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Original article

Gender bias in individual radiosensitivity and the association with genetic polymorphic variations

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ABSTRACT

Purpose: To assess the extent of variation in radiosensitivity between individuals, gender-related dissimilarity and impact on the association with single nucleotide polymorphisms (SNPs).

Materials and methods: Survival curves of 152 fibroblast cell strains derived from both gender were generated. Individual radiosensitivity was characterized by the surviving fraction at 2 Gy (SF2). SNPs in 10 radiation responsive genes were genotyped by direct sequencing.

Results: The wide variation in SF2 (0.12–0.50; mean = 0.33) was significantly associated with 3 SNPs: *TP53* G72C ($P = 0.007$), *XRCC1* G399A ($P = 0.002$) and *ATM* G1853A ($P = 0.01$). Females and males differed significantly in radiosensitivity ($P = 0.004$) that impacted genetic association where only *XRCC1* remained significant in both gender ($P < 0.05$). Meanwhile, discordant association was observed for *TP53* that was significant in females ($P = 0.012$) and *ATM* that was significant in males ($P = 0.0006$). When gender-specific SF2-mean (0.31 and 0.35 for females and males; respectively) was considered, further discordance was observed where *XRCC1* turned out not to be associated with radiosensitivity in males ($P > 0.05$).

Conclusions: Although the variation in individual radiosensitivity was associated with certain SNPs, gender bias for both endpoints was evident. Therefore, assessing the risk of radiation exposure in females and males should be considered separately in order to achieve the ultimate goal of personalized radiation medicine.

Ionizing radiation (IR) is ubiquitous in nature and living organisms are continuously exposed to variable level of low radiation doses from natural radioactive background and escalating doses from medical practices and industrial applications [1]. Although IR has many beneficial applications in modern life, it might cause deleterious effects particularly if it has been misused [2–4].

Individuals, however, do not respond equally to similar doses of IR. Human population heterogeneity in radiosensitivity is illustrated by rare genetic disorders such as ataxia-telangiectasia (A-T), Nijmegen breakage syndrome (NBS), NBS-like, ligase IV deficiency (LIG4 syndrome) and ataxia-telangiectasia like disorder (ATLD). Cells derived from those patients are hypersensitive to IR due to mutations impacting DNA double-strand break (DSB) recognition, signaling, and repair capacity [5,6]. However mutations are rare and present only in a small percentage of hypersensitive individuals [7].

To explain the wide range of radiosensitivity, attention is focused on the more common genetic polymorphic variations between individuals. Unlike genetic mutations that disrupt the function of the encoded protein, single nucleotide polymorphisms (SNPs) may only cause subtle changes that can influence the rate of mRNA transcription, mRNA stability, its rate of translation to protein and/or the protein–protein interactions resulting in sub-optimum protein function leading to different degrees of susceptibility to IR, environmental factors, infectious agents, diseases and individual response to pharmacological agents [8,9]. Furthermore, there are variations between human populations, so a SNP allele that is common in one geographical or ethnic group may be infrequent in another [10].

The association between SNPs and radiosensitivity in the general population has not been systematically studied. This is important because IR poses accentuated health hazard particularly with the continuous increase in the applications of radiation

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technologies in various aspects of health and diseases. The consequent increase in the collective doses received by the population particularly in radiosensitive individuals [11] may cause an increase in the cumulative deleterious effects in humans which in its turn may be translated to increase in the long term appearance of certain types of complications and cancers [12]. The main deterministic and stochastic health effects of radiation exposure are the induction of toxicity in organs and tissues, neoplastic transformation in addition to potential hereditary consequences [4].

The term "radiogenomics" has initially been applied to identify candidate genetic biomarkers to individualize risk of developing morbidity in radiotherapy patients [13], which gained momentum with the advent of genome wide association studies [14,15]. Similarly, it seems tempting to hypothesize that "radiogenomics" can also apply to individual variations in radiosensitivity in the populace. Currently health protection policies do not take into account any contribution of genetic variations to individual risk of radiation exposure [16]. Such contribution would help to develop more refined approaches to assess radiation health risk in humans.

