Published in final edited form as:

Neurotoxicology. 2017 September ; 62: 258–264. doi:10.1016/j.neuro.2017.08.006.

Reduced expression of *PARK2* in manganese-exposed smelting workers

Ximin Fan^a, Ying Luo^a, Qiyuan Fan^{b,*}, and Wei Zheng^{c,*}

^aSchool of Public Health, Zunyi Medical College, Zunyi, Guizhou, China

^bDepartment of Health Management, Zunyi Medical and Pharmaceutical College, Zunyi, Guizhou, China

^cSchool of Health Sciences, Purdue University, West Lafayette, IN, United States

Abstract

Manganese (Mn) is widely used in modern industries. Occupational exposure to Mn is known to cause clinical syndromes similar, but not identical to, Parkinson's disease. This human cohort study was designed to investigate if workers exposed to Mn altered the *PARK2* gene expression, leading to Mn-induced neurotoxicity. Workers (n = 26) occupationally exposed to Mn were recruited from a Mn-iron (Fe) alloy smelter, and control workers (n = 20) without Mn-exposure were from an Fe smelter from Zunyi City in China. Subjects were matched with socioeconomic status and background for environmental factors. Metal concentrations were determined by atomic absorption spectrophotometry (AAS). Total RNA from the blood samples was isolated and analyzed by RT-PCR to quantify *PARK2*. The data showed that Mn concentrations in plasma, red blood cell (RBC) and saliva, and the cumulative Mn-exposure were about 2.2, 2.0, 1.7 and 3.0 fold higher, respectively, in Mn-exposed workers than those in control subjects (p < 0.01). The expression of *PARK2* in Mn-exposed workers was significantly decreased by 42% as compared to controls (p < 0.01). Linear regression analysis further established that the expression of *PARK2* mRNA was inversely correlated with Mn levels in plasma, RBC and saliva, as well as the cumulative Mn exposure (p < 0.01). Taken together, it seems likely that Mn exposure among smelters may lead to a reduced expression of *PARK2*, which may partly explain the Mn-induced Parkinsonian disorder.

Keywords

Manganese; Occupational exposure; Human; Smelter; *PARK2*; Biomarker

1. Introduction

Manganese (Mn) is an abundant, naturally occurring element in the Earth's crust; it is a ubiquitous micronutrient required for normal growth, development and cellular homeostasis

*Corresponding authors at: School of Public Health, Zunyi Medical College, Zunyi, Guizhou, China.

Conflict of interest

None of the authors declare conflicts of interest.

(Aschner and Aschner, 2005; Benedetto et al., 2009; Crossgrove and Zheng, 2004). Despite being essential for metabolic functions, exposure to high doses of Mn is known to cause a neurological disease clinically known as Manganism, which is characterized by symptoms that are similar, but not identical to idiopathic Parkinson's disease (IPD) (Benedetto et al., 2009; Racette et al., 2012; Roth, 2009). The disease is characterized by extra-pyramidal syndrome, such as gait disturbance ("cock-walk"), bradykinesia, tremor, limb rigidity, slurred speech and mask-like face (Racette et al., 2012). In the early stages of Mn poisoning, the symptoms could be reversible given that a reduction or elimination of Mn in the body is made possible. The late stage of Mn poisoning, however, usually displays an irreversible damage (Cersosimo and Koller, 2006; Crossgrove and Zheng, 2004). Therefore, the key to the treatment of Mn poisoning is to understand the mechanism by which Mn causes neurodegenerative damage.

Excessive exposure to Mn occurs mainly in occupational and environmental settings. Occupational exposure to Mn often happens among miners and smelters who are exposed to Mn-containing dust and fumes during ferroalloy smelting, mining, dry-cell battery production, or in manufacturing glass and ceramics (Bowler et al., 2007; Jiang et al., 2007; Lu et al., 2005; Montes et al., 2008; Qiu et al., 2013). The environmental exposure to Mn includes sceneries such as airborne Mn due to uses of the antiknock agent methylcyclopentadienyl manganese tricarbonyl (MMT), the application of fungicide-containing Mn pesticides such as Maneb, and the use of permanganate as a drinking water purifier (Sikk et al., 2007; Burton and Guilarte, 2009).

