



ORIGINAL ARTICLE

# Allelic prevalence of intron 3 insertion/deletion genetic polymorphism of DNA double-strand break repair gene *XRCC4* in four healthy Iranian populations



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## KEYWORDS

Ins/Del;  
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**Abstract** *Background and purpose:* The X-ray cross-complementing group 4 (*XRCC4*; OMIM: 194363), plays an important role in repair of DNA double-strand breaks via non-homologous end joining pathway. In order to find the allelic prevalence of an insertion/deletion polymorphism in intron 3 of *XRCC4* (Ins/Del; rs28360071) among Iranian populations, the present study was carried out.

*Subjects and methods:* The total study subjects consisted of 662, 200, 291, and 200 individuals from Shiraz (Fars province; belong to Persians), Abarku (Yazd province; belong to Persians), Tabriz (East Azerbaijan province; belong to Azaris), and Yasuj (Kohgiluyeh va Boyer-Ahmad province; belong to Lurs), respectively. Genotypic analysis of the Ins/Del *XRCC4* polymorphism was detected by the PCR method.

*Results:* The prevalence of the Del allele in Shiraz, Abarku, Tabriz, and Yasuj was estimated 44.4%, 37.3%, 53.6% and 45.0%, respectively. Tabriz (Azaris) showed the significant difference with other populations ( $\chi^2 = 20.06$ ,  $df = 1$ ,  $P < 0.001$ ). Also there was a significant difference between Persians of Shiraz and Abarku ( $\chi^2 = 6.43$ ,  $df = 1$ ,  $P = 0.011$ ).

*Conclusion:* Although there is a significant heterogeneity between Iranian populations, the Del allele shows high prevalence among Iranian populations, which is much higher than the allelic prevalence among Asians.

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## 1. Introduction

Eukaryote cells use at least two pathways in order to repair DNA double-strand breaks: homologous recombination and non-homologous end joining (NHEJ). The X-ray

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cross-complementing group 4 (*XRCC4*; OMIM: 194363), a ubiquitously expressed product, plays an important role in core of the NHEJ machinery [1]. The *XRCC4*-deficient phenotype in mice is associated with embryonic lethality, neuronal apoptosis, and impaired cellular proliferation [2].

An insertion/deletion genetic polymorphism (Ins/Del; rs28360071) described in the *XRCC4*. This polymorphism occurred by an insertion or deletion of a 30 bp sequence (GAT GAG GAA ACT AAC TCT CAG TGG TGT TTA) in intron 3 on the *XRCC4* gene (<http://www.ncbi.nlm.nih.gov/snp/?term=rs28360071>). There are a few studies concerning the Ins/Del *XRCC4* polymorphism and multifactorial disease [3–11]. Therefore, there is limited data concerning the geographic distribution of this polymorphism.

It is well established that Iranian population is one of the most heterogeneous populations of the world [12–18]. After Persians, Azaris and Lurs are major ethnic groups of Iranian population. The Turkish-speaking Azaris is one of the most important Iranian ethnic groups. They are living in several provinces of Iran including West and East-Azerbaijan, Ardabil and Zanjan, which are mainly located to west-north of Iran. Lurs is distributed in several provinces which mainly located in central, south-west and west parts of the country. Their language is a particular dialect of Persian. They are living in “Lorestan”, “Chaharmahal va Bakhtiari”, “Khuzestan”, “Isfahan”, “Fars”, and “Kohgiluyeh va Boyer-Ahmad” provinces. To the best of our knowledge, there is no published data about the Ins/Del genetic polymorphism of *XRCC4* in Iranian populations. Therefore the present study was carried out.

## 2. Subjects and methods

### 2.1. Participants

The total study subjects consisted of 662, 200, 291, and 200 individuals from Shiraz (Fars province; belong to Persians), Abarku (Yazd province; belong to Persians), Tabriz (East Azerbaijan province; belong to Azaris), and Yasuj (Kohgiluyeh va Boyer-Ahmad province; belong to Lurs), respectively. All individuals were healthy as assessed by medical history. Data on ethnicity were collected using a simple questionnaire including simple questions like the parental and grandparental ethnicity of each participant. Participants and their parents (and also their grandparental) did not belong to same ethnic groups were excluded.

Because the genetic polymorphisms of other DNA repair genes showed significant association with several multifactorial diseases [19–23], we excluded the participants with positive history for diagnosed cancers, psychiatric disorders, asthma, cataract and cardiovascular diseases. Informed consent was

obtained from each subject before the study. This study was approved by the Shiraz University ethics committee. The work has been carried out in accordance with The Code of Ethics of the World medical association (Declaration of Helsinki) for experiments in humans.

