ATP13A2 (PARK9) polymorphisms influence the neurotoxic effects of manganese

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Key words: *SPCA1*, Parkinson, motor, odor, tremor, gene-environment, neurophysiological, ferro-manganese

Abstract

Introduction: A higher prevalence of individuals affected by Parkinsonism was found in Valcamonica, Italy. This may be related to ferro-alloy smelters in the area, releasing manganese (Mn) in the air, soil and water for about a century. There exists individual susceptibility for Mn neurotoxicity.

Aim: To analyse how polymorphism in genes regulating Mn metabolism and toxicity can modify neurophysiological effects of Mn exposure.

Materials and Methods: Elderly (N=255) and adolescents (N=311) from Northern Italy were examined for neuromotor and olfactory functions. Exposure to Mn was assessed in blood and urine by atomic absorption spectroscopy and in soil by a portable instrument based on X-Ray fluorescence technology. Polymorphisms in the Parkinson-related gene ATPase type 13A2 (*ATP13A2*, also called *PARK9*: rs3738815, rs2076602, rs4920608, rs2871776, rs2076600), and in the secretory pathway Ca^{2+}/ Mn^{2+} ATPase isoform 1 gene (*SPCA1*: rs218498, rs3773814, rs2669858) were analysed by TaqMan probes.

Results: For both adolescents and elderly, negative correlations between Mn in soil and motor coordination (R_s=-0.20, p<0.001, R_s=-0.13, p=0.05 respectively) were demonstrated. Also among adolescents, negative correlations were seen between Mn in soil with odor identification (R_s=-0.17, p<0.01). No associations were seen for Mn in blood or urine. *ATP13A2* polymorphisms rs4920608 and rs2871776 significantly modified the effects of Mn exposure on impaired motor coordination in elderly (p for interaction= 0.029, p= 0.041 respectively), also after adjustments for age and gender. The rs2871776 altered a binding site for transcription factor Insulinoma-associated 1.

Conclusions: ATP13A2 variation may be a risk marker for neurotoxic effects of Mn in humans.

1. Introduction

Although being an important essential element, manganese (Mn) is also a recognized neurotoxic metal. This has been shown for more than one century in the occupational literature starting from sir James Couper (1837) and is now assessed extensively also in the general population. Studies on adolescents exposed to high Mn in drinking water (Bouchard et al., 2011) and airborne particulate (Riojas-Rodríguez et al., 2010, Menezes-Filho et al., 2011) have shown impairment of cognitive functions. Adults with environmental exposure to Mn have shown motor impairment regarding position changes in hand movements (Rodríguez-Agudelo et al., 2006). Impairment of postural balance was also observed across different age groups of an environmentally exposed population (Standridge et al., 2008).

Similar to other elements, such as arsenic, lead and beryllium (Nordberg et al., 2007; Scinicariello et al., 2007; Engström, 2011) the genetic background of an individual probably determines Mn metabolism (Curran et al., 2009) and Mn toxicity, but so far the susceptibility genes for Mn are unknown. On the other hand, the genetics of juvenile Parkinson may provide information for Mn susceptibility. Genetic mutations play an important role in causing juvenile form of Parkinsonism, and one of the target genes is *ATP13A2* (Myhre et al., 2008). ATP13A2 belongs to a large group of lysosomal transport proteins in the 5-P type ATPase family. The exact function of ATP13A2 is still unclear, but there is evidence that this protein is involved in the transport of multiple cations (Mn, nickel, cadmium and selenium) from the cytosol to the lysosomal lumen (Schmidt et al., 2009; Santoro et al., 2011). Dopaminergic loss caused by α-synuclein overexpression in animal and neuronal Parkinson disease model is avoided by co-expression of ATP13A2, and a yeast ortholog of ATP13A2 helps to protect cells from Mn toxicity (Gitler et al., 2009). These findings suggest that reduced function, such as caused by polymorphisms, of ATP13A2 is associated with more Mn toxicity and less protection against Parkinsonian changes.

