Genetic polymorphisms in XRCC1, OGG1, APE1 and XRCC3 DNA repair genes, ionizing radiation exposure and chromosomal DNA damage in interventional cardiologists

Maria Grazia Andreassi a,*, Ilenia Foffa a,b, Samantha Manfredi a, Nicoletta Botto a, Angelo Cioppa c, Eugenio Picano a,c

a CNR Institute of Clinical Physiology, National Research Council, Pisa, Italy
b The Sant’Anna School of Advanced Studies, Pisa, Italy
c Clinica Cardiologica “Montevergine”, Mercogliano, Italy

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ABSTRACT

Interventional cardiologists working in high-volume cardiac catheterization laboratory are exposed to significant occupational radiation risks. Common single-nucleotide polymorphisms (SNPs) in DNA repair genes are thought to modify the effects of low-dose radiation exposure on DNA damage, the main initiating event in the development of cancer and hereditary disease. The aim of this study was to determine the relationship between XRCC1 (Arg194Trp and Arg399Gln), OGG1 (Ser326Cys), APE1 (Asp148Glu) and XRCC3 (Thr241Met) SNPs and chromosomal DNA damage.

We enrolled 77 subjects: 40 interventional cardiologists (27 male, 41.3 ± 9.4 years and 13 female, 37.8 ± 8.4 years) and 37 clinical cardiologists (26 male, 39.4 ± 9.5 years and 11 female, 35.0 ± 9.8 years) without radiation exposure as the control group. Micronucleus (MN) assay was performed as biomarker of chromosomal DNA damage and an early predictor of cancer. MN frequency was significantly higher in the exposed group, individuals carrying a XRCC3 Met241 allele had higher frequency than homozygous XRCC3 Thr241 (21.2 ± 7.8‰ vs. 16.6 ± 6.3‰, p = 0.0003). Within the exposed group, individuals carrying a XRCC3 Met241 allele had higher frequency than heterozygous XRCC3 Thr241 (21.2 ± 7.8‰ vs. 16.6 ± 7.1‰, p = 0.03). Individuals with two or more risk alleles showed a higher MN frequency when compared to subjects with one or no risk allele (18.4 ± 6.6‰ vs. 14.4 ± 6.1‰, p = 0.02). An interactive effect was found between smoking, exposure >10 years and the presence of the two or more risk alleles on the MN frequency (F = 6.3, p = 0.02). XRCC3 241Met alleles, particularly in combination with multiple risk alleles of DNA repair genes, contribute to chromosomal DNA damage levels in interventional cardiologists.

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1. Introduction

Invasive cardiovascular procedures currently expose interventional cardiologists to significant occupational radiation risks [1]. For the most experienced (and most exposed) staff working in the cardiac catheterization laboratory, with a range of exposures in between 2 and 5 mSv per year, a typical cumulative 15-year radiological exposure around the equivalent of 50 mSv is associated with a non-negligible lifetime attributable risk of cancer in the order of magnitude of 1 cancer in 200 exposed subjects [2].

Ionizing radiation exposure, an established class 1 human carcinogen, exerts health risk through damage to cellular DNA, producing oxidized bases, bulky DNA adducts, and DNA strand breaks [3]. Several studies showed the occurrence of chromosomal DNA damage in hospital workers chronically exposed to ionizing radiation, although the majority of these studies failed to establish a dose–effect relationship for low doses [4–10]. Recently, we showed that contemporary interventional cardiologists have an increased rate of chromosomal somatic DNA damage, reflected in higher frequency of micronuclei versus clinical cardiologists [11]. Micronuclei are an intermediate endpoint of carcinogenesis and a long-term predictor of cancer [12]. However, the amount of this damage varies and is only weakly related to the duration of professional exposure, suggesting that an individual predisposition may play an important role in the cellular response to radiation exposure. The mechanisms responsible for individual differences in response to ionizing radiation are largely attributed to inherited variations.
in genes involved in repair of DNA damage [13]. The DNA repair system consists of several distinct pathways; such as the base excision repair (BER) pathway and double-strand break repair process (DSB), which are considered the most important pathways involved in repair of radiation-induced DNA damage [3,14,15]. In particular, common single-nucleotide polymorphisms (SNPs) in the 8-oxoguanine glycosylase-1 (OGG1), X-ray repair cross-complementing-1 (XRCC1) and the AP-endonuclease-1 (APE1) genes in the BER pathway, and the X-ray repair cross-complementing-3 (XRCC3) gene in the DSB process have been most extensively studied for their influences in the individual sensitivity to radiation exposure and induction of DNA damage [13,16–21].

