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RESEARCH ARTICLE

ROLE OF TMPRSS6 (V736A) GENE POLYMORPHISM IN THE HEMATOLOGICINDEXES AND THE RISK OF TYPE 2 DIABETES IN AN IRANIAN POPULATION

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ABSTRACT

Background: Transmembrane protease serine 6 (TMPRSS6) regulates iron homeostasis by inhibiting the expression of Hepcidin. Multiple common variants in TMPRSS6 were significantly associated with serum iron and hematology Indexes. Recent genome wide association studies indicated that hematologic Indexes differenced in Type 2 diabetes (T2D). The present study aimed to investigate the possible associations between TMPRSS6 polymorphism, rs855791, and susceptibility to T2D.
Design: The study set to determine whether the TMPRSS6 single nucleotide polymorphism (SNP) rs855791 (V736A) was associated with blood hemoglobin, Hematocrit, serum iron concentrations, and risk of T2D in individuals in Southeast of Iran. To investigate genetic polymorphism, 500 unrelated (250 T2D and 250 Healthy controls (HCs)) Iranians were selected. Genotyping of rs855791 (V736A) in the TMPRSS6 gene variant was performed using Allele-Specific Polymerase Chain Reaction (AS-PCR).
Results: The TMPRSS6 rs855791 (V736A) was not significantly associated with T2D (P =0.41, P=0.34 for TC, CC genotype, respectively). But, in evaluation between genotypes (TT and TC + CC) of this SNP and the clinical and demographic data were showed significant difference in Sex, hemoglobin (Hb) and Hematocrit in T2D(0.025, 0.025 and 0.027 respectively) and Hb (0.013)in HCs.
Conclusions: These findings suggest that TMPRSS6 variant were not significantly associated with type 2 diabetes in a sample of Iranians thus further studies with various ethnics and larger sample sizes are necessary to verify our findings.

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both that exacerbated by bad diet. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Gavin, 1997). Symptoms of marked hyperglycemia include polyuria, polydipsia, sometimes with polyphagia, and blurred vision. Several pathogenic processes are involved in the development of diabetes. This ranges from autoimmune destruction of the cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action (Organization, 1985). Iron is a nutrient essential for many physiologic functions, and iron balance is tightly regulated. Disorders of iron homeostasis are among the most common afflictions of humans, but both excessive and insufficient iron intake can be harmful (Andrews, 2000). Blood hemoglobin concentrations and plasma iron concentrations are therefore used as clinical indicators of body iron status (Organization, 2007). In addition, heritability estimates suggest that genetic factors contribute 20–30% of the variation in blood iron concentrations (Whitfield, 2000; Njajou, 2006; Pilia, 2006 and Marroni, 2008). The harmful effects of excessive iron have been shown in hereditary hemochromatosis that can result in abnormal glucose metabolism (Acton, 2006).

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There is also an observation that iron overload is associated with an increased incidence of T2D (Merle, 2007). Hematocrit is the most important determinant of whole blood viscosity (Chien, 1977). Blood viscosity and vascular resistance affect total peripheral resistance to blood flow (Bristow, 1969), which is abnormally high in the established phase of primary hypertension (Tibblin, 1966). Correspondingly, clinical studies have reported that increase of hematocrit is followed by increase in blood pressure and possibly onset of hypertension in anemic patients (Eschbach, 1987; Schaefer, 1988; Satoh, 1990).Various Reports publish showed some of Single Nucleotide Polymorphism (SNPs) were associated with Blood cell phenotypes (Ganesh, 2009; Finberg *et al*., 2011). Tran membrane Protease, Serine 6 (TMPRSS6) has important role in inhibit hepcidin transcription (Gan, 2012). The rs855791 gene polymorphism of TMPRSS6 with a no synonymous substitution demonstrated the strong association with hematologic indexes and iron status among different population. This SNP effects on hepcidin transcription with reduces the ability of TMPRSS6 enzyme (Pei, 2014).

Subjects

The current study set to determine the association of TMPRSS6 SNP rs855791 (V736A) with blood hemoglobin, serum iron, and hematocrit concentrations in a population-based sample of Iranians. Also, the association of TMPRSS6 variant with risk of T2D was investigated.

