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Introducing a novel allele for the polymorphism of variable number of tandem repeats in the promoter region of *XRCC5*

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ABSTRACT

Polymorphism of variable number of tandem repeats (VNTR) in the promoter region of X-ray repair cross-complementing 5 (*XRCC5*; MIM: 194364, rs6147172) has been reported. The main aim of the present study is to introduce a novel allele for the VNTR polymorphism in the promoter region of *XRCC5*. The participants of the present study were of 535 (140 males, 395 females), unrelated, adult, healthy Iranian blood donors (Caucasians/Muslims). Genotypes of *XRCC5* VNTR were determined by a high resolution melting analysis, and confirmed by DNA sequencing. Based on the sequencing of new bands upper than the 2R allele band, a novel allele was introduced (named 3R allele). The promoter region of *XRCC5* contains several copies of Sp1 recognition *cis* regulatory elements. The novel 3R allele is capable of expanding the number of *cis* regulatory elements to eight. The prevalence of the 0R, 1R, 2R and 3R alleles in our sample was 0.0645, 0.5439, 0.3794 and 0.0122, respectively. The study group was at the Hardy–Weinberg equilibrium for the genotypic frequencies ($\chi^2 = 3.95$, $df = 6$, $P = 0.73$). It is suggested that the prevalence of the novel allele (3R allele) among European populations may be higher than its prevalence among Iranians.

1. Introduction

It is well established that the non-homologous end joining (NHEJ) pathway is a DNA double strand break (DSB) repair mechanism that is crucial for maintaining chromosomal stability. Ku70 and Ku80 form a Ku complex which is a heterodimeric DNA-binding complex involved in the repair of DSBs as a member of the NHEJ pathway [1]. Ku80 is encoded by the *XRCC5* gene (X-ray repair cross-complementing 5; MIM: 194364) [1].

Several polymorphisms in the *XRCC5* have been reported, including a variable number of tandem repeats of a 21 bp (VNTR, rs. 6147172) polymorphism in its promoter region. This polymorphism has three alleles: 2R, 1R, and 0R [2]. The main aim of the present study is to introduce a novel allele for the VNTR polymorphism in the promoter region of *XRCC5*.

2. Materials and methods

The present study was performed in Shiraz (Fars province, southern Iran). The participants were 535 (140 males, 395 females), unrelated, adult, healthy blood donors. All individuals were healthy as assessed by medical history. Considering the high heterogeneity of the Iranian population [3,4], the participants were selected from Persian Muslims (Caucasians) living in Fars province (southern Iran). Informed consent was obtained from each subject before the study, which was approved by the institutional review board of our department.

Peripheral blood samples were collected from the participants. Genomic DNA was isolated from EDTA treated blood samples. Genotyping for the *XRCC5* VNTR polymorphism was carried out using high resolution melting analysis (HRMA) following a pre-amplification step by a Rotor-Gene 6000 instrument (Corbett Life Science). In a recent report, the primers and conditions for HRMA were described [5].

PCR amplification for selected samples was performed and the desired bands were 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 the BLAST tool on the NCBI website [6].

A Chi-square test was performed for the *XRCC5* polymorphism to determine if the sample groups demonstrated the Hardy–Weinberg equilibrium. A probability of $P < 0.05$ was considered as statistically significant.

3. Results and discussion

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

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normalized fluorescence, respectively. Fig. 1C shows the normalized fluorescence minus the 1R/1R genotype. Results were also shown as derivative plots (d*F*/d*T* versus *T* graphs) of the melting curve data (Fig. 1D). Among the 535 samples genotyped by melting curves, 13 revealed three new curves. Conventional PCR amplification for these samples was performed, and products were separated by 2% agarose gel electrophoresis. A new band upper than the band of 2R allele was detected. The bands were excised from the gel. According to sequencing results, all newly identified sequences were the same and contained a new fragment similar to previously identified *cis* regulatory elements in this region of the *Ku80* gene [7]. The novel allele was named 3R (Fig. 2). Sequencing results according to this new allele have been deposited in the NCBI dbSNP database (ss522928856). Due to the lack of the 3R/3R genotype in our samples, the melting curve of this genotype is not shown. The accuracy of the HRMA with a conventional PCR method was compared for genotyping. The results showed 100% concordance between these two methods.