### 2.2. Genotyping analysis

Blood samples were obtained from the participants. Immediately after collection, whole blood was stored at  $-20^{\circ}\text{C}$  until use. Genomic DNA for PCR was extracted from whole blood. Genotypic analysis of the Ins/Del *XRCC4* polymorphism was carried out by PCR as described previously [10].

### 2.3. Statistical analysis

The difference in genotypic frequencies between ethnic groups was determined using the Chi-square test of goodness of fit. Data analysis was performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of  $P < 0.05$  was considered statistically significant. All  $P$ -values were two-tailed.

## 3. Results

The participants were initially divided into two gender groups. Since no statistical difference was observed between gender groups for the frequencies of the genotypes, the gender groups were pooled (data not shown).

The prevalence of the Del allele in Shiraz, Abarku, Tabriz, and Yasuj was 44.4%, 37.3%, 53.6% and 45.0%, respectively (Table 1). For each population, the genotypic frequencies were consistent with those expected from the Hardy–Weinberg equilibrium (Table 1). In overall, prevalence of the Del allele was estimated 0.4542 (95% CI: 0.4350–0.4733) in our country.

Statistical analysis showed that there was a significant difference between these populations for the prevalence of the Del allele ( $\chi^2 = 27.08$ ,  $df = 3$ ,  $P < 0.001$ ). Tabriz (Azaris) showed the significant difference with other populations ( $\chi^2 = 20.06$ ,  $df = 1$ ,  $P < 0.001$ ). On the other hand, there was a significant difference between Persians of Shiraz and Abarku ( $\chi^2 = 6.43$ ,  $df = 1$ ,  $P = 0.011$ ).

## 4. Discussion

Table 2 shows the prevalence of the Del allele in several populations. Although there are no published data from Western countries and African populations, the Del allele showed specific geographical distribution. The frequency of the Del

**Table 1** Distribution of the Ins/Del *XRCC4* genotypes among healthy blood donors in four Iranian populations.

Populations	Ins/Ins	Ins/Del	Del/Del	Total	Del allele (%)	HWE ( $\chi^2$ ) $df = 1$	$P$ -value
Shiraz	199	338	125	662	44.4	0.76	0.380
Abarku	83	85	32	200	37.3	1.65	0.198
Tabriz	62	146	83	291	53.6	0.02	0.882
Yasuj	55	110	35	200	45.0	2.46	0.116

Note: Hardy–Weinberg equilibrium (HWE); Chi-square test ( $\chi^2$ ); degree of freedom (df).

**Table 2** Distribution of the Del allele of Ins/Del *XRCC4* polymorphism in several populations.

Country/ethnic	Del allele (%)	References
Taiwan	17.6	[3–6]
China	17.4	[7]
India	12.7	[8]
Turkey	49.4	[9]
Iran (Persians, Shiraz)	44.4	Present study
Iran (Persians, Abarkuh)	37.2	Present study
Iran (Lurs, Yasuj)	45.0	Present study
Iran (Azaris, Tabriz)	53.7	Present study

allele increased from East to the West. The Del allele has low prevalence among Asian populations (about 15–20%) [3–8]. Although there are differences between Iranian populations for the prevalence of the Del allele, the Del allele shows high prevalence among Iranian populations, which is very higher than among Asian populations.

Based on a report, the Del allele showed high frequency (49.4%) in Turkey [9]. Comparing with our present data, the prevalence of the Del allele shows no significant difference between Iranian Azaris (Tabriz) and Turkey ( $\chi^2 = 1.98$ ,  $df = 1$ ,  $P = 0.159$ ). On the other hand, there is very distinct difference between Iranian populations and Asians for prevalence of the Del allele. It should be noted that the allelic prevalence of other genetic polymorphism among Iranian populations (including Azaris) and Turkey are more similar to Caucasians than Asians [17–19]. Taken together, it might be concluded that although Azaris (and also Turkey population) speak Turkish, they belong to Caucasians.

Finally although there are no published data from European populations for the study polymorphism it might be suggested that the Del allele has high prevalence in those populations. Further researches on other ethnic groups are necessary to find the geographical distribution of the polymorphism of Ins/Del in intron 3 of *XRCC4*.

#### Disclosure statement

The authors declare no conflict of interest.

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