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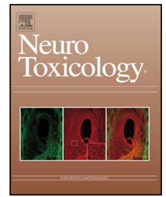

Full Length Article

Exposure to ambient ultrafine particulate matter alters the expression of genes in primary human neurons

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ABSTRACT

Exposure to ambient particulate matter (PM) has been associated with the onset of neurodevelopmental and neurodegenerative disorders, but the mechanism of toxicity remains unclear. To gain insight into this neurotoxicity, this study sought to examine global gene expression changes caused by exposure to ambient ultrafine PM. Microarray analysis was performed on primary human neurons derived from fetal brain tissue after a 24 h exposure to 20 μg/mL of ambient ultrafine particles. We found a majority of the changes in noncoding RNAs, which are involved in epigenetic regulation of gene expression, and thereby could impact the expression of several other protein coding gene targets. Although neurons from biologically different lot numbers were used, we found a significant increase in the expression of metallothionein 1A and 1F in all samples after exposure to particulate matter as confirmed by quantitative PCR. These metallothionein 1 proteins are responsible for neuroprotection after exposure to environmental insult but prolonged induction can be toxic. Epidemiological studies have reported that *in utero* exposure to ultrafine PM not only leads to neurodevelopmental and behavioral abnormalities, but may also predispose the progeny to neurodegenerative disease later in life by genetic imprinting. Our results pinpoint some of the PM-induced genetic changes that may underlie these findings.

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1. Introduction

Emerging evidence has implicated air pollution exposure as a risk factor for both neurodevelopmental and neurodegenerative abnormalities (Block et al., 2012; Costa et al., 2014; Davis et al., 2013; Genc et al., 2012). Maternal exposure to ultrafine particulate matter (PM) can alter fetal brain development in a manner that predisposes the progeny to neurodevelopmental disorders (Allen et al., 2014; Hougaard et al., 2015). Numerous reports have linked prenatal exposure to air pollution with an increased incidence of autism spectrum disorder (ASD) among children (Kalkbrenner et al., 2015; Raz et al., 2015; Volk et al., 2013). Correlations have been made between air pollution exposure and several other behavioral disorders including anxiety, depression, and schizophrenia (Pedersen et al., 2004; Perera et al., 2012, 2011). Cognitive deficits and structural brain alterations have also been found in children exposed to high levels of air pollution (Calderon-Garciduenas et al., 2011; Freire et al., 2010). Early life exposure may result in genetic imprinting that increases the risk of neurodegenerative disease later in life (Lahiri et al., 2009) including decreased cognitive function, stroke, Parkinson's disease, and Alzheimer's disease (AD) (Block et al., 2012; Chen and Schwartz, 2009; Moulton and Yang, 2012; Ritz et al., 2016). The effects of air pollution on the aging brain may also be direct and a recent population-based cohort study in Taiwan found a 138% increased risk for developing AD when living in areas containing high PM (Jung et al., 2015).

Despite our current knowledge of the adverse CNS effects of air pollution and despite existing EPA standards in the USA, it is still estimated that over 100 million people live in areas that exceed the recommended air quality levels (United States. Environmental Protection Agency, Office of Air Quality Planning and Standards and United States, Environmental Protection Agency, Air Quality Trends Analysis Group). Living under such circumstances is especially prevalent in urban areas. PM is generally the air pollutant that exceeds the recommended air quality standards (Moulton and

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Yang, 2012). PM is a complex mixture of solid particles and liquid droplets suspended in the air. It originates from a variety of sources including, but not limited to, vehicle emissions and industrial by-products (Hasheminassab et al., 2013). PM is characterized by its size, aerodynamic properties, and chemical composition, which can all influence its biological effects. Ultrafine particles (UFP) in particular are not filtered out during their passage through the nose and bronchioles but can penetrate to the alveoli. From here they are able to enter the blood circulation where they can be distributed throughout the body (Genc et al., 2012). Because of the small size, it is believed that UFP are able to cross the blood brain barrier (Block and Calderon-Garciduenas, 2009). Their large surface area, can enhance both oxidant capacity and greater inflammatory potential. Together with their ability to access the CNS these properties allow ultrafine particles to contribute to the pathogenesis of CNS diseases (Poon et al., 2004).