PARK2 (Parkin gene) is located on the chromosome 6 between base pairs 161, 768, 589 and 163, 148, 833 (6q25.2–q27), and contains 12 exons surrounded by large intron regions and spans about 1.5 Mb (Matsumine et al., 2017). Loss-of-function mutations in the *PARK2* gene have been associated with the autosomal recessive juvenile parkinsonism (AR-JP), which is characterized by an early onset (Gasser, 2009; Hauser et al., 2016; Hunn et al., 2015; Illarioshkin et al., 2003; Kim et al., 2011; Mata et al., 2004; Song et al., 2016). The *PARK2* encodes the Parkin protein, an E3 ubiquitin protein ligase, and ubiquitinates a variety of substrates (Fallon et al., 2006; Sarraf et al., 2013; Trempe et al., 2009). Parkin contains 465 amino acids, with molecular mass of about 52 kD. Parkin consists of N-terminal ubiquitin-like (UBL) domain, C-terminal RING-between-RING (RBR) domain and unique Parkin domain (UPD) (Hristova et al., 2009). The RBR domain comprises three RING (RING0, RING1 and RING2) domains and a 51-residue in-between-ring (IBR) domain. Parkin-mediated ubiquitination promotes mitochondrial autophagy or degradation of target proteins (Gegg et al., 2010; Geisler et al., 2017; Poole et al., 2010; Sarraf et al., 2013).

Our preliminary in vivo animal experiments and in vitro cell culture studies have confirmed that Mn exposure can result in a decreased expression level of *PARK2* (Deng et al., 2011; Luo et al., 2016). Since rat *PARK2* is analogy to humans, we hypothesized that the *PARK2* expression level may be reduced among workers who were occupationally exposed to Mn in their daily work, which may contribute, at least partially, to the mechanism of Mn-induced Parkinsonian disorder. Thus, the purpose of this human studies was to conduct a cross-sectional study in a smelter cohort in Zunyi City in China (Cowan et al., 2009a,b), to

determine the expression of *PARK2* in the control and Mn-exposed groups using RT-PCR, and to establish the relationship between the expression of *PARK2* and Mn levels in blood. The data from human studies shall be useful for mechanistic understanding of how Mn exposure leads to the Parkinsonian disorder. It is also possible that *PARK2* expression may be used as a new exposure biomarker for Mn neurotoxicity.

2. Materials and methods

2.1. Study population

This cross-sectional study was conducted in Mn-exposed ferroalloy smelting workers. The ferroalloy manufacturer is located in Guizhou Province, a mountainous region in the southwest China. The workers in the control group were recruited from an iron smelting factory, which is about 20 miles away from the ferroalloy factory in Zunyi City. The smelting process and labor intensity of the two factories are similar. The demographic information on all subjects is summarized in Table 1.

Subjects in both groups were matched with socioeconomic status (wages, education, etc.) and background environmental factors (place of residence, etc.). At the time of the interview, the subjects had no reported exposures to other toxic substances, radiation therapy or substance abuse. The subjects who took drugs that may interfere with Fe metabolism, such as vitamin D, aspirin or herbal supplements were excluded from the study. This study was approved by the Office of Clinical Investigation at the Zunyi Medical College and the Human Research Institutional Review Board at Purdue University.

2.2. Determination of airborne Mn levels in work places

The air Mn level was measured by a personal air sampler with a SKC pump (Model 224-44XR, calibrated at 2 L/min), a polyethylene tube, and a sample box with a proximal MCE filter (37 mm, pore size: 0.8 mm) was in the worker's breathing zone. Time-weighted the average concentration was calculated after each 8-h shift. Airborne Mn and Fe concentrations were determined by atomic absorption spectrophotometry (AAS) as described in our published paper (Cowan et al., 2009a, 2009b) and summarized below.

2.3. Collection of biological samples

Written informed consent was obtained from all subjects before the interview and the physical examination. All participants agreed to use their biological samples for this study. Participants were asked to fast over night before the study. They were invited to the Zunyi Medical College (ZMC) to collect biological samples (blood and saliva). Blood samples for the analysis of Mn and Fe were placed in a heparinized tube and allowed to stand at room temperature for 30 min. After centrifuged at 600 g/min for 5 min, the supernatant and pellet were collected as the plasma and RBC, respectively. Another aliquots of blood samples (0.5 mL venous blood) were collected into an autoclave tube (pre-loaded with 0.7 mL Trizol) for gene expression analysis.

Saliva samples were collected by requiring the subject to rinse the mouth three times with distilled, deionized (DDI) water, followed by expectorating of saliva into a 2-mL test tube. All samples were stored at -80°C until analysis.

