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ENVIRONMENTAL MANGANESE EXPOSURE IN RESIDENTS LIVING NEAR A FERROMANGANESE REFINERY IN SOUTHEAST OHIO: A PILOT STUDY

Erin N. Haynes, DrPH, MS, Pamela Heckel, PhD, PE, Patrick Ryan, PhD, Sandy Roda, Yuet-Kin Leung, PhD, Kelly Sebastian, and Paul Succop, PhD

University of Cincinnati, Department of Environmental Health

Abstract

Manganese (Mn) is an essential element, yet is neurotoxic in excess. The majority of Mn research has been conducted on occupationally exposed adults with few studies focused on an environmentally exposed population. Marietta, Ohio is home to one of the largest airborne Mn emission sources in the United States, a ferromanganese refinery. In preparation for a community-based participatory research study, a preliminary pilot study was initiated to characterize the community's exposure to Mn in ambient air and to evaluate the relationship between biological indices of Mn exposure and genes associated with Mn metabolism in Marietta area residents. Participants in the pilot study were recruited through newspaper advertisement, fliers and direct mailing. Exposure to ambient Mn was estimated using an air pollution dispersion model, AERMOD. A total of 141 residents participated in the pilot study ranging in age from 2-81 years. Estimated annual average ambient air Mn concentrations in the study area obtained from AERMOD varied from 0.02-2.61 $\mu\text{g}/\text{m}^3$. Mean blood and hair Mn values were 9.12 $\mu\text{g}/\text{L}$ (SD 3.90) and 5.80 $\mu\text{g}/\text{g}$ (SD 6.40 $\mu\text{g}/\text{g}$), respectively and were significantly correlated ($r=0.30$, $p<0.01$). Blood and hair Mn was significantly associated within families ($r=0.27$, $p<0.02$ and $r=0.43$, $p<0.01$), respectively. The relationship between hair Mn and estimated ambient air Mn became significant when genes for iron metabolism were included in linear models. The preliminary ambient air and biological concentrations of Mn found in this population demonstrate the need for further research into potential health effects. A comprehensive study of neurobehavioral performance and environmental exposure to Mn in children residing in Marietta and a control community is currently underway.

Keywords

manganese; lead; community-based; exposure; dispersion model; air pollution

Introduction

Ingested manganese (Mn) is essential for normal metabolic processes; yet, inhaled Mn can bypass normal biliary excretion mechanisms and directly enter the brain along olfactory

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Correspondence to: Erin N. Haynes, DrPH, MS, 3223 Eden Ave Cincinnati, OH 45267-0056, Tel: 513-558-5427; Fax: 513-558-4838, Erin.Haynes@uc.edu.

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neurons [1-3]. As a documented neurotoxin, Mn causes a dose-related continuum of central nervous system (CNS) dysfunctions [4]. At high levels of inhalation exposure, Mn can produce a neurologic psychiatric disorder, manganism, which resembles Parkinsonian syndrome [5]. These extrapyramidal and neuropsychiatric symptoms progress even after cessation of exposure [5-7]. Industrial and environmental exposures to Mn have been associated with neurobehavioral dysfunction including deficits in reaction time and motor function, changes in mood and affect, and increased risk of delinquency [7-10]. Public health concern regarding exposure to Mn is related to the use of Mn in gasoline as an antiknock agent, methycyclopentadienyl manganese tricarbonyl (MMT) [1] and exposure from industrial sources such as iron and steel production plants, ferromanganese refineries, battery production and welding [11].