Although growing evidence implicated air pollution as contributing to adverse health effects on the brain, the underlying mechanisms and sequence of events that culminate in neurotoxicity remain poorly understood (Block et al., 2012; Lucchini et al., 2012). In this study, we evaluated gene expression profiles in human neurons after exposure to UFP. The UFP were collected from a sampling site in downtown Los Angeles, which is heavily impacted by pollution from vehicle emissions. The use of normal primary neurons serves as a good model system for identifying changes in gene expression in the human brain, particularly when observing gene expression changes that may be species-specific. We performed a whole genome wide expression analysis upon neuronal cells derived from fetal tissue of three different samples. Understanding how UFP affects gene expression may help identify early genetic markers of dysregulated pathways that ultimately contribute to CNS abnormalities (Block et al., 2012; Cooper-Knock et al., 2012). This exploratory report may help explain the mechanism by which exposure to UFP can contribute to the etiology of several neurodevelopmental and neurodegenerative diseases.

2. Materials and methods

2.1. UFP collection & characterization

Ambient ultrafine particles (UFP, diameter $Dp < 0.18\ \mu m$) were collected during January and February of 2014, on Zefluor filters (supported PTFE, 3.0 μm pore size, Pall Life Sciences) using a High-Volume Ultrafine Particle (HVUP) Sampler (Misra et al., 2002) at 400 L/min near downtown Los Angeles, about 120 m downwind of the CA-110 Freeway. The particles were then extracted into an aqueous suspension by 5 min of soaking in Milli-Q ultrapure water (resistivity 18.2 MΩ), followed by vortexing (5 min) and sonication (30 min). The dose of the PM solution was determined by gravimetric analysis. Briefly, the filters were weighed prior to the extraction using a microbalance (Sartorius, model LA 130-F). After the extraction process was completed the filters were dried and weighed again. The difference of the two gravimetric measurements was the mass of the PM that was extracted into the solution. Elemental content of the slurries was quantified using a high-resolution sector field inductively coupled plasma mass spectrometry (ICP-MS, Thermo Finnigan Element 2). Details of this method are described in Herner et al. (Herner et al., 2006). Total organic carbon was also quantified using a Sievers 900 organic carbon analyzer, following the method of Stone et al. (Stone et al., 2009). To investigate whether airborne size characteristic is preserved after extraction in ultrapure water using vortexing and sonication, we measured the aqueous-phase size distribution of PM using Dynamic Light Scattering (DLS). The size distribution of particles in the aqueous-phase by both number and surface area were partitioned in a size range similar to the airborne ultrafine PM, indicating that size distribution is overall well-preserved after water extraction, and the impact of particle agglomeration is trivial.

2.2. Primary neuron cell culture and treatment

Primary human neurons (HN, Cat. #1520) were purchased from ScienCell Research Laboratories and maintained at 37 °C in 5% CO_2 in neuronal medium (NM, Cat. #1521) supplemented with 1% neuronal growth supplement (NGS, Cat. #1562) and 1% penicillin/streptomycin solution (P/S, Cat. #0503). Neurons were plated at a density of 5×10^5 cells per well on 6-well cell culture plates that had been pre-treated with 15 μg/mL poly-L-lysine (PLL, Cat. #0413). The neurons were fully differentiated before treatments. Cells were determined to be differentiated by distinct morphological features that are characteristic of normal neurons we have previously reported (Campbell et al., 2014). Neurons were treated for 24 h with either an ultrapure aqueous solution (control) or with 20 μg/mL ultrafine particulate matter suspended in the ultrapure aqueous solution. In our previous studies we demonstrated that ROS and TNF-alpha levels change in primary human neurons exposed to PM within 24 h, and thus continued to use this time point for our current study (Campbell et al., 2014). Primary human neurons came from 3 different fetal samples and were identified by their lot numbers: 10866, 12732 and 13879. UFP were used for exposures because of their unique ability to penetrate the blood-brain-barrier (BBB) and gain access to neurons. In our previous experiments, we had used UFP in the range of 2–20 μg/mL. In this study, the higher dose was selected to better reflect the concentrations used by other investigators. These are in the range of 1–500 μg/mL (Bhavaraju et al., 2014; Campbell et al., 2014; Han et al., 2012).

2.3. Cell viability assays

Cells were lysed and an ATP-dependent cell viability assay was used to determine the viability of the cells after treatments with ultrafine PM. Cell Titer Glo reagent was used according to the manufacturers protocols (Promega, Madison, Wisconsin).