Secretory pathway Ca2+/ Mn2+ ATPase isoform 1 (SPCA1: encoded by *ATP2C1*) has become known as a Ca2+/ Mn2+ transporter pump, and is localized in the membrane of the Golgi apparatus in mammalian cells (Murín et al., 2006). The Golgi apparatus participates in Ca2+ and Mn2+ transport from cytosol to the Golgi lumen (Sepúlveda et al., 2009). These cations are used for specific luminal processes such as protein sorting and endosome fusion (Dode et al., 2006). SPCA1 plays an important role in Mn2+ homeostasis and is the only known P-type ATPase, which with high affinities can transport Mn2+. Silencing of SPCA1

results in the deficiency of Ca and/or Mn uptake, which impairs Ca2+ homeostasis inside the Golgi stores and disturbs alteration of neuronal polarity (Sepúlveda et al., 2009).

The purpose of this study was to analyse, in a population of adolescents and elderly from Northern Italy, how genetic variation in *ATP13A2* and *SPCA1* modifies neurotoxic effects of environmental exposure to Mn.

2. Materials and methods

2.1 Study population

This cross-sectional study comprises a population of 311 adolescent boys and girls (11-14 years), and 255 elderly men and women (63-80 years) from Italy with varying Mn exposure through ferroalloy foundries in the area. We considered here adolescents and elderly as potentially susceptible population strata to Mn exposure. Two different areas of the province of Brescia in Italy have been studied: the Valcamonica valley as exposed area, and the Garda Lake as reference area. Three ferroalloy plants have been operating in Valcamonica for a century until 2001. The emission in the atmosphere, soil and water of this area has caused contamination with various metals including Mn, lead, iron, chromium, zinc, and copper that has overlapped the naturally occurring geological composition. Comprehensive assessment of environmental exposure has been conducted on deposited dust (Zacco et al., 2009) and airborne particles that are still present due to re-suspension related to car traffic and construction work (Borgese et al., 2011). Further description of the methodology concerning neuropsychological testing and exposure assessment available in a separate publication (Lucchini et al., this issue).

All subjects underwent an extended assessment of neurological testing on motor, cognitive sensory and behavioral functions. None of the subjects was a confirmed patient or had a medical-proved diagnosis of Parkinson's disease-like syndromes. Data were collected also for alcohol-drinking (estimated g per week) and smoking habit (number of cigarettes per day). All blood samples were collected in EDTA vacutainers (Sarstedt monovette) during the same week of the neurological testing and stored at -20°C for subsequent DNA extraction and analysis.

2.2 Exposure assessment

Mn was measured in blood and urine by atomic absorption spectroscopy at the Laboratory of Industrial Toxicology of the University of Brescia. Due to the strong homeostatic regulations of Mn in the body, it is difficult to assess the exposure to Mn with human biomarkers. Soil is considered as a potential indicator of cumulative exposure to heavy metals and has been observed as predictive of neurological effects in children (Liu et al., 2010). Thus, environmental exposure to manganese in these individuals was also assessed by the concentration of Mn in surface soil. Concentrations were analyzed with a portable instrument based on X-Ray Fluorescence (XRF) (Thermo Scientific Niton, model XLt) equipped with GPS geo-referencing capability. Kringing GIS interpolation was used to estimate the soil levels for the houses without family gardens.

2.3 Neuropsychological testing

Motor coordination, odor identification, tremor, and sway intensity was examined in the study populations. Motor coordination was explored with five subtests of the Luria Nebraska Motor Battery (Golden et al., 1980): dominant hand clench, non dominant hand clench, alternative hand clench, finger-thumb touching with dominant hand, finger-thumb touching with non dominant hand. In the first set, the subject had to close and open, as fast as possible, the dominant and non-dominant hand and also the two hands alternatively. In the second set, the subject had to touch thumb (of dominant and non-dominant hand) with each finger as fast as possible (each exercise took 10 seconds). We used the Sniffin' Sticks-Olfactory test-Screening 12 to assess olfactory identification. The subject smell the tip of 12 fiber sticks filled with different scents and must identify the odors (e.g. coffee, orange) from a list (Hummel et al., 2001). Tremor was assessed as the subject held a stylus for about 10 seconds. The hand vibrations were recorded and displaced in a time axis plot on the computer screen (Tremor 7.0 DPD, Denmark). The accelerations were analyzed by methods drawn from vibrations measurements. Body sway (motor-balance) was recorded by a balance plate, producing signals from three sensors to map the position of the force centre during the test period. This centre is defined as the center of equilibrium of the three vertical forces, recorded at the three supports of the sway plate. During the test, subject stands erect on the sway plate, which is visualized on the computer screen, where the journey of the force centre can be observed in an X-Y coordinate system.

