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The Combined Effect of ACE, TCF7L2, and PPARGC1A Gene Polymorphisms in Diabetic Nephropathy

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THIS study was performed for investigation the relationship between variants of ACE, TCF7L2 and PPARGC1A gene polymorphisms individually or in combination with the development of nephropathy in T2DM. The study was included 85 T2DM patients (45 with nephropathy and 40 without nephropathy), and 45 healthy control subjects. The I/D polymorphism of ACE gene was evaluated by PCR method. The polymorphisms rs7903146 (C/T) of TCF7L2 gene and Gly482Ser (G/A) and Thr394Thr (G/A) of PPARGC1A gene were evaluated by PCR-RFLP analysis.The frequency of ACE DD genotype and D allele was significantly higher in DN patients when compared to diabetic without nephropathy. The frequency of TCF7L2 rs7903146 TT genotype and T allele were significantly associated with DN patients compared to T2DM. Moreover, a significant association in A allelic frequencies was observed in DN cases compared to T2DM patients without nephropathy. No differences in the genotypic and allelic frequencies between T2DM patients with and without nephropathy were found for the Thr394Thr polymorphism.Our study suggested that candidate gene polymorphisms I/D of ACE, rs7903146 of TCF7L2 and Gly482Ser of PPARGC1A individually or in combination may act as susceptibility biomarkers for nephropathy in T2DM.

Keywords: Diabetic nephropathy (DN), Type 2 diabetes mellitus (T2DM), Angiotensin-converting enzyme (ACE), Transcription factor 7–like 2 (TCF7L2), Peroxisome proliferator activated receptor gamma coactivator-1 alpha (PPARGC1A).

Introduction

The pathogenesis of diabetic nephropathy (DN) has many genetic and environmental factors contributing to its developing and progression. The ACE gene polymorphism is insertion/deletion (I/D) of a 287-bp sequence of DNA in the intron 16 that could be relating to circulating ACE level which involved in the etiology of DN [1,2].

The transcription factor 7–like 2 (TCF7L2) is located on chromosome 10q25.3 which considered the main susceptibility gene for T2DM [3]. Wu [4] was reported that the TCF7L2 gene may be contributed to the etiology of DN in combination with other genes. However, Hussain [5] showed an association between TCF7L2 gene and DN, but this association is not independent of T2DM.

Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A) gene is located on chromosome 4p15.1 that is a biological and positional candidate for T2DM progression [6]. Furthermore, PPARGC1A Gly482Ser polymorphism is associated with diabetic nephropathy [7].

There was no enough research available for the genetic combination of ACE, TCF7L2 and PPARGC1A gene polymorphisms in DN. Therefore the aim of this study was to assess the genetic interaction between ACE, TCF7L2 and PPARGC1A polymorphisms in DN development.

Subjects and Methods

Study subjects

This study recruited 85 T2DM patients diagnosed at least 5 years before, the patients consecutively attended the diabetes clinic of Internal Medicine Department; Kasr El-Aini

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Hospital affiliated to Cairo University. Forty five of them (12 males and 33 females) with nephropathy, while the remaining 40 (9 males and 31 females) without nephropathy. The mean age of T2DM patients with nephropathy was 49.5 ± 8.7 years compared to 55.5 ± 8 years for the T2DM patients without nephropathy. The diagnosis of T2DM was based on the American Diabetes Association criteria, a fasting plasma glucose level >126 mg/dL and glycated hemoglobin (HbA1c) > 6.5% and/or treated by oral hypoglycemic agents and/or insulin to achieve glycemic control. Diabetic nephropathy was defined by persistent albuminuria (albumin-creatinine ratio [ACR] > 30 mg/g creatinine) on at least two consecutive occasions over the previous six months. While patients with ACR < 30 mg/g creatinine were normoalbuminuric and had no nephropathy. To avoid misclassification diabetic individuals as having; abnormal liver function, abnormal thyroid function, advanced renal diseases other than diabetes, cardiovascular disease were excluded.

Beside 45 healthy subjects (17 males and 28 females) with mean age 48.47 ± 5.7 years and without any previous history of diabetes or renal disorders were enrolled as control group. The study protocol was approved by the Research Ethics Committee, Faculty of Pharmacy- Cairo University (REC-FOPCU). A written informed consent was taken from each participant prior to the study.