2.4. Determination of Mn or Fe concentrations in air, blood and saliva samples

Tools and glassware for AAS analysis of metal concentrations were immersed in 10% HNO_3 for 8 h and washed several times with DDI water in order to prevent metal contamination. For metals in air, the filters from the personal air sampler were removed and dried in a desiccator at room temperature for 48 h. Filter were then digested with 5 mL of a HClO_4 - HNO_3 mixture (1: 9 vol/vol). For metals in the plasma, an aliquots (0.5 mL) of plasma were mixed with 5 mL of the digestion solution (HClO_4 : HNO_3 ; 3:7). For metals in the erythrocytes, approximately 1.5 g of precipitated blood cells was mixed with saline to a volume of 4 mL. An aliquot of the fraction (2 mL) was mixed with 10 mL of the digestion solution prior to AAS.

The saliva sample was centrifuged to remove the large particles; an aliquot (0.5 mL) of the supernatant was mixed with 0.5 mL of 100% HNO_3 for digestion. The digested sample was heated to dryness, followed by addition of 1% HNO_3 to the final volume of 0.5 mL. All samples were diluted appropriately within the range of the AAS standard curve. Mn and Fe concentrations were measured by AA240FS Varian AAS (Australia Pty Ltd.). The China National Standard Operation Protocol (GB/T16018-1995) was used for metal quantitation. The detection limits for Mn and Fe in graphite furnace AAS were 0.1 ng/mL and 0.09 ng/mL, respectively.

The method used to calculate the cumulative Mn exposure was based on the following assumption: the respiratory rate among normal subjects is about 20 times per minute or 1200 times per hour; with 750 mL per inspiration and 8 h per working day and 360 days per year, a normal person breathes 2592 cubic meters a year. The respiratory rate in active workers double the rate in the resting normal subjects. Thus, the smelting workers breathe 5184 cubic meters ($2592 \times 2 = 5184$) per year. These values, which are multiplied by the airborne Mn level, yield the cumulative Mn exposure.

2.5. RT-PCR analysis

The transcriptional levels of mRNA encoding *PARK2* and *Actb* (β -actin, an internal control) were quantified using qPCR. The total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA), according to the Manufacturer's recommendations, followed by purification process (Qiagen, Valencia, CA). The purified RNA was converted to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The forward and reverse primer sequences for selected genes were designed with the ABI Primer Express software (Foster City, CA) and listed in Table 2. A Power SYBR Green Master Mix (Applied Biosystems, Cheshire, UK) was used for RT-PCR analysis. The relative differences in expression between groups were expressed using cycle time (C_t) values as follows: the C_t values of the interested genes were first normalized with β -actin of the same sample, and then the relative differences between control and treatment groups were calculated and expressed as relative increases.

2.6. Statistical analysis

All demographic and personal data were abstracted from the survey questionnaires. All the data were converted to logarithms, and the symmetric distribution and linearity of the log transformed variables are valid. The data were analyzed by an Independent-Samples t Test using SPSS17.0 software. All data are expressed as mean $\pm$ SEM unless otherwise noted. Correlation coefficients was used to assess the association between the indicators. The difference between two means is considered significant, when the p value is less than 0.05.

3. Results

3.1. Analysis of demographic data and airborne Mn levels

Among a total of 46 subjects recruited in the study, the Mn-exposed smelters were slightly younger than the controls (Table 1). The control group had two females out of 20 subjects, while the Mn-exposed group had six women out of 26 subjects. Noticeably, the difference in the proportion of male to female subjects between two groups was not statistically significant. The average airborne Mn level in the smelter's working place was 0.31 mg/m³, which is higher than the ACGIH TLV of 0.2 mg/m³, and also about 1.6 fold greater than the level in the control group (p <0.001).

3.2. Expression of *PARK2* mRNA and concentrations of Mn and Fe among workers

Quantifying the expression of *PARK2* mRNA in blood samples showed that the relative expression of *PARK2* mRNA in the Mn-exposed smelters was significantly lower than that in the control workers, about 42% of reduction (p <0.01; Table 3). The distribution pattern of collected data is presented in Fig. 1.

The AAS data further revealed that Mn concentrations in plasma, RBC, and saliva were about 2.2, 2.0, 1.7 fold higher, respectively, in the Mn-exposed smelters than in control workers (p <0.01; Table 3; Fig. 2A–C). By multiplying the airborne Mn level with estimated working days, the cumulative Mn-exposure among smelters was about 3 fold higher than that in controls (p <0.01; Fig. 2D).

