


## RESEARCH ARTICLE

# Metallothionein-I/II expression associates with the astrocyte DNA damage response and not Alzheimer-type pathology in the aging brain

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**Funding information**

MRC, Grant/Award Number: MRC/G0900582; Alzheimer's Society, Grant/Award Number: AS386, AS242, and AS248; Sheffield charity Neurocare; UK NIHR Biomedical Research Centre for Ageing and Age - awarded to Newcastle-upon-Tyne Hospitals Foundation Trust; NIHR Cambridge Biomedical Research Centre; Nottingham University Hospitals NHS Trust; Sheffield Teaching Hospitals NHS Foundation Trust and Sheffield NIHR Biomedical Research Centre; Oxford Biomedical Research Centre; Walton Centre NHS Foundation Trust

**Abstract**

Oxidative stress and oxidative DNA damage are early features of mild cognitive impairment and Alzheimer's disease (AD), occurring before the formation of classical AD neuropathology, and resulting from an imbalance between pro- and anti-oxidants. Astrocytes play a major neuroprotective role, producing high levels of anti-oxidants including metallothionein-I and -II (MT-I/II). In the present study we characterized the immunoreactive profile of MT-I/II in the temporal cortex of the Cognitive Function and Ageing Study (CFAS) aging population-representative neuropathology cohort, and examined H<sub>2</sub>O<sub>2</sub>-modulation of MT transcription by human astrocytes. MT-I/II is primarily expressed by astrocytes in the aging brain, but is also associated with pyramidal neurons in a small proportion of cases. Astrocyte expression of MT-I/II does not correlate with Alzheimer-type pathology (A $\beta$  plaques and neurofibrillary tangles) but does relate to astrocyte oxidative DNA damage ( $r_s = .312$ ,  $p = .006$ ) and the astrocyte response to oxidative DNA damage *in vivo* ( $r_s = .238$ ,  $p = .04$ ), and MT gene expression is significantly induced in human astrocytes response to oxidative stress *in vitro* ( $p = .01$ ). In contrast, neuronal MT-I/II does not relate to oxidative DNA damage or the neuronal DNA damage response, but is significantly higher in cases with high levels of local tangle pathology ( $p = .007$ ). As MT-I/II is neuroprotective against oxidative stress, modulation of MT-I/II expression is a potential therapeutic target to treat the onset and progression of cognitive impairment.

1. MT-I/II is expressed by astrocytes in the aging brain.
2. MT-I/II expression does not correlate with Alzheimer pathology but to astrocyte oxidative DNA damage *in vivo*.
3. In response to oxidative stress MT genes are induced in human astrocytes *in vitro*.

**KEYWORDS**

Alzheimer's disease, astrocyte, metallothionein-I/II, oxidative stress

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## 1 | INTRODUCTION

Oxidative stress is a feature of brain aging which gives rise to the oxidative modification of proteins, lipids, DNA and RNA and plays a key role in several neurodegenerative pathologies, including Alzheimer's

disease (AD). Both oxidative stress and oxidative DNA damage are early features of mild cognitive impairment (MCI) and AD, occurring before the formation of classical AD neuropathology, including extracellular deposits of  $\beta$ -amyloid peptides (A $\beta$  plaques) and intracellular deposits of hyperphosphorylated tau (neurofibrillary tangles, NFT; Keller et al., 2005; Nunomura et al., 2006; Nunomura et al., 2001).

Oxidative DNA damage, in particular DNA double-strand breaks, is a potent inducer of a DNA damage response (DDR), which is characterized by the activation of sensor kinases, including DNA-protein kinase catalytic subunit (DNA-PKcs), and regions of DNA damage incorporating phosphorylated histone  $\gamma$ H2AX. We have previously shown an association between the neuronal DDR and cognitive impairment, independent of AD pathology in the ageing brain (Simpson et al., 2015), and demonstrated that increased levels of oxidative stress and the astrocyte DDR are an early event and not simply a secondary response to established AD pathology (Simpson, Ince, Haynes, et al., 2010).