Sampling

Venous blood was obtained from patients and controls after an overnight fast of 8 h and was divided into two portions. One portion of blood (2 ml) was added to EDTA and stored at −20 °C for detection of polymorphism of ACE, TCF7L2 and PPARGC1A genes and glycated hemoglobin assay. A second portion of 1 ml blood was added to fluoride and centrifuged at 1000 ×g for 10 min; the plasma was then separated for determination of fasting plasma glucose.

Morning urine samples were collected under aseptic conditions from patients and controls. Urine was centrifuged at 500 ×g to get rid of the cells and salts then the supernatant was used for determination of albumin.

Fasting blood glucose, glycated hemoglobin and urinary albumin were measured using commercially available kits following the manufacturer's instructions.

Isolation of genomic DNA

Genomic DNA was isolated from peripheral white blood cells using Qia-amplification extraction kit (Qiagen-USA) according to the manufacturer's instructions.

Genotyping

An I/D polymorphism of ACE gene was examined by the polymerase chain reaction (PCR) using forward primer5'-CTGGAGACCACTCCCATCCTTTCT-3'and reverse primer 5'-GATGTCGCCATCA-CATTCGTCAGAT-3' according to the method of Rigat [1]. To avoid the mistyping of ID genotype as DD, an additional PCR was performed in all the DD samples [8]. Single band products with size of 190 and 490 bp were considered as homozygous DD and II genotypes respectively; whereas, two bands of 190 and 490 bp confirmed heterozygous ID genotype. The genotyping of TCF7L2 rs7903146 (C/T) was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. A 266 bp fragment of the gene was amplified with primer sequences fwd: 5' CTGAACAATTAGAGAGCTAA-GCACTTTTTAGGTA-3'; rev: 5' T T T C A C T A T G T A T T G T T G - C C A G T CAGCAAACAC-3' [9] followed by the digestion of PCR product with RsaI enzyme (New England Biolabs, Hitchin, UK). Allele T was characterized by the presence of a 266-bp fragment, which was digested further into 233 and 33 bp fragments in allele C carriers. Thr394Thr and Gly482Ser polymorphisms of PPARGC1A gene were genotyped by PCR-RFLP method, the sequences of the primers to detect Thr394Thr polymorphism were 5'-GCCAGTCAATTA-ATTCCAAACC-3' and 5'-TTGGAGCTGTTTTCTTGTGC-3' [10] while the sequences of the primers that used to detect Gly482Ser polymorphism were 5'-CAAGTCCTCAGTC-CTCAC-3' and 5'-GGGGTCTTTGAGAAAATAAGG-3' [11]. The PCR products were digested with *Msp*I for Thr394Thr and *HpaII* for Gly482Ser polymorphisms. There were three genotypes, homozygous AA (203 bp), GG (182 bp and 21 bp) and heterozygous GA (203 bp, 182 bp and 21 bp) for Thr394Thr polymorphism while, homozygous AA (608 bp), GG (376 bp and 232 bp) and heterozygous GA (608 bp, 376 bp and 232 bp) for Gly482Ser polymorphism.

Statistical analysis

Data are expressed as means ± standard deviation for quantitative variables, frequency for qualitative variables. Qualitative variables were compared using chi square ($\chi 2$) test or Fischer's exact test. One-way ANOVA was used to compare the clinical and laboratory characteristics of patients divided according to genotypes. The Statistical Package for the Social Sciences software (SPSS 17.0, Chicago, IL, USA) was used. P< 0.05 was considered significant.

Results

Table 1 shows the genotype distribution of ACE, TCF7L2, PPARGC1A genes in diabetic with nephropathy (DN), diabetic without nephropathy, and control groups as follow:

For ACE gene, the frequency of the mutant DD and wild II genotypes was significantly different within the studied groups with *P*-values = 0.0011 and 0.014, respectively. The distribution of DD *vs* II genotypes was significantly different in the studied groups (P = 0.000). Moreover, there was a significant different between different genotypes of ACE gene (DD, ID, II) within the studied groups P = 0.000.

For TCF7L2 gene, the TT mutant genotype frequency was significantly different between different groups of P= 0.046. The *P*-value for genotypic distribution of TT *vs* CC within the different groups = 0.014. Furthermore, a significant different was observed between different genotypes of TCF7L2 (TT, CT, CC) in the studied groups (P = 0.05).

Regarding PPARGC1A: for Gly482Ser

TABLE 1. Genotype distribution of the ACE, TCF7L2 rs7903146, PPARGC1A Thr394Thr, and PPARGC1A Gly482Ser gene polymorphisms in the studied group.

