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Expression of JAZF1, ABCC8, KCNJ11and Notch2 genes and vitamin D receptor polymorphisms in type 2 diabetes, and their association with microvascular complications

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Abstract

Background: We studied JAZF1, ABCC8, KCNJ11and Notch2 gene expression and vitamin D receptor (VDR) polymorphisms (Fok1 and Bsm1) in patients with type 2 diabetes mellitus (T2DM) and tried to find out their association with microvascular complications in these patients.

Methods: The study was conducted on 180 patients (93 complicated and 87 noncomplicated) and 150 healthy subjects. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to assess gene expression and real-time PCR was used to detect VDR genotypes. Serum vitamin D was assessed using Elisa technique.

Results: After adjustment for age, sex, body mass index and glycated hemoglobin, altered Notch2 gene expression was found between patients and controls and between complicated and noncomplicated cases ($p = 0.001$ and 0.001, respectively) and ABCC8 gene expression showed significant difference between patients and controls only ($p = 0.003$), while JAZF1and KCNJ11 expression showed no significant difference between the studied groups ($p = 0.3$ and 0.4, respectively). Serum vitamin D level was decreased in patients compared with controls ($p = 0.001$), while no difference was detected between complicated and noncomplicated cases ($p = 0.1$). Our results revealed no significant difference in VDR Fok1 and Bsm1 genotype distributions ($p = 0.7$ and 0.1, respectively) and allele frequencies ($p = 0.4$ and 0.1, respectively) between patients and controls. Patients with complications showed increased frequencies of Fok1GG genotype and G allele, while patients without complications showed increased frequencies of AA, then AG Fok1 genotype and A allele ($p = 0.001$ and 0.001, respectively). In addition, the frequencies of CC Bsm1 genotype and C allele were significantly higher among patients with complications, while frequencies of TT Bsm1 genotype and T allele were significantly higher among patients without complications ($p = 0.02$ and 0.003, respectively).

Conclusion: Altered expression of Notch2 and ABCC8 genes may play a role in the pathogenesis of T2DM. Altered expression of Notch2 and VDR polymorphisms may play a role in the development of microvascular complications in diabetic patients. These results may assist in early identification and management of diabetic complications.

Keywords: gene expression, microvascular complications, type 2 diabetes mellitus, VDR, vitamin D

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Introduction

Type 2 diabetes mellitus (T2DM) is the most chronic life-threatening, metabolic disease, characterized by hyperglycemia, resulting from defect in insulin secretion and sensitivity.[1] Its prevalence has been increasing dramatically over recent years, reaching 8.8% globally and 11.5% in Egypt.[2] Long-standing T2DM can affect the vascular system leading to microvascular complications. The pathologic landmark of T2DM is microvascular complications, including nephropathy, retinopathy, neuropathy and microangiopathic cardiovascular disease (CVD). Interestingly, several studies report that tight glycemic control doesn't lower the incidence and progression of microvascular complications.[3] Thus, presence of long-standing hyperglycemia doesn't explain microvascular complications in T2DM, and other factors would be contributing to the pathogenesis of such complications; identification of these factors should minimize incidence of these complications and improve their management.[1]

However, T2DM is multifactorial disease influenced by several environmental and genetic factors.[3] Change in lifestyle, lowering other cardiovascular risk factors and maintaining blood glucose levels within the normal range are recommendations for T2DM management but to date, there is no effective treatment for this serious disease.[2] Early prediction, identification and management of complications associated with diabetes are still challenging. The increased incidence of T2DM and the importance of early identification and management of its complications have highlighted identification of the genetic factors that increase risk of T2DM and its related complications.[4]

Several genes have been reported to contribute to T2DM pathogenesis. These genes, such as juxtaposed-with-another-zinc-finger protein 1 (JAZF1), potassium voltage-gated channel subfamily J member 11 (KCNJ11), adenosine triphosphate (ATP)-binding cassette transporter sub-family C member 8 (ABCC8) and neurogenic locus notch homolog protein 2 (Notch2) are known to play a role in insulin secretion and sensitivity, and growth and development of the pancreas.[5,6] However, there is limited information available on the role of these genes in pathogenesis of microvascular complications in T2DM. Most studies demonstrated association of these genes with T2DM at a deoxyribonucleic acid (DNA) level *via* identification of risk alleles, but very few studies assessed their expression in the serum of diabetic patients.

Vitamin D plays an important role in glucose metabolism by stimulating insulin exocytosis and uptake of glucose by peripheral tissues, and decreasing insulin resistance.[7] It also has anti-angiogenic, immunomodulatory and anti-inflammatory effects. Thus, vitamin D may contribute in pathogenesis of T2DM and its complications and the vitamin D receptor (VDR) gene may be candidate gene for T2DM. However, the link between vitamin D and VDR polymorphisms with T2DM is still unclear and results regarding this link are still controversial.[8]

To highlight the molecular mechanisms of T2DM and associated microvascular complications, we studied JAZF1, ABCC8, KCNJ11 and Notch2 gene expression, VDR polymorphisms (Fok1 and Bsm1) and vitamin D levels in patients with T2DM and tried to establish an association between these markers and the susceptibility to microvascular complications in these patients.

