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HERITABILITY IN RADIATION RESPONSE INVESTIGATION OF H2AX AND MDM2

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ABSTRACT

Radiation response is closely related to individual cancer risk and it vary due to genetic and environmental factors. There are only a few quantitative estimates of genetic factor impact, also known as heritability, in radiation protection studies. In the presented work, the estimation of heritability for H2AX and MDM2, which are radiation related biomarkers, was performed. To achieve these results the most powerful tool of signal variance decomposition by the structural equation modeling method on a group of dizygotic and monozygotic twins was performed. The results of heritability estimation were accompanied by genome-wide association study. It was shown that, narrow-sense heritability for H2AX equals $\sim$46%, while for MDM2 the genetic factor does not have significant impact on phenotype variation under irradiation. The equivalent outcomes were observed for single nucleotide polymorphism investigation via genome-wide association study. In case of H2AX, the 2,816 polymorphisms were detected as related to signal level, while for MDM2 none of polymorphism was detected. In the group of statistically significant SNPs for H2AX, the rs9298170 located EYA1 gene was marked as SNP of special interest, while EYA1 directly interact with H2AX. The presented study suggests that genetic factors in H2AX activity contribute significantly to human variation of radiation response. Furthermore, those outcomes can potentially be incorporated in radiation risk assessment in public health radiation protection, as H2AX was previously shown to be a potential biomarker of radiation response.

INTRODUCTION

The human body is exposed to a variety of harmful factors starting from environmental and finishing at medical or industrial influences. One of them, is ionizing radiation (IR), which occurs in natural environment (e.g. radiation of outer space), medical application (e.g. x-rays, computed tomography) and industrial application (e.g. radiation disasters). It is well known that exposure to IR leads to cancer via a variety of causes, like double strand breaks of DNA, disorders in apoptosis or cell cycle [1]. People react to IR in different ways from the ability to stand high doses of radiation to fast toxic reaction. This individual reaction to irradiation is called radiosensitivity

and represents adaptation of our organism to environmental background through genetic variation. Over the years several biomarkers of radiosensitivity were pointed out e.g. H2AX, BBC3, SENS1, GADD45A, FDXR, CDKN1A or MDM2 [2],[3],[4]. However, during the investigation of radiation response effects, health protection standards in general do not consider the contribution of genetic variation in individual risk. Nevertheless, it is of great importance to assess the influence of genetic factors which could refine approaches to assess radiation health risk and radiosensitivity in humans.

The most powerful data for the variance decomposition of trait, are twin studies based on dizygotic (DZ) and monozygotic (MZ) twin types. Twin studies have been used with success for the estimation of genetic factors (heritability) in cancers e.g. prostate cancer (42%)[5] but also in other diseases like Alzheimer (74%)[6]. Two types of heritability can be distinguished: broad-sense heritability (H^2) and narrow-sense heritability (h^2). Broad-sense heritability is defined as a ratio of genetic variance (additive and dominant; A+D) over the whole observed phenotype variance, while narrow-sense heritability is the ratio of additive genetic variance over the total phenotype variance [7]. The methods of genetic and environmental effects estimation started with the work of Ronald Fisher [8] were he proposed the usage of phenotype intra-class correlation of individuals with different genetic sharing. This concept was further extended to classical twin study approach proposed by Douglas Falconer. Knowing that MZ twins are genetically identical he states that correlation within twin pairs represent the sum of additive genetic effects (A) and common environment effects (C), for DZ twins who share 50% of their genetic material correlation coefficient represents 50%A+C. Based on this information Falconer said that heritability can be calculated as $2[r_{MZtwins}\text{-}r_{DZtwins}]$ [7]. This simple method gives only a rough estimate of heritability level without any statistical testing process. What is more, such heritability estimation is not clear with the understanding of the metric itself. Over the years several adjustments of heritability calculations were proposed, which mostly based on phenotype variance decomposition [9], [10]. However, nowadays the gold standard is the method of structural equation modeling (SEM) for variance decomposition introduced by Neal and Cardon [11], where each effect contributed to total variance (genetic and environmental) is estimate with the usage of maximum likelihood function.

In the presented study, the variance decomposition to genetic and environmental effects was performed for two biomarkers: H2AX and MDM2, both previously associated with radiation response [2]. The H2AX is known as H2A histone family and is necessary for starting the process of DNA double-strand break repair, which are commonly observed after irradiation [3]. The MDM2 (Murine double minute 2) mainly controls activity of the p53 tumor suppressor gene. The expression of MDM2 similar to H2AX is induced by DNA damage, which is the leading effect of ionizing radiation [4]. The assessment of heritability for both of those markers using SEM is of great importance for understanding the impact of genetic factors on gene expression level under irradiation. Additionally, the presented study is accompanied by validation processes in the form of seeking single nucleotide polymorphisms for genes with significant part of genetic factors. In summary, the aim of the study is to investigate the heritability of H2AX phosphorylation level and M2DM gene expression and to relate them to the genetic background expressed by set of potentially associated Single Nucleotide Polymorphisms (SNPs).