MATERIALS AND METHODS

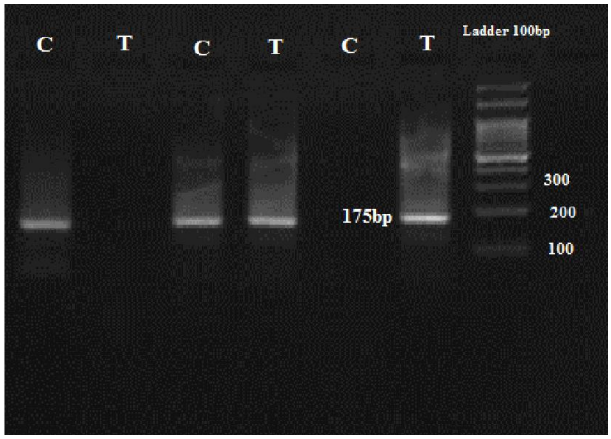
Sample collection

After we received ethical approval from Zahedan medical university Ethics Board, and ethical approval was obtained from all participants (with code 7248)subjects diagnosed with T2D were selected to biochemical parameters including: Fasting blood Sugar (FBS), HbA1C (FBS≥126mg/dL and HbA1C>6.7%) and healthy controls had no previous history of diabetes and without any relation with T2D group with normal biochemical parameters (FBS=70-100mg/dL and HbA1C ≤6.7%), finally both of groups were confirmed by a physician. After the subjects signed written informed consents, from either T2D or Healthy controls, 2 mL of peripheral blood in an Ethylene DiamineTetra Acetic Acid (EDTA) tube and 2 mL for the tubes without anticoagulant for preparation of serum were used, then the samples were sent to the laboratory and stored at -20 ° C until examination.. A total of 500 blood samples were collected (250 subjects with T2D and 250 healthy controls).

Genotyping

DNA extraction was accomplished using salting out method, after estimation of the quality of DNA by spectrophotometer (260/280). Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method was used to analyze the genotyping of rs855791 (V736A) T/C using Allele-Specific Primers (Table 1). The PCR conditions for the amplification of TMPRSS6 (rs855791) were as follows: initial phase comprised 5 min at 95°C as denaturation, then 30 cycles (30 sec at 95°C for denaturation, 30 sec at 62°C for annealing, and 30 sec at 72°C for extension); final extension comprised for 5 min at 72°C, then, stored at 4°C until electrophoresis.

All products of PCR with 175 bp were checked out under UV-ray (Figure 1).



Product size: 175 bp, sample 1: C, sample 2: TC, sample 3: T.

Figure 1. Electrophoresis Pattern of the Allele-Specific Primer (ASP)- Polymerase Chain Reaction (PCR) for Detection of TMPRSS6 SNP rs855791(V736A) C/T Polymorphism

Table 1. Allele-Specific Polymerase (ASP), Polymerase Chain Reaction Primers sequences

Primer 5'-3'	Product	Method
rs855791 Fw: CACAGGACCTGTGCAGCGAGGT Fm: CACAGGACCTGTGCAGCAAGGC R: GATGTGAGCAAAGGGCCAGAC	175bp	ARMS

Assessment hematologic indexes and iron level

For all the tests, Hemoglobin and Hematocrit were measured using the automatic Cell Counter (Sysmex, KX-21N, Japan). serum iron was measured using a commercially available kit Pars Azmon, Iran (Biotecnlca instrument, auto-analyzer BT-1500, Italy).The hemoglobin concentration, 120-160 g/L for women and, 140-180 g/L for men, also normal serum iron was 40-120 µg/dL in men and 39-149 µg/dL in women, and hematocrit concentrations were 34.9-44.5% and 38-50% in women and men, respectively.

Statistical analysis

SPSS version 16.0 (SPSS, Chicago) was used for all the statistical analyses. These included categorical data (Pearson's $\chi 2$), adjustment of P-values with variables using the binary logistic regression test for estimation of the odds ratios (OR), 95% confidence intervals, and for the relationship between TMPRSS6 (rs855791) gene polymorphism and parametric hematology and serum iron used Independent-Samples T Test. The significance level was set at $P \leq 0.05$.

RESULTS

The T/T, T/C, and C/C genotypes were found in 30%, 45.2%, and 24.8% of healthy controls (HCs), in comparison with 27.2%, 46.8%, and 26% of T2D people, respectively. The allele frequencies of (T/C) were 52.6% (T) and 47.4% (C) in HCs and 50.6% (T) and 49.4% (C) in diabetic patients, respectively. The distribution of polymorphism in rs855791 (T/C) was not significantly different between patients and controls for TC (OR =1.19(0.78-1.82), P =0.41), CC (OR =1.27(0.78-2.04), P =0.34) genotypes, and C (OR = 1.08(0.85-1.39), P =0.57) allele (Table 2).