In this study, we have explored this hypothesis using 152 fibroblast cell cultures established from normal individuals. Cellular radiosensitivity was measured by the gold-standard clonogenic survival assays. Genetic variations were determined by direct genotyping of 10 selected SNPs in genes known to be involved in radiation response (*CDKN1A (p21)* C31A (Ser/Arg) rs1801270, *TP53 (p53)* codon G72C (Arg/Pro) rs1042522, *HDM2 (MDM2)* promoter T309G rs2279744, *ATM* G1853A (Asp/Asn) rs1801516, *XRCC1* G399A (Arg/Gln) rs25487, *XRCC3* G241A (-strand C/T) (Thr/Met) rs861539, *LIG4 (DNA-Ligase 4)* C9T (Thr/Ile) rs1805388, *PRKDC (DNA-PKcs)* T3434C (-strand A/G) (Ile/Thr) rs7830743, *TGFB1* C10T (Lue/Pro) rs1982073 and *XRCC5 (KU80)* A2790G 3′ UTR rs1051685).

Materials and methods

Cell strains and culture conditions

A total of 152 non-transformed fibroblast cell strains were used from our cell strain collections established from phenotypically normal individuals. The institutional review board (IRB) has approved the study. Donors have voluntarily participated and signed an informed consent. The method of establishing the fibroblast cell strains was described elsewhere [17]. Cells were maintained in DMEM culture medium supplemented with 15% fetal bovine serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin and incubated at 37 °C in 5% CO2 humidified atmosphere.

Cellular radiosensitivity measurements

Experiments were carried out using previously described methodology with minor modifications [18]. Briefly, to minimize experimental variations due to cell cycle differences, contact-inhibited cultures were used. Clonogenic survival was assessed using fixed number of seeded cells (tested + feeder) of 1000 cells/cm^2. Feeder cells, from the same cell strain tested, were irradiated with a single irradiation dose of 30 Gy (to prevent any cell division) and seeded in appropriate numbers 24 h before receiving the tested cells. The tested confluent fibroblast cultures were trypsinized, counted, diluted and seeded in an appropriate number to yield at least 50 colonies in each of 3 replicated flasks. Irradiation, with a single dose that ranged between 0 and 4 Gy, was delayed for 4–6 h after plating to allow the cells to attach to the surface of the flasks. The cells were incubated for 2–3 weeks, then they were fixed and stained using crystal violet. Colonies of at least 50 cells were scored as survivors. Three to five independent experiments were carried out for each cell strain.

DNA extraction, amplification and sequencing

DNA was extracted from cultured fibroblasts using Puregene DNA Purification Kit (Gentra System, Qiagen, Minneapolis, MN, USA) according to the manufacturer's instruction. PCR primers of the selected SNPs are available upon request. Relevant segments of DNA were amplified by thermal cycling as described previously [19]. The amplified fragment was directly sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instruction, and were run on the MegaBase 1000 sequencer (Applied Biosystems, Waltham, MA, USA). Sequencing results were aligned to the corresponding reference sequence and the SNPs were genotyped using SeqManII sequence analysis software (DNASTAR Inc., Madison, WI, USA).

Irradiation

Irradiation was performed using X-RAD 320 (Precision X-ray, CT, USA) biological irradiator at a maximum energy of 320 keV filtered with 2 mm Al, and a dose rate of 1.33 Gy/min. In addition to ionizing chamber (PTW, Freiburg, Germany), the absorbed dose was also measured using a GAFCHROMIC film, EBT2 model (International Specialty Products, Wayne, NJ, USA) as described previously [20].

Data analysis

Survival data from replicate experiments were pooled and fitted to the linear quadratic model of cell killing [SF = exp $(-\alpha D - \beta D^2)$, where α and β are constant and D is the dose], to generate cellular survival curves. The well-established parameter of the surviving fraction at 2 Gy (SF2) was used to characterize the radiosensitivity of each cell strain [21]. SF2 was computed from the whole survival curve and used as a unique measure of cellular sensitivity to radiation. The mean SF2 of the 152 cell strains was used to separate cell strains to 2 groups, radiosensitive (cases) and normal (controls).

The association between radiosensitivity groups (SF2), SNPs genotype and allelic frequency were measured by the odds ratio (OR) with its 95% confidence interval (95% CI). Significance of OR was assessed by the Chi-square (χ^2) test. A P-value of 0.05 or less is considered statistically significant. The alleles showing statistically significant ($P \leqslant 0.05$) association with increased radiosensitivity (decreased SF2) were considered as risk allele and given a score of 1. Therefore, cell strains homozygous for a risk allele have a score of 2, heterozygous have a score of 1, while those which do not harbor the risk allele have a score of zero. The number of risk alleles for each individual was calculated by summing the scores of the different SNPs significantly associated with radiosensitivity. Difference between groups was assessed by the non-parametric Mann–Whitney Rank Sum test. Correction for multiple comparisons was carried out using the Bonferroni method, which indicates statistical significance when the P-value is lower than the type I error (0.05) divided by the number of comparisons declared significant. Statistical analysis was carried out using the SigmaPlot platform (Version 12.5, Systat Software, Inc., San Jose, CA, USA) and the free online software, *Case Control Studies*, Institute of Human Genetics, Helmholtz Center Munich, Germany (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Results

