

Prevalence of the rs7903146C>T polymorphism in *TCF7L2* gene for prediction of type 2 diabetes risk among Iranians of different ethnicities

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Background: Pharmacogenetics is the study of genetic polymorphisms affecting responses to drug therapy. The common rs7903146 (C>T) polymorphism of the *TCF7L2* gene has recently been associated with type 2 diabetes (T2D). In this study, prevalence of the rs7903146 (C>T) polymorphism in the *TCF7L2* gene for prediction of T2D risk was examined in an Iranian population of different ethnicities.

Methods: The prevalence of rs7903146 (C>T) and the predicted phenotypes, including extensive metabolizers, intermediate metabolizers, and poor metabolizers were investigated in blood samples of 300 unrelated healthy individuals in an Iranian population, including Fars, Turk, Lure, and Kurd, using polymerase chain reaction restriction fragment length polymorphism and direct genomic DNA sequencing.

Results: The homozygous wild-type (C/C), heterozygous (C/T), and homozygous (T/T) allelic frequencies of rs7903146 (C>T) in the *TCF7L2* gene were 29% (extensive metabolizers), 66.34% (intermediate metabolizers), and 4.66% (poor metabolizers), respectively. The C/C, C/T, and T/T genotypic frequencies of the rs7903146 (C>T) allele were significantly different ($P<0.01$) among Iranians of different ethnicities. The frequency of the homozygous T/T variant of the rs7903146 (C>T) allele was significantly low in the Lure ($P<0.01$) and high in the Fars ($P<0.001$) ethnicities. Additionally, the frequency of the T/T variant of the rs7903146 (C>T) allele in the South of Iran was the highest ($P<0.04$), while the East of Iran had the lowest frequency ($P<0.01$).

Conclusion: The prediction of rs7903146 (C>T) is required in drug research and routine treatment, where the information would be helpful for clinicians to optimize therapy and adverse drug reactions and predict drug response in individuals at risk of T2D.

Keywords: pharmacogenetics of type 2 diabetes (T2D), transcription factor 7-like 2 gene, *TCF7L2*, rs7903146 (C>T) SNP, Fars Turk Lure and Kurd, Iranian populations of different ethnicities

Introduction

Pharmacokinetics is the basis of personalized medicine to determine the activity of drug-metabolizing enzymes, where the occurrence of mutations in genes that are associated with drug metabolism alters drug efficacy.¹ Type 2 diabetes (T2D) is a common complex disorder resulting from the interplay of genetic and environmental factors.^{2,3}

A strong association was identified between T2D and a single nucleotide rs7903146C>T polymorphism, located within intron 4 of the *TCF7L2* gene.^{4,5} *TCF7L2* gene consists of 17 exons and is located on chromosome 10q25.3, where the rs7903146C>T polymorphism is the most important of these improved variants with negative effects on metabolism. Certain variants of *TCF7L2* gene increase the risk

of T2D and reduce insulin secretion.^{6,7} Prevalence of T2D is increasing in an epidemic fashion worldwide, attributed to a collision between genes and increasing globalization of the Western lifestyle, and a few genetic variants consistently associated with T2D.^{8,9} Using gene-locus genotyping, this variant has been consistently associated with T2D in European, Asian, and African populations.¹⁰ In this study, the prevalence of the rs7903146C>T polymorphism in the *TCF7L2* gene for the prediction of T2D risk was investigated among Iranians of different ethnicities, and in comparison with populations of other countries.

Materials and methods

In this study, 300 blood samples of unrelated and healthy donors from Iranian populations of different ethnicities, including 178 Fars, 75 Turk, 29 Lure, and 18 Kurd, were obtained from the Special Medical Centre, Tehran, Iran. Blood samples (2 mL) were taken and collected in tubes with ethylenediaminetetraacetic acid. Written informed consent, including consent to participate in the study for genetic analysis and consent to publish, was obtained from individuals, and the Medical Ethics Committee of the Special Medical Center specifically approved this study (approval number AA-25-2008). The exclusion criterion for the healthy control group was any history of cancer, metabolic diseases and nuclear and mitochondrial DNA-related diseases that may affect the DNA.