2.4. RNA collection and quantitative real-time PCR

RNA was isolated using RNeasy Mini columns (Qiagen) according to the manufacturer's instructions. Collected RNA was either directly used for microarray analysis, or saved for subsequent confirmation of microarray results by quantitative real time PCR (qPCR) analysis. Briefly, reverse transcription of 1 μg of total RNA was performed with SuperScript III Reverse Transcriptase (Life Technologies) according to the manufacturer's protocols using random hexamer primers (Life Technologies). Synthesized cDNA was then used as a template for qPCR at a 1:10 dilution and quantitative PCR analysis was performed using SYBR Green PCR Master Mix (Applied Biosystems) according to standard protocols on a CFX96 Touch (Bio-Rad) real-time PCR machine. Primers were designed with Primer Express 3.0 (Table S1) using the Transcript Cluster sequence used on the Affymetrix microarray, and then purchased from Fisher Scientific (Pittsburgh, PA). Data was normalized to the HMBS housekeeping gene. We previously determined that the expression of this housekeeping gene was not influenced by exposure to PM, and out of 14 well known housekeeping genes tested HMBS appeared to be best suited to use in primary neurons (data not shown). The housekeeping gene primers came from a set of validated primers whose sequences were proprietary (Prime PCR plate, Bio-Rad, cat# 10025217). Where applicable, a student's 2-tailed *t*-test was performed to

determine statistical significance, and data are presented as mean ± SD.

2.5. Microarray analysis

Isolated total RNA was processed using the Affymetrix GeneChip Whole Transcript Sense Target Labeling Assay according to the manufacturer's recommendations (Affymetrix, Inc., Santa Clara, CA). Eluted total RNA was quantified by NanoDrop (ThermoScientific, Wilmington, DE). Quality of the total RNA from each sample was assessed by an RNA 6000 Nano LabChip and evaluated on an Agilent Biolanalyzer 2100 (Agilent Technologies, Palo Alto, CA). The Affymetrix GeneChip WT plus reagent expression kit (Affymetrix, Inc., Santa Clara, CA) was used to prepare RNA samples for whole transcriptome microarray analysis. Briefly, random hexamers that were tagged with a T7 promoter were used in first strand synthesis of cDNA followed by second strand synthesis with the use of the T7 promoter. In vitro transcription was then performed with the double stranded cDNA template in order to generate many copies of antisense cRNA. 15 μg of antisense cRNA was used as a template for a second cycle cDNA reaction, with reverse transcriptase and random primers, to produce single stranded DNA in the sense orientation form. The single-stranded DNA was fragmented to an average length of 70 bases and then labeled using a recombinant terminal deoxynucleotidyl transferase (TdT) and an Affymetrix proprietary DNA labeling reagent that is covalently linked to biotin. 2 μg of the labeled, fragmented single-stranded cDNA was hybridized at 45 °C with rotation for 17 h (Affymetrix GeneChip Hybridization Oven 640) to more than 48,000 probe sets present on an Affymetrix GeneChip 2.0ST array. Noncoding RNA (ncRNA), including long noncoding (lncRNA) and intergenic noncoding RNA (lincRNA) transcripts, were present in these probe sets because recent evidence has pointed to the fact that differential expression of these transcripts plays an important role in the origin and progression of disease. The GeneChip arrays were washed and then stained with streptavidin-phycoerythrin on an Affymetrix Fluidics Station 450 (Fluidics protocol FS450_007). Arrays were scanned using GeneChip Scanner 3000 7G and Command Console Software v. 3.2.3 to produce CEL intensity files. The probe cell intensity files (*.CEL) were analyzed in Affymetrix Expression Console software v1.1.1 using the PLIER algorithm to generate probe level summarization files (*.CHP). The settings used were algorithm-PLIER v2.0; quantification scale-Linear; quantification type-signal and detection p-value; background-PM-GCBG; normalization method-sketch-quantile. Probe level summarization files (*.CHP) were then analyzed with the use of Transcriptome Analysis Console (TAC) Software from Affymetrix to determine differences in expression between the 3 different samples and 2 treatment groups. Targets were selected for further analysis if they were significantly up or down regulated with a one-way ANOVA p-value of ≤0.05, and/or had a fold-change in expression of >2.0.