Adult and childhood studies suggest that Mn exposure through inhalation and consumption of water contaminated with Mn may lead to neurotoxic effects. Individuals residing near a former manganese alloy production plant in Southwest Quebec with blood Mn concentrations >7.5 µg/L had deficits in neuromotor skills, learning, and memory relative to the study participants with lower blood manganese levels [4]. In addition, a recent non-occupational study found a significant association between air Mn concentrations (mean value 0.42 µg/m³) and motor tests assessing the coordination of two movements and position changes in hand movements in adults living near a mining district in Mexico [12]. Cross-sectional studies of children residing in different geographical regions of South Africa where MMT is used as a gasoline additive found significant variation in blood Mn levels in children residing in that nation's cities [13]. Gulson et al. [14] explored the relationship between dustfall Mn and blood Mn concentrations in children in Australia where MMT is also used in gasoline. Blood Mn values ranged from 1.8 to 45 µg/L with a mean of 12 µg/L.

Mn is under tight homeostatic control. Iron and Mn share similar uptake and transport mechanisms including hemochromatosis (*HFE*) and transferring (*TF*). Two predominant variants of the *HFE* gene account for most cases of hereditary hemochromatosis (HH), characterized by increased intestinal iron absorption and excessive iron stores [15,16]. The C282Y and H63D variants are frequent in the U.S. population, with a prevalence of 7-17% and 10-32%, respectively [17]. Transferrin is involved with the stabilization of *HFE* and facilitating iron transfer [18]. In the general population, the missense variant P570S among the *TF* gene has a prevalence rate of about 15% [19].

Few locations in the world offer the ability to study the health effects of manganese exposure on the general population. The purpose of this pilot research study was to characterize ambient air Mn exposure and evaluate the relationship between biological measures of Mn exposure in blood and hair, modeled exposure, and genetic variants in *HFE* and *TF* in an Appalachian American community in Southeast Ohio exposed to air Mn from an active ferromanganese refinery.

Materials and Methods

Study design and recruitment

Marietta, Ohio is home to the only Mn refinery in North America. The refinery is the world's leading producer of Mn alloys for the steel industry in addition to producing Mn-based products for applications including batteries, ferrites, fertilizers and animal feeds. The refinery has been in operation since 1951 and, on average, has reported 450,000 pounds/year of Mn fugitive air emissions since 1998 [20]. The Marietta community has a population of 14,515; 53% are female, 96.3% are Caucasian. The median family income in Marietta in 1999 was \$36,042 and 13.6% of families were below the poverty level [21]. In preparation for a community-based participatory research study in collaboration with concerned community members and the

Neighbors for Clean Air (NCA) a pilot study was initiated. Participants in this pilot study were identified using a Community Profile Survey (CPS) distributed throughout the community as suggested by the NCA. The study was advertised on local radio stations and newspaper articles and the CPS was distributed by NCA through their member distribution list. The CPS collected demographic information including age, years lived in Marietta, number of children, occupation, former residential addresses and length of time lived at those former residences. Adult respondents were queried regarding the potential participation of their children in the research study. Surveys were returned either by self-addressed envelopes through the postal mail or by fax. Respondents were eligible for study inclusion if they reported living within a 20-mile radius of Marietta. Respondents were contacted by phone, screened for eligibility and scheduled for a study visit. All participants signed a University of Cincinnati Institutional Review Board approved consent or assent form prior to data collection.

Biological Markers of Manganese Exposure

Mn concentration was assessed in blood and hair provided by each participant. A trained phlebotomist collected 20 ml of blood obtained by venipuncture using single-use syringes and techniques to ensure minimal extraneous Pb contamination [22]. Mn was analyzed from whole blood using methods described previously [23,24]. Analysis was performed on a Perkin Elmer, model 5100 Zeeman Graphite Furnace Atomic Absorption Spectrometer using Triton-X 100 and a Magnesium Nitrate modifier.

