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The influence of polymorphisms of xenobiotic-metabolizing and DNA repair genes in DNA damage, telomere length and global DNA methylation evaluated in open-cast coal mining workers

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ABSTRACT

Coal plants represent one of the main sources of environmental pollution due to the combustion process of this mineral and the consequent release of gases and particles which, in significant quantities, can lead to a potential risk to health and the environment. The susceptibility of individuals to the genotoxic effects of coal mining can be modulated by genetic variations in the xenobiotic detoxification and DNA repair processes. The aim of this study was to evaluate if xenobiotic metabolism polymorphism, base excision repair polymorphisms and non-homologous end joining repair polymorphism, could modify individual susceptibility to genomic instability and epigenetic alterations induced in workers by occupational exposure to coal. In this study, polymerase chain reaction was used to examine the polymorphic sites. The sample population comprising 70 coal mine workers and 71 workers non-exposed to coal. Our results demonstrated the effect of individual genotypes on different biomarkers evaluated. Significant decrease in % of global DNA methylation were observed in *CYP1A1 Val/-* exposed individuals compared to *CYP1A1 Ile/Ile* individuals. Coal workers who carried the XRCC4 Ile/Ile genotype showed decrease NBUD frequencies, while the *XRCC4 Thr/-* genotype was associated with decrease in Buccal micronucleus cells for the group not exposed. No influence of *GSTM1 null*, *GSTT1 null*, *GSTP1 Ile105Val*, *hOGG1 Ser*326Cys, *XRCC1 Arg194Trp* polymorphisms was observed. Thus, the current study reinforces the importance of considering the effect of metabolizing and repair variant genotypes on the individual susceptibility to incorporate DNA damage, as these processes act in a coordinated manner to determine the final response to coal exposure.

1. Introduction

Coal remains the main source of electricity worldwide, reaching 40% of the energy matrix (World Energy Council - WEC, 2013). Coal mining and combustion can generate environmental pollutants, from extraction to combustion, causing significant environmental impacts that affect soil, topography, air and water resources of surrounding areas (Bian et al., 2010). The mineral is one of the greatest natural sources of hydrocarbons and coal plants are a source of gases and particles capable of penetrating water fountains in significant amounts, providing a potential risk to human health and to the environment (Celik et al., 2007).

Occupational exposure to coal mine dust and products of combustion may lead to an increased risk by inhaling complex mixtures containing substances such as heavy metals, ash, iron, polycyclic aromatic hydrocarbons (PAHs) and sulfur (Une et al., 1995; Beckman and Ames,

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1997; Pinho et al., 2004; Matzenbacher et al., 2017). Therefore, coal workers may be more susceptible to developing several disorders, including pneumoconiosis, non-melanoma skin carcinoma, progressive massive fibrosis, asbestosis, silicosis, bronchitis, loss of lung function, emphysema and even stomach, liver or lung cancer (IARC - International Agency for Research on Cancer, 1997, IARC - International Agency for Research on Cancer, 2014; Pesch et al., 2002; Howarth et al., 2011; Jenkins et al., 2013).

Chemical mixtures represent one of the main health and safety hazards to coal industry's workers due to potential synergistic effects of the resulting combinations (White, 2002). Overall, studies suggest that responses resulting from exposure to complex mixtures are varied and complex. DNA damage induced by coal and byproducts is commonly related to disruption of metal ion homeostasis that may lead to oxidative stress (Da Silva, 2016). A number of studies have shown that parameters of oxidative damage in workers are altered following exposure to coal and particles from its combustion (León-Mejía et al., 2011; Rohr et al., 2013a; Espitia-Pérez et al., 2016).

Numerous reports have suggested that exposure to coal is associated with genotoxicity biomarkers such as chromosomal aberrations, sister chromatid exchange, micronucleus (MN) formation, and DNA damage, evaluated through Comet assay (reviewed by Da Silva, 2016; Souza et al., 2019). Currently, researchers are attempting to elucidate the relationship between coal and cancer; however, plenty variables are involved, leading to somewhat subjective results (reviewed by Jenkins et al., 2013). Among these variables we can mention epigenetic mechanisms, which can alter the level of DNA methylation consequently changing gene expression and this process can lead to the development of diseases (Wang et al., 2012). Wang et al. (2012) study shows a relationship between increased global DNA methylation and exposure to particulate matter associated with manganese and zinc emitted by coal combustion. Despite these findings, coal dust continues to be classified as non-carcinogenic to humans by the International Cancer Research agency (IARC - International Agency for Research on Cancer, 2014), which is also associated with considerable individual variation in DNA damage observed in response to exposure to this agent (Da Silva et al., 2016). This wide range of responses can be attributed to the genetic susceptibility of individuals to the genotoxic effects by genetic variations in gene coding proteins acting in metabolization and detoxification of xenobiotics and in DNA repair (Wilkinson and Clapper, 1997; Singh et al., 2007).

Studies of polymorphisms involved in the development of genotoxic and carcinogenic effects can provide useful information on the role of individual genetic propensity and the relationship with environmental and occupational exposure to xenobiotics. Variations in different polymorphic genes can be unfavorable when they are associated with an augmented activation of xenobiotics or decrease of hazardous substances detoxification, leading to increased susceptibility to mutations, cancer and diseases (Bolognesi, 2003).The aim of this study was to evaluate if polymorphisms of xenobiotic metabolism (GSTP1 Ile105Val rs1695, GSTT1, GSTM1 and CYP1A1 Ile462Val rs1048943), base excision repair (BER) (hOGG1 Ser326Cys rs1052133 and XRCC1 Arg194Trp rs1799782), and non-homologous end joining (NHEJ) repair pathway (XRCC4 Ile401Val rs28360135), could modify individual susceptibility to genomic instability and epigenetic alterations induced in workers by occupational exposure to coal.

2. Materials and methods

2.1. Study population and sample collection

This study involved 141 individuals, including 70 who were exposed to coal as part of their occupation (exposed group) and 71 who were not exposed to coal (non-exposed group/control group). The exposed workers were sampled in an open-cast coal mine in Candiota (Rio Grande do Sul, Brazil), where they were involved in extraction and transport of coal to storage centers at least for a year. Rio-Grandense Mining Company (CRM) maintains a mining staff of approximately 230 company workers in direct activity with coal. To calculate the sample size, the procedure proposed by Miot (2011) was used. Considering a 95% confidence level in a 10% margin of error, the calculated size was 69 individuals to be collected. Individual samplings were performed during the Rohr et al. (2013a; 2013b), and some new samples were included in this study.

The non-exposed group consisted of individuals from the same region (cities of Candiota and Bagé, Rio Grande do Sul, Brazil), who had not been exposed to genotoxic agents including coal, radiation and chemicals. This study included individuals at least 18 years old who had no previous chronic pathology. Smokers with a history of cancer who are taking medication did not participate in the study. Blood samples were collected by venipuncture with EDTA or heparin, maintained at or below 8 °C during transport to the laboratory and processed within 24 h of collection. All participants written informed consent was obtained from each individual before the research began and asked to answer a Portuguese-language version of a questionnaire from the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano and Natarajan, 1988), applied in a face-to-face interview. Was included questions demographic data (e.g., age and gender), medical issues (e.g., exposure to X-rays, vaccinations and medications), lifestyle (e.g., smoking, coffee and alcohol consumption and diet) and occupational habits (e.g., number of working hours per day, protective measures adopted). All workers reported the use of some type of protection while working (gloves, breathing masks, glasses, waterproof boots, among others). Subjects from Candiota and Bagé were sampled between March 2009 and March 2012. This study was approved by the Committee on Research Ethics at Universidade Federal do Rio Grande do Sul (N.2007978).

