



## Cohort Profile: The Guangxi manganese exposure workers healthy cohort (GXMEWHC)

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005070
Article Type:	Protocol
Date Submitted by the Author:	17-Feb-2014
Complete List of Authors:	Lv, Yingnan; Guangxi Medical University, Occupational Health and Environmental Health Zou, Yunfeng Liu, Jing chen, kangcheng huang, damin shen, yuefei; The 1st affiliated hospital, Neurology zhong, yaoqiu Zhihao, Zhihao Jiang, Bei li, qin Qing, Li zhang, wei chen, lang Wang, Fenfen Xia, Bing Yang, Li yang, xiaobo
<b>Primary Subject Heading</b>:	Occupational and environmental medicine
Secondary Subject Heading:	Epidemiology, Genetics and genomics
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, EPIDEMIOLOGY, GENETICS

SCHOLARONE™  
Manuscripts

**Cohort Profile: The Guangxi manganese exposure workers healthy cohort (GXMEWHC)**

Yingnan Lv<sup>1</sup>, Yunfeng Zou<sup>2</sup>, Jing Liu<sup>1</sup>, Kangcheng Chen<sup>1</sup>, Damin Huang<sup>1</sup>, Yuefei Shen<sup>3</sup>, Yaoqiu Zhong<sup>1</sup>, Zhihao Liu<sup>4</sup>, Bei Jiang<sup>4</sup>, Qin Li<sup>2</sup>, Li Qing<sup>5</sup>, Wei Zhang<sup>3</sup>, Lang Chen<sup>3</sup>, Fenfen Wang<sup>1</sup>, Bing Xia<sup>1</sup>, Li Yang<sup>1</sup>, Xiaobo Yang<sup>1,6,\*</sup>

<sup>1</sup> Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>2</sup> Department of Toxicology, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>3</sup> Department of Neurology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

<sup>4</sup> Baise Center for Disease Control and Prevention, Baise, Guangxi, China

<sup>5</sup> Department of Epidemiology and Health Statistics, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>6</sup> Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning, Guangxi, China

Yingnan Lv and Yunfeng Zou are contributed equally.

\* Corresponding Author: Dr. Xiaobo Yang, Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Shuangyong Road 22, Nanning, Guangxi, 530021, P. R. China; [yxbo21021@163.com](mailto:yxbo21021@163.com)

## Abstract

**Introduction:** Manganese (Mn) is an essential element for growth and maintenance of human health. Inhaled manganese can be excrete by normal homeostatic mechanisms, but it also can be accumulate in the bodily organ when excess the ability of metabolism. Occupational exposure to Mn in air can cause the adverse health effects in the human bodies. Consequently, we established the Guangxi manganese exposure workers healthy cohort (GXMEWHC) to explore the early healthy effect and the long-term related disease of occupational manganese exposure by the biomarkers of exposure, effect and susceptibility.

**Methods and analysis:** The GXMEWHC is a prospective study. We recruited the workers in Ferro-manganese refinery Factory and presently conducted the baseline surveys including epidemiological investigation, neurological function test, occupational health examination and environmental monitoring. The genome-wide association study (GWAS) are also implemented further. We will follow up the participators every three years and ultimately the appropriate measures will be taken to prevent and control the early healthy injure and the related disease.

**Ethics and dissemination:** The study has been approved by Medical ethics committee of Guangxi Medical University. The results will be published by peer-reviewed publications and presented at international conferences.

## Keywords

Manganese; Occupational exposure; Manganese toxicity; Genetic Susceptibility

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47 **Strengths and limitations of this study**

- 48 ■ In this study, we can collect an abundant database because of large samples in the  
49 heavy metals cohort.
- 50 ■ The Ferro-manganese refinery Factory is the largest metallurgical factory of  
51 manganese processing in China so that it can provide an extremely rich dataset of  
52 analysis.
- 53 ■ The GXMEWHC is the longitudinal study which can continuous follow up and  
54 repeated investigate the participants. We can explore the relations between  
55 occupational manganese exposure and the early health injure.
- 56 ■ The GWAS are implemented for seeking the susceptibility genes of chronic  
57 low-level manganese exposure, and exploring the interactions between genetic  
58 factors and environmental factors. Those provide an important opportunity to  
59 identify the more susceptible individuals so that prevent the early health injure of  
60 workers.
- 61 ■ Potential limitations are that loss of follow up may be a weakness with our study.  
62 There are some temporary workers in the factory and they may leave the factory  
63 after a period of time working in factory. We can reduce the probability of the  
64 loss of follow up through strict controlled the inclusion criteria when established  
65 the cohort.
- 66  
67  
68  
69

## INTRODUCTION

Mn is an essential nutrient and it is necessary to inhaled manganese for maintain the daily life. In addition to food intake, environmental exposure to Mn was the way to absorption of Mn, especially occupational exposure. Mn was accumulated in some bodily organ and induced the adverse effects when the Mn concentration in *vivo* over the capacity of human metabolism.<sup>1</sup>

Many studies showed that Mn can caused neurological abnormalities when it accumulated in brain in human bodies,<sup>2-4</sup> such as early impaired Finger Tapping speed<sup>5</sup> or cognitive deficits, terminal Parkinsonian-like symptoms,<sup>6</sup> and Manganism.<sup>7</sup> The values of Mn in the human bodies were detected through some internal biomarkers, neurobehavioral tests and functional neuroimaging.<sup>8,9</sup> The concentrations of Mn in kidney were increased in the manganese exposure workers because the kidney is a way to excrete manganese.<sup>4</sup> In addition, the repeated respiratory exposed to Mn resulted in accumulation in the lung. One study showed that it was a dose-effect relationship between occupational manganese exposures and the reduction of pulmonary function.<sup>10</sup> Compared with the non-exposure workers, the pulmonary function in the manganese exposure workers were evaluated by the spirometry test and the values of them were a significant decrease in FEV<sub>1</sub>, FVC and FEV<sub>1</sub>% values.<sup>11</sup> Increased manganese levels in blood serum ascribed to that liver is a mainly organ to store, biotransformation and detoxify the poisonous substance.<sup>12</sup> Over exposure to manganese can cause liver toxicity as well as exacerbate liver dysfunction.<sup>13, 14</sup> Chronic manganese exposures lead to a series of significant cardiovascular toxicities including the abnormal electrocardiogram (ECG) and

inhibition of myocardial contraction which can alters the blood pressure (BP).<sup>15</sup>

Besides, the manganic cytotoxicity could induce cell apoptosis and the DNA damage of bird immune cells.<sup>16</sup> Low Mn<sup>2+</sup> can induce oxidative DNA damage via an apoptotic pathway so that the DNA damage could be reduced using antioxidants. A research conducted a risk assessment of inhaled manganese through incorporating genetics and genomics to identify genetically based biomarkers of exposure, disease and susceptibility.<sup>17</sup>

From the above, manganic toxicity in humans played a significant role in several systems. Currently, most studies were explored separately the effect of manganese exposure for different system in the human body. To explore further the effect and the interaction of manganese exposure in various systems, we will establish a prospective cohort study which includes the situation of individual manganese exposure and regular occupational examination. Simultaneously, we will detect the biological exposure indicators by means of hair, urine and the blood samples. Blood and urine can reflect the extents of manganese exposure for a short term so that they can as the biomarker of manganese exposure. Previous research has shown that hair also can act as a biomarker of manganese exposure because that it may reflect the levels of manganese exposure for longer timeframes.<sup>18</sup> Moreover, a suitable Mn-biomonitoring including Mn-citrate can be used to determine the early onset of Mn concentrations in human bodies and therefore it can prevent the early onset of manganism or Mn-induced Parkinsonism as far as possible.<sup>19</sup> Accordingly, the risk of manganese exposure in sensitive effective biomarkers and the effect of health injure are also the main emphasis of this study.

## 116 METHODS AND ANALYSIS

### 117 Establishing a cohort

118 To explore the early healthy effect, the potential biomarkers of exposure,  
119 susceptibility and disease, as well as the related disease of occupational manganese  
120 exposure, we establish the GXMEWHC. The cohort consists of the workers in the  
121 Ferro-manganese refinery Factory. It is a long-term prospective cohort study of  
122 manganese exposure workers. The study investigates a variety of lifestyle,  
123 socio-economic status, environmental and occupational factors as well as genetic  
124 factors in relation to the early health injure for manganese exposure. This is an  
125 opportunity to explore the relations between various kinds of risk factors and the early  
126 health injure of manganese exposure, particularly the genetic and environmental  
127 factors and their interactions.

### 129 Sample source

130 The entire samples in this study were collected from a Ferro-manganese refinery  
131 Factory. The workers who participated in physical examination every year and  
132 accorded with the following conditions were recruited. The study was approved by  
133 the Ethics Committee. Inclusion criteria included the age of 18-60 years, living in the  
134 local, working in this company for a long time (at least one year) and being able to  
135 long term follow-up, the inexistence of obvious diseases for each system, outing of  
136 touch with other risk factors except manganese (such as Cu, Pb, Cr, Hg, et al) and  
137 voluntary participating after informed consent. Exclusion criteria contained the  
138 presence of obvious diseases for any system (such as a serious neurologic disease,

139 hepatic disease, kidney disease and cardiovascular disease) in the beginning of work,  
140 the contact of various occupational risk factors (such as Cu, Pb, Cr, Hg, et al) and  
141 unable to provide informed consent (psychiatric disease, language barrier, mental  
142 deficiency). All participants were divided into different exposed groups according to  
143 the type of work.

145 **Follow-up**

146 We will follow up the participants every three years and collect the data of  
147 questionnaire interview, physical examination, biological specimens and  
148 environmental monitoring repeatedly. **Fig. 1** shows the complete study plan of the  
149 GXMEWHC. The retrospective survey are establishing and perfecting the  
150 GXMEWHC by collecting baseline data on demographic information, lifestyle,  
151 biological specimens as well as history of environmental and occupational exposure.  
152 The short-term objectives are researching the early healthy effect of occupational  
153 manganese exposure interact with environmental influences. In addition, preliminary  
154 exploring the effects of manganese exposure on genetic field are also one of our  
155 studies. In the future, our long-term and final objectives are exploring the early  
156 healthy injure on various systems in human body by gene-environmental interactions  
157 for long-term and continuous low levels of manganese exposure.

159 **Building database**

160 **Questionnaire**

161 The trained interviewers used a specifically designed questionnaire to collect the



162 baseline data after obtaining written informed consent. In order to obtain real and  
163 accurate information, we take face-to-face interviews during the physical examination.  
164 The self-reported diseases are tested and verified through the diagnosis of specialists,  
165 which is based on recognized international standards. The questionnaires consist of  
166 demographic information, socio-economic status, smoking history and alcohol  
167 consumption and occupational history.

### 169 **Occupational health examination**

170 The occupational health examination was implemented at the same time. All  
171 participants took part in the general health examination and were checked by trained  
172 physicians, nurses and the medical technicians.

173 The physical measurements covered height, weight, blood pressure (BP) and  
174 pulmonary function. The pulmonary function was estimated using a spirometry test  
175 which comprised the test value of forced vital capacity (FVC), forced expiratory  
176 volume at one second (FEV<sub>1</sub>), the ratio of forced expiratory volume at one second  
177 (FEV<sub>1</sub>%), maximal medexpiratory flow curve (MMEF), peak expiratory flow ratio  
178 (PEFR), maximal voluntary ventilation (MVV), the predicted value and the ratio  
179 percentage of all above.

180 The clinical examinations included high kilovar chest radiograph (HKV),  
181 Neurology inspection, ECG, Uncorrected visual acuity (UCVA), pure tone audiometry  
182 and physical examination of the heart, lungs, liver, spleen and abdomen.

183 The laboratory tests included blood routine tests, urine routine tests and liver  
184 function tests. The blood routine tests were measured in the laboratory covering white

185 blood cell count(WBC), lymphocyte ratio(LYR), neutrophile granulocyte  
186 ratio(GRANR), middle cell ratio (MIDR), lymphocyte count(LYC), neutrophile  
187 granulocyte count(NGC), middle cell count (MIDC), red blood cell count (RBC),  
188 hemoglobin (Hb), platelet count (PLT), hematokrit (HCT), mean corpuscular volume  
189 (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin  
190 concentration (MCHC), thrombocytocrit (THR), erythrocyte hemoglobin distribution  
191 width (RDW), platelet volume distribution width (PDW) and mean platelet volume  
192 (MPV). The urine routines were detected by urobilinogen(URO), bilirubin,(BIL),  
193 ketobody(KET), blood(BLD), protein(PRO), nitrite(NIT), white blood cell(WBC),  
194 glucose(GLU), specific gravity(SG), power of hydrogen(PH) and vitamin C.  
195 Furthermore, we examined the content of manganese in urine. The liver function test  
196 contained total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL),  
197 alanine aminotransferase (ALT), aspartate aminotransferase (AST) and AST/ALT.

199 **Neurological function test**

200 The neurological function test consists of neurocognitive function test,  
201 neurobehavioral function tests, and neuropsychological test. The Montreal Cognitive  
202 Assessment (MoCA) is a neurocognitive function test and it is an assessment method  
203 which rapid screen the Mild Cognitive Impairment (MCI) with high sensitivity and  
204 specificity.<sup>20-22</sup> We assessed the influence of manganese exposure on nervous system  
205 using the MoCA as a cognitive screening tool.<sup>23</sup> The Non-Motor Symptoms scale  
206 (NMSS) and the Scales for Outcomes in Parkinson's disease-Autonomic  
207 (SCOPA-AUT) are neurobehavioral function tests. NMSS is an acceptable and valid

assessment means for non-motor symptoms in Parkinson's disease (PD).<sup>24 25</sup>

SCOPA-AUT is a self-administered scale and can be used for the screening of autonomic symptoms in PD.<sup>26 27</sup> We used NMSS and SCOPA-AUT to evaluate the neurobehavioral function of workers who exposed to occupational manganese. The Profile of Mood States (POMS) is a neuropsychological test and is a scale to assess the emotions of manganese exposed workers.<sup>28 29</sup> We used POMS as an assessment instrument for assessing neuropsychological of workers.

### Database of biological specimens

The biological specimens were composed blood samples, urine specimen and the hair samples. Three vacuum tubes (two ethylene diamine tetraacetic teraacetic (EDTA) anticoagulant tubes and a coagulation tube) filled with five milliliters of fasting blood respectively through intravenous access. The blood sample in the coagulation tube was used to detect the liver function and one of the EDTA anticoagulant tubes was measured the blood routine. The blood sample in another EDTA anticoagulant tube was separated into blood plasma and blood corpuscle which was extracted the DNA from it as soon as possible. All the blood specimens were stored in -80°C refrigerators. In addition, a minimum of 10 milliliter of the urine were collected in the urine bottles by the participants and then as the urine specimen stored in 4°C refrigerators. A tuft of hair of proximate 0.5 cm diameter was cut off with a stainless steel scissor and collected in the special sacks which close to the scalp in the occipital region about 2 cm.<sup>30</sup> All the hair specimens were stored in a cool and dry area.