Patients and methods

Patients

This case-control type of study included 180 cases of T2DM randomly selected from the endocrinology outpatient clinic of the El-Kasr Al-Ainy Hospital, Cairo University and the outpatient clinic of the National Research Centre. Inclusion criteria: all the patients belonged to the Egyptian ethnic group and were unrelated to each other. All patients met the diagnostic criteria of the American Diabetes Association (ADA) in 2000[9] and the updated ADA diagnostic criteria for T2DM.[10] The patients were either simple T2DM without further complications (87 patients) or complicated T2DM (93 patients). Patients of T2DM with complications fulfilled diagnostic criteria of at least one of the following complications: nephropathy, retinopathy, peripheral neuropathy or microangiopathic CVD,[11–14] respectively. Vitamin D deficiency was defined as level <20 ng/ml and insufficiency, 20–29 ng/ml in accordance with WHO definition.[15]

Exclusion criteria included other acute or chronic metabolic, systemic, endocrine and autoimmune inflammatory diseases, cancer, diseases that affect calcium or vitamin D levels, vitamin D

supplementation and use of medications known to affect serum vitamin D.

This study also included 150 apparently healthy volunteers of comparable body mass index (BMI), sex, age and socioeconomic status with the patient group. They had no evidence of T2DM, hypertension, previous or current acute or chronic systemic or metabolic illness, family history of diabetes or history of vitamin supplementation.

The protocol for this study was approved by National Research Centre Ethics Committee according to the ethical guidelines of the declaration of Helsinki, with informed consent taken from all participating individuals. The study was part of project no. 10010321 (10th plan of the National Research Centre, Egypt).

Assessment of serum vitamin D level

Assessment of vitamin D level in serum was done using competitive Elisa kit (DRG, Malburg Germany) with 100% specificity and intra- and interassay precision: 4.7% and 9.7%, respectively.[16]

Vitamin D receptor genotyping

DNA was extracted using the QIAamp DNA Blood Mini Kit–50 (QIAGEN, Hilden, Germany. The FokI and Bsm1 polymorphisms were detected by real-time polymerase chain reactions (PCRs) using the Quantistudio 12 Flex real-time PCR system (Applied Biosystems, Foster City, CA, USA). FokI A/G (rs2228570) and Bsm1 C/T (rs1544410), were performed using the TaqMan® genotyping protocol (Applied Biosystems, Foster City, CA, USA).

PCR reactions were set up in 20 µl reaction volumes, including 20–30 ng DNA and 10 µl TaqMan® Universal PCR Master Mix (Applied) in 96-well PCR plates. The PCR assay was carried out according to the manufacturer's instructions, including one step of 10 min at 95°C, followed by 40 cycles of DNA denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Final products were analyzed by TaqManGenotyper software (Applied).

Assessment of gene expression using reverse-transcriptase polymerase chain reaction

Ribonucleic acid extraction from human whole blood. Ribonucleic acid (RNA) was extracted using QIAamp RNA Blood Mini kits (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Samples were extracted on the same day. The final RNA concentration was determined using a spectrophotometer (Nanodrop 2000, Therom Fisher, Walthm, USA) and RNA purity was verified by an average A260/A280 ratio of 1.98 (range, 1.97–2.01).

Reverse transcription or complementary deoxyribonucleic acid synthesis. RNA was reverse transcribed to complementary deoxyribonucleic acid (cDNA) using a high-capacity cDNA reverse transcription kit (Applied Biosystems®, Branchburg, New Jersey, USA) in a final volume of 20 µl. Negative control samples were included in each set of reactions. Reactions were incubated at 25°C for 10 min, followed by 37°C for 120 min and final denaturation at 85°C for 5 min. The reaction was carried out in the Veriti Thermal Cycler (Applied Biosystems®, Branchburg, New Jersey, USA). cDNA was stored at −20°C.

Real-time polymerase chain reaction. Gene expression of JAZF1, KCNJ11, ABCC8 and Notch2 was measured by using TaqMan® Amplification System (Applied Biosystems®, Branchburg, New Jersey, USA). All samples were run in a final reaction volume of 20 µl. The reaction mix was combined using 10 µl TaqMan® Universal PCR Master Mix, 3 µl of cDNA, 6 µl of DNase-free water and 1 µl of specific primers and probes 20x20 (Applied Biosystems®, Branchburg, New Jersey, USA). Expression of genes JAZF1 Hs00697777_m1, KCNJ11 Hs00265026_s1, ABCC8 Hs01093752_m1 and Notch2 Hs01050702_m1, were normalized using the Glyceraldhyde 3-phosphate dehydrogenase (GADPH) housekeeping gene. The PCR run was carried out using the thermal profile 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min on the Quantistudio 12K Flex (Applied Biosystems®, Branchburg, New Jersey, USA).

Data analysis. The data from TaqMan® gene expression assays obtained at the end of the run were analyzed by viewing the amplification plots for all samples followed setting the baseline and threshold values. The relative standard curve or the comparative cycle threshold (CT) methods were used to analyze data. $-\Delta\Delta CT$ = (CT target gene, test sample – CT endogenous control, test sample) – (CT target gene, calibrator sample – CT endogenous control, calibrator sample).

Statistical analysis

Analysis was performed using SPSS version 17 software for Windows. Data are reported as means ± standard deviation (SD) or number (percentage). The differences between the two groups were tested by the *t* test for independent samples with normal data distribution or by the Man–Whitney nonparametric test. One-way ANOVA analysis was used for multiple group comparisons. The least significant difference (LSD)-*t* test was used in pairwise comparison of averages among groups. Pearson's and Spearman's correlation tests (r = correlation coefficient) were used for correlating normal and nonparametric variables, respectively. A p value of <0.05 was considered statistically significant. Linear regression analysis was performed to confirm association of significant gene expression with the disease after adjustment of associated covariates. Logistic regression model was used to assess the odds ratios (ORs) and 95% confidence intervals (CIs) of the association of VDR genotypes and alleles with microvascular complications, after adjusting for multiple factors. Power calculation was analyzed for significant results, using the Power and Sample Size Calculations Program (PS Version 3.1.2).

