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Polymorphisms in metabolism and repair genes affects DNA damage caused by open-cast coal mining exposure

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ABSTRACT

Increasing evidence suggest that occupational exposure to open-cast coal mining residues like dust particles, heavy metals and Polycyclic Aromatic Hydrocarbons (PAHs) may cause a wide range of DNA damage and genomic instability that could be associated to initial steps in cancer development and other work-related diseases. The aim of our study was to evaluate if key polymorphisms in metabolism genes *CYP1A1*$_{Msp1}$, *GSTM1*$_{Null}$, *GSTT1*$_{Null}$ and DNA repair genes *XRCC1*$_{Arg194Trp}$ and *hOGG1*$_{Ser326Cys}$ could modify individual susceptibility to adverse coal exposure effects, considering the DNA damage (Comet assay) and micronucleus formation in lymphocytes (CBMN) and buccal mucosa cells (BMNCyt) as endpoints for genotoxicity. The study population is comprised of 200 healthy male subjects, 100 open-cast coal-mining workers from "El Cerrejón" (world's largest open-cast coal mine located in Guajira – Colombia) and 100 non-exposed referents from general population. The data revealed a significant increase of CBMN frequency in peripheral lymphocytes of occupationally exposed workers carrying the wild-type variant of *GSTT1* $_{(+)}$ gene. Exposed subjects carrying *GSTT1*$_{null}$ polymorphism showed a lower micronucleus frequency compared with their positive counterparts (FR: 0.83; P = 0.04), while BMNCyt, frequency and Comet assay parameters in lymphocytes: Damage Index (DI) and percentage of DNA in the tail (Tail % DNA) were significantly higher in exposed workers with the *GSTM1*$_{Null}$ polymorphism. Other exfoliated buccal mucosa abnormalities related to cell death (Karyorrhexis and Karyolysis) were increased in *GSTT/M1*$_{Null}$ carriers. Nuclear buds were significantly higher in workers carrying the *CYP1A1*$_{Msp1}$ *(m1/m2, m2/m2)* allele. Moreover, BMNCyt frequency and Comet assay parameters were significantly lower in exposed carriers of *XRCC1*$_{Arg194Trp}$ *(Arg/Trp, Trp/Trp)* and *hOGG1*$_{Ser326Cys}$ *(Ser/Cys, Cys/Cys)*, thereby providing new data to the increasing evidence about the protective role of these polymorphisms. This modulation may involve specific and differentiated pathways in different tissues that also may cause a differential sensitivity related to differential induction of some enzymes.

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1. Introduction

The coal-mining region in northern Colombia is one of the largest open pit mining regions of the world. In 2009, there were eight mining companies in operation with an approximate coal production of 70 Mt/year. In 2010, more than 33,372 workers were employed full time in coal mines in the country and more than 25,000 individuals worked in open-cast systems [1,2]. In this particular extraction method, large amounts of dust particles are released into the atmosphere as fugitive particulate matter [3]. In addition to coal, which is the main component, this mixture can also contain oxygen, nitrogen, hydrogen, trace elements, and several inorganic minerals. The trace elements may include silica, copper, aluminum, nickel, cadmium, boron, antimony, iron, lead, and zinc [3]. Table 1 shows some of the main environmental pollutants and chemical substances detected in coal, ashes and combustion processes in coal mining systems around the world.

Excess occupational exposure to metals, particularly in mining is considered to be major cause of metal-related cancer [4]. Additionally, in open-cast mines from north Colombia extracted coal is stored under the sunlight at high ambient temperatures, where spontaneous and incomplete coal combustion may lead in Polycyclic Aromatic Hydrocarbons (PAHs) emission [5], most of which exhibit well-known mutagenic and carcinogenic activity [6]. Particularly, in open-cast mining facilities these toxic substances are released in the atmosphere where they can form complex mixtures [7]. Such mixtures represent one of the most important health and safety hazards to this industry's workers due to potential synergistic effects of the resulting combinations [8]. For most chemical compounds found in complex mixtures generated in open cast mines, metabolic activation is required for the formation of electrophilic intermediates capable of binding to cellular macromolecules and DNA [9,10]. As consequence of cellular metabolism, some of these intermediates and some heavy metals found in blood samples from exposed individuals could be involved in the generation of oxidatively damaged DNA and proteins [11,12]. In this order, susceptibility to the hazardous action of this chemicals may derive from genetic or acquired characteristics of the individual, and may be associated with variations in genes encoding for carcinogen or xenobiotic-metabolizing enzymes, such as Cytochrome P-450 *(CYP)* in Phase I and Glutathione *S*-transferases *(GST)* in Phase II [13] and also in DNA repair genes majorly involve in oxidative damage repair such as X-ray repair complementing defective in Chinese hamster 1 *(XRCC1)* and 8-oxo guanine-DNA glycosylase 1 *(hOGG1).*

