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The response to oxidative stress and metallomics analysis in a twin study: The role of the environment

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ABSTRACT

Inefficient response to oxidative stress has been associated with ageing and health risk. Metals are known to inhibit DNA repair and may modify the antioxidant response. How genetic variability and lifestyle factors modulate the response to oxidative stress is poorly explored. Our study aims to disentangle the contribution of genetics and environmental exposures to oxidative stress response using data from twin pairs. The non-enzymatic antioxidant capacity (NEAC), the repair capacity of 8-oxo-7,8-dihydroguanine (OGG activity) and the levels of 12 metals were measured in blood of 64 monozygotic and 31 dizygotic twin pairs. The contributions of genetic and environmental effects were assessed using standard univariate twin modelling. NEAC and OGG activity significantly decreased with age. Gender-, age- and body mass index-associated differences were identified for some metals. Principal Component Analysis identified two groups of metals whose levels in blood were highly correlated: As, Hg, Pb, Se, Zn and Al, Co, Cr, Mn, Ni. The environmental influence was predominant on OGG activity and NEAC variance whereas for most metals the best-fitting model incorporated additive genetic and unique environmental sources of variance. NEAC and OGG activity were both inversely correlated with blood levels of various metals. The inhibition of OGG activity by Cd was largely explained by smoking.

Our data show a substantial role of environmental factors in NEAC and OGG activity variance that is not explained by twins' age. Exogenous environmental factors such as metals contribute to oxidative stress by decreasing NEAC and inhibiting repair of oxidatively-induced DNA damage.

1. Introduction

Reactive oxygen radicals (ROS) are produced in the body, primarily as a result of aerobic metabolism, and are involved in many vital cell activities such as signal transduction and gene transcription therefore playing beneficial effects on the organisms. Antioxidants defence systems co-evolved along with aerobic metabolism to counteract oxidative damage (reviewed in [1]). Antioxidants, such as glutathione and vitamins, together with antioxidant enzymes, such as superoxide dismutases, catalases and glutathione peroxidases, help to regulate the endogenous ROS. The maintenance of the redox balance is mostly seen as a genetic trait with the transcription factor Nrf2 as master regulator of the antioxidant response modulating the expression of hundreds of genes (reviewed in [2]). These latter include not only the antioxidant enzymes, but also genes involved in the immune and inflammatory responses, tissue remodelling, carcinogenesis, and cognitive dysfunction. However, humans are constantly exposed to ROS from environmental sources such as pollutants and cigarette smoke that may act independently and concomitantly of genetic factors. Among these environmental threats, metals are an important category that present as unifying factor, in determining their toxicity, the generation of ROS and nitrogen species (reviewed in [3,4]). Metals may impact on the redox status either by undergoing redox-cycling reactions or by depletion of major antioxidants and bonding to sulfhydryl groups of proteins. Moreover, environmental factors can induce the release of harmful "free" metals from storage proteins thus initiating ROS-producing reactions [5,6]. Alterations in metal homeostasis have been suggested

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as a key factor in the development of several disorders including neurological diseases [7]. Exposure to metals may also impact on metabolism [8], such as by substituting for essential micronutrients and for other metals, or by inducing oxidative stress [9].

When the overproduction of ROS exceeds the cell defence mechanisms, free radicals and singlet oxygen may damage DNA, RNA and proteins. The control of genomic stability is of utmost importance for the organism and cells are endowed of DNA repair mechanisms that maintain a low level of oxidatively-induced DNA damage. Among these mechanisms the base excision repair (BER) is the most relevant and within BER the 8-oxoguanine DNA glycosylase, OGG1, is the primary enzyme responsible for the excision of 8-oxo-7,8-dihydroguanine (8-oxoGua), a mutagenic base that occurs as a result of exposure to the hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$) and one-electron oxidants [10]. Several studies indicate that OGG1 activity is highly sensitive to inactivation by oxidizing agents [11,12]. Toxic metal ions may also interfere with DNA repair processes by inactivating the enzyme(s) directly, for example by reaction with histidine or cysteine residues, or by competing with and displacing essential metal ions (reviewed in [13]). Recent studies indicate that the inhibition of DNA repair by metals may also influence the choice of the DNA repair pathway thus favouring "error-prone" mechanisms [14]. By interference with cellular redox regulation, inhibition of DNA repair and/or deregulation of cell proliferation, metals may induce genetic instability and possibly carcinogenic effects (reviewed in [15]).