Genotype	T2DM with nephropathy (n=45)	T2DM without nephropathy (n=40)	Control (n=45)	P^r	P	P*
ACE						
II	7 (15.5%)	10 (25%)	21 (46.7%)	**0.014**		
ID	13 (28.9%)	20 (50%)	18 (40%)	0.465	0.17	**0.000**
DD	25 (55.6%)	10 (25%)	6 (13.3%)	**0.001**	**0.000**	
TCF7L2						
CC	13 (28.9%)	18 (45%)	24 (53.3%)	0.191		
CT	23 (51.1%)	20 (50%)	18 (40%)	0.732	0.179	**0.05**
TT	9 (20%)	2 (5%)	3 (6.7%)	**0.046**	**0.014**	
Gly482Ser						
GG	14 (31.1%)	20 (50%)	21 (46.7%)	0.458		
GA	23 (51.1%)	17 (42.5%)	24 (53.3%)	0.511	0.375	**0.025**
AA	8 (17.8%)	3 (7.5%)	0 (0%)	0.132	**0.005**	
Thr394Thr						
GG	23 (51.1%)	25 (62.5%)	24 (53.3%)	0.959		
GA	15 (33.3%)	12 (30%)	15 (33.3%)	0.807	0.792	0.769
AA	7 (15.6%)	3 (7.5%)	6 (13.4%)	0.444	0.438	

Data are number (%), variables were compared using chi square ($\chi 2$) test or Fischer's exact test.
P^r values for the frequency of each genotype within the studied groups.
P values for comparison the mutant *vs* the wild genotypes and the hetero *vs* the wild genotypes within the studied groups.
P* values for comparison the different genotypes (mutant, hetero, and wild) within the studied groups.
Bold values indicate significant difference P value ≤ 0.05 was considered significant.

polymorphism, the genotype distribution of the mutant AA *vs* the wild GG and the distribution of different genotypes (AA, GA, GG) were significantly different between DN, T2DM and controls (P = 0.005 and 0.025, respectively). However in the Thr394Thr polymorphism, there was no significant difference found between the different groups.

Table 2 shows that the genotypic distribution of ACE gene significantly differed between the two diabetic groups (with and without nephropathy) P-value = 0.017. Furthermore, the frequency of the mutant D allele was significantly higher in the DN group in comparison with the diabetic without nephropathy group (OR = 0.429, 95% CI: 0.229-0.804, P = 0.008). Using genetic models we found that the recessive model (DD+ID *vs* II) had no significant difference when the DN compared to diabetic without nephropathy patients (OR = 1.81, 95% CI: 0.616–5.318, P = 0.277). But we observed significant differences in the co-dominant model (II *vs* DD) (OR = 0.28, 95% CI: 0. 0.083-0.942, P = 0.035), dominant model (II+ID *vs* DD) (OR = 0.267, 95% CI: 0. 0.106-0.673, P = 0.004), and over dominant model (II+DD *vs* ID) (OR = 2.462, 95% CI: 1.007-6.02, P = 0.046).

The allele frequency of TCF7L2 gene in Table 2 revealed that there was a significant difference between the diabetic with and without nephropathy groups P = 0.024 (OR = 2.074, 95% CI: 1.096-3.923). Moreover in the co-dominant model (CC *vs* TT) and dominant model (CC+CT *vs* TT) significant differences were detected between the two diabetic groups P = 0.023 and 0.04, respectively.

Table 2 also shows the frequency of the mutant A allele of Gly482Ser in PPARGC1A gene was significantly increased in the diabetic nephropathy patients compared to diabetic patients without nephropathy (OR = 0.528, 95% CI: 0.279-1, P = 0.049). On the other hand, we did not find any significant difference in the allele and genotype frequencies of Thr394Thr polymorphism for the PPARGC1A gene between the two diabetic groups (P = 0.421 and P = 0.157, respectively).

To assess the interaction between the genotypes, the combination effect of ACE, TCF7L2, Gly482Ser and Thr394Thr genotypes was examined (Table 3). The frequency of DD/CT/GA/GG combined genotypes is higher in DN group (15.6%) than in the diabetic without nephropathy (5%). Furthermore, the DD/TT/AA/AA combined mutant genotypes is increased in diabetic nephropathy (8.9%) compared to diabetic without nephropathy (0%). The heterozygous ID, CT and GA and homozygous GG is higher in diabetic without nephropathy group (15%) than in diabetic nephropathy group (0%) where individuals with genotypes ID+CT+GA+GG have a protecting effect for nephropathy. The wild II+CC+GG+GG genotypes had significantly lower combination in T2DM with nephropathy patients (8.9%) compared to T2DM without nephropathy patients (25%).

