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ARTICLE

Occupational radiation exposure and genetic polymorphisms in DNA repair genes

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Abstract – Radiation sensitivity appears to be influenced by genetic polymorphisms in some DNA repair genes and individuals with low DNA repair capacity can be at increased risk of developing cancer. The aim of the present study was to determine the impact of gene polymorphisms on DNA damages in the form of micronuclei (MN) in circulating lymphocytes of interventional cardiologists (ICs), occupationally exposed to ionizing radiation. MN frequency was significantly higher in the ICs than in the controls (27.7 ± 17.2‰ *vs.* 8.0 ± 2.6‰, $p < 0.0005$). MN values were higher in the ICs with exposure of ≥ 10 years and ≥ 3 mSv in comparison to the related referents. The analysis of different interactions showed significantly higher MN frequencies in ICs exposed to ≥ 10 mSv carrying the variant-type XRCC3 241Met genotype compared to those of wild-type XRCC3 (Thr/Thr) genotype. In the ICs carrying both wild-type alleles for XRCC3 and MSH3 polymorphisms showed a significantly lower MN frequency compared to the exposed individuals with one or two variant alleles for XRCC3 and MSH3 polymorphisms. In conclusion, ICs showed significantly higher MN frequencies than in the control, and this effect was higher in individuals carrying the variant-type XRCC3 241Met genotype. These results not only show the need for improvement of safety and training programs for ICs but also may be helpful in developing amenable screening tests for identifying radiosensitive workers and applying suitable radiation protection job-specific strategies for effective dose reduction.

Keywords: occupational exposure / cytogenetic / DNA / genetic effect / radiation / low doses

1 Introduction

Radiation exposure causes a variety of DNA damages including single strand breaks (SSB) and double-strand breaks (DSB) leading to lethal events, cancers and non-cancers. There are specific pathways for the repair of different types of DNA damages such as base excision repair (BER), homologous recombination repair (HRR), non homologous endjoining (NHEJ), nucleotide excision repair (NER) and mismatch repair (MMR). Oxidative base damage and strand breaks induced by ionizing radiation are mainly repaired by BER, NHEJ and HRR pathways (Friedberg *et al.*, 1995) but may also involve to a lesser extent MMR and NER. Therefore any defect in these pathways may cause radiation sensitivity and elevated cancer risk (Hu *et al.*, 2001). Genetic polymorphisms in DNA repair genes may alter the functional properties of DNA repair enzymes and consequently influence inter-individual variation in DNA repair capacity and susceptibility to cancer (Lunn *et al.*, 1999).

The cytokinesis-block micronucleus assay (CBMN) is a reliable method for detection of chromosome breakage and loss in human peripheral blood lymphocytes and also is a standardized technique for biodosimetry in radiation protection (Thierens and Vral, 2009). Micronuclei (MN) have been known as intermediate endpoints of carcinogenesis and a predictor of cancer (Fenech, 2006; Fenech *et al.*, 2016). There are association studies that show the link between DNA repair gene polymorphisms and MN induction (Angelini *et al.*, 2005). XRCC1 (X-ray cross-complementation group 1) has a role in BER, and XRCC3 (X-ray cross-complementation group 3) acts in the HRR pathway. Cells with mutation in XRCC1 and XRCC3 show increased and moderate sensitivity to ionizing radiation, respectively, therefore single nucleotide polymorphisms (SNPs) in these two genes are very important in radiation sensitivity (Angelini *et al.*, 2005). Also a possible role for NER proteins such as Xeroderma pigmentosum; XP in the repair of some types of oxidative damage induced by ionizing radiation has been shown and polymorphisms in the XPD and XPG genes have been associated with lung and breast cancers (Angelini *et al.*, 2005).

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MMR has a role in the repair of post-DNA replication base pairing errors by the MutSα complex (composed of the MMR proteins MSH2 and MSH6) or MutSβ complex (containing MSH2 and MSH3) (Angelini *et al.*, 2005).

Invasive cardiology procedures can deliver high radiation doses to the staff capable of inducing significant health effects such as occurrence of higher DNA damages, cataracts and higher incidence of brain cancer (Zakeri *et al.*, 2010). Due to the impact of radiosensitivity on enhanced DNA damage and risk of developing different types of cancer, there is a need for identifying sensitive individuals particularly in work environments where occupational exposure doses may be significant. Therefore in the present study, we investigated the potential links between genetic polymorphisms in genes XRCC1 Arg194Trp, XRCC3 Thr241Met, XPD Lys751Gln and MSH3 Ala1036Thr coding DNA repair enzymes in different pathways and the levels of DNA damage investigated by micronucleus assay in interventional cardiologists.

2 Methods

The present study included 129 subjects: 89 interventional cardiologists (58 males, 42.5 ± 8.1 years and 31 females, 39.6 ± 6.0 years) who worked in cardiac catheterization laboratories of heart hospitals, and 40 individuals (27 males, 41.93 ± 9.46 years and 13 females, 40.3 ± 8.4 years) working in the administrative departments of the same hospitals without radiation exposure as the control group.