Subjects and cellular radiosensitivity

The age of the 152 subjects included in this study ranged between 18 and 79 (median = 48) years old. There were 63 males and 89 females. The survival curves of the 152 fibroblast cell

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strains derived from those subjects showed a wide range of radiosensitivity (Fig. 1, panel A). The SF2 ranged between 0.12 and 0.50 (Fig. 1 panel B) with a mean of 0.33 (SD = 0.089). The mean SF2 was used to separate the cell strains according to radiosensitivity to 2 groups: normal (control, SF2 > 0.33, $n = 83$) and radiosensitive (cases, SF2 ⩽ 0.33, $n = 69$). The mean SF2 of the control and the radiosensitive groups were 0.40 (SD = 0.039) and 0.25 (SD = 0.062); respectively. A gender related differences in radiosensitivity was observed. The average SF2 of the survival curves of females donors (SF2 = 0.31, C.I. 95% = 0.019) was slightly lower than that of males (SF2 = 0.35, C.I. 95% = 0.020). Box plot analysis of the relationship between SF2 and gender showed lower median SF2 for females (0.33) than for males (0.36) donors (Fig. 2). The Mann–Whitney sum rank test showed that differences between the median SF2 was statistically significant ($P = 0.004$).

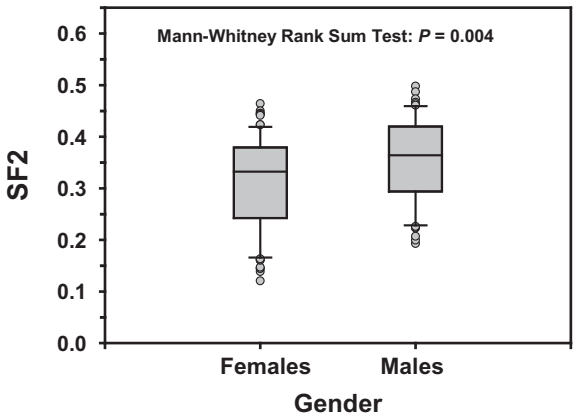

Fig. 2. Box plot analysis of the relationship between radiosensitivity (SF2) and gender of donors. The lines within the boxes represent the median SF2 of females and males. The lower and the upper boundaries of the box indicate the 25th and the 75th percentiles, respectively. The bars above and below the box indicate the 90th and 10th percentiles. Data points represent the outliers.

SNPs genotypes and alleles analysis

The genotype distribution of the assessed polymorphisms and its relationship with radiosensitivity (SF2) are given in Fig. 3. Although a wide range of variations were observed, there were apparent patterns between the mean SF2 per genotype and the polymorphisms studied. The pattern was gene dependent. The *CDKN1A* C31A and *ATM* G1853A showed a trend toward increased radiosensitivity with the presence of the variant genotype. Conversely, the *TP53* G72C, the *XRCC1* G399A, *XRCC5* A2790G and *PRKDC* T3434C showed a trend toward decreased sensitivity with the presence of the variant genotype. The remaining SNPs (*HDM2* T309G, *TGFB1* C10T, *XRCC3* G241A, and *LIG4* C9T) could hardly reveale any trend toward dependence of SF2 on genotypes (Fig. 3).

Statistically significant associations between genotypes and groups of radiosensitivity were observed for *TP53* G72C, *ATM* G1853A and *XRCC1* G399A, particularly when comparing homozygous variants with majority genotypes (Table 1). These associations were more obvious with the allelic analysis. Both variant alleles of *TP53* 72C (Pro) and *XRCC1* 399A (Gln) were significantly associated with more radio-resistant (higher SF2) phenotype [$P = 0.007$, Odds Ratio = 0.52 (C.I. 95%: 0.33–0.84) and $P = 0.002$, Odds Ratio = 0.41 (C.I. 95%: 0.23–0.74); respectively]. In contrast, the variant allele of *ATM* 1853A (Asn) was significantly associated with radio-sensitive (lower SF2) phenotype [$P = 0.01$, Odds Ratio = 2.96 (C.I. 95%: 1.24–7.04)]. Borderline ($P > 0.05$; ⩽0.10) allelic association was observed for *TGFB1* rs1982073 where the variant allele 10C (Pro) tended to be associated with lower SF2 [$P = 0.09$, Odds Ratio = 1.48 (C.I. 95%: 0.93–2.36)].