The Fe concentrations in plasma among the Mn-exposed smelters was significantly decreased by 17% as compared to controls (p <0.01; Fig. 3A); the finding was in a good agreement with our previous report (Cowan et al., 2009a). However, Fe concentration in RBC did not change significantly in Mn-exposed workers compared with controls (p >0.05; Fig. 3B).

3.3. Associations between *PARK2* mRNA expression and Mn and Fe levels

Altered *PARK2* mRNA expression could be due to increased Mn levels in body fluids and/or decreased Fe levels in the blood. The linear regression analyses revealed that the relative *PARK2* mRNA expression was statistically significantly associated with the Mn levels in plasma (r = -0.53, p <0.01), in RBC (r = -0.32, p <0.05), and in saliva (r = -0.65, p <0.01) (Fig. 4A–C). Most significantly, the *PARK2* mRNA expression was decreased as the cumulative Mn-exposure increased (r = -0.73, p <0.01) (Fig. 4D).

When the association was sought for blood Fe, it appeared that the *PARK2* mRNA expression was increased as the Fe level in plasma and RBC increased; this positive correlation, however, did not reach the statistical significance ($r = 0.24$, $p > 0.05$) (Fig. 5).

4. Discussion

This cross-sectional study, based on the study cohort from the manganese-iron alloy smelters, demonstrated that the *PARK2* expression among Mn-exposed workers was significantly reduced by 42% as compared to control workers. More importantly, our data established an inverse association between the expression of *PARK2* and Mn levels in plasmas, RBC and saliva. The most significant correlation was seen between the *PARK2* expression and the cumulative Mn exposure. The data provide the direct evidence to support the hypothesis that Mn-induced Parkinsonian disorder is likely associated with the altered *PARK2* regulatory pathway.

The *PARK2* gene encodes Parkin, a protein that is a component of tightly regulated E3 ubiquitin ligase complex. Parkin is known to promote the survival of dopaminergic neurons in both IPD and Parkinsonian disorders induced by acute exposures to neurotoxic agents (Duong et al., 2014). A loss of function of the Parkin protein has been shown to lead to the dopaminergic cell death in IPD (Romani-Aumedes et al., 2014). Studies on drosophila demonstrate that co-expression of human Parkin (*hParkin*) prevents against toxicities induced by over-expression of dNrdp1; the latter causes the loss of dopaminergic neurons in brain (Tan et al., 2011). While the mechanism remains unclear, Parkin is thought to be genetically linked to the glial cell line-derived neurotrophic factor receptor RET (Rearranged during Transfection); by converging with the RET signaling cascade, the Parkin controls mitochondrial structure and function, and maintains the integrity of dopaminergic neurons in substantia nigra and their innervation in striatum (Corti and Brice, 2013; Meka et al., 2015). In cell models, expression of Parkin can restore the mitochondrial function in DNF/RET-deficient cells (Meka et al., 2015). Literature data also show that the cell-permeable Parkin can compensate for intrinsic limitations in the Parkin response and provide a therapeutic strategy to augment Parkin activity in vivo (Duong et al., 2014). Toxicologically, induction of Parkin expression can protect striatal dopaminergic terminals against methamphetamine (METH)-induced neurotoxicity (Liu et al., 2013a). Logically, a reduction in Parkin expression, as observed in this study, could weaken the protective effect of Parkin in the pathoetiology of Parkinson's disease or related disorders.

Our previous studies show that subchronic exposure to Mn in rats causes a significant decrease of the *PARK2* expression in striatum, cortex, and blood cells, accompanying with a declined motor activity among Mn-exposed animals (Deng et al., 2011; Luo et al., 2016). Tyrosine hydroxylase (TH) immunohistochemical staining further demonstrate that Mn decreases dopaminergic neurons in substantia nigra, which is possibly associated with the decreased *PARK2* (Luo et al., 2016). Our in vitro studies using dopaminergic PC12 cells also show that the reduced dopamine level is associated with the down-regulated *PARK2* expression (Qiu et al., 2013). The current results from the Mn-exposed human cohort appeared to verify these animal data and demonstrated that the relative *PARK2* mRNA expression was indeed decreased following occupational exposure to Mn.