All subjects in this study filled-in questionnaires included information about smoking habits, medical history, drug intake and diagnostic medical irradiation. They were healthy individuals without current infections and medications in the last 6 months, and were mostly non-smokers. All participants' rights were protected and written informed consent was obtained from all donors. The study protocol was approved by the national ethical committee and also conformed to the ethical guidelines of the World Medical Association (Declaration of Helsinki).

The official personal dosimetry records based on bimonthly film dosemeters were collected for each radiation worker for the last 1 and 5 years.

The micronuclei were prepared in cytokinesis blocked cells using cytochalasin B (Cyt-B Sigma) as suggested by (Fenech, 1993). Whole blood cultures were performed by adding 0.5 ml blood to 4.5 ml RPMI-1640 culture medium supplemented with 20% fetal calf serum,100 UI/ml penicillin and 0.1 mg/ml streptomycin, 1.0% l-glutamine and 1.0% phytohemagglutinin (PHA) for mitogenic stimulation. Two separate cultures from each sample prepared and incubated at 37 °C, 5% CO2 for 72 hours. After 44 hours of incubation cytochalasin B (6 μg/ml) was added to the cultures to block cytokinesis. After collection of the cells by centrifugation, cells were treated with a hypotonic solution (0.075 M KCl) for 3 min. After centrifugation and removal of the supernatant, the cells were fixed with a fresh mixture of methanol/acetic acid (3:1) (all materials purchased from Gibco BRL). After three times centrifugation and resuspension, slides were made under warm and humid conditions, air-dried and stained with 5% Giemsa as previously described (Rastkhah *et al.*, 2016).

To determine the total number of MN in binucleated cells, according to the IAEA handbook (EPR Biodosimetry IAEA, 2011) a total of 1000 binucleated cells with well-preserved cytoplasm (500 cells per replicate) were scored for each subject and the nuclear division index (NDI) was calculated.

Blood specimens were taken from all participants by using EDTA-containing tubes, and genomic DNA was extracted from peripheral blood by using QIAgen kit, Germany. All DNA materials were protected in 4 °C refrigerators for later analyses. Genetic polymorphisms were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. For each of the studied parameters, the appropriate primers were used to amplify the corresponding gene by PCR and the reaction products were digested by using the specific restriction enzymes (Fermentas, USA) that recognized and cut either the wild-type or variant sequence site. Information about primers sequence, annealing temperature, restriction pattern and restriction enzymes used for each genotyping assay are given in Table 1. The digested PCR products were analyzed on 10% polyacrylamide gels and 4% ultra-pure agarose gel and stained with Gel red (Biotium, USA). Genotype results were confirmed by random repetition of 30% of the samples, and all uncertain results were rechecked with the same technique.

The SPSS package, version 17 was used for statistical analyses of the data. The data are shown as mean ± SD. Because of the over-dispersed nature of the MN distribution, logarithmic transformation of data was used for analyses. The Student's *t*-test and $\chi 2$ analysis were used for evaluation of the differences between the means of the two continuous and non-continuous variables, respectively. Multinomial logistic regression was used to test the existence of a different genotype distribution between controls and ICs. Different gene interactions were investigated with exposure doses, age, smoking, gender and time exposure (years). The data for more than two different groups were analyzed by ANOVA, $p < 0.05$ was considered as the significant level for all analyses.

3 Results and discussion

The characteristics of the study groups are shown in Table 2. The genotype distribution at each locus in the total population was consistent with the Hardy–Weinberg equilibrium for the Caucasian population. There were no statistically significant differences between any genotypes or alleles in the control and exposed groups for XRCC1 Arg194Trp, XRCC3 Thr241Met and MSH3 Ala1036Thr (Tab. 2) except in the wild-type and variant genotypes for XPD Lys751Gln ($p = 0.003$).

The mean cumulative dose exposures during the 1 and 5 last years received by ICs were 3.5 ± 3.4 mSv and 11.1 ± 10.0 mSv, respectively. They were occupationally exposed to ionizing radiation for 9.5 ± 6.7 years.

It has shown that the doses received by interventional cardiologists may well be above the annual limits of 20 mSv proposed by ICRP. ICs are at high risk of posterior subcapsular cataracts and the risk increased with duration of activity but no clear relationship with workload was observed (Jacob *et al.*, 2013). Approaches have developed in the European ELDO project for retrospective assessment of eye lens doses for interventional cardiologists that will be of great benefit for ongoing epidemiological studies (Domienik *et al.*, 2016). In a

Table 1. Details of PCR and RFLP procedures for each genotyping assay.