The promoter region of *Ku80* contains several copies of Sp1 recognition *cis* regulatory elements (Ku promoter binding element). A previous study has reported 3 alleles (0R, 1R, 2R) for VNTR of *XRCC5* [2]. The alleles 2R, 1R and 0R possess seven, six and five copies of *cis* regulatory elements, respectively. However, the new allele (the 3R) identified in the present study was capable of expanding the number of *cis* regulatory element to eight (Fig. 2).

Table 1 shows the genotype distribution of the VNTR polymorphism of *XRCC5* among healthy controls. Prevalence of the 0R, 1R, 2R and 3R alleles in our sample was 0.0645, 0.5439, 0.3794 and 0.0122, respectively. The study group was at the Hardy–Weinberg equilibrium for genotypic frequencies ($\chi^2 = 3.95$, d$f = 6$, $P = 0.73$).

To the best of the authors' knowledge, the genotypic and allelic frequencies of *XRCC5* VNTR are only reported for the Chinese population [2] and are far from what we observed in our control group. The prevalence of the 1R allele in our sample was higher and the 0R allele lower than those from China. Also, we found the novel 3R allele which was absent in the Chinese population.

Previous reports for other genetic polymorphisms such as *GSTM1*, *GSTT1*, *GSTO2*, *XRCC1*, *CC16* and *GRIN1* [3,4,8–16], showed that the Iranian gene pool showed intermediate frequency in comparison with European Caucasians and Asians. Although no report from other countries (except for China and Iran) exist regarding the frequency of VNTR XRCC5 polymorphism [2,5, present study], it is