A clear inverse association between the *PARK2* expression and Mn levels in plasma, RBC, and saliva is evident; more impressive is a great correlation of the *PARK2* expression with the cumulative Mn exposure. Thus, the *PARK2* expression seemed likely to be sensitive to the Mn exposure. The question as to how the change in *PARK2* expression may ultimately contribute to the clinically defined manganism remains elusive. Since the clinical syndromes of manganism overlap, to some degree, with the IPD, it is highly likely that Mn-induced alteration in *PARK2* expression may partly underlie some of the clinical Parkinsonian signs and symptoms observed in manganism patients. Noticeably also, a recent finding from this group has shown that subacute, low-level Mn exposure indeed disrupts multiple neurotransmitter systems including dopaminergic system in a rat model (O'Neal et al., 2014).

Research efforts on searching for an effective biomarker for Mn intoxication are plentiful (O'Neal and Zheng, 2015). While the blood Mn level could partly differentiate groups of occupational Mn exposure from those non-Mn exposed controls, the large variation prevents it from being used as a reliable biomarker for individual monitoring (O'Neal and Zheng, 2015; Smith et al., 2007). One earlier study among welders shows that saliva Mn levels are higher in welders than in control subjects (Wang et al., 2008); but the larger variation in saliva than in serum renders the saliva assessment less applicable in real-life assessment. Mn levels in bone are the most promising approach for noninvasive assessment of body burden of Mn for cumulative exposure (Liu et al., 2013b, 2017). However, the detection system has not yet been made available for general populational research. In our previous study, we have attempted to establish the manganese-iron ratio (MIR) in blood as the biomarker for Mn health effect assessment (Cowan et al., 2009a,b). From the same cohort, we have also investigated the expression of some iron transport proteins such as DMT1, transferrin and hepcidin (Fan et al., 2016). But then again the concept has not yet been further verified in larger study cohorts. The current study has identified an excellent correlation between the reduction of *PARK2* and the cumulative Mn exposure. Thus, the possibility to use blood *PARK2* as the useful biomarker for Mn exposure deserves the future in-depth investigation.

In summary, this study demonstrated that *PARK2* expression decreased as the cumulative Mn exposure increases among smelters who were occupationally exposed to Mn. The finding suggests that Mn-induced Parkinsonian syndromes may be due partly to the altered *PARK2* expression. Furthermore, the possibility to use the altered *PARK2* expression in blood as a biomarker for Mn exposure assessment deserves further investigation.

Acknowledgments

This study was partially supported by the National Natural Science Foundation of China Grant #81260420 (QYF), Scientific and Technology Department of Guizhou Province Grant #SY (2012) 3140 (QYF), and NIH/National Institute of Environmental Health Science Grant #ES08146-18 (WZ).

References

- Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. *Mol Aspects Med.* 2005; 26:353–362. [PubMed: 16099026]