Gene	SNPs	Primers 5'–3'	Annealing Temp (°C)	Restriction Enzymes	Fragment sizes (bp)
XRCC1	Arg194Trp	F: GCC AGG GCC CCT CCT TCA A R:TAC CCT CAG ACC CAC GAG T	62	Pvu II	485(R/R) 396 + 89(W/W) 485 + 396 + 89(R/W)
XRCC3	Thr241Met	F:GGT CGA GTG ACA GTC CAA AC R:TGC AAC GGC TGA GGG TCT T	60	Hin1 II	315 + 140(T/T) 210 + 140 + 105(M/M) 315 + 210 + 140 + 105(T/M)
XPD	Lys751Gln	F:CCC CTC TCC CTT TCC TCT GTT R:GCT GCC TTC TCC TGC GAT TA	60	Pst I	245 + 28(K/K) 182 + 63 + 28(Q/Q) 245 + 182 + 63 + 28(K/Q)
MSH3	Ala1036Thr	F:TCT AAC AGG CAA GTA GGA AC R:TAG CCA CAT TTA ATC CAT AAC	52	Hha I	138 + 87(A/A) 225(T/T) 225 + 138 + 87(A/T)

Table 2. General characteristics and genotype distributions of studied genes in the ICS and control groups.

	ICs[a]	Control	*p* value
Gender, n (%)			
Male	58 (65.2)	27 (67.5)	0.48
Female	31 (34.8)	13 (32.5)	
Mean age, years ± S.D.	41.5 ± 7.5	41.4 ± 9.0	0.93
Smoking habits, n (%)			
Yes	3 (3.5)	4 (10.0)	0.13
No	83 (96.5)	36 (90.0)	
Mean time exposure, years ± S.D.	9.5 ± 6.7	13.8 ± 7.7	0.21
Mean last year exposure mSv ± SD	3.5 ± 3.4	–	
Mean last five years exposure mSv ± SD	11.1 ± 10.0	–	
Mean MN frequency ± SD (‰)	27.7 ± 17.2 (89)	8.0 ± 2.6 (40)	< 0.0005
Nuclear division index (NDI)	1.7 ± 0.1	1.7 ± 0.1	0.32
XRCC1 codon 194, n (%)			0.82
Wild: *Arg/Arg*	77 (86.5)	34 (85)	
Variant: *Trp/ Trp*	0	0	
Variant: *Arg/ Trp*	12 (13.5)	6 (15)	
XRCC3 codon 241, n (%)			0.72
Wild: *Thr/ Thr*	42 (47.2)	17 (42.5)	
Variant:*Met/Met*	11 (12.4)	7 (17.5)	
Variant: *Thr/Met*	36 (40.4)	16 (40)	
XPD codon 751, n (%)			0.003
Wild: *Lys/Lys*	39 (43.8)	7 (17.5)	
Variant:*Gln/Gln*	16 (18.0)	17 (42.5)	
Variant: *Lys/ Gln*	34 (38.2)	16 (40)	
MSH3 codon 1036, n (%)			0.4
Wild: *Ala/ Ala*	8 (9.0)	1 (2.5)	
Variant :*Thr/ Thr*	50 (56.2)	25 (62.5)	
Variant: *Ala/ Thr*	31 (34.8)	14 (35.0)	

[a] Interventional cardiologists.

study the estimated cumulative left and right eye lens dose ranged from 8 to 264 mSv and 6 to 225 mSv, respectively and calculations showed annual eye lens doses sometimes exceeding the new ICRP annual limit of 20 mSv (Farah *et al.*, 2014).

MN frequency was significantly higher in the ICs than in controls (27.7 ± 17.2‰ *vs.* 8.0 ± 2.6‰, $p < 0.0005$). This result agrees with several cytogenetic investigations by CBMN assay in radiation workers (Maluf *et al.*, 2000; Aka *et al.*, 2004; Dias *et al.*, 2007). The MN frequency in interventional cardiologists with the mean cumulative radiation dose of 1.7 ± 2.3 mSv (range 0.2–8.3 mSv) over the last 6 months, was significantly higher than in clinical physicians; 19.7 ± 7.8‰ *vs.* 13.5 ± 6.3‰, $p = 0.0003$ (Andreassi *et al.*, 2009). In another

