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Original Article

Association of CpG-SNP and 3'UTR-SNP of WFS1 with the Risk of Type 2 Diabetes Mellitus in an Iranian Population

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Type 2 diabetes mellitus (T2DM) is one of the most common multifactorial disorders in Iran. Recent genome wide association studies (GWASs) and functional studies have suggested that *WFS1* may predispose individuals to T2DM. However, to date, the possible association of such variants with T2DM in Iranians remained unknown. Here, we investigated the association of the two polymorphisms of *WFS1* (rs1801214 a CpG-SNP, and rs1046320 a 3'UTR-SNP) with T2DM in an Iranian population. The study population comprised 432 unrelated Iranian individuals including 220 patients with T2DM, and 211 unrelated healthy control subjects. Genotyping was performed using PCR-RFLP, and confirmed with sequencing. In a logistic regression analysis, the rs1801214-T allele was associated with a significantly lower risk of T2DM assuming the log-additive model (OR: 0.68, 95% CI: 0.52-0.91, P= 0.007539). Moreover, the G allele of rs1046320 was associated with a lower risk of T2DM assuming the log-additive model (OR: 0.68, 95% CI: 0.50- 0.91, P= 0.008313). Haplotype analysis revealed that haplotypes that carry at least one protective allele are associated with a lower risk of T2DM. This is a first evidence for the association of *WFS1* rs1801214, and rs1046320 with T2DM in an Iranian population.

Key words: WFS-1, type 2 diabetes, genome wide association study, single nucleotide polymorphisms

Type 2 diabetes mellitus (T2DM), the seventh cause of worldwide mortality is a complex disorder resulting from the absolute or relative lack of insulin (1). Prevalence of diabetes is greater than

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14% in Iranians aged above 30 years, and according to the International Diabetes Federation (IDF) website, more than 4.5 million Iranians were diagnosed as diabetic cases in 2014 (1,2). Sufficient insulin secretion from pancreas beta cells is essential to maintain blood glucose within normal range, and destroy beta cells during T2D development upon insufficient insulin secretion and diabetes progression (3,4).

A variety of environmental, epigenetic, genetic factors. and also interplay between these factors contribute to the development of T2DM. Genetic susceptibility to T2DM has been extensively explored by genome- wide association studies (GWASs), and accordingly a number of loci have been identified to be associated with T2DM (5-7). Among these, variants in the Wolferamin (*WSF1*) have been shown to modify the susceptibility to T2DM in different studies (8, 9).

Wolferamin is a 890 amino-acid transmembrane polypeptide located on chromosome 4p16 that is ubiquitously expressed, but has high expression rate in specific neurons and pancreatic beta cells (10). This protein is a novel endoplasmic reticulum (ER) transmembrane glycoprotein that plays a role in regulating calcium fluxes, and hemostasis in the ER (11,12). Mutations in *WFS1* are known to cause the Wolfram syndrome (WFS; OMIM 222300), an autosomal recessive neurodegenerative disorder that is clinically defined by diabetes insipidus, young-onset non-immune insulin-dependent diabetes mellitus, optic atrophy, and deafness. Therefore, WFS is also known as DIDMOAD syndrome (13). A growing body of evidence suggested that highly developed ER structure in beta cells had important roles in insulin production, and secretion in response to blood glucose levels (14-17). Impaired insulin secretion due to epigenetic modifications, and pathogenic variants in the *WSF1* results in progressive rodent and human glucose intolerance, and insulin deficiency due to ER stress and apoptosis of beta cells (11,18,19). Polymorphisms of the *WFS1* was suggested as a minor modulator of gene function, and susceptibility to polygenic forms of diabetes. To the best of our knowledge, there are no data regarding the possible contribution of the GWAS-identified locus at this gene to T2DM in Iranian population.

In this study, we evaluated the association of two *WFS1* variants with T2DM in an Iranian population. These variants include *WFS1* rs1801214, an index variant of the GWAS identified locus that removes a cytosine-phosphate-guanine (CpG) dinucleotide, and rs1046320 at 3'UTR of *WFS1* that is a candidate functional variant in high linkage disequilibrium (r^2= 0.97) with the index variant.