3. Results

3.1. PM Characterization

Fig. 1A shows the contribution of Total Organic Carbon (TOC), as well as trace elements and metals to the overall mass of the ultrafine particles. TOC, accounting for 43 ± 2% of the total ultrafine mass, consisted of a great number of primary organic compounds (including but not limited to Polycyclic Aromatic Hydrocarbons (PAHs), Hopanes, Steranes and Alkanes), in addition to water soluble organic compounds originating from secondary organic aerosol formation processes (Hasheminassab et al., 2013). Total elements and metals, were calculated as the cumulative mass fraction of 50 species (Table S2) and constituted 16 ± 1% of the ultrafine mass (Fig. 1A). Despite their relatively small contribution to ultrafine mass, certain metals are of utmost toxicological importance due to their potential to induce oxidative stress in cells (Shi et al., 2003; Valavanidis et al., 2005). Fig. 1B, shows mass fraction (ng/μg PM) of 12 metal species (including Fe, Zn, Cu, Mn, Ni, Cr, Pb, V, As, Co, La and Cd) that were previously found to be associated with the ultrafine-induced oxidative potential (Saffari et al., 2013a, 2014; Verma et al., 2009). Fe is the most predominant redox-active metal, with mass fraction of 9.5 ± 0.08 ng/μg PM, followed by Zn, Cu, Mn, Ni and Cr (mass fractions ranging between 0.2 and 3.5 ng/μg PM). These metals originate from a variety of sources in the Los Angeles area, most notably vehicular emissions, ship emissions and industrial activities (such as fuel oil combustion in refineries and metal plating) (Saffari et al., 2013b). It is important to note that the chemical composition of ultrafine particles is strongly affected by temporal and spatial variations and therefore the organic carbon and metal mass fractions presented here is specific to this sample and would likely be different for particles in other locations or collected during a different season.

3.2. Exposure to UFP alters expression of several noncoding RNAs

The entire set of 48,226 probes on the Affymetrix microarray was analyzed to determine expression changes between the control and the 20 μg/mL UFP treated primary human neurons with the use of Expression Console® and Transcriptome Analysis Console software from Affymetrix. We previously determined that there were no significant changes in cell viability in these cells after UFP exposure at this dose (Fig. S1). From this microarray analysis it

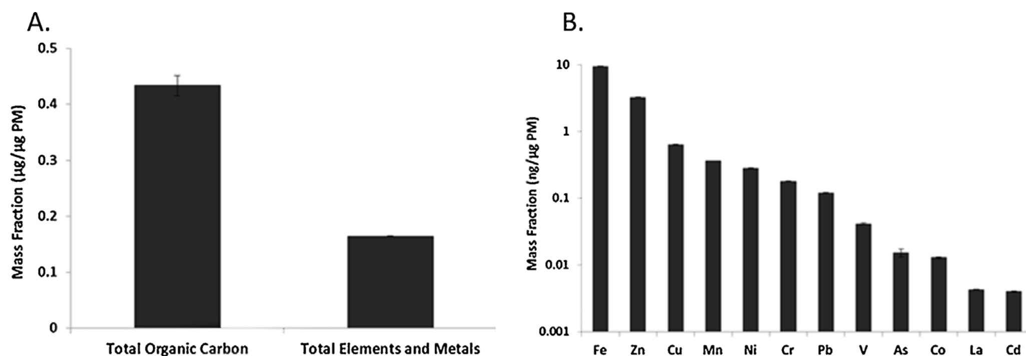
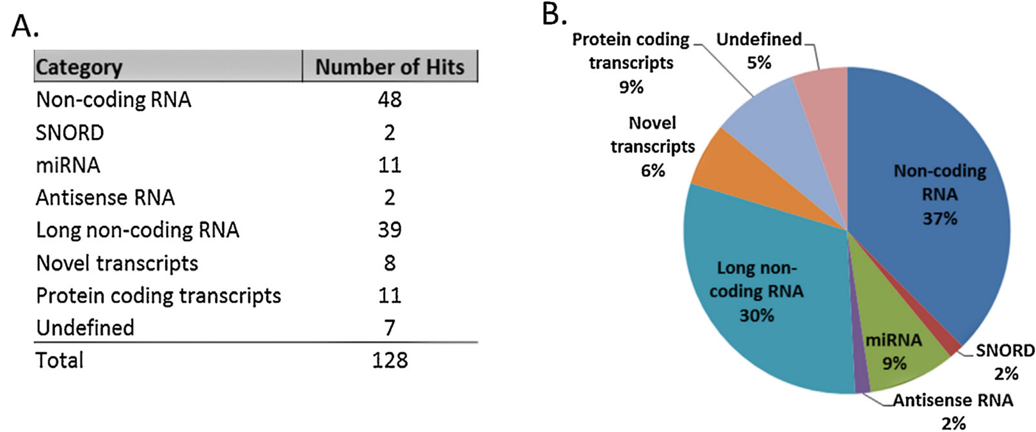


