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RAD51 homologous recombination repair gene haplotypes and risk of acute myeloid leukaemia☆

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Abstract

Homologous recombination (HR) is one of the main pathways for the repair of DNA double strand breaks (DSBs). To investigate whether inherited variants in genes encoding proteins that repair DSBs by HR modulate acute myeloid leukaemia (AML) risk, we have examined the frequency of two variants in the 5′ untranslated region (UTR) of *RAD51* (*RAD51 135 G>C* and the *RAD51 172 G>T*) in a large case–control study of acute myeloid leukaemia (AML). Inheritance of a *RAD51 135 C* allele was associated with a reduced risk of estimate for AML (odds ratio (OR) 0.56, 95% confidence intervals (CI), 0.38–0.83), while the *RAD51 172 T* allele was not associated with AML. The *RAD51 135* and *172* variants were in strong linkage disequilibrium, with three out of the four possible haplotypes being observed in the population. The protective effect associated with the *RAD51 135 C* allele was found to be associated with inheritance of the *RAD51 135–172 C–G* haplotype (cases 3.9% versus controls 6.5%, OR 0.61, 95% CI 0.42–0.90). These data suggest that variants in the *RAD51* HR gene may modulate genetic predisposition to AML.

Keywords: Acute myeloid leukaemia; DNA repair; Double strand break; Homologous recombination; Non-homologous end joining; Case–control study

1. Introduction

DNA damage in the haemopoietic precursor cell is thought to be an essential prerequisite for the development of acute myeloid leukaemia (AML). Double strand breaks (DSBs) are one of the most deleterious forms of DNA damage, representing a mechanism by which chromosomal translocations and other common molecular events in AML may occur. Due to the threat posed by DSBs, eukaryotic cells have evolved two main pathways for the repair of DSBs; non-homologous end joining (NHEJ) and homologous recombination (HR). Studies in mice and yeast have shown that the absence of either pathway leads to genomic instability [1,2], while cell lines defective in HR are known to have high rates of spontaneous chromosomal abnormalities [3]. In humans, inherited defects in HR pathways are known to predispose to AML, an example of this, Fanconi anemia (FA) [4] is characterized by spontaneous and mutagen-induced chromosome instability. Recently BRCA2, was identified as an FA protein, linking this pathway to HR through the interaction of BRCA2 with RAD51 [5].

HR effects DNA repair through the interaction of free DNA ends with a homologous DNA sequence that is used as a template for the high fidelity repair of the DSB. HR is

Abbreviations: AML, acute myeloid leukaemia; DSB, double strand break; HR, homologous recombination; NHEJ, non-homologous end joining; FA, Fanconi's anemia; OR, odds ratio; CI, confidence interval

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thought to be particularly important in DNA repair occurring during cellular replication [6–8]. RAD51 is one of the key proteins for HR, and functions by forming nucleoprotein filaments on single stranded DNA, mediating homologous pairing and strand exchange reactions between single and double stranded DNA during repair [9]. The central role of RAD51 in maintaining genomic stability is supported by the fact that null mice are embryo lethal [10], while in the chicken DT40 system disruption of the *RAD51* gene results in chromosome instability [11], a molecular feature commonly seen in AML. In contrast, over-expression results in increased resistance to apoptosis induced by ionizing radiation [9], a well defined cause of DNA DSBs. The emerging role of HR in myeloid precursor cells [12] combined with the phenotypic features of cells associated with over- and under-expression of RAD51 suggest that genetic variation within the gene may mediate risk of AML.

Two polymorphisms have been described in the 5′ UTR of *RAD51*, a G > C substitution at position +135 bp, and a G > T substitution at position +172 bp from the start of the cDNA sequence (NCBI accession number D14134). While alone neither polymorphism has been associated with risk of breast or ovarian cancer [13,14], nor an effect on breast cancer survival [15], the *RAD51 135 C* allele has been associated with an increased risk of breast cancer and a reduced risk of ovarian cancer amongst individuals that carry germline mutations in *BRCA1* or *BRCA2* [16,17]. To examine the effects of polymorphisms in *RAD51* on AML risk, we have examined the frequency of the *RAD51 135* and *RAD51 172* variants in a large case–control study of AML.