Materials and methods

Subjects

The study population comprised 432 unrelated Iranian individuals, including 220 patients with T2DM and 211 unrelated healthy control subjects, matched for age and gender. The diagnosis of T2DM was based on the WHO criteria (20, 21). T2DM was diagnosed as fasting plasma glucose (FPG) levels of ≥ 126 mg/dl, and 2-hour glucose concentrations of ≥ 200 mg/dl after a 75 g oral glucose tolerance test or HbA_{1c} > 6.5%. Written informed consent was taken from all participants. This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Control subjects were those without any past documented history of glucose intolerance or family history of diabetes that have glucose concentrations below the thresholds for T2DM.

DNA extraction

Peripheral blood samples were collected in EDTA tubes, and transferred to Medical Genetics department laboratory for DNA extraction. The DNA was extracted by use standard salting out method. The quality and quantity of extracted DNA was measured spectrophotometri cally at 260nm/

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280 nm wave length.

Genotyping

Genotyping was performed using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analysis (22). The PCR reaction consisted of 30 cycles at annealing 64 C for rs1801214 and 60 C for rs1046320. Restriction enzymes and primer sequences are presented in table 2. To confirm the accuracy of the genotyping method, twenty samples were randomly selected and the assigned genotypes were confirmed by Sanger sequencing of the genomic regions encompassing the polymorphisms (Table 2).

Statistical analysis

Genotyping results were tested for significant departure from Hardy-Weinberg equilibrium among patients and controls using χ^2 and all statistical analyses were performed by R programming language (version 3.1.0) (23). Differences in clinical variables and demographic characteristics between patients and controls were evaluated using *t*-test for continuous variables and χ^2 for categorical variables. Multivariate logistic regression analysis was undertaken in order to control age, sex and BMI category. The association of rs1801214, and rs1046320 with T2DM was implemented in the SNPassoc package (version 1.9-2). Odds ratios (OR), and respective 95% confidence intervals (95% CI) were calculated considering codominant, dominant, recessive, overdominant, and log-additive models. A p value <0.05 was considered to be statistically significant in this study .

Table 1. Characteristics of the study population.

	Patients (N=220)	Controls (N=211)	P value
Age (years)/Median	61.78 ± 9.71	60.71 ± 8.50	0.2396
Sex, Male (%)	43.69	46.44	-
BMI (Kg/m^2)	31.01 ± 4.79	25.95 ± 1.68	**> 0.001**
FPG (mg/dl)	148.36 ± 27.50	88.47 ± 9.69	**> 0.001**

BMI: body–mass index; FPG: fasting plasma glucose. Quantitative variables are shown as mean ± standard deviation, and qualitative variable is shown as percent. P values less than 0.05 are shown in bold face, and are statistically significant.

Table 2. The primer sequences of RFLP, sequencing, and PCR conditions for the studied SNPs.

SNP	Type of primers	Primer sequences 5' → 3'	Enzyme	PCR condition (ºC/s) Den.	Ann.	Ext.	DNA/ fragment size (bp)
rs1801214	PCR-RFLP	F:TTAGCCACCTGG TCGTCGTCAA R:AGGGCACAAGGT AGCAGTAGGTGC	Hind II	95/30	64/30	72/30	123/106+17
	Sequencing	F:GCAACCTCACCA TCGACTTC R:AGGGCACAAGGT AGCAGTAG		95/30	59/30	72/30	600
rs1046320	PCR-RFLP	F:CTTTCACCAGTGC CGCCTGTG R:GTATTCCCTTTGT CGGGGTGCA	*Sph I*	95/30	60/30	72/30	96/77+19
	Sequencing	F:GATCGAGTTCAG CACCATCCT R:AAAGGGGAAGA GCTGCTAAGG		95/30	60/30	72/30	679

Den: denaturation; Ann: annealing; Ext: extension.

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Results

Population characteristics

Clinical characteristics and demographic data are shown in table 1. The patients and controls were matched for age, and sex in this study. The patients had higher levels of BMI and FPG than those of controls (Table 1).