Astrocytes play a major neuroprotective role, producing high levels of anti-oxidants (Ben Haim, Carrillo-de Sauvage, Ceyzeriat, & Escartin, 2015; Liddell, Robinson, Dringen, & Bishop, 2010). Metallothioneins (MT) are low molecular weight cysteine-rich proteins with a high metal content that can protect against reactive oxygen species (ROS; Vasak & Meloni, 2011). They comprise four subfamilies: MT-I and MT-II are present in most tissues, with MT-III and MT-IV expression confined to the central nervous system (CNS) and stratified squamous epithelium, respectively. While MT-I and -II have a similar structure, differing only by one amino acid and their ability to bind different metal ions, they are generally expressed in response to the same stimuli and perform the same function, so they are commonly grouped together and referred to as MT-I/II (Artells, Palacios, Capdevila, & Atrian, 2013). Within the CNS, MT-I/II is predominantly expressed by astrocytes, but has also been detected in neurons, microglia and endothelial cells (Pedersen et al., 2009; West, Hidalgo, Eddins, Levin, & Aschner, 2008), and is highly inducible in response to a variety of stimuli, including oxidative stress, neuroinflammation and toxic levels of metal ions (Andrews, 2000; Hidalgo et al., 2006; Manso, Adlard, Carrasco, Vasak, & Hidalgo, 2011). *MT-I/II* gene expression is regulated by antioxidant response elements, and reduced expression of *MT-I/II* in cell models or knock out animals results in heightened sensitivity to oxidative stress (Pitt et al., 1997; Qu, Pi, & Waalkes, 2013). *MT-I/II* is upregulated in AD (Adlard, West, & Vickers, 1998; Zambenedetti, Giordano, & Zatta, 1998) and is primarily associated with A $\beta$  plaques in the hippocampus of several AD animal models (Carrasco et al., 2006; Hidalgo et al., 2006).

Given the major role oxidative stress plays in the pathogenesis of neurodegenerative pathologies the present study evaluated *MT-I/II* expression in the temporal cortex of the Cognitive Function and Ageing Study (CFAS) neuropathology cohort, a well characterized large, ageing population-representative cohort which facilitates unbiased assessment of relationships between pathology and dementia, with the aim of assessing the relationship between *MT-I/II* and Alzheimer-type pathology and the association with the astrocyte DDR in the aging human brain. In addition, the direct effect of oxidative stress on *MT-I/II* expression by human astrocytes was also assessed.

## 2 | MATERIALS AND METHODS

### 2.1 | Human CNS cases

Human autopsy brain tissue was obtained from one center of the Cognitive Function and Ageing Study (CFAS), following multi-center research ethics committee (REC) approval (REC Reference number 15/SW/0246; Wharton et al., 2011). Neuropathological lesions were assessed as part of the core CFAS neuropathology study using a modified protocol from the Consortium to Establish a Registry of Alzheimer's Disease (CERAD; www.cfas.ac.uk; Mirra, 1997) and Braak neurofibrillary tangle staging (Braak & Braak, 1991). The cases were categorized into three groups representing entorhinal stages (Group 1; Braak Stages 0–II; 21 cases), limbic stages (Group 2; Braak Stages III–IV; 14 cases) and isocortical stages of tangle pathology (Group 3; Braak Stages V–VI; 43 cases). The mean age of death was 86.8 standard error of the mean (SEM 6.98) years. The median post-mortem delay was 16 hr, interquartile range (IQR 9–35.5 hr) and brain pH 6.49 (IQR 6.2–6.7). Formalin-fixed lateral temporal cortex samples (superior/middle temporal gyrus, Brodmann areas 22/21) were available for all cases. The astrocyte DDR ( $\gamma$ H2AX nuclear immunoreactivity), oxidative DNA damage (8-hydroxydeoxyguanosine, 8-OHdG), astrogliosis (GFAP), and local measures of AD-type pathology (A $\beta$  and AT8) were previously assessed in these cases (Simpson, Ince, Haynes, et al., 2010; Simpson, Ince, Lacey, et al., 2010; Wharton et al., 2009). A total of 78 participants were included in these analyses, where 48 were female.

### 2.2 | Immunohistochemistry

Immunohistochemistry was performed using a standard avidin-biotin complex (ABC) method. Sections were deparaffinized, rehydrated to water and endogenous peroxidase activity quenched by placing the sections in 0.3% H<sub>2</sub>O<sub>2</sub>/methanol for 20 min at room temperature (RT). Sections were subjected to antigen retrieval (Access Super pH9.5, pressure cooker [Menarini Diagnostics, UK]). Following incubation with 1.5% normal serum for 30 min at RT, the sections were incubated for 60 min at RT with the well characterized, commercially available mouse monoclonal antibody against *MT-I/II* (1:100; DakoCytomation, UK). To visualize antibody binding, the horse-radish peroxidase avidin biotin complex (ABC-HRP) was used (Vectastain Elite kit, Vector Laboratories, UK) with 3,3'-diaminobenzidine (DAB) as the chromogen (Vector Laboratories, UK; brown). Negative controls, either omission of the primary antibody or a mouse isotype control, were included in every run.