2.4 Genotype analyses

TagSNPs that carry the information about the genetic variation in a larger segment of the gene were selected from *ATP13A2* and *SPCA1,* using the CEPH population as a reference and the Hapmap data (www.hapmap.org). The selected polymorphisms in *ATP13A2* were rs3738815 (C_2190590_20: assay number for polymorphism in the TaqMan® SNP Genotyping Assays of Applied Biosystems, Foster city, CA, USA), rs2076602 (C_15866733_10), rs4920608 (C_2190586_10), rs2871776 (C_2190583_10) and rs2076600 (C_15866734_10). The three SNPs studied in *SPCA1* were rs218498 (C_2966605_10), rs3773814 (C_27482612_10), and rs2669858 (C_16051815_10).

DNA was extracted from whole blood by QIAmp DNA mini kit (Qiagen, Heidelberg) according to the instructions of the manufacturer. SNPs for *ATP13A2* and *SPCA1* were analysed by TaqMan® allelic discrimination assay (ABI 7900 instrument, Applied Biosystems, Foster City, CA, USA). The reaction volume was 10 μL, and the reaction consisted of 1X TaqMan® Universal Master Mix (Applied Biosystems), 1 X TaqMan® SNP Genotyping Assay Mix, and 8 ng DNA). In each run, negative controls were included. Genotyping analysis was repeated for 5 % of individuals with 100% concordance.

2.5 Statistics

Hardy-Weinberg equilibrium (HWE) analysis was undertaken using the χ^2-test. In order to evaluate the associations between Mn exposure markers and various neurobehavioral functions, we performed a Spearman r correlation. We chose associations between Mn exposure markers and outcome that were strongest for further evaluation of genetic effect modification by multivariate linear regression analysis. The multivariate model was run with a cross product interaction term between Mn in soil and genotype. If no effect modification was to be seen in the interaction model, the model was performed without the interaction term in order to assess main effects. When the number of variant homozygotes was very few this group was combined with the heterozygous carriers. All statistical analyses were performed using the software PASW (Statistical Packages for Social Sciences, version 18; SPSS, Chicago, IL USA). A p-value <0.05 denotes statistical significance. Bioinformatic analysis was performed for potential disruption of transcription factor binding sites by the programme from Genomatix (www.genomatix.de).

3. Results

Demographic information of the study population and exposure markers are presented in Table 1. The exposure markers in blood and urine showed that Mn in blood was similar, but Mn in urine was higher in elderly compared to the adolescents. For the elderly, the exposure marker Mn in soil (MnS) was negatively associated with Mn in blood (MnB; Spearman R correlation R_s=-0.136, P = 0.036), but no significant associations were found for MnS with MnU (R_s =-0.033, P = 0.61), or MnB with MnU (R_s=0.12, P = 0.076). Among adolescents no significant associations were found between the exposure markers (MnS with MnB R_s=0.009, P = 0.88; MnS with MnU R_s=0.094, P = 0.10; MnB with MnU R_s=0.094, P = 0.10).

Median scores and range of motor coordination test, sway, tremor and odor identification of the two study groups are shown in the Table 1. Age influenced negatively the olfactory function and sway in the elderly, whereas motor and olfactory functions improved with age among the adolescents. A gender influence was found in both age groups, with males showing higher tremor intensity, centre frequency and body sway, and decreased odor identification compared to females. Elderly men had better motor coordination than women. Smoking habits did not influence the health outcomes in elderly, whereas alcohol intake increased tremor intensity and sway among the elderly and centre frequency of the left hand among adolescents. There were too few smokers among the adolescents to calculate correlation coefficients. Excluding those adolescents that had not clearly indicated that they neither consumed alcohol nor tobacco (N=18) did not change our findings.