Twin studies have been widely used to estimate the heritability of complex traits and, more recently, to quantify the contributions that the genes, the shared environment, the individual-specific environment and their interactions make to human complex traits [16]. The comparison of the similarity between pairs of identical twins (monozygotic, MZ) and fraternal twins (dizygotic, DZ) allows to measure the contribution of genetics, as opposed to environment, to a given trait or condition of interest [17].

In this study we have addressed the question of the relative contribution of genetic and environmental factors to the body's redox homeostasis by analysing in blood samples from MZ and DZ pairs the non-enzymatic antioxidant activity (NEAC), as a biomarker of in vivo antioxidant status [18], and OGG activity, as a biomarker of oxidatively-induced DNA damage repair [19], together with a descriptor of metal burden, i.e. metallomics of blood [20]. This study has revealed the importance of environmental factors in the control of the redox balance.

2. Materials and methods (see also Supplemental material)

2.1. Study subjects

Same sex twin pairs were selected from the Italian Twin Registry [21] aged over 18 and a final number of 190 twins were characterized (Fig. S1). Sixty ml of blood were drawn from each subject in lithium heparin, EDTA or serum separator tubes, 5 ml were frozen at −20 °C for metal determination, and the remaining volume was immediately processed to obtain lymphocytes and plasma and stored as required.

2.2. Metallomics analysis

One ml of blood was microwave digested (ETHOS-Mega II oven, FKV, Bergamo, Italy) in 15-ml plastic tubes (Falcon, Becton, Franklin Lakes, NJ) with the addition of 2 ml of suprapur concentrated HNO_3 (Romil Ltd, Cambridge, UK). Aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn) were measured by sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) (ThermoFischer, Bremen, Germany) as previously reported [22–24].

2.3. NEAC assay

Plasma was obtained from venous blood collected into lithium heparin vacuum tubes, centrifuged at 1500 rcf for 15 min and stored at −80 °C prior to assay. NEAC in plasma samples was determined in 116 twins using a spectrophotometric assay based on the reduction of Cu^{++} to Cu^{+} by the activity of all non enzymatic reducing species present in the sample [25].

2.4. OGG assay

Peripheral blood mononuclear cells (PBMC) were separated by centrifugation with Ficoll Paque PLUS (GE Healthcare, Milan, Italy) from heparinised venous blood and stored in liquid nitrogen. OGG activity was measured in protein extracts from PBMC by using as substrate a 6-carboxyfluorescein (6-FAM) 3'end-labelled 30 base pairs duplex containing a single 8-oxoGua [26].

2.5. Statistical analysis

Subjects were analysed both as individuals and as twin pairs. Descriptive analysis (mean ± standard deviation for continuous variables, percentage for categorical variables) for investigated characteristics was conducted using Stata Software (version 11.2, StataCorp, College Station, TX). Robust regression was used to identify variables associated with NEAC or OGG activity taking into account the dependence of twin data within pairs. Twin analyses were conducted using standard univariate twin modelling based on linear structural equations [17].

Principal Component Analysis (PCA) was performed on blood metal data to study the relationships among measured levels of different metals and to single out, if any, common sources [27].

A mediation analysis was performed to estimate the relationship of blood Cd levels to smoke habits and OGG activity. Mediation was evaluated by multiple regression [28].

3. Results

3.1. Study population

Characteristics of the study population are presented in Table S1. The 190 Italian twins included 64 monozygotic (MZ) and 31 dizygotic (DZ) pairs. There were no significant differences between MZ and DZ twins for demographic, clinical and biochemical characteristics. MZ and DZ twins were also similar regarding the smoking habits. This population was analysed for blood metal levels, OGG activity and NEAC.

3.2. Metallomics, NEAC and OGG activity profile in the twin population

In searching for environmental factors that could impact on oxidative stress response, chronic metal burden in blood was measured by SF-ICP-MS in the twin population (Table 1). The blood metal levels are within the range of the reference values obtained in Italian human biomonitoring surveys conducted on healthy adult subjects (18–65 years) [29,30]. To test whether there are correlations between the concentrations of different elements, PCA was conducted to identify groups of interrelated variables (Principal Components, PCs). The first five PCs accounted for 65% of the metal total variation (Table 2) suggesting that the original variables were not highly correlated. The remaining seven PCs