To determine if *MT-I/II*<sup>+</sup> astrocytes colocalized with A $\beta$  plaques, NFT or the astrocyte DDR, sequential dual labeling was performed with *MT-I/II* and either A $\beta$ , AT8 or  $\gamma$ H2AX. Briefly, amyloid plaques (mouse monoclonal A $\beta$  1:100; DakoCytomation, UK), tau tangles (mouse monoclonal AT8 1:400; Endogen, UK) or the astrocyte DNA damage response (rabbit polyclonal  $\gamma$ H2AX 1: 1,000, R&D Systems, UK) were first visualized using the standard ABC-HRP protocol with DAB as substrate described above. For A $\beta$  immunohistochemistry, antigen retrieval was carried out following pre-treatment with formic acid for 40 min at RT. Sections were then incubated with the

avidin-biotin blocking kit (Vector Laboratories, UK), according to the manufacturer's instructions. The tissue was incubated overnight at 4 °C with anti-MT-I/II (1:100), followed by biotinylated secondary antibody, and visualized with the alkaline-phosphatase-conjugated avidin-biotin complex and alkaline phosphatase substrate 1 (Vector Laboratories, UK, red). Every immuno-run included a single-labeled section, which showed the same pattern and intensity of immunoreactivity as seen in the dual-labeling experiments.

### 2.3 | Quantitative analysis of MT-I/II

Assessment of MT-I/II-specific immunoreactivity was performed by capturing bright-field microscopic images in three adjacent 350  $\mu\text{m}$ -wide cortical ribbons, consisting of contiguous fields to cover the total cortical thickness through the apex of the gyrus, using a 20 $\times$  objective (Nikon Eclipse Ni-U microscope, Nikon, UK) and analyzed using the Analysis D software (Olympus Biosystems, Watford, UK). The image was thresholded and the immunoreactive area of the field determined per total area examined.

### 2.4 | Primary human astrocytes

Previously characterized (Garwood et al., 2015), human primary astrocytes were obtained from ScienCell Research Laboratories (Carlsbad, CA). Astrocytes were cultured in Human Astrocyte media (ScienCell Research Laboratories) supplemented with 2% fetal bovine serum (FBS), 1% penicillin streptomycin and 1% Astrocyte Growth Supplement (ScienCell Research Laboratories). To characterize the effect of oxidative stress in MT-I/II expression, astrocytes were cultured in the presence and absence of 50  $\mu\text{M}$  hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) for 1 or 24 hr at passage 7. Cell viability tests were not performed, however previous studies have shown that this concentration of  $\text{H}_2\text{O}_2$  is sublethal to astrocytes (Crowe et al., 2016).

### 2.5 | Immunocytochemistry

Cells were fixed in 4% paraformaldehyde in PBS for 5 min at 37 °C, then permeabilized (0.3% Triton X-100 in PBS) and blocked with 3% bovine serum albumin for 20 min at RT. Cells were incubated with MT-I/II (1:100) for 60 min at RT, followed by biotinylated secondary antibody, and visualized with streptavidin alexa fluor 488 (1:500). Nuclei were counterstained with Hoechst 33342, and the staining visualized using a Nikon Eclipse Ni-U microscope (Nikon, Kingston upon Thames, UK).

### 2.6 | Quantitative real-time polymerase chain reaction (qPCR)

Cultured astrocytes were washed with PBS and lysed in 110  $\mu\text{l}$  (1 ml/10  $\text{cm}^2$ ) Trizol (Thermoscientific, UK). RNA was isolated using the Direct-Zol RNA Miniprep Kit with Zymo-Spin IIC Columns (Zymo, Irvine, CA) and total RNA (~500 ng) was incubated at 25 °C for 5 min and reverse transcribed at 42 °C for 30 min followed by incubation at 85 °C for 5 min in a reaction mix containing qScript (Quanta Biosciences, Gaithersburg, MD). qPCR was performed using IDT PrimeTime qPCR assays (Integrated DNA Technologies, UK) containing 50 ng

cDNA, 500 nM primers, 250 nM probe, and Brilliant qPCR mix (Agilent, UK). Primers for MT2A and  $\beta$ -actin were designed to span between adjacent exons (MT2A Primer 1: 5'-AGCTTTTCTTGCAAGAGGTG-3', Primer 2: 5'-GCAACCTGTCCCGACTCTA-3', probe: 5'-FAM-AGCTCGC-CATGGATCCCAACT-3';  $\beta$ -actin Primer 1: 5'-GTCCCCAACTTGA-GATGTATG-3', Primer 2: 5'-AAGTCAGTGTACAGGTAAGCC-3', probe: 5'-FAM-CCATGTACGTTGCTATCCAGGCTGT-3'). Following denaturation at 95 °C for 3 min the products were amplified (40 cycles at 95 °C for 10 s and 60 °C for 30 s) using a CFX384 Touch RT PCR detection system (Bio-Rad, UK).  $\beta$ -actin was amplified on each plate to normalize expression levels of target genes between different samples using the  $\Delta\Delta\text{Ct}$  calculation (ABI) and to assess assay reproducibility.

#### 2.6.1 | Statistical analysis

As the immunohistochemistry data was skewed, median and interquartile range (IQR) was used for descriptive analyses. Spearman's correlation coefficient ( $r_s$ ) was calculated to verify the strength of correlations between continuous variables, and Kruskal-Wallis (KW) to test the correlation with parametric data. All tests were two-tailed. Student's paired t test was used to analyze the qPCR data. Statistical analyses were performed using statistical package IBM SPSS Statistics version 23 and GraphPad Prism version 7.