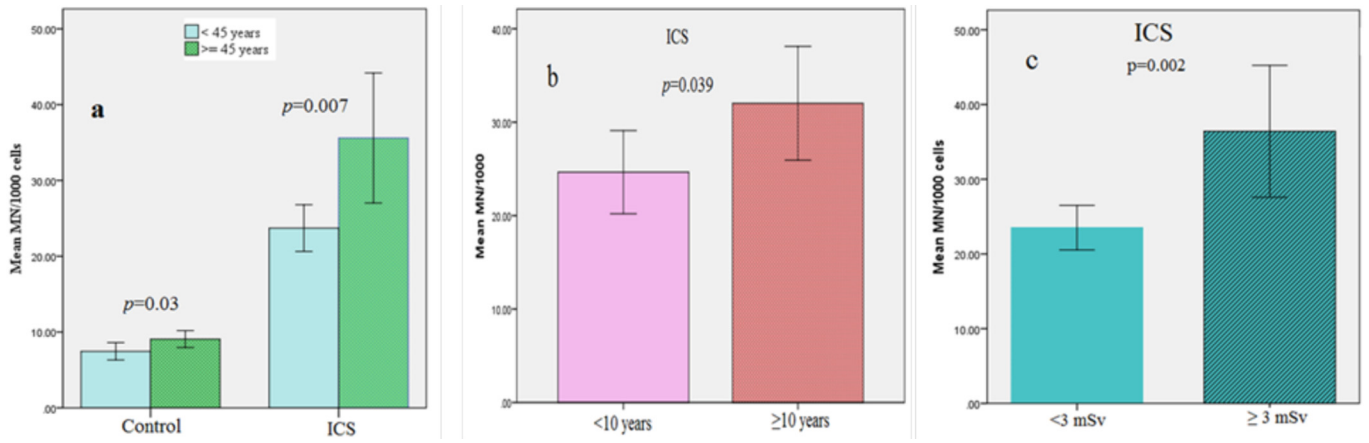

Fig. 1. MN frequencies in the study population subdivided by age (a); significantly higher MN frequencies were found in the older age groups (>45 years) than in the younger age groups (<45 years) in the both groups, time exposure year (b); MN values were significantly higher in the ICS group with time exposure of >10 years in comparison to <10 years ($p=0.039$) and exposure status (c); significant difference in MN frequency was also observed between occupational radiation doses of ≥ 3 mSv and <3 mSv during last year of exposure ($p=0.002$).

study the mean frequencies of micronuclei in the 30 cardiology department workers (25.57–4.79‰) and 30 radiology department workers (21.90–4.23‰) were significantly higher than that in the 27 controls (10.78–1.47‰) (Sakly *et al.*, 2013).

The MN frequency in the 67 healthcare workers occupationally exposed to ionizing radiation below the permissible dose of 50 mSv/year showed a significant higher MN frequency compared to the control group (13.63 ± 4.9‰ *vs.* 6.52 ± 4.21‰, $p<0.05$). Also the (C–MN) frequency was significantly higher in the exposed subjects than in the controls (9.04 ± 4.57‰ *vs.* 1.17 ± 0.77‰) pointing to the clastogenic effects of radiation. There was a lack of data on individual physical doses of radiation in this study. However, the time of exposure to ionizing radiation had a significant effect on the level of MNs and C–MN (Bouraoui *et al.*, 2013).

In our recent in vitro study blood samples from 16 healthy donors (eight males and eight females in two age ranges of 20–34 and 35–50 years) irradiated up to 4 Gy (0.25–4 Gy) of gamma rays (with a dose rate of 0.83 Gy/min). The micronucleus assay was employed to obtain the frequencies of micronuclei and the data were used to construct the calibration curves for men and women in two age groups, separately. The results showed the average baseline frequency of 11.75 ± 1.48‰ (8–14) micronuclei for the donors in ages between 35–50 years and after 0.25 Gy in vitro irradiation, the average frequency of 35.37 ± 6.34‰ (28–49) micronuclei was observed (Rastkhah *et al.*, 2016).

Most micronuclei induced by ionizing radiation are formed of acentric fragments because they are the result of chromosome breakage that consequently may cause radiation sensitivity and genetic susceptibility to cancer. Other factors such as nutritional status, genotoxins and genetic predisposition, may influence MN induction and cause variable MN background frequency. However, the studied groups in this study are healthy individuals from the same city and culture and are socioeconomically matched, and this technique is used as a sensitive and validated technique for systematic biomonitoring of radiation workers exposed to low-doses of ionizing radiation (Thierens and Vral, 2009).

No statistically significant differences in MN frequencies were also observed between males and females in the ICs (25.8 ± 15.1‰ *vs.* 31.1 ± 20.4‰; $p=0.16$) and in the control group (7.7 ± 2.6‰ *vs.* 8.6 ± 2.6‰; $p=0.36$) respectively. However, MN frequency in the females was slightly higher than in the males in both groups. It has been reported that women have a 19–40% higher baseline micronucleus frequencies than men (Rastkhah *et al.*, 2016).

Multiple regression analysis showed that age had a significant effect on the MN frequency in control group (standardized $\beta=0.313$, $p=0.049$), whereas the effects of age in ICs were not significant (standardized $\beta=0.199$, $p=0.062$). As shown in Figure 1a, significantly higher MN frequencies were found in the subgroups of older age (≥ 45 years) than in the younger age group (<45 years) (23.7 ± 11.8‰ *vs.* 35.6 ± 22.9‰, $p=0.007$ in the ICs and 7.4 ± 2.8‰ *vs.* 9.0 ± 1.9‰; $p=0.03$ in the control group). Forty five years were used for the age subdivision and for calculating the cumulative percentage. The increasing effect of age on background MN frequencies are due to the decline in DNA repair capacity and attributed to increased chromosome loss with age, related to X-chromosome (Thierens and Vral, 2009). The observed result in the control group is because of age. However, in the ICs it is also affected by the higher duration of work and the doses received.