The Biochemical parameters of T2DM with and without nephropathy with different genotypes in ACE, TCF7L2 and PPARGC1A genes were shown in Fig. 1, 2, 3 and 4. In T2DM with nephropathy patients, the glycohemoglobin percent showed higher significance in ID and DD mutant genotypes (10.2%) compared to that of II wild genotype (8.58%) of ACE gene. Moreover in T2DM with nephropathy patients, there was significant difference between the mutant GA and AA genotypes compared to the wild GG genotype of Gly482Ser PPARGC1A regarding the fasting plasma glucose (317.6±15 and 226.78±26, respectively) as well as urinary albumin concentration (97.4±14 and 72.4±5.2, respectively). However, the biochemical data of Thr394Thr genotypes in PPARGC1A and rs7903146 in TCF7L2 gene showed no significant differences between different genotypes of T2DM patients with and without nephropathy.

Discussion

In an attempt to study I/D polymorphism in ACE gene, rs7903146 (C/T) polymorphism in TCF7L2 gene, and Thr394Thr (G/A) and Gly482Ser (G/A) polymorphisms in PPARGC1A gene, and find out their impact on susceptibility to nephropathy in T2DM patients, the genotype distribution and allele frequencies of the forementioned genes were evaluated in controls, as well as T2DM patients with and without nephropathy. In addition, the effect of different genotypes on the assessed biochemical characteristics of the patients was evaluated.

The distribution of mutant DD genotype and D allele was significantly higher in diabetic nephropathy than in T2DM without nephropathy. Our finding could be explained on the basis that the deletion polymorphism is coupled with increased serum levels of ACE, while the insertion polymorphism has lowest ACE levels [12-14].

TABLE 2. Co-dominant, Dominant, Recessive, and Over-dominant Models for ACE I/D, TCF7L2 rs7903146, PPARGC1A Gly482Ser, and PPARGC1A Thr394Thr gene polymorphisms in T2DM patients with and without nephropathy.