Hair samples for measuring Mn were collected by cutting approximately 20 strands of hair from each subject with ceramic scissors using methods described previously [23,25]. All glassware and plasticware were cleaned by soaking in 20% HNO₃ for 24 hours and rinsing several times with distilled-deionized water. Hair samples were placed in a clean 15 ml culture tube and submerged and mixed for 30 minutes in a 10% solution of 7-X-Omatic Cleanser. Hair samples were then rinsed using a gravity filtration system with at least 100 ml of distilled-deionized water. Samples were dried at room temperature and covered with clean wipes to prevent contamination for approximately 48 hours. Each sample was then weighed (approximately 10 to 50 mg was used for the analysis) and transferred into a 25 ml beaker. The samples were dissolved in 2 ml of trace metal-free, concentrated nitric acid on a hot plate at 125°C for 30 minutes. Samples were allowed to cool before they were quantitatively transferred and brought to a final volume of 10 ml with distilled-deionized water. Samples were analyzed by a Perkin Elmer model 5100 Zeeman Graphite Furnace Atomic Absorption Spectrometer. Results were interpreted with the instrument's Winlab Version 3.2 Software. A reagent blank and two spiked reagent samples were incorporated into the preparation of each set of hair samples [26,27]. Mn analysis in blood and hair were analyzed by the Hematology and Environmental Laboratory at the University of Cincinnati, Department of Environmental Health.

Exposure Assessment

Sources of Mn within a 15-mile radius of the confluence of the Muskingum and Ohio Rivers in Marietta were identified through the United States Environmental Protection Agency (USEPA) Toxic Release Inventory (TRI) electronic database [20]. Annual emissions of Mn from each industry were identified from annual reports filed with the West Virginia and Ohio EPAs [28]. A detailed characterization of the industrial Mn sources included the physical location (latitude and longitude), the annual Mn emissions (pounds/year), exhaust gas temperature (°C), stack height (m), and stack diameter (m) [29].

Residential estimates of Mn exposure were obtained using the USEPA air dispersion modeling software AERMOD, a steady-state Gaussian dispersion model designed for near field (up to 50 kilometers) applications. In addition to AERMID, three AERMOD preprocessors

AERMAP, AETMET and AERSURFACE generated a more accurate assessment of area characteristics that affect pollutant dispersion, deposition, and exposure estimates. AERMOD includes input data on sources, emissions, receptors, terrain, and meteorology to estimate Mn concentrations within the study area. AERMAP extracted the terrain elevations from US Geological Survey digital maps. AERSURFACE computed the seasonal surface albedo and Bowen ratio in 12 wind directions. Meteorological data was obtained from the National Oceanic and Atmospheric Agency (NOAA) Climate Data Center for the year 2006. AERMET developed the stability factors using the following parameters: AERMAP and AERSURFACE outputs, the local surface weather data and the upper air meteorological data, including variables that influence the behavior of the plume after it enters the atmosphere including ambient temperature (°C), dew point (°C), wind direction (0-360 degrees), wind speed (mph), cloud cover (%), cloud layer(s), ceiling height (km), visibility (km), precipitation (cm/hr.), surface roughness and elevation (m). The model assumes that the plume emitted from the source is at steady state; pollutants are dispersed according to a straight-line Gaussian model; meteorological conditions including wind, temperature, and precipitation affect the rate of deposition. Once the model was built, each residential address was geocoded using EZLocate (Teleatlas, Inc) to determine each participant's modeled Mn exposure estimate. Several addresses were not identifiable through TeleAtlas due to road name changes. A study team member visited these locations and identified their latitude and longitude by using a handheld global positioning system (GPS).

HFE and TF genotyping

Genomic DNA was extracted from whole blood using Qiagen blood genomic DNA extraction kits (Qiagen, Valencia, CA). The quality and quantity of the extracted genomic DNA was assessed with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Denver, U.S.A) and by agarose gel electrophoresis respectively. In this pilot study, three single nucleotide polymorphism (SNP) were analyzed by Taqman SNP Genotyping assays (ABI, Foster City, CA): hemochromatosis (*HFE*) C282Y (rs1800562) and H63D (rs1799945), and transferrin (*TF*) P570S (rs1049296). Taqman Universal PCR Master Mix without AmpErase UNG was used as a master mix for these assays. The reactions were carried out in a total of 25 µl consisting of 12.5 µl of master mix, 1.25 µl of TaqMan SNP Genotyping Assay Mix and 20ng of genomic DNA. Amplification conditions were performed per the manufacturer's protocol, with an initial step of 10 min at 95° C, followed by 40 cycles of 15s at 92° C and 1 min annealing at 60° C. These assays were performed in ABI PRISM 7900 HT Sequence Detection system as per the manufacturer's protocol.