Results

Demographic, clinical and laboratory data of studied groups are shown in table 1.

The expression of the JAZF and KCN genes did not show any differences between studied groups (p = 0.3 and 0.4, respectively). ABCC8 showed decreased expression in patient groups compared with controls (p = 0.001), while no differential expression was detected between studied patient groups (p = 0.9). Altered Notch2 expression was detected between patient groups and healthy subjects and between complicated and noncomplicated patients (p = 0.001 and 0.001, respectively) (Table 2). Furthermore, after adjusting for age, sex, BMI, glycated hemoglobin and lipid parameters, Notch2 and ABCC8 expression remained associated with T2DM (p = 0.001 and 0.003, respectively). Association of Notch2 with microvascular complications was confirmed using linear regression analysis after adjustment of age, sex, frequency of hypertension, BMI, glycated hemoglobin, lipid parameters and disease duration (p = 0.003). There was no significant correlation detected between Notch expression in patient group, and age, BMI, glycated hemoglobin, cholesterol, triglycerides, HDL and disease duration (r = 0.06, 0.08, 0.01, 0.02, –0.02, 0.09 and 0.1, respectively and p = 0.3, 0.2, 0.8, 0.7, 0.7, 0.2 and 0.09, respectively). There was no significant association between method of treatment and expression of JAZF, KCN, ABCC and Notch2 genes, using linear regression analysis (p = 0.5, 0.6, 0.6 and 0.4, respectively).

Serum vitamin D level was significantly lower in patients compared with controls (p = 0.001), while no significant difference was detected between patients with complications and those without (p = 0.1) (Table 1).

Distributions of VDR Fok1 and Bsm1 genotype and allele frequencies in patients and controls are summarized in Table 3. Our results revealed no significant difference in VDR Fok1 and Bsm1 genotype distributions (p = 0.7 and 0.1, respectively) and allele frequencies (p = 0.4 and 0.1, respectively) between patients and controls.

Conversely, VDR Fok1 and Bsm1 genotype distributions and allele frequencies showed significant differences between patients without complications and those with complications (Table 4). Patients with complications showed increased frequencies of Fok1GG genotype and G allele, while patients without complications showed increased frequencies of AA, then AG Fok1 genotype and A allele. In addition, the frequencies of CC Bsm1 genotype and C allele were significantly higher among patients with complications, while frequencies of TT Bsm1 genotype and T allele were significantly higher among patients without complications. Regarding these results, odds ratios and confidence intervals were calculated for each genotype and allele with regards to associated complications in patient groups (Table 4). The logistic regression model revealed no significant interaction between different VDR Fok1 and Bsm1 genotypes in association with microvascular complications (p = 0.7).

Power analysis of the study yielded a statistical power of >90% for Notch2 and ABCC8 gene expression with T2DM and 86% For Notch2 gene expression with microvascular complications. Power analysis for significant results regarding VDR genotypes showed 87% and 53% for Fok1 and Bsm1 polymorphisms' association with microvascular complications, respectively, in T2DM.

Table 1. Demographic, clinical and laboratory characteristics of patient groups and controls.

Variable	Controls ($n = 150$)	Patients without complications ($n = 87$)	Patients with complications ($n = 93$)	*p* value
Age (years)	44.3 ± 4.1	42.8 ± 6.5	44.1 ± 6.6	0.1
Sex (%) Male	83 (55.3%)	45 (51.7%)	55 (59.1%)	
Female	67 (44.7%)	42 (48.3%)	38 (40.9%)	0.6
BMI (kg/m^2)	26.9 ± 3.5	27.4 ± 3.2	27.2 ± 3.1	0.6
Disease duration (years)	–	12.4 ± 1.6	12.5 ± 2.4	0.8
Hypertension (%) Yes	0	38 (43.7%)*	59 (63.4%)**	
No	150 (100%)	49 (56.3%)	34 (36.6%)	0.001
Complications:				
Nephropathy			24 (25.8%)	
Retinopathy	–	–	54 (58%)	–
Neuropathy			85 (91%)	
CVD			59 (63.4%)	
Treatment for diabetes (%)				
Oral hypoglycemics	–	33 (38%)	34 (36.6%)	
Oral hypoglycemic and insulin	–	11 (12.6%)	13 (14%)	0.9
Insulin	–	43 (49.4%)	46 (49.4%)	
Total cholesterol (mg/dl)	128.6 ± 19	184.4 ± 47*	201 ± 45**	0.001
Triglycerides (mg/dl)	106.7 ± 13	136.4 ± 70*	155 ± 84**	0.001
HDL-C (mg/dl)	43.9 ± 5.5	38 ± 5*	34.8 ± 4.4**	0.001
LDL-C (mg/dl)	63 ± 20	118.5 ± 46*	135 ± 42**	0.001
Glycated Hb%	4.8 ± 0.6	7.7 ± 0.5*	7.8 ± 0.6	0.001
Vitamin D (ng/ml)	21.9 ± 6.9	16.1 ± 7*	17.9 ± 9.3	0.001

BMI, body mass index; Hb, hemoglobin; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *significant *p* value between cases and controls; **significant *p* value between complicated and noncomplicated patients.

Table 2. Studied gene expression in patient groups and controls.