XRCC1 plays a pivotal role in DNA single-strand break (SSB) repair by interacting with DNA ligase III, polymerase beta (polyB) and poly(ADP-ribose) polymerase (PARP) in the BER pathway. Several variants of XRCC1 have been described, including one affecting codon 194 in exon 6 that results in an arginine (Arg) to tryptophan (Trp) substitution and one affecting codon 399 in exon 10 that results in arginine (Arg) to glutamine (Gln) change. The Arg194Trp is located between the binding domains of polyB and PARP, while Arg399Gln occurs in the vicinity of the PARP binding domain. The presence of the variant 399Gln has been shown to be associated with measurable reduced DNA repair capacity and increased risk of several types of cancers [13,16,17]. Consistent functional information of XRCC1 Arg194Trp is not yet well established; however, some studies have suggested that B144Trp variant may be associated to risk reduction for cancer [13,17].

hOGG1 is specifically concerned with the removal of 8-hydroxyguanine resulting from the action of reactive oxygen species that can be formed via ionization radiation-induced hydrolysis of H2O. An amino acid change from serine to cysteine at codon 326 (Ser326Cys) is the most frequently studied SNP. It has been shown that individuals with Ser/Cys or Cys/Cys OGG1 genotypes showed slower DNA repair capacity compared to those with the Ser/Ser OGG1 genotype [13,17,20].

APE1 is involved in the excision of abasic sites formed in DNA cleavage by OGG1. Several sequence variants were identified in APE1 gene, including an amino acid change from aspartic acid to glutamic acid (Asp148Glu) in exon 5 that may be associated with hypersensitivity to ionizing radiation [13].

XRCC3, which participates in DNA double-strand break via homologous recombinational repair, presents a Thr241Met substitution in exon 7, which was found to be associated with an increase in chromosome deletions using an in vitro cytogenetic challenge assay [22] and chromosomal translocations in healthy nonsmoker subjects carefully characterized for indoor radon exposure [23].

Therefore, the aim of this study was to determine the relationship between XRCC1 (Arg194Trp and Arg399Gln), OGG1 (Ser326Cys), APE1 (Asp148Glu) and XRCC3 (Thr241Met) SNPs and chromosomal damage in interventional cardiologists working in high-volume cardiac catheterization laboratory.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>SNPs</th>
<th>Annealing temperature (°C)</th>
<th>Enzyme</th>
<th>Fragment sizes (pb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1</td>
<td>Exon 6</td>
<td>Arg194Trp</td>
<td>58</td>
<td>Pvu II</td>
<td>490 (Arg/Arg)</td>
</tr>
<tr>
<td></td>
<td>Exon 10</td>
<td>Arg399Gln</td>
<td>56</td>
<td>MspI</td>
<td>294 + 196 (Trp/Trp)</td>
</tr>
<tr>
<td></td>
<td>Exon 5</td>
<td>Asp148Glu</td>
<td>54</td>
<td>Bgl II</td>
<td>269 + 133 (Arg/Arg)</td>
</tr>
<tr>
<td>APE1</td>
<td>Exon 7</td>
<td>Ser326Cys</td>
<td>58</td>
<td>Ita I</td>
<td>402 (Glu/Glu)</td>
</tr>
<tr>
<td>OGG1</td>
<td>Exon 7</td>
<td>Thr241Met</td>
<td>60</td>
<td>Nla III</td>
<td>164 (Asp/Asp)</td>
</tr>
<tr>
<td>XRCC3</td>
<td>Exon 7</td>
<td>Thr241Met</td>
<td>60</td>
<td>116 (Ser/Ser)</td>
<td>144 + 20 (Glu/Glu)</td>
</tr>
</tbody>
</table>