Statistical analysis

Descriptive statistics were calculated for the blood and hair Mn concentrations. In order to assess environmental exposure, subjects who reported ever having worked in the ferromanganese refinery were excluded from the analyses. Pearson bivariate correlations and linear regression models were used to determine an association between blood and hair Mn concentration and various independent variables, including residential distance from the refinery and estimated average annual air Mn concentration. The SAS GLM procedure was used to estimate and test for associations between biological measures of Mn (blood and hair) and categorical predictors (e.g, gender and distance from the primary Mn source). Hair color was classified on a scale of 1 to 7 where 1 was light hair and 7 was black hair to evaluate the influence of hair color on hair Mn.

The intra-class correlation was estimated to determine the within-family correlation of blood and hair Mn measurements. Blood and hair Mn concentrations were transformed to their natural logarithm to attain approximate normality of their distributions. An α level of 0.05 was used

to judge statistical significance. We examined the distribution of the *HFE* and *TF* alleles and genotypes and their relationship with biological measures of Mn and modeled air Mn.

Results

Study Population Characteristics

In total, 172 CPS were completed. Of these, 141 individuals were eligible and participated in the pilot study. The 31 who were unable to attend ranged in age from nine months old to 83 years of age with an average age of 54 years. Table 1 describes the characteristics of the study population. The age of participants ranged from 2 years to 81 years with an average age of 44 years (SD 19.2 years). Approximately 57% of the study population was female and 99% were Caucasian. The length of time lived in the area ranged from 1 to 79 years (mean, 31 years; SD, 20.2 years). The length of time participants had resided at their current address ranged from two months to 79 years (mean, 18 years; SD, 17 years); the mean residential distance from the ferromanganese refinery was 13 miles, ranging from 0.26 to 18 miles.

Of the 141 participants, 139 (98.6%) had levels of blood Mn above the LOD and 74 (52%) had hair Mn above the LOD. Study participants who reported ever having worked at the ferromanganese refinery were removed from further analysis ($n=4$). The mean blood Mn for these four workers was 11.8 $\mu\text{g/L}$ (SD 2.0). Overall, mean blood Mn was 9.12 $\mu\text{g/L}$ (range 1.8 $\mu\text{g/L}$ to 22 $\mu\text{g/L}$) (Table 1). Mean blood Mn was higher in children (10.48 $\mu\text{g/dL}$ (Std 4.0 $\mu\text{g/L}$), although this was not significant (Table 1). Blood Mn in children ranged from 5.3 $\mu\text{g/L}$ to 22 $\mu\text{g/L}$ (mean, 10.5 $\mu\text{g/L}$, std, 4.05 $\mu\text{g/L}$) and did not significantly differ by gender in adults and children.

Hair Mn ranged from 0.64 $\mu\text{g/g}$ to 41.1 $\mu\text{g/g}$ (mean, 5.8 $\mu\text{g/g}$; SD, 6.42 $\mu\text{g/g}$) (Table 1). In addition, one outlier was observed (hair Mn of 366 $\mu\text{g/g}$) and removed from the analysis. Further investigation revealed this subject to be a female who reported having applied a medium blonde hair dye 20 days before her hair was collected. Sufficient amounts of hair were collected for 12 children: the mean of these was 4.0 $\mu\text{g/g}$ (range, 0.64 $\mu\text{g/g}$ – 7.8 $\mu\text{g/g}$). Hair Mn did not significantly differ by gender in both adults and children. There was not a significant relationship between the biological measures of Mn and gender ($p = 0.33$ for $\log[\text{hair Mn}]$ and 0.48 for $\log[\text{blood Mn}]$).