Genes (mean ± SD)	Controls ($n = 150$)	Noncomplicated cases ($n = 87$)	Complicated cases ($n = 93$)	*p* value
JAZF1	1.1 ± 0.9	1.3 ± 0.9	1.1 ± 0.9	0.3
KCNJ11	0.5 ± 0.7	0.5 ± 0.7	0.6 ± 0.9	0.4
ABCC8	0.5 ± 0.6	0.007 ± 0.012*	0.009 ± 0.015	0.001
Notch2	0.01 ± 0.02	0.92 ± 0.6*	0.5 ± 0.6**	0.001

JAZF1, juxtaposed with another zinc finger protein 1; KCNJ11, potassium voltage-gated channel subfamily J member 11; ABCC8, adenosine triphosphate-binding cassette transporter sub-family C member 8; Notch2, neurogenic locus notch homolog protein 2; *significant *p* between cases and controls; **significant *p* between complicated and noncomplicated patients.

Discussion

Although diabetic patients often develop microvascular complications, the susceptibilities of these patients to these complications are different. It is suggested that diabetes not only predisposes to these complications but other factors

Table 3. Genotype distributions and allele frequencies of vitamin D receptor in patients and controls.

VDR polymorphism	Patients ($n = 180$)	Controls ($n = 150$)	*p* value
Fok 1 genotypes			
AA	17 (9.4%)	11 (7.3%)	
AG	57 (31.7%)	47 (31.3%)	0.7
GG	106 (58.9%)	92 (61.4%)	
Fok1 allele			
A (2n)	91 (25.3%)	69 (23%)	0.4
G (2n)	269 (74.7%)	231 (77%)	
Bsm1 genotypes			
CC	72 (40%)	57 (38%)	
CT	74 (41.1%)	52 (34.7%)	0.1
TT	34 (18.9%)	41 (27.3%)	
Bsm1 alleles			
C (2n)	218 (60.6%)	166 (55.3%)	0.1
T (2n)	142 (39.4%)	134 (44.7%)	

VDR, vitamin D receptor.

Table 4. Genotype distributions and allele frequencies of vitamin D receptor in patient groups with regards to associated microvascular complications.

VDR polymorphism	Noncomplicated patients ($n = 87$)	Complicated patients ($n = 93$)	*p* value	OR (CI)
Fok 1 genotypes				
AA	12 (13.8%)	5 (5.4%)		Reference
AG	38 (43.7%)	19 (20.4%)	0.001	0.23 (0.07–0.6)
GG	37 (42.5%)	69 (74.2%)		0.26 (0.1–0.5)
Fok1 allele				
A (2n)	62 (35.6%)	29 (15.6%)	0.001	Reference
G (2n)	112 (64.4%)	157 (84.4%)		2.9 (1.8–4.9)
Bsm1 genotypes				
CC	28 (32.2%)	44 (47.3%)		3.2 (1.3–7.7)
CT	36 (41.4%)	38 (40.9%)	0.02	2.2 (0.9–5.1)
TT	23 (26.4%)	11 (11.8%)		Reference
Bsm1 alleles				
C (2n)	92 (52.9%)	126 (67.7%)	0.003	1.8 (1.2–2.8)
T (2n)	82 (47.1%)	60 (32.3%)		Reference

VDR, vitamin D receptor; OR, odds ratio; CI, confidence interval.

also determine the susceptibility of complications in these diabetic subjects.[17] However, early prediction, identification and management of complications associated with diabetes are still challenging. In this study, we tried to highlight candidate genes known to play roles in glucose

homeostasis *via* different mechanisms and attempted to establish associations between these genes and diabetic microvascular complications.

JAZF1 encodes for a nuclear protein that functions as a transcriptional repressor of the transcription factor NR2C2 (nuclear receptor subfamily 2, group C, member 2).[18] JAZF1 is expressed in the pancreas, playing an important role in maintaining pancreatic β-cell mass and function and improving insulin sensitivity.[19] Moreover, Lee at al. demonstrated that deletion of the JAZF1 gene is associated with increased insulin resistance.[20] Different studies reported associations between impairment of pancreatic β-cell function and diabetes, and risk alleles in the JAZF1 gene.[18,19] Several reports have demonstrated altered JAZF1 expression in T2DM.[21,22] In 2012, Taneera *et al.* performed a map-linking genetic study, where they ranked genes according to single nucleotide polymorphism (SNP) associated with T2DM and their expression correlation with HbA1c and insulin secretion in islet cells of donors with T2DM compared with healthy donors. JAZF1 was top ranked among all studied genes. They reported decreased JAZF expression in islet cells of diabetics compared with control group. Interestingly, silencing of JAZF1 gene didn't show on glucose-stimulated insulin secretion. However, they explained this finding either due to failure of the experimental situation or because difference in this gene expression could be a consequence, not a cause, of chronic hyperglycemia or insulin secretion and also requires networks of coexpressed genes. Moreover, in a study performed on myogenic C2C12 cells, Yuasa *et al.* reported that aberrant JAZF expression (either increased or decreased) is associated with T2DM pathogenesis.[23] However, previous studies couldn't conclude if this reduced expression is a consequence or a cause of hyperglycemia. In our study, we demonstrated insignificant difference in JAZF expression between cases and controls and between simple cases and complicated cases. This finding may have different explanations. First, certain JAZF alleles may carry risk of T2DM but affect a splice isoform pattern rather than total gene expression[22]; second, most of the previous studies reported reduction of JAZF expression either in human pancreatic cells or experimental cells, but we studied it in serum; third, presence of other epigenetic modifications may affect gene expression and mediate the influence of the environment on it.[24]