2. Methods

2.1. Study population

The study population comprised 77 subjects: 40 interventional cardiologists (27 male, 41.3 ± 9.4 years and 13 female, 37.8 ± 8.4 years) who operate in high-volume cardiac catheterization laboratories, and 37 clinical cardiologists (26 male, 39.4 ± 9.5 years and 11 female, 35.0 ± 9.8 years) working in the same hospital without radiation exposure as the control group. Exclusion criteria for both exposed and control subjects were personal medical history of disease, cancer, or recent infectious state. All subjects gave their informed consent before entering the study. The Ethical Committee approved the study. All participants were asked to fill in a standardized questionnaire including items concerning smoking habits, alcohol intake, drug consumption, medical history, and years of employment.

Staff dosimetry for the last 6 months was obtained by a thermoluminescent dosimeter, with monthly measurement. Dosimeter was located under lead apron, at the waist or over the chest. Dosimetry results were converted to effective dose as suggested by the International Commission on Radiological Protection [24]. Blood samples were coded and laboratory analyses were performed without any knowledge of patient identity and exposure status.

2.2. Cytokinesis-block micronucleus test

Two separate cultures from each sample were set up by mixing 0.3 mL of whole blood with 4.7 mL of RPMI 1640 medium: the cultures were incubated at 37 °C for 72 h. Cytochalasin B (6 μg/mL) was added 44 h after culture initiation. Cells were then harvested and fixed according to the standard methods [11]. For each culture, 1000 binucleated cells were scored under optical microscope (final magnification 400 ×) for micronucleus analysis, following the criteria for micronucleus acceptance [25]. We evaluated the MN frequency as the number of micronucleated cells per 1000 cells (%).

2.3. PCR-RFLP genotyping assays

Genomic DNA was extracted from peripheral blood leukocytes. Genetic polymorphisms were analyzed by PCR combined with restriction fragment length polymorphism (RFLP) as described earlier [16–20]. The primer pairs used were: (a) XRCC1: Arg194Trp, F 5′-GCCCTCGGTGTTTTGC-3′ and R5′-AGCCAAGACCTTCACT-3′; (b) XRCC1: Arg399Gln, F 5′-AGTACTGCAGGTTCTTG-3′ and R5′-TCTCTTCTGTTGCTC-CAACTCT-3′; for Arg399Gln; (c) XRCC3 Thr241Met, F 5′-GGTCTGAGCATCAGTACCCAAAC-3′ and R5′-TCAACGGCTCACTCTGCTC-3′; (d) APE1 Asp148Glu, F 5′-CTTCTCTTCTCTATGCAA-3′ and R5′-ACAATGAGAACAGGCTTC-3′; (e) OGG1 Ser326Cys, F 5′-AGTCGACATGGAGACCAAG-3′ and R5′-CTTCTCGGCGTTCGGAC-3′. PCR products were digested with specific restriction enzymes that recognized and cut either the wild-type or variant sequence site. Details of annealing temperature, restriction pattern, and restriction enzymes used for each genotyping assay are listed in Table 1. The digested PCR products were analyzed on 10% polyacrylamide gels and stained with ethidium bromide. Genotype results were regularly confirmed by random repetition of the samples. All uncertain results were reanalyzed with the same technique, and usually one more assay was sufficient to clarify any doubts.

2.4. Statistical analysis

Statistical analyses of the data were conducted with the Stat view statistical package, version 5.0.1 [Abacus Concepts, Berkeley, CA, USA]. Data are expressed as mean (±S.D.).

On the basis of our previous study [11], the power to detect a 50% increased MN frequency in radiation-exposed physicians compared with unexposed physicians with a level of significance of p = 0.05 was >80%. In addition, the number of subjects studied was sufficient to detect, with a statistical power of 80% and significance value of 0.05, an increased MN frequency of moderate magnitude in radiation-exposed physicians compared with unexposed physicians with a level of significance of p = 0.05 was >80%. In addition, the number of subjects studied was sufficient to detect, with a statistical power of 80% and significance value of 0.05, an increased MN frequency of moderate magnitude in heterozygous carriers, assuming an allele frequency of over 40%.

