NeuroToxicology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Polymorphisms in manganese transporters show developmental stage and sex specific associations with manganese concentrations in primary teeth

Karin Wahlberg[a,*], Manish Arora[b], Austen Curtin[b], Paul Curtin[b], Robert O. Wright[b], Donald R. Smith[c], Roberto G. Lucchini[b,d], Karin Broberg[a,e], Christine Austin[b]

[a] Division of Occupational and Environmental Medicine, Lund University, Klinikgatan 21, 221 85 Lund, Sweden
[b] Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10029-5674, USA
[c] Microbiology and Environmental Toxicology, University of California, 156 High Street, Santa Cruz, CA 95064, USA
[d] Occupational Health Institute, University of Brescia, Viale Europa, 11, 25123 Brescia BS, Italy
[e] Institute of Environmental Medicine, Karolinska Institutet, Nobels väg 13, 171 65 Solna, Sweden

ARTICLE INFO

Article history:
Received 8 September 2017
Accepted 12 September 2017
Available online xxx

Keywords:
Manganese homeostasis
Manganese neurotoxicity
Manganese transporter genetics
Teeth biomarkers
SLC30A10
SLC39A8

ABSTRACT

Background: Manganese (Mn) is an essential metal that can become neurotoxic at elevated levels with negative consequences on neurodevelopment. We have evaluated the influence of single nucleotide polymorphisms (SNPs) in Mn transporter genes *SLC30A10* and *SLC39A8* on Mn concentrations in dentine, a validated biomarker that reflects Mn tissue concentrations early in life.
Methods: The study included 195 children with variable environmental Mn exposure. Mn concentrations in dentine representing fetal, early postnatal and early childhood developmental periods were measured using laser ablation-inductively coupled plasma mass spectrometry. *SLC30A10* rs12064812 (T/C) and *SLC39A8* rs13107325 (C/T) were genotyped by TaqMan real time PCR and *SLC30A10* rs1776029 (G/A) by pyrosequencing; and SNPs were analyzed in association with Mn in dentine.
Results: SLC39A8 rs13107325 rare allele (T) carriers had significantly higher Mn concentrations in postnatal dentine (110%, p = 0.008). For all SNPs we also observed non-significant associations with Mn concentrations in dentine in opposite directions for fetal and early postnatal periods. Furthermore, there were significant differences in the influence of *SLC30A10* rs1776929 genotypes on Mn concentrations in dentine between sexes.
Discussion: The findings from this study indicate that common SNPs in Mn transporters influence Mn homeostasis in early development and may therefore be important to consider in future studies of early life Mn exposure and health effects. Our results also suggest that the influence of these transporters on Mn regulation may differ by developmental stage, as well as between girls and boys.

1. Introduction

Manganese (Mn) is an essential nutrient involved in a number of physiological processes in humans (Jitrapakdee et al., 2008; Reddi et al., 2009; Takeda, 2003). Mn is required for normal brain function but can become neurotoxic at elevated concentrations (Tuschl et al., 2013) and there appears to be a narrow window between deficiency, essential dose and toxicity in early neurological development (Claus Henn et al., 2010). Elevated internal Mn can occur via exposure from a range of sources including naturally elevated levels in drinking water (Rahman et al., 2016) and exposure from soil, air and dust as a consequence of industrial pollution (Boudissa et al., 2006; Pavilonis et al., 2015). Animal and human studies have shown that Mn crosses the placenta (Krachler et al., 1999) and maternal exposure may therefore reach the fetus.

Several studies have suggested that environmental Mn exposure can interfere with children's neurodevelopment and has been linked to cognitive, motor and behavioral deficits (Zoni and Lucchini, 2013), and may also modify the neurotoxic effects of

* Corresponding author at: Lund University, Division of Occupational and Environmental Medicine, Scheelevägen 8, 223 81 Lund, Sweden. Tel.: +46 462221643; fax: +46 46173180.
E-mail address: karin_e.wahlberg@med.lu.se (K. Wahlberg).

http://dx.doi.org/10.1016/j.neuro.2017.09.003
0161-813X/

other metals, including lead (Claus Henn et al., 2012). Several studies have also shown sex differences in the health effects of Mn exposure (Gunier et al., 2015; Menezes-Filho et al., 2011; Riojas-Rodriguez et al., 2010), suggesting that there may be important differences in Mn homeostasis and toxicity between girls and boys. Furthermore, studies in animals and humans have indicated differences in the effects of exposure related to the developmental stage at which the exposure was experienced (Betharia and Maher, 2012; Gunier et al., 2015; Mora et al., 2015).

In addition to the effects of exposure, internal manganese concentrations can be influenced by variations in genes involved in Mn regulation. Recent studies have shown that common single nucleotide polymorphisms (SNPs) in Mn transporter genes *SLC30A10* (efflux transporter) and *SLC39A8* (influx transporter) modify Mn concentrations in adults and are also associated with neurological symptoms (Ng et al., 2015; Pickrell et al., 2016; Wahlberg et al., 2016). These finding suggests that SNPs in Mn transporters may influence the brain, possibly via interference with Mn homeostasis.

In the presents study we have investigated the influence of common SNPs in transporter genes *SLC30A10* and *SLC39A8* on Mn concentrations in tooth dentine from early childhood and evaluated potential differences in genetic influence between different developmental stages (prenatal, early postnatal and early childhood) and between sexes. For this purpose we have applied newly-developed and validated tooth-matrix Mn biomarkers that can directly measure prenatal and early childhood Mn uptake and which has been shown to reflect early-life Mn exposure (Arora et al., 2012). We undertook this study in a well-characterized study of Italian children (n = 195) with different background exposures to Mn.