The KCNJ11 gene encodes a voltage-gated potassium ion channel (Kir6.2), which together with the high-affinity sulfonylurea receptor 1 (SUR1), forms the ATP-sensitive K(+) channel (KATP). SUR1 is encoded by the ABCC8 gene, located near the KCNJ11 gene.[1] The pancreatic β-cell KATP channels play a critical role in glucose homeostasis and insulin secretion. Mutations in KCNJ11 and ABCC8 genes have been identified as the most common cause of neonatal T2DM,[25] and patients carrying identified mutations in these genes can replace insulin by oral sulfonylureas, which offer better glycemic control and improve quality of life.[26] The genes ABCC8 and KCNJ11 have received intense focus in T2DM, and previous studies indicated that variants in these genes encoding Kir6.2 and SUR1 are associated with susceptibility to T2DM.[27] Although Taneera *et al.* reported reduced KCN expression in the islet cells of diabetic patients and the relationship between its expression and lower HbA1c, they ranked it as the eighth gene in the list of genes associated with T2DM. However, they explained the presence of KCN further down in the ranking list because the effect of KCN genotypes on the risk of T2DM affects a specific splice isoform pattern and function, rather than total gene expression.[22] This is in agreement with Zhou *et al.*, who observed that the underlying mechanism by which KCN can affect insulin sensitivity is unknown, but they suggested that KCN SNPs influenced gene expression through the introduction or removal of islands rich in cytosine followed by guanine nucleotide and linked by one phosphate bond only (CPG).[28] This finding partially disagrees with our study that revealed no differential expression of the KCN gene between cases and controls and between simple cases and complicated cases. However, our results can be explained by the concept mentioned before, that KCN genotypes affect T2DM through a specific splice isoform pattern and function, rather than total gene expression.[22] Our results are in agreement with Marselli *et al.*, who reported no differential expression of KCN gene in frozen sections obtained from cadaver pancreases of 10 control and 10 T2DM human subjects.[21] Although we reported no differential expression of KCN gene in the current study, ABCC8 showed significantly reduced expression in diabetic patients compared with the control group. Our results are in agreement with Marselli *et al.* and Taneera *et al.*, who reported reduced ABCC8 expression in the islet cells of diabetic patients.[21,22] Our findings could be explained by several reports that describe the critical role of KATP channels and its SUR1 subunit in insulin secretion, and any aberrant expression disrupts the normal stoichiometry (4 Kir6:4

SUR1) of the two subunits and will disrupt pancreatic insulin secretion, contributing to hyperglycemia associated with T2DM. Koblas *et al*. also demonstrated that reprogramming of pancreatic exocrine cells into insulin-producing cells, induced by synthetic messenger RNAs encoding pancreatic transcription factors and SUR1 protein, represents a promising therapeutic strategy for diabetes.[29] Different studies have demonstrated important insights into analysis of gene expression in peripheral blood mononuclear cells (PBMNCs) of T2DM patients. It is suggested that analysis of PBMNC gene expression may potentially serve as a noninvasive and effective marker of diabetes because it is postulated that PBMNCs express 80% of the genes previously restricted to nonblood tissues and that these genes' expression responds to changes occurring in the micro- and macro-environment.[30] Therefore, PBMNCs change their genomic expression in response to the disease activity and reflect changes in tissue gene expression. Safi and his colleagues performed a comprehensive analysis of the gene expression profiles of PBMNCs among offspring of one T2DM parent with normal glucose tolerance and impaired glucose tolerance in comparison to newly diagnosed diabetics and normal controls. Surprisingly, they reported increased expression of ABCC8 gene expression in first-degree relatives with impaired glucose tolerance *versus* controls, while, ABCC8 showed weak decreased expression in newly diagnosed T2DM *versus* controls and weak expression in first-degree relatives with normal glucose tolerance *versus* controls.[31] Kaviarasan *et al*. performed a gene expression array study that included 84 candidate genes in six T2DM patients *versus* three normal patients and reported decreased ABCC8 gene expression in patients *versus* controls.[32] On the other hand, we demonstrated no significant difference in ABCC8 expression between simple and complicated patients, but this finding doesn't exclude the role of ABCC in pathogenesis of diabetic complications because it was demonstrated that identified ABCC alleles may be associated with an alternative splice form affecting protein function, rather than gene expression.[22] However, results regarding gene expression are affected by sample size, sample ascertainment, tissues chosen for analysis and associated genetic and epigenetic factors.[21,22,25]

Notch signaling pathway is a conserved pathway that includes four Notch receptors and is essential for cell–cell communication, cell cycle regulation and tissue homeostasis under both physiologic and pathologic conditions.[33] Notch 2 is a protein shown to be essential for endothelial and smooth muscle function, and pancreatic and nephron development.[34,35] Any dysregulation in Notch signaling is linked to different pathologies.[36,37] In the current study, Notch 2 showed overexpression in diabetic patients compared with the control group. These results are in accordance with with previous studies that have linked certain polymorphisms of Notch2 gene to the development of T2DM.[38] Interestingly, an experimental study suggested that altered notch function, either by gain or loss of function, strongly affects pancreatic β cells.[35] Although Notch 2 is essential for differentiation of pancreatic precursor cells, Marselli *et al*. found no differential expression in Notch 2 of islet cells in diabetic patients compared with those of normal subjects.[21] In our study, we found that patients without complications showed Notch 2 overexpression compared with patients who had complications, in agreement with different studies that reported association between altered Notch expression and diabetic complications.[34,35] Yoon *et al*. reported in an experimental study that Jagged1 (Notch2 ligand) overexpression and suppression of Notch signaling in adult mice endothelial cells, led to diabetic microangiopathy. Furthermore, blocking Jagged1, even after 4 weeks of complications, could reverse these complications and normalize retinal vasculature.[39] Another experimental study undertaken by Bonegio *et al*. demonstrated that altered Notch function, either by gain or loss, strongly affected pancreatic β cell and nephron endowment, suggesting a role of Notch 2 in diabetic nephropathy.[35] In humans, recent studies also reported altered expression of Notch proteins in kidneys of patients with diabetic nephropathy.[34] Ahn *et al*. suggested that altered Notch expression in the kidney of patients with diabetic nephropathy is a valuable translational finding that provides a new therapeutic strategy for the cure of diabetic nephropathy, by blocking Notch activation. They also reported that the ability of hyperglycemia to regulate Notch expression in different cells will raise the possibility of Notch activation contributing to the development of diabetic complications.[33]