2.2. Comet assay

The alkaline Comet assay was performed as described by Singh et al. (1988) with the modification suggested by Tice et al. (2000). Blood samples were embedded in low melting point agarose, placed in lysis buffer and after the DNA was submitted to electrophoresis, finally the DNA was stained with ethidium bromide. The efficiency of each electrophoresis and the slides reading was assessed as described by Rohr et al. (2013b). The images of 100 cells from each individual were analyzed using a fluorescence microscope. Damage index was evaluated as described in detail by Collins (2004) following parameters: each cell was assigned to one of five classes (from no damage = 0 to maximum damage = 4) according to tail size and shape). The international guidelines and recommendations for the Comet assay consider the visual scoring of comets to be a well-validated evaluation method (Collins, 2004). More details can be found in Rohr et al. (2013b).

2.3. Cytokinesis block micronucleus (CBMN) assay

The CBMN assay was performed as described by Fenech (2007). Briefly, for each blood sample, lymphocyte cultures were established and incubated until adding cytochalasin B. After the incubation the lymphocytes were harvested via centrifugation, fixed in methanol/acetic acid, placed on a microscope slide and stained with Giemsa. For each blood sample, 2000 binucleated cells were scored for the presence of MN, nucleoplasmic bridges (NPB) and nuclear buds (NBUD) using optical microscopy. More details can be found in Rohr et al. (2013b).

2.4. Buccal micronucleus cytome (BMCyt) assay

Buccal cells samples collected in cold saline were centrifuged and the pellet of buccal cells was washed twice with saline and once with Carnoy's fixative. The cell suspension was dropped onto slides and allowed to air dry. Slides were stained with Giemsa solution. For each

individual, the frequency of MN was evaluated in 2000 cells, in blind analysis as described by Thomas et al., (2008). More details can be found in Rohr et al. (2013a).

2.5. DNA isolation

Genomic DNA was isolated from total blood through a salting-out method (Lahiri and Nurnberger, 1991) and then used for telomere length measurement, global DNA methylation analysis and genotyping.

2.6. Quantitative polymerase chain reaction (qPCR) for telomere length (TL) measurement

Quantitative polymerase chain reaction was performed for TL measurement following the protocol described by Cawthon (2002), with slight modifications by Kahl et al. (2018). A standard curve was established by the serial dilution of known quantities of a DNA pool sample. The single copy gene 36B4 was used for amplification control. All the samples were analyzed in triplicate, with negative and positive controls, and standard curves in each plate run. The reactions were performed using telomere and 36B4 specific primers in a 96-well plate containing a reference sample. Master mix solution and cycle conditions were done according to Kahl et al. (2018). Values generated by qPCR software were used to calculate total telomere in base pairs (bp) per human diploid genome. More details can be found in Souza et al. (2018).

2.7. Global DNA methylation analysis

Global DNA methylation levels were measured by relative quantitation of 5-methyl-deoxycytidine (5-mdC) in isolated DNA using high performance liquid chromatography (HPLC) as described by Berdasco et al. (2009). DNA was hydrolyzed with P1 nuclease and alkaline phosphatase to yield 2′-deoxomononucleosides, which were separated by HPLC and detected by ultraviolet light (UV). A mixture of deoxyadenosine, deoxythymidine, deoxyguanosine, deoxycytidine, 5-methyl-2′-deoxycytidine and deoxyuridine were used as standard. Methylation percentages of global genomic DNA were calculated by integrating the 5-mdC peak area with global cytidine methylated or not. More details can be found in Souza et al. (2018).

2.8. Genotyping of xenobiotic-metabolizing GSTP1 Ile105Val, GSTT1, GSTM1, CYP1A1 Ile462Val and DNA repair hOGG1 Ser326Cys, XRCC1 Arg194Trp and XRCC4 Ile401Thr

Polymorphic sites were examined by Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP). Assays were performed on each DNA sample using the primer sequences and restriction enzymes as described in the literature. Digested product was analyzed by electrophoresis on a polyacrylamide gel and visualized after staining with silver nitrate 0.04%.

The Detection of *GSTM1, GSTT1*, and *GSTP1 Ile105Val* gene variants (location: 22q11.23, 1p13.3 and 11q13-rs1695, respectively) was developed using multiplex PCR. The primers used and conditions for PCR fragment of 480 bp, 215bp and 176 bp, respectively, were indicated by Pemble et al. (1994); Bell et al. (1993) and Harries et al. (1997). PCR reactions were carried out in 50 µ1 consisting of 100 ng of genomic DNA, 1 µmol of each primer, 1X buffer, 5 mM $MgC1_2$, 1 mM dNTPs and 1 U of Taq polymerase. PCR conditions were 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 69 °C for 30 s, 72 °C for 30 s and another 5 min final extension at 72 °C. The alleles of GSTP1were detected following digestion with the enzyme *BsmaI,* following manufacturer's instructions.

The *CYP1A1 Ile462Val* polymorphism (Cytochrome P450 family 1 subfamily A member 1; location: 15q24.1- rs1048943) was genotyped using the primers and conditions for PCR fragment of 204 bp indicated by Cascorbi et al. (1996). The amplification reaction was performed in a total volume of 25 µL consisting of 100 ng of genomic DNA, 1 µmol of each primer, 1X buffer, 2.5 mM $MgCl_2$, 0.1 mM of dNTP, 0.5 U *Taq* polymerase. Amplification conditions: 94 °C for 5 min initial denaturation, 35 cycles of 30 s at 94 °C, 30 s at 63 °C, 30 s at 72 °C and another 5 min final extension at 72 °C. The alleles were detected following digestion with the enzyme *BsrDI,* following manufacturer's instructions.

The *hOGG1 Ser3*26Cys polymorphism (human 8-oxoguanine DNA glycosylase; location: 3p26.2- rs1052133) was genotyped using the primers and conditions indicated by De Ruyck et al. (2005) with slight modifications to fit laboratory conditions. The reaction for amplification of the 672 bp fragment of the gene was performed in a total volume of 25 µL consisting of 100 ng of genomic DNA, 2 µmol of each primer, 1X Buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTP, 1 U *Taq* DNA polymerase. Amplification conditions: 5 min initial denaturation at 94 °C, 35 cycles of 40 s at 94 °C, 40 s at 58 °C, 40 s at 72 °C and another 5 min final extension at 72 °C. After amplification, the fragments were digested with restriction enzyme Fnu4HI, following manufacturer's instructions.

The *XRCC1 Arg194Trp* polymorphism (X-ray repair cross-complementing protein 1; location: 19q13.2- rs1799782) was genotyped according to De Ruyck et al. (2005) for amplification of the 504 bp fragment. For amplification of the fragment of 504 bp of the *XRCC1* gene, a reaction in a total volume of 25 µL was performed including of 100 ng of genomic DNA, 2 µmol of each primer, 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTP, 1 U *Taq* polymerase. Amplification conditions were: 4 min initial denaturation at 95 °C, 12 cycles of touchdown of 20 s at 95 °C, 15 s at 67 °C and 1 min at 72 °C, followed by 24 cycles of 95 °C for 40 s at 58 °C, 40 s at 72 °C and 10 min final extension at 72 °C. The *XRCC1 194Arg* and *XRCC1 194Trp* alleles were detected after digestion with *Alwl* enzyme, following manufacturer's instructions. The *XRCC4 Ile4*01Thr polymorphism (X-ray repair cross-complementing protein 4; location: 5q14.2- rs28360135) was genotyped according to Relton et al. (2004). The amplification reaction of the 277 bp fragment was performed in a total volume of 25 µL consisting of 100 ng of genomic DNA, 2 µmol of each primer, 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U *Taq* polymerase. Amplification conditions were: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 30 s at 52 °C, 30 s at 72 °C, and final extension at 72 °C for 5 min. For genotyping, the fragment was digested with the restriction enzyme *BsrDI,* following manufacturer's instructions.