Smoking status had not significant effects on MN frequency in both groups (in control: standardized $\beta=-0.007$, $p=0.964$ and in ICs: standardized $\beta=0.153$, $p=0.159$).

Multiple regression analysis showed that last year exposure (mSv) (standardized $\beta=0.150$, $p=0.304$) and last 5 year exposures (mSv) (standardized $\beta=0.033$, $p=0.827$) and occupational time of exposure (standardized $\beta=0.132$, $p=0.219$) had not significant effects on the MN frequency in the ICs.

After 10 years of study on 200 nuclear dockyard workers, an increased frequency of chromosomal aberrations and dose effect relationship were reported on the basis of accumulated dose (Evanse *et al.*, 1979). In some other studies higher frequencies of MN and chromosomal aberration have been

Table 3. MN frequencies in genotypes of different DNA repair genes polymorphisms the ICS and control groups.

Gene	Codon	Genotype	Control			ICS		
			n	Mean MN ± SD	*p* value	n	Mean MN ± SD	*p* value
XRCC1	194	Wild	34	7.8 ± 2.6	0.39	77	28.6 ± 18.3	0.2
		Variant	6	8.8 ± 2.6		12	22.0 ± 5.3	
XRCC3	241	Wild	17	7.5 ± 3.0	0.174	42	24.3 ± 13.6	0.09
		Variant	23	8.3 ± 2.3		47	30.7 ± 19.5	
XPD	751	Wild	7	7.2 ± 2.1	0.56	39	27.1 ± 15.7	0.99
		Variant	33	8.1 ± 2.7		50	28.1 ± 18.4	
MSH3	1036	Wild	1	12.0	–	8	25.0 ± 22.1	0.21
		Variant	39	7.9 ± 2.6		81	27.9 ± 16.8	

reported in people occupationally exposed to X-rays than in normal controls without a dose-dependent increase in yield as a function of duration of exposure (Zakeri and Assaei, 2004; Sari-Minodier *et al.*, 2007). This can be explained by the fact that during long term exposure part of the DNA damage is not detected because of the death of lymphocytes.

By division of the ICs into time exposure sub-sets of less than, or more than, 10 years the results showed that MN frequencies were higher in the ICs with time exposure of ≥ 10 years in comparison to < 10 years (32.0 ± 18.2‰ *vs.* 24.6 ± 15.9‰, $p = 0.039$) (Fig. 1b) due to their higher exposure levels (6.4 ± 4.5 mSv *vs.* 2.8 ± 2.7 mSv, respectively).

As shown in Figure 1c significant difference in MN frequency was also observed between occupational radiation doses of ≥ 3 mSv and < 3 mSv during last year of exposure (36.4 ± 23.2‰ *vs.* 23.5 ± 11.5‰, $p = 0.002$). However, for last five years of exposure there was no significant difference between radiation doses of ≥ 10 mSv and < 10 mSv ($p = 0.11$), may be due to the unstable nature of the MN aberrations.

The radiation doses in the studied groups of interventional cardiologists have been routinely monitored by film badges they wore under lead aprons. However, it has been reported in ICRP (2011), that personal dosimeters are not used by some professionals or their use is irregular and as a consequence, occupational doses in several fluoroscopically guided practices are largely unknown.

As shown in Table 3, none of the polymorphisms was associated with MN frequency in the whole population. However, the 'variant' of the XRCC3 gene at $p = 0.09$ is not far from significance.

Considering the XRCC3 codon 241 genotypes, significantly higher MN frequency was found in ICS exposed to ≥ 10 mSv carrying the variant-type XRCC3 241Met alleles compared to those carrying wild-type XRCC3 (Thr/Thr) allele (exposed to ≥ 10 mSv) and also to the referent counterparts carrying either the variant or wild-type XRCC3 genotypes with exposure < 10 mSv (40.5 ± 24.0‰ *vs.* 22.1 ± 16.4‰, 25.1 ± 12.8‰, 25.1 ± 14.2‰; $F = 4.5$, $p = 0.002$) (Tab. 4).

The small sample size of individuals per category in our study is an important limitation that may affect the statistical power of findings. Therefore, larger scale studies are needed for assessing the interaction between genes and environmental factors and confirming these results.