In the current study, mean vitamin D levels showed statistical difference between cases and controls but no difference was detected between patients with complications and patients without. Bajaj *et al*. reported increased prevalence of

vitamin D deficiency among diabetic patients compared with controls and demonstrated an association between vitamin D deficiency and diabetic microvascular complications.[7] However, results regarding vitamin D in T2DM and associated complications are conflicting. Usluogullari *et al.* reported no association of vitamin D deficiency with T2DM and microvascular complications except diabetic nephropathy.[40] Herrmann *et al.* reported no association between vitamin D and diabetes or microvascular complications.[41] A meta-analysis of three studies with adjusted variables showed that vitamin D deficiency was associated with increased risk of diabetic nephropathy in patients with T2DM.[42] Zoppini *et al.* found an inverse relationship between vitamin D levels and the prevalence of microvascular complications in patients with T2DM.[43] However, all these cross-sectional studies proved association, but could not prove causality. Discrepancy between these studies can be explained by differences in sample size, geographical environment, sun-avoidance behaviors, prevalence of vitamin D deficiency, even among healthy subjects, skin color, dietary intake and genetic predisposition.[44]

Vitamin D acts *via* a VDR that plays a crucial role in regulating insulin secretion. VDR gene polymorphisms have been associated with altered gene expression or disturbed gene function.[45] Despite the efforts contributed for years, the role of VDR polymorphisms in T2DM pathogenesis has been unclear. Reports regarding the association between VDR gene polymorphisms and T2DM are inconsistent. In our study, we did not find any association between Fok1 and Bsm1 genotypes with susceptibility of T2DM among Egyptian patients. Similar to our results, Malecki *et al.* and Bid *et al.* demonstrated no association between Fok1 and Bsm1 genotypes and T2DM in Polish Whites and Indians.[45,46] Conversely, our results disagree with Wang *et al.*, who reported association of Fok1 and Bsm1 genotypes and T2DM susceptibility.[47] In a meta-analysis performed by Li *et al.*, no association was found between Bsm1 with T2DM among Asian population, while Fok1 showed strong association with T2DM.[48] In spite of the previously mentioned results concerning VDR genotypes and T2DM susceptibility, our study demonstrated significant differences in Fok1 and Bsm1 genotype distributions and allele frequencies between complicated and noncomplicated patients. These findings agree with studies demonstrating that diabetic microvascular complications result from several mechanisms, including hyperglycemia, endothelial dysfunction and altered oxidative processes. Vitamin D is a known player in maintaining normal glucose metabolism and normal endothelial function. Besides, it has the ability to control the apoptosis and inflammatory processes. VDR polymorphisms may be associated with altered vitamin D level or function. Therefore, it is postulated that VDR polymorphism may influence the development of diabetic microvascular complications.[8] However, Maia *et al.* reported no significant association between VDR Fok1 polymorphism and T2DM and its complications in Brazilian postmenopausal women.[49] Also, Vedralová *et al.* failed to link Fok1 and Bsm1 genotypes with T2DM and associated diabetic nephropathy in the German population.[50] Lui *et al.*, in a meta-analysis study, reported no associations between VDR polymorphisms and microvascular complications in T2DM.[51] On the other hand, Hong *et al.* suggested that Bsm1 polymorphisms could be used as a susceptibility marker to predict the risk of diabetic complications in the Korean population.[8] A multinational prospective study demonstrated that Bsm1 genotypes were associated with an increased risk of microangiopathic CVD in T2DM patients.[52] Our explanations for the discrepancy between the results are differences in gene–gene and gene–environment interactions, enrolled characteristics of patients among studies, ethnicity and associated environmental risk factors, especially differences in lifestyles (diet, smoking and physical activity).

Some limitations should be considered in this study. Although power of Fok1 association with microvascular complications is 87%, the small sample size has limited the power to detect the Bsm1 polymorphism effect on microvascular complications associated with T2DM (53%). This cross-sectional study could not exclude noncomplicated T2DM patients who will develop these complications later in the course of the disease. Our results were also limited by the absence of dietary information and sun exposure behavior for our study participants, which may affect vitamin D level. Moreover, most of the patients were already receiving treatment with insulin or oral hypoglycemic drugs, and we cannot exclude the possibility that these drugs could affect levels of expression.

Conclusions

Altered expression of Notch2 and ABCC8 genes and vitamin D deficiency may play a role in

T2DM pathogenesis. Altered expression of Notch2 and VDR polymorphisms may play a role in increased susceptibility to microvascular complications in diabetic patients. These results may contribute to early identification and management of diabetic complications. Our findings provide new insight into better therapeutic strategies in patients with altered gene expression. Furthermore, more detailed genetic and experimental studies are needed to clarify associations between gene expression, genetic alleles, and levels and functions of proteins of the studied genes.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