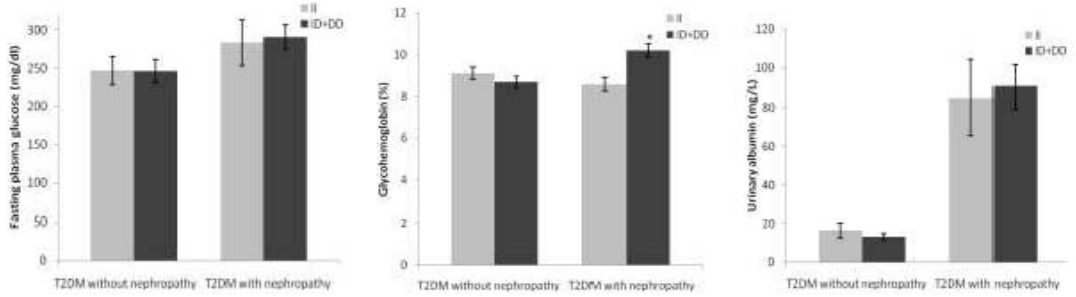
		T2DM with Nephropathy	T2DM without Nephropathy	P value	OR (95% CI)
ACE					
Genotype	**DD**/ID/II	25/13/7	10/20/10	**0.017**	
Allele	**D**/I	63/27	40/40	**0.008**	0.429 (0.229-0.804)
Co-dominant model	II	7 (15.6%)	10 (25%)		1
	ID	13 (28.8%)	20 (50%)	0.903	1.077 (0.327-3.546)
	DD	25 (55.6%)	10 (25%)	**0.035**	0.28 (0.083-0.942)
Recessive model	ID+DD	38 (84.4%)	30 (75%)	0.277	1.81 (0.616-5.318)
	II	7 (15.6%)	10 (25%)		1
Dominant model	DD	25 (55.6%)	10 (25%)	**0.004**	0.267 (0.106-0.673)
	II+ID	20 (44.4%)	30 (75%)		1
Over-Dominant model	ID	13 (28.9%)	20 (50%)	**0.046**	2.462 (1.007-6.02)
	II+DD	32 (71.1%)	20 (50%)		1
TCF7L2					
Genotype	**TT**/CT/CC	9/23/13	2/20/18	0.074	
Allele	**T**/C	41/49	23/57	**0.024**	2.074 (1.096-3.923)
Co-dominant model	CC	13 (28.9%)	18 (45%)		1
	CT	23 (51.1%)	20 (50%)	0.327	0.628 (0.247-1.594)
	TT	9 (20%)	2 (5%)	**0.023**	0.16 (0.03-0.87)
Recessive t model	CT+TT	32 (71.1%)	22 (55%)	0.123	2.014 (0.822-4.936)
	CC	13 (28.9%)	18 (45%)		1
Dominant model	TT	9 (20%)	2 (5%)	**0.04**	0.211 (0.043-1.041)
	CC+CT	36 (80%)	38 (95%)		1
Over-Dominant model	CT	23 (51.1%)	20 (50%)	0.919	0.957 (0.408-2.242)
	CC+TT	22 (48.9%)	20 (50%)		1
Gly482Ser					
Genotype	**AA**/GA/GG	8/23/14	3/17/20	0.139	
Allele	**A**/G	39/51	23/57	**0.049**	0.528 (0.279-1)
Co-dominant model	GG	14 (31.1%)	20 (50%)		1
	GA	23 (51.1%)	17 (42.5%)	0.162	0.517 (0.205-1.307)
	AA	8 (17.8%)	3 (7.5%)	0.069	0.263 (0.059-1.167)
Recessive model	GA+AA	31 (68.9%)	20 (50%)	0.076	2.214 (0.914-5.363)
	GG	14 (31.1%)	20 (50%)		1
Dominant model	AA	8 (17.8%)	3 (7.5%)	0.159	0.375 (0.092-1.525)
	GG+GA	37 (82.2%)	37 (92.5%)		1
Over-dominant model	GA	23 (51.1%)	17 (42.5%)	0.427	0.707 (0.3-1.666)
	GG+AA	22 (48.9%)	23 (57.5%)		1
Thr394Thr					
Genotype	**AA**/GA/GG	7/15/23	3/12/25	0.421	
Allele	**A**/G	29/61	18/62	0.157	0.611 (0.307-1.213)
Co-dominant model	GG	23 (51.1%)	25 (62.5%)		1
	GA	15 (33.3%)	12 (30%)	0.525	0.736 (0.258-1.897)
	AA	7 (15.6%)	3 (7.5%)	0.2	0.394 (0.91-1.708)
Recessive model	GA+AA	22 (48.9%)	15 (37.5%)	0.29	1.594 (0.67-3.793)
	GG	23 (51.1%)	25 (62.5%)		1
Dominant model	AA	7 (15.6%)	3 (7.5%)	0.25	0.44 (0.106-1.832)
	GG+GA	38 (84.4%)	37 (92.5%)		1
Over-dominant model	GA	15 (33.3%)	12 (30%)	0.742	0.857 (0.343-2.145)
	GG+AA	30 (66.7%)	28 (70%)		1

Data are number (%), variables were compared using Chi square ($\chi 2$) test or Fischer's exact test.
P values for comparison between T2DM with and without nephropathy.
OR: odd ratio; Cl: confidence interval.
Bold values indicate significant difference at $P \leq 0.05$.

TABLE 3. The combined effect of ACE, TCF7L2 rs7903146, PPARGC1A Gly482Ser, and PPARGC1A Thr394Thr genotype frequencies in T2DM patients with and without nephropathy.