Fig. 1. Ultrafine PM characterization.
Mass fraction (μg/μg PM) of total organic carbon (TOC) and total elements and metals in the ultrafine slurry (a), and mass fraction (ng/μg PM) of selected metals (b). Error bars represent the method uncertainties.



A.

Category	Number of Hits
Non-coding RNA	48
SNORD	2
miRNA	11
Antisense RNA	2
Long non-coding RNA	39
Novel transcripts	8
Protein coding transcripts	11
Undefined	7
Total	128

Fig. 2. Summary of microarray results based on Affymetrix Expression Console software v1.1.1.
Summary of the number of hits per category (a) and percentage of hits per category (b) obtained from using the Transcriptome Analysis Console (TAC) software by Affymetrix. Noncoding RNA includes miRNA, SNORDs (small nucleolar RNA), long noncoding RNA, and antisense RNA making up a total of 80% of the hits.

was found that following an exposure to 20 μg/mL ultrafine particulate matter for 24 h, primary human neurons had a significant change in the expression of 128 transcripts. This includes a decrease in the expression of 67 transcripts and an increase in the expression of 61 transcripts. Several of the significant changes were in transcript cluster IDs with no associated gene names. 102 of the 128 genes showing significant change were transcripts for noncoding RNA. The expression of 51 of these were decreased after exposure to UFP and 51 were increased. These noncoding RNAs included long noncoding RNAs, microRNAs, small nucleolar RNA (SNORDs), and antisense RNA. Based on the Affymetrix Expression Console software v1.1.1, noncoding RNAs made up less than 22% of the transcripts on the array, but made up 80% of our 128 hits that were identified based on differences in expression between 3 samples and 2 treatment group (Fig. 2).

3.3. Expression of proteins upregulated upon exposure to UFP

The TAC Affymetrix software revealed 11 protein coding transcript genes which were significantly up or down regulated in response to UFP by 2 fold or greater and with a p-value ≤ 0.05 (Table 1). We followed up on those changes reflecting increase gene expression by quantitative PCR analysis. OR4D11, MT1A, OR5B3, and CFHR3 transcripts are upregulated in response to UFP (Table 2). The samples used for our experiments came from 3 different lot numbers, and as such, the baseline gene expression pattern from each lot number varied widely. These samples not only had different baseline gene expression patterns in control samples, but also had different degrees of response to particulate matter as demonstrated by our principal component analysis (PCA) (Fig. S2). Despite this variation that was present in our human samples, the expression of MT1A was significantly increased in all 3 samples (Fig. 3). We also looked at the effects of UFP on the expression of several other metallothionein 1 (MT1) isoforms involved in xenobiotic metabolism (including MT1JP, MT1CP, MT1F, MT1G, MT1X, and MT1E) in order to determine the specificity of this MT1A induction in response to UFP. MT1A and MT1F expression were the only isoforms that increased in all 3 donor samples treated with UFP (Fig. 3), indicating that exposure to ultrafine PM has a significant impact on specific isoforms of these neuroprotective genes.

4. Discussion

4.1. Non coding RNAs

Following an acute exposure to ultrafine particulate matter for 24 h, human primary neurons had a significant change in the expression of 128 transcripts. This included 102 noncoding RNAs, 11 protein coding transcripts, and 15 undefined or novel transcripts. This study is the first to report such a considerable change in the expression of noncoding RNAs in human CNS cells after exposure to UFP. Noncoding RNAs are transcripts that have no apparent protein coding function (Rinn and Chang, 2012). We now know that only 2% of the human genome is translated into protein, yet more than 50% of the genome is transcribed into RNA (Enciu et al., 2012). This means that most transcripts are noncoding. Cells can use these noncoding RNAs to repress or activate gene

Table 1
Protein Coding Transcript Hits Based on Affymetrix Software.