The alleles that showed statistically significant association with increased radiosensitivity [majority alleles *TP53* G72 (Arg), *XRCC1* G399 (Arg) and variant *ATM* 1853A (Asn)] have been counted to calculate the total number of risk alleles for each cell strain. The relationship between the number of risk alleles and SF2 has been analyzed by Box Plot (Fig. 4). To obtain sufficient numbers for meaningful analysis, the total risk alleles have been grouped as follows: 0 + 1, 2, 3, 4, and 5 + 6. Although there were variations, the median value of SF2 showed a clear trend to decrease (higher radiosensitivity) with increasing number of risk alleles. The Kruskal–Wallis One Way Analysis of Variance on Ranks confirmed this trend and showed a statistically significant difference in the median number of risk alleles (ANOVA on ranks: $P = 0.012$).

Discussion

The objective of this study was to evaluate the extent of variation in radiosensitivity between individuals and to seek genetic

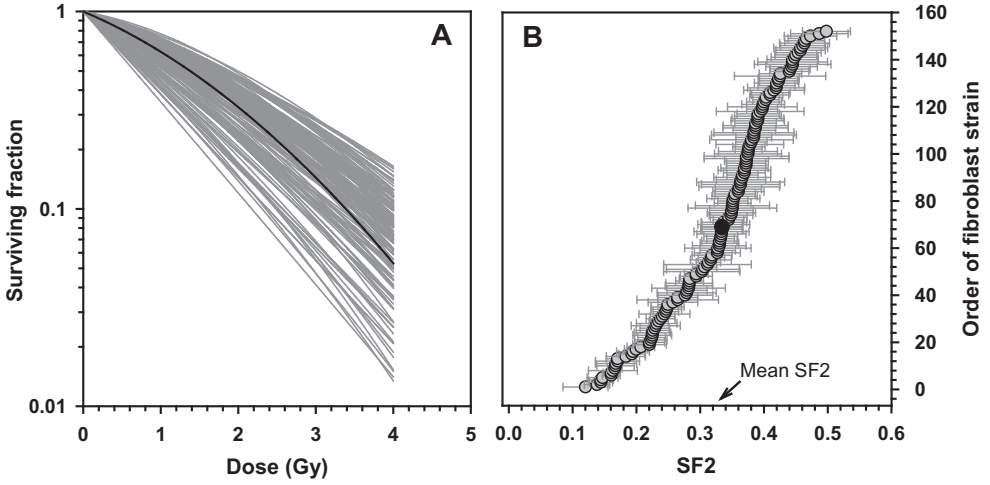

Fig. 1. (A) Clonogenic survival curves of the 152 fibroblast cell strains fitted to the linear quadratic model of cell killing by ionizing radiation. The bold survival curve represents the average survival curve with a mean surviving fraction at 2 Gy (SF2) of 0.33. The cell strains were divided into 2 groups according to the mean (SF2): radiosensitive (below the bold curve) and normal (above the bold curve). The data points and the error bars were removed for clarity. (B) Cumulative distribution of SF2 of the 152 cell strains. Solid symbol position the cut-off of the mean SF2. Error bars represent the 95% confidence intervals.