The relationship between Mn in urine, blood and soil and neurophysiological outcomes was investigated using Spearman R correlation coefficient (Table 1). There was a negative correlation between Mn in soil and motor coordination for both adolescents and elderly and odor identification for adolescents. Mn in soil demonstrated a stronger correlation with the neurological outcomes compared with the measured data of blood and urine. Thus, the further analysis on genetic effect modification focused on Mn in soil as an exposure marker for Mn exposure.

The genotype frequencies for *ATP13A2,* and *SPCA1* were all in Hardy Weinberg equilibrium (data not shown). The allelic frequencies were almost the same in elderly and adolescents (Table 2). The allele frequencies were very similar to those in a European population (CEU population: from http://www.ncbi.nlm.nih.gov/SNP) (< 17 % difference).

Polymorphisms in rs4920608 and rs2871776 in *ATP13A2* significantly modified the association between Mn in soil and motor coordination in elderly (p=0.029, p=0.041

respectively; Table 3, Figures 1 & 2). This was still significant when adjusted for age and gender (p=0.032, p=0.044). Smoking and alcohol consumption did not contribute significantly to the adjusted model and were therefore excluded. None of the polymorphisms alone had a significant influence on motor coordination (Supplemental Material, Table 1) only in combination with Mn exposure as shown in Table 3. Figure 1 shows that with increasing Mn exposure, the rs4920608 CT and CC carriers had a decreased motor coordination, compared with TT carriers. In Figure 2, motor coordination was reduced for rs2871776 GG and GA carriers, compared to the AA individuals with increasing Mn exposure. There was modest linkage disequilibrium between the two polymorphism [r^2=0.48 in the CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) + TSI (Tuscan in Italy) populations from the Hapmap database (www.hapmap.org)].

There was no genetic effect modification among adolescents for *ATP13A2*. Also, there was no effect of *SPCA1* in neither group on the association between Mn and neurophysiological outcomes.

Bioinformatic analysis revealed that the *ATP13A2* rs2871776 G allele deleted a binding site for the transcription factor Insm1 (Insulinoma-associated 1). No binding site was modified by the different alleles of the rs4920608 SNP.

4. Discussion

This study reveals impairment of motor function in individuals living in the vicinity of ferroalloy emissions and associated with the Mn content in deposited dust, which is particularly relevant in an area were previous research has shown a high prevalence of Parkinsonism, (Lucchini et al., 2007). In addition to exposure-related changes of neurological functions, this is the first study to show that genetic predisposition can further amplify these effects in susceptible individuals. Common genotypes of two *ATP13A2* polymorphisms were associated with reduced motor coordination of Mn, but only among the elderly. Hypothetically, the genetic profile causes increased risk only after long-term exposure, such as in elderly long-term residents from this region.

There are some limitations in this study. One concern is the marker of Mn exposure. We here analysed the interrelation between Mn exposure and motor-sensory functions with different exposure biomarkers for Mn, with the strongest effect seen for soil Mn and motor

coordination, as assessed with the Luria Nebraska motor test, and odor identification. Soil can also be considered as a suitable indicator of lifetime and cumulative exposure to metals that are stable and long-lived in the environment, and accumulate in soils over time (Aelion et al., 2009). This is particularly important for Mn that causes long-term effects on the nervous system with a cumulative mechanism of toxicity (Lucchini and Zimmerman, 2009). Few studies have considered soil in the assessment of health effects due to the environment. Liu et al. (2010) have found an association between arsenic in soil and mental retardation in adolescents. Soil may be able to integrate different exposure sources (airborne particles, water, foods) and absorption routes in a longitudinal fashion. This may lead to the assessment of the total body load of chemicals like metals that can accumulate in the organism and especially in the brain, causing long-term effects. However, limited information is available in the literature on soil background levels of heavy metals specific to these study regions.
It is likely that there are no associations between B-Mn or U-Mn and neuromotor functions as Mn is also an essential element and thus, there are strict homeostatic mechanisms for the regulation of the levels of Mn in blood and in urine. We assume that the exposure route to the central nervous system is from inhaled dust through absorption and translocation in the olfactory nerve(Lucchini et al., 2011), which may explain the positive finding between Mn in soil and motor coordination.