## 3 | RESULTS

### 3.1 | MT-I/II predominantly immunolabels astrocytes in the aging human temporal cortex

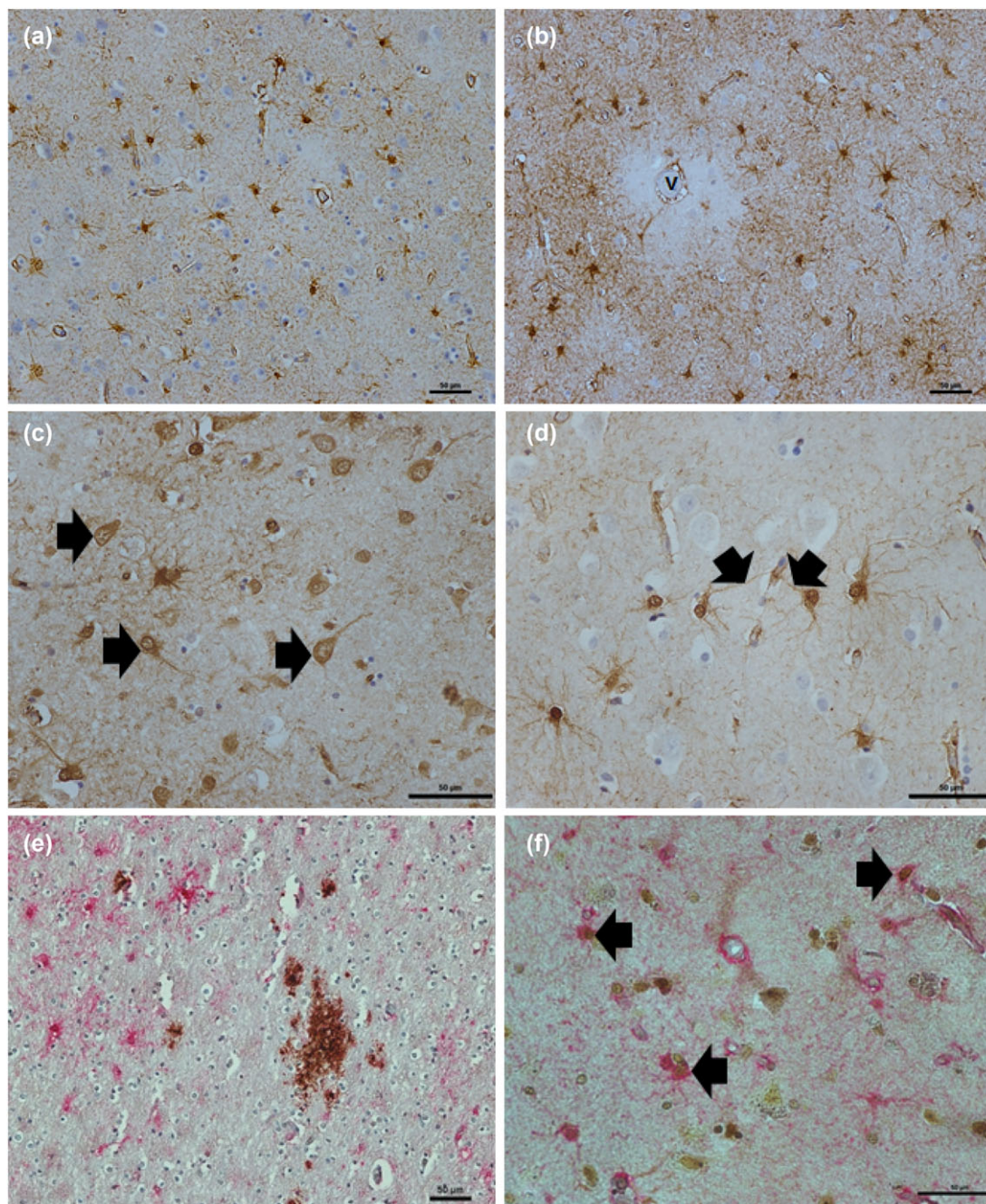
MT-I/II was associated with astrocytes, predominantly staining the astrocyte cell body and their primary radiating processes throughout layers II–VI of the temporal cortex (Figure 1a). A punctate pattern of staining was observed throughout the parenchyma, likely reflecting immunolabeling of cross-sections of the astrocyte processes. The pattern and intensity of immunoreactivity varied greatly across the cohort (median 7.31%, IQR 5.77), with some cases showing reduced staining, particularly surrounding blood vessels (Figure 1b).

While MT-I/II predominantly labeled astrocytes, weak immunoreactivity was also associated with a subpopulation of neurons in 16.6% of cases (13 out of 78 cases, Figure 1c), but no microglial, oligodendrocyte, or endothelial staining was detected. The immunoreactivity associated with capillaries and blood vessels likely represents MT-I/II<sup>+</sup> astrocytes, whose foot processes line the blood brain barrier (Figure 1d). No specific immunoreactivity was observed in the negative controls.