For statistical analysis, smoking status was defined on the basis of smokers/non-smokers approach; the smoker category consisted of individual who...
smoked and former smokers who stopped smoking ≤5 years before sampling. XRCC1 (Arg194Trp and Arg399Gln), XRCC3 (Thr241Met), OGG1 (Ser326Cys) and APE1 (Asp148Glu) polymorphisms were categorized into wild-type or variant (presence of at least one mutant allele).

Because of the skewness of the distributions of MN values, analyses were performed by using logarithmic transformation of data. Differences between the means of the two continuous variables were evaluated by the Student’s t-test. Differences in non-continuous variables were tested by χ² analysis. The data for three different groups were analyzed by ANOVA, and significant differences among pairs of means were tested by the Scheffe’s test. The Scheffe’s test has been chosen for multiple comparisons since it is very robust for the violations of the assumptions that are typically associated with multiple comparisons procedures. Interaction terms were also introduced in the model to examine the interaction of genotypes with exposure or smoking.

Regression analysis with Pearson’s test was also used to evaluate the relationship between the two continuous variables. Multiple regression analyses were carried out with MN values as dependent variables, and relevant parameters (exposure, age, smoking, enzyme genotype, etc.) as independent variables. The level of significance was set at p < 0.05 was considered for all statistical analyses.

3. Results

3.1. Demographic characteristics, genetic findings and dose exposure

The main characteristics of the study subjects are described in Table 2. There were no statistically significant differences in the genotype distribution between control and exposed populations. Allele frequencies obtained for the analyzed genes were consistent with literature data obtained for Caucasian population. There was no difference in age and gender between exposed and control physicians. The exposed group had a larger number of smokers (p = 0.0002). Over the last 6 months, the mean cumulative radiation dose recorded by monthly dosimeters under apron was 1.7 ± 2.3 mSv (range 0.2–8.3 mSv) for subjects in the exposed group. Significant difference in mean annual doses was recorded between females and males (0.2 ± 0.1 mSv vs. 2.3 ± 2.8 mSv, p = 0.03). The subjects enrolled in this study were occupationally exposed to ionizing radiation for 12.0 ± 9.9 years (range 1–35 years). No difference in mean dose was recorded between the smokers and non-smokers (1.3 ± 2.1 mSv vs. 2.3 ± 3.1 mSv, p = 0.3).

3.2. Determinants of MN frequency

Concerning MN frequency, MN had a significant positive correlation with donor age (r = 0.359, p = 0.001). MN frequencies were also higher in smokers than in non-smokers (19.1 ± 7.9‰ vs. 15.0 ± 7.3‰, p = 0.02). MN frequency was significantly higher in exposed physicians than in controls (19.7 ± 7.8‰ vs. 13.5 ± 6.3‰, p = 0.0003), even when possible gender-related effects were taken into account (Fig. 1). No significant correlation was observed between occupational radiation doses and MN frequency (r = 0.265, p = 0.18).

However, MN values were higher in physicians with exposure > 10 years in comparison to exposed physicians with hospital work ≤10 years (22.3 ± 4.8‰ vs. 17.5 ± 6.9‰, p = 0.05). Multiple regression analysis showed that occupational exposure status (standardized β = 0.342, p = 0.004) and age (standardized β = 0.311, p = 0.004) had a significant effect, whereas the effects of smoking status were not significant (standardized β = 0.053, p = 0.6).

3.3. Genotypes and MN frequency

Distribution of MN frequencies by genetic polymorphisms is shown in Table 3. None of the polymorphisms was associated with MN frequency in the whole population. In the exposed group, individuals carrying an XRCC3 Met241 allele had higher frequency than homozygous XRCC3 Thr241 (21.2 ± 7.8‰ vs. 16.6 ± 7.1‰, p = 0.03).