231 **Determining manganese exposure in the workplace**

232 We will track the levels of manganese exposure workers who participated in the  
233 present cohort study by means of the workplace monitoring. We will record the basic  
234 information of the factory, the technological processes of production, and the  
235 distributions of occupational risk factors, the work mode and the situation of  
236 manganese in this company. The concentrations of manganese dust and fume in the  
237 workplace were detected through the air point sampler. At the same time, we monitor  
238 the individual levels of manganese by the individual sampler in their working time.  
239 Permissible concentration-time-weighted average (PC-TWA) is the average  
240 permissible exposure levels on the regulation eight hours working day weighting by  
241 time. The Permissible concentration-Short Term Exposure Limit (PC-STEL) is the  
242 permissible exposure levels on no more than 15 minutes any time weighting by time  
243 within a working day. The cumulative exposure index (CEI) is calculated through  
244 TWA, STEL and the seniority in working. The CEI as an external exposure index of  
245 manganese and was calculated for each job combining the airborne monitoring with  
246 the individual monitoring both at working time and break time.

247

248 **Genetic determination**

249 GWAS is implemented in this study which researched the effect of manganese  
250 exposure on genetic side. The GWAS of Quantative Trait Loci (QTL) and Binary Trait  
251 Loci (BTL) are also performed for the exposed workers using the Infinium  
252 HumanExome BeadChip from Illumina Company (Illumina Infinium HumanExome

v1.0 BeadChips, 12-sample HD). The Illumina's HumanExome BeadChips covered emphatically human exonic regions and the exonic content contains more than 240,000 variant markers. The markers represented a variety of common diseases and the different groups which contained the individuals of China, Europe, Africa and Spain. We will focus on the potential interaction of environmental manganese exposure and genetics which based on the significant effects of Mn on the targeted phenotypes. Furthermore, the potential gene-environment interaction is explored through the genomes of the Manganism patients and healthy individuals who exposed manganese in workplace.

### Statistical analyses

After collecting the complete questionnaire, the data of physical examination and neurological function test, the trained investigators enter all the above data into the computer twice using the EpiData software. The GXMEWHC study database is established and it is gradually improved in later follow-up. Simultaneously, the experimental data is contained by the database. All the data is analyzed by the SPSS 16.0 software. The data of genetic determination is obtained and analyzed through the Illumina's GenomeStudio which is an integrated software platform for data visualization and analysis. The GenomeStudio Genotyping Module is an application for extracting genotyping data from the Illumina iScan systems. We use the Efficient and Parallelizable Association Container Toolbox (EPACTS) which can perform various statistical tests for identifying genome-wide association. The Quantative Trait

are calculated by the efficient mixed-model association eXpedited (EMMAX) program which can correct for sample structure within human GWASs by taking an expedited mixed linear model approach<sup>31</sup> The Binary Trait are calculated through the Logistic Score Test (LST) which can test with rare variants and relate the enriched genetic information to disease phenotypes through Logistic regression models.<sup>32</sup> When the Gene-wise or Group-wise Tests are conducted, the optimal sequence kernel association tests (SKAT-O) are used<sup>33</sup>

282

283 **Preliminary result**

284 **Demographic description of the cohort**

285 The 1991 individuals were recruited from the Ferro-manganese refinery Factory. After  
286 completing the questionnaire, a total of 1888 participants entered into the  
287 GXMEWHC who accord with standard and the effective rate is 94.8%.

288 In the cohort, 63.4% were male and 36.6% were female. The mean age was  
289 40.31 years and the percent of the four-stages were similar. Thereinto, 34.5%, 31.0%,  
290 and 34.5% of the participants had the seniority of <10, 10-20, and >20 years,  
291 respectively. In the factory, 31.2%, 15.7%, 20.1% and 33.0% were smelter, raw  
292 material processor, high exposed auxiliary and low exposed auxiliary, respectively.  
293 The mean seniority was 15.34 years. The mean Body Mass Index (BMI) was normal  
294 (22.47kg·m<sup>-2</sup>). Among the participants, 48.5% was Han Chinese. A majority of the  
295 participants (83.7%) were married. In the midst of the participants, 43.9% graduated  
296 from middle school, 45.0% had finished high school and 11.1% achieved college or  
297 higher education. In the cohort, 38.6% was current smoker, 7.0% was former smoker

and 54.4% was never smoker. Current passive smoking rates were 87.3%. The proportion of current drinker was 48.1%, the former drinker was 15.9% and the never drinker was 36.0%. Detailed information of the demographic characteristics of this cohort is provided in **Table 1**. Among the participators, 31.5% was smelters, 16.9% was human crushing workers and 6.8% was welder. The other types of work, the proportion of them, the mean age and seniority show in **Table 2**.

### **Determining manganese exposure in the workplace**

All the participators were divided according to different the types of work in the factory. Then the extents of the manganese exposure were confirmed using the working positions combine with the results of workplace detection. The CEI is calculated through TWA or STEL. Finally, all workers were classified into four exposed groups on the basis of the Mn-CEI which are respectively the internal control group ( $\text{Mn-CEI} < 1.0 \text{ mg/m}^3 \cdot \text{year}$ ), the low exposed group ( $1.0 \leq \text{Mn-CEI} < 2.0 \text{ mg/m}^3 \cdot \text{year}$ ), the medium exposed group ( $2.0 \leq \text{Mn-CEI} < 5.0 \text{ mg/m}^3 \cdot \text{year}$ ) and the high exposed group ( $\text{Mn-CEI} \geq 5.0 \text{ mg/m}^3 \cdot \text{year}$ ). The percent of internal control group, low exposed group, medium exposed group and high exposed group were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The median of total Mn-CEI was 1.85  $\text{mg/m}^3 \cdot \text{year}$  and the range was  $0.01 \text{ mg/m}^3 \cdot \text{year} \sim 9.77 \text{ mg/m}^3 \cdot \text{year}$ . The details of Mn-CEI are show in **Table 3**.

### **Main results of occupational health examination of the cohort**

The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) was



125.43 and 78.81 mmHg, respectively. The median of uric Mn was 2.63µg/L, the male was 3.67µg/L, and the female was 2.84µg/L. The values of the Blood Routine, Hepatic Function and Pulmonary Function are show in the **Table 4**.

**Detection of biological specimens and GWAS of the cohort**

We pay attention to the potential gene-environment interaction. Therefore, we performed the GWAS of QTL and BTL using the Illumina Infinium HumanExome BeadChip for 500 exposed workers, such as uric Mn and various kinds of index of pulmonary function, liver function and blood routine. The Illumina's GenomeStudio Genotyping Module was used for genotyping and data analysis which is an integrated platform for data visualization and analysis. About twenty –five thousand locus was involved in the analysis after Quality Control (QC). And then the QTL, BTL and Gene-wise or Group-wise Tests were conducted by EMMAX, LST and SKAT-O, respectively. We will analyze the differential gene expression further. The results of GWAS and other indexes will be reported in separate articles. We plan to conduct GWAS in a larger number of manganese exposure workers for exploring the genic risk factors and the gene-environment interaction.

**ETHICS AND DISSEMINATION**

The study has been approved via the Medical ethics committee of Guangxi Medical University. All the original files and data are maintained and stored at the research office, in the Department of Occupational Health and Environmental Health, School



of Public Health, Guangxi Medical University, Nanning, China. Electronic materials are stored in a safe system file and accessible by the data manager. All the biological samples are marked in a sequential order and stored in secure freezer. The results will be disseminated to relevant scientific forums which included publishing in peer-reviewed journals and presenting at international conferences.

349

350

**Acknowledgements** We thank all participants who volunteered to take part in this study, all members of the GXMEWHC research team, the nurses and administrators in the Ferro-manganese refinery Factory.

**Contributors** Xiaobo Yang and Yunfeng Zou contributed in conception and design; Jing Liu, Kangcheng Chen, Yingnan Lv, Damin Huang, Yuefei Shen, Yaoqiu Zhong, Zhihao Liu, Bei Jiang, Qin Li, Li Qing, Wei Zhang, Lang Chen, Fenfen Wang, Bing Xia and Li Yang contributed in acquisition of the data; Yingnan Lv analysed the data and drafted the manuscript; all authors contributed to review and revision of the manuscript and approved the final version.

**Funding** This study was supported by National Natural Science Foundation of China (81060234, 21167004, and 81160339); Guangxi Science Fund for Distinguished Young Scholars (2012jjFA40011); Guangxi Natural Science Foundation (2011jjA40294); Guangxi science and technology development project (1355007-1); and Program for New Century Excellent Talents in University (NCET-12-0653).

**Competing interests** All authors declare that they have no conflict of interest.

**Ethics approval** Medical ethics committee of Guangxi Medical University.

**Patient consent** Obtained.

367 **Provenance and peer review** Not commissioned; externally peer reviewed.

368 **Data sharing statement** No additional data are available.

369

370

371 **References**

372 1 Erikson KM, Syversen T, Aschner JL et al. Interactions between excessive manganese  
373 exposures and dietary iron-deficiency in neurodegeneration. *Environ Toxicol Pharmacol*  
374 2005;19:415-21.

375 2 Erikson KM, Aschner M. Manganese neurotoxicity and glutamate-GABA interaction.  
376 *Neurochemistry International* 2003;43:475-480.

377 3 Bowler RM, Roels HA, Nakagawa S et al. Dose-effect relationships between manganese  
378 exposure and neurological, neuropsychological and pulmonary function in confined space  
379 bridge welders. *Occup Environ Med* 2007;64:167-77.

380 4 Sriram K, Lin GX, Jefferson AM et al. Manganese accumulation in nail clippings as a  
381 biomarker of welding fume exposure and neurotoxicity. *Toxicology* 2012;291:73-82.

382 5 Ellingsen DG, Konstantinov R, Bast-Pettersen R et al. A neurobehavioral study of current and  
383 former welders exposed to manganese. *Neurotoxicology* 2008;29:48-59.

384 6 Summers MJ, Summers JJ, White TF et al. The effect of occupational exposure to manganese  
385 dust and fume on neuropsychological functioning in Australian smelter workers. *J Clin Exp*  
386 *Neuropsychol* 2011;33:692-703.

387 7 Rivera-Mancia S, Rios C, Montes S. Manganese accumulation in the CNS and associated  
388 pathologies. *Biometals* 2011;24:811-25.

389 8 Roels HA, Bowler RM, Kim Y et al. Manganese exposure and cognitive deficits: a growing  
390 concern for manganese neurotoxicity. *Neurotoxicology* 2012;33:872-80.

391 9 Kim EA, Cheong HK, Choi DS et al. Effect of occupational manganese exposure on the  
392 central nervous system of welders: 1H magnetic resonance spectroscopy and MRI findings.  
393 *Neurotoxicology* 2007;28:276-83.

394 10 Yang Y, Huang J, Liu J et al. Long-Term Effect of Occupational Exposure to Manganese on  
395 Pulmonary Ventilation Function. *Journal of Environmental & Occupational Medicine*  
396 2013;30:29-31.

397 11 Boojar MM, Goodarzi F. A longitudinal follow-up of pulmonary function and respiratory  
398 symptoms in workers exposed to manganese. *J Occup Environ Med* 2002;44:282-90.

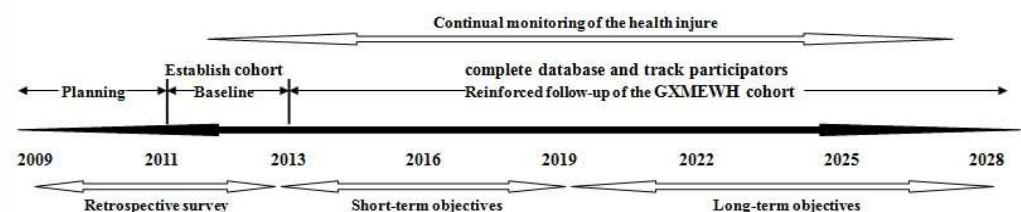
399 12 McKinney AM, Filice RW, Teksam M et al. Diffusion abnormalities of the globi pallidi in  
400 manganese neurotoxicity. *Neuroradiology* 2004;46:291-5.

401 13 Aschner M, Erikson KM, Dorman DC. Manganese Dosimetry: Species Differences and  
402 Implications for Neurotoxicity. *Critical Reviews in Toxicology* 2005;35:1-32.

403 14 Deng Q, Liu J, Li Q et al. Interaction of occupational manganese exposure and alcohol  
404 drinking aggravates the increase of liver enzyme concentrations from a cross-sectional study  
405 in China. *Environ Health* 2013;12:30.

406 15 Jiang YM, Zheng W. Cardiovascular toxicities upon manganese exposure. *Cardiovascular*  
407 *Toxicology* 2005;5:345-354.

- 408 16 Liu XF, Li ZP, Tie F et al. Effects of manganese-toxicity on immune-related organs of cocks.  
409 *Chemosphere* 2013;90:2085-100.
- 410 17 Curran CP, Park RM, Ho SM et al. Incorporating genetics and genomics in risk assessment for  
411 inhaled manganese: from data to policy. *Neurotoxicology* 2009;30:754-60.
- 412 18 Eastman RR, Jursa TP, Benedetti C et al. Hair as a biomarker of environmental manganese  
413 exposure. *Environ Sci Technol* 2013;47:1629-37.
- 414 19 Michalke B, Fernsebner K. New insights into manganese toxicity and speciation. *J Trace Elem  
415 Med Biol* 2013.
- 416 20 Nasreddine ZS, Phillips NA, Bedirian V et al. The Montreal Cognitive Assessment, MoCA: a  
417 brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53:695-9.
- 418 21 Fisekovic S, Memic A, Pasalic A. Correlation between moca and mmse for the assessment of  
419 cognition in schizophrenia. *Acta Inform Med* 2012;20:186-9.
- 420 22 Freitas S, Simoes MR, Alves L et al. Montreal Cognitive Assessment (MoCA): validation  
421 study for frontotemporal dementia. *J Geriatr Psychiatry Neurol* 2012;25:146-54.
- 422 23 Zou Y, Qing L, Zeng X et al. Cognitive function and plasma BDNF levels among  
423 manganese-exposed smelters. *Occup Environ Med* 2014;71:189-94.
- 424 24 Martinez-Martin P, Rodriguez-Blazquez C, Abe K et al. International study on the  
425 psychometric attributes of the non-motor symptoms scale in Parkinson disease. *Neurology*  
426 2009;73:1584-91.
- 427 25 Chaudhuri KR, Martinez-Martin P, Brown RG et al. The metric properties of a novel  
428 non-motor symptoms scale for Parkinson's disease: Results from an international pilot study.  
429 *Mov Disord* 2007;22:1901-11.
- 430 26 Visser M, Marinus J, Stiggelbout AM et al. Assessment of autonomic dysfunction in  
431 Parkinson's disease: the SCOPA-AUT. *Mov Disord* 2004;19:1306-12.
- 432 27 Rodriguez-Blazquez C, Forjaz MJ, Frades-Payo B et al. Independent validation of the scales  
433 for outcomes in Parkinson's disease-autonomic (SCOPA-AUT). *Eur J Neurol*  
434 2010;17:194-201.
- 435 28 Laohaudomchok W, Lin X, Herrick RF et al. Neuropsychological effects of low-level  
436 manganese exposure in welders. *Neurotoxicology* 2011;32:171-9.
- 437 29 Niu Q, Shuchang H, Sheng W et al. Neurobehavioral functions, serum prolactin and plasma  
438 renin activity of manganese-exposed workers. *Int J Immunopathol Pharmacol* 2004;17:17-24.
- 439 30 Menezes-Filho JA, Paes CR, Pontes AM et al. High levels of hair manganese in children  
440 living in the vicinity of a ferro-manganese alloy production plant. *Neurotoxicology*  
441 2009;30:1207-13.
- 442 31 Kang HM, Sul JH, Service SK et al. Variance component model to account for sample  
443 structure in genome-wide association studies. *Nat Genet* 2010;42:348-54.
- 444 32 Lin DY, Tang ZZ. A general framework for detecting disease associations with rare variants in  
445 sequencing studies. *Am J Hum Genet* 2011;89:354-67.
- 446 33 Lee S, Wu MC, Lin X. Optimal tests for rare variant effects in sequencing association studies.  
447 *Biostatistics* 2012;13:762-75.
- 448
- 449
- 450



**Fig. 1 The complete study plan of the GXMEWHC**

The baseline survey was completed preliminary in 2013 and collected the epidemiological information, biological sample, the data of occupational health examination and workplace monitoring. Simultaneously, the database of GWAS was performed for 500 manganese exposed workers. The participants will be following up every three years and the databases were reinforced gradually in the future.