As the XRCC3 gene acts in the homologous recombination repair pathway of DNA double strand breaks, this is an interesting result. The scaffolding protein XRCC3 is a paralog of the very important RAD51 gene involved in the first step of homologous recombination repair of DNA double-strand breaks. The XRCC3 Thr241Met polymorphism has been associated with an increase in DNA damage (Matullo *et al.*, 2001; Au *et al.*, 2003) although the reason is not clear. It has been reported earlier that the Met/Met variant of XRCC3 results in an increased frequency of micronuclei in workers exposed to ionizing radiation (Andreassi *et al.*, 2009). The effect of the XRCC3 codon 241 variants on the frequency of MN may be due to the chromosome loss caused by malsegregation and centrosome amplification and relatively low DNA repair capacity (Yoshihara *et al.*, 2004; Lindh *et al.*, 2006). XRCC3 acts in the HRR pathway for correct chromosome segregation and stability (Wildinga *et al.*, 2005). XRCC3 241Met causes a deficiency in the structure of the DNA repair protein and therefore in the HRR pathway and the repair mechanism may shift toward NHEJ, with chromosome instability and repair deficiency (Song *et al.*, 2015).

Further analysis of different interactions between genotypes revealed by ANOVA analysis showed significantly higher MN frequencies in the following subsets:

- Control individuals with one variant allele for XRCC1 and XRCC3 polymorphisms compared to controls carrying both wild-type alleles for XRCC1 (Arg/Arg) and XRCC3 (Thr/Thr) polymorphisms (8.6 ± 2.5‰ *vs.* 7.0 ± 2.8‰, $p = 0.04$), (Fig. 2).
- Control individuals carrying both variant alleles for XRCC3 241Met and XPD 751Gln polymorphisms compared to controls with one variant alleles for XRCC3 and XPD polymorphisms (8.7 ± 2.2‰ *vs.* 7.1 ± 2.8‰, $p = 0.04$) (Fig. 3). Apparently, a combination of adverse genotypes may increase the risk of DNA damage. XPD in NER repair pathway senses the distortion caused by a base and is involved in the removal of cyclopyrimidine dimers, photoproducts produced by UV light and bulky lesions in human DNA induced by environmental physical or chemical agents (Friedberg *et al.*, 1995). XPD gene defects are involved in genetic disorders of cancer-prone syndrome xeroderma pigmentosum complementation group D, trichothiodystrophy and Cockayne syndrome. The possible role for XPD in the repair of oxidative damages induced by ionizing radiation has been also reported (Angelini *et al.*, 2005). In our study, the relatively

Table 4. Associations of different DNA repair gene polymorphisms with the MN frequencies in the ICs group subdivided by exposure status.

Cardiology staff		Exposure (last year)		Exposure (last 5 years)	
		< 3 mSv	≥ 3 mSv	< 10 mSv	≥ 10 mSv
XRCC1 codon 194	W(n)	23.6 ± 12.0* (54)	**40.2 ± 24.5 (23)**	25.5 ± 14.2 (53)	35.3 ± 24.1 (24)
	M(n)	22.3 ± 5.2 (6)	21.6 ± 5.9** (6)	22.5 ± 5.4 (8)	21.0 ± 5.7 (4)
	p value	**$p < 0.0005$*** **$p = 0.04$****		NS	
XRCC3 codon 241	W(n)	22.2 ± 9.5* (33)	32 ± 22.5 (9)	25.1 ± 12.8 (31)	22.1 ± 16.4 (12)
	V(n)	25.0 ± 13.6** (27)	**38.4 ± 23.8 (20)**	25.1 ± 14.2 (30)	40.5 ± 24.0 (17)
	p value	**$p = 0.001$*** **$p = 0.01$****		**$F = 4.5$**	**$p = 0.002$**[a]
XPD codon 751	W(n)	23.6 ± 12.4 (28)	36.0 ± 20.2 (11)	25.2 ± 15.0 (29)	32.5 ± 17.2 (10)
	V(n)	23.4 ± 10.9 (32)	**36.6 ± 25.4 (18)**	25.0 ± 11.9 (32)	33.7 ± 25.9 (18)
	p value	**$F = 3.48$** **$p = 0.019$**[a]		NS	
MSH3 codon 1036	W(n)	16.0 ± 9.2 (6)	52.0 ± 32.5 (2)	17.8 ± 9.0* (5)	37.0 ± 34.6 (3)
	V(n)	24.3 ± 11.5 (54)	**35.2 ± 22.8 (27)**	25.8 ± 13.5 (56)	**32.8 ± 22.0 (25)**
	p value	**$F = 5.54$** **$p = 0.002$**[a]		**$p = 0.044$***	

W: wild type, V: variant type. ∗or ∗∗ Mean ± SD *vs.* bold variable is significant at the 0.05 level by least significant difference (LSD) post hoc test.
[a] ANOVA *p* value among groups.

