Neurotox Res (2017) 32:172–174
DOI 10.1007/s12640-017-9736-7

ORIGINAL ARTICLE

On the Role of Mining Exposure in Epigenetic Effects in Parkinson's Disease

Sebastian Castillo[1] · **Patricia Muñoz**[1] · **Maria Isabel Behrens**[1] · **Fernando Diaz-Grez**[1] · **Juan Segura-Aguilar**[1]

Received: 11 April 2017 / Revised: 13 April 2017 / Accepted: 18 April 2017 / Published online: 29 April 2017

Abstract To explore the possible influence of heavy metal mining on incidence of Parkinson's disease (PD), global DNA methylation was assessed in blood samples from a population of PD patients ($n = 45$) and control subjects ($n = 52$) in Antofagasta neighborhood, a Chilean city built for exclusive use of mining companies. Comparisons were made with PD subjects ($n = 52$) and control subjects ($n = 59$) from Santiago Chile, a city having little association with mining. All subjects were assessed by two neurologists and PD diagnosis was based on UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria. From blood samples obtained from each individual, a decrease in global DNA methylation was observed in PD patients either exposed (49% of control, $P < 0.001$) or not exposed (47% of control, $P < 0.001$) to mining activity. Although there was no difference in levels of DNA methylation between PD patients from the two cities, there was a lower level of DNA methylation in control subjects from Santiago versus Antofagasta.

Keywords Mining · Parkinson's disease · Epigenetics · Environmental factors · DNA methylation

Introduction

Antofagasta is a city of 390,832 inhabitants located in the north of Chile with a large mining activity. The Antofagasta region accounts for 51% of the mineral production in the country, producing copper, lithium, natural nitrates, iodine, molybdenum, gold, silver, and borates. However, mining activity is present in all of the country and Santiago accounts for 0.7% of the mineral production in Chile. The mining companies have built the entire neighborhoods for the exclusive use of their employees. Interestingly, we found several mining neighborhoods with very high incidence of Parkinson's disease (PD) (nine PD cases in 0.25 km^2, Fig. 1). A possible link between mining activity exposure and PD is probably not due to Parkinsonism induced by metals, such as manganese and copper, that has been observed in young workers (Gunnarsson and Bodin 2017; Caviedes and Segura-Aguilar 2001; Bouabid et al. 2015), since the majority of PD patients in Antofagasta have a late onset of the disease. Epigenetic alterations such as DNA methylation have been suggested to account for the individual susceptibility to PD (Wüllner et al. 2016; Matsumoto et al. 2010). Epigenetic changes in alpha synuclein intron 1 have been reported to regulate the expression of this gene, where hypomethylation increases this gene expression (Jowaed et al. 2010). It seems plausible that the environment of Antofagasta city with high mining activity can induce epigenetic changes such as DNA methylation, which can be related to the high incidence of PD cases observed in several neighborhoods. Therefore, the aim of this study was to test the possible association between DNA methylation and the high incidence of PD in Antofagasta neighborhoods constructed for the exclusive use of mining workers.

Patients and Methods

Materials and Methods

For this study, there were 97 PD patients, 45 from Antofagasta and 52 from Santiago, as a comparative city with very low

✉ Juan Segura-Aguilar
jsegura@med.uchile.cl

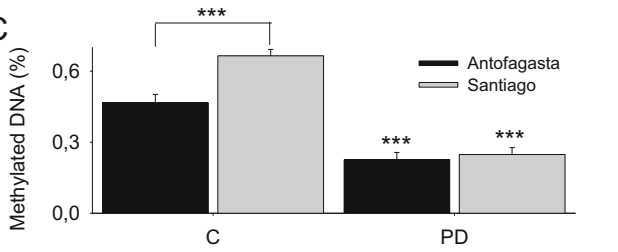
[1] Molecular and Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile

A

B

	Control Antofagasta	PD Antofagasta	Control Santiago	PD Santiago
n=	52	45	54	52
Age	63 ± 11	72 ± 13	60 ± 5	65 ± 7
Sex distribution (male/female, % male)	16/36 (30,1%)	23/22 (51%)	25/29 (46,3%)	33/23 (63,5%)
worked at the mine	8	7	0	0