### 3.2 | Astrocyte expression of MT-I/II does not relate to global or local Alzheimer-type pathology

To analyze the relationship of MT-I/II<sup>+</sup> astrocytes with measures of global Alzheimer-type pathology the variation of expression was determined between Braak and Braak neurofibrillary tangle groups (entorhinal, Stages I–II; limbic, Stages III–IV; isocortical, Stages V–VI). There were no differences in MT-I/II expression between Braak and Braak groups (Kruskal-Wallis  $p = .314$ ) (Figure 2a). We also compared





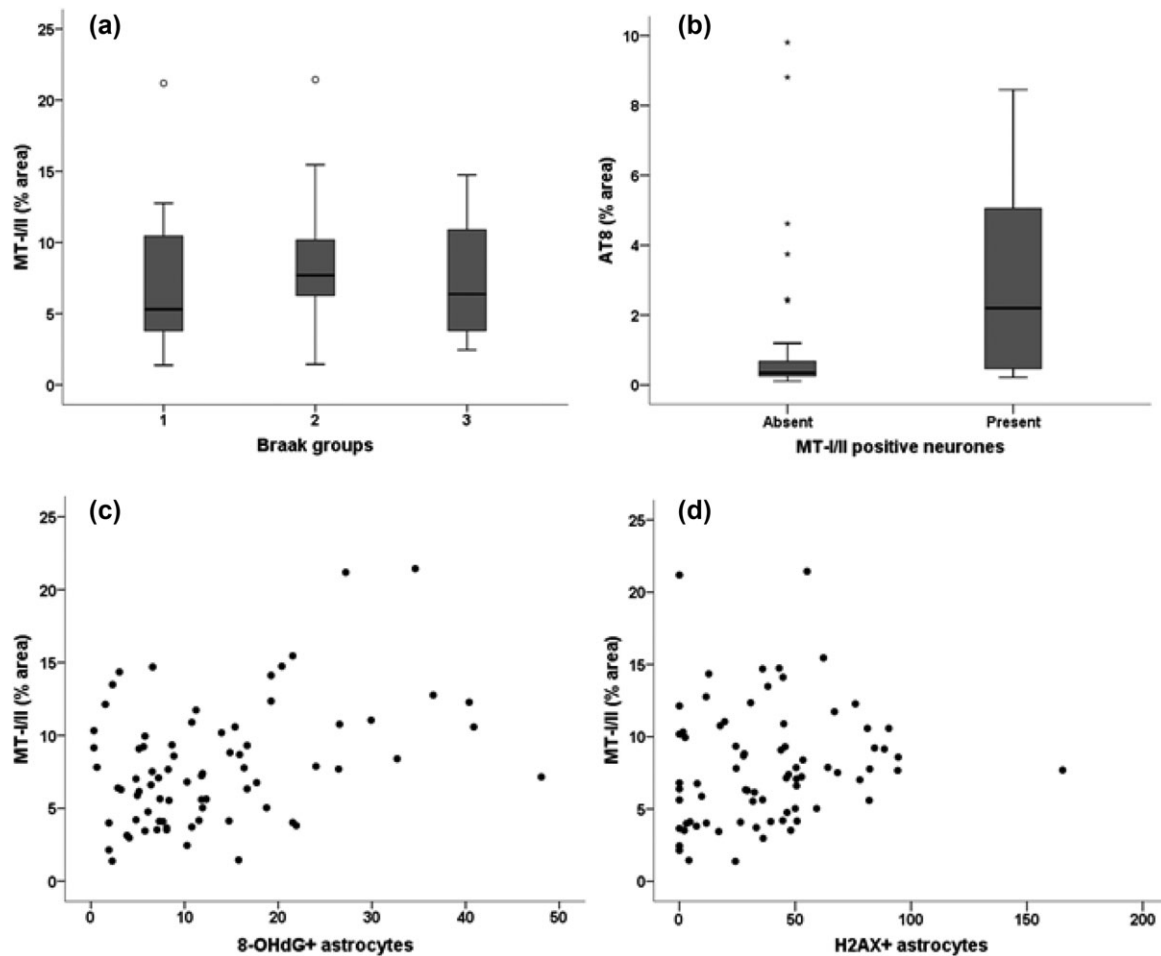
**FIGURE 1** MT-I/II expression in the aging brain. (a) MT-I/II predominantly immunolabeled astrocyte cell bodies and primary radiating processes. (b) A reduced pattern of staining was observed in some cases, particularly surrounding blood vessels (v). (c) MT-I/II<sup>+</sup> neurons were present in some cases (indicated by the arrows). (d) The staining associated with blood vessels likely represents MT-I/II<sup>+</sup> astrocyte foot processes (indicated by the arrows). (e) MT-I/II<sup>+</sup> astrocytes (red) were not associated with A $\beta$  plaques (brown). (f) In contrast, nuclear immunoreactivity for  $\gamma$ H2AX (brown) was detected in a proportion of MT-I/II<sup>+</sup> astrocytes (red). Scale bar represents 50  $\mu$ m [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