Table 2

<table>
<thead>
<tr>
<th>Gender, n (%)</th>
<th>Interventional cardiologists</th>
<th>Clinical cardiologists</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>27 (67.5)</td>
<td>26 (70.3)</td>
<td>0.8</td>
</tr>
<tr>
<td>Female</td>
<td>13 (32.5)</td>
<td>11 (29.7)</td>
<td></td>
</tr>
<tr>
<td>Mean age, years ± S.D.</td>
<td>41.6 ± 9.7</td>
<td>39.2 ± 9.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Smoking habits, n (%)</td>
<td>24 (60)</td>
<td>7 (18.9)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Yes</td>
<td>16 (40)</td>
<td>30 (81.1)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8 (20)</td>
<td>7 (18.9)</td>
<td></td>
</tr>
<tr>
<td>Mean dose, mSv ± S.D.</td>
<td>1.6 ± 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean time exposure, years ± S.D.</td>
<td>12.0 ± 9.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XRCC1 codon 399, n (%)</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>16 (40)</td>
<td>19 (51.4)</td>
<td></td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>20 (50)</td>
<td>16 (43.2)</td>
<td></td>
</tr>
<tr>
<td>Glu/Gln</td>
<td>4 (10)</td>
<td>2 (5.4)</td>
<td></td>
</tr>
<tr>
<td>XRCC1 codon 194, n (%)</td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>34 (85)</td>
<td>31 (83.8)</td>
<td></td>
</tr>
<tr>
<td>Arg/Trp</td>
<td>6 (15)</td>
<td>5 (13.5)</td>
<td></td>
</tr>
<tr>
<td>Trp/Trp</td>
<td></td>
<td>1 (2.7)</td>
<td></td>
</tr>
<tr>
<td>APE1 codon 148, n (%)</td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Asp/Asp</td>
<td>20 (50)</td>
<td>13 (35.1)</td>
<td></td>
</tr>
<tr>
<td>Asp/Glu</td>
<td>16 (40)</td>
<td>23 (62.2)</td>
<td></td>
</tr>
<tr>
<td>Glu/Glu</td>
<td>4 (10)</td>
<td>1 (2.7)</td>
<td></td>
</tr>
<tr>
<td>OGG1 codon 326, n (%)</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>28 (70)</td>
<td>27 (73)</td>
<td></td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>12 (30)</td>
<td>9 (24.3)</td>
<td></td>
</tr>
<tr>
<td>Cys/Cys</td>
<td></td>
<td>1 (2.7)</td>
<td></td>
</tr>
<tr>
<td>XRCC3 codon 241, n (%)</td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>13 (32.5)</td>
<td>9 (24.3)</td>
<td></td>
</tr>
<tr>
<td>Thr/Met</td>
<td>22 (55)</td>
<td>19 (51.3)</td>
<td></td>
</tr>
<tr>
<td>Met/Met</td>
<td>5 (12.5)</td>
<td>9 (24.3)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3
MN frequencies by genotype distribution.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Whole group (‰)</th>
<th>p</th>
<th>Exposed</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1</td>
<td>Arg/Arg</td>
<td>15.9 ± 8.9</td>
<td>0.2</td>
<td>19.7 ± 10.4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Arg/Gln-Gln/Gln</td>
<td>17.6 ± 6.6</td>
<td></td>
<td>20.0 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>XRCC1</td>
<td>Arg/Arg</td>
<td>16.5 ± 6.8</td>
<td>0.8</td>
<td>19.0 ± 6.6</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Arg/Trp-Trp/Trp</td>
<td>17.7 ± 10</td>
<td></td>
<td>23.7 ± 11</td>
<td></td>
</tr>
<tr>
<td>APE1</td>
<td>Asp/Asp</td>
<td>16.6 ± 7.5</td>
<td>0.6</td>
<td>18.7 ± 8.9</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Asp/Glu-Glu/Glu</td>
<td>16.9 ± 8.2</td>
<td></td>
<td>20.8 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>OGG1</td>
<td>Ser/Ser</td>
<td>16.4 ± 7.9</td>
<td>0.7</td>
<td>18.7 ± 8.4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Ser/Cys-Cys/Cys</td>
<td>17.4 ± 7.6</td>
<td></td>
<td>20.1 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>XRCC3</td>
<td>Thr/Thr</td>
<td>17.3 ± 6.9</td>
<td>0.5</td>
<td>16.6 ± 7.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Thr/Met-Thr/Met</td>
<td>16.5 ± 8.1</td>
<td></td>
<td>21.2 ± 7.8</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. MN levels in exposed control group, in all participants (a) and stratified for gender (b). The results are expressed as boxes with five horizontal lines, displaying the 10th, 25th, 50th (median), 75th, and 90th percentiles of the MN. All values above the 90th and below the 10th are plotted separately as squares.