471  
472

Table 1

## Demographic characteristics of the GXMEWHC

Variables	Number (n=1888)	Percent (%)
Sex		
Male	1197	63.4
Female	691	36.6
Age, years (mean $\pm$ SD)	40.31 $\pm$ 7.85	
<35	482	25.5
35~40	402	21.3
40~45	440	23.3
$\geq 45$	564	29.9
Seniority, years (mean $\pm$ SD)	15.34 $\pm$ 9.63	
<10	652	34.5
10~20	585	31
>20	651	34.5
BMI, kg/m <sup>2</sup> (mean $\pm$ SD)	22.47 $\pm$ 2.8	
<18.5	95	5
18.5~24	1289	68.3
24~28	422	22.4
$\geq 28$	74	3.9
Missing	8	0.4
Race/ethnicity		
Han Chinese	916	48.5
Other ethnic groups	972	51.5
Marital status		
Single	233	12.3
Married	1580	83.7
Windowed or divorced	75	4
Education or lower		
Middle school	829	43.9
High school	850	45
University or college or higher	209	11.1
Smoking status		
Current smoker	729	38.6
Former smoker	132	7
Never smoker	1027	54.4
Drinking status		
Current drinker	907	48.1
Former drinker	301	15.9
Never drinker	680	36

473  
474  
475  
476  
477  
478  
479  
480

**Table 2**  
**Different types of work of the GXMEWHC**

Types of work	Number (n)	Percent (%)	Age (years)	Seniority (years)
			Mean± SD	Mean± SD
Smelter	594	31.5	38.95 ± 8.20	15.82 ± 9.02
Human Crushing Worker	320	16.9	41.08 ± 5.30	9.04 ± 6.00
Craneman	74	3.9	37.15 ± 8.76	16.24 ± 8.88
Finishing Machining Worker	99	5.2	40.36 ± 6.10	10.20 ± 8.79
Scaleman	105	5.6	42.30 ± 4.92	17.53 ± 6.88
Sampleman	21	1.1	45.75 ± 7.02	23.07 ± 6.57
Welder	128	6.8	40.75 ± 10.13	18.29 ± 10.76
Chemical Analyst	54	2.9	45.52 ± 7.02	24.29 ± 8.37
Repairman	151	8.0	41.63 ± 9.10	19.19 ± 10.64
Electrician	91	4.8	40.28 ± 7.31	19.45 ± 8.00
Alkali Recovery Worker	133	7.0	40.89 ± 6.33	13.74 ± 8.74
Car Driver	118	6.2	39.01 ± 9.96	15.09 ± 12.07
Total	1888	100	40.31 ± 7.85	15.23 ± 9.60

481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493

**Table 3**

The Mn-CEI of the GXMEWHC

Mn-CEI (mg/m <sup>3</sup> ·year)	Number (n)	Percent (%)	Median (Interquartile Range)	Range
Internal Control Group (Mn-CEI <1.0)	651	34.5	0.51 (0.55)	0.01~0.99
Low Exposed Group (1.0≤Mn-CEI<2.0)	333	17.6	1.49 (0.46)	1.01~1.99
Medium Exposed Group (2.0≤Mn-CEI<5.0)	710	37.6	3.04 (1.20)	2.00~4.98
High Exposed Group (Mn-CEI≥5.0)	194	10.3	5.99 (2.47)	5.01~9.77
Total	1888	100	1.85 (2.58)	0.01~9.77

494  
495  
496

**Table 4**  
The results of occupational health examination of the GXMEWHC

Variables	Male(n=1197)	Female(n=691)	Total(n=1888)
	Mean ± SD	Mean ± SD	Mean ± SD
Systolic blood pressure, mmHg	127.68 ± 12.11	121.54 ± 11.53	125.43 ± 1 2.26
Diastolic blood pressure, mmHg	79.93 ± 8.29	76.86 ± 7.93	78.81 ± 8.29
Blood routine			
WBC, 10 <sup>9</sup> /L	6.91 ± 1.51	6.32 ± 1.52	6.69 ± 1.54
RBC, 10 <sup>12</sup> /L	5.13 ± 0.52	4.61 ± 0.44	4.94 ± 0.55
Haemoglobin, g/L	148.69 ± 12.73	128.8 ± 14.37	141.38 ± 16.44
Platelet count, 10 <sup>9</sup> /L	241.76 ± 54.13	256.29 ± 62.86	247.1 ± 57.9
Hepatic function			
Total bilirubin, µmol/l	12.48 ± 5.3	11.94 ± 4.49	12.28 ± 5.02
Direct bilirubin, µmol/l	3.98 ± 2.16	3.66 ± 2.19	3.86 ± 2.17
Indirect bilirubin, µmol/l	8.5 ± 3.44	8.24 ± 2.51	8.4 ± 3.13
ALT, U/L	25.35 ± 17.62	17.23 ± 14.74	22.38 ± 17.07
AST, U/L	27.06 ± 15.7	23.32 ± 21.75	25.69 ± 18.24
Pulmonary function			
Test value of FVC, L	4.25 ± 0.86	3.18 ± 0.64	3.86 ± 0.94
Test value of FEV1, L	3.61 ± 0.72	2.71 ± 0.54	3.28 ± 0.79
Uric Mn, µg/L			
Median (Interquartile Range)	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)



# BMJ Open

## Rationale, design and baseline results of the Guangxi manganese-exposed workers healthy cohort (GXMEWHC) study

Journal:	BMJ Open
Manuscript ID:	bmjopen-2014-005070.R1
Article Type:	Research
Date Submitted by the Author:	31-May-2014
Complete List of Authors:	<p>Lv, Yingnan; Guangxi Medical University, Occupational Health and Environmental Health</p> <p>Zou, Yunfeng; Guangxi Medical University, Department of Toxicology</p> <p>Liu, Jing; Guangxi Medical University, Department of Occupational Health and Environmental Health</p> <p>chen, kangcheng; Guangxi Medical University, Department of Occupational Health and Environmental Health</p> <p>huang, damin; Guangxi Medical University, Department of Occupational Health and Environmental Health</p> <p>shen, yuefei; The 1st affiliated hospital, Neurology</p> <p>zhong, yaoqiu; Guangxi Medical University, Department of Occupational Health and Environmental Health</p> <p>Zhihao, Zhihao; Baise Center for Disease Control and Prevention, office</p> <p>Jiang, Bei; Baise Center for Disease Control and Prevention, Department of Occupational Health</p> <p>li, qin; Guangxi Medical University, Department of Toxicology</p> <p>Qing, Li; Guangxi Medical University, Department of Epidemiology and Health Statistics</p> <p>zhang, wei; The First Affiliated Hospital of Guangxi Medical University, Department of Neurology</p> <p>chen, lang; The First Affiliated Hospital of Guangxi Medical University, Department of Neurology</p> <p>Wang, Fenfen; Guangxi Medical University, Department of Occupational Health and Environmental Health</p> <p>Xia, Bing; Guangxi Medical University, Department of Occupational Health and Environmental Health</p> <p>Yang, Li; Guangxi Medical University, Department of Occupational Health and Environmental Health</p> <p>yang, xiaobo; Guangxi Medical University, Occupational Health and Environmental Health</p>
<b>Primary Subject Heading</b>:	Occupational and environmental medicine
Secondary Subject Heading:	Epidemiology, Genetics and genomics
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, EPIDEMIOLOGY, GENETICS



For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

# **Rationale, design and baseline results of the Guangxi manganese-exposed workers healthy cohort (GXMEWHC) study**

Yingnan Lv<sup>1</sup>, Yunfeng Zou<sup>2</sup>, Jing Liu<sup>1</sup>, Kangcheng Chen<sup>1</sup>, Damin Huang<sup>1</sup>, Yuefei Shen<sup>3</sup>, Yaoqiu Zhong<sup>1</sup>, Zhihao Liu<sup>4</sup>, Bei Jiang<sup>4</sup>, Qin Li<sup>2</sup>, Li Qing<sup>5</sup>, Wei Zhang<sup>3</sup>, Lang Chen<sup>3</sup>, Fenfen Wang<sup>1</sup>, Bing Xia<sup>1</sup>, Li Yang<sup>1</sup>, Xiaobo Yang<sup>1,6,\*</sup>

<sup>1</sup> Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>2</sup> Department of Toxicology, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>3</sup> Department of Neurology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

<sup>4</sup> Baise Center for Disease Control and Prevention, Baise, Guangxi, China

<sup>5</sup> Department of Epidemiology and Health Statistics, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>6</sup> Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning, Guangxi, China

Yingnan Lv and Yunfeng Zou are contributed equally.

\* Corresponding Author: Dr. Xiaobo Yang, Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University,

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

24 Shuangyong Road 22, Nanning, Guangxi, 530021, P. R. China; [yxbo21021@163.com](mailto:yxbo21021@163.com)

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

For peer review only

## Abstract

**Objective:** To explore the early health effect and long-term related diseases of occupational manganese (Mn) exposure according to biomarkers of exposure, effect and susceptibility.

**Design:** The baseline survey of a longitudinal cohort study from a Ferromanganese Refinery.

**Participants:** A total of 1888 individuals (1197 men, 691 women) involved in Guangxi manganese-exposed worker healthy cohort (GXMEWHC) study. Participants were aged between 18 and 60 years (average age 40.31 years) and worked in Ferromanganese Refinery at least one year and lived in the local area.

**Results:** The baseline survey was completed and the GXMEWHC study was established, which included 1888 workers (average seniority 15.34 years). All participants were divided into four groups according to the levels of Mn cumulative exposure index (Mn-CEI), which included internal control group (Mn-CEI <1.0 mg/m<sup>3</sup>•year), low exposure group (1.0 mg/m<sup>3</sup>•year ≤ Mn-CEI <2.0 mg/m<sup>3</sup>•year), medium exposure group (2.0 mg/m<sup>3</sup>•year ≤ Mn-CEI <5.0 mg/m<sup>3</sup>•year), and high exposure group (Mn-CEI ≥5.0 mg/m<sup>3</sup>•year). GWAS of quantitative trait loci (QTL) and binary trait loci (BTL) were performed using Illumina Infinium HumanExome BeadChip for 500 manganese-exposed workers. Stored plasma, DNA, hair and urine are available in further study. Participants will be followed up every three years.

**Conclusions:** The GXMEWHC study provides abundant data to explore the multi-organ health effects of occupational Mn exposure by biomarkers of exposure, response and susceptibility, respectively.

70 **Strengths and limitations of this study**

- 71 ■ In this study, we can collect an abundant database because of the large samples in  
72 the heavy metals cohort.
- 73 ■ The Ferromanganese Refinery is the largest metallurgical factory of Mn  
74 processing in China; therefore, it can provide an extremely rich dataset for  
75 analysis.
- 76 ■ The GXMEWHC is a longitudinal study that can continuously follow up and  
77 repeatedly investigate the participants. We can explore the relationships between  
78 occupational Mn exposure and the early health effects.
- 79 ■ GWAS was implemented to determine the susceptibility genes related to chronic  
80 low-level Mn exposure, and to explore the interactions between genetic factors  
81 and environmental factors. These data will provide an important opportunity to  
82 identify more susceptible individuals to prevent early health effect in workers.
- 83 ■ The potential limitations are that loss to follow-up may be a weakness with our  
84 study. There are some temporary workers in the factory and they may leave the  
85 factory after a period of time. We can reduce the probability of the loss to follow  
86 up through strictly controlled inclusion criteria when establishing the cohort.

## INTRODUCTION

Manganese (Mn) is an essential nutrient and is necessary to inhale Mn for maintaining daily life. In addition to food intake, environmental exposure to Mn allows the absorption of Mn, including occupational exposure. Mn accumulated in some organs and induces adverse effects when the Mn concentration *in vivo* exceeds the capacity of human metabolism.<sup>1</sup>

Many studies have shown that Mn can cause neurological abnormalities when it accumulates in the human brain,<sup>2-4</sup> such as early impaired finger tapping speed<sup>5</sup> or cognitive deficits, terminal Parkinsonian-like symptoms,<sup>6</sup> and manganism.<sup>7</sup> The level of Mn in the human body can be detected through some internal biomarkers, neurobehavioral tests and functional neuroimaging.<sup>8,9</sup> Increased concentrations of Mn in the kidney have been found in the manganese-exposed workers because the kidney is a pathway of Mn excretion.<sup>4</sup> In addition, repeated respiratory exposure to Mn may cause impaired lung function. In one study, there was a dose-effect relationship between occupational Mn exposures and a reduction in pulmonary function.<sup>10</sup> Compared with the non-exposed workers, the pulmonary function in the manganese-exposed workers evaluated by the spirometry tests showed a significant decrease in forced vital capacity (FVC), forced expiratory volume at one second (FEV<sub>1</sub>), and the ratio of forced expiratory volume at one second (FEV<sub>1</sub>%) values.<sup>11</sup> Increased Mn levels in blood serum ascribed to that liver is the main organ to store, biotransformation and detoxify Mn.<sup>12</sup> Overexposure to Mn can cause liver toxicity and exacerbate liver dysfunction.<sup>13, 14</sup> Chronic Mn exposure leads to a series of significant cardiovascular toxicities including an abnormal electrocardiogram (ECG)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

116 and the inhibition of myocardial contraction which can alter the blood pressure  
117 (BP).<sup>15</sup> Additionally, Mn cytotoxicity has been shown to induce cell apoptosis and  
118 DNA damage in avian immune cells.<sup>16</sup> Low Mn<sup>2+</sup> levels can induce oxidative DNA  
119 damage via an apoptotic pathway, but this DNA damage can be reduced using  
120 antioxidants. A risk assessment of inhaled Mn incorporating genetics and genomics  
121 identified genetically based biomarkers of exposure, disease and susceptibility.<sup>17</sup>

122 Thus, Mn toxicity in humans plays a significant role in several systems.  
123 Currently, most studies have explored separately the effect of Mn exposure on  
124 different systems of the human body. To further explore the effects and the interaction  
125 of Mn exposure in various systems, we will establish a prospective cohort study  
126 which includes individual Mn exposure and regular occupational examinations.  
127 Simultaneously, we will determine biological exposure indicators by means of hair,  
128 urine and blood samples. Blood and urine can reflect the extent of Mn exposure in the  
129 short term so they can be used as biomarkers of Mn exposure. Previous research has  
130 shown that hair can also act as a biomarker of Mn exposure because it may reflect the  
131 levels of Mn exposure over longer timeframes.<sup>18</sup> Moreover, suitable Mn  
132 biomonitoring including Mn-citrate can be used to determine the early onset of excess  
133 Mn concentrations in the human body and can prevent the early onset of manganism  
134 or Mn-induced Parkinsonism as far as possible.<sup>19</sup> Accordingly, the risk of Mn  
135 exposure using sensitive effective biomarkers and the associated health effects are  
136 also the main emphasis of this study.



