Development of a High-Resolution Melting Method for Screening *R188H* Polymorphism in *XRCC2* Gene

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ABSTRACT

Background: The High Resolution Melting (HRM) method is a new scanning method for detecting unknown changes in DNA and its advantages have persuaded researchers to recruit it as a screening method.

Objectives: Here, we developed a HRM method to screen R188H SNP (rs3218536) of XRCC2 and compared the results with a well known PCR-RFLP technique.

Materials and Methods: Genomic DNA samples from 350 healthy individuals were obtained. PCR-HRM analysis and PCR-RFLP method were performed simultaneously.

Results: Three different melting profiles corresponding to three different genotypes recognized by HRM analysis. The results of PCR-RFLP showed no discrepancy.

Conclusions: We concluded that the HRM technique can be used as a screening method for rapid discrimination of R188H genotypes in XRCC2 gene.

Keywords: HRM; *XRCC2*; Genotyping

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1. Background

DNA repair genes are involved in the maintenance of genomes integration. Defects in DNA repair genes may contribute to the development of various types of cancers (1-4). X-ray repair cross-complementing group 2 (*XRCC2*), is one of the DNA repair genes which works in the homologous repair pathway (5). There are plenty of reported SNPs in this gene (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?geneId=7516) where *R188H* (rs3218536) is the most studied (6). This non-synonymous polymorphism (G to A) causes a non-conservative change in the level of protein expression which may alter its DNA repair functional activity (7). Many studies have been conducted to show possible associations of this polymorphism with various cancers (8-10). Various techniques have been used for *R188H* genotyping but most of them include mutation detection screening methods, such as PCR-RFLP and TaqMan allele discrimination techniques. However, these techniques have some potential disadvantages: PCR-RFLP technique is time consuming and in this case uses a relatively expensive restriction enzyme. Taqman probes are expensive and setting up the technique may require considerable effort. As a large number of samples should be tested in molecular-epidemiological studies, high throughput techniques are needed. To address this demand, we used a high resolution melting (HRM) method to discriminate *R188H* genotypes. This rapid and relatively new mutation scanning method has some advantages over similar gel based scanning methods (i.e. DGGE, SSCP and CSGE). For example, compared to a modified and rapid CSGE technique (11, 12), HRM method is much faster. Also, HRM has been successfully used as a mutation detection screening technique in other genes (13).

2. Objectives

Assessment of HRM method to discriminate *R188H* genotypes as a fast and high throughput screening method and compare it to PCR-RFLP method.