An interactive effect \((F = 3.9, p = 0.03)\) was found between the presence of XRCC3 Met241 variant and exposure >10 years (Fig. 2).

Within the exposed group, we found that individuals with two or more risk alleles showed a higher MN frequency when compared to subjects with one or no risk allele \((18.4 ± 6.6‰ vs. 14.4 ± 6.1‰, p = 0.02)\). No detectable influence of the two or more risk alleles on the MN frequency was observed in the control group \((p = 0.9)\). ANOVA analysis revealed an interactive effect \((F = 6.3, p = 0.02)\) between smoking, exposure >10 years and the presence of the two or more risk alleles on the MN frequency (Fig. 3). Multiple regression analysis of the exposed cohort revealed the presence of risk allele ≥2 as a significant predictor of MN frequency (standardized \(\beta = 0.357, p = 0.03\)).

4. Discussion

Occupational exposure to ionizing radiation in invasive cardiologists is associated to an increased chromosomal DNA damage expressed as MNs in human lymphocytes, that are early predictors of cancer [11,12].

Furthermore, we found that the presence of XRCC3 Thr241Met genetic polymorphism is associated with a higher MN, suggesting that this amino acid substitution variant may alter the repair of DNA double-strand breaks.

4.1. Ionizing radiation exposure, DNA damage and repair

Of the many types of DNA damage that ionizing radiation exposure induces within the cell, the most dangerous is the DNA double-strand breaks [26,27]. Indeed, the most of radiation-induced micronuclei in human lymphocytes are caused by DSB [17,28]. DSBs are considered to be particularly biologically important because their repair is intrinsically more difficult than that of other types of DNA damage [27]. They may lead to either induction of gene mutations, the loss or amplification of chromosomal leading to tumourigenesis if, for example, the deleted chromosomal region encodes a tumour suppressor or if an amplified region encodes a protein with oncogenic potential [29].

It is also important to note that the stable chromosome translocations produced by ionizing radiation persist long after exposure and tends to cumulate with repeated genetic injuries, showing the persistence of elevated long-term health risks and emphasizing the importance to consider chromosome translocations for biodosimetry of cumulative exposure to external ionizing radiation [30].

Our results suggest that XRCC3 241Met variant influences chromosomal DNA damage expressed as MN in human lymphocytes of exposed cardiologists.
The functional consequence of XRCC3 Thr241Met polymorphism on the overall function of the protein is yet not clear. It has previously been shown that carriers of XRCC3 Met241 variant have relatively high-DNA adduct levels in lymphocytes, indicating that the polymorphism is associated with relatively low-DNA repair capacity [31]. However, functional data suggested that the XRCC3 Thr241Met variant protein complements defects in HR repair in XRCC3-deficient Chinese ovary hamster cells and human colon cancer cell line (HCT116) [32,33]. Anyway, our observation is consistent with previous studies reporting that the XRCC3 Thr241Met is associated with an increased chromosomal DNA damage [22,23].

Of a particular interest, a study showing that lymphocytes from carriers of the Met allele show an elevated yield of chromosome-type deletions in blood lymphocyte after an in vitro X-ray exposure [22]. Furthermore, a previous paper showed that the MN frequency significantly increased with age, radiation exposure to γ-rays from a nuclear power plant and in workers with Thr/Met or Met/Met XRCC3 genotypes [17].

Finally, a recent study has shown that XRCC3 Met241 variant influence radiosensitivity of human fibroblasts and that more risk allele of susceptible genes have a combined effect on cellular radiation response, suggesting that individuals with multiple risk alleles could be more susceptible to radiation effects than those with fewer risk alleles [34].