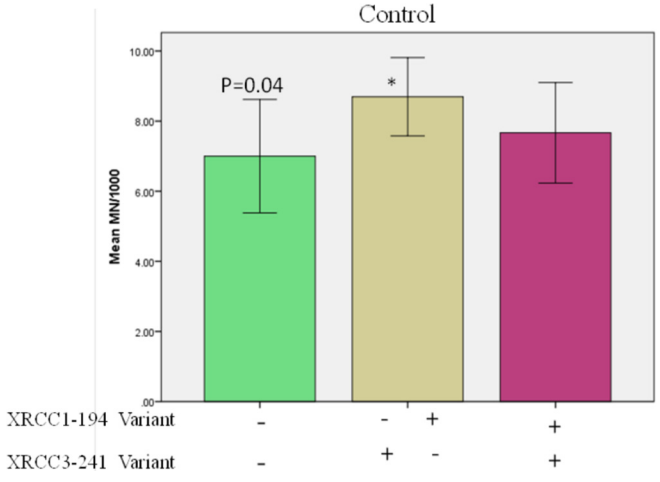

Fig. 2. Interactive effects of *XRCC1* codon 194 and *XRCC3* codon 241 genes in the control group. Control individuals with one variant allele for XRCC1 and XRCC3 polymorphisms showed significantly higher MN frequencies compared to controls carrying both wild-type alleles for XRCC1 and XRCC3 polymorphisms.*Significant at $p = 0.04$ *vs.* homozygous wild (−−) genotypes (ANOVA analysis). (−−): both wild genotypes, (−+ or +−): one variant genotypes and (++): homozygous variant genotypes.

small sample size of subjects having this genotype may affect the result, however, the strength of this study is the analysis of a study group of individuals exposed to significant occupational radiation doses.

– However, in the ICs, individuals carrying both wild-type alleles for XRCC3 (Thr/Thr) and MSH3 (Ala/Ala) polymorphisms showed a significantly lower MN frequency or a better repair compared to exposed individuals with one or two variant alleles for XRCC3 and MSH3 polymorphisms (16.0 ± 9.2‰ *vs.* 27.1 ± 15.7‰ and 29.7 ± 18.8‰, $F = 3.28$, $p = 0.04$) (Fig. 4).

This observed association in the radiation exposed individuals is the first report and needs to be confirmed in other studies with larger population exposed to radiation. However, one possible explanation for this result is that combination of these wild type genotypes and their effects on DSBs repair and correction of mispaired bases during DNA replication errors may contribute to the lower MN frequencies. MMR initially corrects single base-pair mismatches and small insertion-deletion loops during replication recognized by the MSH2–MSH6 heterodimer (MutSa) and larger insertion-deletion loops are recognized by the MSH2–MSH3 heterodimer (MutSb) (Berndt *et al.*, 2007). Song *et al.* (2006) reported an increased ovarian cancer risk with single nucleotide polymorphisms in MSH6 and MSH3. The

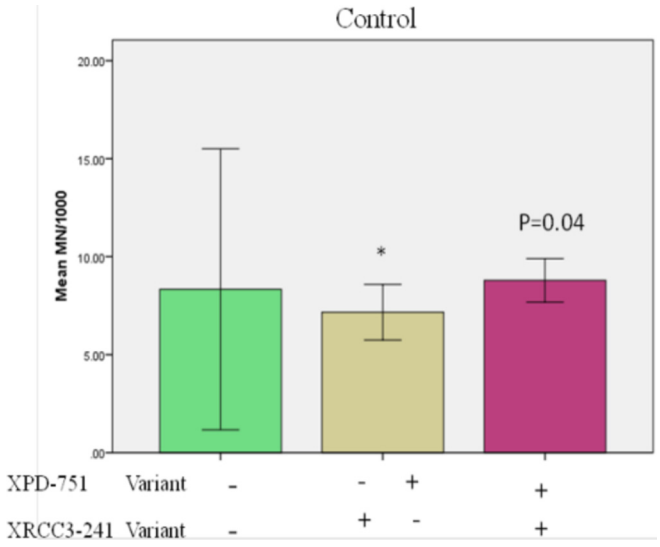

Fig. 3. Interactive effects of *XPD* codon 751 and *XRCC3* codon 241 genes in the control group. Control individuals carrying both variant alleles for XRCC3 241Met and XPD 751Gln polymorphisms showed significantly higher MN frequencies compared to controls with one variant alleles for XRCC3 and XPD polymorphisms. *Significant at $p = 0.04$ *vs.* homozygous variant (++) genotypes (ANOVA analysis). (−−): both wild genotypes, (−+ or +−): one variant genotypes and (++): homozygous variant genotypes.

