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High resolution melting analysis for detection of variable number of tandem repeats polymorphism of *XRCC5*

Mehrdad Rajaei, Iraj Saadat, Mostafa Saadat *

Department of Biology, College of Sciences, and Institute of Biotechnology, Shiraz University, Shiraz, Iran

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ABSTRACT

Several polymorphisms in the *XRCC5* (X-ray repair cross-complementing 5; OMIM: 194364) were reported. Polymorphism of variable number of tandem repeats (VNTR) in the promoter region of *XRCC5* (rs6147172) was reported. The main aim of the present study is to introduce the high resolution melting analysis (HRMA) method for genotyping of the polymorphism of *XRCC5* VNTR. Genotypes of *XRCC5* VNTR were determined by HRMA and conventional PCR method, and confirmed by DNA sequencing. The results for genotyping using HRMA and conventional PCR showed 100% concordance. All genotypes of the *XRCC5* VNTR polymorphism could be accurately detected by HRMA.

1. Introduction

The non-homologous end joining (NHEJ) pathway is a DNA double strand break (DSB) repair mechanism that is crucial for maintaining the chromosomal stability. Ku, a heterodimeric DNA-binding complex, is directly involved in repair of DSBs as a member of the NHEJ pathway. Ku consists of two subunits, Ku70 and Ku80, which are encoded by the *XRCC6* and *XRCC5* genes, respectively [1].

Several polymorphisms in the *XRCC5* (X-ray repair cross-complementing 5; MIM: 194364) were reported. One of them is a variable number of tandem repeats of a 21 bp (VNTR, rs. 6147172) polymorphism in the promoter region of *XRCC5*. This polymorphism has three alleles: 2R, 1R and 0R [2].

Previous studies have used conventional PCR and agarose gel electrophoresis for genotyping the *XRCC5* VNTR polymorphism. High resolution melting analysis (HRMA) is a recent development in real-time PCR and is a fast, sensitive and powerful single-tube method for genotyping, mutation scanning and sequence matching [3,4]. The HRMA is mostly used for detection of point mutations, single nucleotide polymorphisms and subtle variations in nucleic acid sequences [5]. The aim of the present study is to test the feasibility of HRMA for detection of a large sequence variation of *XRCC5* VNTR polymorphism.

2. Materials and methods

Genomic DNA was isolated from EDTA treated blood samples. Genotyping for the *XRCC5* VNTR polymorphism was carried out using HRMA following a pre-amplification step by Rotor-Gene 6000 instrument (Corbett Life Science). The reaction employed SYBR Premix Ex Taq II PCR master mix (Takara Bio Inc.) and previously described primers, 5′-AGG CGG CTC AAA CAC CAC AC-3′ (forward), and 5′-CAA GCG GCA GAT AGC GGA AAG-3′ (reverse) [4]. The PCR was performed in a total volume of 20 μl containing 100 ng genomic DNA, 10 μl SYBR Premix Ex Taq II, 0.8 μl (0.4 μM) of each forward and reverse primers, and the total reaction volume was brought to 20 μl with dH_2O. Real-time PCR conditions were as follows: one cycle at 95 °C for 1 min followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 67 °C for 20 s and extension at 72 °C for 20 s. Melting curve data were collected from 85 °C to 95 °C at a ramping rate of 0.1 °C per second. Melting curve analysis was performed using SYBR green I channel, employing Rotor Gene 6000 software (version 1.7). Conventional PCR was performed for 141 participants with above-mentioned primers.

PCR amplification for selected samples was performed and the desired bands excised from the gel and purified. Isolated nucleic acid was purified and sequenced by Eurofins MWG Operon (Ebersberg, Germany). Sequencing results were compared to each other and to the genome database, using BLAST tool on the NCBI website [6].

Peripheral blood samples were collected from 141 healthy blood donors. Considering the heterogeneity of Iranian population [7,8], our participants were selected from Persian Muslims (Caucasians) living in Shiraz (Fars province, southern Iran). Informed consent was obtained from each subject before the study. This study was approved by the local ethics committee. Informed consent was obtained from all participants.

3. Results and discussion

Results of HRMA for *XRCC5* VNTR polymorphism are illustrated in Fig. 1. The genotypes were detected in the region of 90.2–92.8 °C

* Corresponding author. Fax: +98 711 2280926.
E-mail addresses: saadat@susc.ac.ir, msaadat41@yahoo.com (M. Saadat).

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by HRMA. Fig. 1A and B show the raw fluorescence and normalized fluorescence, respectively. Fig. 1C shows the normalized fluorescence minus 1R/1R genotype. Results were also shown as derivative plots (dF/dT versus T graphs) of the melting curve data (Fig. 1D). Conventional PCR amplification for these samples was performed, and products separated by 2% agarose gel electrophoresis. In order to compare the accuracy of the HRMA with conventional PCR method for genotyping the VNTR, 141 randomly selected PCR products were analyzed by electrophoresis. The results showed 100% concordance between these two methods.

Compared to conventional PCR, HRMA has some advantages such as elimination of mutagenic reagents and reduction of contamination probability [9]. HRMA has been previously used to detect Internal Tandem Duplications (ITDs) with 6–102 bp long in the juxtamembrane domain of the FLT3 gene [5]. However, there is still a doubt about the ability of HRM for complete and efficient genotyping of short tandem repeats and to eliminate the dependence on electrophoresis in repeat genotyping [4]. Based on the present finding, all genotypes of the so called VNTR could be accurately detected by HRMA.