- Benedetto A, Au C, Aschner M. Manganese-induced dopaminergic neurodegeneration: insights into mechanisms and genetics shared with Parkinson's disease. *Chem Rev.* 2009; 109:4862–4884. [PubMed: 19534496]
- Bowler RM, Nakagawa S, Drezgic M, Roels HA, Park RM, Diamond E, Mergler D, Bouchard M, Bowler RP, Koller W. Sequelae of fume exposure in confined space welding: a neurological and neuropsychological case series. *Neurotoxicology.* 2007; 28:298–311. [PubMed: 17169432]
- Burton NC, Guilarte TR. Manganese neurotoxicity: lessons learned from longitudinal studies in nonhuman primates. *Environ Health Perspect.* 2009; 117:325–332. [PubMed: 19337503]
- Cersosimo MG, Koller WC. The diagnosis of manganese-induced parkinsonism. *Neurotoxicology.* 2006; 27:340–346. [PubMed: 16325915]
- Corti O, Brice A. Mitochondrial quality control turns out to be the principal suspect in parkin and PINK1-related autosomal recessive Parkinson's disease. *Curr Opin Neurobiol.* 2013; 23:100–108. [PubMed: 23206589]
- Cowan DM, Fan QY, Zou Y, Shi XJ, Chen J, Rosenthal FS, Aschner M, Zheng W. Manganese exposure among smelting workers: blood manganese-iron ratio as a novel tool for manganese exposure assessment. *Biomarkers.* 2009a; 14:3–16.
- Cowan DM, Zheng W, Zou Y, Shi XJ, Chen J, Rosenthal FS, Fan QY. Manganese exposure among smelting workers: relationship between blood manganese-iron ratio and early onset neurobehavioral alternations. *Neurotoxicology.* 2009b; 30:1214–1222. [PubMed: 19963104]
- Crossgrove J, Zheng W. Manganese toxicity upon over exposure. *NMR Biomed.* 2004; 17:544–553. [PubMed: 15617053]
- Deng Y, Sui DP, Xu J, Qiu J, Zhang H, Fan QY. Park2 expression alteration in the blood of manganese exposed animal and human beings. *ZUNYI YIXUE YUAN XUEBAO Acta Academiae Med Zunyi.* 2011; 34:567–569.
- Duong T, Kim J, Ruley HE, Jo D. Cell – permeable parkin proteins suppress Parkinson disease – associated phenotypes in cultured cells and animals. *PLoS One.* 2014; 9:e102517. [PubMed: 25019626]
- Fallon L, Bélanger CM, Corera AT, Kontogiannia M, Regan-Klapisz E, Moreau F, Voortman J, Haber M, Rouleau G, Thorarindottir T, Brice A, van Bergen E, Henegouwen PM, Fon EA. A regulated interaction with the UIM protein Eps15 implicates parkin in EGF receptor trafficking and PI(3)K? Akt signalling. *Nat Cell Biol.* 2006; 8:834–842. [PubMed: 16862145]
- Fan QY, Zhou Y, Yu CY, Chen J, Shi XJ, Zhang YS, Zheng W. Cross-sectional study of expression of divalent metal transporter-1, transferrin, and hepcidin in blood of smelters who are occupationally exposed to manganese. *Peer J.* 2016; 4:e2413. [PubMed: 27635361]
- Gasser T. Mendelian forms of Parkinson's disease. *Biochim Biophys Acta.* 2009; 1792:587–596. [PubMed: 19168133]
- Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, Taanman JW. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum Mol Genet.* 2010; 19:4861–4870. [PubMed: 20871098]
- Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol.* 2017; 12:119–131.
- Hauser DN, Primiani CT, Cookson MR. The effects of variants in the PARK2 (parkin), PINK1, and PARK7 (DJ-1) genes along with evidence for their pathogenicity. *Curr Protein Pept Sci.* 2016; 11(March)
- Hristova VA, Beasley SA, Rylett RJ, Shaw GS. Identification of a novel Zn²⁺-binding domain in the autosomal recessive juvenile Parkinson-related E3 ligase parkin. *J Biol Chem.* 2009; 284:14978–14986. [PubMed: 19339245]
- Hunn BH, Cragg SJ, Bolam JP, Spillantini MG, Wade-Martins R. Impaired intracellular trafficking defines early Parkinson's disease. *Trends Neurosci.* 2015; 38:178–188. [PubMed: 25639775]
- Illarioshkin SN, Periquet M, Rawal N, Lücking CB, Zagorovskaya TB, Slominsky PA, Miloserdova OV, Markova ED, Limborska SA, Ivanova-Smolenskaya IA, Brice A. Mutation analysis of the parkin gene in Russian families with autosomal recessive juvenile parkinsonism. *Mov Disord.* 2003; 18:914–919. [PubMed: 12889082]