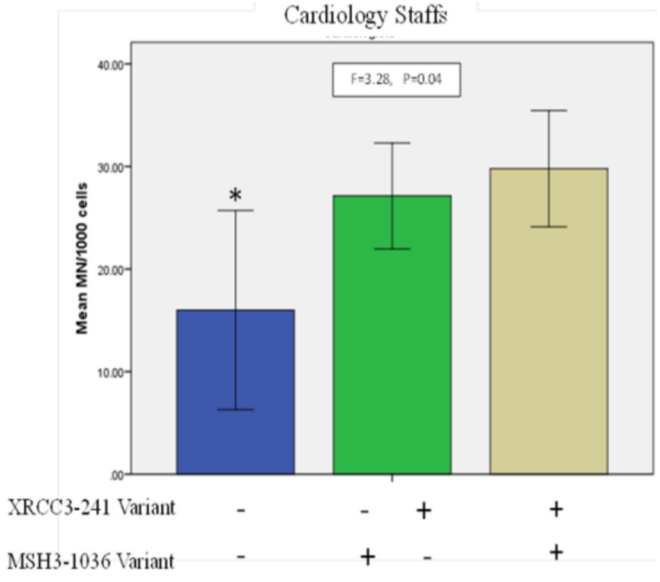

Fig. 4. Interactive effects *of XRCC3* codon 241 and *MSH3* codon 1036 genes in the ICS group. Individuals carrying both wild-type alleles for XRCC3 and MSH3 polymorphisms showed a significantly lower MN frequency compared to exposed individuals with one or two variant alleles for XRCC3 and MSH3 polymorphisms. *Significant at $p = 0.04$ *vs.* one variant genotypes (−+ or +−) and at $p = 0.01$ *vs.* homozygous variant (++) genotypes. (−−): both wild genotypes, (−+ or +−): one variant genotypes and (++): homozygous variant genotypes.

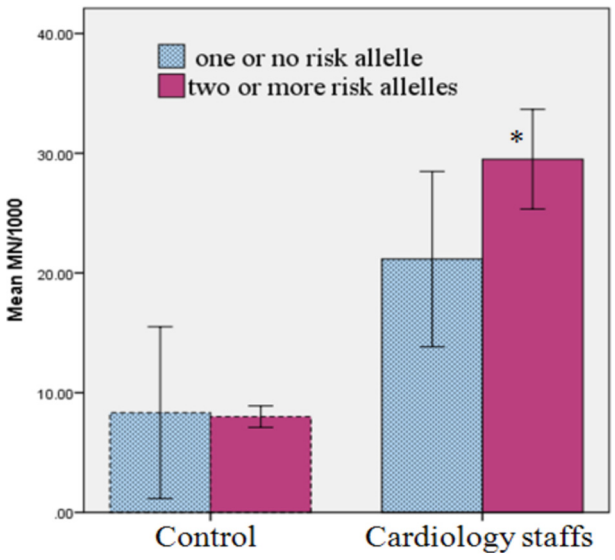

Fig. 5. MN frequency of individuals with one or no risk allele compared to those with two or more risk alleles in ICS and control groups. *Significant at $p = 0.01$ *vs.* one or no risk allele.

polymorphism in MSH3 and p53 codon 72 may increase the risk for prostate cancer (Hirata et al., 2008).

Individuals carrying polymorphisms of genes in two important DNA repair pathways (XRCC1 of BER, XRCC3 of HRR; XRCC3 of HRR and XPD of NER; XRCC3 of HRR and MSH3 of MMR) are very likely affected in their repair of specific DNA lesions involving these different repair pathways. This is especially of importance if the inducing agent consists of ionizing radiation inducing multiple types of DNA lesions.

– Multiple regression analysis of the ICs revealed the presence of risk allele ≥ 2 as a significant predictor of MN frequency (standardized $\beta = 0.269$, $p = 0.01$). As shown in Figure 5 in the ICs individuals with two or more risk alleles compared to those with one or no risk allele showed significantly higher MN frequencies (29.5 ± 17.4‰ *vs.* 21.1 ± 15.1‰, $p = 0.01$) (Fig. 5).

In conclusion, our results indicate a relationship between MN frequency as a marker of genotoxic effect and genetic polymorphisms in genes coding for DNA repair enzymes. Our results showed that even low doses of ionizing radiation exposure could have genotoxic effects and that genetic polymorphisms in susceptible individuals occupationally exposed to low doses of ionizing radiation might contribute to the increased DNA damage. This study may be helpful in developing genetic screening tests to identify radiosensitive workers. Identification of radiosensitive workers may help in providing better medical surveillance programs and job-specific strategies to reduce dose. Additional protections, more restricted dose limits, especial education and training should be made available for sensitive individuals working in places where occupational radiation exposures may be significant. Moreover, it is unethical and unwise to put radiosensitive individuals in situations where they might receive a large dose (ICRP Publication 118, 2012). However, for better understanding of

the effects of genetic polymorphisms on MN induction further larger population studies with considering individual DNA repair capacity are recommended. The present study confirmed the usefulness of MN analysis for cytogenetic biomonitoring of radiation workers and increased levels of MN in lymphocytes of interventional cardiologists showed the need for improvement of their safety and training programs.

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