2.9. Statistical analysis

The agreement of genotype frequencies with Hardy–Weinberg expectations for *GSTM1*, *GSTT1*, *GSTP1 Ile105Val*, *CYP1A1 Ile462Val*, *hOGG1 Ser3*26Cys, *XRCC1 Arg194Trp,* and *XRCC4 Ile4*01Thr was performed using a $\chi 2$ test with 1 degree of freedom. Effects of genotypes in the exposed and non-exposed groups were investigated using unpaired Student's t-test. The critical level for rejection of the null hypothesis was considered a P-value of 0.05. Statistical analysis was performed with the Graphpad PRISM software, version 5.01 (Graphpad Inc., San Diego, CA).

3. Results

Among individuals from the non-exposed group, 34 were male and 37 were female, age ranging from 18 to 76 years, with an average of 41 years (± 15.2 SD). Of the exposed group, all 70 individuals were male, ranging in age from 26 to 60 years, with an average of 43 years (± 8.4 SD) and 14 years of exposure to coal and byproducts (± 8.1 SD). There was no significant difference between both groups as regard mean age (P = 0.0657, Student's t-test).

The genotoxic and epigenetic effects of occupational coal exposure in open-cast mine workers were analyzed in peripheral blood lymphocytes and buccal cells and compared with results from non-exposed group, and significant differences were found for all parameters

Table 1
Comet assay (DI = damage index), lymphocyte micronucleus test (MN = micronucleus; NBUD = nuclear buds; NPB = nucleoplasmic bridges), buccal micronucleus test (MN), telomere length, and % global DNA methylation results in non-exposed and exposed individuals divided by gender and for entire group [mean ± S.D.; number of individuals (n)].

Groups	Comet assay (n)	Lymphocytes micronucleus test (n)			MN in buccal cells (n)	Telomere length / bp (n)	% Global DNA methylation (n)
	DI	MN	NBUD	NPB			
Non-exposed							
Male	13.4 ± 1.3 (22)	3.3 ± 0.5 (26)	6.5 ± 0.8 (26)	5.8 ± 0.8 (26)	0.2 ± 0.1 (29)	9189 ± 726.4 (31)	2.8 ± 0.1 (29)
Female	17.0 ± 2.0 (24)	2.9 ± 0.5 (36)	6.9 ± 0.9 (36)	5.3 ± 0.7 (36)	0.2 ± 0.1 (34)	7999 ± 575.0 (29)	2.8 ± 0.1 (27)
Entire Group	15.3 ± 1.2 (46)	3.1 ± 0.4 (62)	6.8 ± 0.6 (62)	5.5 ± 0.5 (62)	0.2 ± 0.1 (63)	8614 ± 469.5 (60)	2.8 ± 0.1 (56)
Exposed							
Entire Group[a]	33.7 ± 3.5*** (68)	7.5 ± 0.7*** (39)	3.3 ± 0.7*** (39)	12.3 ± 1.2*** (39)	1.9 ± 0.3*** (69)	7437 ± 332.7* (66)	3.1 ± 0.1**(59)

* Significant difference in relation to non-exposed group at $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$ (Student's t-test).
[a] Entire Group is compound only by men. Data from Rohr et al. (2013a, b) and Souza et al. (2018).

analyzed (Table 1). study. No difference was observed between males and females from the non-exposed group. Workers occupationally exposed presented higher levels of Damage Index (DI), lymphocyte MN, and NPB, buccal MN and % of global DNA methylation. In addition, they also showed significantly shorter TL (Rohr et al., 2013a, 2013b; Souza et al., 2018). Therefore, these data were compared with data on genotyping from the current study.

Table 2 shows the results of the genotype and allele frequencies observed in the sample studied. No deviation from Hardy-Weinberg expectations was detect for *GSTM1*, *GSTT1*, *GSTP1*, *CYP1A1, hOGG1*, *XRCC1* and *XRCC4* genotypes, either relative to different polymorphisms or within sample group. Variant alleles frequencies agreed with the literature values reported previously in Brazilian subpopulations (Gaspar et al., 2004; Heuser et al., 2007; Rohr et al., 2011; Da Silva et al., 2012).

Table 2
Genotypes distribution and variant alleles frequencies in studied subjects.

Genotypes	Groups		p[a]	Total Frequency observed	p[a]	Allele/ Genotype frequency
	Non-exposed	Exposed				
	n (%)	n (%)		n (%)		
CYP1A1*462			0.33		0.91	*Ile:* 0.84
Ile/Ile	45(62.5)	56 (81.2)		101 (71.7)		
Ile/Val	24(33.3)	12 (17.4)		36 (25.5)		*Val:* 0.15
Val/Val	3(4.2)	1(1.4)		4 (2.8)		
OGG1 *326			0.64		0.48	*Ser:* 0.74
Ser/Ser	34 (54.0)	40(61.5)		74 (57.8)		
Ser/Cys	22 (34.9)	20(30.8)		42 (32.8)		*Cys:* 0.26
Cys/Cys	7 (11.1)	5(7.7)		12 (9.4)		
XRCC1*194			0.41		0.59	*Arg:* 0.95
Arg/Arg	61(87.1)	64 (91.4)		125 (89.3)		
Arg/Trp	9(12.9)	6 (8.6)		15 (10.7)		*Trp:* 0.05
Trp/Trp	0	0				
XRCC4*401			0.78		0.66	*Ile:* 0.96
Ile/Ile	64 (94.1)	63 (91.3)		127 (92.7)		
Ile/Thr	3 (4.4)	5 (7.2)		8 (5.8)		*Thr:* 0.04
Thr/Thr	1 (1.5)	1 (1.5)		2 (1.5)		
GSTP1			0.92		0.33	*Ile:* 0.68
Ile/Ile	31(44)	29(42)		60 (43)		
Ile/Val	36(50)	35(51)		71 (50)		*Thr:* 0.31
Val/Val	4(6)	5(7)		9 (6)		
GSTT1			0.39			
Presence	56(55)	51(63)		127 (92.7)		
Absence	18(33)	14(28)		8 (5.8)		
GSTM1			0.80			
Presence	35(55)	35(63)		127 (92.7)		
Absence	37	34(28)		8 (5.8)		

[a] Statistical significance of genotype frequency differences between non-exposed and exposed groups and differences between expected and observed values determined by χ^2 test.

Table 3 shows the effect of individual genotypes on different biomarkers (DI, MN, NBUD and NPB in lymphocytes, buccal MN, telomere length, and % of global DNA methylation) evaluated in the non-exposed and exposed groups. Significant decrease in % of global DNA methylation were observed in *CYP1A1 Ile/Val* or *Val/Val* exposed individuals compared to *CYP1A1 Ile/Ile* individuals ($P = 0.0369$). Coal workers who carried the *XRCC4 Ile/Ile* genotype showed decrease NBUD frequencies ($P = 0.0085$), while the *XRCC4 Thr/-* genotype was associated with decrease in Buccal MN cells for the group not exposed ($P = 0.0029$). No influence of *GSTM1*, *GSTT1*, *GSTP1*, *XRCC1* and *hOGG1* polymorphisms was observed for the studied biomarkers.

4. Discussion

Biotransformation of environmental xenobiotics may play an important role in their possible carcinogenic activity. Large inter-individual variation to exposures has been reported and genetic polymorphisms in xenobiotic metabolizing and DNA repair enzymes can in part explain, to some extent, the differences found (Weiss et al., 2005). Numerous studies have assessed coal miners and their results demonstrate variation on the health when compared to non-exposed individuals. Exposure to coal dust associated with coal burn products induced significant increase in DNA damage by comet assay and MN frequency (Pavanello and Clonfero, 2000; Leon-Mejia et al., 2011; Rohr et al. 2013a, 2013b; Da Silva, 2016; Espitia-Pérez et al., 2016). In addition, our recent study demonstrated telomere shortening and hypermethylation in individuals exposed to coal compared with non-exposed ones (Souza et al., 2018). The composition of coal and ash from Candiota contributed to DNA damage observed in these studies, which includes PAH (naphthalene, acenaphthalene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, benzo(b)fluoranthene, dibenzo(a,h)anthracene, benzo(k)fluoranthene, indene(1,2, 3−cd) pyrene, and benzo(g, h, i)perylene), inorganic elements (e.g. Al, S, Si, K, and Ti) and minerals such as quartz (Matzenbacher et al., 2017).