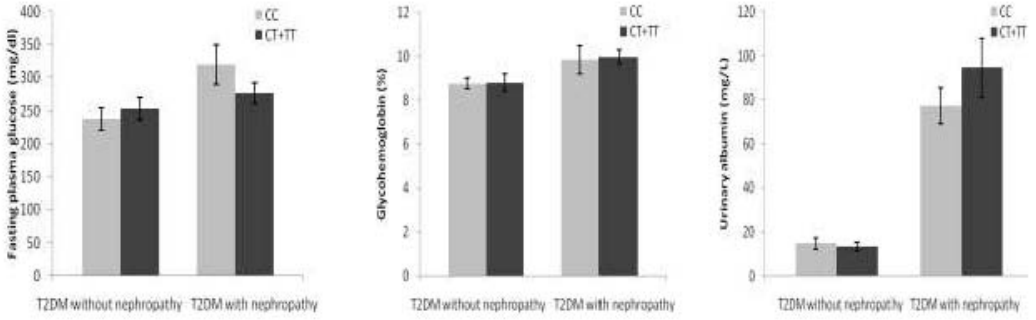
ACE+TCF7L2+Gly482Ser+Thr394Thr genotypes				T2DM without nephropathy	T2DM with nephropathy
ACE	TCF7L2	Gly482Ser	Thr394Thr		
II	CC	GG	GG	**10 (25%)**	**4 (8.9%)***
II	CC	GA	GG	0 (0%)	1 (2.2%)
II	CT	AA	GG	0 (0%)	1 (2.2%)
II	TT	AA	GA	0 (0%)	1 (2.2%)
ID	CC	GG	GG	3 (7.5%)	1 (2.2%)
ID	CC	GG	GA	1 (2.5%)	0 (0%)
ID	CC	GA	GG	0 (0%)	1 (2.2%)
ID	CC	GA	GA	2 (5%)	2 (4.4%)
ID	CT	GG	GG	1 (2.5%)	1 (2.2%)
ID	CT	GG	GA	2 (5%)	0 (0%)
ID	CT	GG	AA	0 (0%)	1 (2.2%)
ID	CT	GA	GG	**6 (15%)**	**0 (0%)***
ID	CT	GA	GA	3 (7.5%)	5 (11.1%)
ID	CT	GA	AA	1 (2.5%)	0 (0%)
ID	CT	AA	GG	1 (2.5%)	0 (0%)
ID	CT	AA	AA	0 (0%)	1 (2.2%)
ID	TT	GG	GG	0 (0%)	1 (2.2%)
DD	CC	GG	GG	1 (2.5%)	2 (4.4%)
DD	CC	GG	GA	1 (2.5%)	0 (0%)
DD	CC	GA	GA	0 (0%)	1 (2.2%)
DD	CC	AA	GG	0 (0%)	1 (2.2%)
DD	CT	GG	GG	0 (0%)	2 (4.4%)
DD	CT	GG	GA	1 (2.5%)	2 (4.4%)
DD	CT	GA	GG	**2 (5%)**	**7 (15.6%)***
DD	CT	GA	GA	1 (2.5%)	3 (6.7%)
DD	CT	GA	AA	1 (2.5%)	0 (0%)
DD	CT	AA	GA	1 (2.5%)	0 (0%)
DD	TT	GA	GG	0 (0%)	1 (2.2%)
DD	TT	GA	GA	0 (0%)	1 (2.2%)
DD	TT	GA	AA	1 (2.5%)	1 (2.2%)
DD	TT	AA	GG	1 (2.5%)	0 (0%)
DD	TT	AA	AA	**0 (0%)**	**4 (8.9%)***

Data are expressed as number (percentage).
*Significantly different from T2DM without nephropathy at $P \leq 0.05$.
The missed genotypes combinations are not found in the studied groups.



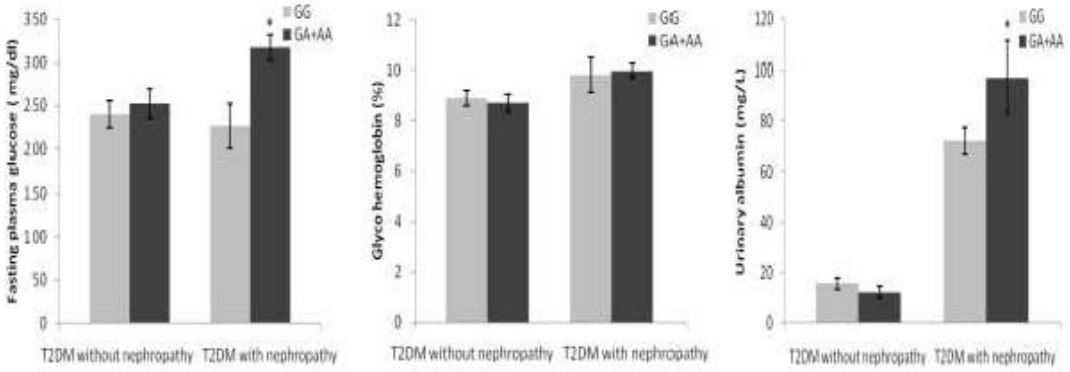
Data are expressed as mean ± SE.
*Significant difference from II wild genotype group at P<0.05.