Similarly, our observation that harbouring two or more risk alleles in exposed cardiologists lead to an increased MN frequency, is consistent with the view that chromosome breakage induced by ionizing radiation is probably due to the combined effect of the conversion of oxidized bases and single-strand breaks which were not repaired by base excision (deficiency of XRCC1, OGG1 and/or APE1) and, further replicated into double-strand breaks during the next S-phase if not repaired (deficiency of XRCC3), giving rise to micronuclei in the following mitosis [17,19].

It is also worth noticing that smokers or invasive cardiologists with a longer duration of employment with XRCC3 Thr241Met genetic polymorphism showed a higher yield of chromosomal damage, again suggesting that this polymorphisms may contribute to increase the genetic damage in susceptible individuals who are long-term exposed to carcinogenic substances.

4.2. XRCC3 Thr241Met polymorphism and cancer risk

The XRCC3 gene is localised on chromosome 14q32.3 and codes for a protein participating in involved in homologous recombination repair pathway repairing radiation-induced DSB DNA damage [35]. XRCC3-deficient cells demonstrated genetic instability and increased sensitivity to DNA damaging agents [36].

The Thr241Met substitution is the most investigated polymorphism in XRCC3 due to a C18067T transition at exon 7 [37]. It was suggested that conversion from one with a neutral hydrophilic hydroxyl group (Thr) to a hydrophobic one with a methyl sulphur group (Met) could represent a substantial change in protein characteristics which could affect protein structure and integrity [38].

A large number of molecular epidemiologic studies have been performed in order to evaluate the role of XRCC3 polymorphisms on the risk of various cancers. However, the results remain fairly conflicting rather than conclusive [39,40].

A positive association between the XRCC3 Met241 variant allele and various types of cancer has been observed in several investigations, including bladder cancer [41], melanoma skin cancer [38], gastric cancer [42] and cancer in patients of varying radiosensitivity [43]. A recent meta-analysis support that the XRCC3 Thr241Met polymorphism might represent a low-penetrance susceptible gene especially for cancer of breast, bladder, head and neck, and non-melanoma skin cancer [40]. However, the effect of XRCC3 Thr241Met polymorphisms on risk of cancer may be more apparent in the presence of environmental factors, such as tobacco smoke or ionizing radiation exposure [40].

4.3. Limitations of the study

The genetic susceptibility to radiation exposure is likely to result from the contribution of many genetic variants in multiple repair pathways that may have a joint or additive effect on repair diverse DNA damages. An undoubted limitation of our study is the lack of a more comprehensive genetic analysis of genes involved in the different mechanisms of the cellular response to radiation, not only DNA damage detection and repair, but also apoptosis, inflammation, and proliferation [44,45]. Another limitation of our study is that the sample size has limited power for assessing gene–gene interactions and/or interactions between genes and multiple environmental factors (e.g., smoking, ionizing radiation). The small sample size is the crucial study limitation and, therefore, it is possible that some statistical associations may be change findings, whereas other were not found because of small number of individuals per category.

A more comprehensive, larger scale genetic study is needed to further explore the effects of gene–environment interaction on genetic susceptibility to ionizing radiation exposure.

However, the strength of this study includes a homogeneous group of highly exposed subjects. In fact, interventional cardiologists have an exposure per-head per year two to three times higher than that of radiologists [46]. Ranges of staff radiation effective dose equivalent in cardiac catheterization laboratories averages 2–10 mSv/year based on direct measurements with thermoluminescent dosimeters worn below protective aprons [1]. Cumulative doses after 30 years of working life cab be as high as 100–250 mSv, corresponding to a whole body dose equivalent of 5000–12,500 chest X-rays. This dose gives an estimated lifetime attributable risk of cancer incidence in the range of 1 cancer in 100 to 1 in 200 exposed subjects [2,47].

Identification and biological characterization of the genes that have potential impact on the development of cancer is crucial and will help in developing better radiation prevention programme [13].

Our current data show a significant association between MN frequency and XRCC3 Thr241Met polymorphism, particularly in combination with multiple risk alleles of BER repair genes, in
this population of interventional cardiologists. Further larger population-based studies are needed in order to confirm our findings as well as to evaluate whether genotyping for DNA repair polymorphisms could represent a useful tool for a better medical surveillance of subjects exposed to significant occupational radiation risks.

Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work.

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References