A further concern is that multiple comparisons were made in this study; some of the reported associations may be false positive and the results of *ATP13A2* need to be verified. Also, other genes involved in Mn metabolism and toxicity are probably important. For example, there is *in vitro* evidence that the wildtype of Parkinson-associated mutation G2019S of leucine-rich repeat kinase 2 *(PARK8)* has a dramatically reduced catalytic activity in the presence of Mn while the catalytic activity of the variant is unchanged (Lovitt et al., 2010; Covy and Giasson, 2011; Covy and Giasson, 2010).
The protective role of ATP13A2 of the toxicity induced by alpha-synuclein in animal models of Parkinson disease, as well as the toxic effects of Mn exposure (Gitler et al., 2009) suggest that polymorphisms in this gene may render susceptibility to Parkinson disease and Mn toxicity. Our study lends some support to this notion. Both rs4920608 and rs2871776 SNPs are located in intron regions of *ATP13A2*: rs2871776 is located in the upper half of the gene and rs4920608 towards the end of the gene, but the functional impacts of both SNPs are still unknown. The SNPs included in this study were selected because they are tagging genetic variation for larger segments of the *ATP13A2* gene. Their role in parkinsonism is still

unknown apart from rs3738815, which was not associated with Parkinson disease (Vilarino-Guell et al., 2009), in a candidate gene study of *ATP13A2*. The SNPs in our study have not been been identified as parkinsonism-susceptibility markers in genome-wide associations studies (Fung et al., 2006, Maraganore et al., 2005).

The rs2871776 G allele that was associated with adverse effect of manganese on motor coordination was associated with destroying a binding site for the transcription factor INSM1 that plays an important role in the developing central nervous system as shown in mouse (Farkas et al., 2008; Rosenbaum et al., 2011) and human embryos (Duggan et al., 2008). In adult tissues the expression seems to be limited to areas of neurogenesis (the hippocampus, rostral migratory pathway and the olfactory bulb) (Duggan et al., 2008). Absence of the binding site for this transcription factor on the *ATP13A2* gene hypothetically might explain why adult carriers in our population perform more poorly in the motor coordination test upon Mn exposure.

In conclusion, this study showed early effects (i.e. below the threshold for the definition of clinical effects) on motor coordination and odor identification associated with lifetime environmental exposure to Mn. Further progression of reduced motor coordination (Almeida et al., 2002), and olfactory impairment (Haehner et al., 2009) are seen in Parkinson disease. The effect of Mn exposure on the nervous system was, in the elderly, influenced by genetic polymorphism of *PARK9*, a susceptibility gene for Parkinson disease. Further studies are warranted for elucidating mechanisms for susceptibility to Mn exposure.

Acknowledgements

The authors would like to thank Mrs. Karin Paulsson for help with genotyping. This study was supported by funding from the European Union through its Sixth Framework Programme for RTD (contract no FOOD-CT-2006- 016253). It reflects only the authors' views, and the European Commission is not liable for any use that may be made of the information contained therein. The project described was supported also by Award Number R01ES019222 from the National Institute Of Environmental Health Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Environmental Health Sciences or the National Institutes of Health.

Conflict of interest: none declared.