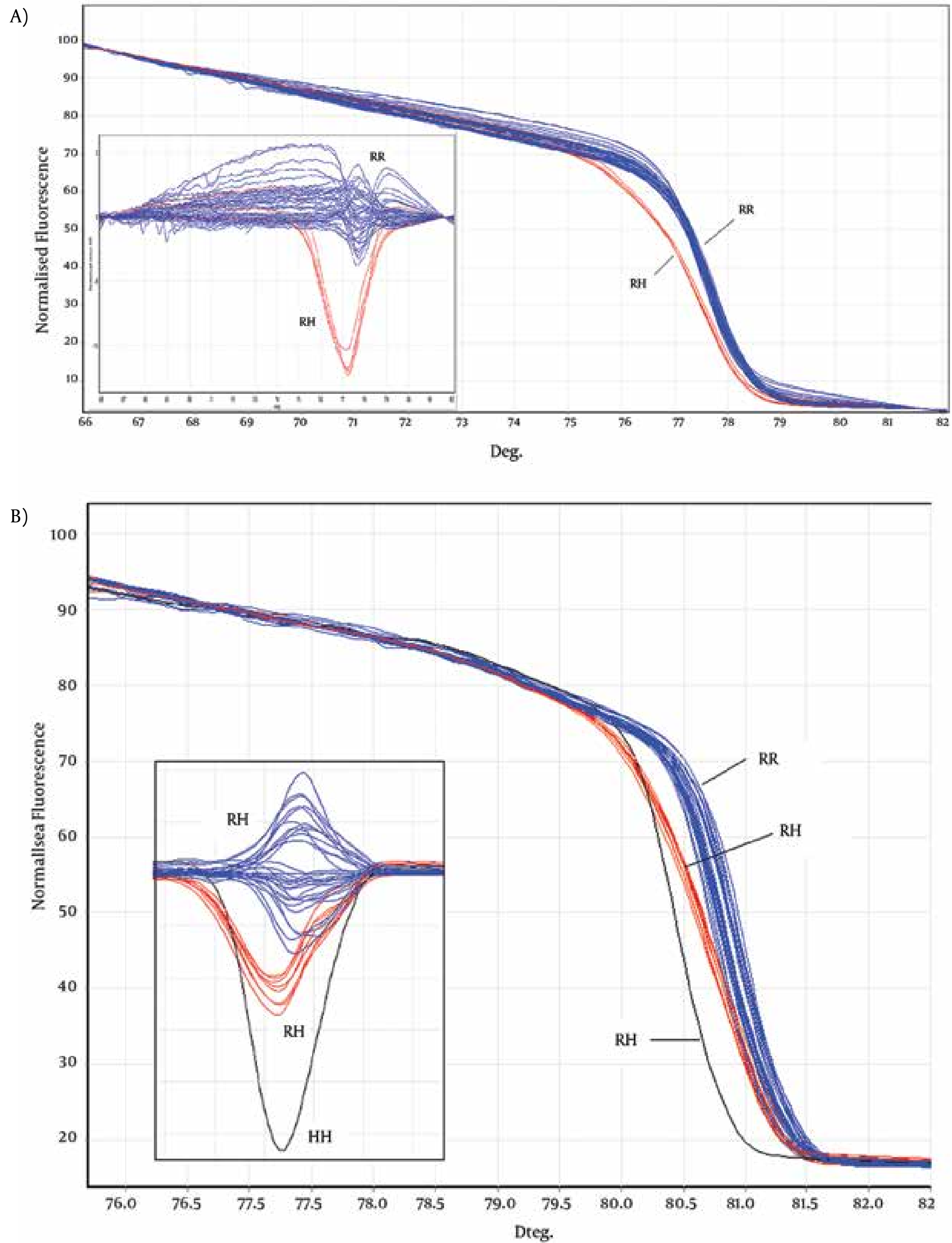
3. Materials and Methods

In this study 350 human genomic DNA samples were genotyped for *R188H* polymorphism in *XRCC2* gene using PCR-HRM method and the results were compared with PCR-RFLP results obtained from the same samples. Samples were collected from healthy volunteers from two academic centers. After signing the genetic test's consent form by all candidates, peripheral blood samples were collected in EDTA tubes and genomic DNA of white blood cells were extracted using a previously described salting out method (14). *R188H* Genotyping: *R188H* alleles of *XRCC2* gene from each sample were screened using polymerase chain reaction followed by high resolution melting (PCR-HRM) analysis using Rotor- Gene™ 6000 real time rotary analyzer (Corbett Research, Qiagen). Four previously known genotype samples were included in each run (two for each 188RR and 188RH genotypes). These control samples were used as unique analysts in every run to test reproducibility. Forward and reverse primers were designed. A 104 bp fragment of exon 3 from *XRCC2* gene containing codon 188 was amplified using GGA AAT GTT CTC AGT GCT TAG AG and TTC TTC TGA TGA GCT CGA GG primers. The PCR-HRM reactions were carried out in a 10 µl volume using Type-it HRM PCR Kit (Qiagen) containing 10 pmol of each primer and 20-50 ng genomic DNA. The PCR cycling temperature was: 95°C for 5 min; 45X (95°C 10 s, 59°C 30 s, 72°C 10 s); 72°C for 4 min; followed by Pre-HRM heteroduplex enrichment: 95°C for 10 s; 65°C for 5 min and HRM protocol was: Ramp, from 65°C to 95°C; increasing by 0.05°C at each step and waiting for 10 s in each round. After PCR-HRM, the same samples were treated with SexA1 restriction enzyme (RE) (Fermentas) using the manufacturer's protocol and were subjected to 3.5% agarose gel electrophoresis and ethidium bromide visualization.