There was a significant correlation between the blood Mn and hair Mn in all participants ($r=0.30$, $p=0.01$) (Table 2). Likewise, length of time lived at their current residence and the number of years lived in the area were significantly correlated with blood Mn (Table 2). The number of years lived in the Marietta area was significantly correlated with the years lived at their current address. In total, nineteen families, defined as a minimum of one parent and one child, participated in the study. Four families consisted of a grandparent, parent and child. Biological measures of Mn were strongly correlated within families. Of the 19 families, 14 had sufficient hair collected on all family member participants for analysis of Mn. After adjusting for age, the within-family intra-class correlation for blood Mn was 0.27 ($p<0.02$) and the intra-class correlation for hair Mn was 0.43 ($p<0.004$).

The genotype frequencies are presented in Table 3. One hundred and twelve individuals were genotyped and 25, 33, and 27 individuals carried the *HFE* C282Y, *HFE* H63D, and *Tf* P570S variants. There was no statistical relationship between these variants and blood or hair Mn in the study population. Individuals with the *HFE* C282Y variant (*GA/AA*) had higher hair Mn concentrations (7.1 $\mu\text{g/g}$) than the individuals with the wildtype (*GG*) (4.9 $\mu\text{g/g}$). The difference between hair Mn concentrations of individuals with the other genotypes did not widely differ between the wildtype and variant genotypes (Table 3).

Manganese Sources and Exposure

There are 9 Mn emission sources within a 20 mile radius of Marietta (Figure 1). By law, industries must report their emissions to the EPA. Mn emissions from coal-fired power plants are automatically calculated as a small percentage of steam produced. Emissions from metal refineries are determined using a mass balance equation which considers the amount of raw materials purchased, the number of hours of operation for each process, and the process efficiency [30]. The ferromanganese refinery was the largest emitter of Mn with 326,900 lb/yr total on-site and fugitive emissions reported in 2006 (73% of total Mn emissions within a 20 mile radius of the city of Marietta). The other leading Mn emission sources in 2006 were a power plant located near the refinery (100,536 lbs/yr, 22%) and a battery manufacturing facility in close proximity to the refinery (17,539 lbs/yr, 4%). Geographic coordinates were obtained for each facility using a handheld GPS as coordinates reported to the Ohio Emission Inventory System (OEIS) were determined to be inaccurate. Stack location was identified via satellite images (Google Maps).

The air dispersion model estimated an annual average air Mn concentration of $0.13 \mu\text{g}/\text{m}^3$ within the 15 mile grid surrounding the Marietta community (Figure 1). Mn air concentrations are estimated to range from $0.01 - 18.13 \mu\text{g}/\text{m}^3$ within the area. The highest annual average ambient Mn concentration, $18.13 \mu\text{g}/\text{m}^3$, was slightly north of the refinery. Within the city limits of Marietta, the modeled air Mn concentrations range from $0.08 - 0.19 \mu\text{g}/\text{m}^3$. All Ohio and West Virginia residents living within a 10 mile radius of the ferromanganese refinery were estimated to have exposures that surpass the US EPA reference concentration of for Mn: $0.05 \mu\text{g}/\text{m}^3$.

Blood Mn and hair Mn were not significantly correlated with modeled air concentration; however, residential distance from the refinery was significantly related with modeled Mn air concentration. Although individuals with the wildtype and variant genotypes did not differ in terms of their blood or hair Mn concentrations, when they were included in the exposure model, a significant relationship between hair Mn and estimated ambient air Mn was observed (Table 2).

