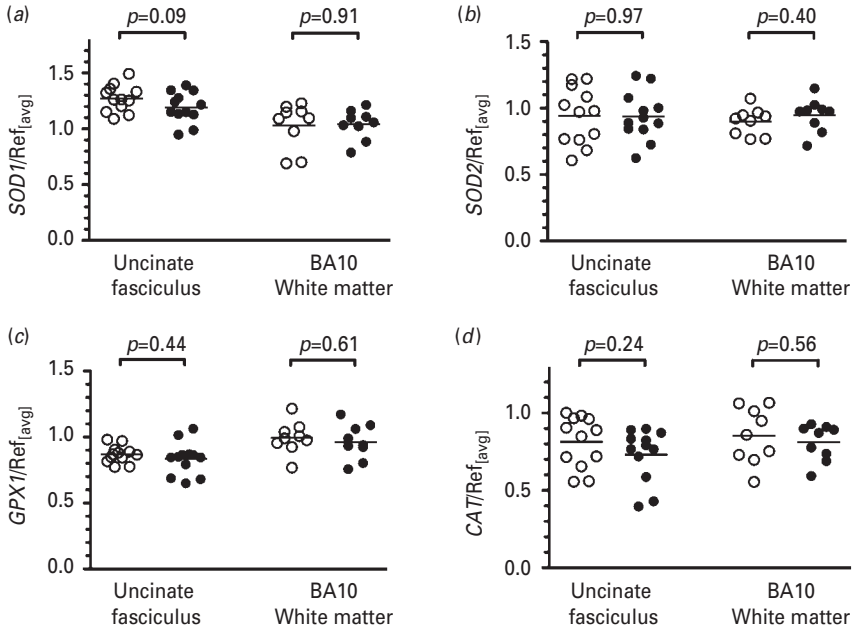

Fig. 4. Gene expression of oxidative stress defence enzymes in astrocytes laser captured from uncinate fasciculus (UF) and BA10 white matter of psychiatrically normal control donors (open symbols) and MDD donors (closed symbols). Gene expression levels are normalized to the average of stable reference genes as noted in Fig. 1. *p* values from the multivariate analysis of variance are shown.

correlation between duration of illness and the gene expressions of *TERT* and the antioxidant enzymes in both cell types and in both brain regions (Table S4). Given the reductions in gene expressions and relative telomere lengths in MDD subjects, the potential correlation of relative telomere length to expression levels of *TERT* and the antioxidant enzymes was examined, but there were no significant correlations (Table S5).

As noted in the Method section above, three MDD subjects had alcohol-related disorders. To consider the possibility that chronic alcohol ingestion might affect telomere length or gene expressions, all data were re-analysed removing the three subjects with alcohol-related comorbidity. The statistical conclusions as reported above were not affected by removal of these subjects (see Table S6).

Discussion

Relative telomere lengths were significantly lower in oligodendrocytes captured from MDD donors in both the UF and white matter of the prefrontal cortex (BA10) as compared to healthy age-matched control donors. In contrast, relative telomere lengths in astrocytes from the same brain regions were similar in MDD and control donors. Recent studies demonstrate shortened telomeres in leukocytes of MDD patients, and also in subjects exposed to extended periods of psychological stress (Hoen et al., 2011; Wolkowitz et al., 2011a; Garcia-Rizo et al., 2013; Puterman et al., 2013). However, telomere shortening was not observed previously in post-mortem brain tissues from MDD (Teyssier et al., 2010; Zhang et al., 2010). These past brain findings have been used to reason that brain cells may not be susceptible to the same factors that contribute to telomere shortening in leukocytes. However, tissues examined in these earlier brain studies were grossly dissected and would be expected to contain many cell types (e.g. neurons, glia, vascular and immune cells). Different cell types are unequally vulnerable to factors, such as oxidative stress, that contribute to shortened telomeres. The present study is the first to examine telomere length in single cell populations from post-mortem human brain in any psychiatric disorder, and our findings demonstrate that telomere shortening occurs in brain white matter oligodendrocytes in MDD as has been shown in leukocytes.