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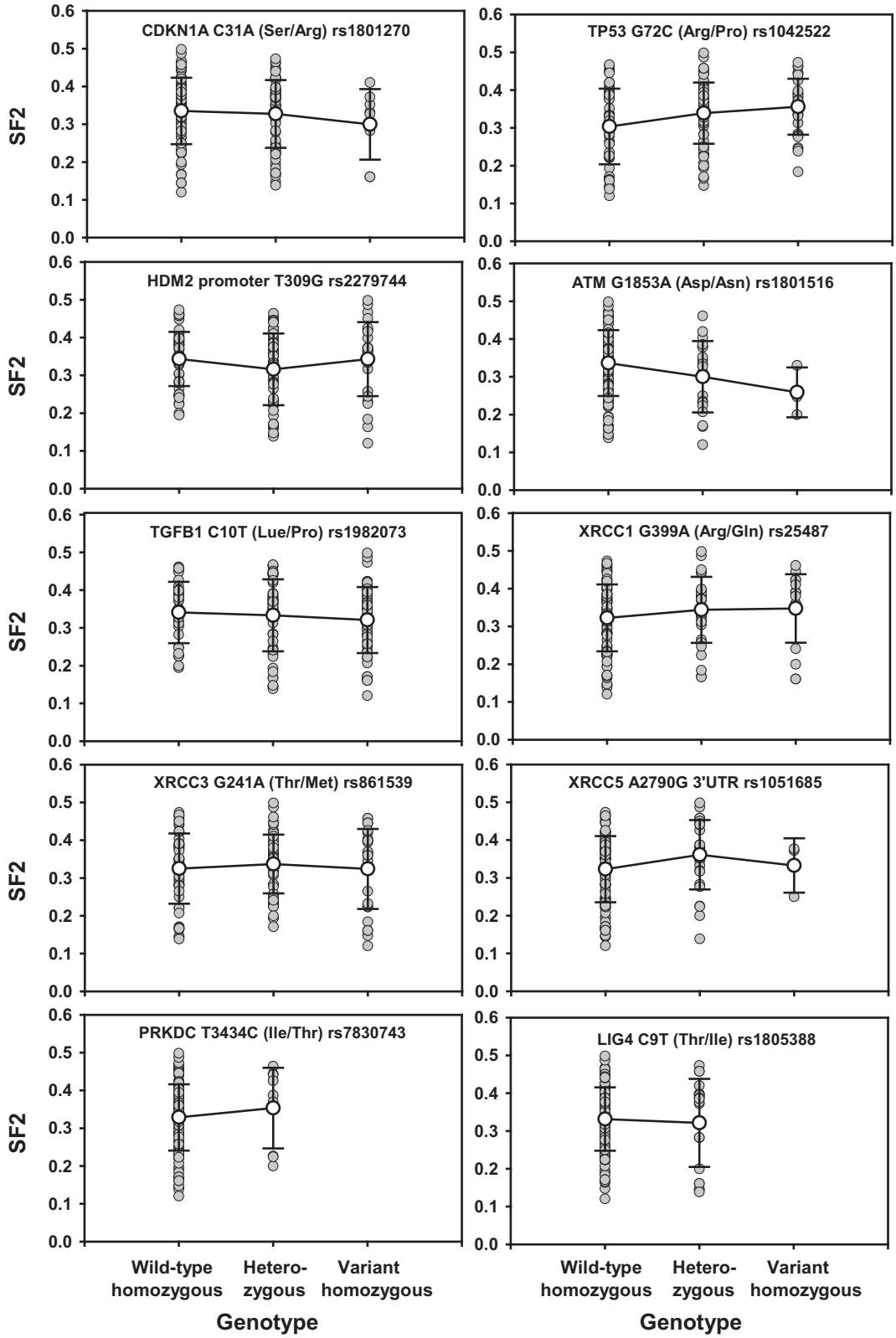

Fig. 3. The relationship between radiosensitivity (SF2) and genotype distribution of the 10 assessed polymorphisms in 152 fibroblast cell strains (gray circles). The open circles represent the mean SF2 by genotype. The error bars are the standard deviation from the mean.

polymorphic biomarkers that can predict cellular response and assess potential risk of radiation exposure [22]. The presence of differences in radiation sensitivity between individuals is expected to render subjects more or less susceptible to radiation-induced injuries [23]. Thus some individuals may be more vulnerable and are at increased risk of sustaining acute, short-term reactions or latent, long-term radiation consequences.

To our knowledge, this is one of the largest cohort of radiation sensitivity study involving fibroblast cell strains derived from normal individuals [18,24,25]. The age of donors ranged between 18

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Table 1
Genotype and allele frequencies of 10 assessed polymorphisms in 152 individuals who either having normal (controls, SF2 > 0.33) or increased (cases, SF2 ⩽ 0.33) cellular radiosensitivity.

