Original Article

Association of vitamin D receptor gene polymorphisms with type 2 diabetes mellitus in Taif population: a case-control study

Associação de polimorfismos do gene do receptor de vitamina D com diabetes mellitus tipo 2 na população Taif: um estudo de caso-controle

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Abstract

Several reasons may underlie the dramatic increase in type2 diabetes mellitus. One of these reasons is the genetic basis and variations. Vitamin D receptor polymorphisms are associated with different diseases such as rheumatoid arthritis and diabetes. The aim of this study is to investigate the possible association of two identified mutations ApaI (rs7975232) and TaqI (rs731236). Eighty-nine healthy individuals and Fifty-six Type 2 Diabetic (T2D) patients were investigated using RFLP technique for genotyping and haplotyping as well. The distribution of Apal genotypes was not statistically significant among the control (P=0.65) as well as for diabetic patients (P=0.58). For Taql allele frequencies of T allele was 0.61 where of G allele was 0.39. The frequency distribution of Taql genotypes was not statistically significant among the control (P=0.26) as well as diabetic patients (P=0.17). Relative risk of the allele T of Apa1 gene is 1.28 and the odds ratio of the same allele is 1.53, while both estimates were < 1.0 of the allele G. Similarly, with the Taq1 gene the relative risk and the odds ratio values for the allele T are 1.09 and 1.27 respectively and both estimates of the allele C were 0.86 for the relative risk and 0.79 for the odds ratio. The pairwise linkage disequilibrium between the two SNPs Taq1/apa1 was statistically significant in control group (D = 0.218, D' = 0.925 and P value < 0.001) and similar data in diabetic groups (D = 0.2, D' = 0.875 and P value < 0.001). These data suggest that the T allele of both genes Apa1 and Taq1 is associated with the increased risk of type 2 diabetes. We think that we need a larger number of volunteers to reach a more accurate conclusion.

Keywords: type 2 diabetes, vitamin D receptor, polymorphisms, Taq1, Apa1.

Resumo

Várias razões podem estar subjacentes ao aumento dramático da diabetes mellitus tipo 2. Um desses motivos é a base genética e variações. Os polimorfismos do receptor da vitamina D estão associados a diferentes doenças, como artrite reumatoide e diabetes. O objetivo deste estudo é investigar a possível associação de duas mutações identificadas Apal (rs7975232) e TaqI (rs731236). Oitenta e nove indivíduos saudáveis e 56 pacientes com diabetes tipo 2 (T2D) foram investigados usando a técnica RFLP para genotipagem e haplotipagem também. A distribuição dos genótipos Apal não foi estatisticamente significativa entre o controle (P = 0,65), bem como para os pacientes diabéticos (P = 0,58). Para as frequências do alelo Taql, o alelo T foi de 0,61, onde o alelo G foi de 0,39. A distribuição de frequência dos genótipos Taql não foi estatisticamente significativa entre o controle (P = 0,26), bem como os pacientes diabéticos (P = 0,17). O risco relativo do alelo T do gene Apa1 é 1,28 e a razão de chances do mesmo alelo é 1,53, enquanto ambas as estimativas foram < 1,0 do alelo G. Da mesma forma, com o gene Taq1, os valores de risco relativo e razão de chances para o alelo T são 1,09 e 1,27, respectivamente, e ambas as estimativas do alelo C foram de 0,86 para o risco relativo e 0,79 para o *odds ratio*. O desequilíbrio de ligação par a par entre os dois SNPs Taq1 / apa1 foi estatisticamente significativo no grupo de controle (D = 0,218, D' = 0,925 e valor P < 0,001) e dados semelhantes em grupos diabéticos (D = 0,2, D' = 0,875 e valor P < 0,001). Esses dados sugerem que o alelo T de ambos os genes Apa1 e Taq1 está associado ao aumento do risco de diabetes tipo 2. Achamos que precisamos de um número maior de voluntários para chegar a uma conclusão mais precisa.