Association of *WFS1* polymorphisms with T2DM

The genotype frequencies of rs1801214, and rs1046320 were not significantly deviated from Hardy–Weinberg equilibrium among controls (P= 0.42 and 0.24, respectively). The genotype frequencies of the studied SNPs in patients and controls are presented in table 3. *WFS1* rs1801214 was associated with T2DM assuming codominant, recessive and log-additive modes of inheritance (Table 3). In the codominant model, individuals carrying the TT genotype had a significantly lower risk of T2DM in comparison with those who carry the CC genotype (TT vs. CC, OR: 0.41, CI: 0.22-0.77, P=0.016909). In the recessive model, individuals carrying the TT genotype had a lower risk of T2DM in comparison with those with CC+CT genotypes (TT vs. CC+CT, OR: 0.47, CI: 0.26 – 0.83, P= 0.008355). However, the model with the lowest P value was log-additive (OR: 0.68, CI: 0.52-0.91, P= 0.007539).

Logistic regression analysis revealed that rs1046320 was associated with T2DM risk in different modes of inheritance. In codominant model, individuals carrying the GG genotype had a lower risk of T2DM in comparison to those with AA genotype (OR: 0.47, CI: 0.23-0.93, P= 0.030530). The association was also statistically significant assuming a dominant model (AG+GG vs AA, OR: 62, CI: 0.42- 0.91, P=0.014049), recessive

Table 3. The distribution of genotypes in T2DM cases and controls.

Model	Genotypes	Patients N(%)	Controls N (%)	OR (95% CI)	P
rs1801214					
Codominant	CC	102 (45.9)	78 (37)	1.00	0.016909
	CT	100 (45.0)	96 (45.5)	0.80 (0.53-1.20)	
	TT	20 (9.0)	37 (17.5)	0.41 (0.22-0.77)	
Dominant	CC	102 (45.9)	150 (50.0)	1.00	0.057837
	CT+TT	120 (54.1)	78 (37)	0.69 (0.47-1.01)	
Recessive	CC+CT	202 (91.0)	174 (82.5)	1.00	0.008355
	TT	20 (9.0)	37 (17.5)	0.47 (0.26– 0.83)	
Overdominant	CC+TT	122 (55.0)	115 (54.5)	1.00	0.924656
	CT	100 (45.0)	96 (45.5)	0.98 (0.67–1.43)	
log-Additive	-	-	-	0.68 (0.52-0.91)	**0.007539**
rs1046320					
Codominant	AA	134 (60. 4)	100 (48.5)	1.00	0.030530
	AG	73 (32.9)	82 (39.8)	0.66 (0.44- 1.00)	
	GG	15 (6.8)	24 (11.7)	0.47 (0.23-0.93)	
Dominant	AA	134 (60.4)	100 (48.5)	1.00	0.014049
	AG+GG	88 (39.6)	106 (51.5)	0.62 (0.42-0.91)	
Recessive	AA+AG	207(93.2)	182 (88.3)	1.00	0.078039
	GG	15 (6.8)	24(11.7)	0.55 (0.28-1.08)	
Overdominant	AA+GG	149 (67.1)	124 (60.2)	1.00	0.136481
	AG	73 (32.9)	82 (39.8)	0.74 (0.50-1.10)	
log-Additive	-	-	-	0.68 (0.50-0.91)	**0.008313**

CI: confidence interval; N/A: not applicable; OR: odds ratio. P values for the most probable genetic models are indicated with bold face, and are statistically significant.

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Table 4. Haplotype analysis of rs1801214, and rs1046320 in Iranian population.

Haplotype	rs1801214-rs1046320	Haplotype Frequency	OR (95% CI)	P value
1	[C;A]	0.4028	1.00	
2	[C;G]	0.2374	0.48 (0.32-0.70)	**0.0002**
3	[T;A]	0.3250	0.48 (0.33- 0.70)	**0.0002**
4	[T;G]	0.0348	**0.35 (0.14- 0.88)**	**0.0252**

OR: odds ratio. P values less than 0.05 are shown in bold face. Bold values are statistically significant

model (GG vs AA+AG, OR: 0.55, CI: 0.28-1.08, P= 0.078039), over dominant model (AG vs AA+GG, OR: 0.74, CI: 0.50- 1.10, P= 0.136481) or a log additive model (OR: 0.68, CI: 0.50- 0.91, P= 0.008313). The log-additive model had the lowest P value (Table 3).

Haplotype analysis

We further estimated the frequencies of haplotypes for these SNPs, and evaluated the association of haplotypes with T2DM. Table 4 represents the frequency of haplotypes in the studied population along with the results of association analysis. The most frequent haplotype (haplotype 1 in the table 4, consisting of rs1801214-C, and rs1046320-A) was used as a reference haplotype. The results showed that haplotypes that contain at least one protective allele (either rs1801214-T or rs1046320-G) had a lower risk of T2DM in comparison to the reference haplotype (Table 4).