4. Results

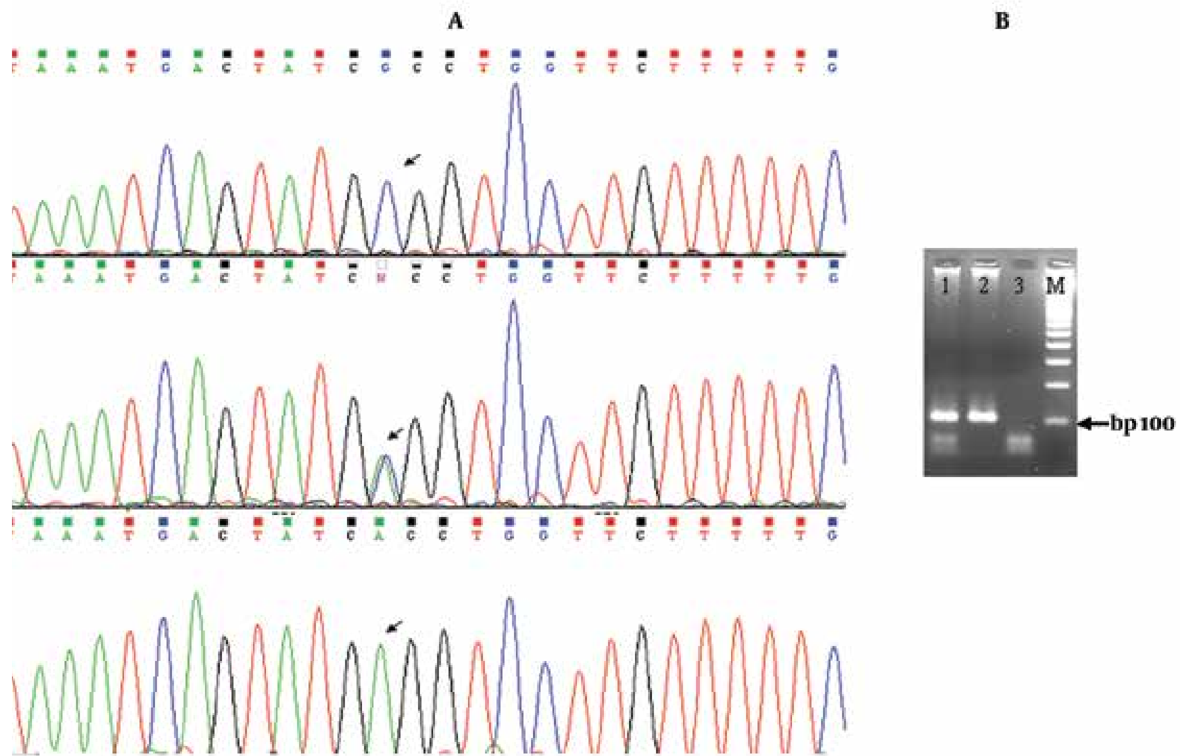
After RE treatment, PCR products carrying the wild type allele (188R) remained intact, while presence of the 188H allele produced two fragments of 61 bp and 53 bp. Simultaneously, based on melting profiles, three main groups were retrieved (see below) and four samples from each group were randomly selected and subjected to DNA sequencing. The third group consisted of two samples and both samples were sequenced. HRM runs for the 65 samples are shown in *Figure 1* A, B. Three different melting profiles could be recognized (*Figure 1* B). The profile of each control sample fell in a separate group. DNA sequencing of a few samples, each from a control-containing group confirmed *R188H* heterozyosity and 188R homozygosity in the same relevant group (*Figure 2* A). Also, DNA sequencing of the samples belonging to the third group showed 188H homozygosity. PCR-RFLP results are shown in *Figure 2* B. No discrepancy was seen between the resultant alleles from PCR-HRM and PCR-RFLP techniques.

Figure 1. HRM of a Segment of Exon 3 of XRCC2 Gene



HRM of a segment of exon 3 of XRCC2 gene in 49 (A) and 16 (B) samples including 188RR and 188RH control samples. RR-type, RH-type and HH-type curves are shown as RR, RH and HH respectively. Small boxes show difference graphs. No HH-type was found in the 49 samples (A).

Figure 2. Electropherograms and RFLP Results



(A) Electropherograms of 188RR (up), 188RH (middle) and 188HH (down) genotypes. (B) RFLP samples: lane 1, 188RH; lane 2, 188RR; lane 3, 188HH and M, 100 bp DNA ladder.

5. Discussion

The importance of DNA repair genes in modifying the risk of developing cancer is not a subject that could be neglected. There are more than 20 DNA repair genes in different DNA repair pathways (15) in which plenty of single nucleotide polymorphisms are recognized. Therefore, using rapid SNP detection methods is crucial in genotyping studies. *R188H* polymorphism in *XRCC2* gene is one of the changes and its association with different cancers has been shown to lead to various outcomes. For example presence of the 188RH allele has been associated with decreased risk of breast cancer (16), but an increased risk of pancreatic cancer (9). In spite of the general view that HRM should be used as a scanning method for the detection of an unknown DNA polymorphism, we suggest that it can also be used as a screening method for the detection of specific polymorphisms, where no frequent SNPs have been reported in their proximity. Accordingly, we found that *R188H* polymorphism in *XRCC2* gene was a good candidate. Indeed, we designed primers to amplify a small PCR fragment to minimize the chance for the presence of other polymorphisms around *R188H*. In the present study we analyzed 350 genomic DNA for *R188H* polymorphisms by PCR-HRM technique. To our knowledge, this was the first time that the HRM technique was implicated for genotyping of *R188H* changes in *XRCC2* gene. No discrepancy was found between these results and those obtained from the PCR-RFLP method. The simplicity, speed and accuracy of the PCR-HRM method convinced us that it could be used for screening *R188H* polymorphism in *XRCC2* gene. Moreover, the probable power of the PCR-HRM method for screening of other single nucleotide polymorphisms in other sites of the genome is a subject not far from imagination.

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Authors' Contribution

Pezhman Fard-Esfahani designed and directed this study, Shima Fayaz did HRM and RFLP analysis and wrote the manuscript, Shahnaz Khaghani contributed to data analysis.

Financial Disclosure

The authors stated that they had no interests which might be posing a conflict.

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