DNA extraction and primer sequences

Genomic DNA was extracted using an Molecular Biological System Transfer kit (salting-out method) from 2 mL of whole blood. Primer sequences for the rs7903146 (C>T) were ordered as described by Palizban et al.^{11,12} The oligonucleotide forward and reverse primers that were used to amplify the rs7903146C>T are as follows: F: 5'-TTA GAG AGC TAA GCA CTT TTT AGGTA-3', R: 5'-AGA GAT GAA ATG TAG CAG TGA AGTG-3'.

Restriction fragment length polymorphism (RFLP)

Genotyping of the rs7903146 (C>T) alleles was performed by polymerase chain reaction RFLP (PCR-RFLP). The PCR amplification was carried out with 20–60 ng of genomic DNA, 0.3 U of *Taq* DNA polymerase, 20 mM of each primers, 10× of PCR buffer, 2.5 mM of MgCl₂, and 10 mM of deoxynucleotide triphosphate (dNTP). The reaction mixture was initially denatured at 95°C for 3 minutes, followed by 35 cycles of 95°C for 1 minute, 58.3°C

for 50 seconds, 72°C for 50 seconds, and a final extension at 72°C for 10 minutes. The 201-bp amplified fragment was analyzed on 1.5% agarose gel stained with ethidium bromide before restriction digestion. One unit of *RsaI* restriction enzyme was added to each PCR product (5 μL) and incubated at 37°C for 16 hours. Digested products were analyzed on 8% polyacrylamide gel electrophoresis. The PCR product with a length of 201 bp was subsequently digested with the restriction endonuclease *RsaI*. The digestion of the homozygous wild type (C/C) leads to 176-bp and 25-bp fragments, a fragment of 201 bp for the homozygous mutant (T/T) and three fragments of 201 bp, 176 bp, and 25 bp for the heterozygous (C/T) genotype (Figures 1 and 2).

Sequencing analysis

The PCR products were examined for specificity using 1.5% agarose gel electrophoresis. Double-stranded DNA automated sequencing was performed using an ABI 3100 sequencing machine (Applied Biosystems, Kavosh Fanavaran Kawsar Company, Iran). All fragments were sequenced in both the forward and reverse directions for confirmation of any nucleotide variation. Sequence variants were analyzed using FinchTV software (<http://www.geospiza.com/finchtv/>).

Calculation of genotypic and allelic frequencies

Genotypic and allelic frequencies were calculated among Iranians of different ethnicities using the equation which is available online.¹³

Statistical analysis

The statistical analyses were conducted using SPSS 17.0 to perform the chi-square and confidence interval (95%) tests

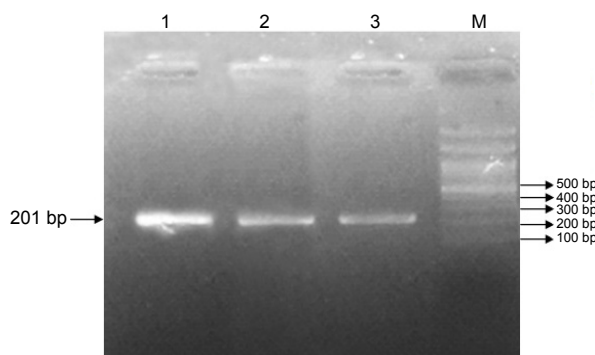


Figure 1 PCR analysis of the rs7903146 (C>T) allele on 1.5% agarose gel.

Note: Lanes 1, 2, and 3 represent a 201-bp fragment, indicating the homozygous T/T genotype.

Abbreviations: PCR, polymerase chain reaction; M, molecular size marker (100 bp ladder).