## 139 METHODS

### 140 Establishing a cohort

141 To explore the early healthy effects, potential biomarkers of exposure, susceptibility  
142 and disease, as well as the related disease of occupational Mn exposure, we  
143 established the GXMEWHC. The prospective cohort study started in 2011 and the  
144 targeted population was the workers aged 18 years or older working in the  
145 Ferromanganese Refinery. The study investigates a variety of lifestyle,  
146 socio-economic status, environmental and occupational factors as well as genetic  
147 factors in relation to the early health effects of Mn exposure. This is an opportunity to  
148 explore the relationships between various risk factors and the early health effects of  
149 Mn exposure, particularly genetic and environmental factors and their interactions.

150

### 151 Sample source

152 The entire sample of this study was collected from a Ferromanganese Refinery. The  
153 workers who participated in physical examinations every year and met the following  
154 conditions were recruited. The study was approved by the local Ethics Committee.  
155 Inclusion criteria were age of 18-60 years, living in the local area, working in the  
156 factory for a long time (at least one year) and being able to complete long-term  
157 follow-up, the lack of obvious diseases in each system, absence if other risk factors  
158 except Mn exposure (such as Cu, Pb, Cr, or Hg) and voluntary participation after  
159 providing informed consent. People were excluded if they had an obvious diseases in  
160 any system (such as a serious neurological disease, hepatic disease, kidney disease or  
161 cardiovascular disease), contact with other occupational risk factors (such as Cu, Pb,

162 Cr, or Hg) and inability to provide informed consent (psychiatric disease, language  
163 barrier, or mental deficiency). All participants were divided into different exposure  
164 groups according to the type of work.

165

166 **Follow-up**

167 We will follow up the participants every three years. The information collected will be  
168 the same as the baseline data and will be collected by questionnaire interviews,  
169 physical examinations, biological specimens and environmental monitoring. **Fig. 1**  
170 shows the complete study plan of the GXMEWHC. The retrospective survey are  
171 establishing and perfecting the GXMEWHC by collecting baseline data on  
172 demographic information, lifestyle, biological specimens as well as history of  
173 environmental and occupational exposure. The short-term objectives are to explore  
174 the early health effects of occupational Mn exposure interact with environmental  
175 influences. In addition, preliminary exploring the effects of Mn exposure on genetics  
176 are also one of our studies. Our long-term and final objectives are to explore the early  
177 health effects on various systems of the human body by gene-environmental  
178 interactions for long-term and continuous low levels of Mn exposure.

179

180 **Building database**

181 **Questionnaire**

182 Trained interviewers used a structured questionnaire to collect the baseline data after  
183 obtaining written informed consent. In order to obtain real and accurate information,  
184 we conducted face-to-face interviews during the physical examination. Self-reported

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

185 diseases were tested and verified through the diagnosis of specialists, based on  
186 recognized international standards. The questionnaire consisted of demographic  
187 information, socio-economic status, smoking history, alcohol consumption and  
188 occupational history.

### 189 190 **Occupational health examination**

191 The occupational health examination was conducted at the same time. All participants  
192 took part in the general health examination and were checked by trained physicians,  
193 nurses, and medical technicians.

194 The physical measurements covered height, weight, BP, and pulmonary function.  
195 The pulmonary function was estimated using a spirometry test which included FVC ,  
196 FEV<sub>1</sub>, FEV<sub>1</sub>%, maximal mid-expiratory flow (MMEF), the peak expiratory flow ratio  
197 (PEFR), maximal voluntary ventilation (MVV), the predicted value of them and the  
198 ratio percentage of all above.

199 The clinical examinations included a high kilovar chest radiograph (HKV),  
200 neurology inspection, ECG, uncorrected visual acuity (UCVA) test, pure tone  
201 audiometry and physical examination of the heart, lungs, liver, spleen and abdomen.

202 The laboratory tests included routine blood tests, routine urine tests and liver  
203 function tests. The routine blood tests were measured in the laboratory and included  
204 the white blood cell count (WBC), lymphocyte ratio (LYR), neutrophil granulocyte  
205 ratio (GRANR), middle cell ratio (MIDR), lymphocyte count (LYC), neutrophil  
206 granulocyte count (NGC), middle cell count (MIDC), red blood cell count (RBC),  
207 hemoglobin (Hb), platelet count (PLT), hematocrit (HCT), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocytocrit (THR), erythrocyte hemoglobin distribution width (RDW), platelet volume distribution width (PDW), and mean platelet volume (MPV). The routines urine tests included urobilinogen (URO), bilirubin (BIL), ketobodies (KET), blood (BLD), protein (PRO), nitrite (NIT), white blood cells (WBC), glucose (GLU), specific gravity (SG), pH and vitamin C. Furthermore, we examined the content of Mn in urine. The liver function tests contained total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the AST/ALT ratio.

**Neurological function test**

The neurological function test consisted of a neurocognitive function test, neurobehavioral function test, and neuropsychological test. The Montreal Cognitive Assessment (MoCA) is a neurocognitive function test and is an assessment method which rapidly screens for Mild Cognitive Impairment (MCI) with high sensitivity and specificity.<sup>20-22</sup> We assessed the influence of Mn exposure on the nervous system using the MoCA as a cognitive screening tool.<sup>23</sup> The Non-Motor Symptoms scale (NMSS) and the Scales for Outcomes in Parkinson's disease-Autonomic (SCOPA-AUT) are neurobehavioral function tests. NMSS is an acceptable and valid assessment means for non-motor symptoms in Parkinson's disease (PD).<sup>24 25</sup> SCOPA-AUT is a self-administered scale and can be used for the screening of autonomic symptoms in PD.<sup>26 27</sup> We used NMSS and SCOPA-AUT to evaluate the neurobehavioral function of workers who were exposed to occupational Mn. The

Profile of Mood States (POMS) is a neuropsychological test and is a scale to assess the emotions of manganese-exposed workers.<sup>28 29</sup> We used POMS as an assessment instrument for assessing neuropsychological of workers.

### **Database of biological specimens**

The biological specimens were composed blood samples, urine specimen and the hair samples. Three vacuum tubes (two ethylene diamine tetraacetic teraacetic (EDTA) anticoagulant tubes and a coagulation tube) were filled with 5 mL of fasting blood obtained through intravenous access. The blood sample in the coagulation tube was used to assess liver function and one of the EDTA anticoagulant tubes was used for routine blood tests. The blood sample in the other EDTA anticoagulant tube was separated into blood plasma and blood cell from which was DNA extracted as soon as possible. All the blood specimens were stored at -80°C. In addition, a minimum of 10 mL of the urine were collected in a urine bottle and stored at 4°C. A tuft of hair close to the scalp in the occipital region, about 2 cm in length and approximately 0.5 cm in diameter was cut off with stainless steel scissors and collected in a special bag.<sup>30</sup> All hair specimens were stored in a cool and dry area.

### **Determining Mn exposure in the workplace**

We will track the levels of manganese-exposed workers who participated in the present cohort study by means of workplace monitoring. We will record the basic information of the factory, the technological processes of production, the distributions of occupational risk factors, the work mode, and the level of Mn in this factory. The

254 concentrations of Mn dust and fumes in the workplace are detected through an air  
255 point sampler. At the same time, we will monitor the individual levels of Mn using  
256 individual samplers during working hours. The permissible  
257 concentration-time-weighted average (PC-TWA) is the average permissible exposure  
258 levels during the regulation eight-hour working day, weighted by time. The  
259 permissible concentration-short term exposure limit (PC-STEL) is the permissible  
260 exposure levels in no more than 15 minutes at any time, weighted by time within a  
261 working day. The cumulative exposure index (CEI) is calculated through TWA, STEL,  
262 and workplace seniority in working. The CEI as an external exposure index of ma Mn  
263 nganese and was calculated for each job, combining the airborne monitoring with the  
264 individual monitoring both during work time and break time.

265

266 **Database of biomarkers**

267 The database of biomarkers included biomarkers of exposure, effect and susceptibility.  
268 The biomarkers of exposure will be detected through atomic absorption spectrometry  
269 (AAS) which are the levels of Mn and Fe in plasma, urine and hair. The levels of  
270 plasma brain-derived neurotrophic factor (BDNF), dopamine (DA) were determined  
271 by Sandwich ELISA kits which are biomarkers of effect. The biomarkers of  
272 susceptibility are also assessed by GWAS which are shown in the following Genetic  
273 assessments part in detail.

274

275

## Genetic assessments

A sub-cohort of GWAS is implemented in this study to assess the effect of Mn exposure on genetics. The GWAS of quantitative trait loci (QTL) and binary trait loci (BTL) will also be performed for exposed workers using the Infinium HumanExome BeadChip from Illumina Company (Illumina Infinium HumanExome v1.0 BeadChips, 12-sample HD). Illumina's HumanExome BeadChips are covered with human exonic regions, the exonic content contains more than 240,000 variant markers. The markers represent a variety of common diseases. The different groups include individuals from China, Europe, and Africa. We will focus on the potential interactions of environmental Mn exposure and genetics based on the significant effects of Mn on the targeted phenotypes. Furthermore, potential gene-environment interactions will be explored through the genomes of manganism patients and healthy individuals exposed to Mn in the workplace.

## Statistical analyses

After collecting the complete questionnaires, the physical examination results and neurological function test data, trained investigators will enter the above data into the computer twice using EpiData software. The GXMEWHC study database is established and will be gradually improved in subsequent follow-up. Simultaneously, the experimental data will be contained in the database. All the data will be analyzed by SPSS 16.0 software. The genetic determination data will be obtained and analyzed through Illumina's GenomeStudio, which is an integrated software platform for data



298 visualization and analysis. The GenomeStudio Genotyping Module is an application  
299 for extracting genotyping data from the Illumina iScan systems. We will use the  
300 Efficient and Parallelizable Association Container Toolbox (EPACTS), which can  
301 perform various statistical tests for identifying genome-wide associations. The  
302 quantitative traits will be calculated by the efficient mixed-model association  
303 eXpedited (EMMAX) program, which can correct for sample structure within human  
304 GWAS by taking an expedited mixed linear model approach.<sup>31</sup> The binary traits will  
305 be calculated through the Logistic Score Test (LST) which can test rare variants and  
306 relate the enriched genetic information to disease phenotypes through Logistic  
307 regression models.<sup>32</sup> When the gene-wise or group-wise tests are conducted, optimal  
308 sequence kernel association tests (SKAT-O) will be used<sup>33</sup>

310 **PRELIMINARY RESULTS**

311 **Demographic description of the cohort**

312 In total, 1,991 individuals were recruited to participate in the study from the  
313 Ferromanganese Refinery. After completing the questionnaire, a total of 1,888  
314 participants who met the standards were entered into the GXMEWHC study, with an  
315 effective rate of 94.8%.

316 **Table 1** summarizes the baseline characteristics of the cohort. Of the cohort,  
317 63.4% were male and 36.6% were female. The mean age was 40.31 years and the  
318 percent of the age distribution was similar. 34.5%, 31.0%, and 34.5% of the  
319 participants had seniority of <10, 10-20, and >20 years, respectively. In the factory,  
320 31.2%, 15.7%, 20.1% and 33.0% were smelters, raw material processors, high



321 exposure auxiliary, and low exposure auxiliary, respectively. The mean seniority was  
322 15.34 years. The mean body mass index (BMI) was normal ( $22.47\text{kg}\cdot\text{m}^{-2}$ ). Among the  
323 participants, 48.5% were Han Chinese. A majority of the participants (83.7%) were  
324 married. Of these participants, 43.9% graduated from middle school, 45.0% finished  
325 high school and 11.1% completed college or higher education. In the cohort, 38.6%  
326 were current smokers, 7.0% were former smokers and 54.4% were never smokers.  
327 Current passive smoking rates were 87.3%. The proportion of current drinkers was  
328 48.1%, former drinkers was 15.9% and never drinkers was 36.0%. Among the  
329 participants, 31.5% were smelters, 16.9% were human crushing workers and 6.8%  
330 were welder. The other types of work, the proportion of them, the mean age, and  
331 seniority are shown in **Table 2**.

332

### 333 **Determining Mn exposure in the workplace**

334 All the participants were divided according to different types of work in the factory.  
335 Then, the extent of the Mn exposure was confirmed using the working positions  
336 combined with the results of workplace detection. The CEI was calculated through  
337 TWA or STEL. Finally, all workers were classified into four exposure groups on the  
338 basis of the Mn-CEI results, including the internal control group ( $\text{Mn-CEI} < 1.0$   
339  $\text{mg}/\text{m}^3\cdot\text{year}$ ), the low exposure group ( $1.0 \text{ mg}/\text{m}^3\cdot\text{year} \leq \text{Mn-CEI} < 2.0 \text{ mg}/\text{m}^3\cdot\text{year}$ ),  
340 the medium exposure group ( $2.0 \text{ mg}/\text{m}^3\cdot\text{year} \leq \text{Mn-CEI} < 5.0 \text{ mg}/\text{m}^3\cdot\text{year}$ ) and the  
341 high exposure group ( $\text{Mn-CEI} \geq 5.0 \text{ mg}/\text{m}^3\cdot\text{year}$ ). The percentages of the internal  
342 control group, low exposure group, medium exposure group and high exposure group  
343 were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The median of total Mn-CEI was

1  
2 344 1.85 mg/m<sup>3</sup>·year and the range was 0.01 mg/m<sup>3</sup>·year-9.77 mg/m<sup>3</sup>·year. The details of  
3  
4 345 Mn-CEI are shown in **Table 3**.  
5  
6  
7 346  
8  
9

10 347 **Main results of the occupational health examination of the cohort**

11  
12 348 The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were  
13  
14 349 125.43 and 78.81 mmHg, respectively. The median urine Mn level was 2.63µg/L,  
15  
16 350 3.67µg/L in males, and 2.84µg/L in females. The results of the routine blood tests,  
17  
18 351 hepatic function tests and pulmonary function tests are shown in the **Table 4**.  
19  
20 352  
21  
22  
23  
24

25 353 **Assessment of biomarkers**

26  
27 354 The liver function was analyzed between different manganese-exposed groups of the  
28  
29 355 cohort in 2013. Our conclusions in this study were that occupational Mn exposure can  
30  
31 356 cause a dose-dependent increase of liver enzyme levels and interact with alcohol  
32  
33 357 drinking to potentially aggravate the liver damage.<sup>14</sup> The plasma BDNF levels and  
34  
35 358 cognitive function of different manganese-exposed groups were also measured. Our  
36  
37 359 results showed that occupational Mn exposure may be related to decreased plasma  
38  
39 360 BDNF levels and cognition impairment.<sup>23</sup>  
40  
41  
42  
43  
44  
45  
46  
47  
48

49 362 **Assessment of GWAS in the cohort**

50 363 We greatly paid attention to potential gene-environment interactions. Therefore, we  
51  
52 364 performed GWAS of QTL and BTL using the Illumina Infinium HumanExome  
53  
54 365 BeadChip for 500 exposed workers, including urine Mn and various indices of  
55  
56 366 pulmonary function, liver function and blood detection. Illumina's GenomeStudio  
57  
58  
59  
60

Genotyping Module was used for genotyping and data analysis, using an integrated platform for data visualization and analysis. About 25,000 loci were involved in the analysis after quality control (QC). The QTL, BTL, gene-wise and group-wise tests were conducted by EMMAX, LST, and SKAT-O, respectively. We will further analyze differential gene expression. The results of GWAS and other indices will be reported in ongoing articles. We plan to conduct GWAS in a larger number of manganese-exposed workers to explore the genic risk factors and gene-environment interactions.