Palavras-chave: diabetes tipo 2, receptor de vitamina D, polimorfismos, Taq1, Apa1.

1. Introduction

The worldwide poor lifestyle is a very concern that underlies a dramatic increase in some health, metabolic disorders, and chronic diseases such as obesity, hypertension, cancers, and diabetes mellitus. Nowadays, the number of people suffering from type 2 diabetes mellitus (T2D) is exceeding 400 million according to World Health Organization (WHO, 2018). In the Kingdom of Saudi Arabia the prevalence of diabetes in adults is about 18.5% (Gosadi et al., 2018). This very concern numbers pushed governments, research centers and thousands of researchers to study the exact causes of this phenomenon and the possible treatments and solutions. A very important part that may guide scholars to improve treatments or to attenuate this increase is looking through the genetic basis in both patients and healthy individuals and to study the susceptibility extent of people to have type 2 diabetes or not, according to their own genetic variation.

Vitamin d receptor is an intracellular small protein that binds to hormonal vitamin d (D3) and translocated to bind with a specific sequence on the promoter of genes that how to the activated vitamin d works. In genetic polymorphic studies, there are three famous genetic variation loci of vitamin d receptor ApaI, TaqI and BsmI which are named according to the enzyme that cuts the mutation point at each one. These loci have attracted the scientists to study the possible association between these variants and some diseases among different societies. In a study conducted on Taiwanese population scholars have reported that vitamin d receptor polymorphisms were associated with type1 diabetes (Chang et al., 2000). That study was followed by others that emphasized the link between vitamin d receptor gene variations and type 1 diabetes (Sahin et al., 2017). Furthermore, vitamin d receptor polymorphisms have been reported to be associated with different kind of cancers such as epithelial ovarian cancer, prostate cancer and breast cancer (Habuchi et al., 2000; John et al., 2005, 2007; Lurie et al., 2011). In Taif city in the Kingdom of Saudi Arabia, besides vitamin d deficiency the number of diabetic patients is increasing as a reflection of lifestyle. Therefore, this study is aimed to investigate the possible association of genetic variation of both vitamin d receptor in ApaI, TaqI loci with the onset of type 2 diabetes among Taif population.

2. Methods and Materials

2.1. Material

Whole blood DNA extraction kit (QIAamp DNA Blood Mini Kit (250)) was purchased from Qiagen Inc, Germantown, MD 20874, USA. ApaI and TaqI were purchased from New England BiolabsInc,240 County Road, Ipswich, USA. Master mix kits for PCR reactions were purchased from Promega Corporation, Madison, USA. Routine work chemicals (Boric acid, Agarose, Tris-base and Ethidium bromide) were purchased from Sigma Aldrich, USA.

2.2. Sample collection

Eighty-nine healthy individuals and Fifty-six Type 2 Diabetic (T2D) patients were voluntarily participated in this study after understanding and signing the consent form. The ethical protocol was approved by the Scientific Research and Ethical Committee (SREC) of University College of Turbah, Taif University for research project # No, 1/441/107. Whole blood samples were extracted on EDTA from each participant for DNA extraction.