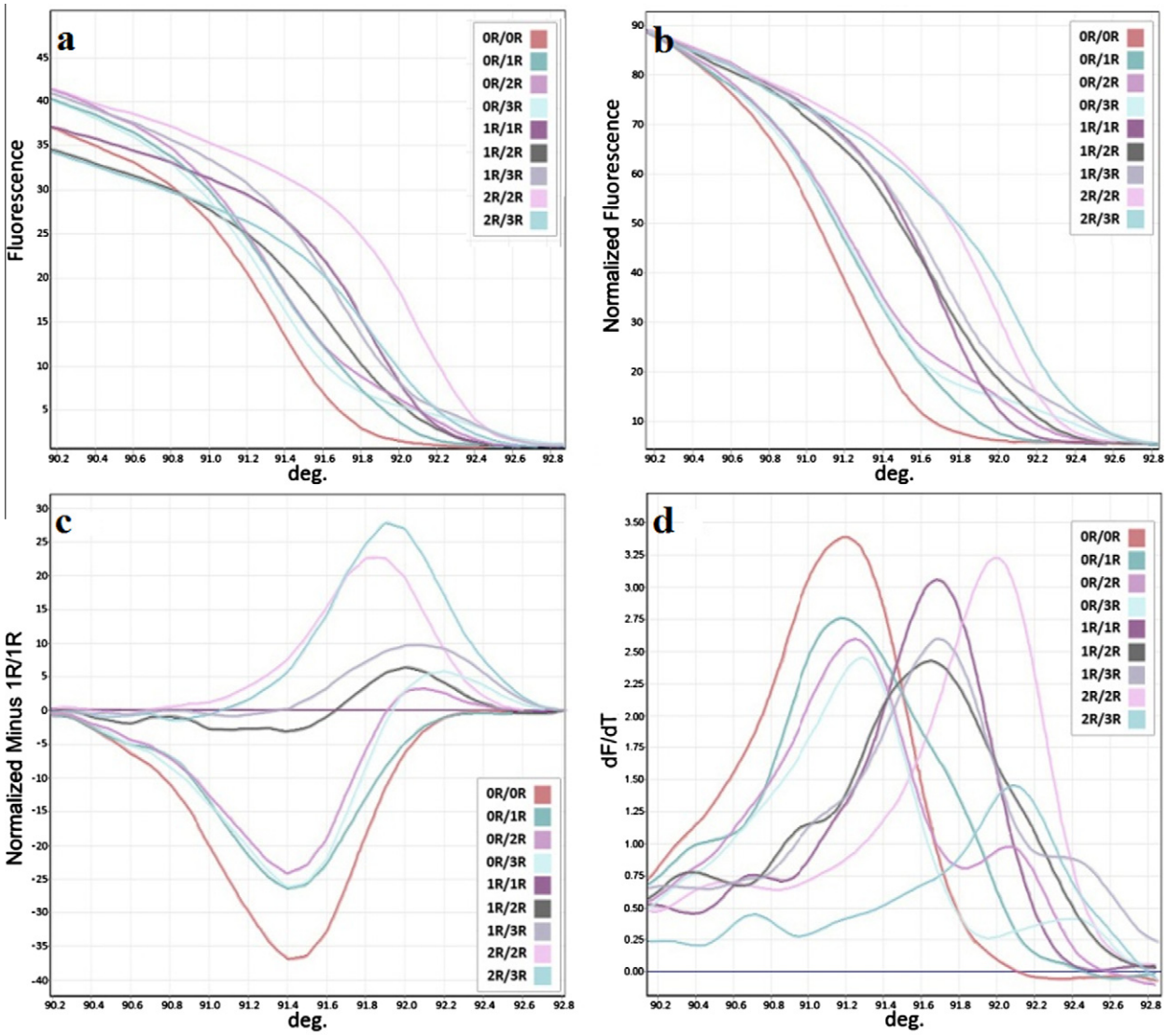

Fig. 1. Detection of the *XRCC5* VNTR polymorphism (rs6147172) by high resolution melting analysis. Raw fluorescences (A) and normalized fluorescences (B) versus temperature for 9 genotypes considering the 3 newly identified genotypes. Fluorescence difference plots of the normalized data (C). The 1R/1R genotype is used for the baseline and the curves for the remaining genotypes cluster around the baseline. Derivative (−d*F*/d*T* versus *T*) melting curves of the normalized fluorescence (D).

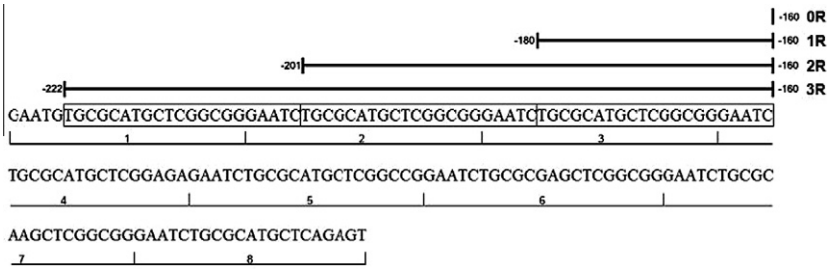

Fig. 2. A schematic diagram illustrating the VNTR polymorphism and *cis* regulatory elements in the *XRCC5* promoter. The rectangular box includes three 21-bp repeats of VNTR (2 previously identified and 1 newly identified repeats), result in formation of 4 allele: 3R with three 21-bp repeats (−222 to −160), 2R with two 21-bp repeats (−201 to −160), 1R with one 21-bp repeat (−180 to −160) and 0R without repeat. This diagram also shows the 8 copies of *cis* regulatory elements in the promoter region of *KU80* gene (7 previously identified and 1 newly identified elements).

Table 1
Prevalence of *XRCC5* VNTR polymorphism (rs6147172) among healthy Iranian blood donors.

Genotypes	Number	Percent
0R/0R	3	0.56
0R/1R	33	6.17
1R/1R	168	31.40
0R/2R	29	5.42
1R/2R	205	38.32
2R/2R	84	15.70
0R/3R	1	0.17
1R/3R	8	1.50
2R/3R	4	0.75

suggested that the 3R allele might be present among Caucasian populations. Also it is suggested that the frequency of the 3R allele among European populations might be higher than its prevalence among the Iranian population.

Conflict of interest statement

No conflicts of interest exist.

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