Using of SYBR Green is not suggested for HRMA by some researchers [4]. There are some limitations in using this dye for detection of small variations [3]. Our present study, however, indicates that SYBR Green is a highly suitable dye for HRMA, at least for detection of VNTR in the promoter of *XRCC5*. This finding confirmed some studies [10,11].

Prevalence of the 0R, 1R, and 2R alleles in our study group was 0.06, 0.56, and 0.38, respectively. To the best of our knowledge, the genotypic and allelic frequencies of *XRCC5* VNTR are only reported form Chinese population [2] and are far from what we observed in

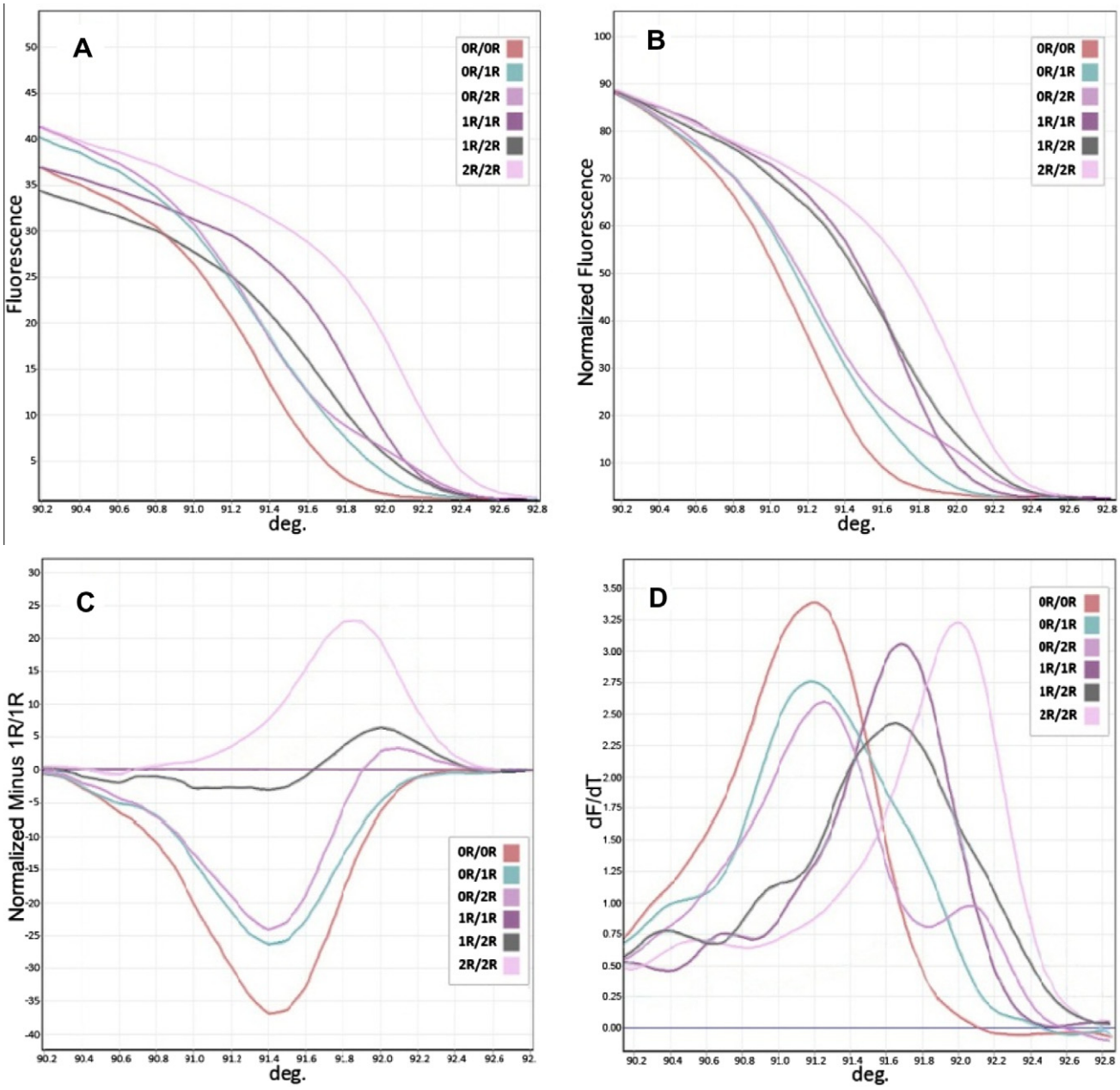

Fig. 1. Detection of the *XRCC5* VNTR polymorphism (rs6147172) by high resolution melting analysis. Raw fluorescences (A) and normalized fluorescences (B) versus temperature for 6 genotypes. Fluorescence difference plots of the normalized data (C). 1R/1R genotype is used for the baseline and the curves for the remaining genotypes cluster around the baseline. Derivative (−dF/dT versus T) melting curves of the normalized fluorescence (D).

our control group. The prevalence of 1R allele in our sample was higher and 0R allele was lower than that from China.

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