- Jiang Y, Zheng W, Long L, Zhao W, Li X, Mo X, Lu J, Fu X, Li W, Liu S, Long Q, Huang J, Pira E. Brain magnetic resonance imaging and manganese concentrations in RBCs of Smelting workers: search for biomarkers of manganese exposure. *Neurotoxicology*. 2007; 28:126–135. [PubMed: 16978697]
- Kim HJ, Kim HJ, Lee JY, Yun JY, Kim SY, Park SS, Jeon BS. Phenotype analysis in patients with early onset Parkinson's disease with and without parkin mutations. *J Neurol*. 2011; 258:2260–2267. [PubMed: 21625934]
- Liu B, Traini R, Killinger B, Schneider B, Moszczynska A. Overexpression of parkin in the rat nigrostriatal dopamine system protects against methamphetamine neurotoxicity. *Exp Neurol*. 2013a; 247:359–372. [PubMed: 23313192]
- Liu Y, Koltick D, Byrne P, Wang H, Zheng W, Nie LH. Development of a transportable neutron activation analysis system to quantify manganese in bone in vivo: Feasibility and methodology. *Physiol Meas*. 2013b; 34:1593–1609. [PubMed: 24165395]
- Liu Y, Mostafaei F, Sowers D, Blake S, Hsieh M, Zheng W, Nie LH. Customized portable neutron activation analysis system to quantify manganese (Mn) in bone in vivo. *Physiol Meas*. 2017; 38:452–465. [PubMed: 28060775]
- Lu L, Zhang L, Li G, Guo W, Liang W, Zheng W. Alteration of serum concentration of manganese iron, ferritin, transferring receptor following exposure to welding fumes among career welders. *Neurotoxicology*. 2005; 26:257–265. [PubMed: 15713346]
- Luo Y, Xu J, Fan XM, Wu Q, Liu J, FANQY. [The effect of PARK2 gene on motor activity deficit in rats induced by manganese]. *DU LI XUE ZA ZHI* [J Toxicol. 2016; 30:27–30.
- Mata IF, Lockhart PJ, Farrer MJ. Parkin genetics: one model for Parkinson's disease. *Hum Mol Genet*. 2004; 13:R127–R133. [PubMed: 14976155]
- Matsumine H, Saito M, Shimoda-Matsubayashi S, Tanaka H, Ishikawa A, Nakagawa-Hattori Y, Yokochi M, Kobayashi T, Igarashi S, Takano H, Sanpei K, Koike R, Mori H, Kondo T, Mizutani Y, Schaffer AA, Yamamura Y, Nakamura S, Kuzuhara S, Tsuji S, Mizuno Y. Localization of a gene for autosomal recessive form of juvenile parkinsonism (AR-JP) to chromosome 6q25. 2-27. *Am J Hum Genet*. 2017; 60:588–596.
- Meka DP, Müller-Rischart AK, Nidadavolu P, Mohammadi B, Motori E, Ponna SK, Aboutalebi H, Bassal M, Annamneedi A, Finckh B, Miesbauer M, Rotermund N, Lohr C, Tatzelt J, Winklhofer KF, Kramer ER. Parkin cooperates with GDNF/RET signaling to prevent dopaminergic neuron degeneration. *J Clin Invest*. 2015; 125:1873–1885. [PubMed: 25822020]
- Montes S, Riojas-Rodriguez H, Sabido-Pedraza E, Rios C. Biomarkers of manganese exposure in a population living close to a mine and mineral processing plant in Mexico. *Environ Res*. 2008; 106:89–95. [PubMed: 17915211]
- O'Neal S, Zheng W. Manganese toxicity upon overexposure: a decade in review. *Curr Env Health Rep*. 2015; 2:315–328. [PubMed: 26231508]
- O'Neal OL, Lee JW, Zheng W, Cannon JR. Subacute manganese exposure in rats is a neurochemical model of early manganese toxicity. *Neurotoxicology*. 2014; 44:303–313. [PubMed: 25117542]
- Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L. The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/parkin pathway. *PLoS One*. 2010; 5:e10054. [PubMed: 20383334]
- Qiu J, Fan L, Tao D, Sui DP, Deng Y, Fan QY. Modification of dopamine secretion in PC12Cells by manganese induced PARK2 gene expression. *HUANJING YU ZHIYE YIXUE J Environ Occup Med*. 2013; 30:176–179.
- Racette BA, Aschner M, Guilarte TR, Dydak U, Criswell SR, Zheng W. Pathophysiology of manganese-associated neurotoxicity. *Neurotoxicology*. 2012; 33:881–886. [PubMed: 22202748]
- Romani-Aumedes J, Canal M, Martin-Flores N, Sun X, Perez-Fernandez V, Wewering S, Fernandez-Santiago R, Ezquerra M, Pont-Sunyer C, Lafuente A, Alberch J, Luebbert H, Tolosa E, Levy OA, Greene LA, Malagelada C. Parkin loss of function contributes to RTP801 elevation and neurodegeneration in Parkinson's disease. *Cell Death Dis*. 2014; 5:e1364. [PubMed: 25101677]
- Roth JA. Are there common biochemical and molecular mechanisms controlling manganese and parkinsonism. *Neuromol Med*. 2009; 11:281–296.

- Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, Gygi SP, Harper JW. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature*. 2013; 496:372–376. [PubMed: 23503661]
- Sikk K, Taba P, Haldre S, Bergquist J, Nyholm D, Zjablov G, Asser T, Aquilonius SM. Irreversible motor impairment in young addicts – ephedrone, manganism or both? *Acta Neurol Scand*. 2007; 115:385–389. [PubMed: 17511846]
- Smith D, Gwiazda R, Bowler R, Roels H, Park R, Taicher C, Lucchini R. Biomarkers of Mn exposure in humans. *Am J Ind Med*. 2007; 50:801–811. [PubMed: 17924418]
- Song P, Trajkovic K, Tsunemi T, Krainc D. Parkin modulates endosomal organization and function of the endo-Lysosomal pathway. *J Neurosci*. 2016; 36:2425–2437. [PubMed: 26911690]
- Tan Y, Yu F, Pereira A, Morin P, Zhou J. Suppression of Nrdp1 toxicity by Parkin in *Drosophila* models. *Biochem Biophys Res Commun*. 2011; 416:18–23. [PubMed: 22086177]
- Trempe JF, Chen CX, Grenier K, Camacho EM, Kozlov G, McPherson PS, Gehring K, Fon EA. SH3 domains from a subset of BAR proteins define a Ubl-binding domain and implicate parkin in synaptic ubiquitination. *Mol Cell*. 2009; 36:1034–1047. [PubMed: 20064468]
- Wang DX, Du XQ, Zheng W. Alteration of saliva and serum concentrations of manganese, copper, zinc, cadmium and lead among career welders. *Toxicol Lett*. 2008; 176:40–47. [PubMed: 18054180]

Fig. 1.

Expression of mRNA encoding PARK2 in blood. Blood samples were collected from smelters or control workers in Zunyi, China. Lines represent the group mean and dots represent each individual determinant; $n = 20$ for controls and $n = 26$ for Mn-exposed smelters.

Fig. 2.

Mn concentration in plasma, RBC and saliva, and cumulative Mn-exposure. Mn concentrations were measured by AAS. (A). Mn levels in plasma. (B). Mn levels in RBC. (C). Mn levels in saliva. (D). Cumulative Mn exposure.

Fig. 3. Fe concentration in plasma and RBC. Fe concentrations were determined by AAS. (A). Fe levels in plasma. (B). Fe levels in RBC.

Fig. 4.

Correlations between *PARK2* expression and Mn concentrations. Stars represent the data from the control group and circles represent those from the Mn-exposure group. (A). *PARK2* and Mn in plasma ($r = -0.53$; $p < 0.01$). (B). *PARK2* and Mn in RBC ($r = -0.32$; $p < 0.05$). (C) *PARK2* and Mn in saliva ($r = -0.65$; $p < 0.01$). (D) *PARK2* and cumulative Mn-exposure ($r = -0.73$; $p < 0.01$).

Fig. 5. Correlation between PARK2 expression and Fe concentrations. (A). PARK2 and Fe in plasma ($r = 0.24$; $p > 0.05$). (B). PARK2 and Fe in RBC ($r = 0.24$; $p > 0.05$).

Table 1

Summary of Demographic Information.

	Control Group		Mn-Exposed group		P values
	n	Mean (SEM)	n	Mean (SEM)	
Age (years)	20	39.8 ± 1.72	26	34.4 ± 1.11	<0.05
Years of employment	20	2.48 ± 0.39	26	4.04 ± 0.52	<0.05
Gender (male/female)	20	18/2	26	20/6	>0.10
Airborne Mn level (mg/m ³)	20	0.002 (0.002–0.002) [#]	26	0.308 (0.015–0.800) [#]	<0.001

Data represent mean ± SEM.

[#] : values in parentheses represent the range.

Table 2

Forward and Reverse Primer Sequences for Selected Genes.

Gene Product	Forward Primer	Reverse Primer
β -actin(NM_001099771)	GGGGCATGCATCAGAAAGAG	GCAGCTCGTTGTAGAAGGTG
<i>PARK2</i> (NM_013987)	AAAGGCCCTGTCAAAGAGT	TTGTTGCGATCAGGTGCAA

Notes. The forward and reverse primer sequences for selected genes were designed with the ABI Primer Express software (Foster City, CA).

Table 3Levels of *PARK2*, Mn, Fe and Cumulative Mn Exposure.

	Control Group	Mn-Exposed Group	% Change
log(Relative PARK2 mRNA)	3.087 ± 0.108	1.788 ± 0.113 **	-42
log(Mn-plasma)	0.698 ± 0.117	1.530 ± 0.050 **	+119
log(Mn-RBC)	0.528 ± 0.106	1.074 ± 0.064 **	+103
log(Mn-saliva)	0.819 ± 0.070	1.382 ± 0.051 **	+68
log(cumulative Mn-exposure)	1.016 ± 0.000	3.088 ± 0.067 **	+204
log(Fe-plasma)	0.943 ± 0.046	0.783 ± 0.025 **	-17
log(Fe-RBC)	2.966 ± 0.033	2.907 ± 0.015	-2

Data represent mean ± SEM.

**
p <0.01 compared with control.

% Change = 100 x (values in the Mn-exposed group – values in the control group)/values in the control group.