Table 2. Genotype and allelic frequencyofrs855791SNP in patients and control subjects

SNP (rs855791)	Genotype, alleles	T2D n (%)	HCs n (%)	*OR (95% CI)	*P*-value
Codominant	TT	68 (27.2%)	75 (30%)	Ref	-
	TC	117(46.8%)	113(45.2%)	1.19(0.78-1.82)	0.41
	CC	65 (26%)	62 (24.8%)	1.27(0.78-2.04)	0.34
Alleles	T	253(50.6%)	263(52.6%)	Ref	-
	C	247(49.4%)	237(47.4%)	1.08(0.85-1.39)	0.57
Dominant	TT	68 (27.2%)	75 (30%)	Ref	
	TC + CC	182 (72.8%)	175 (70%)	1.15(0.78-1.69)	0.49
Recessive	TT + TC	185 (74%)	188 (75.2%)	Ref	
	CC	65 (26%)	62 (24.8%)	1.06 (0.71-1.59)	0.76

Table 3. Association between TMPRSS6 polymorphism with clinical demographic and characteristics of T2D patients and HCs

rs855791	Sex(Female/male)	Age	Hb	Iron	HCT
T2D					
TT	34(F)/30(M)	48.28 ± 7.83	12.84±2.07	70.24±35.26	41.17±6.27
TC+CC	128(F)/58(M)	49.64±8.85	12.71±1.48	73.21±29.13	40.17±4.43
P-value	0.025	0.345	0.025	0.264	0.027
HCs					
TT	49(F)/26(M)	48.79±8.44	13.74±2.35	95.42±22.98	41.74±5.61
TC+CC	114(F)/61(M)	48.49±8.49	13.81±1.81	104.13±21.50	42.23±4.81
P-value	0.977	0.565	0.013	0.677	0.071

We tested SNP (rs855791) for its associations with the concentrations of hemoglobin, hematocrit and serum iron. In evaluation between genotypes (TT and TC + CC) of this SNP and the clinical and demographic data were showed significant difference in Sex, hemoglobin (Hb) and Hematocrit in T2D(0.025, 0.025 and 0.027 respectively) and Hb (0.013)in HCs. (Table 3). In evaluation between demographic and clinical data between T2D and HCs, we found hemoglobin (p=0.006) and serum iron (P=0.000) were significantly associated with type 2 diabetes; in contrast; Hematocrit (HCT) (P=0.090) did not have such association (Table 4).

Table 4. Demographic characteristics of T2D patients and controls

Data	HCs	T2D	P-Value
Sex(Female/Male)	163/87	161/89	0.899
Age	48.58±8.46	49.28±8.60	0.824
Hb	13.79±1.98	12.75±1.65	0.006
Iron	101.52±22.27	72.44±30.80	0.000
HCT	42.08±5.05	40.42±4.97	0.090

DISCUSSION

TMPRSS6 gene encoding serine protease Matriptase-2, is serine protease that has negative regulator role in hepcidin expression and important task in maintaining iron hemostasis. Previous studies on both human and animal showed mutation on catalytic domain of Mastriptase-2 leads the loss of inhibiting effect of this enzyme on hepcidin expression, thus increase hepcidin transcription, iron deficiency anemia and reduced absorption of dietary iron (He, 2012). In Present study, we evaluated association between TMPRSS6 rs855791 polymorphism and T2D. We found no statistically association between TC and CC genotypes and T2D as well as C allele of this SNP, while in evaluation clinical and demographic data between T2D and HCs, we observed significant association in hemoglobin and serum Iron. Another result of current investigation, the data showed significant association between genotypes of rs855791 polymorphism with hematologic indexes (Hb and HCT) and sex in T2D group and Hb in HCs.

There is little data regarding the role of TMPRSS6 polymorphism and T2D risk. Gan *et al* have found that TMPRSS6 rs855791 (V736A) variant increased the risk of T2D in Chinese Hans (Gan, 2012) but in another study no significant association was found between either CT or TT genotypes this variant (rs855791) of TMPRSS6 with T2D in American women although normally significant was showed in men as decreased risk of T2D (He, 2012). M He *et al* have investigated TMPRSS6 rs855791 (V736A) polymorphism in T2D. They found no significant association between this SNP and heme iron intake and ferritin (He, 2012) Although in another study were done by X Guo *et al* have observed significant association between ferritin and T2D, however they found no statistically association between Hb and T2D (Guo, 2013). IjKullo *et al* have studied TMPRSS6 rs855791 in peripheral arterial disease. They found that the rs855791 variant have significant association with RBC traits (Kullo, 2010) in another study in Africa (rural southern Rwanda) have investigated relation between the role of iron deficiency and of the TMPRSS6 (rs855791) T allele, data showed to reduce iron status and Hb levels (Danquah, 2014). In conclusion, our finding showed that TMPRSS6 rs855791 (V736A) polymorphism was not associated with T2D in our study population also statistically association between this SNP and hematologic indexes (Hb and HCT) in T2D and Hb in HCs. The clinical and demographic data between T2D and HCs have showed significant association between serum iron and Hb with T2D. However, these observations need to be confirmed in populations with larger sample sizes and other ethics.

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Conflict of Interests

The authors declare that there is no conflict of interests to disclose.

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