The influence of *CYP1A1 Ile462Val* polymorphism was observed in individuals with the variant allele *CYP1A1 Ile/Ile-*, who showed significantly higher % of DNA methylation, suggesting that this polymorphism may influence the effect of coal exposure in occupational settings. Analyzing the literature of the DNA global methylation and relation with polymorphisms, we employed the CRAB3 tool (http://crab3.lionproject.net). Briefly, only 29 articles were found, and only MTHFR (methylenetetrahydrofolate reductase), MTRR (methionine synthase reductase), DNMT1 (DNA methyltransferase 1), DNMT3L (DNA methyltransferase 3 L), PON1 (Serum paraoxonase/arylesterase 1), IL1B (interleukin 1, beta), RFC1 (Replication Factor C Subunit 1) and CDA (cytidine deaminase) demonstrated influence on methylation. Regarding CYP1A1, its influence on global methylation is first reported (Ren et al., 2018; Arakawa et al., 2012; Bednarska-Makaruk et al.,

Table 3
Effect of the genotypes on comet assay (DI = damage index), micronucleus test (lymphocytes and buccal cells; MN = micronucleus; NBUD = nuclear buds; NPB = nucleoplasmic bridges), telomere length and % global DNA methylation in non-exposed and exposed groups [mean ± S.D. and number of individuals (n)].

Biomarkers	Genotypes	Non-exposed (n)	Exposed (n)
Comet Assay - DI	*CYP1A1 462 Ile/Ile*	17.33 ± 1.70 (27)	35.18 ± 3.94 (55)
	CYP1A1 462 Val/-	12.29 ± 1.75 (17)	28.45 ± 8.61 (11)
	P	0.0557	0.4872
	hOGG1 326 Ser/Ser	14.75 ± 1.77 (24)	34.65 ± 4.80 (40)
	hOGG1 326 Cys/-	15.61 ± 2.05 (18)	26.96 ± 4.53 (23)
	P	0.7521	0.2905
	XRCC1 194 Arg/Arg	15.00 ± 1.44 (38)	32.89 ± 3.65 (61)
	XRCC1 194 Trp/-	16.20 ± 2.31 (5)	42.67 ± 13.95 (6)
	P	0.7702	0.4333
	XRCC4 401 Ile/Ile	14.74 ± 1.22 (38)	33.95 ± 3.72 (60)
	XRCC4 401 Thr/-	29.00 ± 15.00 (2)	36.17 ± 13.44 (6)
	P	0.5171	0.8597
	GSTP1 105 Ile/Ile	15.27 ± 1.85 (22)	28.93 ± 4.81 (28)
	GSTP1 105 Val/-	16.10 ± 1.96 (20)	37.84 ± 5.06 (38)
	P	0.7606	0.2203
	GSTT1 Non-null	16.15 ± 1.55 (33)	36.36 ± 4.32 (50)
	GSTT1 Null	14.88 ± 2.73 (8)	26.88 ± 5.73 (16)
	P	0.7114	0.2585
	GSTM1 Non-null	16.11 ± 1.94 (19)	35.00 ± 5.22 (33)
	GSTM1 Null	14.96 ± 1.81 (24)	33.12 ± 4.95 (33)
	P	0.6704	0.7949
MN in Lymphocytes	*CYP1A1 462 Ile/Ile*	3.057 ± 0.48 (35)	7.879 ± 0.83 (33)
	CYP1A1 462 Val/-	3.320 ± 0.62 (25)	5.167 ± 1.40 (6)
	P	0.7350	0.1914
	hOGG1 326 Ser/Ser	3.742 ± 0.55 (31)	7.435 ± 0.98 (23)
	hOGG1 326 Cys/-	2.609 ± 0.62 (23)	7.364 ± 1.43 (11)
	P	0.1764	0.9674
	XRCC1 194 Arg/Arg	3.30 ± 0.41 (50)	7.59 ± 0.80 (34)
	XRCC1 194 Trp/-	2.22 ± 1.06 (9)	6.60 ± 2.09 (5)
	P	0.3163	0.6625
	XRCC4 401 Ile/Ile	3.11 ± 0.40 (54)	7.72 ± 0.78 (36)
	XRCC4 401 Thr/-	1.50 ± 1.50 (2)	4.33 ± 2.03 (3)
	P	0.4466	0.2288
	GSTP1 105 Ile/Ile	3.333 ± 0.45 (45)	7.781 ± 0.81 (32)
	GSTP1 105 Val/-	3.083 ± 0.80 (12)	6.000 ± 1.90 (7)
	P	0.7973	0.3644
	GSTT1 Non-null	2.731 ± 0.46 (26)	7.150 ± 1.02 (20)
	GSTT1 Null	3.576 ± 0.58 (33)	7.789 ± 1.11 (19)
	P	0.2769	0.6728
	GSTM1 Non-null	3.308 ± 0.60 (26)	8.000 ± 1.21 (13)
	GSTM1 Null	3.219 ± 0.50 (32)	7.192 ± 0.95 (26)
	P	0.9098	0.6148
NBUD in Lymphocytes	*CYP1A1 462 Ile/Ile*	4.293 ± 0.68 (41)	6.769 ± 1.17 (26)
	CYP1A1 462 Val/-	5.500 ± 0.99 (24)	8.200 ± 2.94 (5)
	P	0.3077	0.6312
	hOGG1 326 Ser/Ser	4.033 ± 0.77 (30)	7.316 ± 1.38 (19)
	hOGG1 326 Cys/-	4.643 ± 0.89 (28)	6.778 ± 1.86 (9)
	P	0.6062	0.8228
	XRCC1 194 Arg/Arg	6.68 ± 0.73 (50)	3.62 ± 0.80 (34)
	XRCC1 194 Trp/-	6.11 ± 1.46 (9)	1.00 ± 0.77 (5)
	P	0.7567	0.2269
	XRCC4 401 Ile/Ile	6.778 ± 0.77 (45)	2.882 ± 0.70 (34)
	XRCC4 401 Thr/-	3.750 ± 1.44 (4)	10.00 ± 3.46 (3)
	P	0.1371	**0.0085**
	GSTP1 105 Ile/Ile	6.756 ± 0.74 (45)	2.844 ± 0.67 (32)
	GSTP1 105 Val/-	6.333 ± 1.66 (12)	5.286 ± 2.59 (7)
	P	0.8007	0.1950
	GSTT1 Non-null	6.769 ± 1.08 (26)	4.000 ± 1.17 (20)
	GSTT1 Null	6.455 ± 0.81 (33)	2.526 ± 0.80 (19)
	P	0.8130	0.3106
	GSTM1 Non-null	5.346 ± 0.87 (26)	4.154 ± 1.33 (13)
	GSTM1 Null	7.656 ± 0.94 (32)	2.846 ± 0.85 (26)
	P	0.0829	0.3971

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Table 3 (*continued*)