## ETHICS AND DISSEMINATION

The study was approved via the medical ethics committee of Guangxi Medical University. All the original files and data are maintained and stored at the research office, in the Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Nanning, China. Electronic materials are stored in a safe system file and are accessible only by the data manager. All the biological samples are marked in sequential order and stored in secure freezers. The results will be disseminated to relevant scientific forums which include publishing in peer-reviewed journals and presentation at international conferences.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Acknowledgements** We thank all participants who volunteered to take part in this study, all members of the GXMEWHC research team, the nurses and administrators in the Ferro-manganese refinery Factory.

**Contributors** Xiaobo Yang and Yunfeng Zou contributed in conception and design; Jing Liu, Kangcheng Chen, Yingnan Lv, Damin Huang, Yuefei Shen, Yaoqiu Zhong, Zhihao Liu, Bei Jiang, Qin Li, Li Qing, Wei Zhang, Lang Chen, Fenfen Wang, Bing Xia and Li Yang contributed in acquisition of the data; Yingnan Lv analysed the data and drafted the manuscript; all authors contributed to review and revision of the manuscript and approved the final version.

**Funding** This study was supported by National Natural Science Foundation of China (81060234, 21167004, and 81160339); Guangxi Science Fund for Distinguished Young Scholars (2012jjFA40011); Guangxi Natural Science Foundation (2011jjA40294); Guangxi science and technology development project (1355007-1); and Program for New Century Excellent Talents in University (NCET-12-0653).

**Competing interests** All authors declare that they have no conflict of interest.

**Ethics approval** Medical ethics committee of Guangxi Medical University.

**Participant consent** Obtained.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** No additional data are available.

408  
409  
410  
411  
412

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

413 **Fig. 1 The complete study plan of the GXMEWHC**

414 The preliminary baseline survey was completed in 2013 and collected  
415 epidemiological information, biological samples, data from the occupational health  
416 examination and workplace monitoring. Simultaneously, the GWAS database was  
417 established for 500 manganese-exposed workers. The participants will be followed up  
418 every three years and the databases will be amended in the future.

436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479

References

1 Erikson KM, Syversen T, Aschner JL et al. Interactions between excessive manganese exposures and dietary iron-deficiency in neurodegeneration. *Environ Toxicol Pharmacol* 2005;19:415-21.

2 Erikson KM, Aschner M. Manganese neurotoxicity and glutamate-GABA interaction. *Neurochemistry International* 2003;43:475-480.

3 Bowler RM, Roels HA, Nakagawa S et al. Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. *Occup Environ Med* 2007;64:167-77.

4 Sriram K, Lin GX, Jefferson AM et al. Manganese accumulation in nail clippings as a biomarker of welding fume exposure and neurotoxicity. *Toxicology* 2012;291:73-82.

5 Ellingsen DG, Konstantinov R, Bast-Pettersen R et al. A neurobehavioral study of current and former welders exposed to manganese. *Neurotoxicology* 2008;29:48-59.

6 Summers MJ, Summers JJ, White TF et al. The effect of occupational exposure to manganese dust and fume on neuropsychological functioning in Australian smelter workers. *J Clin Exp Neuropsychol* 2011;33:692-703.

7 Rivera-Mancia S, Rios C, Montes S. Manganese accumulation in the CNS and associated pathologies. *Biometals* 2011;24:811-25.

8 Roels HA, Bowler RM, Kim Y et al. Manganese exposure and cognitive deficits: a growing concern for manganese neurotoxicity. *Neurotoxicology* 2012;33:872-80.

9 Kim EA, Cheong HK, Choi DS et al. Effect of occupational manganese exposure on the central nervous system of welders: 1H magnetic resonance spectroscopy and MRI findings. *Neurotoxicology* 2007;28:276-83.

10 Yang Y, Huang J, Liu J et al. Long-Term Effect of Occupational Exposure to Manganese on Pulmonary Ventilation Function. *Journal of Environmental & Occupational Medicine* 2013;30:29-31.

11 Boojar MM, Goodarzi F. A longitudinal follow-up of pulmonary function and respiratory symptoms in workers exposed to manganese. *J Occup Environ Med* 2002;44:282-90.

12 McKinney AM, Filice RW, Teksam M et al. Diffusion abnormalities of the globi pallidi in manganese neurotoxicity. *Neuroradiology* 2004;46:291-5.

13 Aschner M, Erikson KM, Dorman DC. Manganese Dosimetry: Species Differences and Implications for Neurotoxicity. *Critical Reviews in Toxicology* 2005;35:1-32.

14 Deng Q, Liu J, Li Q et al. Interaction of occupational manganese exposure and alcohol drinking aggravates the increase of liver enzyme concentrations from a cross-sectional study in China. *Environ Health* 2013;12:30.

15 Jiang YM, Zheng W. Cardiovascular toxicities upon manganese exposure. *Cardiovascular Toxicology* 2005;5:345-354.

16 Liu XF, Li ZP, Tie F et al. Effects of manganese-toxicity on immune-related organs of cocks. *Chemosphere* 2013;90:2085-100.

17 Curran CP, Park RM, Ho SM et al. Incorporating genetics and genomics in risk assessment for inhaled manganese: from data to policy. *Neurotoxicology* 2009;30:754-60.

18 Eastman RR, Jursa TP, Benedetti C et al. Hair as a biomarker of environmental manganese exposure. *Environ Sci Technol* 2013;47:1629-37.

- 19 Michalke B, Fernsebner K. New insights into manganese toxicity and speciation. *J Trace Elem Med Biol* 2013.
- 20 Nasreddine ZS, Phillips NA, Bedirian V et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53:695-9.
- 21 Fisekovic S, Memic A, Pasalic A. Correlation between moca and mmse for the assessment of cognition in schizophrenia. *Acta Inform Med* 2012;20:186-9.
- 22 Freitas S, Simoes MR, Alves L et al. Montreal Cognitive Assessment (MoCA): validation study for frontotemporal dementia. *J Geriatr Psychiatry Neurol* 2012;25:146-54.
- 23 Zou Y, Qing L, Zeng X et al. Cognitive function and plasma BDNF levels among manganese-exposed smelters. *Occup Environ Med* 2014;71:189-94.
- 24 Martinez-Martin P, Rodriguez-Blazquez C, Abe K et al. International study on the psychometric attributes of the non-motor symptoms scale in Parkinson disease. *Neurology* 2009;73:1584-91.
- 25 Chaudhuri KR, Martinez-Martin P, Brown RG et al. The metric properties of a novel non-motor symptoms scale for Parkinson's disease: Results from an international pilot study. *Mov Disord* 2007;22:1901-11.
- 26 Visser M, Marinus J, Stiggelbout AM et al. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. *Mov Disord* 2004;19:1306-12.
- 27 Rodriguez-Blazquez C, Forjaz MJ, Frades-Payo B et al. Independent validation of the scales for outcomes in Parkinson's disease-autonomic (SCOPA-AUT). *Eur J Neurol* 2010;17:194-201.
- 28 Laohaudomchok W, Lin X, Herrick RF et al. Neuropsychological effects of low-level manganese exposure in welders. *Neurotoxicology* 2011;32:171-9.
- 29 Niu Q, Shuchang H, Sheng W et al. Neurobehavioral functions, serum prolactin and plasma renin activity of manganese-exposed workers. *Int J Immunopathol Pharmacol* 2004;17:17-24.
- 30 Menezes-Filho JA, Paes CR, Pontes AM et al. High levels of hair manganese in children living in the vicinity of a ferro-manganese alloy production plant. *Neurotoxicology* 2009;30:1207-13.
- 31 Kang HM, Sul JH, Service SK et al. Variance component model to account for sample structure in genome-wide association studies. *Nat Genet* 2010;42:348-54.
- 32 Lin DY, Tang ZZ. A general framework for detecting disease associations with rare variants in sequencing studies. *Am J Hum Genet* 2011;89:354-67.
- 33 Lee S, Wu MC, Lin X. Optimal tests for rare variant effects in sequencing association studies. *Biostatistics* 2012;13:762-75.



520  
521  
522  
523

Table 1

Demographic characteristics of the GXMEWHC

Variables	Number (n=1888)	Percent (%)
Sex		
Male	1197	63.4
Female	691	36.6
Age, years (mean± SD)	40.31 ± 7.85	
<35	482	25.5
35~40	402	21.3
40~45	440	23.3
≥45	564	29.9
Seniority, years (mean± SD)	15.34 ± 9.63	
<10	652	34.5
10~20	585	31
>20	651	34.5
BMI, kg/m <sup>2</sup> (mean± SD)	22.47 ± 2.8	
<18.5	95	5
18.5~24	1289	68.3
24~28	422	22.4
≥28	74	3.9
Missing	8	0.4
Race/ethnicity		
Han Chinese	916	48.5
Zhuang Minority	885	46.9
Other ethnic groups	80	4.2
Marital status		
Single	233	12.3
Married	1580	83.7
Widowed or divorced	75	4
Education or lower		
Middle school	829	43.9
High school	850	45
University or college or higher	209	11.1
Smoking status		
Current smoker	729	38.6
Former smoker	132	7
Never smoker	1027	54.4
Drinking status		



Current drinker	907	48.1
Former drinker	301	15.9
Never drinker	680	36

**Table 2**

Different types of work of the GXMEWHC

Types of work	Number (n)	Percent (%)	Age (years)	Seniority (years)
			Mean± SD	Mean± SD
Smelter	594	31.5	38.95 ± 8.20	15.82 ± 9.02
Human crushing worker	320	16.9	41.08 ± 5.30	9.04 ± 6.00
Craneman	74	3.9	37.15 ± 8.76	16.24 ± 8.88
Finishing machining worker	99	5.2	40.36 ± 6.10	10.20 ± 8.79
Scaleman	105	5.6	42.30 ± 4.92	17.53 ± 6.88
Sampleman	21	1.1	45.75 ± 7.02	23.07 ± 6.57
Welder	128	6.8	40.75 ± 10.13	18.29 ± 10.76
Chemical analyst	54	2.9	45.52 ± 7.02	24.29 ± 8.37
Repairman	151	8.0	41.63 ± 9.10	19.19 ± 10.64
Electrician	91	4.8	40.28 ± 7.31	19.45 ± 8.00
Alkali recovery worker	133	7.0	40.89 ± 6.33	13.74 ± 8.74
Car driver	118	6.2	39.01 ± 9.96	15.09 ± 12.07
Total	1888	100	40.31 ± 7.85	15.23 ± 9.60

530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542

Table 3

The Mn-CEI of the GXMEWHC

Mn-CEI (mg/m <sup>3</sup> ·year)	Number (n)	Percent (%)	Median (Interquartile Range)	Range
Internal control group (Mn-CEI <1.0)	651	34.5	0.51 (0.55)	0.01~0.99
Low exposure group (1.0≤Mn-CEI<2.0)	333	17.6	1.49 (0.46)	1.01~1.99
Medium exposure group (2.0≤Mn-CEI<5.0)	710	37.6	3.04 (1.20)	2.00~4.98
High exposure group (Mn-CEI≥5.0)	194	10.3	5.99 (2.47)	5.01~9.77
Total	1888	100	1.85 (2.58)	0.01~9.77

**Table 4**

The results of occupational health examination of the GXMEWHC

Variables	Male(n=1197)	Female(n=691)	Total(n=1888)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Systolic blood pressure, mmHg	127.68 $\pm$ 12.11	121.54 $\pm$ 11.53	125.43 $\pm$ 12.26
Diastolic blood pressure, mmHg	79.93 $\pm$ 8.29	76.86 $\pm$ 7.93	78.81 $\pm$ 8.29
Blood routine			
WBC, $10^9/L$	6.91 $\pm$ 1.51	6.32 $\pm$ 1.52	6.69 $\pm$ 1.54
RBC, $10^{12}/L$	5.13 $\pm$ 0.52	4.61 $\pm$ 0.44	4.94 $\pm$ 0.55
Hemoglobin, g/L	148.69 $\pm$ 12.73	128.8 $\pm$ 14.37	141.38 $\pm$ 16.44
Platelet count, $10^9/L$	241.76 $\pm$ 54.13	256.29 $\pm$ 62.86	247.1 $\pm$ 57.9
Hepatic function			
Total bilirubin, $\mu\text{mol}/L$	12.48 $\pm$ 5.3	11.94 $\pm$ 4.49	12.28 $\pm$ 5.02
Direct bilirubin, $\mu\text{mol}/L$	3.98 $\pm$ 2.16	3.66 $\pm$ 2.19	3.86 $\pm$ 2.17
Indirect bilirubin, $\mu\text{mol}/L$	8.5 $\pm$ 3.44	8.24 $\pm$ 2.51	8.4 $\pm$ 3.13
ALT, U/L	25.35 $\pm$ 17.62	17.23 $\pm$ 14.74	22.38 $\pm$ 17.07
AST, U/L	27.06 $\pm$ 15.7	23.32 $\pm$ 21.75	25.69 $\pm$ 18.24
Pulmonary function			
Test value of FVC, L	4.25 $\pm$ 0.86	3.18 $\pm$ 0.64	3.86 $\pm$ 0.94
Test value of FEV1, L	3.61 $\pm$ 0.72	2.71 $\pm$ 0.54	3.28 $\pm$ 0.79
Uric Mn, $\mu\text{g}/L$	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Median (interquartile range)

547

For peer review only

# **Rationale, design and baseline results of tCohort Profile:**

## **The Guangxi manganese-exposed workers healthy cohort (GXMEWHC) study**

Yingnan Lv<sup>1</sup>, Yunfeng Zou<sup>2</sup>, Jing Liu<sup>1</sup>, Kangcheng Chen<sup>1</sup>, Damin Huang<sup>1</sup>, Yuefei Shen<sup>3</sup>, Yaoqiu Zhong<sup>1</sup>, Zhihao Liu<sup>4</sup>, Bei Jiang<sup>4</sup>, Qin Li<sup>2</sup>, Li Qing<sup>5</sup>, Wei Zhang<sup>3</sup>, Lang Chen<sup>3</sup>, Fenfen Wang<sup>1</sup>, Bing Xia<sup>1</sup>, Li Yang<sup>1</sup>, Xiaobo Yang<sup>1,6,\*</sup>

<sup>1</sup> Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>2</sup> Department of Toxicology, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>3</sup> Department of Neurology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

<sup>4</sup> Baise Center for Disease Control and Prevention, Baise, Guangxi, China

<sup>5</sup> Department of Epidemiology and Health Statistics, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

<sup>6</sup> Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning, Guangxi, China

Yingnan Lv and Yunfeng Zou are contributed equally.