References

1. Haghvirdizadeh P, Mohamed Z, Abdullah NA, *et al*. KCNJ11: genetic polymorphisms and risk of diabetes mellitus. *J Diabetes Res* 2015; 2015: 908152.
2. Motawi T, Salman T, Shaker O, *et al*. Association of polymorphism in adiponectin (+45 T/G) and leptin (-2548 G/A) genes with type 2 diabetes mellitus in male Egyptians. *Arch Med Sci* 2015; 11(5): 937–944.
3. Chawla A, Chawla R and Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian J Endocrinol Metab* 2016; 20(4): 546–551.
4. Nair AK and Baier LJ. Complex genetics of type 2 diabetes and effect size: what have we learned from isolated populations? *Rev Diabet Stud* 2015; 12(3–4): 299–319.
5. Jonsson A, Ladenvall C, Ahluwalia TS, *et al*. Effects of common genetic variants associated with type 2 diabetes and glycemic traits on α- and β-cell function and insulin action in humans. *Diabetes* 2013; 62(8): 2978–2983.
6. Alharbi KK, Ali Khan I, Syed R, *et al*. Association of JAZF1 and TSPAN8/LGR5 variants in relation to type 2 diabetes mellitus in a Saudi population. *Diabetol Metab Syndr* 2015; 7: 92.
7. Bajaj S, Singh R, Dwivedi N, *et al*. Vitamin D levels and microvascular complications in type 2 diabetes. *Indian J Endocrinol Metab* 2014; 18(4): 537–541.
8. Hong YJ, Kang ES, Ji MJ, *et al*. Association between Bsm1 polymorphism in vitamin D receptor gene and diabetic retinopathy of type 2 diabetes in Korean population. *Endocrinol Metab* 2015; 30(4): 469–474.
9. American Diabetes Association. Standards of medical care for patients with diabetes mellitus. *Diabetes Care* 2000; 23(Suppl. 1): S32–S42.
10. American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In Standards of Medical Care in Diabetes 2015. *Diabetes Care* 2015; 38(Suppl. 1): S8–S16.
11. Mehrabzadeh M, Pasalar P, Karimi M, *et al*. Association between ELMO1 gene polymorphisms and diabetic nephropathy in an Iranian population. *J Diabetes Metab Disord* 2016; 15: 43.
12. Lima VC, Cavalieri GC, Lima MC, *et al*. Risk factors for diabetic retinopathy: a case-control study. *Int J Retina Vitreous* 2016; 2: 21.
13. Miric DJ, Kisic BM, Filipovic-Danic S, *et al*. Xanthine oxidase activity in type 2 diabetes mellitus patients with and without diabetic peripheral neuropathy. *J Diabetes Res* 2016; 2016: 4370490.
14. El-Lebedy D, Kafoury M, Abd-El Haleem D, *et al*. Paraoxonase-1 gene Q192R and L55M polymorphisms and risk of cardiovascular disease in Egyptian patients with type 2 diabetes mellitus. *J Diabetes Metab Disord* 2014; 13(1): 124.
15. Prevention and management of osteoporosis. World Health Organ Tech Rep Ser 2003; 921: 1–164.
16. El Rifai NM, Abdel Moety GA, Gaafar HM, *et al*. Vitamin D deficiency in Egyptian mothers and their neonates and possible related factors. *J Matern Fetal Neonatal Med* 2014; 27(10): 1064–1068.
17. Xu X, Sun J, Chang X, *et al*. Genetic variants of nuclear factor erythroid-derived 2-like 2 associated with the complications in Han descents with type 2 diabetes mellitus of northeast China. *J Cell Mol Med* 2016; 20(11): 2078–2088.
18. Staiger H, Machicao F, Kantartzis K, *et al*. Novel meta-analysis-derived type 2 diabetes risk loci do not determine prediabetic phenotypes. *PLoS One* 2008; 3(8): e3019.
19. Grarup N, Andersen G, Krarup NT, *et al*. Association testing of novel type 2 diabetes

risk alleles in the JAZF1, CDC123/CAMK1D, TSPAN8, THADA, ADAMTS9, and NOTCH2 loci with insulin release, insulin sensitivity, and obesity in a population-based sample of 4516 glucose-tolerant middle-aged Danes. *Diabetes* 2008; 57(9): 2534–2540.

20. Lee HY, Hui LI, Guigni B, *et al*. Early growth retardation and insulin resistance in JAZF1 KO Mice. *Diabetes* 2011; 60: A103.

21. Marselli L, Thorne J, Dahiya S, *et al*. Gene expression profiles of Beta-cell enriched tissue obtained by laser capture microdissection from subjects with type 2 diabetes. *PLoS One* 2010; 5(7): e11499.

22. Taneera J, Lang S, Sharma A, *et al*. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. *Cell Metab* 2012; 16(1): 122–134.

23. Yuasa K, Aoki N and Hijikata T. JAZF1 promotes proliferation of C2C12 cells, but retards their myogenic differentiation through transcriptional repression of MEF2C and MRF4-implications for the role of JAZF1 variants in oncogenesis and type 2 diabetes. *Exp Cell Res* 2015; 336(2): 287–297.

24. Sanghera DK and Blackett PR. Type 2 diabetes genetics: beyond GWAS. *J Diabetes Metab* 2012; 3: 198.

25. Remedi MS, Friedman JB and Nichols CG (2016). Diabetes induced by gain-of-function mutations in the Kir6.1 subunit of the KATP channel. *J Gen Physiol* 2016; pii: jgp.201611653.

26. Holstein A, Hahn M, Stumvoll M, *et al*. The E23K variant of KCNJ11 and the risk for severe sulfonylurea-induced hypoglycemia in patients with type 2 diabetes. *Horm Metab Res* 2009; 41(5): 387–390.

27. Sokolova EA, Bondar IA, Shabelnikova OY, *et al*. Replication of KCNJ11 (p.E23K) and ABCC8 (p.S1369A) association in Russian diabetes mellitus 2 type cohort and meta-analysis. *PLoS One* 2015; 10(5): e0124662.