2. Methods

2.1. Study population

The full study cohort consists of 721 adolescents, aged 10–14 years, residing in the three different districts of Valcamonica valley, Bagnolo Mella and Lake Garda within the Province of Brescia, Italy. Valcamonica has a long history of ferromanganese alloy plants which were active in the period 1902–2001, while Bagnolo Mella has ongoing exposure from a plant that has been active since 1973. In contrast, Lake Garda has no history of ferroalloy plants. A first round of recruitment including 311 children from Valcamonica and Lake Garda was completed in 2010 and included sampling of air, water,soil and dust and biomarkers blood, urine, saliva, hair and nails. A second round of recruitment of 410 children from Valcamonica, Lake Garda and Bagnolo Mella took place 2010–2014 and for some of these children shed baby teeth were collected for analysis for metals.

Mn exposure of these children has been carefully monitored by measurements of Mn concentrations in samples from the their home environment and shown increased Mn concentrations in samples from the Valcamonica and Bagnolo Mella compared to Lake Garda (Borgese et al., 2011, 2013; Lucas et al., 2015). The children have also undergone comprehensive neurological and neuropsychological testing at adolescent age, including tests for IQ, motor function and behaviour; however, neurological outcomes were not in included in the present study. Sex of the child was reported in connection with neurological testing. The study design, information about study aims and forms for informed consents were reviewed and approved by the ethics committees of the local Public Health agencies of Valcamonica and Brescia, and carefully explained to participants before recruitment. The present study involves a sub-cohort of 195 children for which Mn concentrations in teeth were measured.

2.2. Tooth metal analysis

Our approach to measuring metals in teeth using laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) and assigning developmental times has been detailed elsewhere (Arora and Austin, 2013; Austin et al., 2013). Briefly, we used the neonatal line (a histological feature formed in enamel and dentine at birth) and daily growth incremental markings to assign temporal information to sampling points. The neonatal line (or birth line) provides a landmark that distinguishes the prenatal and postnatal compartments of teeth. Additional microscopic analysis allows us to identify specific regions of teeth that develop at different ages. These regions of teeth are then sampled using a laser coupled with a mass spectrometer (Arora and Austin, 2013; Austin et al., 2013). Prenatal dentine was sampled from primary dentine (cuspal to the neonatal line) that forms during the second trimester until birth. Postnatal dentine was sampled from primary dentine (cervical to the neonatal line) that forms from birth to approximately 1.5–11 months of age depending on tooth type. Early childhood dentine was sampled from secondary dentine that forms from 1.5–10 years of age depending on tooth type (Berkovitz et al., 2009).

The laser ablation unit used was a New Wave Research NWR-193 system (ESI, USA) equipped with an excimer argon-fluoride laser emitting a nanosecond laser pulse with a wavelength of 193 nm. An approximately 1 m length of Tygon® tubing (i.d. 3 mm) connected the laser ablation unit to an Agilent Technologies 8800 ICP-MS. The instrument was fitted with an 's' lens system for enhanced sensitivity. The system was tuned daily for sensitivity using NIST SRM 612 (trace elements in glass). Polyatomic oxide interference was evaluated and minimized by monitoring the Th^+/ThO^+ (m/z 232/248) ratio. Typical oxide formation was consistently under 0.3%. The NIST SRM 612 glass standard was run every day to tune the instrument and multiple times during the day to check for signal stability. Ca was also used as an internal standard to account for variation in the mineral content of teeth and any signal drift between runs.

Using the laser we sampled 30 sampling points in primary dentine and 10 sampling points in secondary dentine. Data were analyzed as ^{55}Mn:^{43}Ca ratios to control for any variations in mineral content within a tooth and between samples. The limit of detection (LOD) was 0.001 ^{55}Mn:^{43}Ca.

2.3. Genetic analysis

Briefly, DNA was extracted from whole blood using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). Genotyping of rs12064812 and rs13107325 was performed by TaqMan Real Time PCR using predesigned assays (Thermo Scientific assay IDs C_32155052_10 and C___1827682_10 respectively) as previously described (Wahlberg et al., 2016). Reactions were performed in 5 μl total volumes containing 10 ng of DNA, and analysed on the ABI 7900HT Fast Real Time PCR System (Applied Biosystems, Thermo Fisher, Waltham, USA), using manufacturer's recommended standard conditions.

Rs1776029 was genotyped using pyrosequencing due to its location in a repeat region. This SNP is highly linked to rs2275707 in this cohort, and due to its location in a genomic region with signatures of regulatory elements (i.e. histone acetylation and DNAse I hypersensitivity), it is the more likely causative variant of the two and was therefore selected in this study. The assay was designed by PyroMark Assay Design 2.0 software (Qiagen, Germany) with primers targeting sequences flanking the repeat, which allowed the generation of a specific PCR product for sequencing. The following primer sequences were used: forward primer 5′TTAGTCATACCATGGGTCATGTCT, reverse primer (biotinylated) 5′ACTCTTGGAAGGCATGATGATT and sequencing primer

Please cite this article in press as: K. Wahlberg, et al., Polymorphisms in manganese transporters show developmental stage and sex specific associations with manganese concentrations in primary teeth, Neurotoxicology (2017), http://dx.doi.org/10.1016/j.neuro.2017.09.003