\* Corresponding Author: Dr. Xiaobo Yang, Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University,

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
55  
56  
57  
58  
59  
60

Shuangyong Road 22, Nanning, Guangxi, 530021, P. R. China; [yxbo21021@163.com](mailto:yxbo21021@163.com)

For peer review only

## Abstract

**Objective:** To explore the early health effect and long-term related diseases of occupational manganese (Mn) exposure according to biomarkers of exposure, effect and susceptibility.

**Design:** The baseline survey of a longitudinal cohort study from a Ferromanganese Refinery.

**Participants:** A total of 1888 individuals (1197 men, 691 women) involved in Guangxi manganese-exposed worker healthy cohort (GXMEWHC) study. Participants were aged between 18 and 60 years (average age 40.31 years) and worked in Ferromanganese Refinery at least one year and lived in the local area.

**Results:** The baseline survey was completed and the GXMEWHC study was established, which included 1888 workers (average seniority 15.34 years). All participants were divided into four groups according to the levels of Mn cumulative exposure index (Mn-CEI), which included internal control group (Mn-CEI <1.0 mg/m<sup>3</sup>•year), low exposure group (1.0 mg/m<sup>3</sup>•year ≤ Mn-CEI <2.0 mg/m<sup>3</sup>•year), medium exposure group (2.0 mg/m<sup>3</sup>•year ≤ Mn-CEI <5.0 mg/m<sup>3</sup>•year), and high exposure group (Mn-CEI ≥ 5.0 mg/m<sup>3</sup>•year). GWAS of quantitative trait loci (QTL) and binary trait loci (BTL) were performed using Illumina Infinium HumanExome BeadChip for 500 manganese-exposed workers. Stored plasma, DNA, hair and urine are available in further study. Participants will be followed up every three years.

**Conclusions:** The GXMEWHC study provides abundant data to explore the multi-organ health effects of occupational Mn exposure by biomarkers of exposure, response and susceptibility, respectively.

Formatted: Highlight

Formatted: Font: Bold, Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Superscript, Highlight

Formatted: Highlight

Formatted: Superscript, Highlight

Formatted: Highlight

Formatted: Superscript, Highlight

Formatted: Highlight

Formatted: Superscript, Highlight

Formatted: Highlight

Formatted: Superscript, Highlight

Formatted: Highlight

Formatted: Superscript, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

Formatted: Font: Not Bold, Highlight

Formatted: Highlight

**Introduction:** Manganese (Mn) is an essential element for growth and maintenance of human health. Inhaled manganese can be excrete by normal homeostatic mechanisms, but it also can be accumulate in the bodily organ when excess the ability of metabolism. Occupational exposure to Mn in air can cause the adverse health effects in the human bodies. Consequently, we established the Guangxi manganese exposure workers healthy cohort (GXMEWHC) to explore the early healthy effect and the long term related disease of occupational manganese exposure by the biomarkers of exposure, effect and susceptibility.

**Methods and analysis:** The GXMEWHC is a prospective study. We recruited the workers in Ferro manganese refinery Factory and presently conducted the baseline surveys including epidemiological investigation, neurological function test, occupational health examination and environmental monitoring. The genome wide association study (GWAS) are also implemented further. We will follow up the participants every three years and ultimately the appropriate measures will be taken to prevent and control the early healthy injure and the related disease.

**Ethics and dissemination:** The study has been approved by Medical ethics committee of Guangxi Medical University. The results will be published by peer-reviewed publications and presented at international conferences.

**Keywords**

Manganese; Occupational exposure; Manganese toxicity; Genetic Susceptibility



## Strengths and limitations of this study

- In this study, we can collect an abundant database because of the large samples in the heavy metals cohort.
- The Ferro-manganese ~~refinery~~ Refinery Factory is the largest metallurgical factory of manganese-Mn processing in China ~~so that; therefore~~, it can provide an extremely rich dataset ~~for~~ of analysis.
- The GXMEWHC is ~~the a~~ longitudinal study ~~which that~~ can continuously ly follow up and repeatedly ly investigate the participants. We can explore the relationships between occupational manganese-Mn exposure and the early health ~~injure~~ effects.
- ~~The~~ GWAS ~~are was~~ implemented ~~for seeking to determine~~ the susceptibility genes ~~of related to~~ chronic low-level manganese-Mn exposure, and ~~to exploring explore~~ the interactions between genetic factors and environmental factors. ~~These These~~ data will provide an important opportunity to identify ~~the~~ more susceptible individuals ~~so that to~~ prevent ~~the~~ early health ~~injure effect of in~~ workers.
- ~~The p~~ Potential limitations are that loss ~~of to follow follow~~ up may be a weakness with our study. There are some temporary workers in the factory and they may leave the factory after a period of time ~~working in factory~~. We can reduce the probability of the loss ~~of to~~ follow up through strictly controlled ~~the~~ inclusion criteria when ~~established~~ establishing the cohort.

INTRODUCTION

Manganese (Mn) is an essential nutrient and it is necessary to inhaled manganese-Mn for maintaining the daily life. In addition to food intake, environmental exposure to Mn was allows the way to absorption of Mn, especially including occupational exposure. Mn was accumulated in some bodily organs and induced induces the adverse effects when the Mn concentration in vivo over exceeds the capacity of human metabolism.<sup>1</sup>

Many studies have showed shown that Mn can caused neurological abnormalities when it accumulated accumulates in the human brain in human bodies,<sup>2-4</sup> such as early impaired Finger finger Tapping tapping speed<sup>5</sup> or cognitive deficits, terminal Parkinsonian-like symptoms,<sup>6</sup> and Manganismmanganism.<sup>7</sup> The values level of Mn in the human bodies body were can be detected through some internal biomarkers, neurobehavioral tests and functional neuroimaging.<sup>8,9</sup> The Increased concentrations of Mn in the kidney were increased have been found in the manganese manganese-exposure exposed workers because the kidney is a way to pathway of excrete-Mnmanganese excretion.<sup>4</sup> In addition, the repeated respiratory exposured to Mn may cause impaired resulted in accumulation in the lung function. In oOne study, showed that it there was a dose-effect relationship between occupational manganese Mn exposures and the a reduction of in pulmonary function.<sup>10</sup> Compared with the non-exposure exposed workers, the pulmonary function in the manganese manganese-exposure exposed workers were evaluated by the spirometry tests and the

Formatted: Highlight

Formatted: Font: Italic

Formatted: Highlight

~~values of them were showed~~ a significant decrease in forced vital capacity (FVC),  
forced expiratory volume at one second (FEV1), and the ratio of forced expiratory  
volume at one second (FEV<sub>1</sub>/FVC and FEV<sub>1</sub>%) values.<sup>11</sup> Increased manganese-Mn  
 levels in blood serum ascribed to that liver is ~~a-the~~ mainly organ to store,  
 biotransformation and detoxify ~~the poisonous substance Mn~~.<sup>12</sup> Over-exposure to  
manganese-Mn can cause liver toxicity ~~as well as and~~ exacerbate liver dysfunction.<sup>13 14</sup>  
 Chronic manganese-Mn exposures leads to a series of significant cardiovascular  
 toxicities including ~~the an~~ abnormal electrocardiogram (ECG) and ~~the~~ inhibition of  
 myocardial contraction which can alters the blood pressure (BP).<sup>15</sup>  
~~Additionally Besides, the manganie Mn~~ cytotoxicity ~~could has been shown to~~ induce  
 cell apoptosis and ~~the~~ DNA damage ~~of bird in avian~~ immune cells.<sup>16</sup> Low Mn<sup>2+</sup> levels  
 can induce oxidative DNA damage via an apoptotic pathway, ~~so that the but this~~ DNA  
 damage ~~could can~~ be reduced using antioxidants. A ~~research conducted a risk~~  
 assessment of inhaled manganese-Mn ~~through~~ incorporating genetics and genomics ~~to~~  
 identifyed genetically based biomarkers of exposure, disease and susceptibility.<sup>17</sup>  
~~From the above Thus, manganie Mn~~ toxicity in humans ~~played plays~~ a significant  
 role in several systems. Currently, most studies ~~were have~~ explored separately the  
 effect of Mn manganese exposure ~~for on~~ different systems ~~in of~~ the human body. To  
further explore ~~further the effects~~ and the interaction of manganese-Mn exposure in  
 various systems, we will establish a prospective cohort study which includes ~~the~~  
~~situation of individual manganese-Mn~~ exposure and regular occupational  
 examinations. Simultaneously, we will determine ~~et the~~ biological exposure indicators  
 by means of hair, urine and ~~the~~ blood samples. Blood and urine can reflect the extens

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

of ~~manganese-Mn~~ exposure ~~for in a the~~ short term so ~~that~~ they can be used as ~~the~~ biomarkers of ~~manganese-Mn~~ exposure. Previous research has shown that hair ~~also~~ can also act as a biomarker of ~~Mnmanganese~~ exposure because ~~that~~ it may reflect the levels of ~~Mnmanganese~~ exposure ~~for over~~ longer timeframes.<sup>18</sup> Moreover, ~~a~~ suitable Mn-biomonitoring including Mn-citrate can be used to determine the early onset of excess Mn concentrations in the human ~~bodies-body~~ and ~~therefore it~~ can prevent the early onset of manganism or Mn-induced Parkinsonism as far as possible.<sup>19</sup> Accordingly, the risk of ~~manganese-Mn~~ exposure ~~in using~~ sensitive effective biomarkers and the associated health effects ~~of health injure~~ are also the main emphasis of this study.

**METHODS ~~AND ANALYSIS~~**

**Establishing a cohort**

To explore the early healthy effects, ~~the~~ potential biomarkers of exposure, susceptibility and disease, as well as the related disease of occupational ~~Mnmanganese~~ exposure, we established the GXMEWHC. The prospective cohort study started in 2011 and the targeted population was consists of the workers aged 18 years or older working in the Ferro-manganese ~~refinery-Refinery~~Factory. ~~It is a long term prospective cohort study of manganese exposure workers.~~ The study investigates a variety of lifestyle, socio-economic status, environmental and occupational factors as well as genetic factors in relation to the early health ~~injure~~ effects for of ~~manganese-Mn~~ exposure. This is an opportunity to explore the

relationships between various kinds of risk factors and the early health injure-effects of manganese-Mn exposure, particularly the genetic and environmental factors and their interactions.

### Sample source

The entire samples in-of this study were-was collected from a Ferro-manganese refinery-RefineryFactory. The workers who participated in physical examinations every year and accoeded-withmet the following conditions were recruited. The study was approved by the local Ethics Committee. Inclusion criteria included-were the-age of 18-60 years, living in the local area, working in this-the company-factory for a long time (at least one year) and being able to complete long-long-term follow-up, the inexistence-lack of obvious diseases for-in each system, absence ifouting-of touch with other risk factors except manganese-Mn exposure (such as Cu, Pb, Cr, or Hg, et-al) and voluntary participating-participation after providing informed consent. People were excluded if they had Exclusion-criteria-contained-the-presence-ofan obvious diseases for-in any system (such as a serious neurological disease, hepatic disease, kidney disease and-or cardiovascular disease)-in the beginning of work, the contact of-various-with other occupational risk factors (such as Cu, Pb, Cr, or Hg, et-al) and unable-inability to provide informed consent (psychiatric disease, language barrier, or mental deficiency). All participants were divided into different exposed-exposure groups according to the type of work.

**Follow-up**

We will follow up the participants every three years. ~~The information collected will be the same as the baseline data~~ and ~~will be collected the data by~~ questionnaire interviews, physical examinations, biological specimens and environmental monitoring ~~repeatedly~~. Fig. 1 shows the complete study plan of the GXMEWHC. The retrospective survey are establishing and perfecting the GXMEWHC by collecting baseline data on demographic information, lifestyle, biological specimens as well as history of environmental and occupational exposure. The short-term objectives are ~~to researching-explore~~ the early healthy effects of occupational ~~Mnmanganese~~ exposure interact with environmental influences. In addition, preliminary exploring the effects of ~~Mnmanganese~~ exposure on genetic ~~fields~~ are also one of our studies. ~~In the future,~~ Our long-term and final objectives are ~~to exploring-explore~~ the early healthy ~~injure~~ effects on various systems ~~in-of the~~ human body by gene-environmental interactions for long-term and continuous low levels of ~~manganese-Mn~~ exposure.

**Building database**

**Questionnaire**

~~The-t~~Trained interviewers used a ~~structuredspecifically-designed~~ questionnaire to collect the baseline data after obtaining written informed consent. In order to obtain real and accurate information, we ~~take-conducted~~ face-to-face interviews during the physical examination. ~~The-s~~Self-reported diseases ~~are-were~~ tested and verified through the diagnosis of specialists, ~~which-is~~ based on recognized international standards. The questionnaires consisted of demographic information, socio-economic

status, smoking history ~~and~~, alcohol consumption and occupational history.

### Occupational health examination

The occupational health examination was ~~implemented~~ conducted at the same time.

All participants took part in the general health examination and were checked by trained physicians, nurses, ~~and the~~ medical technicians.

The physical measurements covered height, weight, ~~blood pressure (BP)~~, and pulmonary function. The pulmonary function was estimated using a spirometry test which ~~comprised the test value of forced vital capacity~~ included (FVC), ~~forced expiratory volume at one second (FEV<sub>1</sub>), the ratio of forced expiratory volume at one second (FEV<sub>1</sub>%)~~, maximal ~~med~~ expiratory mid-expiratory flow ~~curve~~ (MMEF), ~~the~~ peak expiratory flow ratio (PEFR), maximal voluntary ventilation (MVV), the predicted value of them and the ratio percentage of all above.

The clinical examinations included a high kilovar chest radiograph (HKV), ~~Neurology-neurology~~ inspection, ECG, ~~Uncorrected-uncorrected~~ visual acuity (UCVA) test, pure tone audiometry and physical examination of the heart, lungs, liver, spleen and abdomen.

The laboratory tests included ~~blood~~ routine blood tests, ~~urine~~ routine urine tests and liver function tests. The ~~blood~~ routine blood tests were measured in the laboratory ~~covering and included the~~ white blood cell count (WBC), lymphocyte ratio (LYR), neutrophile granulocyte ratio (GRANR), middle cell ratio (MIDR), lymphocyte count (LYC), neutrophile granulocyte count (NGC), middle cell count (MIDC), red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT), ~~hematokrit-hematocrit~~



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

(HCT), mean corpuscular volume (MCV), ~~mean corpuscular hemoglobin (MCH),~~  
mean corpuscular hemoglobin concentration (MCHC), thrombocytocrit (THR),  
erythrocyte hemoglobin distribution width (RDW), platelet volume distribution width  
(PDW), and mean platelet volume (MPV). The ~~urine~~-routines urine tests were  
~~detected by~~included urobilinogen (URO), bilirubin, ~~(BIL), ketobodyketobodies~~  
(KET), blood (BLD), protein (PRO), nitrite (NIT), white blood cells (WBC), glucose  
(GLU), specific gravity (SG), ~~power of hydrogen (pH)~~ and vitamin C. Furthermore,  
we examined the content of ~~Mn~~manganese in urine. The liver function tests contained  
total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), alanine  
aminotransferase (ALT), aspartate aminotransferase (AST), and the AST/ALT ratio.