C



D

Direct exposure to metals	Control Antofagasta	Control Antofagasta	PD Antofagasta	PD Antofagasta
	No	Yes	No	Yes
n=	5	5	5	5
Age	65 ± 9	66 ± 13	75 ± 10	77 ±7
Tabaco	16 %	16 %	33 %	16 %
Diabetes Mellitus	33 %	16 %	16 %	33 %
Hypertension	66 %	33 %	50 %	33 %
Alcohol consume	0%	0%	0%	16%

E

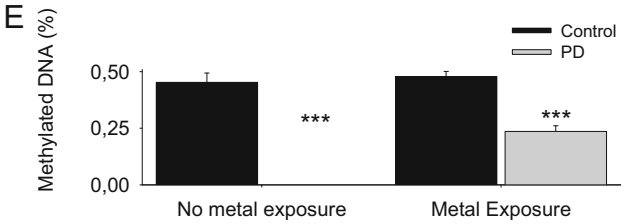


Fig. 1 Global methylation in control and PD patients from Antofagasta and Santiago. **a** Incidence of PD in a mining workers' neighborhood in Antofagasta, Chile. The *black square* represents individual PD patients' houses. **b** Data from patients and controls from Antofagasta and Santiago. **c** A significant decrease in global DNA methylation is observed in PD patients both from Antofagasta and Santiago. **c** A significant decrease in global DNA methylation was observed both in Antofagasta and Santiago PD patients. **d** The data of controls and PD patients directly exposed to metals who participate in this study is shown. **e** Global methylation in controls and patients from Antofagasta directly exposed to metals under mining work. The statistical significance was determined as described under "Materials and Methods" (***$P < 0.001$)

mining activity, and 111 healthy seniors (52 from Antofagasta and 59 from Santiago). Patients and controls were examined by two neurologists. PD diagnosis was based on the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria (Hughes et al. 1992). All patients signed a written informed consent according to the Declaration of Helsinki. The study was approved by the local ethics committee. Patients with cognitive decline or other neurodegenerative disease features were excluded by clinical neurological examination. A blood sample was obtained for the study of global DNA methylation.

DNA Preparation

Genomic DNA was obtained by using the kit Tissue DNA Kit (D3396-02, EZNA). Samples were quantified by using El NanoDrop® ND-1000 spectrophotometer. Some samples were concentrated by using DNA Clean & Concentrator −5 Kit (catalog no. D4003s, Zymo research).

Global DNA Methylation Determination

Global DNA methylation level was determined by using Methylamp Global DNA Methylation Quantification Ultra Kit (Epigenetik, catalog number P-1014B, USA). A sample of 100 ng genomic DNA was used for 5-methylcytosine quantification according to the manufacturer's protocol. Antibodies against 5-ethylcytosine were used to quantify the global DNA methylation through an ELISA-like reaction.

Bisulphite Treatment

All samples were treated with bisulfite conversion using the EZ DNA Methylation-Lightning™ Kit (Zymo Research), according to the manufacturer's instructions.

Statistical Analysis

For statistical analysis, we used the IBM SPSS program. First, through the analysis of variance (ANOVA), we analyzed differences between the mean percentages of methylation, either in their intergroup analysis as in the intragroup. Subsequently, by Tukey HSD test, we compared the average methylation of each group in the form of pairs, showing the existence or absence of differences in the contrast binomial.

Results

A significant decrease in global DNA methylation was observed in PD patients from both Antofagasta (48% $P < 0.001$) and Santiago (37% $P < 0.001$) with respect to healthy control subjects from the corresponding cities. DNA methylation in healthy controls from Antofagasta was also significantly decreased with respect to healthy controls from Santiago (70%; $P < 0.001$; Fig. 1b, c). The high incidence of PD in neighborhoods built for the exclusive use of mining workers opens the question about a possible role of mining activity on this high PD incidence. We compared the global

DNA methylation in healthy controls and PD patients in Antofagasta, comparing those directly exposed versus those not directly exposed to mining activity (Fig. 1c, d). There was no significant difference in global DNA methylation, neither in PD patients nor in healthy controls, comparing those directly exposed to mining activity to those not exposed. However, a significant decrease in global DNA methylation was observed in PD patients either exposed (49% of control; $P < 0.001$) or not exposed (47% of control; $P < 0.001$) to mining activity compared to controls in Antofagasta (Fig. 1c, d).