Gene Symbol	Description	Transcript Cluster ID	Fold Change	ANOVA p-value
MMP13	matrix metallopeptidase 13 (collagenase 3)	16743764	−4.67	0.0447
HIST1H3C	histone cluster 1, H3c; histone cluster 1, H3f; histone cluster 1, H3b	17005539	−3.24	0.0223
OR1L4	olfactory receptor, family 1, subfamily L, member 4	17088819	−2.78	0.0464
NXPE2	neurexophilin and PC-esterase domain family, member 2	16731515	−2.27	0.0452
TAS2R40	taste receptor, type 2, member 40	17052746	−2.09	0.0326
OR2F2	olfactory receptor, family 2, subfamily F, member 2	17052881	−2.06	0.0216
DCAF4L1	DDB1 and CUL4 associated factor 4-like 1	16966389	2.2	0.0196
OR4D11	olfactory receptor, family 4, subfamily D, member 11	16725097	2.22	0.0161
MT1A	metallothionein 1A	16819233	2.7	0.0407
OR5B3	olfactory receptor, family 5, subfamily B, member 3	16738613	3.08	0.0452
CFHR3	complement factor H-related 3	16675428	4.33	0.0004

List of protein coding transcripts found to be either significantly upregulated or significantly downregulated upon exposure to UFP in primary human neurons. Results are filtered by fold change (±2.0) and ANOVA p-value ($\leq$0.05). A negative value denotes downregulation of the gene expression.

Table 2
Microarray Results Confirmed by qPCR.

Gene Symbol	Fold Change (Microarray)	Fold Change (qPCR)
OR4D11	2.22	7.4
MT1A	2.7	1.8
OR5B3	3.08	19.5
CFHR3	4.33	2.2

Confirmation of microarray changes with an upward change in expression after exposure to UFP in primary human neurons by qPCR.

expression at a specific locus, and thereby fine tune gene expression in response to environmental stimuli.

We and others have shown that exposure to ambient PM upregulates inflammatory processes in the brain (Campbell et al., 2005, 2009; Levesque et al., 2011; Morgan et al., 2011). Noncoding RNAs such as miRNA and lncRNA play an important role in regulating the magnitude of inflammatory responses (Elling et al., 2016; O'Connell et al., 2012; O'Neill et al., 2011). Considering that our findings are in differentiated human neurons, it is plausible that these cells have the intrinsic capability of determining the degree of local neuroinflammation by control of noncoding RNA expression. This may be a mechanism of protection against uncontrolled inflammatory processes that are known to be neurotoxic. In fetal derived human neurons, the appropriate modulation of the expression levels of noncoding RNA may reflect successful protection against environmental stressors such as UFP. The susceptibility to developmental and degenerative changes may be associated with the capability to modulate noncoding RNA gene expression necessary for neuronal survival. Considering that 90% of disease-associated single nucleotide polymorphisms are located in noncoding DNA elements (Kumar et al., 2013), adds to the plausibility of this contention.

Noncoding RNAs were initially studied extensively because the dysregulation of expression has been shown to contribute to carcinogenesis (Haemmerle and Gutschner, 2015). However, recently noncoding RNA dysfunction has also been associated with brain abnormalities. In the developing brain, antisense RNA corresponding to the SYNGAP1 locus (SYNGAP1-AS) is differentially expressed in brain regions of patients with ASD when compared to control individuals (Velmeshev et al., 2013). In postmortem CNS tissue from ASD patients microarray analysis shows that over 200 lncRNAs are differentially expressed (Ziats and Rennert, 2013). lncRNA have the ability to change the accessibility of gene promoters by recruiting proteins to the promoter or by blocking their access to the promoter (Batista and Chang, 2013). Few studies have looked at the effects of PM on the expression of noncoding RNA, and none to date have looked at these effects in the human brain. With the rapidly increasing identification of, and functional analysis of, new noncoding RNAs, understanding of the association between these noncoding RNAs and CNS abnormalities will be increasingly facilitated (Guennewig and Cooper, 2014).