Biomarkers	Genotypes	Non-exposed (n)	Exposed (n)
NPB in Lymphocytes	*CYP1A1*462 Ile/Ile*	8.073 ± 1.07 (41)	6.308 ± 0.94 (26)
	CYP1A1 462 Val/-	8.958 ± 1.22 (24)	8.200 ± 2.15 (5)
	P	0.6015	0.4282
	hOGG1 326 Ser/Ser	10.10 ± 1.21 (30)	5.579 ± 0.84 (19)
	hOGG1 326 Cys/-	7.071 ± 1.24 (28)	7.111 ± 1.52 (9)
	P	0.0857	0.3500
	XRCC1 194 Arg/Arg	5.60 ± 0.59 (50)	12.68 ± 1.28 (34)
	XRCC1 194 Trp/-	3.78 ± 0.99 (9)	10.00 ± 3.46 (5)
	P	0.2178	0.4622
	XRCC4 401 Ile/Ile	5.60 ± 0.63 (45)	12.53 ± 1.22 (34)
	XRCC4 401 Thr/-	7.75 ± 5.51 (4)	6.367 ± 3.15 (3)
	P	0.7243	0.2094
	GSTP1 105 Ile/Ile	5.692 ± 0.82 (26)	13.30 ± 1.33 (20)
	GSTP1 105 Val/-	4.970 ± 0.69 (33)	11.32 ± 2.03 (19)
	P	0.5012	0.4148
	GSTT1 Non-null	4.269 ± 0.75 (26)	12.77 ± 2.10 (13)
	GSTT1 Null	5.906 ± 0.71 (32)	12.12 ± 1.48 (26)
	P	0.1209	0.8007
	GSTM1 Non-null	5.289 ± 0.59 (45)	12.03 ± 1.39 (32)
	GSTM1 Null	5.083 ± 1.21 (12)	13.71 ± 2.07 (7)
	P	0.8755	0.5963
MN in Buccal cells	*CYP1A1 462 Ile/Ile*	0.1316 ± 0.05 (38)	2.127 ± 0.31 (55)
	CYP1A1 462 Val/-	0.3043 ± 0.01(23)	0.750 ± 0.463 (12)
	P	0.1032	0.0536
	hOGG1 326 Ser/Ser	0.2000 ± 0.07 (30)	1.718 ± 0.32 (39)
	hOGG1 326 Cys/-	0.2174 ± 0.09 (23)	2.208 ± 0.48 (24)
	P	0.8799	0.3793
	XRCC1 194 Arg/Arg	0.22 ± 0.06 (51)	1.82 ± 0.26 (62)
	XRCC1 194 Trp/-	0.11 ± 0.11 (9)	2.50 ± 1.45 (6)
	P	0.4781	0.6661
	XRCC4 401 Ile/Ile	0.20 ± 0.05(54)	1.97 ± 0.29 (62)
	XRCC4 401 Thr/-	0.02 ± 0 (5)	1.00 ± 0.45 (6)
	P	**0.0029**	0.3136
	GSTP1 105 Ile/Ile	0.2414 ± 0.08 (29)	1.941 ± 0.41 (34)
	GSTP1 105 Val/-	0.1613 ± 0.07 (31)	1.818 ± 0.37 (33)
	P	0.4469	0.8247
	GSTT1 Non-null	0.1667 ± 0.08 (24)	1.679 ± 0.41 (28)
	GSTT1 Null	0.2286 ± 0.07 (35)	2.026 ± 0.37 (39)
	P	0.5696	0.5369
	GSTM1 Non-null		1.843 ± 0.32 (51)
	GSTM1 Null		2.000 ± 0.52 (16)
	P		0.8096
Telomere length	*CYP1A1 462 Ile/Ile*	8355 ± 567.1 (37)	7499 ± 383.7 (52)
	CYP1A1 462 Val/-	9032 ± 826.5 (23)	7469 ± 707.8 (12)
	P	0.4883	0.9724
	hOGG1 326 Ser/Ser	8493 ± 602.5 (30)	7684 ± 443.5 (38)
	hOGG1 326 Cys/-	9301 ± 812.2 (25)	7395 ± 546.6 (22)
	P	0.4190	0.6878
	XRCC1 194 Arg/Arg	8579 ± 506.0 (52)	7537 ± 347.0 (59)
	XRCC1 194 Trp/-	9581 ± 1296 (7)	7147 ± 1185 (6)
	P	0.4952	0.7363
	XRCC4 401 Ile/Ile	8707 ± 498.0 (55)	7573 ± 340.0 (58)
	XRCC4 401 Thr/-	6282 ± 1342 (3)	7463 ± 1394 (6)
	P	0.2685	0.9236
	GSTP1 105 Ile/Ile	8659 ± 707.3 (27)	7177 ± 524.1 (33)
	GSTP1 105 Val/-	8577 ± 637.5 (33)	7831 ± 414.5 (31)
	P	0.9317	0.3358
	GSTT1 Non-null	8145 ± 740.0 (26)	7404 ± 522.9 (24)
	GSTT1 Null	9014 ± 625.0 (33)	7547 ± 442.4 (40)
	P	0.3707	0.8389
	GSTM1 Non-null	8402 ± 512.4 (49)	7465 ± 401.7 (47)
	GSTM1 Null	9665 ± 1424 (9)	7573 ± 629.1 (17)
	P	0.3493	0.8882

(*continued on next page*)

Table 3 (*continued*)

Biomarkers	Genotypes	Non-exposed (n)	Exposed (n)
% Global DNA Methylation	*CYP1A1 462 Ile/Ile*	2.878 ± 0.09 (34)	3.115 ± 0.07 (47)
	CYP1A1 462 Val/-	2.697 ± 0.09 (22)	2.770 ± 0.12 (11)
	P	0.1703	**0.0369**
	hOGG1 326 Ser/Ser	2.725 ± 0.10 (28)	2.998 ± 0.06 (37)
	hOGG1 326 Cys/-	2.883 ± 0.08 (27)	3.129 ± 0.16 (19)
	P	0.2281	0.4557
	XRCC1 194 Arg/Arg	2.82 ± 0.07 (50)	3.04 ± 0.07 (55)
	XRCC1 194 Trp/-	2.71 ± 0.18 (6)	3.09 ± 0.13 (4)
	P	0.6146	0.8528
	XRCC4 401 Ile/Ile	2.78 ± 0.07 (52)	3.05 ± 0.07 (52)
	XRCC4 401 Thr/-	2.42 ± 0.02 (2)	2.97 ± 0.13 (6)
	P	0.2906	0.6982
	GSTP1 105 Ile/Ile	2.780 ± 0.09 (25)	3.066 ± 0.12 (28)
	GSTP1 105 Val/-	2.829 ± 0.09 (31)	3.034 ± 0.06 (30)
	P	0.7111	0.8126
	GSTT1 Non-null	2.872 ± 0.10 (23)	3.132 ± 0.14 (22)
	GSTT1 Null	2.768 ± 0.08 (32)	2.999 ± 0.06 (36)
	P	0.4328	0.3255
	GSTM1 Non-null	2.778 ± 0.07 (46)	3.067 ± 0.08 (43)
	GSTM1 Null	3.043 ± 0.19 (8)	2.999 ± 0.08 (15)
	P	0.1560	0.6536

Bold represent significant difference (Student's t-test).

2016; Moruzzi et al., 2017; Falk et al., 2013).

Cytochrome P-450 (CYP450) is an important class of genes that encode metabolizing enzymes involved in the metabolism of carcinogens such as PAHs (Wormhoudt et al., 1999). The dihydrodiols derived from PAHs, present in coal from Candiota (Matzenbacher et al., 2017), may be further transformed by specific CYP450 enzymes into still more reactive species, such as dihydrodiol epoxides, the ultimate mutagenic and carcinogenic metabolites of the PAH. The members of subfamily 1 of the CYP450 play a major role in the catalysis of such metabolic activation (Nebert et al., 1991). Among them, CYP1A1 is a key enzyme that catalyzes oxidative reactions and activates xenobiotics like benzo (a)pyrene to carcinogenic reactive metabolites, thereby involving it in the pathogenesis of various malignancies (Gajecka et al., 2005). *CYP1A1* polymorphism transition leads to an amino-acid substitution of *Val* for *Ile* in exon 7 and is significantly associated with CYP1A1 enzyme induction (Crofts et al., 1994). Therefore, it is reasonable to hypothesize that genetic polymorphisms of *CYP1A1* may play a role in individual susceptibility to genetic damage and developing various types of cancers. The *CYP1A1* gene polymorphisms were examined extensively to evaluate their possible role in DNA damage and cancer promotion in populations exposed to PAHs (Chen et al., 2006). In agreement with our results, Kumar et al. (2011) also found a significant association of *CYP1A1* variants with genetic damage in coal-tar workers, suggesting that these polymorphisms may modulate the effects of exposure in occupational settings (Kumar et al., 2011). *CYP1A1* polymorphisms have also been shown to be associated with moderate to high risk of lung cancer in Asian (Hayashi et al., 1991), in Caucasian and Hawaiian populations (Kawajiri et al., 1990).

