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Influence of DNA repair polymorphisms on biomarkers of genotoxic damage in peripheral lymphocytes of healthy subjects

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Abstract

DNA repair polymorphisms may represent susceptibility factors affecting DNA integrity, and possibly cancer risk, in human population. In order to elucidate the influence of a few widely studied DNA repair polymorphisms on individual levels of DNA damage and their possible interaction with lifestyle and environmental exposures, 171 subjects from a well-characterized human population enrolled in a previous study on genetic effects of air pollution were genotyped for the *XRCC1* Arg280His and Arg399Glu, *XRCC3* Thr241Met and *ERCC2* Lys751Gln polymorphisms. The association between DNA repair genotype, alone or in combination with metabolic genotype, on the levels of SCE, micronuclei and tail moment values in peripheral lymphocytes was evaluated. A significant influence of the *ERCC2* genotype on SCE frequency was observed. Subjects with *ERCC2* 751 Gln/Gln genotype had significantly higher risk of high (above the median) SCE/cell with respect to Lys/Lys referents (OR 4.55, 95% CI 1.48–13.99). A non-significantly elevated OR was also observed in Gln/Lys heterozygotes, suggesting a gene dosage effect. When subjects were categorized by smoking habits and professional exposure, the variant *ERCC2* 751 Gln/Gln genotype was associated with elevated SCE rates in non-smokers and in exposed subjects, but not in smokers. The results of this study support the hypothesis that some DNA repair polymorphisms exert a modifying effect on individual levels of DNA damage in healthy subjects, possibly also modulating cancer risk.

Keywords: DNA repair; Genetic polymorphism; Biomarkers; Genetic damage; Tobacco smoke

1. Introduction

Prospective epidemiological studies show that, in healthy subjects, higher levels of cytogenetic damage in peripheral lymphocytes are associated with an increased risk of cancer development and mortality [1]. This association is independent of external exposures to genotoxic agents (either occupational or related to life style), pointing to the involvement of intrinsic factors affecting genomic stability as risk determinants [2].

Among genetic traits able to modify individual levels of DNA damage, genetic variation in xenobiotics metabolism and DNA repair is likely to play a central role. Single nucleotide polymorphisms (SNP) have been identified for a number of genes involved in the activation/detoxification of genotoxic compounds and in the subsequent repair of induced DNA lesions [3]. Even

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though in most cases the phenotypic expression of such variants is not known, an association has been demonstrated between certain metabolic and DNA repair alleles and cancer risk [4,5]. Moreover, an increased susceptibility to *in vitro* induced DNA damage has been associated with both kinds of polymorphisms [3].

These observations suggest that polymorphisms with low phenotypic expression may modulate the interaction of exogenous or endogenous genotoxins with cellular targets, affecting the extent of induced DNA damage in surrogate and target cells, and ultimately cancer risk. Common variant alleles determine only slight changes in the catalytic activity of the protein and hence, on individual basis, the possible increase in cancer risk is low; however, due to their polymorphic frequency, they may account for a sizeable number of cancer cases in the general population.

The greater health impact is expected for genetic polymorphisms which affect genes with key functions in DNA repair: among these *XRCC1* Arg280His and Arg399Gln, *XRCC3* Thr241Met and *ERCC2/XPD* Lys751Gln have received greater consideration in recent years. The X-ray cross complementation group 1 protein (XRCC1) is a scaffolding protein which interacts with several BER components [6]; the X-ray cross complementation group 3 protein (XRCC3), one of the RAD51 parologue proteins, is involved in the repair of double strand breaks by homologous recombination (HR) [7]; the excision repair cross complementation group 2 protein (ERCC2/XPD) is a helicase belonging to the TFIIH complex, which participates in DNA unwinding during both global genome and transcription-coupled nucleotide excision repair (NER) and transcription initiation [8].

Some studies suggest that polymorphisms in these genes can modulate spontaneous and/or *in vitro* induced DNA damage and/or cancer risk [3–5]. However, the overall weight of such evidence is low, because of the minimal incremental risk associated to variant alleles and the contradictory findings reported from other studies [9]. Indeed, due to the complexity of repair pathways, which require the cooperation of multiple gene products, it is conceivable that the influence of single, low penetrance variants be hardly detectable. In this respect the combination of multiple variant alleles may be more effective than single SNP in modulating DNA repair capacity, and theoretically more easily detectable, provided that adequately large populations for stratification and analysis are available [10,11].

In order to assess the influence of DNA repair polymorphism on individual DNA damage, in this work a well-characterized human population enrolled in a previous biomonitoring study has been genotyped for the above-mentioned four SNPs. The study population consisted in a group of traffic wardens of the municipality of Rome for which detailed information on occupational exposure to air pollutants, lifestyle and metabolic polymorphisms were available, together with the results of the analysis of SCE, micronuclei and tail moment in peripheral lymphocytes [12–14]. The influence of each DNA repair polymorphism, alone or in combination with metabolic genotype, on biomarkers of genetic damage was assessed by regression analysis after adjustment for possible confounders. The results obtained indicate an association of the *ERCC2* variant genotype with higher SCE rates, and a possible gene dosage effect modulating the genotoxicity of tobacco smoking.

2. Materials and methods

2.1. Study population

The study population consisted of 192 officers from the Municipality of Rome (141 males and 51 females, 126 non-smokers and 65 smokers, mean age 43.4 years) enrolled during the period December 1999–February 2001 in a biomonitoring study on genetic effects of the occupational exposure to urban air pollutants. Detailed data on individual exposure to benzene and other aromatic hydrocarbons were published elsewhere [15]. Information on smoking habits, alcohol consumption, diagnostic X rays, chemical exposure during occupational or recreational activities, and family history of cancer were collected by questionnaire. All subjects gave an informed written consent to their participation in the study. All analyses were carried out on anonymous, coded samples.

2.2. Samplings

All subjects contributed to the study with a single blood donation. Blood (approximately 40 ml) was taken by venipuncture and aliquoted into heparinized tubes for genotoxicity and haematochemical analyses, and into tubes devoid of anticoagulant for DNA extraction and amplification.

2.3. Genotypic analysis

Genomic DNA was isolated from whole blood samples, stored at −80 °C, using Instagene Matrix (Bio-Rad, Hercules, CA, USA) according to manufacturer's instructions. Genotypes were attributed based on restriction fragment length polymorphism, after gel electrophoresis of the amplified fragments. *XRCC1* Arg280His and Arg399Gln were analysed according to [16,17], respectively. *XRCC3* Thr241Met was characterized according to [16] and *ERCC2* Lys751Gln according to [18]. A referent DNA sample with known restriction pattern profile, processed in parallel with study samples, was used as internal control in all experiments. A variable fraction of the study