2.3. DNA extraction and genetic screening of the ApaI (rs7975232) and TaqI (rs731236)

The whole DNA genomes were extracted from the blood samples following the protocol provided from supplier Qiagen Inc. Vitamin D receptor genotyping for both(rs7975232) and (rs731236) was performed via a multiplex PCR-based method and two sets of primers. The sequences for the ApaI polymorphisms -(rs7975232) forward and reverse primers were 5′-GGG ACG CTG AGG GAT GGC AGA GC-3′ and 5′-GGA AAG GGG TTA GGT TGG ACA GGA-3′ respectively. The sequences for the TaqI polymorphisms (rs731236) forward and reverse primers were 5′-GGG ACG CTG AGG GAT GGC AGA GC-3′ and5′-GGA AAG GGG TTA GGT TGGACAGGA-3′ respectively. PCR reaction was performed in a total volume50 µl reaction mixture containing: 50-100 ng of genomic DNA; 1.6 mM dNTPs; 200 nM of each specific primers; 10 mM Tris_/HCI (pH 9.2); 50 mM KCl; 2 mM MgCl2; and 1.5 units of Taq polymerase. Cycling conditions are an initial 4 min at 93 0C for loading and denaturation, followed by 45 s at 61 0C and 90 s at 72 0C. An additional 36 cycles of 93 0C for 30 s, 63 0C for 45 s, and 72 0C for 90 s. a negative control, will be included in all amplification a reaction mixture that contains all components except the DNA template. PCR products were incubated overnight with a specific restriction enzyme at 37°C. The restriction digestion products were detected visually on a 2% agarose gel and electrophoresed at 100 V for 30-60 minutes. Gels were stained in 0.1µg/ml ethidium bromide solution for 10-15 minutes and visualized under UV light in Gel Documentation System (GDS).

2.4. Statistical analysis

Statistical analysis were carried out under R statistical environment (Team, 2019). The edegnet R package and ape R package to estimate allele frequencies, genotype frequencies and carriage rates of the alleles in all the groups were compared by using Fisher's exact test (Jombart and Ahmed, 2011). Relative risk and odds ration analysis were carried out using epiR.

3. Results

For Apal allele frequencies of T allele was 0.6 and of G allele was 0.4. The frequency distribution of Apal genotypes was not statistically significant among the control (P=0.65) as well as for diabetic patients (P=0.58). For Taql allele frequencies of T allele was 0.61 where of G allele was 0.39. The frequency distribution of Taql genotypes was

not statistically significant among the control (P=0.26) as well as diabetic patients (P=0.17) Table 1. As shown in Table 1 both heterozygosity and polymorphic information content (PIC) gave equal values for both genes and that was due to the fact that estimation of PIC is mainly based on the values of allele frequencies rather than genotypic frequencies. Frequencies of alleles of both Apa1 and Taq1 were equal although.

Table 2 shows two measures (Relative risk and Odds ratio) that are commonly used to determine to what extent the allelic frequency may be associated with a certain trait as shown in Table 2 relative risk of the allele T of Apa1 gene is 1.28 and the odds ratio of the same allele is 1.53, while both estimates were < 1.0 of the allele G. Similarly, with the Taq1 gene the relative risk and the odds ratio values for the allele T are 1.09 and 1.27 respectively and both estimates of the allele C were 0.86 for the relative risk and 0.79 for the odds ratio.

Table 3 shows three estimates of Linkage disequilibrium LD along with 95% confidence intervals. The basic component of all LD statistics (D) is the difference between observed and expected haplotype frequencies, r2 is the square correlation coefficient between two loci. D' is scaled based on the observed allele frequencies. Results show that pairwise linkage disequilibrium between the two SNPs Taq1/apa1 was statistically significant in control group (D = 0.218, D' = 0.925 and P value < 0.001) and similar data in diabetic groups (D = 0.2, D' = 0.875 and P value < 0.001) as shown in Table 3.

4. Discussion

Type 2 Diabetes Mellitus is one of some reasons underlay the increase of mortality rate especially in developed countries. In the kingdom of Saudi Arabia, the situation is similar particularly in Taif city where there is a dramatic increase in T2DM patients and that may because of either the high altitude and the oxygen levels decrease or because of some genetic variations among the population. Several studies have reported the association between vitamin D receptor (VDR) polymorphisms and different diseases among different societies. In a study conducted two years ago, researchers have reported that VDR polymorphisms were associated with arthritis development in a Pakistani society (Mukhtar et al., 2019). Another study has suggested that VDR polymorphisms have a protective link against Multiple sclerosis in Egyptian society (Hassab et al., 2019).

Table 1. Number of individuals (n) frequency distribution of genotypes (f), level of significance, Heterozygosity and Polymorphic information content (PIC) for Apal and Taql among patients and controls.