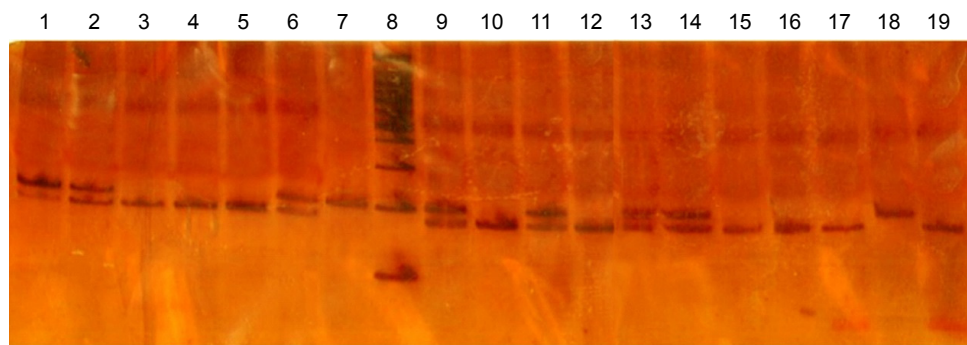


Figure 2 PCR-RFLP analysis of the rs7903146 (C>T) allele using *RsaI*.

Notes: This enzyme generated two fragments of 176 bp and 25 bp in length in the wild-type C/C genotype, a fragment of 201 bp in the homozygous T/T genotype, and three fragments of 201 bp, 176 bp, and 25 bp in the heterozygous C/T genotype, respectively. Lanes 3, 4, 5, 10, 12, 15, 16, 17, and 19 indicate the wild genotype. Lanes 1, 2, 6, 9, 11, 13, and 14 represent the heterozygous genotype, while lane 18 represents the homozygous (mutant) genotype. Lanes 7 and 8 show the control and ladder (100 bp), respectively.

Abbreviation: PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism.

to calculate the rs7903146C>T frequencies in different ethnicities and different geographical regions of Iran. A *P*-value of <0.01 is considered statistically significant.

Results

Allele and genotype frequency distributions of the rs7903146 (C>T) variants were analyzed from the blood samples of 300 unrelated healthy individuals in an Iranian population of different ethnicities. The allele frequencies of the homozygous (T/T), heterozygous (C/T), and wild type (C/C) variants of the rs7903146 (C>T) were observed in 14 (4.66%), 199 (66.34%), and 87 (29%) individuals, respectively (Table 1 and Figure 3). The frequency of the homozygous (T/T) variant of rs7903146 (C>T) was significantly high in the Fars ethnicity and low in the Lure ($\chi^2=13$,

$P<0.01$). However, the frequency of the homozygous (T/T) variant of rs7903146 (C>T) in Kurd ethnicity was similar to that of Turk (Table 1). The frequency of the heterozygous (C/T) variant of rs7903146 (C>T) was significantly high in Fars and low in Kurd ($\chi^2=198$, $P<0.01$). Additionally, the frequency of the wild type (C/C) variant of rs7903146 (C>T) was significantly highest in Fars, yet lowest in Lure ($\chi^2=86$, $P<0.01$) (Table 1).

The results indicated that the frequency of the homozygous (T/T) variant of rs7903146 (C>T) was significantly highest in the South of Iran ($\chi^2=2.571$, $P<0.01$) with poor metabolizers, while the homozygous (T/T) genotype was not detected in the East of Iran. The frequency of the heterozygous (C/T) variant of rs7903146 (C>T) had the highest frequency in central Iran with intermediate metabolizers, while

Table 1 Statistical analysis of the rs7903146 genotypes and metabolic phenotypes among Iranians of different ethnicities