**Neurological function test**

The neurological function test consisted of a neurocognitive function test,  
neurobehavioral function tests, and neuropsychological test. The Montreal Cognitive  
Assessment (MoCA) is a neurocognitive function test and ~~it~~ is an assessment method  
which rapidly screens for the Mild Cognitive Impairment (MCI) with high sensitivity  
and specificity.<sup>20-22</sup> We assessed the influence of ~~manganese~~ Mn exposure on the  
nervous system using the MoCA as a cognitive screening tool.<sup>23</sup> The Non-Motor  
Symptoms scale (NMSS) and the Scales for Outcomes in Parkinson's  
disease-Autonomic (SCOPA-AUT) are neurobehavioral function tests. NMSS is an  
acceptable and valid assessment means for non-motor symptoms in Parkinson's  
disease (PD).<sup>24 25</sup> SCOPA-AUT is a self-administered scale and can be used for the  
screening of autonomic symptoms in PD.<sup>26 27</sup> We used NMSS and SCOPA-AUT to



evaluate the neurobehavioral function of workers who were exposed to occupational  
~~Mnmanganese~~. The Profile of Mood States (POMS) is a neuropsychological test and  
 is a scale to assess the emotions of ~~manganese-manganese~~-exposed workers.<sup>28 29</sup> We  
 used POMS as an assessment instrument for assessing neuropsychological of workers.

### Database of biological specimens

The biological specimens were composed blood samples, urine specimen and the hair  
 samples. Three vacuum tubes (two ethylene diamine tetraacetic teraacetic (EDTA)  
 anticoagulant tubes and a coagulation tube) were filled with ~~five-5 milliliters-mL~~ of  
 fasting blood ~~respectively-obtained~~ through intravenous access. The blood sample in  
 the coagulation tube was used to ~~detect-assess the~~ liver function and one of the EDTA  
 anticoagulant tubes was ~~measured-used for the blood~~ routine blood tests. The blood  
 sample in ~~the another~~ EDTA anticoagulant tube was separated into blood plasma and  
 blood ~~corpusele-cell from~~ which was DNA extracted ~~the DNA from it~~ as soon as  
 possible. All the blood specimens were stored ~~in-at -80°C-refrigerators~~. In addition,  
 a minimum of 10 ~~milliliter-mL~~ of the urine were collected in ~~the-a~~ urine bottles ~~by the~~  
~~participants~~ and ~~then as the urine specimen~~ stored ~~in-at 4°C-refrigerators~~. A tuft of  
 hair close to the scalp in the occipital region, about 2 cm in length and of  
approximately 0.5 cm in diameter was cut off with ~~a~~-stainless steel scissors and  
 collected in ~~the-a~~ special ~~sacks-bag~~ which close to the scalp in the occipital region  
~~about 2 cm.~~<sup>30</sup> All ~~the~~ hair specimens were stored in a cool and dry area.

**Determining Mnmanganese exposure in the workplace**

We will track the levels of ~~manganese-manganese-exposure-exposed~~ workers who participated in the present cohort study by means of ~~the~~-workplace monitoring. We will record the basic information of the factory, the technological processes of production, ~~and~~ the distributions of occupational risk factors, the work mode, and the ~~situation-level~~ of ~~manganese-Mn~~ in this ~~company~~factory. The concentrations of ~~manganese-Mn~~ dust and fumes in the workplace ~~were-are~~ detected through ~~the-an~~ air point sampler. At the same time, we will monitor the individual levels of ~~manganese~~ ~~byMn~~ using ~~the~~-individual samplers ~~in-theirduring~~ working ~~time~~hours. The ~~Permissible-permissible~~ concentration-time-weighted average (PC-TWA) is the average permissible exposure levels ~~on-during~~ the regulation ~~eight-eight~~-hours working day, ~~weight~~ed by time. The ~~Permissible-permissible~~ concentration-~~Short~~ ~~short Term-term Exposure-exposure Limit-limit~~ (PC-STEL) is the permissible exposure levels ~~on-in~~ no more than 15 minutes ~~at~~ any time, ~~weighting-weighted~~ by time within a working day. The cumulative exposure index (CEI) is calculated through TWA, STEL, and ~~the-workplace~~ seniority in working. The CEI as an external exposure index of ma Mn nganese and was calculated for each job, combining the airborne monitoring with the individual monitoring both ~~at-during~~ working ~~ing~~ time and break time.

**Database of biomarkers**

The database of biomarkers included biomarkers of exposure, effect and susceptibility.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

The biomarkers of exposure will be detected through atomic absorption spectrometry (AAS) which are the levels of Mn and Fe in plasma, urine and hair. The levels of plasma brain-derived neurotrophic factor (BDNF), dopamine (DA) were determined by Sandwich ELISA kits which are biomarkers of effect. The biomarkers of susceptibility are also assessed by GWAS which are shown in the following Genetic assessments part in detail.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Font: Not Bold

### Genetic ~~determination~~ assessments

A sub-cohort of GWAS is implemented in this study ~~which researched to assess~~ the effect of ~~manganese-Mn~~ exposure on genetic ~~sides~~. The GWAS of ~~Quantative~~ ~~quantitative Trait-trait Loci-loci~~ (QTL) and ~~Binary-binary Trait-trait Loci-loci~~ (BTL) ~~will also are-be also~~ performed for ~~the~~ exposed workers using the Infinium HumanExome BeadChip from Illumina Company (Illumina Infinium HumanExome v1.0 BeadChips, 12-sample HD). ~~The~~ Illumina's HumanExome BeadChips ~~are~~ covered ~~with emphatically~~ human exonic regions ~~and~~ the exonic content contains more than 240,000 variant markers. The markers represented a variety of common diseases, ~~and~~ ~~the~~ different groups ~~which contained the~~ include individuals ~~of from~~ China, Europe, ~~and~~ Africa ~~and Spain~~. We will focus on the potential interactions of environmental ~~manganese-Mn~~ exposure and genetics ~~which~~ based on the significant effects of Mn on the targeted phenotypes. Furthermore, ~~the~~ potential gene-environment interactions ~~is~~ ~~will be~~ explored through the genomes of ~~the~~ ~~Mn~~ anganism patients and healthy individuals ~~who~~ exposed ~~to manganese-Mn~~ in ~~the~~

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

workplace.

**Statistical analyses**

After collecting the complete questionnaires, the ~~data of~~ physical examination results and neurological function test data, ~~the~~ trained investigators will enter ~~all~~ the above data into the computer twice using ~~the~~ EpiData software. The GXMEWHC study database is established and ~~it is~~ will be gradually improved in ~~later~~ subsequent follow-up. Simultaneously, the experimental data ~~is~~ will be contained ~~by~~ in the database. All the data ~~is~~ will be analyzed by ~~the~~ SPSS 16.0 software. The ~~data of~~ genetic determination data is will be obtained and analyzed through ~~the~~ Illumina's GenomeStudio, which is an integrated software platform for data visualization and analysis. The GenomeStudio Genotyping Module is an application for extracting genotyping data from the Illumina iScan systems. We will use the Efficient and Parallelizable Association Container Toolbox (EPACTS), which can perform various statistical tests for identifying genome-wide associations. The ~~Quantative~~ quantitative ~~Trait~~ traits are will be calculated by the efficient mixed-model association eXpedited (EMMAX) program, which can correct for sample structure within human GWASs by taking an expedited mixed linear model approach.<sup>31</sup> The ~~Binary~~ binary ~~Trait~~ traits are will be calculated through the Logistic Score Test (LST) which can test ~~with~~ rare variants and relate the enriched genetic information to disease phenotypes through Logistic regression models.<sup>32</sup> When the ~~Gene~~ gene-wise or ~~Group~~ group-wise ~~Tests~~ tests are conducted, ~~the~~ optimal sequence kernel association tests (SKAT-O) ~~are~~ will

be used<sup>33</sup>

## **Preliminary resultPRELIMINARY RESULTS**

### **Demographic description of the cohort**

The In total, 1,991 individuals were recruited to participate in the study from the FerroFerro-manganese refinery-RefineryFactory. After completing the questionnaire, a total of 1,888 participants who met the standards were entered into the GXMEWHC study, who accord with standard and the with an effective rate is of 94.8%.

Table 1 summarizes the baseline characteristics of the cohort. In Of the cohort, 63.4% were male and 36.6% were female. The mean age was 40.31 years and the percent of the four-stagesage distribution was were similar. Thereinto, 34.5%, 31.0%, and 34.5% of the participants had the-seniority of <10, 10-20, and >20 years, respectively. In the factory, 31.2%, 15.7%, 20.1% and 33.0% were smelters, raw material processors, high exposed-exposure auxiliary, and low exposed-exposure auxiliary, respectively. The mean seniority was 15.34 years. The mean Body-body Mass-mass Index-index (BMI) was normal ( $22.47\text{kg}\cdot\text{m}^{-2}$ ). Among the participants, 48.5% was-were Han Chinese. A majority of the participants (83.7%) were married. In the midst-of-theOf these participants, 43.9% graduated from middle school, 45.0% had-finished high school and 11.1% achieved-completed college or higher education. In the cohort, 38.6% was-were current smokers, 7.0% was-were former smokers and 54.4% was-were never smokers. Current passive smoking rates were 87.3%. The proportion of current drinkers was 48.1%, the-former drinkers was 15.9% and the never drinkers was 36.0%. Detailed information of the demographic characteristics of

Formatted: Font: Not Bold

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

~~this cohort is provided in Table 1.~~ Among the ~~participants~~participants, 31.5% ~~was~~  
~~were~~ smelters, 16.9% ~~was-were~~ human crushing workers and 6.8% ~~was-were~~ welder.  
The other types of work, the proportion of them, the mean age, and seniority are  
shown in Table 2.

**Determining Mnmanganese exposure in the workplace**

All the ~~participants~~participants were divided according to different ~~the~~ types of work  
in the factory. Then, the extents of the ~~manganese-Mn~~ exposure ~~were-was~~ confirmed  
using the working positions combined with the results of workplace detection. The  
CEI ~~is-was~~ calculated through TWA or STEL. Finally, all workers were classified into  
four ~~exposed-exposure~~ groups on the basis of the Mn-CEI ~~results, including which are~~  
~~respectively~~ the internal control group ( $\text{Mn-CEI} < 1.0 \text{ mg/m}^3 \cdot \text{year}$ ), the low ~~exposed~~  
~~exposure~~ group ( $1.0 \text{ mg/m}^3 \cdot \text{year} \leq \text{Mn-CEI} < 2.0 \text{ mg/m}^3 \cdot \text{year}$ ), the medium ~~exposed~~  
~~exposure~~ group ( $2.0 \text{ mg/m}^3 \cdot \text{year} \leq \text{Mn-CEI} < 5.0 \text{ mg/m}^3 \cdot \text{year}$ ) and the high ~~exposed~~  
~~exposure~~ group ( $\text{Mn-CEI} \geq 5.0 \text{ mg/m}^3 \cdot \text{year}$ ). The percentages of ~~the~~ internal control  
group, low ~~exposed-exposure~~ group, medium ~~exposureexposed~~ group and high  
~~exposureexposed~~ group were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The  
median of total Mn-CEI was  $1.85 \text{ mg/m}^3 \cdot \text{year}$  and the range was  $0.01 \text{ mg/m}^3 \cdot \text{year}$ –  
 $9.77 \text{ mg/m}^3 \cdot \text{year}$ . The details of Mn-CEI are shown in Table 3.

**Main results of the occupational health examination of the cohort–**

The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) ~~was-were~~  
125.43 and 78.81 mmHg, respectively. The median ~~of-urine~~ Mn level was  $2.63 \mu\text{g/L}$ ,

~~the male was~~ 3.67µg/L ~~in males~~, and ~~the female was~~ 2.84µg/L ~~in females~~. The ~~values~~ results of the ~~Blood R~~ routine ~~blood tests~~, ~~Hepatic-hepatic Function-function tests~~ and ~~Pulmonary-pulmonary Function-function tests~~ are shown in the Table 4.

### Assessment of biomarkers

The liver function was analyzed between different manganese-exposed groups of the cohort in 2013. Our conclusions in this study were that occupational Mn exposure can cause a dose-dependent increase of liver enzyme levels and interact with alcohol drinking to potentially aggravate the liver damage.<sup>14</sup> The plasma BDNF levels and cognitive function of different manganese-exposed groups were also measured. Our results showed that occupational Mn exposure may be related to decreased plasma BDNF levels and cognition impairment.<sup>23</sup>

### Detection-Assessment of biological specimens and GWAS of in the cohort-

We ~~greatly pay-paid~~ attention to ~~the~~ potential gene-environment interactions. Therefore, we performed ~~the~~ GWAS of QTL and BTL using the Illumina Infinium HumanExome BeadChip for 500 exposed workers, ~~such as including urie-urine~~ Mn and various ~~kinds of indexindices~~ of pulmonary function, liver function and blood ~~routine detection~~. The Illumina's GenomeStudio Genotyping Module was used for genotyping and data analysis, ~~which is using~~ an integrated platform for data visualization and analysis. About ~~twenty-five thousand~~ 25,000 loci ~~was-were~~ involved in the analysis after ~~Quality-quality Control-control~~ (QC). ~~And then t~~The QTL, BTL ~~and~~, ~~Gene~~gene-wise ~~or and Groupgroup~~-wise ~~Tests-tests~~ were conducted



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

by EMMAX, LST<sub>a</sub> and SKAT-O, respectively. We will further analyze ~~the~~ differential gene expression ~~further~~. The results of GWAS and other ~~indexes~~ indices will be reported in ~~separate~~ ongoing articles. We plan to conduct GWAS in a larger number of ~~manganese-manganese-exposure-exposed~~ workers ~~for-to~~ exploring-explore the genic risk factors and ~~the~~ gene-environment interactions.

**ETHICS AND DISSEMINATION**

The study ~~has-been~~ was approved via the ~~Medical-medical~~ ethics committee of Guangxi Medical University. All the original files and data are maintained and stored at the research office, in the Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Nanning, China. Electronic materials are stored in a safe system file and are accessible only by the data manager. All the biological samples are marked in ~~a~~ sequential order and stored in secure freezers. The results will be disseminated to relevant scientific forums which included publishing in peer-reviewed journals and ~~presenting~~ presentation at international conferences.

**Acknowledgements** We thank all participants who volunteered to take part in this study, all members of the GXMEWHC research team, the nurses and administrators in the Ferro-manganese refinery Factory.

**Contributors** Xiaobo Yang and Yunfeng Zou contributed in conception and design; Jing Liu,



459 Kangcheng Chen, Yingnan Lv, Damin Huang, Yuefei Shen, Yaoqiu Zhong, Zhihao Liu, Bei Jiang,  
460 Qin Li, Li Qing, Wei Zhang, Lang Chen, Fenfen Wang, Bing Xia and Li Yang contributed in  
461 acquisition of the data; Yingnan Lv analysed the data and drafted the manuscript; all authors  
462 contributed to review and revision of the manuscript and approved the final version.