Genotype		Apal			P-value	Taql			P-value
		TT	TG	GG		TT	TC	CC	
Control	(n)	33	39	15		30	48	11	
	(f)	0.38	0.45	0.17	0.65	0.34	0.54	0.12	0.26
Heterozygosity			0.48				0.48		
PIC			0.36				0.36		
Patients	(n)	22	24	9		18	32	6	
	(f)	0.40	0.44	0.16	0.58	0.32	0.57	0.11	0.17
Heterozygosity			0.48				0.48		
PIC			0.36				0.36		

Table 2. Relative risk and odds ratio associated with Apal and Taql genes along with 95% confidence interval (Cl).

Gene	Allele	Frequency		Relative Risk (Cl)	Odds Ratio (Cl)
		Diabetic	Control		
Apa1	T	34	37	1.28 (0.84, 1.94)	1.53 (0.75, 3.13)
	G	21	35	0.78 (0.52, 1.19)	0.65 (0.32, 1.33)
Taq1	T	43	54	1.09 (0.86, 1.92)	1.27 (0.65, 2.46)
	C	22	35	0.86 (0.56, 1.31)	0.79 (0.40, 1.54)

Table 3. Pairwise linkage disequilibrium of TaqI and Apal polymorphism.

	SNP1	SNP2	D	D'	Correlation	P-value
Control	TaqI	Apal	0.218	0.925	0.914	<0.001
Diabetic	TaqI	Apal	0.200	0.875	0.841	<0.001

In addition, Barbara Angel and her colleagues reported that VDR polymorphisms are associated with type 2 diabetes in older individuals among the Santiago de Chile population (Angel et al., 2018). Further studies have concluded that there is an association between VDR polymorphisms and type 2 diabetes in different societies (Angel et al., 2018; Gendy et al., 2019; Khan et al., 2019).

Herein, the current study shows that Apa1 allele frequencies were 0.6 of T allele and 0.4 of G allele respectively. Similar allelic frequencies were found with Taq1 allele 0.61 and 0.39 of T and C alleles respectively. As shown in Table 1 the frequency distribution of either genotype Apa1 and Taq1 were not significant in both investigated groups, normal and diabetic participants. In such study, two popular estimates are used to determine the association between a certain allele frequency with a certain traits or disease (the relative risk and the odds ratio). As shown in Table 2, both relative risk and odds ratio revealed that T allele of Apa1 gene to be more associated with type 2 diabetes in which both measures gave values more than 1 (1.28 and 1.53 respectively). While the G allele frequency shows a protective value (< 1.0) as shown in Table 2. Similarly, the T allele of Taq1 gene gave more than one in both relative risk and odds ratio as well (1.09 and 1.27 respectively) which means that T allele is more associated with diabetes than the C allele or in other words the C allele frequency shows protective values < 0.1 as shown in Table 2.

Linkage Disequilibrium (LD) or that known as the allelic association is nonrandom association of alleles at different loci. LD is a property employed by haplotype analysis that illustrate the degree to which recombination redistributes genetic diversity. In the present study, LD was employed to estimate the association of both genes (rs7975232) and (rs731236) together in either type 2 diabetes or healthy people. Data demonstrated in Table 3 clearly show a statistically significant LD between the two studied genes Taq1 and Apa1. These data are in accordance with several previous studies (Fronczek et al., 2021; Imani et al., 2019; Karonova et al., 2018; Mukhtar et al., 2019).

5. Conclusion

In conclusion, the current data suggest that the T allele of (rs7975232) Apa1 and the T allele of (rs731236) Taq1 of vitamin d receptor are statistically associated with type 2 diabetes while the G allele of (rs7975232) Apa1 and the C allele of (rs731236) Taq1 of vitamin d receptor are protective alleles. Moreover, the haplotype analysis suggests there is a significant linkage disequilibrium between the two genes Taq1 and Apa1. We think that we need a larger number of volunteers to reach a more accurate conclusion.

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