MT-I/II to measures of local AD neuropathology in temporal cortex. For comparisons with CERAD scores of diffuse and neuritic plaques and neurofibrillary tangles, the moderate-severe score groups were combined. MT-I/II expression was not associated with neuritic plaques (Kruskal-Wallis  $p = .697$ ), diffuse plaques ( $p = .871$ ) or neurofibrillary tangles ( $p = .891$ ) and there were no correlations with area immunoreactivity for phospho-tau (AT8; Spearman's rank,  $r_s = .081$ ,  $p = .498$ ) or A $\beta$  ( $r_s = .001$ ,  $p = .99$ ). MT-I/II expression did not correlate with post-mortem delay ( $r_s = .113$ ,  $p = .37$ ) or brain pH ( $r_s = .038$ ,

$p = .771$ ). Furthermore, dual labeling demonstrated that while MT-I/II<sup>+</sup> astrocytes were a prominent feature of the aging brain, they did not intimately associate with the majority of A $\beta$  plaques (Figure 1e).

### 3.3 | Cases with MT-I/II + neurons have higher levels of local tau pathology

MT-I/II<sup>+</sup> neurons were present in a small number of the cases assessed in this study (13 out of 78 cases). To determine if MT-I/II<sup>+</sup>



**FIGURE 2** MT-I/II expression is associated with the astrocyte response to DNA damage. (a) MT-I/II expression did not associate with global Alzheimer-type pathology (Braak stage). (b) In contrast, cases with MT-I/II + neurons had significantly higher levels of tau pathology (AT8% area immunoreactivity,  $p = .007$ ). (c) MT-I/II expression significantly correlates with the number of 8OHdG + glia ( $r_s = .312$ ,  $p = .006$ ) and (d)  $\gamma$ H2AX + astrocytes in the aging temporal cortex ( $r_s = .238$ ,  $p = .04$ )

neurons related to AD pathology, the cases were dichotomized into low (Braak and Braak Stages I–III) and high Braak stages (Braak and Braak stages IV–VI). The proportion of cases with MT-I/II<sup>+</sup> neurons was significantly higher in the high Braak stage group (28%) compared with the low Braak stage group (7%; Chi Square 1df,  $\chi^2 = 5.94$ ,  $p = .015$ ). We further tested whether cases with MT-I/II<sup>+</sup> neurons had higher levels of local AD-type pathology. AT8 immunoreactivity (% area) was higher in cases with MT-I/II<sup>+</sup> neurons compared with cases without (median 2.2% vs. 0.35%,  $p = .007$ ; Figure 2b), whereas there was no difference in A $\beta$  immunoreactivity (% area). Additionally, there was no difference in the proportion of  $\gamma$ H2AX<sup>+</sup> neurons in cases with MT-I/II<sup>+</sup> neurons compared with cases without, demonstrating that MT-I/II<sup>+</sup> neurons are not associated with the neuronal DNA damage response.

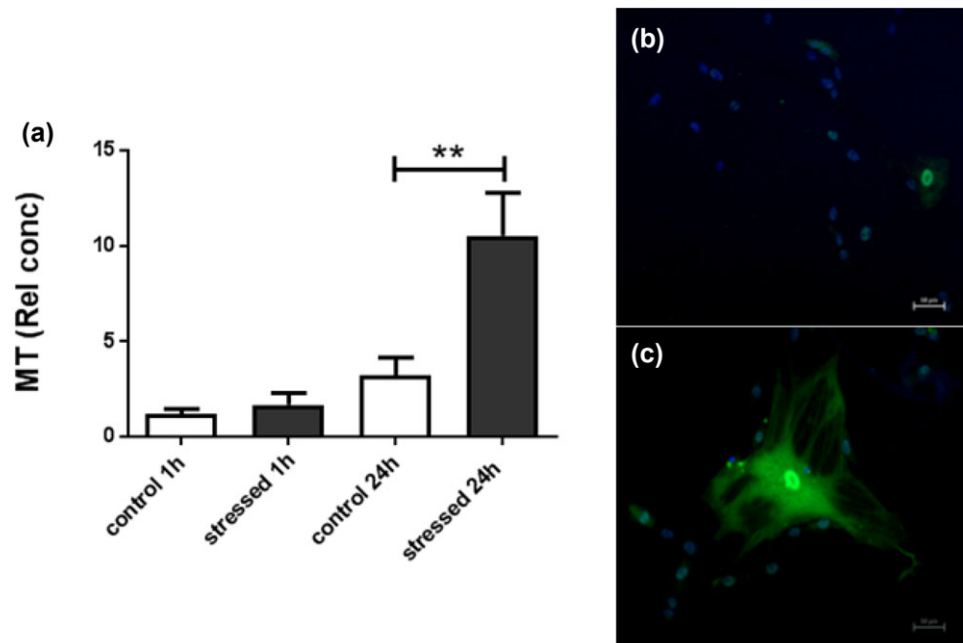
### 3.4 | Astrocyte expression of MT-I/II correlates with the astrocytic DNA damage response