4.2. Changes in specific protein coding mRNAs

We also confirmed by qPCR that the expression of two metallothioneins, MT1A and MT1F, are significantly up-regulated upon exposure to UFP in all three of our different samples. Although there was significant variation among gene expression patterns in response to exposure to PM, metallothionein 1 isoforms A and F were upregulated in all three samples derived from different lot numbers. Since the metallothionein isoforms are such small similar proteins, there is an absence of available assays that are capable of accurately distinguishing between the different MT isoforms. Thus, we were limited in our ability to determine the effects of MT1A and MT1F at the protein level in this study. Metallothioneins are low molecular weight neuroprotective proteins that play a major role in metal detoxification by sequestering metals like zinc and copper (Stankovic et al., 2007; Stelmashook et al., 2014). They are induced in response to environmental stressors, and provide protection in the aging brain through zinc-mediated transcriptional regulation of genes involved in cell growth, proliferation, and differentiation (Cherian and Apostolova, 2000; Sharma and Ebadi, 2014). The homeostasis of metallothioneins is crucial, as dysregulation of $Zn2+$ and $Cu2+$ in the brain can be damaging for neurons. Metal-ion dyshomeostasis and metallothionein dysregulation are likely to be critical elements in neurodegenerative diseases of aging such as AD (Bonda et al., 2011; Hozumi, 2013). In fact, mouse models of neurodegenerative diseases which cannot express MTI/II express accelerated neurodegeneration (Stankovic et al., 2007). A metal-ion imbalance has also been noted in many studies of children with autism, and it has been suggested that this may occur because of a dysfunction in metallothionein (MT) proteins (Bjorklund, 2013).

Since the UFP collected from the Los Angeles ambient air are rich in metals, most notably iron, zinc, and copper, this may lead to induction in MT1 expression followed by subsequent metal detoxification. This is consistent with the reports of an increase in MT1 expression in the brains of dogs living in highly polluted regions of Mexico (Calderon-Garciduenas et al., 2003). In the CNS,

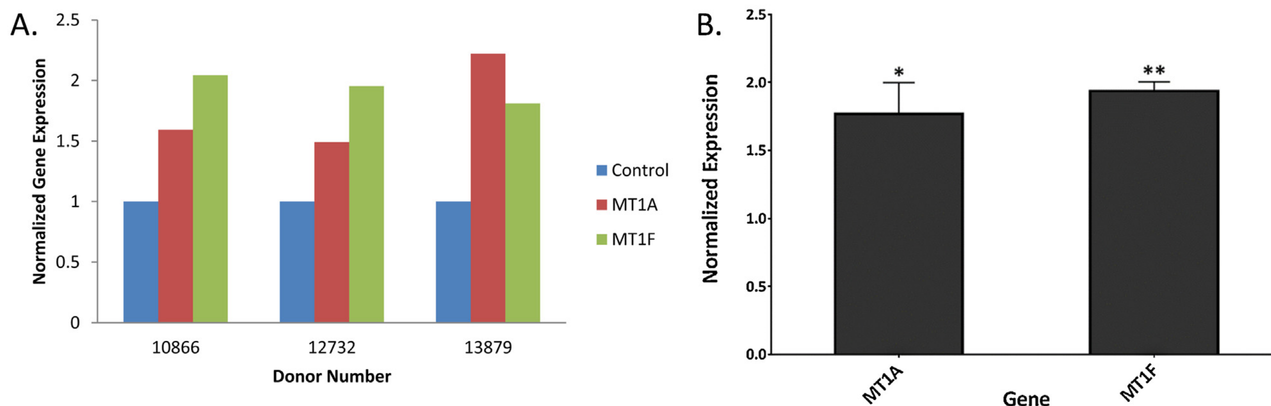


Fig. 3. Expression of MT1A and MT1F is significantly increased in human neurons exposed to UFP.
Quantitative PCR analysis of human primary neurons confirms results from microarray analysis. A. Normalized gene expression profile of MT1A and MT1F based on specific samples. B. Normalized expression of MT1A and MT1F in all lot numbers. Bars represent the gene expression normalized to HMBS housekeeping gene and relative to cells from the same sample treated with a water control. Expression of metallothionein isoforms MT1A and MT1F are significantly increased in response to ultrafine particulate matter in primary human neurons from 3 distinct lot numbers. *p-value $\leq$ 0.05; **p-value $\leq$ 0.01

high expression of MT1 is thought to confer protection against heavy metal induced oxidative stress related damage and cell death through anti-inflammatory and antioxidant mechanisms (Sharma et al., 2013; Stankovic et al., 2007). Even though MTs are known to be protective when induced, their enhanced induction by PM may also signify the toxicity of these particles. Although the mechanism is not clearly understood, prolonged elevated MTI/MTII expression in the brain has been consistently shown to be correlated with the onset and progression of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and motor neuron disease (Manso et al., 2011; Stankovic et al., 2007). In the case of AD, animal studies support the hypothesis that there is an interaction between MTI/II induction and amyloid-beta deposition (Carrasco et al., 2006). Future studies are needed to determine whether the prolonged induction of MT1A and MT1F isoforms by PM specifically contributes to the progression of AD or is a secondary protective response triggered by the underlying pathology.