Discussion

T2DM is one of the most worldwide health problems of the 21th century, and many genetic variants were shown to be associated with disease susceptibility. *WFS1* is a new susceptible diabetes gene, which has been confirmed recently with genome wide association, and replication studies (8, 9, 24-26). We showed that rs1801214 was significantly associated with T2DM in the studied Iranian population. Moreover, this is the first replication study for this SNP and the results were in line with the original GWAS with the T allele being associated with a lower risk of T2DM (8). Subjects with at least one T allele had a lower risk of T2DM. *WFS1* rs1801214 is located in the coding sequence of *WFS1*, removing a CpG dinucleotide site that has the potential for DNA methylation. It has been shown that different methylation pattern of CpGs overlapping with SNP may affect the expression of target genes (27). Analyzing 19 CpG overlapping SNPs that were identified by T2DM GWASs, Dayeh et al. showed that all CpG-SNPs were associated with different DNA methylation pattern of CpG sites at islet tissue of diabetic donors. Furthermore, they demonstrated that rs1801214 was associated not only with differential DNA methylation of surrounding CpG sites, but also with differential exon expression, and alternative splicing (19). Therefore, this genetics-epigenetics interaction may interfere with DNA binding of regulatory proteins that, in turn, may influence local DNA methylation or alternative splicing, and expression of target genes (27).

Rs1046320 is located at the 3'UTR of *WFS1* that may affect miRNA binding site, and has been another investigated variant in this study that was found to be significantly associated with T2DM risk. Previous studies demonstrated that genetic variations within or proximal to miRNA binding sites in target genes have the potential to either destroy or create miRNA binding sites which affect on target genes expression, phenotypes or causes disease (28). We identified that GG genotype or the G allele of rs1046320 had protective effect against T2DM. Several studies have been performed to

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elucidate the role of rs1046320 SNP in T2DM pathogenesis. Katherine et al. has performed a fine mapping experiment in UK and showed that rs1046320 has a protective effects against T2DM. This protective effect was also confirmed in a meta-analysis performed by the same authors (24). Another study on Hungarian population has evaluated the association of *WFS1* 3'UTR variants potentially affecting miRNA binding including rs1046320 with T2DM, and confirmed the protective role of the G allele (25). Besides, rs1046320 may potentially alter miR-204-5p and miR-211-5p binding to *WFS1* transcript that may imply a mechanism by which this SNP contribute to T2DM risk.

Furthermore, we estimated the frequencies of haplotypes for these SNPs and evaluated the association of haplotypes with T2DM, and identified that haplotypes carrying at least one protective allele of either SNPs were associated with a lower risk of T2DM. Different experimental and meta-analysis studies have investigated the association of *WFS1* variants located at this haplotype (rs10010131, and rs6446482) with the risk of T2DM and many lines of evidence indicate that polymorphisms of *WFS1* had important effects on insulin secretion, insulin sensitivity and the risk of hyperglycemia in T2DM which need to be confirmed in different functional analysis studies (29-31).

In conclusion, we have replicated the association between two SNPs of *WFS1*, and risk of T2M in an Iranian case-control study. Our results are a first report of significant association of the T allele of rs1801214 polymorphism and G allele of rs1046320 polymorphism with reduced risk of T2DM in Iranian population, and also a first replication study of rs1801214. Further studies are needed to fully elucidate the role of *WFS1* SNPs in T2DM susceptibility.

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Conflict of interest

The authors declared no conflict of interest

References

1. American Diabetes Association. Statistics About Diabetes. 2017; Available from: http://www.diabetes.org/diabetes-basics/statistics/.

2. Haghdoost AA, Rezazadeh-Kermani M, Sadghirad B, et al. Prevalence of type 2 diabetes in the Islamic Republic of Iran: systematic review and meta-analysis. East Mediterr Health J 2009;15:591-9.

3. Cerf ME. Beta cell dysfunction and insulin resistance. Front Endocrinol (Lausanne) 2013;4:37.

4. Dooley J, Tian L, Schonefeldt S, et al. Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes. Nat Genet 2016;48:519-27.

5. Esparza-Castro D, Andrade-Ancira FJ, Merelo-Arias CA, et al. Genome-wide association in type 2 diabetes and its clinical application. Rev Med Inst Mex Seguro So 2015;53:592-9.

