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The genetics study of gestational diabetes in Iranian women and DIO2 gene

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ABSTRACT

Introduction: Gestational diabetes mellitus (GDM) is defined as abnormal glucose tolerance that is first identified or diagnosedduring pregnancy.Deiodinase D2 is essential for the local production of T_3 through deiodination of triiodothyroxine (T4). Several polymorphisms in D2 have been described. The single-nucleotide polymorphism (SNP) in D2 Thr92Ala is associated with decreased enzyme activity and greater insulin resistance in non-diabetic and in type 2 diabetes mellits the purpose of this study was to investigate an association of rs225014 deiodinase 2 gene polymorphism with GDM.

Methods: In this case-control study, 100 patients with GDM and 100 healthy control non-GDM, with mean age 29 were consecutively recruited from the endocrinology clinic of Arak University of Medical Sciences. We analyzed D2 Thr92Ala polymorphism by Tetra-Arms PCR method. The distribution of alleles and genotypes of this polymorphism in two groupswere determined and the data were analyzed by Chi-square and logistic regression analysis.

Results: In the 100 of case group, the frequency of TT, TC and CC genotypes of rs225014 polymorphism were 6 (6%), 82 (82%) and 12 (12%), respectively that indicated asignificant difference compared with 100 of control group: TT 10 (10%), TC 87 (87%), CC 3 (3%) (P=0.03).

Conclusion: The results demonstrated that homozygosis for Ala/Ala polymorphism has increased risk for GDM. Totally, this polymorphism is significantly associated with GDM disease in Iranian population as reported by previous studies.

Key words: Gestational Diabetes, DIO2 Gene, Genetic Study

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Introduction:

Gestational diabetes mellitus (GDM) is defined as abnormal glucose tolerance that is first identified or diagnosedduring pregnancy (1). In addition to increasing the risk of adverse infant outcomes, GDM also has high predictive value for later development of type 2 diabetes (T2D) in the mother and in her off spring.Women who experience GDM have increased risk of developing T2D after pregnancy, ranging from 17% to 63% within 5-16 years after pregnancy depending on the population (2).

Thyroid hormones play an essential role in organism development and in the maintenance of metabolic homeostasis (5). Most actions of thyroid hormone are mediated by the active form of thyroid hormone, T_3 (6). Deiodinase D2 is essential for the local production of T_3 through deiodination of triiodothyroxine (T4) (6). Several polymorphisms in D2 have been described (6-8). The single-nucleotide polymorphism (SNP) in D2 Thr92Alais associated with decreased enzyme activity and greater insulin resistance in non-diabetic and in type 2 diabetes mellits (6-8). In this work, the association of GDM with SNP of Rs225014 deiodinase 2 gene was evaluated.

Methods:

Subject

In this case-control study, 100 patients with GDM and 100 healthy control non-GDM (The case and control were matched by age.), with mean age 29 were consecutively recruited from the endocrinology clinic of Arak University of Medical Sciences. The diagnosis of GDM was based on a 75g oral glucose tolerancetest (OGTT). This OGTT was performed at 24-28th weeks of gestation using a standardized glucose solution and based on thecriteria of the International Association of Diabetes Pregnancy StudyGroup (IADPSG). Also, non-GDM group was diagnosed when all plasma glucose levels were below the threshold values. A written consent was obtained from all cases. The difference in frequency of the DIO2 genotypes between the GDM patients and the control group were analyzed using the chi-square test. The odds ratios (ORs) and 95% confidence intervals (CIs) for the DIO2 genotype were calculated using logistic regression analysis after adjustment for age. A P-value b 0.03 was regarded statistically significant. All data were analyzed using SPSS 16.0 software. The sample size of study was calculated based on following formula (9):

P1=0.35
P2=0.18
α =0.05
β =0.2
n1=n2=102

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \left[P_1(1-P_1) + P_2(1-P_2)\right]}{(P_1 - P_2)^2}$$

Genetic evaluation

Peripheral blood samples about 5cc in tubes containing EDTA (ethylene demine tetraacetic) were taken, was frozen and stored at -20 °C until thetime of DNA extraction. Genomic DNA was extracted based on instructionsof manufacturer by a genomic DNA purification kit (DNG plus DNA Extraction Kit, Sinaclon Co., Iran) and was held at 4°C until performing PCR. Alleles and genotypes were assessed by Tetra-Arms PCR (Tetra-primer amplification refractory mutation system-polymerase chain). Primers were designed by Primer Blast tool.

PCR reactions were carried out in a volume of 25µL containing 12.5µL of 2x PCR master mix (Sinaclon Co, Iran), 0.5µL (10 pmol/µL) of internal primers, 0.7µL (10 pmol/µL) of outer primers, 0.5µL (10Mm) $MgCl_2$, 1µL (~100 ng/µL) template DNA and 8.57µL deionized distilled water. Amplification was carried out in a Peqlab thermal cycler. PCR tests were done under the following program with Initial denaturation (4 min at 94°C) followed by 35 cycles (45 sec at 94°C, 45 sec at 55°C, then 45 sec at 72°C) and final extension 10 min at 72°C.