A mechanism that may contribute to telomere shortening in brain oligodendrocytes in MDD is altered expression of telomerase. Telomerase is a nuclear enzyme that maintains telomere length through the addition of nucleotides. *TERT* is the reverse transcriptase enzyme component of telomerase. We found the expression of *TERT* in oligodendrocytes, but not astrocytes, to be significantly lower in MDD subjects as compared to control subjects. Lowered *TERT* expression could translate to reduce telomerase activity, ultimately resulting in shorter telomeres. However, it is worth noting that Wolfkowitz and co-workers demonstrated elevation of telomerase activity in peripheral blood mononuclear cells from MDD patients as compared to control subjects (Wolkowitz et al., 2012), despite shorter telomeres in those MDD patients (Wolkowitz et al., 2011a). Unfortunately, we

were unable to perform telomerase activity measurements given the minute amounts of tissue obtained from LCM of oligodendrocytes. Nevertheless, the lower expression of *TERT* in MDD implicates perturbed regulation of telemorase in MDD oligodendrocytes.

Telomere shortening is considered a biomarker for accelerated cellular aging resulting from cumulative stress exposure (Von Zglinicki and Martin-Ruiz, 2005; Hartmann et al., 2010; Wolkowitz et al., 2010, 2011a; Hoen et al., 2011; Malan et al., 2011; Wikgren et al., 2012). The molecular mechanisms contributing to telomere shortening in leukocytes in MDD patients are uncertain, but are assumed to be similar to mechanisms at play in brain oligodendrocytes. Elevated exposure to ROS may facilitate leukocyte telomere shortening in depressive disorders. Guanine nucleotides, abundant in telomeric DNA, are particularly susceptible to oxidation reactions induced by ROS, resulting in activation of DNA damage repair mechanisms that contribute to telomere shortening (Rhee et al., 2011). Numerous studies demonstrate evidence of elevation of ROS in depressive disorders (Leonard and Maes, 2012), including increased oxidation of guanine nucleotides (Irie et al., 2005; Forlenza and Miller, 2006; Maes et al., 2009), in peripheral tissues of depressed patients.

To investigate the potential role of oxidative mechanisms in telomere shortening in MDD oligodendrocytes, the expression of oxidative stress defence genes *SOD1, SOD2, GPX1* and *CAT* were measured. SOD1 and SOD2 catalyze dismutation of superoxide anions to H_2O_2 and O_2 in the cytosol and mitochondria, respectively. H_2O_2 is further detoxified by CAT and GPX1 to H_2O and O_2. Gene expression levels of all four oxidative stress defence enzymes were significantly lower in oligodendrocytes from MDD donors as compared to normal control donors. GPX and SOD activities are reduced by glucocorticoids (Pereira et al., 1995; McIntosh et al., 1998a, b; Schmidt et al., 2005; Verhaeghe et al., 2009; You et al., 2009) and it is conceivable that reduced *GPX* and *SOD* expression in MDD oligodendrocytes is secondary to elevated cortisol or related stress hormones widely believed to accompany depression. However, an explanation for down-regulation of *GPX* and *SOD* expression in oligodendrocytes but not astrocytes cannot be provided. Regardless of the potential association with stress hormones, white matter oligodendrocytes in MDD subjects demonstrate accelerated aging through telomere shortening that may have resulted from pathologic compensation of cellular mechanisms designed to protect the telomere from oxidative stress-induced degradation.

Brain astrocytes may be more resistant to oxidative stress damage when compared to oligodendrocytes. Greater resistance of astrocytes to reactive oxygen species has been demonstrated previously and is thought to be derived from low iron content, high glutathione concentration, and high glutathione peroxidase activity (Juurlink et al., 1998). In contrast, oligodendrocytes appear to be vulnerable to intracellular free radicals produced during intensive cell respiration. Oligodendrocytes have extensive lipid membranes that are a primary target of free radicals (Kim and Kim, 1991; Thorburne and Juurlink, 1996). Furthermore, production of lipids during myelination requires peroxisome activity that generates H_2O_2 and increases the oxidative load on oligodendrocytes, cells with lower glutathione reductase enzyme activity than astrocytes (French et al., 2009).