Discussion

The concentration of blood Mn in the residents of Marietta, OH is within the ATSDR range of normal blood Mn ($4-14 \mu\text{g}/\text{L}$) [31]; however, blood Mn concentrations of $7.5 \mu\text{g}/\text{L}$ and greater have been associated with neuromotor deficits in adults residing near a former ferromanganese refinery in Quebec [32]. In this pilot study, approximately 75% of the blood samples had Mn concentration $\geq 7.5 \mu\text{g}/\text{L}$.

The mean hair Mn concentration in our pilot study was $5.8 \mu\text{g}/\text{g}$ (range, $0.64 \mu\text{g}/\text{g}$ to $41.1 \mu\text{g}/\text{g}$). Though no neurobehavioral data was collected as part of this pilot study, a recent study in Quebec found positive and significant associations for teacher-reported behavioral problems (Oppositional and Hyperactivity subscales), as measured in the Revised Conners' Teachers Rating Scale, with children who had hair Mn concentrations $>3.0 \mu\text{g}/\text{g}$ [33]. In our pilot study, 58% ($n=34$) had hair Mn values $>3.0 \mu\text{g}/\text{g}$; the geometric mean of hair Mn in children ($n=12$) was $3.42 \mu\text{g}/\text{g}$. In addition, an inverse association between hair Mn (mean $0.472 \mu\text{g}/\text{g}$) and IQ was found in 6th grade children living near a hazardous waste site in Oklahoma [26]. The mean hair Mn levels for children in our pilot study were 12 times higher than the hair Mn levels found in the hair of these Oklahoma children. The potential relationship between motor deficits and biological Mn values in this study population was examined by Standridge and colleagues [34]. A significant relationship between postural balance and hair Mn was observed after adjusting for age, gender, and height to weight ratio [34].

We found a significant relationship between blood Mn and hair Mn. Hair analysis for Mn has also been used by others to ascertain environmental exposure to Mn and its relationship to other parameters of health. Menezes-Filho et al [35] recently found mean concentrations of hair Mn of 15.20 $\mu\text{g/g}$ (1.10-95.0 $\mu\text{g/g}$) in children exposed to Mn from a ferromanganese alloy production plant in Bhaia, Brazil. The limitations of using hair as a biological marker for metals including the analysis methods have been discussed and evaluated [36]. There is reportedly a wide variation in normal hair concentrations of most minerals, which may be attributed to age, sex, race, geographic location, hair color, and use of hair treatments [36]. Laboratory inconsistencies, such as cleaning procedures, can also add to the variability of the measurement [36]. We considered these variables in our collection and analysis procedures. Hair sample collection was restricted to a site at the posterior vertex of the scalp; a 7-X-Omatic Cleanser was used to safely remove external contamination from the hair without disturbing the internal concentration of Mn. Samples were only analyzed if sufficient sample (>10 mg) was presented to the laboratory. Hair Mn did not significantly differ by gender in adults or children. Hair color did not influence Mn concentration.

The estimated exposure to ambient air Mn derived from the dispersion model of participants in this study ranged from 0.01 – 18.13 $\mu\text{g}/\text{m}^3$ with a mean of 0.13 $\mu\text{g}/\text{m}^3$. Motor alterations were observed in a population in Mexico exposed to air concentrations similar to those modeled for Marietta [12]. Air sampling was not conducted as part of this pilot study. The ASTDR and the Ohio EPA, however, has conducted ambient air sampling in the Marietta airshed [31]. In 2001, the annual average concentration of Mn was 0.23 $\mu\text{g}/\text{m}^3$. The air monitor was located approximately 4.5 miles to the north/northeast of the ferromanganese refinery. In comparison, the ATSDR estimates that background airborne Mn concentration in rural areas is 0.005 $\mu\text{g}/\text{m}^3$ and 0.033 $\mu\text{g}/\text{m}^3$ in urban areas in the United States [31]. The USEPA reference concentration for chronic Mn exposure is 0.05 $\mu\text{g}/\text{m}^3$. Our air model and the ATSDR-EPA sampling indicate that the air in Marietta, OH far exceeds the USEPA reference concentration.