The PCR products were electrophoresed through 8% acrylamide gel, and visualized in the silver nitrate stained gels. Products of Tetra-ARMS-PCR reaction for Rs225014 polymorphism are including 500 bp (base pairs) (from activity of OF and OR primers as control) are present in all genotypes. T allele with 196 bp is result of activity of OF and IR primers and C allele with 324 bp is result of activity of OR and IF primers). Genotypes were defined as TT (500,196), TC (500,196 and 324) and CC (500,324). Primers sequences for detecting alleles and genotypes of Rs225014 (T>C) polymorphisms deiodinase 2 gene are shown in Table 1.

Table 1. Primers used for detecting of alleles and genotypes of Rs225014 of D2.

	Name	5'.....3'		TM
1	Of	GAACTCTTCCACCAGTTTGCG		59.74
2	OR	GTTAACTCCAGTGCCTTCAGTG	CACTGAAGGCACTGGAGTTAAC	59.19
3	IF	CCACTGTTGTCACCTCCTTCTGT		63.32
4	IR	TGTGGTGCATGTCTCCAGTg	CACTGGAGACATGCACCACA	60.25

F1: Forward Internal, RI: Reverse Internal, FO: Forward Outer, RO:Reverse Outer

Table 2. Frequency of genotypes in case and control groups

	Control N (%)	Case N (%)	OR	95% CI		P-value	test	
				Bunder	Upper		X^2	P-value
CC	3 (3)	12 (12)	1.66	33.69	1.319	0.02	6.5	0.03
TC	87 (87)	82 (82)	1.57	4.517	0.546	0.4		
TT	10 (10)	6 (6)	-	-	-			

Table 3. Frequency of genotypes in case and control groups

	Control N (%)	Case N (%)	OR	95% Confidence Interval		Test	
				Bunder	Upper	X^2	P-value
C	93 (46.5)	106 (53)	1.29	1.92	0.876	1.69	0.1
T	107 (53.5)	94 (47)					

Statistical analysis:

Statistical analysis was performed by SPSS 16 (Chicago, USA). The allele and genotype distribution in the control group versus the case group was compared by Chi-squared or Fisher's exact tests. Mean and standard deviation Quantitative variables in the control group versus the case group was compared using t-student Logistic regression was used to evaluate using logistic regression analysis after adjustment for age. The odds ratio (OR) and 95% confidence interval (95% CI) provide a measure of the strength of association.

Results:

In this study, the results were analyzed after detection of genotypes for target point in 200 samples.

In the100 of case group, the frequency of TT, TC and CC genotypes of Rs225014 polymorphism were 6(6%), 82(82%) and 12(12%), respectively that indicated a significant difference compared with100 of control group: TT 10 (10%), TC 87 (87%), CC 3 (3%) (P=0.03).

Also, the frequency of C allele was more in case group than in control group and inversely T allele was more frequency in control group than in case group. The patients with diabetes showed statistically significant upper frequency of TT and CC genotype compared to patients without diabetes. The frequency of genotypes and alleles in case and control groups are shown in table 1,2.

The chance of diabetes in persons who had CC genotype compared to TT genotype is significant (OR=1.66).

Conclusion:

In this study, we have performed a case–control study of genetic association between GDM and Thr92Ala polymorphism, which demonstrated that homozygosis for Ala/Ala polymorphism has increased risk for GDM. The Thr92Ala polymorphism is characterized by the first amino acid substitution 114 located in the 115 instability loop of D2, a site of importance for D2 turnover rate (10,11). Clinical and experimental data support a biologicalplausibility for a role of the D2 Thr92Ala variant in predisposition to Diabetes mellitus type 2 (DM2), a heterogeneous disease withmany environmental and genetic factor interactions (12).

The association of polymorphism of Rs225014 with multiple disorder including insulin resistant, DM2 and thyroid noduledue to decrease of expression level of D2 (deiodinase2) was determined in many studies (13-15). Similar results with our

study were reported by Grarup et al. (16) studied 7342 white subjects from Glostrup and Copenhagen (Denmark), in a mixed case–control and cross-sectional design.

In this study, in the unadjusted analyses, an association was verified between the D2 Ala/Ala genotype and glycemic traits of insulin resistance.

Dora et al. (13) reported that homozygosis for the Ala allele of the single-nucleotide polymorphism Thr/Ala in codon 92 of the D2 is asssociated with increased risk for DM2 reportedthat the carriers of Ala92Ala genotype have high frequency of remission in Graves' disease patients because they had lower activity of the enzyme and less active education of T3 in tissues. Also, Gumieniak et al. (17) reported that Ala92 carriers of Type 2 iodothyronine deiodinase Thr92Ala significantly more frequent in hypertensive subjects compared to normotensive subjects (64.8% versus 47.1%; *P*_0.011). Another similar results with our study have been reported byDora et al (13) that homozygosis for the Ala allele of the single-nucleotide polymorphism Thr/Ala in codon 92 of the D2 isasssociated with increased risk for DM2.

Totally, this polymorphism is significantly associated with GDM disease in Iranian population as reported by previous studies that mentioned above. To explain of this association, there is maybe a role based on other studies for the effect of this polymorphism on T_3 expression. This subject is needed to be more researched in larger samples and further functional analysis.

We found a significant association of and TT genotypes of Rs225014 polymorphismof D2 and the presence of GDM. Although, we suggest further studies with larger sample sizes toconfirm these findings.

Conflict

Authors declare no conflict of interest.

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