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RESEARCH ARTICLE

Vitamin D Metabolism Gene CYP27B1 Promoter Polymorphism and Type 1 Diabetes in the Egyptian Population. A genetic Association Study

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Abstract

Background: Epidemiological studies revealed that higher levels of the active metabolite lα-25 dihydroxy vitamin D3 could protect from immune destruction of the pancreatic B cells which leads to type 1 diabetes, lα-25 dihydroxy vitamin D is derived from 25 hydroxy vitamin D by the enzyme 1α hydroxylase encoded by the CYP27B1 gene which located on chromosome 12- 9. 13.1- 13.3. Our aim was to investigate if there is association between the CYP27B1 gene promoter polymorphisms in the Egyptian Population with type 1 diabetes or not. **Methods:** We studied 90 patients with type 1 diabetes and 90 controls matched for age and sex to evaluate CYP27B1 promoter polymorphism(- 1260) C > A. **Results:** Analysis of the CYP27B1 promoter (-1260 C/A) polymorphism revealed that the CC genotype was significantly more frequent in patients with type 1 diabetes mellitus, than in healthy controls (62. 2% vs 33.3 %, P = 0.0001) and the common C allele of CYP27B1 promoter - 1260 A/C polymorphism was significantly more common in type 1 diabetes group compared to control group. **Conclusion**: The present study may provide evidence that vitamin D metabolism gene CYP27B1 promoter polymorphism increases the risk of developing of type 1 diabetes in Egyptian population. This evidence justifies further studies investigating molecular and cellular actions of vitamin D and mechanisms of its protective effect in type 1 diabetes. Thus vitamin D metabolism gene CYP27B1 promoter polymorphism may be considered as a genetic biomarker for type 1 DM in Egyptian subjects with potential impact on the family counseling and management.

INTRODUCTION

Type 1 diabetes (T1D) is a chronic autoimmune disease that results in the specific immune destruction of insulin producing beta cells (1) Activation of auto-aggressive T cells that are thought to mediate destruction of beta cells in TID is multifactorial involving a genetic predisposition (2) and environmental triggers such as viruses (3).

Vitamin D is a secosteroid that acts via the nuclear vitamin D receptor (VDR). The most active natural Vitamin D metabolite 1α, 25(OH) 2D3 effectively prevents the development of autoimmune diabetes mellitus (4) and autoimmune thyroiditis (5) in animal models. Also, other autoimmune disorders such as experimentally induced autoimmune encephalitis can be favorably influenced by administering 1α, 25(OH) 2D3 (6).

The effect of the vitamin D in type 1 diabetes was first proposed based upon the observation that incidence rates of type 1 diabetes were negatively correlated with sunlight exposure, resulting in higher incidence of the disease at higher latitudes (7), and the distinctive seasonal pattern in type 1 diabetes incidence, with the largest proportion of cases diagnosed during the winter and the lowest during the summer (8). Subsequent evidence includes that type 1 diabetic patients have lower levels of 25(OH)D than age- and sex-matched control subjects (9,10). In addition, vitamin D supplementation is reported to be protective against type 1 diabetes (11) and epidemiological studies have indicated that vitamin D supplementation in early childhood is associated with decreased type 1 diabetes incidence (12-14).

The vitamin D has widespread effects in the immune system (15); lα,25(OH)2D3 has been shown to suppress production of the interleukin (IL)-12, IL-2, tumor necrosis factor-α. and γ-interferon. It has also been shown to activate expression of transforming growth factor-β1 and IL-4, thereby inhibiting T helper 1(Th l)-type responses, and to induce regulatory T-cells (16,17). Furthermore, the active form of vitamin D alter the development of Th1, Th17, and Th9 cells, which are implicated in the pathogenesis of different types of autoimmune diseases including the type 1 diabetes (1, 18). These immunomodulatory effects may explain the reported protective effects of vitamin D in type 1 diabetes (11, 19) Also, direct effects for vitamin D on the normal function of beta cells have been described, with improved beta cell function and survival upon inflammatory or immune attack (20).