463 **Funding** This study was supported by National Natural Science Foundation of China (81060234,  
464 21167004, and 81160339); Guangxi Science Fund for Distinguished Young Scholars  
465 (2012jjFA40011); Guangxi Natural Science Foundation (2011jjA40294); Guangxi science and  
466 technology development project (1355007-1); and Program for New Century Excellent Talents in  
467 University (NCET-12-0653).

468 **Competing interests** All authors declare that they have no conflict of interest.

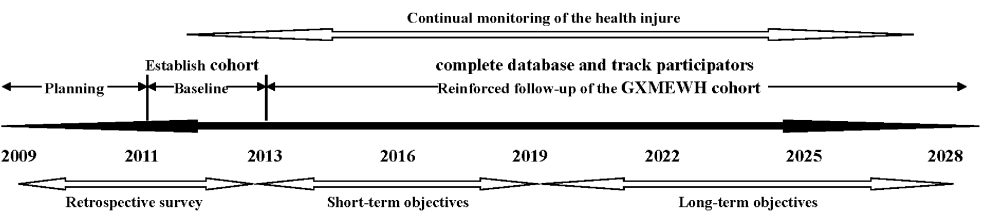
469 **Ethics approval** Medical ethics committee of Guangxi Medical University.

470 **Patient-Participant consent** Obtained.

471 **Provenance and peer review** Not commissioned; externally peer reviewed.

472 **Data sharing statement** No additional data are available.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



**Fig. 1 The complete study plan of the GXMEWHC**

The preliminary baseline survey was completed ~~preliminary~~ in 2013 and collected ~~the~~ epidemiological information, biological samples, ~~the data of from the~~ occupational health examination and workplace monitoring. Simultaneously, the ~~database of~~ GWAS database was ~~established~~performed for 500 ~~manganese-manganese~~-exposed workers. The participants will be ~~following-followed~~ up every three years and the databases ~~were will be amended~~reinforced gradually in the future.

## References

- 1 Erikson KM, Syversen T, Aschner JL et al. Interactions between excessive manganese exposures and dietary iron-deficiency in neurodegeneration. *Environ Toxicol Pharmacol* 2005;19:415-21.
- 2 Erikson KM, Aschner M. Manganese neurotoxicity and glutamate-GABA interaction. *Neurochemistry International* 2003;43:475-480.
- 3 Bowler RM, Roels HA, Nakagawa S et al. Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. *Occup Environ Med* 2007;64:167-77.
- 4 Sriram K, Lin GX, Jefferson AM et al. Manganese accumulation in nail clippings as a biomarker of welding fume exposure and neurotoxicity. *Toxicology* 2012;291:73-82.
- 5 Ellingsen DG, Konstantinov R, Bast-Pettersen R et al. A neurobehavioral study of current and former welders exposed to manganese. *Neurotoxicology* 2008;29:48-59.
- 6 Summers MJ, Summers JJ, White TF et al. The effect of occupational exposure to manganese dust and fume on neuropsychological functioning in Australian smelter workers. *J Clin Exp Neuropsychol* 2011;33:692-703.
- 7 Rivera-Mancia S, Rios C, Montes S. Manganese accumulation in the CNS and associated pathologies. *Biometals* 2011;24:811-25.
- 8 Roels HA, Bowler RM, Kim Y et al. Manganese exposure and cognitive deficits: a growing concern for manganese neurotoxicity. *Neurotoxicology* 2012;33:872-80.
- 9 Kim EA, Cheong HK, Choi DS et al. Effect of occupational manganese exposure on the central nervous system of welders: 1H magnetic resonance spectroscopy and MRI findings. *Neurotoxicology* 2007;28:276-83.
- 10 Yang Y, Huang J, Liu J et al. Long-Term Effect of Occupational Exposure to Manganese on Pulmonary Ventilation Function. *Journal of Environmental & Occupational Medicine* 2013;30:29-31.
- 11 Boobar MM, Goodarzi F. A longitudinal follow-up of pulmonary function and respiratory symptoms in workers exposed to manganese. *J Occup Environ Med* 2002;44:282-90.
- 12 McKinney AM, Filice RW, Teksam M et al. Diffusion abnormalities of the globi pallidi in manganese neurotoxicity. *Neuroradiology* 2004;46:291-5.
- 13 Aschner M, Erikson KM, Dorman DC. Manganese Dosimetry: Species Differences and Implications for Neurotoxicity. *Critical Reviews in Toxicology* 2005;35:1-32.
- 14 Deng Q, Liu J, Li Q et al. Interaction of occupational manganese exposure and alcohol drinking aggravates the increase of liver enzyme concentrations from a cross-sectional study in China. *Environ Health* 2013;12:30.
- 15 Jiang YM, Zheng W. Cardiovascular toxicities upon manganese exposure. *Cardiovascular Toxicology* 2005;5:345-354.
- 16 Liu XF, Li ZP, Tie F et al. Effects of manganese-toxicity on immune-related organs of cocks. *Chemosphere* 2013;90:2085-100.
- 17 Curran CP, Park RM, Ho SM et al. Incorporating genetics and genomics in risk assessment for inhaled manganese: from data to policy. *Neurotoxicology* 2009;30:754-60.
- 18 Eastman RR, Jursa TP, Benedetti C et al. Hair as a biomarker of environmental manganese exposure. *Environ Sci Technol* 2013;47:1629-37.
- 19 Michalke B, Fernsebner K. New insights into manganese toxicity and speciation. *J Trace Elem Med Biol* 2013.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

547 20 Nasreddine ZS, Phillips NA, Bedirian V et al. The Montreal Cognitive Assessment, MoCA: a  
548 brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53:695-9.  
549 21 Fisekovic S, Memic A, Pasalic A. Correlation between moca and mmse for the assessment of  
550 cognition in schizophrenia. *Acta Inform Med* 2012;20:186-9.  
551 22 Freitas S, Simoes MR, Alves L et al. Montreal Cognitive Assessment (MoCA): validation  
552 study for frontotemporal dementia. *J Geriatr Psychiatry Neurol* 2012;25:146-54.  
553 23 Zou Y, Qing L, Zeng X et al. Cognitive function and plasma BDNF levels among  
554 manganese-exposed smelters. *Occup Environ Med* 2014;71:189-94.  
555 24 Martinez-Martin P, Rodriguez-Blazquez C, Abe K et al. International study on the  
556 psychometric attributes of the non-motor symptoms scale in Parkinson disease. *Neurology*  
557 2009;73:1584-91.  
558 25 Chaudhuri KR, Martinez-Martin P, Brown RG et al. The metric properties of a novel  
559 non-motor symptoms scale for Parkinson's disease: Results from an international pilot study.  
560 *Mov Disord* 2007;22:1901-11.  
561 26 Visser M, Marinus J, Stiggelbout AM et al. Assessment of autonomic dysfunction in  
562 Parkinson's disease: the SCOPA-AUT. *Mov Disord* 2004;19:1306-12.  
563 27 Rodriguez-Blazquez C, Forjaz MJ, Frades-Payo B et al. Independent validation of the scales  
564 for outcomes in Parkinson's disease-autonomic (SCOPA-AUT). *Eur J Neurol*  
565 2010;17:194-201.  
566 28 Laohaudomchok W, Lin X, Herrick RF et al. Neuropsychological effects of low-level  
567 manganese exposure in welders. *Neurotoxicology* 2011;32:171-9.  
568 29 Niu Q, Shuchang H, Sheng W et al. Neurobehavioral functions, serum prolactin and plasma  
569 renin activity of manganese-exposed workers. *Int J Immunopathol Pharmacol* 2004;17:17-24.  
570 30 Menezes-Filho JA, Paes CR, Pontes AM et al. High levels of hair manganese in children  
571 living in the vicinity of a ferro-manganese alloy production plant. *Neurotoxicology*  
572 2009;30:1207-13.  
573 31 Kang HM, Sul JH, Service SK et al. Variance component model to account for sample  
574 structure in genome-wide association studies. *Nat Genet* 2010;42:348-54.  
575 32 Lin DY, Tang ZZ. A general framework for detecting disease associations with rare variants in  
576 sequencing studies. *Am J Hum Genet* 2011;89:354-67.  
577 33 Lee S, Wu MC, Lin X. Optimal tests for rare variant effects in sequencing association studies.  
578 *Biostatistics* 2012;13:762-75.  
579  
580  
581  
582  
583  
584  
585

Table 1

## Demographic characteristics of the GXMEWHC

Variables	Number (n=1888)	Percent (%)
Sex		
Male	1197	63.4
Female	691	36.6
Age, years (mean $\pm$ SD)	40.31 $\pm$ 7.85	
<35	482	25.5
35~40	402	21.3
40~45	440	23.3
$\geq 45$	564	29.9
Seniority, years (mean $\pm$ SD)	15.34 $\pm$ 9.63	
<10	652	34.5
10~20	585	31
>20	651	34.5
BMI, kg/m <sup>2</sup> (mean $\pm$ SD)	22.47 $\pm$ 2.8	
<18.5	95	5
18.5~24	1289	68.3
24~28	422	22.4
$\geq 28$	74	3.9
Missing	8	0.4
Race/ethnicity		
Han Chinese	916	48.5
Zhuang Minority	885	46.9
Other ethnic groups	809	42.5
Marital status		
Single	233	12.3
Married	1580	83.7
Widowed or divorced	75	4
Education or lower		
Middle school	829	43.9
High school	850	45
University or college or higher	209	11.1
Smoking status		
Current smoker	729	38.6
Former smoker	132	7
Never smoker	1027	54.4
Drinking status		
Current drinker	907	48.1
Former drinker	301	15.9
Never drinker	680	36

Formatted Table

Formatted: Highlight

Formatted: Indent: First line: 2 ch

Formatted: Highlight

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

588

Table 2

589

Different types of work of the GXMEWHC

Types of work	Number_((n))	Percent_((%))	Age_((years))	Seniority
			Mean± SD	Mean± SD
Smelter	594	31.5	38.95 ± 8.20	15.82 ± 9.02
Human cCrushing wWorker	320	16.9	41.08 ± 5.30	9.04 ± 6.00
Craneman	74	3.9	37.15 ± 8.76	16.24 ± 8.88
Finishing mMachining wWorker	99	5.2	40.36 ± 6.10	10.20 ± 8.79
Scaleman	105	5.6	42.30 ± 4.92	17.53 ± 6.88
Sampleman	21	1.1	45.75 ± 7.02	23.07 ± 6.57
Welder	128	6.8	40.75 ± 10.13	18.29 ± 10.76
Chemical aAnalyst	54	2.9	45.52 ± 7.02	24.29 ± 8.37
Repairman	151	8.0	41.63 ± 9.10	19.19 ± 10.64
Electrician	91	4.8	40.28 ± 7.31	19.45 ± 8.00
Alkali rRecovery wWorker	133	7.0	40.89 ± 6.33	13.74 ± 8.74
Car dDriver	118	6.2	39.01 ± 9.96	15.09 ± 12.07
Total	1888	100	40.31 ± 7.85	15.23 ± 9.60

590

591

592

593

Table 3

## The Mn-CEI of the GXMEWHC

Mn-CEI (mg/m <sup>3</sup> ·year)	Number (n)	Percent (%)	Median (Interquartile Range)	Range
Internal <b>c</b> Control <b>g</b> Group (Mn-CEI <1.0)	651	34.5	0.51 (0.55)	0.01~0.99
Low <b>c</b> Expos <b>ured</b> <b>g</b> Group (1.0≤Mn-CEI<2.0)	333	17.6	1.49 (0.46)	1.01~1.99
Medium <b>c</b> Expos <b>ured</b> <b>g</b> Group (2.0≤Mn-CEI<5.0)	710	37.6	3.04 (1.20)	2.00~4.98
High <b>c</b> Expos <b>ured</b> <b>g</b> Group (Mn-CEI≥5.0)	194	10.3	5.99 (2.47)	5.01~9.77
Total	1888	100	1.85 (2.58)	0.01~9.77

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

607

Table 4

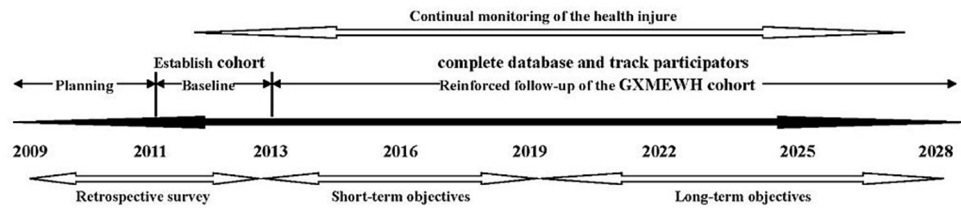
608

The results of occupational health examination of the GXMEWHC

Variables	Male(n=1197)	Female(n=691)	Total(n=1888)
	Mean ± SD	Mean ± SD	Mean ± SD
Systolic blood pressure, mmHg	127.68 ± 12.11	121.54 ± 11.53	125.43 ± 1 2.26
Diastolic blood pressure, mmHg	79.93 ± 8.29	76.86 ± 7.93	78.81 ± 8.29
Blood routine			
WBC, 10 <sup>9</sup> /L	6.91 ± 1.51	6.32 ± 1.52	6.69 ± 1.54
RBC, 10 <sup>12</sup> /L	5.13 ± 0.52	4.61 ± 0.44	4.94 ± 0.55
Haemoglobin, g/L	148.69 ± 12.73	128.8 ± 14.37	141.38 ± 16.44
Platelet count, 10 <sup>9</sup> /L	241.76 ± 54.13	256.29 ± 62.86	247.1 ± 57.9
Hepatic function			
Total bilirubin, µmol/L	12.48 ± 5.3	11.94 ± 4.49	12.28 ± 5.02
Direct bilirubin, µmol/L	3.98 ± 2.16	3.66 ± 2.19	3.86 ± 2.17
Indirect bilirubin, µmol/L	8.5 ± 3.44	8.24 ± 2.51	8.4 ± 3.13
ALT, U/L	25.35 ± 17.62	17.23 ± 14.74	22.38 ± 17.07
AST, U/L	27.06 ± 15.7	23.32 ± 21.75	25.69 ± 18.24
Pulmonary function			
Test value of FVC, L	4.25 ± 0.86	3.18 ± 0.64	3.86 ± 0.94
Test value of FEV1, L	3.61 ± 0.72	2.71 ± 0.54	3.28 ± 0.79
Uric Mn, µg/L			
Median (interquartile range)	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)

609





90x38mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7,8
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7,8
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Non
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	Non
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-13
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	13, 14
		(b) Describe any methods used to examine subgroups and interactions	Non
		(c) Explain how missing data were addressed	Non
		(d) If applicable, explain how loss to follow-up was addressed	Non
		(e) Describe any sensitivity analyses	Non
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14
		(b) Give reasons for non-participation at each stage	Non
		(c) Consider use of a flow diagram	Non
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14-16
		(b) Indicate number of participants with missing data for each variable of interest	Non
		(c) Summarise follow-up time (eg, average and total amount)	Non
Outcome data	15*	Report numbers of outcome events or summary measures over time	14-17
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Non
		(b) Report category boundaries when continuous variables were categorized	Non
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Non
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Non
<b>Limitations</b>			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	4
Generalisability	21	Discuss the generalisability (external validity) of the study results	Non
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).