As MT-I/II plays an anti-oxidant role, we investigated the relationship between MT-I/II expression, oxidative DNA damage and the astrocyte DNA damage response in the ageing brain. MT-I/II expression

significantly correlates with the number of 8OHdG<sup>+</sup> glia ( $r_s = 0.312$ ,  $p = .006$ ; Figure 2c) and  $\gamma$ H2AX<sup>+</sup> astrocytes in the ageing temporal cortex ( $r_s = .238$ ,  $p = .04$ ; Figure 2d). Dual labeling confirmed nuclear immunoreactivity for  $\gamma$ H2AX in a proportion of MT-I/II<sup>+</sup> astrocytes (Figure 1f).

### 3.5 | Oxidative stress induces MT-I/II expression in human astrocytes *in vitro*

Human astrocytes were cultured with a sublethal dose of H<sub>2</sub>O<sub>2</sub> to further investigate the effect of oxidative stress on MT expression. Treatment with 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 1 hr did not significantly impact MT2A mRNA levels, but after 24 hr significantly upregulated MT2A expression was observed ( $n = 3$ ,  $p = .01$ ; Figure 3a). To determine if changes in mRNA impacted translation we evaluated MT-I/II expression by immunocytochemistry. Similar to the qPCR results, low levels of MT-I/II were detected in untreated human astrocytes (Figure 3b). Treatment with 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 24 hr increased the expression of MT-I/II, both in the cytoplasmic and nuclear compartments *in vitro* (Figure 3c).



**FIGURE 3** Oxidative stress induces MT-I/II expression in human astrocytes. (a) Treatment with 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$  for 1 hr did not significantly alter MT2A mRNA levels, but after 24 hr significantly upregulated MT2A expression ( $n = 3$ ,  $p = .01$ ). (b) Unstimulated astrocytes expressed low levels of MT-I/II, (c) but following treatment with 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$  for 24 hr, increased immunostaining for MT-I/II was observed in both the cytoplasmic and nuclear compartments. Scale bar represents 50  $\mu\text{m}$  [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

#### 4 | DISCUSSION

Oxidative stress and the associated oxidative-induced damage plays a key role in the initiation and progression of AD (Keller et al., 2005; Nunomura et al., 2006; Nunomura et al., 2001). In the current study we demonstrate that MT-I/II expression is predominantly associated with astrocytes in the ageing brain. Expression of this anti-oxidant does not correlate with Alzheimer-type pathology but does relate to the astrocyte response to oxidative DNA damage *in vivo*, and is induced in human astrocytes in response to oxidative stress *in vitro*.

Within the aging temporal cortex MT-I/II is predominantly associated with astrocytes, supporting previous studies in AD (Adlard et al., 1998; Zambenedetti et al., 1998). In contrast to these studies which examined a small number of cases and report MT-I/II is exclusively associated with astrocytes, we demonstrate MT-I/II is also weakly associated with pyramidal neurons in cases with high levels of local tau pathology in a small proportion of a much larger cohort. MT-I/II is generally considered an intracellular protein which is primarily localized in the cytoplasm of astrocytes, however MT-I/II can be released into the extracellular matrix and internalized by neurons via low density lipoprotein receptors, decreasing phosphorylation of several intraneuronal kinases implicated in pro-inflammatory processes and apoptosis, and promoting neurite outgrowth and neuronal survival (Ambjorn et al., 2008; Asmussen, Von Sperling, & Penkowa, 2009; Chung et al., 2008; Fitzgerald et al., 2007). Furthermore, MT-I/II expression is induced in neurons in the hippocampus of animal models of multiple sclerosis (Grubic Kezele, Blagojevic Zagorac, Jakovac, Domitrovic, & Radosevic-Stasic, 2017), and in adult rats following systemic administration of kainic acid (Kim et al., 2003). Whether the immunopositive neurons detected in the current study are a result of

neuronal uptake of MT-I/II or induction of MT-I/II expression is unknown.

In the current study we demonstrate MT-I/II expression is not a specific astrocyte response to local Alzheimer type pathology. This finding conflicts with rodent models of AD which show prominent upregulation of MT-I/II associated with  $\text{A}\beta$  plaques (Carrasco et al., 2006; Hidalgo et al., 2006), but supports previous investigation of MT-I/II in AD which also demonstrated no spatial relationship between MT-I/II<sup>+</sup> astrocytes and  $\text{A}\beta$  plaques or tangle pathology (Adlard et al., 1998; Zambenedetti et al., 1998). However, in contrast to the study by Adlard et al, which reported a significant increase in MT-I/II expression in five MCI compared with five AD cases, we did not identify any association between MT-I/II and Braak stage in a large aging-representative neuropathology cohort.