Also confirmed by qPCR was the up-regulation of two olfactory receptor genes, OR4D11 and OR5B3. These are protein coding genes involved in the production of G-protein coupled transmembrane receptors which enable the detection and transmission of olfactory stimuli. Since the initial site of impact of inhaled particles is largely on the olfactory epithelium, these genes are likely to be especially susceptible to modification by PM. CFHR3 (complement factor H related 3) expression was also elevated in PM-treated cells. The protein encoded by this gene is a secreted protein, which belongs to the complement factor H-related protein family. Mutations in this gene are associated with an increased risk of atypical hemolytic-uremic syndrome. Cyclosporine induces endothelial cells to release microparticles that activate the complement system and lead to bystander injury to the kidneys and vasculature (Renner et al., 2013). In a similar manner, modulation of the complement pathway by airborne nanoparticles may account for some of their toxicity. Abnormally high serum levels of the immunoglobulins IgA and IgM, as well as the complement component C3c have been found in adults chronically exposed to high levels of ambient air pollution (Hadnagy et al., 1996).

The studies presented in this report were conducted in CNS cells obtained from human fetuses. Changes in gene expression, such as the ones observed, can persist in a quiescent state but ultimately lead to emergence of neurological deficits. The fetal basis of adult disease hypothesis states that many adult diseases have a developmental origin. Lahiri and colleagues proposed the LEARn (Latent Early-life Associated Regulation) model as a way to explain the onset of idiopathic CNS diseases. In this model, early life exposure to agents that act as stressors can cause epigenetic changes. These changes induce a short-term upregulation of disease-related genes followed by a long latency period of 'normal' levels of gene expression (Lahiri et al., 2009). Ultimately, the latency period ends and the effects of abnormal gene expression become apparent. This may take place at those various stages of the life span that involve shifts in gene expression, such as puberty or senescence. This can form the basis of neurodevelopmental disease in an adolescent, or neurodegenerative disease in the elderly. Neonatal rats that were treated with the heavy metal lead (Pb), which has long been known to have effects on the brain and early developmental exposures were critical in facilitating later brain pathology (Basha et al., 2005). Similar studies conducted in 23-year old primates show elevated expression of AD-related genes if animals were exposed to Pb as infants (Wu et al., 2008). Collectively these studies support the LEARn model and suggest that the pathogenesis of CNS diseases can be strongly influenced by early life exposures.

The genetic variation that was observed in this pilot study in the response to UFP should not be disregarded, as this difference may indicate an individual's susceptibility to, or protection from, developing neurodevelopmental and/or neurodegenerative diseases. Genetic changes induced by UFP may reflect an adaptive response to the presence of specific particle constituents such as toxic metals. Further investigation will reveal if these changes are of significance to the pathogenesis of CNS disorders.

5. Conclusions

The effects of ultrafine particulate matter on global gene expression in primary human neurons was examined. There were significant changes in the expression of several noncoding RNAs in response to exposure to UFP found in the ambient air. Future investigations may determine whether the neurotoxicity that results from exposure to UFP is in part due to alterations in the expression of these noncoding regions. It has been previously proposed that epigenetic factors could influence the ability of environmental contaminants to cause neurotoxicity (Block et al., 2012). Epigenetic variations may clarify why certain subpopulations, such as the very young and elderly, tend to be more susceptible to PM exposure, and may also help explain why some people may be more predisposed to developing CNS abnormalities after exposure to particulate matter. We also observed a significant increase in the expression of neuroprotective metallothioneins MT1A and MT1F. This suggests that some properties of the UFP cause a change in the expression of metallothionein genes in the human brain. The results described here provide insight into the alteration of gene expression that could occur in the brain after exposure to air pollution. A limitation of a simplified cell-culture based study is how well it can be translated to a more biologically relevant *in vivo* system. Thus, further studies are warranted to determine if the findings can help to explain the accumulating evidence linking outdoor air pollution with adverse effects on CNS health, including neurodevelopmental and neurodegenerative disorders.

Declaration of interest statement

The author(s) declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neuro.2016.11.001.

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