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Table 1. Descriptive characteristics and exposure data for the two study populations a) elderly and b) adolescents. [1]

Variable	Distribution	R_s^2 Motor coordination	R_s^2 Odor	R_s^2 Sway Velocity CE	R_s^2 Tremor Intensity L/R	Rs with Tremor Centre frequency L/R
a) Elderly						
Age[3] (Y)	69 (63-80)	-0.11	-0.17*	0.22**	-0.018 / -0.079	-0.084 / -0.15*
Women/ Men (N)	140/115	0.14*	-0.17*	0.34**	0.30** / 0.36**	0.27** / 0.26**
Smoking (N:Never/Yes /Ex)	172/20/63	0.091	-0.078	0.098	0.12 / 0.064	0.02 / 0.046
Alcohol intake[3] (g/day)	12.8 (0-205)	0.014	-0.075	0.21*	0.15* / 0.18*	0.11 / 0.11
MnS[3] (ppm)	786 (313-1724)	-0.13*	-0.093	-0.048	-0.11 / -0.035	-0.027 / 0.026
MnB[3] (μg/L)	9 (3.6 -22)	-0.002	-0.027	0.028	-0.086 / -0.047	0.030 / -0.11
MnU[3] (μg/L)	0.14 (0.06-12)	-0.048	-0.017	0.022	-0.080 / -0.15*	-0.12 / -0.089
Motor coordination[3] (N of correct tasks)	11 (5-18)		0.17*	-0.037	0.048 / 0.062	0.056 / 0.072
Odor identification[3] (N of correct recognitions)	9 (0-12)			-0.11	-0.027 / -0.017	0.002 / -0.030
Sway Velocity CE[3]	15 (3-74)				0.27** / 0.35**	0.12 / 0.18*
b) Adolescents						
Age[3] (Y)	12 (11-14)	0.11*	0.20**	-0.083	-0.17* / -0.14*	0.11 / 0.095
Girls/ Boys (N)	153/158	-0.048	-0.16*	0.22**	0.26** / 0.29**	0.11 / 0.16*
MnS[3] (ppm)	529 (160-1730)	-0.20**	-0.17*	0.065	-0.066 / -0.027	-0.021 / 0.027
MnB[3] (μg/L)	11 (4-24)	0.020	0.082	-0.071	0.062 / 0.065	-0.057 / -0.010
MnU[3] (μg/L)	0.08 (0.05 – 5.4)	-0.10	-0.022	-0.075	-0.098 / -0.083	-0.095 / -0.018
Smoking (N) (Yes/No)	3/301[4]					
Alcohol intake (N) (Yes/No)	9/295	0.006	-0.098	0.001	-0.038 / -0.053	0.13* / 0.074
Motor coordination[3] (N of correct tasks)	13 (6-21)		0.080	-0.010	-0.069 / 0.009	0.11 / -0.014
Odor identification[1] (N of correct recognitions)	10 (4-12)			-0.032	-0.048 / -0.074	0.068 / 0.093
Sway Velocity CE[3]	14 (2-43)				0.27** / 0.24	-0.051 / -0.051

[1]Abbreviations: MnS; manganese in soil, MnB; Mn in blood, MnU; Mn in urine adjusted for mean density 1.020, CE; Closed Eyes.
[2]Spearman R correlations (R_s) between characteristic variables and Mn measurements on the one hand and neurophysiological testing on the other: * denotes $p<0.05$; ** $p<0.001$.
[3] Median and range are given.
[4] too few smokers to calculate correlation

Table 2. Allelic frequencies for *ATP13A2* and *SPCA1* among adolescents and elderly in Northern Italy, as compared to European populations.[1]

ATP13A2	Adolescents %	Elderly %	European population %
rs3738815			
A	26	25	14
G	74	75	86
rs2076602			
A	74	75	86
T	26	25	14
rs4920608			
C	44	42	33
T	56	58	67
rs2871776			
A	47	49	50
G	53	51	50
rs2076600			
C	65	66	82
T	35	34	18
SPCA1			
rs218498			
A	63	63	63
G	37	37	37
rs3773814			
A	85	86	85
C	15	14	15
rs2669858			
C	16	18	16
T	84	82	84

[1]Compared to the CEU + TSI population: from http://www.ncbi.nlm.nih.gov/SNP.