Genotype	Metabolic phenotype	n	Ethnicities				Statistical analysis		
			Fars	Kurd	Lure	Turk	χ^2	P-value	
C/C	EM	87	38 (43.68%)	9 (10.34%)	8 (9.2%)	32 (36.78%)	86	<0.01*	
			95% CI 33.3–54.1	3.9–16.7	3.1–15.3	26.6–46.9			
C/T	IM	199	134 (67.34%)	6 (3.02%)	19 (9.55%)	40 (20.1%)	198	<0.01*	
			95% CI 60.8–73.9	0.6–5.4	5.5–13.6	14.5–25.7			
T/T	PM	14	6 (42.86%)	3 (21.43%)	2 (14.29%)	3 (21.43%)	13	0.005*	
			95% CI 16.9–68.8	0.1–4.2	0.01–3.2	0.1–4.2			
			Geographic						
			East	North	South	West	Central		
C/C	EM	87	2 (2.3%)	26 (29.89%)	17 (19.54%)	7 (8.05%)	35 (17.4%)	41.908	<0.01*
			95% CI 0.0–5.4	20.3–39.5	11.2–27.9	2.3–13.8	29.9–50.5		
C/T	IM	199	24 (12.06%)	45 (22.61%)	12 (6.03%)	20 (10.05%)	98 (39.8%)	121.327	<0.01*
			95% CI 7.5–16.6	16.8–28.4	2.7–9.3	5.9–14.2	42.3–56.2		
T/T	PM	14	0	1 (7.14%)	5 (35.71%)	4 (28.57%)	4 (28.57%)	2.571	<0.01*
			95% CI –	6.3–20.6	10.6–60.8	4.9–52.2	4.9–52.2		

Notes: Data presented as number (percentage). *Statistically significant ($P<0.05$); –not present.

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; CI, confidence interval.

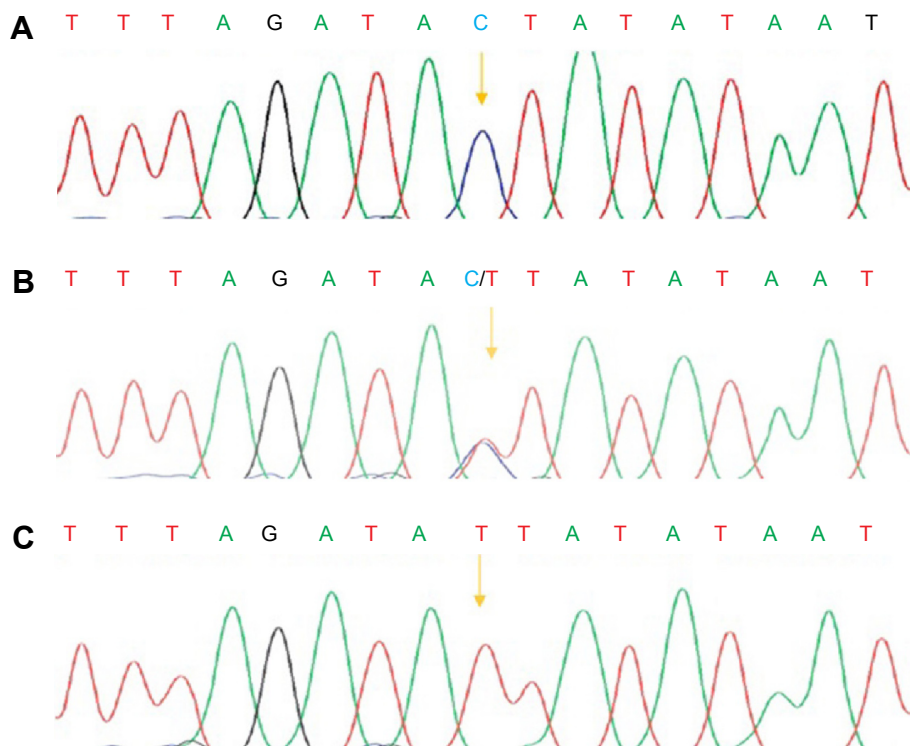


Figure 3 PCR-sequencing of rs7903146C>T.

Notes: The chromatogram shows the wild type (A), heterozygous (B), and mutant (C) alleles. The arrows mark the polymorphisms.

Abbreviation: PCR, polymerase chain reaction.

the South had the lowest frequency ($\chi^2=121.327$, $P<0.01$). Moreover, the frequency of the homozygous (C/C) variant of rs7903146 (C>T) had the highest frequency in the North with extensive metabolizers, while the East ($\chi^2=41.908$, $P<0.001$) had the lowest frequency (Table 1; Figure 4). The sequencing and PCR-RFLP analysis of the rs7903146 (C>T) allele using the *RsaI* enzyme demonstrates wild-type C/C, the heterozygous C/T and homozygous T/T genotypes (Figures 2 and 3).