CYP and *GST* genes play an important role in the detoxification of a wide range of human carcinogens, including several residues as PAHs, and particulate matter from coal mining activities [14]. In general, carcinogenic PAHs, such as benzo (a) pyrene, need to be activated by the phase I enzymes (e.g., Cytochrome P450 1A1) to form ultimate carcinogens, such as B (a) P diol epoxide (BPDE), whereas the phase II enzymes (e.g., glutathione S transferases) generally mediate the conjugation of water-soluble moieties, such as glutathione, which are responsible for detoxification of these reactive metabolites [15]. Hence, the coordinated expression and regulation of phase-I and −II enzymes determines the outcome of carcinogen exposure. The capacity to repair DNA damage induced by activated carcinogens is also a host factor that may influence the risk of cytogenetic instability. Human 8-oxoguanine DNA glycosylase 1 *(hOGG1)* and X-ray repair cross complementing group 1 *(XRCC1)* are BER enzymes involved in the core processes of single-strand break repair. *hOGG1,* a glycosylase, helps in the excision of oxidized guanine, while *XRCC1* stimulates endonuclease action and acts as a scaffold protein in the subsequent restoration of the site.

Cytogenetic endpoints have been extensively employed in surveillance of human genotoxic exposure and increased chromosomal damage has been shown to be predictive of elevated cancer risk [16]. The micronucleus (MN) assay in target as well as non-target cells is used as an indicator of chromosomal damage in interphase cells and is associated with early events in carcinogenesis. Micronuclei in exfoliated cells emerge during mitosis of the basal layers of the epithelium and their absolute quantities could reflect the real situation in target cells [17]. The micronucleus cytome assay applied in buccal exfoliated cells (BMNCyt) provides a complementary method for measuring DNA damage and cytotoxic effects in an easily accessible tissue not requiring in vitro culture [18]. The comet assay is a rapid, sensitive and relatively simple method for measuring DNA damage and has been widely adopted in as a biomarker assay in human biomonitoring studies, in 'biological effect dosing' of occupational and environmental exposures [19,20].

The mining region of "El Cerrejón" includes the world's largest open-cast coal mine located in the northwest Colombian state of Guajira and concentrates most of the country's mining sector workers. In this scenario, the purpose of the present cross-sectional study is to evaluate if the $CYP1A1_{Msp1}$ *(rs.4646903),* $GSTM1_{null}$, $GSTT1_{null}$, $XRCC1_{Arg194Trp}$ *(rs.1799782)* and $hOGG1_{Ser326Cys}$ *(rs. 1052133)* polymorphisms could have an influence on the individual susceptibility to DNA damage caused by coal residues exposure, as previously demonstrated by primary DNA damage in lymphocytes and MN formation in lymphocytes [21] and oral mucosa of exposed workers [11]. Our results will contribute to identify metabolic and DNA-repair polymorphisms involved with the modulation of DNA damage in populations occupationally exposed to open-cast coal mining residues.

2. Methods

2.1. Study population and sample collection

The Committee on Research Ethics of each institution approved this study and a written informed consent was obtained from each individual before sample collection. The study population comprised a total of 200 healthy males (in total). To calculate the size of the sample was considered the minimum necessary to be detected at least 1% of the genetic polymorphism less frequent in the studied population. Within the study population 100 were exposed workers from "El Cerrejón" open-cast mine engaged in surface activities inside several areas of the mining complex showed in Fig. 1, who were exposed to coal dust for at least 5 years. Main chemical and mineralogical properties of coal and coal fly ashes from sampling areas ares described at Table 2. The mean time of service ± standard deviation (SD) of the exposed group was 17.7 ± 6.9 years (range, 5–30 years). The non-exposed reference group consisted of 100 males with no known exposure to genotoxic agents such as coal dust, radiation, chemicals, cigarette, etc., and was selected from the general local population. Exposed workers were matched to non-exposed referents by age (± 2 years) and similar social-economic status. The mean age of exposed group was 44.0 ± 7.5 years (range, 24–60 years), and non-exposed reference group was 43.7± 7.8 years (range, 26–60 years).

Confounding and exclusion factors were collected from all participants who responded to an interviewer-administered, detailed and standard questionnaire which included data on lifestyle, health status, cancer history, other chronic diseases, nutrition and smoking habits, alcohol and medication intake, occupational and time exposure, adoption of protective measurements, and previous exposure to medical X-rays or treatment with known carcinogens. Exclusion criteria for exposed and non-exposed reference groups were age over 60 years or less than 18 years, current and previous smoking habits, medical treatment for up to 3 months or X-ray up to 1 year before sampling, as well as therapeutic drugs intake,