Table 3. Effect modification of *ATP13A2* genotype on the association between manganese (Mn) in soil and motor function (Luria Nebraska Test).[1]

Gene/ *ATP13A2*	Coefficient[2] (95% CI)	P-value interaction	Coefficient[3] (95% CI)	P-value interaction
Elderly				
rs4920608		0.029		0.032
TT	-0.00057 (-0.0012; 0.0023)		0.00044 (-0.0014; 0.0022)	
CT	-0.0018 (-0.0029; -0.00061)		-0.0020 (-0.0032; -0.00085)	
CC	-0.0025 (-0.0042; -0.00076)		-0.0024 (-0.0040; -0.00077)	
rs2871776		0.041		0.046
AA	0.0010 (-0.0012; 0.0033)		0.00073 (-0.0013; 0.0028)	
AG	-0.0016 (-0.0027; -0.00047)		-0.0019 (-0.0030; -0.00071)	
GG	-0.0022 (-0.0037; -0.00068)		-0.0022 (-0.0037; -0.00070)	
Adolescents				
rs4920608		0.69		0.60
TT	-0.0021 (-0.0036; 0.00060)		-0.0023 (-0.0038; -0.00073)	
CT	-0.0013 (-0.0025; -0.000081)		-0.0012 (-0.0024; 0.000034)	
CC	-0.0016 (-0.0039; 0.00071)		-0.0015 (-0.0038; 0.00080)	
rs2871776		0.20		0.17
AA	-0.0030 (-0.0048; -0.0012)		-0.0031 (-0.0049; -0.0013)	
AG	-0.0011 (-0.0022; 0.00080)		-0.0011 (-0.0023; 0.00001)	
GG	-0.0011 (-0.0030; 0.00070)		-0.00088 (-0.0029; 0.0011)	

[1] The model was run for Motor function as outcome and with manganese in soil, genotype and a cross-product term for Mn in soil and genotype. The p-value for the cross term product is shown. In order to obtain effect estimates (beta-coefficients) for the effect of the genotypes, the analysis with motor function as outcome and Mn in soil as dependent variable (beta coefficient given) was run but stratified for the different genotypes.

[2] Unadjusted analysis.

[3] Analysis adjusted for age and gender.

Figure legends

Figure 1. Motor coordination as a function of manganese in soil (MnS). The simple regression was made to illustrate the slope of the different genotypes.

Figure 2. Motor coordination as a function of manganese in soil (MnS). The simple regression was made to illustrate the slope of the different genotypes.