6. Polychronakos C, Alriyami M. Diabetes in the post-GWAS era. Nat Genet 2015;47:1373-4.

7. Gaulton KJ, Ferreira T, Lee Y, et al. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. Nat Genet 2015;47:1415-25.

8. Long J, Edwards T, Signorello LB, et al. Evaluation of genome-wide association study-identified type 2 diabetes loci in African Americans. Am J Epidemiol 2012;176:995-1001.

9. van Hoek M, Dehghan A, Witteman JC, et al. Predicting type 2 diabetes based on polymorphisms from genome-wide association studies: a population-based study. Diabetes 2008;57:3122-8.

10. Takeda K, Inoue H, Tanizawa Y. WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. Hum Mol Genet 2001;10:477-84.

11. Osman AA, Saito M, Makepeace C, et al. Wolframin expression induces novel ion channel activity in endoplasmic reticulum membranes and increases intracellular calcium. J Biol Chem 2003;278:52755-62.

12. Unanue ER, Urano F. Endoplasmic reticulum: an interface

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[DOI: 10.22088/BUMS.6.4.197]

between the immune system and metabolism. Diabetes 2014;63:48-9.

13. Barrett TG, Bundey SE. Wolfram (DIDMOAD) syndrome. J Med Genet 1997;34:838-41.

14. Scheuner D, Kaufman RJ. The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. Endocr Rev 2008;29:317-33.

15. Fonseca SG, Fukuma M, Lipson KL, et al. WFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pancreatic beta-cells. J Biol Chem 2005;280:39609-15.

16. Fonseca SG, Ishigaki S, Oslowski CM, et al. Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. J Clin Invest 2010;120:744-55.

17. Kaufman RJ. Beta-cell failure, stress, and type 2 diabetes. N Engl J Med 2011;365:1931-3.

18. Fonseca SG, Gromada J, Urano F. Endoplasmic reticulum stress and pancreatic beta-cell death. Trends Endocrinol Metab 2011;22:266-74.

19. Dayeh TA, Olsson AH, Volkov P, et al. Identification of CpG-SNPs associated with type 2 diabetes and differential DNA methylation in human pancreatic islets. Diabetologia 2013;56:1036-46.

20. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva2011. p. 25

21. World Health Organization. and International Diabetes Federation., Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia : report of a WHO/IDF consultation Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia : report of a WHO/IDF consultation. Geneva: World Health Organization; 2006.

22. Torkamandi S, Moghbeli M, Farshchian M, et al. Role of Brg1 in progression of esophageal squamous cell carcinoma. Iran J Basic Med Sci 2014;17:912-7.

23. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Austria: Vienna; 2014; Available from: http://www.R-project.org/.

24. Fawcett KA, Wheeler E, Morris AP, et al. Detailed investigation of the role of common and low-frequency WFS1 variants in type 2 diabetes risk. Diabetes 2010;59:741-6.

25. Elek Z, Nemeth N, Nagy G, et al. Micro-RNA Binding Site Polymorphisms in the WFS1 Gene Are Risk Factors of Diabetes Mellitus. PLoS One 2015;10:e0139519.

26. Sandhu MS, Weedon MN, Fawcett KA, et al. Common variants in WFS1 confer risk of type 2 diabetes. Nat Genet 2007;39:951-3.

27. Taqi MM, Bazov I, Watanabe H, et al. Prodynorphin CpG-SNPs associated with alcohol dependence: elevated methylation in the brain of human alcoholics. Addict Biol 2011;16:499-509.

28. Gong W, Xiao D, Ming G, et al. Type 2 diabetes mellitus-related genetic polymorphisms in microRNAs and microRNA target sites. J Diabetes 2014;6:279-89.

29. Cheng S, Wu Y, Wu W, et al. Association of rs734312 and rs10010131 polymorphisms in WFS1 gene with type 2 diabetes mellitus: a meta-analysis. Endocr J 2013;60:441-7.

30. Franks PW, Rolandsson O, Debenham SL, et al. Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. Diabetologia 2008;51:458-63.

31. Florez JC, Jablonski KA, McAteer J, et al. Testing of diabetes-associated WFS1 polymorphisms in the Diabetes Prevention Program. Diabetologia 2008;51:451-7.

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