In support of the Clean Air Act, the USEPA allows the use of a mathematical model to demonstrate that emissions from a new source do not cause the regional air shed to exceed the allowable National Ambient Air Quality Standards. In 2006, AERMOD became the promulgated dispersion model to demonstrate compliance with the Prevention of Significant Deterioration regulations. AERMOD was used to model the transport and dispersion of Mn emissions from nine different local sources to estimate the average ambient concentration of Mn within the study area. The model is recommended for small-scale applications in rough terrain, making it the ideal air dispersion model for Marietta.

We did not identify a relationship between estimated annual Mn air concentration and biological concentrations of Mn in blood and hair until we included the *HFE* and *Tf* variable into the models, suggesting that genetic variation among individuals may modify the effect of Mn exposure measurements and biological Mn concentration. The sample size was too small to demonstrate differences between wildtype and variants of each gene and to include all three genes in the same model. Other genetic variants, such as those found in *DMT1* that decrease iron uptake, have been associated with increased blood levels of Mn [37] suggesting that genetic variation may in part explain the inherent variability of biological Mn concentrations. Although our genetic results and interpretation are limited by sample size, further research is needed to identify those populations that are most susceptible to Mn exposure.

There are potential limitations, however, to using air dispersion models and our modeled results should be interpreted carefully when associating health effects with exposure. First, the USEPA TRI contains self-reported *estimated* annual emissions data in pounds per year from companies who emit hazardous air pollutants. The self-reported data does not include emission particle size. A model assumption is that the particles are of equal size resulting in an overestimation

of exposure to the fine and ultrafine particles that may result in adverse health effects. Airborne particulate matter is a mixture of coarse particles (PM_{10-2.5}) which is often dominated by crustal materials (e.g., wind blown dust, resuspension of road dust, demolition of buildings), whereas the particles in fine (PM_{2.5}) and ultrafine (PM_{0.1}) size fractions are mostly formed through combustion processes. The smallest of the ultrafine particles agglomerate and grow in size; the fine particle fraction is more stable. Furthermore, fine particles have relatively long lifetimes in the air, particularly compared to larger (coarse) particles, and can be easily transported by air currents. The above increases the likelihood of human exposure. The particulate emissions from ferroalloy furnaces, as used in Marietta, OH, have been characterized by EPA studies (EPA-450/4-84-007h March 1984). The particles emitted from the Marietta facility are fine, respirable particles ranging in size from 0.05 microns - 0.4 microns and contain high levels of Mn (33.6% of the total weight). In addition, the biological dose of inhaled Mn will depend upon the inhalable fraction of particles.

Furthermore, as industry does not operate at a constant rate, air emissions vary daily resulting in acute exposures not characterized by estimated annual average emissions. Exposure estimates in our model were calculated based on the outdoor annual air concentration at a residential address and may not reflect personal exposure due to personal activities and home factors [38,39].

As this study was intended to serve as a pilot study for a larger follow-up study our genetic results and interpretation are limited by sample size. Our findings suggest that genetic variation may in part explain the inherent variability of biological Mn concentrations. Further genetic research is needed to identify populations most susceptible to Mn exposure. Based on our biological Mn concentrations and exposure estimates for ambient air Mn in the Marietta community, further research is warranted, including personal air sampling to characterize individual exposure, genetic analyses to identify susceptible populations, and a large-scale study of the neurobehavioral effects of Mn in children in this community, compared to a control community, which is currently underway.