Discussion

A possible relationship between metals and PD has been suggested for a long time but the issue is still not completely clear. Recently, an extensive review concluded that despite a strong association between metal accumulation and PD, it is not yet clear whether metal accumulation leads to PD or if PD leads to metal accumulation (Rasheed et al. 2016).

The remarkable high incidence of PD in several Antofagasta neighborhoods (nine cases in 0.25 km^2; see Fig. 1a) that were built for the exclusive use of the employees of mining companies opens the question about a possible relationship between the environmental exposure to metals in a city with high mining activity and the development of PD. Unfortunately, we do not have epidemiological studies in Chile in order to compare the disease incidence in Antofagasta with other cities. The rates of mortality due to PD in Antofagasta were the lowest in Chile (Chaná et al. 2013) but this low rate conceivably could be explained by the absence of neurologists in this city. We have done an estimation of PD incidence in the area of Fig. 1 by taking into account the total of habitants and house in Antofagasta and number of houses in the Fig. 1 area. According to this estimation, there was an incidence of 437 PD patients/100,000 habitants in this area, accounting for a 2.5-fold higher incidence for the range of 55–64-year-olds globally (Pringsheim et al. 2014).

Our results do not support the possible role of epigenetic changes induced by the mining environmental conditions on this high incidence of PD in mining neighborhoods in Antofagasta since (i) there are no significant differences in global methylation between PD patients from Antofagasta and Santiago Chile and (ii) there are no significant differences in global DNA methylation in PD patients from Antofagasta directly exposed to metals and those not exposed. An alternative possibility is that in PD patients, DNA methylation is already at the lowest level and therefore no further changes can be seen in PD patients from Santiago compared to Antofagasta nor in PD patients directly exposed or not to mining activity in Antofagasta. On the other hand, Santiago is also a city exposed to mining activity in Chile, although at lower levels compared to Antofagasta and in line with this idea, it is interesting that controls in Antofagasta have lower levels of DNA methylation compared to controls in Santiago, therefore suggesting a role for epigenetic changes due to metal exposure.

Acknowledgements We thank the Regional Liga of Parkinson of Antofagasta, especially the president and secretary, for the help in collection of blood samples and for providing patient information. We especially thank Maria Ester Munoz H. for the collection of blood samples. This study was supported by FONDECYT no. 1100165.

References

Bouabid S, Tinakoua A, Lakhdar-Ghazal N, Benazzouz A (2015) Manganese neurotoxicity: behavioral disorders associated with dysfunctions in the basal ganglia and neurochemical transmission. J Neurochem. doi:10.1111/jnc.13442

Caviedes P, Segura-Aguilar J (2001) The price of development in Chile: overcoming environmental hazards produced by heavy industrial exploitation. Neuroreport 12:A25

Chaná P, Jiménez M, Díaz V, Juri C (2013) Parkinson disease mortality rates in Chile. Rev Med Chile 141:327–331

Gunnarsson LG, Bodin L (2017) Parkinson's disease and occupational exposures: a systematic literature review and meta-analyses. Scand J Work Environ Health. doi:10.5271/sjweh.3641

Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease. A clinico-pathological study of 100 cases. J. Neurol Neurosurg Psychiatry 55:181–184

Jowaed A, Schmitt I, Kaut O, Wüllner U (2010) Methylation regulates alpha-synuclein expression and is decreased in Parkinson's disease patients' brains. J Neurosci 30:6355–6359

Matsumoto L, Takuma H, Tamaoka A, Kurisaki H, Date H, Tsuji S, Iwata A (2010) CpG demethylation enhances alpha-synuclein expression and affects the pathogenesis of Parkinson's disease. PLoS One 5: e15522

Pringsheim T, Jette N, Frolkis A, Steeves TD (2014) The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord 29:1583–1590

Rasheed MS, Tripathi S, Mishra S, Singh MP (2016) Coherent and contradictory facts, feats and fictions associated with metal accumulation in Parkinson's disease: epicenter or outcome, yet a demigod question. Mol Neurobiol. 2016 Aug 1

Wüllner U, Kaut O, deBoni L, Piston D, Schmitt I (2016) DNA methylation in Parkinson's disease. J Neurochem 139(Suppl 1):108–120