MATERIAL

The material under investigation comes from FinnTwin16 study [12]. The T-lymphocytes were purified from blood samples collected for 86 healthy Caucasians, among whom there were 28 dizygotic twin pairs (DZ; 37 females & 19 males) and 15 monozygotic twin pairs (MZ; 18 females & 12 males) [12]. MDM2 gene expression was measured using qPCR technique at two experimental conditions: (1) normal (no irradiation, denoted as 0Gy), and (2) just after sample irradiation with a dose of 2Gy. H2AX phosphorylation level via FACS was measured in the same

conditions as for MDM2. The procedures of sample collection and storage, sample irradiation, and qPCR experiments were previously established and described in [2], [13], [14]. The same data collection protocol was performed for 44 healthy working individuals (22 females; 22 males) [13]. Additionally, for all samples (n=130) the genotyping experiment of 567,096 SNPs by Axiom GW Human hg36.1 arrays, was done on samples before irradiation.

METHODS

The data quality control was performed following procedure described in [15]. The standardized ratio between the response at 2Gy and reference 0Gy was calculated for H2AX and MDM2, separately for each individual. The gene expression similarity between twins was assessed by intra-class correlation coefficient (ICC) and the hypothesis on homogeneity of correlation coefficients in DZ and MZ twin groups was tested with the use of z-transform based t-test [16]. The assessment of genetic and environmental impact on phenotype was performed by structural equation model for variance decomposition technique [11].

The following components were considered during the model development: component **A** that represents additive genetic effect; component **D** representing the non-additive genetic effect (named also as dominance variance); component **C** responsible for environmental effect shared by twins brought up in the same family, and component **E** modeling environmental effect unique to the individual. Since MZ twins are genetically identical, while DZ twins on average share 50% of their segregating genes, additive genetic effect **A** is modeled with a weight of 1.0 for MZ twins and 0.5 for DZ twins, while non-additive genetic **D** component is equal to 1.0 for MZ twins and to 0.25 for DZ twins. According to the data available, all twins were brought up together, so the weight in the constructed structural equation model for common environmental effect **C** was assumed to be equal to 1.0. The unshared environmental effect **E** is unique to co-twins and remains uncorrelated. The tested model structure is presented on Fig. 1.

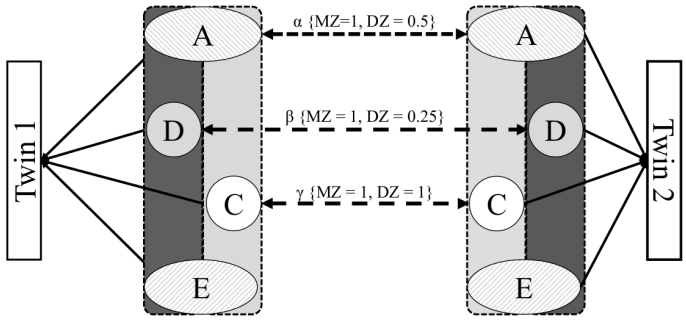

Figure 1. The schema demonstrating the structural model considered in the study.

The model can be transformed into matrix algebra equation as follows:

$$\begin{bmatrix} 1 & 1 & 1 & 0 \\ 0.5 & 0.25 & 1 & 0 \\ 1 & 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} A \\ D \\ C \\ E \end{bmatrix} = \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} \tag{1}$$

where b_1 represents covariance of investigated biomarker level between twin 1 and 2 in MZ twin type, b_2 represents covariance of investigated biomarker level between twin 1 and 2 in DZ twin type, and b_3 represents trait total variance. Seeing that D and C model components are highly correlated and difficult to separate, only models of ADE or ACE type (but no ACDE model) can be considered as the initial ones [17]. The backward feature elimination algorithm supported by Bayesian Information Criterion (BIC) was used for model selection [18]. The analysed structural equation models were implemented in OpenMx R package and their parameters were estimated by

maximum likelihood approach [11], [19]. Based on A and/or D component estimation broad-sense heritability (H^2) and narrow-sense heritability (h^2) were established for both biomarkers [8].

Independently, all samples were analysed for seeking the single nucleotide polymorphisms (SNPs) possibly responsible for system response to irradiation (measured by H2AX and MDM2). At first, quality control for SNP microarray was performed and SNPs with call rate lower than 90% and minor allele frequency (MAF) lower than 0.1 were removed from further analysis [20]. It resulted in dimensionality reduction from 567,096 to 383,322 polymorphisms. Further, for each subgroup (unrelated individuals unR, dizygotic twins DZ and monozygotic twins MZ) the statistical testing the genotype-phenotype interaction based on three models: genotype, dominant and recessive was performed according to the procedure presented in [21]. The significance level in SNP analyses was set to 0.05 in each of tested subgroups.

RESULTS AND DISCUSSION

As a first step, the twin-to-twin biomarker associations were estimated in each twin group (Table 1). The intra-class association was significantly higher among MZ when compared to DZ twins for H2AX phosphorylation level (p=0.0244). The similar effect of response agreement among MZ twins versus DZ twins was observed for MDM2 gene expression, however, due to higher response process complexity, the disagreement is slightly weaker (p=0.0616). We conclude, that for the trait under investigation similarity in response to irradiation weakens with genetic divergences.