Exposed *XRCC4 Thr/-* presented a higher level of NBUDs. Although the MN test was initially developed to measure micronuclei (break and loss of whole chromosome), it is also useful for measuring NPB and NBUD. These nuclear anomalies are commonly seen in cancer and they represent a common phenotype of chromosomally unstable cells (Gisselsson et al., 2001; Caruso et al., 2008). The increase in the frequency of NBUD represents the process of elimination of amplified DNA, DNA repair complexes and possibly excess chromosomes, indicating the performance of the repair system (Fenech et al., 2011). It is likely that the repair mechanism is being activated for individuals carrying the more efficient allele for the *XRCC4* gene, *XRCC4 Ile/Ile*. The shorten telomeres may be recognized as double-strand breaks by the DNA repair machinery, mainly, the NHEJ mechanism (Kahl et al., 2018 and Da Silva, 2016). Therefore, it seems reasonable that individuals who are *XRCC4 Thr/-* present more NBUDs as this allele has reduced enzyme repair activity. The DNA double strand break repair gene *XRCC4*, an important gene of genome stability, is suggested to play a role in the development of human carcinogenesis (Chiu et al., 2008). Besides that Sinitsky et al. (2017) showed XRCC4 associated with high genotoxic risk in coal miners.

5. Conclusion

When the genotoxic and epigenetic effects of occupational coal exposure were analyzed, it was found higher levels of DI, lymphocyte MN, and NPB, buccal MN, % of global DNA methylation and shorter TL when compared with the non-exposed group. The current study demonstrates that the polymorphisms in the metabolizing CYP1A1 gene and the polymorphisms in XRCC4 gene on NHEJ repair mechanism, could modulate the susceptibility to DNA damage caused by coal exposure. Sequence variants in DNA repair genes are also thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk. Nevertheless, these results must be interpreted with caution, owing to the relatively small numbers of individuals in the exposed and non-exposed groups. In addition, the evidence of a genetic hazard related to this exposure suggests the need for health education programs and for the implementation of more effective protective equipment.

Author contributions

Msc. Melissa Souza: Conceptualization, Methodology, Formal analysis, Resources, Writing - Original Draft. Dr. Juliana da Silva: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Supervision. Dr. Paula Rohr: Methodology, Investigation, Writing - Review & Editing. Dr. Kátia Kvitko: Methodology, Writing - Review & Editing, Supervision. Dr. Daniel Simon: Methodology, Writing - Review & Editing, Supervision. Dr. Vivian Kahl: Methodology, Investigation, Writing Original Draft. Dr. Monica Capetta: Methodology, Investigation, Writing - Review & Editing. Dr. Wilner Martinez-Lopez: Writing - Review & Editing, Supervision.

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References

Arakawa, Y., Watanabe, M., Inoue, N., Sarumaru, M., Hidaka, Y., Iwatani, Y., 2012. Association of polymorphisms in DNMT1, DNMT3A, DNMT3B, MTHFR and MTRR genes with global DNA methylation levels and prognosis of autoimmune thyroid disease. Clin. Exp. Immunol. 170 (2), 194–201. https://doi.org/10.1111/j.1365-2249.2012.04646.x.

Beckman, K.B., Ames, B.N., 1997. Oxidative decay of DNA. J. Biol. Chem. 272, 19633–19636. https://doi.org/10.1074/jbc.272.32.19633.

Bednarska-Makaruk, M., Graban, A., Sobczyńska-Malefora, A., Harrington, D.J., Mitchell, M., Voong, K., Dai, L., Łojkowska, W., Bochyńska, A., Ryglewicz, D., Wiśniewska, A., Wehr, H., 2016. Homocysteine metabolism and the associations of global DNA methylation with selected gene polymorphisms and nutritional factors in patients with dementia. Exp. Gerontol. 81, 83–91. https://doi.org/10.1016/j.exger.2016.05.002.

Bell, D.A., Taylor, J.A., Paulson, D.F., Robertson, C.N., Mohler, J.L., Lucier, G.W., 1993. Genetic risk and carcinogen exposure - a common inherited defect of the carcinogen-metabolism gene glutathione-s-transferase M1 (GSTM1) that increases susceptibility to bladder-cancer. J. Natl. Cancer Inst. 85, 1159–1164. https://doi.org/10.1093/jnci/85.14.1159.

Berdasco, M., Fraga, M.F., Esteller, M., 2009. Quantification of global DNA methylation by capillary electrophoresis and mass spectrometry. Methods Mol. Biol. 507, 23–34. https://doi.org/10.1007/978-1-59745-522-0_2.

Bian, Z.F., Inyang, H.I., Daniels, J.L., Otto, F., Struthers, S., 2010. Environmental issues from coal mining and their solutions. Min. Sci. Technol. 20 (2), 215–223. https://doi.org/10.1016/S1674-5264(09)60187-3.

Bolognesi, C., 2003. Genotoxicity of pesticides: a review of human biomonitoring studies. Mut. Res-Rev. Mutat. Res. 543, 251–272. 10.1.1.604.4805&rep=rep1&type=pdf.

Carrano, A.V., Natarajan, A.T., 1998. Considerations for population monitoring using cytogenetic techniques. Mutat. Res. 204, 379–406. https://doi.org/10.1016/0165-1218(88)90036-5.

Caruso, R.A., Fedele, F., Consolo, P., Luigiano, C., Venuti, A., Cavallari, V., 2008. Abnormal nuclear structures (micronuclei, nucleoplasmic bridges, and nuclear buds) in a pleomorphic giant cell carcinoma of the stomach. Ultrastruct. Pathol. 32, 11–15. https://doi.org/10.12691/ajcp-3-6-2.

Cascorbi, I., Brockmöller, J., Roots, I., 1996. A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. Cancer Res. 56, 4965–4969.

Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res. 3, 47. https://doi.org/10.1093/nar/30.10.e47.

Celik, M., Donbak, L., Unal, F., Yuzbasioglu, D., Aksoy, H., Yilmaz, S., 2007. Cytogenetic damage in workers from a coal-fired power plant. Mutat. Res. 627, 158–163. https://doi.org/10.1016/j.mrgentox.2006.11.003.

Chen, Y., Bai, Y., Yuan, J., Chen, W., Sun, J., Wang, H., Liang, H., Guo, L., Yang, X., Tan, H., Su, Y., Wei, Q., Wu, T., 2006. Association of polymorphisms in AhR, CYP1A1, GSTM1, and GSTT1 genes with levels of DNA damage in peripheral blood lymphocytes among coke-oven workers. Cancer Epidemiol. Biomark. 15, 1703–1707. https://doi.org/10.1158/1055-9965.EPI-06-0291.

Chiu, C.F., Wang, H.C., Wang, C.H., Wang, C.L., Lin, C.C., Shen, C.Y., Chiang, S.Y., Bau, D.T., 2008. A new single nucleotide polymorphism in XRCC4 gene is associated with breast cancer susceptibility in Taiwanese patients. Anticancer Res. Jan-Feb 28 (1A), 267–270.

Collins, A.R., 2004. The comet assay for DNA damage and repair - principles, applications, and limitations. Mol. Biotechnol. 26, 249–261. https://doi.org/10.1385/MB:26:3:249.

Crofts, F., Taioli, E., Trachman, J., Cosma, G.N., Currie, D., Toniolo, P., Garte, S.J., 1994. Functional significance of different human CYPlAl genotypes. Carcinogenesis 15, 2961–2963. https://doi.org/10.1093/carcin/15.12.2961.