Figures

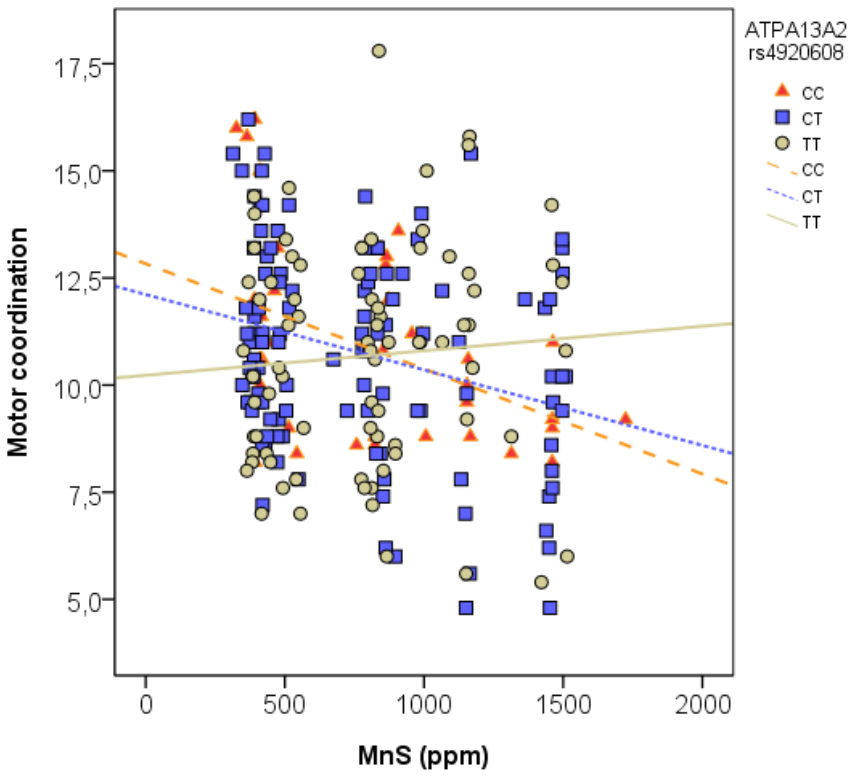

Fig. 1

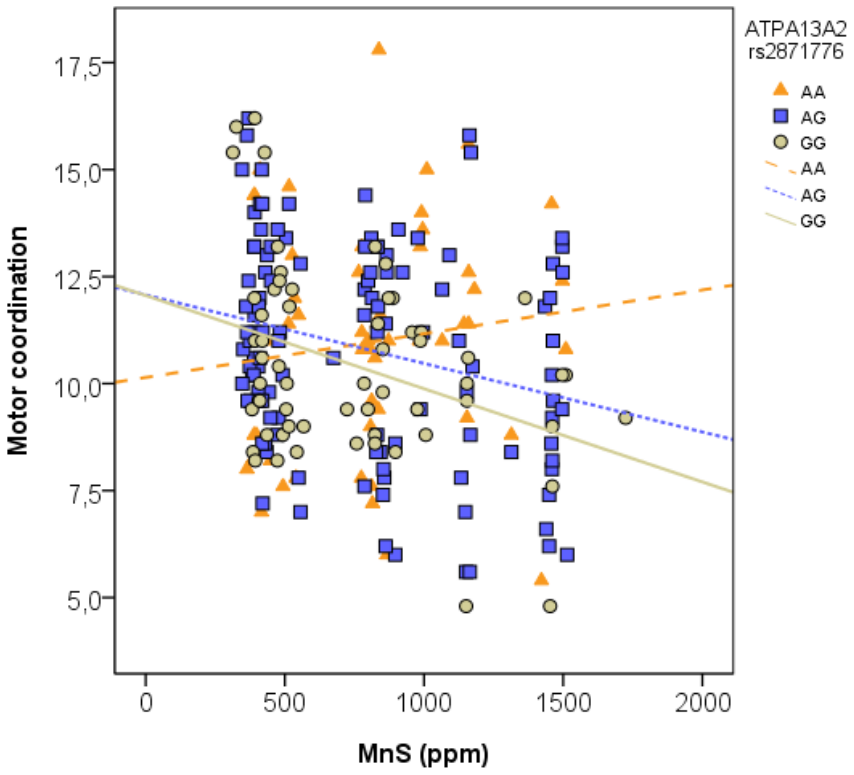
ATPA13A2
rs2871776
AA
AG
GG
AA
AG
GG
Motor coordination
17,5
15,0
12,5
10,0
7,5
5,0
0
500
1000
1500
2000
MnS (ppm)

Fig. 2

Supplemental Material Table 1. Effects on motor function by manganese in soil according to genotype.[1]

	P-value	β	P-value	β
	Adolescents		Elderly	
Gene/ ATP13A2				
MnS	<0.001	- 0.002	0.005	- 0.001
rs3738815	0.44		0.63	
GG[2]				
AG		0.40		- 0.29
AA		0.42		0.17
rs2076602	0.60		0.56	
AA				
AT		0.34		-0.34
TT		0.31		0.11
rs4920608	0.94		0.91	
TT				
CT		0.12		0.06
CC		0.15		0.20
rs2871776	0.24		0.35	
GG				
AG		0.53		0.44
AA		0.70		0.62
rs2076600	0.36		0.08	
CC				
CT		0.15		- 0.75
TT		-0.58		- 0.23
Gene/ SPCA1				
rs218498	0.40		0.38	
GG				
AG		0.29		- 0.32
AA		0.62		- 0.66
rs3773814	0.65		0.84	
AA				
AC+CC		-0.16		0.07
rs2669858	0.8		0.30	
TT				
CT+TT		-0.09		0.36

[1]The linear regression analysis was as follows: Motor coordination= α + β1MnS + β2genotype. The p-value denotes the significance for β2.

[2]The most common genotype was used as a reference category.