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Figure 1.
Air dispersion model of airborne Mn ($\mu\text{g}/\text{m}^3$) from all Mn sources in Marietta, Ohio

Table 1

Descriptive Characteristics of Pilot Study Participants

Characteristic	n	Mean (SD)	Median	Range	GM ^a (GSD ^b)
Age (years)	141	44 (19.2)		2 - 81	
Years Lived in the Area	139	31.5 (20.2)		1 - 79	
Years at Current Address	141	18 (16.8)		0.17 - 79	
Residential Distance from Mn Refinery (miles)	139	13 (87.2)		0.26 - 18	
Blood Mn ^c (µg/L)	135	9.12 (3.89)	8.7	1.8 - 22	8.34 (1.55)
Mn Refinery Workers Blood Mn (µg/L)	4	11.78 (20.5)		10.1 - 14.4	
≤18 years of age Blood Mn (µg/L)	18	10.48 (4.0)	9.05	5.3 - 22	9.88 (1.41)
Hair Mn ^d (µg/g)	73	5.8 (6.42)	3.57	0.64 - 41	3.94 (2.39)
≤18 years of age Hair Mn (µg/g)	12	4.0 (1.96)	3.52	0.64 - 7.86	3.42 (1.9)

^aGM = geometric mean

^bGSD = geometric standard deviation

^cAnalysis excluded the Mn refinery workers

^dAnalysis excluded Mn hair outlier (Mn = 366 µg/g)

Table 2

Correlation^a among Pilot Study Participant Characteristics

	Ln Blood Mn (µg/L)	Ln Hair Mn (µg/g)	Residential distance from Mn refinery	Years at Current address	Years lived in Area
Ln Blood Mn (µg/L) n=139	1.00				
Ln Hair Mn (µg/g) n=73	0.30	1.00			
Residential distance from Mn refinery n=139	-0.07	0.04	1.00		
Years at Current address n=141	-0.20	0.05	-0.23	1.00	
Years lived in Area n=139	-0.16	.10	.01	0.71	1.00
Ln Avg Mn Air Conc (µg/m ³)	0.05	0.17	-0.54	N/A	N/A
Ln Avg Mn Air Conc HFE H63D n=56	0.17	0.30	N/A	N/A	N/A
Ln Avg Mn Air Conc HFE C282Y n=56	0.21	0.29	N/A	N/A	N/A
Ln Avg Mn Air Conc Tj P570S n=58	0.18	0.40	N/A	N/A	N/A

^aValue is bolded if p is ≤ 0.05

Table 3

Genotype frequencies and biological Mn concentration in the study population

Genotype	Blood Mn (µg/L)		Hair Mn (µg/g)	
	n (%)	Mean (SD)	n (%)	Mean (SD)
<i>HFE C282Y^a</i>				
Wildtype <i>GG</i>	80 (76)	8.1 (3.1)	47 (78)	4.9 (4.6)
<i>GA</i>	24 (23)	9.6 (4.0)	12 (20)	7.1 (6.3)
<i>AA</i>	1 (1)		1 (1)	
<i>HFE H63D^b</i>				
Wildtype <i>CC</i>	70 (68)	9.3 (3.9)	38 (66)	5.3 (4.5)
<i>CG</i>	30 (29)	9.3 (3.7)	19 (33)	5.6 (6.2)
<i>GG</i>	3 (3)		1 (2)	
<i>Tf P570S^c</i>				
Wildtype <i>CC</i>	78 (74)	9.0 (3.5)	41 (71)	5.7 (5.6)
<i>CT</i>	24 (23)	8.9 (3.9)	15 (26)	4.3 (3.2)
<i>TT</i>	3 (3)		2 (3)	

^aSeven participants were missing genotype data or blood Mn data; results in Hardy-Weinberg equilibrium: $\chi^2 = 0.3$, $p > 0.05$

^bNine participants were missing genotype data or blood Mn data; results in Hardy-Weinberg equilibrium: $\chi^2 = 0.01$, $p > 0.05$

^cSeven participants were missing genotype data or blood Mn data; results in Hardy-Weinberg equilibrium: $\chi^2 = 0.47$, $p > 0.05$