Da Silva, F.R., Da Silva, J., Allgayer, C.M., Simon, C.F., Dias, J.F., Santos, C., Salvador, M., Branco, C., Schneider, N.B., Kahl, V., Rohr, P., Kvitko, K., 2012. Genotoxic biomonitoring of tobacco farmers: biomarkers of exposure, of early biological effects and of susceptibility. J. Hazard Mater. 225, 81–90. https://doi.org/10.1016/j.jhazmat.2012.04.074.

Da Silva, J., 2016. DNA damage induced by occupational and environmental exposure to miscellaneous chemicals. Mut. Res-Rev. Mutat. Res. 770, 170–182. https://doi.org/10.1016/j.mrrev.2016.02.002.

De Ruyck, K., Van Eijkeren, M., Claes, K., Morthier, R., De Paepe, A., Vral, A., De Ridder, L., Thierens, H., 2005. Radiation-induced damage to normal tissues after radiotherapy in patients treated for gynecologic tumors: association with single nucleotide polymorphisms in XRCC1, XRCC3, and OGG1 genes and *in vitro* chromosomal radiosensitivity in lymphocytes. Int. J. Radiat. Oncol. Biol. Phys. 62, 1140–1149. https://doi.org/10.1016/j.ijrobp.2004.12.027.

Espitia-Pérez, L., Sosa, M.Q., Salcedo-Arteaga, S., León-Mejía, G., Hoyos-Giraldo, L.S., Brango, H., Kvitko, K., da Silva, J., Henriques, J.A.P., 2016. Polymorphisms in metabolism and repair genes affects DNA damage caused by open-cast coal mining exposure. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 808, 38–51. https://doi.org/10.1016/j.mrgentox.2016.08.003.

Falk, I.J., Fyrberg, A., Paul, E., Nahi, H., Hermanson, M., Rosenquist, R., Höglund, M., Palmqvist, L., Stockelberg, D., Wei, Y., Gréen, H., Lotfi, K., 2013. Decreased survival in normal karyotype AML with single-nucleotide polymorphisms in genes encoding the AraC metabolizing enzymes cytidine deaminase and 5'-nucleotidase. Am. J. Hematol. 88 (12), 1001–1006. https://doi.org/10.1002/ajh.23549.

Fenech, M., Kirsch-Volders, M., Natarajan, A.T., Surralles, J., Crott, J.W., Parry, J., Norppa, H., Eastmond, D.A., Tucker, J.D., Thomas, P., 2011. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 26 (1), 125–132. https://doi.org/10.1093/mutage/geq052.

Fenech, M., 2007. Cytokinesis-block micronucleus cytome assay. Nat. Protoc. 2, 1084–1104. https://doi.org/10.1038/nprot.2007.77.

Gajecka, M., Rydzanicz, M., Jaskula-Sztul, R., Kujawski, M., Szyfter, W., Szyfter, K., 2005. CYP1A1, CYP2D6, CYP2E1, NAT2, GSTM1 and GSTT1 polymorphisms or their combinations are associated with the increased risk of the laryngeal squamous cell carcinoma. Mutat. Res. 574, 112–123. https://doi.org/10.1016/j.mrfmmm.2005.01.027.

Gaspar, P., Moreira, J., Kvitko, K., Torres, M., Moreira, A., Weimer, T., 2004. CYP1A1, CYP2E1, GSTM1, GSTT1, GSTP1, and TP53 polymorphisms: do they indicate susceptibility to chronic obstructive pulmonary disease and non-small-cell lung cancer? Genet. Mol. Biol. 27, 133–138. https://doi.org/10.1590/S1415-47572004000200001.

Gisselsson, D., Bjork, J., Hoglund, M., Mertens, F., Dal Cin, P., Akerman, M., Mandahl, N., 2001. Abnormal nuclear shape in solid tumors reflects mitotic instability. Am. J. Pathol. 158, 199–206. https://doi.org/10.1016/S0002-9440(10)63958-2.

Harries, L.W., Stubbins, M.J., Forman, D., Howard, G.C.W., Wolf, C.R., 1997. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis 18, 641–644. https://doi.org/10.1093/carcin/18.4.641.

Hayashi, S., Watanabe, J., Nakachi, K., Kawajiri, K., 1991. Genetic linkage of lung cancer-associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. J. Biochem. 110, 407–411. https://doi.org/10.1093/oxfordjournals.jbchem.a123594.

Heuser, V.D., Erdtmann, B., Kvitko, K., Rohr, P., Da Silva, J., 2007. Evaluation of genetic damage in Brazilian footwear-workers: biomarkers of exposure, effect, and susceptibility. Toxicology 232, 235–247. https://doi.org/10.1016/j.tox.2007.01.011.

Howarth, R.W., Ingraffea, A., Engelder, T., 2011. Natural gas: should fracking stop? Nature 477, 271–275. https://doi.org/10.1038/477271a.

IARC - International Agency for Research on Cancer, 1997. Silica, some silicates, coal dust and para-aramid fibrils. Lyon. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 68 On-line. http://monographs.iarc.fr/ENG/Monographs/vol68/mono68.pdf.

IARC - International Agency for Research on Cancer, 2014. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Report of the advisory group to recommend priorities during 2015-2019. On line:. https://monographs.iarc.fr/wp-content/uploads/2018/08/14-002.pdf.

Jenkins, W.D., Christian, W.J., Mueller, G., Robbins, K.T., 2013. Population cancer risks associated with coal mining: a systematic review. PLoS One 8, e71312. https://doi.org/10.1371/journal.pone.0071312.

Kahl, V.F.S., Dhilon, V.S., Simon, D., da Silva, F.R., Salvador, M., Branco, C.S., Cappetta, M., Martínez-López, W., Thiesen, F.V., Dias, J.F., Souza, C.T., Fenech, M., Da Silva, J., 2018. Chronic occupational exposure endured by tobacco farmers from Brazil and association with DNA damage. Mutagenesis. https://doi.org/10.1093/mutage/gex045.

Kawajiri, K., Nakachi, K., Imai, K., Yoshii, A., Shinoda, N., Watanabe, J., 1990. Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P45 0IA1 gene. FEBS (Fed. Eur. Biochem. Soc.) Lett. 263, 131–133. https://doi.org/10.1016/0014-5793(90)80721-T.

Kumar, A.1, Yadav, A., Giri, S.K., Dev, K., Gautam, S.K., Gupta, R., Aggarwal, N., 2011. Effect of genetic polymorphism of GSTM1 and GSTT1 genotypes on cytogenetic biomarkers among coaltar workers. Environ. Toxicol. Pharmacol. 32 (2), 128–135. https://doi.org/10.1016/j.etap.2011.04.002.

Lahiri, D.K., Nurnberger Jr., J.I., 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 19, 5444. https://doi.org/10.1093/nar/19.19.5444.

Leon-Mejia, G.L., Espitia-Perez, L., Hoyos-Giraldo, S., Da Silva, J., Hartmann, A., Henriques, J.A.P., Quintana, M., 2011. Assessment of DNA damage in coal open-cast mining workers using the cytokinesis-blocked micronucleus test and the comet assay. Sci. Total Environ. 409, 686–691. https://doi.org/10.1016/j.scitotenv.2010.10.049.

Matzenbacher, C.M., Garcia, A.L.H., dos Santos, M.S., Nicolau, C.C., Premoli, S., Corrêa, D.S., de Souza, C.T., Niekraszewicz, L., Dias, J.F., Delgado, T.V., Kalkreuth, W., Grivicich, I., da Silva, J., 2017. DNA damage induced by coal dust, fly and bottom ash from coal combustion evaluated using the micronucleus test and comet assay in vitro. J. Hazard Mater. 324, 781–788. https://doi.org/10.1016/j.jhazmat.2016.11.062.