2. Methods

2.1. Study design

Full details of the Leukaemia Research Fund population-based AML case–control study are given elsewhere [18]. Briefly, acute leukaemia cases aged between 16 and 69 years were recruited from the UK, and diagnoses were pathologically confirmed. For every case that participated in the study, two controls of the same sex, race and year of birth were recruited from the general practice of the case. All subjects included in the study gave informed consent, and ethical approval was obtained for all study subjects. Briefly, the study group comprised 479 cases, of which 424 were classified as *de novo* AML, and 55 as secondary AML (defined as a history of previous malignancy or myelodysplasia (MDS)). The average age of the *de novo* cases was 47.6 years and 54% were male, while for the secondary cases the average age was 51.8 years, and 45.4% were male. The study group also comprised of 952 age sex matched controls, with an average age of 48.1, with 53.5% being male. As the frequency of alleles has been shown to vary with race [19], the genotyping and subsequent analysis was restricted to Caucasian subjects.

2.2. Genotyping and assay validation

Genomic DNA was extracted from whole frozen blood using a proteinase K treatment, followed by a series of phenol:chloroform extractions and ethanol precipitation [20]. Quality checks, using both DNA negative and known genotype control samples, were run with each set of subject DNA for both assays.

Inheritance of the *RAD51 135* and *172* polymorphisms was determined using Taqman™ Allelic Discrimination. Primer and probe sequences were as follows (variant base in lower case), *RAD51 135* forward primer 5′-TCTGGGTTGTGCGCAGA-3′, reverse primer 5′-CCGCGCTCCGACTTCA-3′, G allele probe 5′-FAM-AGCGTAAGCCAgGGGCGTTGG-3′, C allele probe 5′-VIC-GCGTAAGCCAcGGGCGTTGG-3′. *RAD51 172* forward primer 5′-CGAGTAGAGAAGTGGAGCGTAAGC-3′, reverse primer 5′-CCGCGCTCCGACTTCA-3′, T allele probe 5′-FAM-CGTGCCACtCCCGCGGG-3′, G allele probe 5′-VIC-CGTGCCACgCCCGCGGG-3′. All reactions were carried out in 15 μl volumes using 900 pmol of each primer and 100 nmol of each probe, using the 2× Applied Biosystems (ABI) (Foster City, CA) universal master mix. Amplification was carried out on an ABI 9700 using the following amplification conditions for both assays; 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 61 °C for 1 min. Data capture and analysis were carried out using an ABI PRISM 7700 sequence detector and the Sequence Detection Systems software (ABI).

Genotyping results were verified by sequencing 60 randomly chosen samples. A 178 bp fragment of the *RAD51* sequence surrounding the two variants was amplified using the following primers and reaction conditions; 10 pmol each primer (forward 5′-ACCGAGCCCTAAGGAGAGTG-3′, reverse 5′-CCGCGCTCCGACTTCA-3′), 10 ng of DNA, 1× Amplitaq Gold buffer, 200 μmol dNTPs, 1.5 mM $MgCl_2$, 1 unit Amplitaq Gold DNA polymerase, in a final reaction volume of 20 μl. Amplification conditions were as follows; 10 min at 95 °C, followed by 35 cycles of 95 °C 1 min, 56 °C 1 min, and 72 °C 1 min. Products were run out on 1% agarose gels and purified using the QiaQuick system from Qiagen (West Sussex, UK). Samples were sequenced using ABI PRISM® BigDye™ Terminator cycle sequencing, the sequencing products being visualized on an ABI 377 sequencer.

To verify the linkage disequilibrium predicted using statistical analysis between the *RAD51 172* and *RAD51 135* variants, five samples identified as dual *RAD51 172 GT* and *RAD51 135 GC* heterozygotes, were blunt-end cloned into the pT7Blue-3 Blunt vector following the manufactures instructions (Novagen, Germany). Plasmids that contained insert were then amplified using the R-20mer primer (Novogen) in combination with the reverse primer previously used for sequencing, and the products were then gel purified and sequenced as described previously.

Out of the 120 genotypes checked by sequencing, all genotypes agreed for the *RAD51 135*, while 1 genotype (*RAD51*