If vitamin D is a significant factor in type 1 diabetes, then it might be expected that common functional sequence polymorphisms in the genes that influence vitamin D action could predispose to the disease (21). Genes that influence vitamin D action include : a) gene of the vitamin D receptor (VDR), which binds lα,25(OH)2D3 and mediates the effects of vitamin D, b) CYP24Al gene located on chromosome 20ql3.2-ql3.3 that encodes vitamin D 24-hydroxylase, an enzyme that inactivates lα,25(OH)2D c) CYP2R1 gene located on chromosome 11p15.2, that encodes 25- hydroxylase (CYP2R1) which catalyzes the hydroxylation of vitamin D3 to 25-hydroxyvitamin D3 (25(OH)D3), the main circulating vitamin D metabolite, and d) CYP27Bl gene located on chromosome 12q 13.1-ql3.3 (22-26), that encodes lα-hydroxylase (CYP27B1) a mitochondrial P450 enzyme (27), which catalyzes the conversion of 25 - hydroxyvitamin D3 to 1α,25 (OH)2 D3 , the most active natural vitamin D metabolite (28). It is the key enzyme determining the rate of l α, 25 (OH) 2D3 productions (29). The common polymorphisms of the CYP27B1 gene are the, CYP27B1 promoter (-1260) C/A polymorphism and CYP27B1 intron 6 (+2838) C/T polymorphism (30).

Several studies have reported association of type 1 diabetes and other autoimmune diseases with polymorphisms in the CYP27B1 gene (22- 24). However these results have not been verified. In the present study we have investigated if there is association between type 1 diabetes and the CYP27B1 promoter (-1260) C/A polymorphism or not in the Egyptian Population.

SUBJECTS AND METHODS

This cross sectional descriptive study was done on ninety patients with type 1diabetes mellitus, recruited from the endocrine outpatient clinics at Zagazig University Hospitals during the period from January 2013 to October 2013. Type 1 diabetes mellitus was diagnosed according to World Health Organization criteria. Ninety apparently healthy subjects matched with age and sex were included in the study as controls. Full history taking and physical examination including measurement of the body mass index was done for the patients and controls. Laboratory measurement including random blood glucose level, CYP27B1-1260 promoter polymorphism genotype was measured. An informed consent was obtained from all participants.

Genotype analysis:

Blood samples from all subjects were collected in vaccutainers

containing EDTA and stored at -20 ºC. Genomic DNA was extracted from whole blood using the QIAmp Blood Mini kit (Quiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. CYP27B1-1260 promoter polymorphism was genotyped by DNA amplification with polymerase chain reaction (PCR) using specific primer sets, followed by the restriction fragment length polymorphism method. The primers for CYP27B1-1260 were: forward 5` GTGTTCCCTAAGTGTTGTCTC-3`; reverse 5`-GCTGACTC GGTCTCCTCTG-3`. The PCR was performed in a volume of 25µL reaction containing 12.5 µL of 2xTaq PCR Master Mix (Quiagen GmbH, Hilden, Germany), 2 µL template DNA (0.05 µg / µL), 1.0 µL (10 µM) of each primer, and 8.5 µL sterile double - distilled water. The amplification was performed with the following program: the mixture was first heated at 94 ºC for 3 min and then amplified for 30 cycles by denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s and extension at 72 ºC for 1 min in each cycle, and a final extension at 72 ºC for 10 min.

The amplified DNA was digested with the restriction enzyme TfiI (New England Bio Labs, Beverly, MA, USA) according to the manufacturer's instructions for 4 hours. Digested fragments were separated on a 2.5% agarose gel and visualized by SYPR green staining and ultraviolet illumination.

Statistical analysis:

SPSS 17.0 for Windows (SPSS Inc., Chicago, IL) was used to analyze data. Genotype and allele frequencies were compared between patients and controls using the x2 test. Probabilities (P) were considered statistically significant if P-value < 0.05

RESULTS:

This study included 90 patients with type1 diabetes mellitus (42 males and 48 females) and 90 healthy control subjects (40 males and 50 females). There were no significant differences in mean age between the patients and controls (table 1). However, there were a significant differences between the patients and controls regarding random blood glucose level, body mass index (BMI) and positive family history for type 1 diabetes (table 2).

Table 1: Demographic characteristics of patients and controls:

	Cases N=90	Controls N=90	P-value
Age (years) mean ± SD	14.9 ± 3.1	16.3 ±2.8	> 0.05
Gender (no)			
Male	42	40	> 0.05
Female	48	50	

Table 2: Statistical analysis of random blood glucose (mg/dl), BMI and positive family history in cases (N=90) and controls (N=90)

	Cases N=90	Controls N=90	P-value
Random blood glucose (mg/dl) mean ± SD	210.3 ±20.9	114.1 ± 12.0	< 0.05
BM I (kg/m2) mean ± SD	19.3 ± 1.3	23.8 ± 1.4	< 0.05
Positive family history	25	8	< 0.05

Analysis of the CYP27B1 promoter (-1260 C/A) polymorphism (Table 3) revealed that the CC genotype was significantly more frequent in patients with type 1 diabetes mellitus, than in healthy controls (62. 2% vs 33.3 %, P = 0.0001). Patients with type 1 diabetes mellitus also had higher frequencies of the allele C than controls (76.7 % vs 53.3 %, P = 0.000004).