Miot, H.A., 2011. Sample size in clinical and experimental trials. J. Vasc. Bras. 10, 275–278. https://doi.org/10.1590/S1677-54492011000400001.

Moruzzi, S., Guarini, P., Udali, S., Ruzzenente, A., Guglielmi, A., Conci, S., Pattini, P., Martinelli, N., Olivieri, O., Tammen, S.A., Choi, S.W., Friso, S., 2017. One-carbon genetic variants and the role of MTHFD1 1958G > A in liver and colon cancer risk according to global DNA methylation. PLoS One 12 (10), e0185792. https://doi.org/10.1371/journal.pone.0185792.

Nebert, D.W., Nelson, D.R., Coon, M.J., Estabrook, R.W., Feyereisen, R., Fujii-Kuriyama, Y., Gonzalez, F.J., Guengerich, F.P., Gunsalus, I.C., Johnson, E.F., 1991. The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. DNA Cell Biol. 10, 1–14. https://doi.org/10.1089/dna.1991.10.1.

Pavanello, S., Clonfero, E., 2000. Biological indicators of genotoxic risk and metabolic polymorphisms. Mutat. Res-Rev. Mutat. Res. 463, 285–308. https://doi.org/10.

1016/S1383-5742(00)00051-X.
Pemble, S., Schroeder, K.R., Spencer, S.R., Meyer, D.J., Hallier, E., Bolt, H.M., Ketterer, B., Taylor, J.B., 1994. Human glutathione-s-transferase-theta (GSTT1) - cDNA cloning and the characterization of a genetic-polymorphism. Biochem. J. 300, 271–276. https://doi.org/10.1042/bj3000271.
Pesch, B., Ranft, U., Jakubis, P., Nieuwenhuijsen, M.J., Hergemoller, A., Unfried, K., Jakubis, M., Miskovic, P., Keegan, T., Grp, E.S., 2002. Environmental arsenic exposure from a coal-burning power plant as a potential risk factor for nonmelanoma skin carcinoma: results from a case-control study in the district of Prievidza. Slovakia: Am. J. Epidemiol. 155, 798–809. https://doi.org/10.1093/aje/155.9.798.
Pinho, R.A., Bonatto, F., Andrades, M., Frota, M.L.C., Ritter, C., Klamt, F., Dal-Pizzol, F., Uldrich-Kulczynski, J.M., Moreira, J.C.F., 2004. Lung oxidative response after acute coal dust exposure. Environ. Res. 96, 290–297. https://doi.org/10.1016/j.envres.2003.10.006.
Relton, C.L., Daniel, C.P., Hammal, D.M., Parker, L., Tawn, E.J., Burn, J., 2004. DNA repair gene polymorphisms, pre-natal factors and the frequency of somatic mutations in the glycophorin-A gene among healthy newborns. Mutat. Res-Fundam. Mol. Mech. Mutagen. 545, 49–57. https://doi.org/10.1016/j.mrfmmm.2003.09.007.
Ren, J.C., Wu, Y.X., Wu, Z., Zhang, G.H., Wang, H., Liu, H., Cui, J.P., Chen, Q., Liu, J., Frank, A., Cao, J., Xia, Z.L., 2018. MTHFR gene polymorphism is associated with DNA hypomethylation and genetic damage among benzene-exposed workers in southeast China. J. Occup. Environ. Med. 60 (4), e188–e192. https://doi.org/10.1097/JOM.0000000000001288.
Rohr, P., da Silva, J., da Silva, F.R., Sarmento, M., Porto, C., Debastiani, R., dos Santos, C.E.I., Dias, J.F., Kvitko, K., 2013a. Evaluation of genetic damage in open-cast coal mine workers using the buccal micronucleus cytome assay. Environ. Mol. Mutagen. 54, 65–71. https://doi.org/10.1002/em.21744.
Rohr, P., da Silva, J., Erdtmann, B., Saffi, J., Guecheva, T.N., Henriques, J.A.P., Kvitko, K., 2011. BER gene polymorphisms (OGG1 Ser326Cys and XRCC1 Arg194Trp) and modulation of DNA damage due to pesticides exposure. Environ. Mol. Mutagen. 52, 20–27. https://doi.org/10.1590/S1415-47572012000600022.
Rohr, P., Kvitko, K., da Silva, F.R., Simoes Menezes, A.P., Porto, C., Sarmento, M., Decker, N., Reyes, J.M., Allgayer, M.D.C., Furtado, T.C., Salvador, M., Branco, C., da Silva, J., 2013b. Genetic and oxidative damage of peripheral blood lymphocytes in workers with occupational exposure to coal. Mutat Res-Genetic Toxicol. Environ. Mutagen. 758, 23–28. https://doi.org/10.1016/j.mrgentox.2013.08.006.
Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175 (1), 184–191.
Singh, R., Sram, R.J., Binkova, B., Kalina, I., Popov, T.A., Georgieva, T., Garte, S., Taioli, E., Farmer, P.B., 2007. The relationship between biomarkers of oxidative DNA damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans. Mutat. Res. 620, 83–92. https://doi.org/10.1016/j.mrfmmm.2007.02.025.
Sinitsky, M.Y., Minina, V.I., Asanov, M.A., Yuzhalin, A.E., Ponasenko, A.V., Druzhinin, V.G., 2017. Association of DNA repair gene polymorphisms with genotoxic stress in underground coal miners. Mutagenesis 32 (5), 501–509. https://doi.org/10.1093/mutage/gex018.
Souza, M.R., Kahl, V.F.S., Rohr, P., Kvitko, K., Cappetta, M., Lopes, W.M., da Silva, J., 2018. Shorter telomere length and DNA hypermethylation in peripheral blood cells of coal workers. Mutat. Res. 836 (B), 36–41. https://doi.org/10.1016/j.mrgentox.2018.03.009.
Souza, M.R., Dihl, R.R., Da Silva, J., 2019. Use of micronucleus assays to measure DNA damage caused by coal dust and ash. In: Knasmüller, Siegfried, Fenech, Michael (Eds.), The Micronucleus Assay in Toxicology. The Royal Society of Chemistry. https://doi.org/10.1039/9781788013604.
Thomas, P., O'Callaghan, N.J., Fenech, M., 2008. Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease. Mech. Ageing Dev. 129, 183–190. https://doi.org/10.1016/j.mad.2007.12.004.
Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F., 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen. 35, 206–221. https://doi.org/10.1002/(SICI)1098-2280.
Une, H., Esaki, H., Osajima, K., Ikui, H., Kodama, K., Hatada, K., 1995. A prospective-study on mortality among Japanese coal-miners. Ind. Health 33, 67–76. https://doi.org/10.2486/indhealth.33.67.
Wang, T., Garcia, J.G.N., Zhang, W., 2012. Epigenetic regulation in particulate matter-mediated cardiopulmonary toxicities: a systems biology perspective. Curr. Pharmacogenomics Personalized Med. (CPPM) 10, 314–321. https://doi.org/10.3967/bes2016.012.
Weiss, J.M., Goode, E.L., Ladiges, W.C., Ulrich, C.M., 2005. Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. Mol. Carcinog. 42, 127–141. https://doi.org/10.1002/mc.20067.
White, P.A., 2002. The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. Mutat. Res. 515, 85–98. https://doi.org/10.1016/S1383-5718(02)00017-7.
Wilkinson, J.T., Clapper, M.L., 1997. Detoxication enzymes and chemoprevention. Proc. Soc. Exp. Biol. Med. 216 (2), 192–200. https://doi.org/10.3181/00379727-216-44169.
World Energy Council - WEC, 2013. World Energy Resources: 2013 Survey. WEC, London On line: https://www.worldenergy.org/assets/images/imported/2013/09/Complete_WER_2013_Survey.pdf.
Wormhoudt, L.W., Commandeur, J.N.N., Vermeulen, N.P.E., 1999. Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione S-transferase and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Crit. Rev. Toxicol. 29, 59–124. https://doi.org/10.1080/10408449991349186.