It is intriguing to consider the present findings with regard to the role of white matter pathology in the genesis or sustention of MDD. White matter pathologies have been demonstrated using either neuroimaging methods in living MDD patients or post-mortem tissues from MDD donors (Tham et al., 2011). For example, white matter hyper-intensities on MRI scans are more prevalent in patients with MDD (Nobuhara et al., 2006; Potter et al., 2007; Taylor et al., 2007; Köhler et al., 2010; Tham et al., 2011). Low expression of multiple oligodendrocyte genes has been shown in post-mortem temporal cortex from MDD donors (Aston et al., 2005). Reduced myelin staining (Regenold et al., 2007) and reduced density of oligodendrocytes (Uranova et al., 2004) have been observed in post-mortem prefrontal cortex in MDD. The present findings of oligodendrocyte telomere shortening in MDD, possibly induced by oxidative stress, may be aetiologically linked to these white matter pathologies in MDD.

Study limitation

Sample sizes in the present study are relatively small and larger scale studies are warranted. For the analysis of antioxidant enzymes and *TERT*, sufficient amounts of laser captured oligodendrocytes were not obtainable to perform quantitative immunoblotting or to analyse enzyme activities. Hence, interpretations assume that changes gene expression predict biological outcomes, such as changes in enzyme activity. However, changes in gene expression do not always parallel changes in protein levels derived from the same gene, just as changes in protein levels do not always align with changes in the function of that protein because function can be modified by post-translational modifications or by changes in intracellular trafficking. Nevertheless, it seems reasonable to assume that changes in gene expression do predict the presence of a perturbed or abnormal cellular process. We were unable to obtain sufficiently strong immunostains of tissue sections to analyse levels antioxidant enzymes in white matter. It would be interesting to know whether the number of oligodendrocytes in white matter was lower in MDD subjects than controls. However, it is noteworthy that for LCM, reduced gene expressions in MDD cannot be accounted for by reduced cell numbers because the same number of cells are

captured from MDD and control subjects for the RNA isolations. We did not have blood from subjects of this study to determine whether leukocytes from the same MDD subjects had reduced telomere lengths. Finally, while shortened telomere lengths were accompanied by reductions in *TERT* and antioxidant enzyme gene expressions in MDD oligodendrocytes, there were no significant correlations between telomere lengths and levels of *TERT* or antioxidant gene expressions.

Telomere shortening in mononuclear blood cells has been described in normal aging and in numerous psychiatric, neurological and medical disorders (Epel et al., 2006; Simon et al., 2006; Wolkowitz et al., 2010; Armanios and Blackburn, 2012; Wikgren et al., 2012). Similarly, an increased frequency of white matter hyperintensities has been observed in normal aging and a variety of psychiatric, neurological and medical disorders. Whether telomere shortening occurs in brain oligodendrocytes in disorders other than MDD is currently unknown, and was not addressed in the present study. If telomere shortening in oligodendrocytes contributes to the aetiology of white matter hyper-intensities, it seems unlikely that telomere shortening in oligodendrocytes will be found to be specific to MDD. Likewise, it remains undetermined whether telomere shortening occurs in all white matter areas or is restricted to regions of the brain that process emotionally charged information. Furthermore, it remains undetermined whether oligodendrocytes or specific populations of neurons in grey matter regions are similarly affected in MDD. Additional research is required to fill these numerous gaps in knowledge concerning telomere shortening and its role in brain disorders.

Conclusions

The findings of this study demonstrate cell-specific shortening of telomeres in white matter in MDD. The affected cells, oligodendrocytes, also demonstrate evidence of disrupted mechanisms that normally maintain telomere length or protect the telomere from oxidative stress-induced degradation. Since oligodendrocytes myelinate axon fibres traveling through white matter, oligodendrocytes with diminished function as a result of oxidative stress could contribute to disturbed neuronal communication between key brain regions. The precise relationship between telomere lengths, oxidative stress defence capacity and oligodendrocyte function remains to be determined. Nevertheless, it seems reasonable to consider that medicines that preserve telomere length in these cells may be beneficial to mental health.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000698.

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Statement of Interest

None.

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