Table 3: Distribution of the CYP27B1 promoter (-1260 C/A) polymorphism in patients with type 1 diabetes mellitus and healthy controls as regard genotypes and alleles frequency

	Cases n (%)	**Controls n (%)**	**X^2**	**OR (95% CI)**	**P-value**

Genotype					
AA	8(8.9 %)	24 (26.7 %)	9.7	**0.3** (0.1-0.6)	0.001
CA	26 (28.9%)	36 (40%)	2.4	**0.6** (0.3-1.1)	0.11
CC	56 (62.2 %)	30 (33.3 %)	14.9	**3.3** (1.8-6.1)	0.0001
Allele frequencies					
C	138 (76.7 %)	96 (53.3 %)	21.5	**2.8** (1.8-4.5)	0.000004
A	42 (23.3 %)	84(46.7 %)			

DISCUSSION

The present study revealed that the allele C of CYP27B1 promoter (-1260) C/A polymorphism was significantly more common in type 1 diabetes group compared to the control group. Also, the study found that the CC genotype was significantly more frequent in patients with type 1 diabetes compared to control group and that the subjects carrying the CC genotype have 3.3 fold increased risk to develop type 1 diabetes. Based on these results, the CYP27B1 promoter -1260 C polymorphism is associated with type 1diabetes in our study population. On the other hand, our study revealed that the alternate allele CYP27B1(-1260) A might confer protection against type 1 diabetes as the allele A and the genotype AA were more frequent in the control group compared to the type 1 diabetes group.

These results are in agreement with previous studies which investigated the association of CYP27B1 gene polymorphism with type 1 diabetes mellitus (23,31), Addison's disease, Hashimoto's thyroiditis, and Graves' disease (23) . In these genetic studies the CC genotype and the allele C of the CYP27B1 promoter (-1260) C/A polymorphism were found significantly more often in the group of type 1 diabetes (23, 31) and in other autoimmune endocrine disease (23) than in the control group. Also in agreement with our results, Hussein et al. (21) and Lopez et al. (22) found that the C/A polymorphism in the promoter region of CYP27B1gene was significantly associated with an increased risk of type 1 diabetes in their study populations.

In disagreement with our results, Fichna et al. (32) found that the frequencies of allele C and CC genotype of the CYP27B1promoter (-1260) C/A polymorphism did not present significant differences between type 1 diabetes patients and controls in Polish population. Also, Pani et al. (25) suggested that the CYP27B1 (-1260) C/A polymorphism is not associated with type 1 diabetes in German. The reason for this discrepancy may be due to population ethnic differences.

Taking into account prior epidemiological and experimental links between vitamin D and type 1 diabetes (33-37) and the association between CYP27B1gene and type 1 diabetes in previous studies (21-23, 31) and in our study, we can support the suggestion that common inherited variation in the CYP27B1 gene affects vitamin D metabolism and hence can be regarded as an etiological factor predisposing to type 1 diabetes.

This can be explained by the hypothesis that the presence of the CYP27B1–1260 C allele significantly reduces mRNA levels (38) and thereby reduces the level of the active 1α hydroxylase and conversion of 25(OH)D to 1 α,25(OH)2D3 causing low concentrations of 1,25(OH)2D3 (39, 40); the hormonally active form of vitamin D which plays an immunomodulatory role in preventing type 1 diabetes and other autoimmune diseases (41, 42) in addition to its suggested direct role on the pancreatic islet cell (20, 43).

Furthermore, several studies suggested that CYP27B1 promoter polymorphism C/A (-1260) has role in deficient vitamin D signaling within immune cells , as CYP27B1 is expressed in all immune cells providing local supra-physiological concentrations of l,25(OH)2D3 which are needed to modulate immune responses, without affecting systemic levels of this hormone (19).

In Conclusion: The present study may provide evidence that vitamin D metabolism gene CYP27B1 promoter polymorphism increases the risk of developing of type 1diabetes in Egyptian population. This evidence justifies further studies investigating molecular and cellular actions of vitamin D and mechanisms of its protective effect in type 1 diabetes. Thus the vitamin D metabolism gene CYP27B1 promoter polymorphism may be considered as a genetic biomarker for type 1 DM in Egyptian subjects with potential impact on the family counseling and management.

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