Genetic polymorphism	Genotype and allele	SF2 *n* (%)		Odds Ratio (95% CI)	*P*-value
		Cases (*n* = 69)	Controls (*n* = 83)		
CDKN1A (p21) codon 31 C/A (Ser/Arg) rs1801270	C/C	34 (49)	44 (53)		
	C/A	30 (43)	36 (43)	1.07 (0.55–2.08)	0.82
	A/A	5 (07)	3 (04)	2.15 (0.48–9.66)	0.30
	C	98 (71)	124 (75)		
	A	40 (29)	42 (25)	1.20 (0.72–2.00)	0.47
TP53 (p53) codon 72 G/C (Arg/Pro) rs1042522	G/G	32 (46)	21 (25)		
	G/C	27 (39)	42 (51)	0.42 (0.20-0.87)	0.019
	C/C	10 (14)	20 (24)	0.32 (0.12–0.83)	0.017
	G	91 (66)	84 (51)		
	C	47 (34)	82 (49)	0.52 (0.33–0.84)	0.007
HDM2 (MDM2) promoter 309 T/G rs2279744	T/T	22 (32)	30 (36)		
	T/G	38 (55)	35 (42)	1.48 (0.72–3.03)	0.28
	G/G	9 (13)	18 (22)	0.68 (0.25–1.80)	0.43
	T	82 (59)	95 (57)		
	G	56 (41)	71 (43)	0.91 (0.57–1.44)	0.69
ATM codon 1853 G/A (Asp/Asn) rs1801516	G/G	54 (78)	75 (90)		
	G/A	12 (17)	8 (10)	2.08 (0.79–5.44)	0.12
	A/A	3 (04)	0 (0)	9.69 (0.49–191.60)	0.044
	G	120 (87)	158 (95)		
	A	18 (13)	8 (05)	2.96 (1.24–7.04)	0.010
TGFB1 codon 10 C/T (Lue/Pro) rs1982073	C/C	14 (20)	22 (27)		
	C/T	22 (32)	32 (39)	1.08 (0.45–2.55)	0.86
	T/T	33 (48)	29 (35)	1.78 (0.77–4.12)	0.17
	C	50 (36)	76 (46)		
	T	88 (64)	90 (54)	1.48 (0.93–2.36)	0.092
XRCC1 codon 399 G/A (Arg/Gln) rs25487	G/G	53 (77)	50 (60)		
	G/A	12 (17)	18 (22)	0.62 (0.27–1.43)	0.26
	A/A	4 (06)	15 (18)	0.25 (0.07–0.80)	0.014
	G	118 (86)	118 (71)		
	A	20 (14)	48 (29)	0.41 (0.23–0.74)	0.002
XRCC3 codon 241 G/A (-strand C/T) (Thr/Met) rs861539	G/G	30 (43)	28 (34)		
	G/A	27 (39)	40 (48)	0.63 (0.31-1.28)	0.20
	A/A	12 (17)	15 (18)	0.74 (0.29–1.86)	0.53
	G	87 (63)	96 (58)		
	A	51 (37)	70 (42)	0.80 (0.50–1.27)	0.35
XRCC5 (KU80) A2790G 3′ UTR rs1051685	A/A	59 (86)	63 (76)		
	A/G	9 (13)	18 (22)	0.53 (0.22–1.28)	0.15
	G/G	1 (01)	2 (02)	0.53 (0.04–6.04)	0.60
	A	127 (92)	144 (87)		
	G	11 (08)	22 (13)	0.56 (0.26-1.21)	0.14
PRKDC (DNA-PKcs) codon 3434 T/C (-strand A/G) (Ile/Thr) rs7830743	T/T	66 (96)	77 (93)		
	T/C	3 (04)	6 (07)	0.58 (0.14–2.42)	0.45
	C/C	0 (0)	0 (0)	1.16 (0.02–59.54)	1.00
	T	135 (98)	160 (96)		
	C	3 (02)	6 (04)	0.59 (0.14–2.41)	0.51
LIG4 codon 9 C/T (Thr/Ile) rs1805388	C/C	60 (87)	71 (86)		
	C/T	9 (13)	12 (14)	0.88 (0.35–2.25)	0.80
	T/T	0 (0)	0 (0)	1.18 (0.02–60.45)	1.00
	C	129 (93)	154 (93)		
	T	9 (07)	12 (07)	0.89 (0.36–2.19)	0.80

Computed using the free online test for association (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

and 79 with a median age of 48 years. This is the range of age of major workforce for whom safety and health are important to prevent the burden of illnesses and injuries [26]. For example, evidence is emerging that radiation exposure may increase long-term risk of cardiovascular disease [27]. Of particular in this context are those that can emanate from medical procedures and occupational radiation exposure [28].