Discussion

The *TCF7L2* gene is strongly associated with T2D, impaired insulin secretion,^{4,14–16} and cardiovascular risk,¹⁷ encoding for a transcription factor involved in Wnt (wingless and INT-1) signaling.¹⁸ Heterodimerization of *TCF7L2* with β -catenin induces transcription of a number of genes, including intestinal *GCG*.¹⁹ Here, the prevalence of the rs7903146C>T polymorphism in the *TCF7L2* gene for prediction of T2D was investigated in Iranian populations of different ethnicities, with comparisons to populations in other countries. Previous reports indicated that the *TCF7L2* gene is associate with T2D risk in different ethnic groups.^{11,20,21}

The meta-analysis by Dou et al²² indicated that among different single nucleotide polymorphisms of the *TCF7L2* gene, only the rs7903146 polymorphism has a significant association with T2D risk. However, another meta-analysis study indicated that the rs11196218 polymorphism in the *TCF7L2* gene was associated with risk of T2D mellitus among the Asian population.²³ Additionally, a study demonstrated that the rs7903146, rs12255372, and rs290487 polymorphisms of *TCF7L2* were associated with T2D in a Kurdish ethnic group of Iran.²⁴

Several studies demonstrated that the impact of a single rs7903146(T) polymorphism of *TCF7L2* gene in therapeutic response to sulfonylureas and metformin is lower in mutant alleles.^{25,26} In contrast to previous studies,^{27–29} the rs7903146 polymorphism in individuals leads to reduced enzyme activity and decrease of appropriate response to metformin and sulfonylurea drug treatments.^{30–33} The rs7903146 polymorphism in different ethnicities, including Lure, Turk, Fars, and Kurd, is considered as a possibly important determinant for the prevention of the harmful effects of metformin and sulfonylurea drug interactions, resulting in better survival and reduced mortality risk. However, in the present study, T2D patients and enzyme activity responses to metformin