Table 1. Intra-group Pearson correlation coefficients and the result of one-sided testing on higher association in MZ group (p-value).

Trait/biomarker	MZ (n=15)	DZ (n=28)	p-value
H2AX	0.60	0.02	0.0244
MDM2	0.59	0.15	0.0616

The average of H2AX phosphorylation level and MDM2 gene expression within DZ and MZ groups were tested [15]. For both biomarkers, the differences between DZ and MZ twins are not statistically significant (Table 2). Additionally, the hypothesis of no association of the variance of a trait and twin type was tested [15]. Similar to the mean testing, no statistically significant variance inhomogeneity was observed for both markers (Table 2).

Table 2. Trait mean values, their standard errors (SE) and results of testing on mean value equality (p-value) [14].

	MZ (n=15)		DZ (n=28)		p-value	
Trait	Mean value	SE	Mean value	SE	mean testing	variance testing
H2AX	2.63	0.19	2.39	0.12	0.1865	0.3149
MDM2	4.46	0.17	4.41	0.09	0.9157	0.3988

All the above-mentioned results shows similarity of the response to irradiation in DZ and MZ twins for both biomarkers. The lack of inherited differences in twining processes and increasing correlation due to increased genetic similarity allow to seek for the level of heritability of radiation response for investigated biomarkers.

During the process of model construction, the initial full models were established. For both biomarkers, the ADE model was selected following the rule of minimizing BIC criterion (H2AX: BIC_{ACE}=241.37, BIC_{ADE}=240.35; MDM2: BIC_{ACE}=212.54, BIC_{ADE}=211.76). Further, AE and E models were constructed, and similarly to previous step the BIC criterion was used for the best model selection. The results of modeling process are presented in Table 3.

The best obtained model for variance decomposition of H2AX phosphorylation level includes both additive genetic component **A** and environmental component **E** with the weights of 45.55% and 54.45% respectively. It conclude that narrow-sense heritability for H2AX equals 46%. In case

Table 3. Values of Bayesian Information Criterion (BIC) obtained in stepwise procedure of model construction.

Model	H2AX	MDM2
ADE	240.3	211.8
AE	**236.9**	208.1
E	237.5	**206.6**

of MDM2 gene expression, genetic factor does not influence the phenotype and only environmental component E remained in the model.

To answer the question on potential polymorphisms associated with radiation response of H2AX phosphorylation level and MDM2 gene expression the three step analysis was performed as presented in [21]. First, the classical GWAS-type study combined with gene-interaction modeling was done with the use of data on 44 unrelated individuals [22]. Then, the potential trait related polymorphisms were validated on DZ and MZ twins data. The SNP was classified as potentially associated with trait radiation response if it was significantly associated with the trait radiation response in each of the groups under investigation. The final number of potential polymorphisms related to the changes of H2AX phosphorylation level in response to 2Gy irradiation is equal to 2,816, while for MDM2 gene expression response none of potentially related polymorphism was identified. Out of statistically significant polymorphisms in H2AX, 48% of them are located in transcriptionic regions of 849 genes. Out of them, SNP rs9298170 located in EYA1 gene can be distinguished. The EYA1 gene is responsible for dephosphorylation of H2AX in protein position 142 and triggers the phosphorylation in position 139. Phosphorylation of Serine in position 139 in H2AX is necessary for starting the process of DNA damage repair specially under irradiation [23], [24]. To illustrate the relation between H2AX and the closest genes (including EYA1) the interaction network was constructed in STRING tool [25] - Fig. 2.

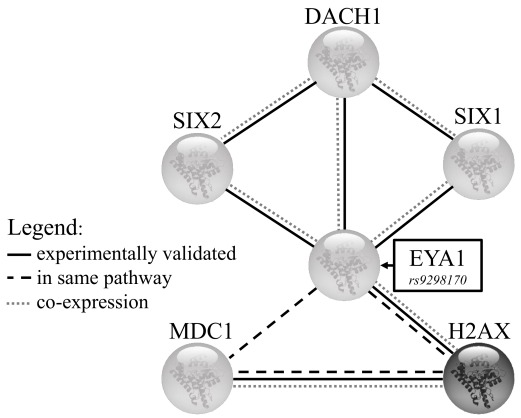

Figure 2. The interaction network of H2AX and closely related genes.

CONCLUSIONS

In this study, variation in H2AX phosphorylation level and MDM2 gene expression in responses to ionizing radiation has been examined. It was shown that for 2Gy irradiation induced changes in H2AX phosphorylation level, genetic effect can explain 46% of phenotype variance, while for MDM2 gene expression response no significant impact of genetic background was noticed. Detailed SNP-phenotype GWAS-type analysis with validation on two independent data sets revealed 2,816 polymorphisms being potentially related to H2AX response none for MDM2. We conclude that existence of genetic factor is directly projected into discoveries in GWAS-type studies.

Presented results give new insight for understanding of genetic impact on radiation response behaviour in investigated biomarkers and can be incorporated to radiation risk assessment in public health radiation protection.

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