Oxidative stress and metal ion dyshomeostasis are key features of AD pathology and may contribute to the pathogenesis of disease. Zinc plays a critical role in memory formation, regulating neuronal communication in the hippocampus (Pan et al., 2011; Wang et al., 2010), and is highly concentrated within  $\text{A}\beta$  plaques, with depleted levels in the surrounding parenchyma (Dong et al., 2003; Lovell, Robertson, Teesdale, Campbell, & Markesbery, 1998; Sayre et al., 2000; Suh et al., 2000). Both zinc deficiency and zinc overload elicit oxidative stress that can lead to neuronal death (Sekler & Silverman, 2012; Wang et al., 2010). MT play a neuroprotective role against ROS damage in AD, scavenging hydroxyl radicals, superoxide anions, hydrogen peroxide and reactive nitrogen radicals, and are generally considered more effective antioxidants than superoxide dismutase, catalase or glutathione peroxidase (Juarez-Rebollar, Rios, Nava-Ruiz, & Mendez-Armenta, 2017; Kumari, Hiramatsu, & Ebadi, 1998). In the current study expression of MT-I/II correlates with the astrocyte DDR and is induced in response to oxidative stress *in vitro*, suggesting that astrocytes play a neuroprotective



role in response to oxidative stress and oxidative DNA damage, and not AD pathology, in the aging brain. In addition to their role in protecting against oxidative stress, astrocytes are actively involved in the neuroinflammatory response in AD (Gonzalez-Reyes, Nava-Mesa, Vargas-Sanchez, Ariza-Salamanca, & Mora-Munoz, 2017). Astrocyte expression of MT-I/II impacts the neuroimmune response in rodent models (Manso et al., 2011; Pankhurst, Bennett, Kirkcaldie, West, & Chung, 2011), suggesting that in addition to their anti-oxidant role, MTs may modify the microglial response in AD.

MT sequester heavy metals, regulating their availability to a range of enzymes and transcription factors. Under normal conditions, MT-I/II is located in the cytoplasm, however nuclear translocation of MT-I/II has been shown to occur in response to ultra-violet damage (Hanada, Tamai, Sawamura, Hashimoto, & Muramatsu, 1998; Jourdan et al., 2002) and cytokine activation (Spahl, Berendji-Grun, Suschek, Kolb-Bachofen, & Kroncke, 2003). In addition to increased expression of MT-I/II in the cytoplasmic compartment of human astrocytes in response to oxidative stress *in vitro*, we also demonstrate increased expression in the nuclear compartment, where it likely plays a role in the requirement of zinc for nuclear metalloenzymes and transcription factors.

While recent research has primarily focused on enhancing MT-III as a therapeutic strategy for AD (Aikins et al., 2017; Roy et al., 2017), the current study highlights the additional potential of targeting MT-I/II expression. In summary we demonstrate that MT-I/II is predominantly expressed by astrocytes in the ageing brain, is associated with the astrocyte DDR and is upregulated in response to oxidative stress *in vitro*. Given the ability of MT-I/II to offer neuroprotection against oxidative stress, modulation of MT-I/II expression is a potential target to treat the onset and progression of cognitive impairment and dementia.

## ACKNOWLEDGMENTS

Work on the CFAS cohort is supported by MRC (MRC/G0900582). RW, CG and AC are supported by Alzheimer's Society UK (AS386, AS242 and AS248). The imaging equipment used in this study was funded by Sheffield charity Neurocare. Work in the individual CFAS centers in this study is supported by the UK NIHR Biomedical Research Centre for Ageing and Age - awarded to Newcastle-upon-Tyne Hospitals Foundation Trust; Cambridge Brain Bank supported by the NIHR Cambridge Biomedical Research Centre; Nottingham University Hospitals NHS Trust; University of Sheffield, Sheffield Teaching Hospitals NHS Foundation Trust and Sheffield NIHR Biomedical Research Centre; The Thomas Willis Oxford Brain Collection, supported by the Oxford Biomedical Research Centre; The Walton Centre NHS Foundation Trust, Liverpool. We would like to acknowledge the essential contribution of the liaison officers, the general practitioners, their staff, and nursing and residential home staff. We are grateful to our respondents and their families for their generous gift to medical research, which has made this study possible.

## CONFLICT OF INTEREST STATEMENT

All authors have seen and approved the manuscript. There are no conflicts of interest.

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**How to cite this article:** Waller R, Murphy M, Garwood CJ, et al. Metallothionein-I/II expression associates with the astrocyte DNA damage response and not Alzheimer-type pathology in the aging brain. *Glia*. 2018;1–8. <https://doi.org/10.1002/glia.23465>