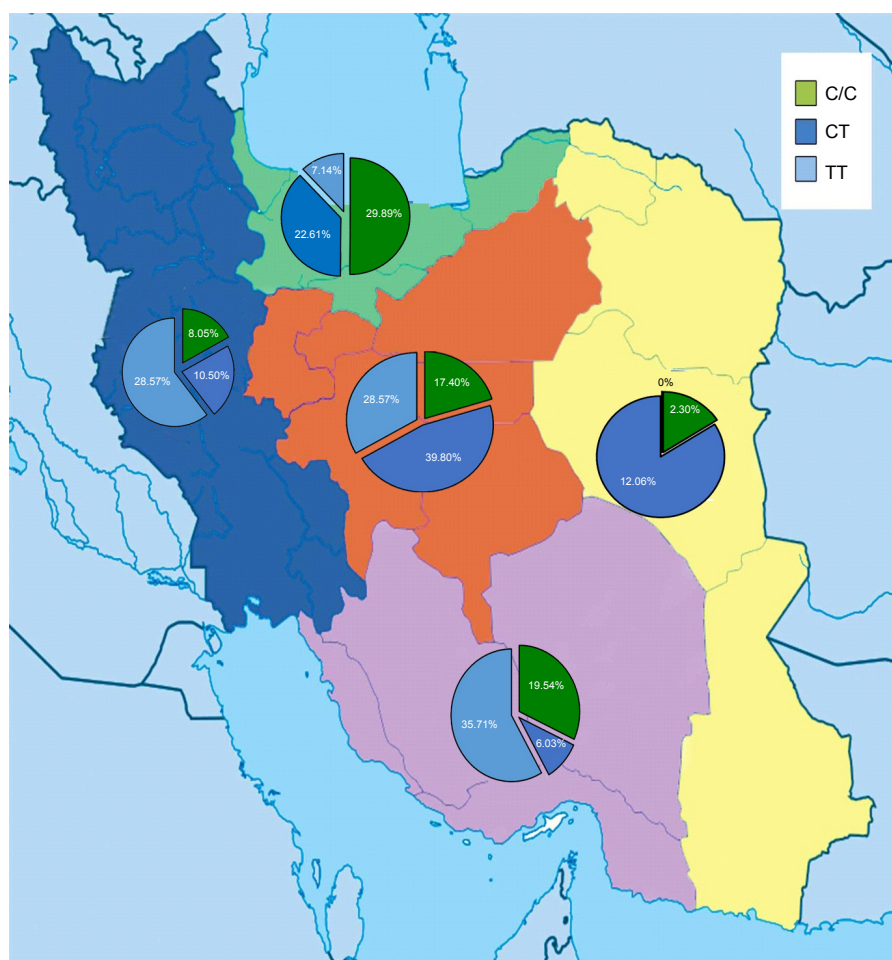


Figure 4 The distribution frequencies of genotype T/T, C/T, and C/C at locus rs7903146 among 300 normal Iranian individuals of different ethnicities were 4.66%, 66.34%, and 29%, respectively.

Notes: It was found that the frequency of the homozygous (T/T) variant of rs7903146 (C>T) was significantly highest in the South of Iran. However, the homozygous (T/T) genotype was not detected in the East of Iran. The frequency of the heterozygous (C/T) and homozygous (C/C) variants of rs7903146 (C>T) had the highest frequency in central and North Iran, respectively. Additionally, the frequency of the heterozygous (C/T) and homozygous (C/C) variants had the lowest frequency in the South and East, respectively. The green, pink, yellow, blue, and red colors indicate the North, South, East, West, and central Iran, respectively.

and sulfonylurea were not examined due to the fact that this study included only healthy individuals among Iranians of different ethnicities.

The frequencies of the rs7903146 polymorphism in our Iranian population with different ethnicities, including Fars, Turk, Lure, and Kurd, were compared with the frequencies seen in other ethnic populations (Table 2). Of 31 provinces of Iran, studies on only two provinces, Isfahan and Rafsanjan, showed that the frequency of the rs7903146 polymorphism was 0.41¹¹ and 0.33,²⁶ respectively. However, in the present study, the samples were selected from all 31 provinces of Iran, where the frequency of the homozygous (T/T) variant of rs7903146 (C>T) was high in the Fars ethnicity, but low in the Lure, similar to that of ethnic Turk and Kurd. The rs7903146 polymorphism was found with a frequency of 0.37 in the Iranian population,

which is higher than the Asian Indians,²⁵ Mexicans,¹² Italians,³⁴ Polish,³⁵ and one province of Iran, Rafsanjan,²⁶ but not as high as what was reported in other populations such as North India,³⁶ and one province of Iran, Isfahan.¹¹

In conclusion, this study demonstrates the presence of the rs7903146 polymorphism in the Iranian population, suggesting susceptibility to T2D and adverse or poor drug responses, which may lead to increase in mortality risk. It is clinically important to identify those individuals who probably have altered pharmacokinetics for the rs7903146 polymorphism in order to avoid adverse drug reactions. The rs7903146 polymorphism analysis in different ethnicities is a very useful forensic tool toward personalized medicine. These data also allow comparisons of pharmacogenetic variation between populations with different ethnicities. Therefore, the

Table 2 The rs7903146 (C>T) allele frequency in different populations

Continent	Population	Method	Numbers	Frequency	Reference
Asia	Iran	PCR-RFLP	300	0.37	Present study
	Isfahan*	PCR-RFLP	80	0.41	11
	Rafsanjan*	PCR-RFLP	168	0.33	25
	North India	PCR-RFLP	184	0.42	35
	Asian Indians	PCR-RFLP	621	0.30	24
America	Mexico	PCR-RFLP	275	0.17	18
Europe	Italian	PCR-RFLP	944	0.35	33
	Polish	PCR-RFLP	195	0.29	34

Note: *Indicates the frequency of only two out of 31 provinces of Iran.

Abbreviation: PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism.

determination of the presence of this allele in individuals is necessary to prevent the harmful effects of drugs.

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Disclosure

The authors declare no conflicts of interest in this work.

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