28. Zhou X, Zhu J, Bao Z, *et al*. A variation in KCNQ1 gene is associated with repaglinide efficacy on insulin resistance in Chinese type 2 diabetes mellitus patients. *Sci Rep* 2016; 6: 37293.

29. Koblas T, Leontovyc I, Loukotova S, *et al*. Reprogramming of pancreatic exocrine cells AR42J into insulin-producing cells using mRNAs for Pdx1, Ngn3, and MafA transcription factors. *Mol Ther Nucleic Acids* 2016; 5: e320.

30. Manoel-Caetano FS, Xavier DJ, Evangelista AF, *et al*. Gene expression profiles displayed by peripheral blood mononuclear cells from patients with type 2 diabetes mellitus focusing on biological processes implicated on the pathogenesis of the disease. *Gene* 2012; 511(2): 151–160.

31. Safi S, Qvist R, Chinna K, *et al*. Gene expression profiling of the peripheral blood mononuclear cells of offspring of one type 2 diabetic parent. *Int J Diabetes Dev Ctries* 2016; 36(4): 407–419.

32. Kaviarasan S, Muniandy S, Qvist R, *et al*. Gene expression profile in leucocytes of type 2 diabetic subjects. *Biomedical Research* 2009; 20(3):158–165.

33. Ahn S and Susztak K. Getting a Notch closer to understanding diabetic kidney disease. *Diabetes* 2010; 59(8): 1865–1867.

34. Walsh DW, Roxburgh SA, McGettigan P, *et al*. Co-regulation of Gremlin and Notch signalling in diabetic nephropathy. *Biochim Biophys Acta* 2008; 1782(1): 10–21.

35. Bonegio R and Susztak K. Notch signaling in diabetic nephropathy. *Exp Cell Res* 2012; 318(9): 986–992.

36. Gridley T. Notch signaling and inherited disease syndromes. *Hum Mol Genet* 2003; 12: R9–R13.

37. Kim Gridley TW, Shin Y, Kim B-J, *et al*. Notch signaling in pancreatic endocrine cell and diabetes. *Biochem Biophys Res Commun* 2010; 392: 247–251.

38. Zeggini E, Scott LJ, Saxena R, *et al*. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 2008; 40(5): 638–645.

39. Yoon CH, Choi YE, Cha YR, *et al*. Diabetes-induced Jagged1 overexpression in endothelial cells causes retinal capillary regression in a murine Model of diabetes mellitus: insights into diabetic retinopathy. *Circulation* 2016; 134(3): 233–247.

40. Usluogullari CA, Balkan F, Caner S, *et al*. The relationship between microvascular complications and vitamin D deficiency in type 2 diabetes mellitus. *BMC Endocr Disord* 2015; 15: 33.

41. Herrmann M, Sullivan DR, Veillard AS, *et al*. Serum 25-hydroxyvitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes. *Diabetes Care* 2015; 38: 521–528.

42. Lv WS, Zhao WJ, Gong SL, *et al.* Serum 25-hydroxyvitamin D levels and peripheral neuropathy in patients with type 2 diabetes: a systematic review and meta-analysis. *J Endocrinol Invest*. 2014; 38(5): 513–518.

43. Zoppini G, Galletti A, Targher G, *et al.* Lower levels of 25-hydroxyvitamin D3 are associated with a higher prevalence of microvascular complications in patients with type 2 diabetes. *BMJ Open Diabetes Res Care* 2015; 3(1): e000058.

44. Ismail M, Abdel Hamid T, Ibrahim A, *et al.* Serum adipokines and vitamin D levels in patients with type 1 diabetes mellitus. *Arch Med Sci* 2016. DOI: https://doi.org/10.5114/aoms.2016.60680.

45. Malecki MT, Frey J, Moczulski D, *et al.* Vitamin D receptor gene polymorphisms and association with type 2 diabetes mellitus in a Polish population. *Exp Clin Endocrinol Diabetes* 2003; 111(8): 505–509.

46. Bid HK, Konwar R, Aggarwal CG, *et al.* Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and type 2 diabetes mellitus: a north Indian study. *Indian J Med Sci* 2009; 63(5): 187–194.

47. Wang Q, Xi B, Reilly KH, *et al.* Quantitative assessment of the associations between four polymorphisms (FokI, ApaI, BsmI, TaqI) of vitamin D receptor gene and risk of diabetes mellitus. *Mol Biol Rep* 2012; 39(10): 9405–9414.

48. Li L, Wu B, Liu JY, *et al.* Vitamin D receptor gene polymorphisms and type 2 diabetes: a meta-analysis. *Arch Med Res* 2013; 44(3): 235–241.

49. Maia J, da Silva AS, do Carmo RF, *et al.* The association between vitamin D receptor gene polymorphisms (TaqI and FokI), type 2 diabetes, and micro-/macrovascular complications in postmenopausal women. *Appl Clin Genet* 2016; 9: 131–136.

50. Vedralová M, Kotrbova-Kozak A, Zelezníková V, *et al.* Polymorphisms in the vitamin D receptor gene and parathyroid hormone gene in the development and progression of diabetes mellitus and its chronic complications, diabetic nephropathy and non-diabetic renal disease. *Kidney Blood Press Res* 2012; 36(1): 1–9.

51. Liu Z, Liu L, Chen X, *et al.* Associations study of vitamin D receptor gene polymorphisms with diabetic microvascular complications: a meta-analysis. *Gene* 2014; 546(1): 6–10.

52. Ferrarezi DA, Bellili-Muñoz N, Dubois-Laforgue D, *et al.* Allelic variations of the vitamin D receptor (VDR) gene are associated with increased risk of coronary artery disease in type 2 diabetics: the DIABHYCAR prospective study. *Diabetes Metab* 2013; 39(3): 263–270.