The clonogenic survival of the 152 fibroblast cell strains revealed a wide range of radiosensitivity that extends to about 4-fold differences between the most sensitive and the most resistant (Fig. 1). This presents sizable differences and could imply that some subjects are more at risk of radiation injuries than others. In addition, gender-related difference in radiosensitivity was observed (Fig. 2). This suggests gender related variation in radiosensitivity where women are slightly but significantly more radiosensitive than men ($P = 0.004$). The relationship between the genotypes of the 10 SNPs studied and SF2 showed various trends indicating increase, decrease and no dependence on radiosensitivity (Table 1). As tendencies toward increased risk from heterozygous to homozygous genotypes was also observed (Fig. 3), this implies that harboring two variant alleles have added influence on radiosensitivity, suggestive of additive model of genetic penetrance [29]. This also indicates that not all variant SNPs are risky, and some of them could be evolutionary advantageous [30].

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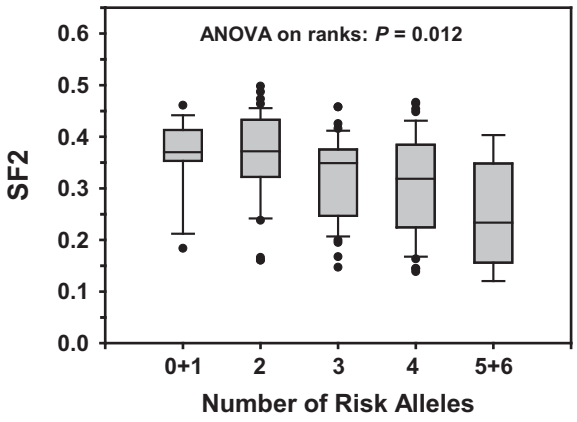

Fig. 4. Box plot analysis of the relationship between radiosensitivity (SF2) and number of risk alleles. The lines within the boxes are the median number of risk alleles. The lower and the upper boundaries of the box indicate the 25th and the 75th percentiles, respectively. The bars above and below the box indicate the 90th and 10th percentiles. Data points represent the outliers.

The analysis of the allelic frequencies provides further insight into the relationship between genotypes and phenotypes. This is particularly true for non-synonymous SNPs where allelic expression becomes an important tool for integrating genome and transcriptome data to characterize various biological phenomena [31]. The association observed for *TP53* rs1042522 ($P = 0.007$), *ATM* rs1801516 ($P = 0.01$) and *XRCC1* rs25487 ($P = 0.002$) remains statistically significant after taking into consideration multiple comparisons using the Bonferroni correction, which in this case equals to 0.017. The collective effects of the number of risk alleles associated with increased radiation sensitivity [the majority alleles *TP53* G72 (Arg) and *XRCC1* G399 (Arg), and the variant allele *ATM* 1853A (Asn)] showed increased cellular radiosensitivity with increasing number of risk alleles (Fig. 4). The significant correlation observed (ANOVA on ranks, $P = 0.012$) indicates that harboring higher number of risk alleles has incremental effect on radiosensitivity. This illustrates that cellular radiation response requires the concerted action of multiple genes and further support the conclusion that radiosensitivity is a complex genetically controlled trait with the outcome being determined by multitude of additive effects. These genes could be candidate biomarkers in occupationally, environmentally or medically exposed groups [22] and possible targets for innovative therapies in radiosensitive individuals.

The gender related discrepancy in radiosensitivity observed in this study is of particular significance. We have separated allelic frequencies of males and females of the 3 SNPs that showed statistically significant associations and we have re-tested their associations with SF2 within each gender. We have observed discordant association for the 3 SNPs tested (Table 2). Using the combined SF2 mean of 0.33, the association between radiosensitivity and *XRCC1* rs25487 remained statistically significant ($P < 0.05$) in both genders. Meanwhile, discordant association was observed for *TP53* rs1042522 that was significant in females ($P = 0.012$) and *ATM* rs1801516 that was significant in males ($P = 0.0006$). Consequently, we have re-tested the association using gender-specific SF2 mean (0.31 and 0.35 for females and males; respectively). *TP53* rs1042522 and *ATM* rs1801516 have maintained the same results mentioned earlier. While, *XRCC1* rs25487 has retained a near-borderline significant association (precisely, $P = 0.0504$) in females, it turned out not to be associated with radiosensitivity in males ($P > 0.05$).

Table 2
Allele frequencies and comparison of gender-related associations of 3 SNPs that showed significant link with radiosensitivity in the 152 individuals. The comparison was carried out in two steps: (A) combined mean SF2 = 0.33 (females: cases = 46, control = 43; males: cases = 23, controls = 40), and (B) gender-related mean SF2 (0.31 for females: cases = 37, controls = 52; and 0.35 for males: cases = 30, controls = 33).

SNPs	Gender	Allele frequencies: *n* (%)		Odds Ratio (95% CI) *P*-value	Concordance F/M
		Cases	Controls		
(A) Combined mean SF2					
TP53 G72C rs1042522	Females	G: 63 (68)	G: 43 (50)	0.46 (0.25–0.84)	No
		C: 29 (32)	C: 43 (50)	0.012	
	Males	G: 28 (61)	G: 41 (51)	0.67 (0.32–1.41)	
		C: 18 (39)	C: 39 (49)	0.29	
ATM G1853A rs1801516	Females	G: 86 (93)	G: 82 (95)	1.43 (0.38–5.25)	No
		A: 6 (7)	A: 4 (5)	0.74	
	Males	G: 34 (74)	G: 76 (95)	6.70 (2.01–22.30)	
		A: 12 (26)	A: 4 (5)	0.0006	
XRCC1 G399A rs25487	Females	G: 77 (84)	G: 59 (69)	0.42 (0.20-0.87)	Yes
		A: 15 (16)	A: 27 (31)	0.017	
	Males	G: 41 (89)	G: 59 (74)	0.34 (0.11–0.98)	
		A: 5 (11)	A: 21 (26)	0.039	
(B) Gender-related mean SF2					
TP53 G72C rs1042522	Females	G: 51 (69)	G: 55 (53)	0.50 (0.27–0.94)	No
		C: 23 (31)	C: 49 (47)	0.031	
	Males	G: 34 (57)	G: 35 (53)	0.86 (0.42–1.74)	
		C: 26 (43)	C: 31 (47)	0.68	
ATM G1853A rs1801516	Females	G: 70 (95)	G: 99 (95)	1.13 (0.29–4.36)	No
		A: 4 (5)	A: 5 (5)	1.00	
	Males	G: 47 (78)	G: 63 (95)	5.80 (1.56–21.54)	
		A: 13 (22)	A: 3 (5)	0.0039	
XRCC1 G399A rs25487	Females	G: 62 (84)	G: 74 (71)	0.47 (0.22–1.01)	No
		A: 15 (16)	A: 30 (29)	0.050	
	Males	G: 47 (78)	G: 53 (80)	1.12 (0.47–2.67)	
		A: 13 (22)	A: 13 (20)	0.78	

Computed using the free online test for association (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

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These are intriguing results and suggest that females and males are two distinct populations and considering them together as one group introduces bias in genetic association studies. Although we do not dispose of mechanistic explanation for these observed differences, it is plausible that it may emanate from genetic and physiological dissimilarities between the two genders where gene regulation and also hormonal factors may play important roles. This conclusion may imply that women are relatively at higher risk of sustaining radiation injuries than men. This is plausible as gender related differences in immunity were also observed and attributed to the sex-specific differences in immune and endocrine systems [32]. Gender-related differences in radiosensitivity in human have occasionally been described for certain endpoints such as proteins expression in preoperative radiotherapy of rectal cancer patients [33], hematopoietic stem cells (HSCs) [34], and DNA double-strand breaks damage and repair [35]. In addition, since some studies have reported individual differences in cellular radiosensitivity at higher doses [36], we have also computed the surviving fraction at 4 Gy (SF4) which had essentially recapitulated the main findings as with SF2.

The projection of these results on clinical radiosensitivity in radiotherapy patients could be important as many studies have reported significant associations with genetic polymorphic variations while other could not ascertain such involvements (see for examples [19,37–41]). The observed gender-related bias may contribute to the inconsistency observed between studies [42]. The advent of genome-wide association (GWAS) and copy number variation (CNVs) studies may further uncover specific genetic differences between males and females. These could be of particular importance for normal tissue complication risk due to gender-related variation in gene regulation and hormonal factors that may impact tissular interaction and affect long-term tissue remodeling and production of extra-cellular matrix. Therefore, gender-specific radiosensitivity should be considered as contributing variable to the development of complications to radiotherapy [43].

Conclusions

Although the wide variation in individual radiosensitivity were significantly associated with *TP53 (p53)* codon G72C (Arg/Pro) rs1042522, *ATM* G1853A (Asp/Asn) rs1801516 and *XRCC1* G399A (Arg/Gln) rs25487, it showed gender-related discrepancies. This gender bias warren against combining data from males and females as their radiosensitivity seems to be slightly but significantly different. Further attention to gender-related dissimilarities in various cellular, genomic and clinical endpoints are essential in order to ascertain the values of biological markers in assessing individual risk of radiation exposure and progress toward personalized radiation medicine.

Conflict of interest statement

The authors declare no conflict of interest related to this manuscript.

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