Fig. 1. Biochemical characteristics of T2DM patients with and without nephropathy with different I/D genotypes in ACE gene.



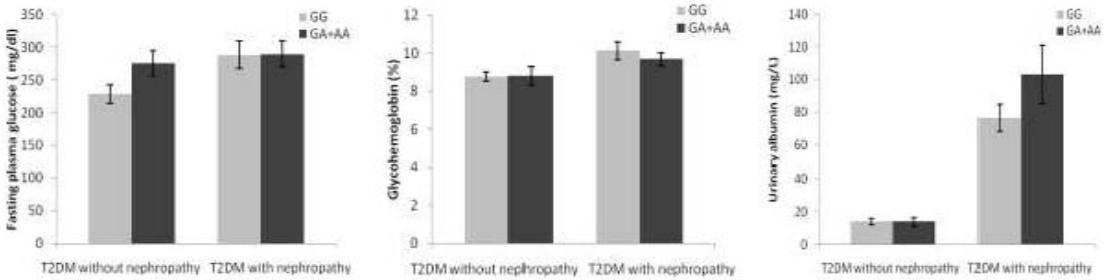
Data are expressed as mean ± SE.

Fig. 2. Biochemical characteristics of T2DM patients with and without nephropathy with different rs7903146 genotypes in TCF7L2 gene.



Data are expressed as mean ± SE
* Significant difference from GG wild genotype group at P<0.05.

Fig. 3. Biochemical characteristics of T2DM patients with and without nephropathy with different Gly482Ser genotypes in PPARGC1A gene.



Data are expressed as mean ± SE.

Fig. 4. Biochemical characteristics of T2DM patients with and without nephropathy with different Thr394Thr genotypes in PPARGC1A gene.

Since ACE catalyses production of angiotensin II from angiotensin I in the diabetic kidney, the angiotensin II raises the intraglomerular pressure and glomerular filtration rate. Also, it stimulates the production or release of several cytokine mediators of glomerulosclerosis [15]. This result raises the thought that the D polymorphism is associated with the development of nephropathy in T2DM patients. Our observations relating to the relationship of ACE polymorphism with diabetic nephropathy were in agreement with other previous studies, a meta-analysis study verified that II genotype has compact the risk of diabetic nephropathy [16]. Our results were also supported by another meta-analysis data, where reported considerable involvement between the ACE DD polymorphism and the possibility of DN [17]. In the same line, several studies in different population have reported that a strong relationship between the ACE DD genotype and the risk for diabetic nephropathy was reported in Malaysian [18], British Caucasian [19], Bahraini [20], Japanese [21], Korean [22], and Americans [14] populations. However, information from other studies in Chinese [23], German [24], Danish [25], Turkish [26], and Tunisian [27] populations were unsuccessful to prove this association. There were conflicting results in Iranian population regarding the association of ACE polymorphism; Nikzamir [28] demonstrated strong relation between the homozygous DD and nephropathy while Rahimi [29] showed no significant association. Furthermore, the statistics from India also showed opposing results by Viswanathan [30], Naresh [31], and Bhavani [32] who reported a positive association for D allele and diabetic nephropathy in South Indian population, whereas Bhaskar [33] in South Indian and Prasad [34] and Kumar [35] in North Indian populations not found this association. These incompatible results may be due to numerous factors, mostly ethnicity.

Regarding the TCF7L2 rs7903146 C/T polymorphism, the mutant TT genotype and/or the T allele were significantly associated with diabetic patients who developed nephropathy when compared to diabetic without nephropathy. Our finding was supported by Buraczynska [35] who showed that mutant T allele in TCF7L2 gene is powerfully linked to nephropathy, particularly in early inception of diabetes. Recently, Buraczynska [36] and Hussain [5] reported that T allele of TCF7L2 gene confers the possibility of developing nephropathy. On contrary, Wu [4] reported that the TCF7L2 gene was not coupled with diabetic nephropathy and the TCF7L2 gene might contribute to the etiology of nephropathy in T2DM patients in an interactive way with other genes. The TCF7L2 might take part in the pathway of diabetic nephropathy development; this could be explained on the basis that the progress of diabetic glomerulosclerosis might be in harmony with the TCF7L2 expression levels [37].

For Gly482Ser and Thr394Thr polymorphisms in the PPARGC1A gene, our results clearly showed a positive association between mutant A allele of Gly482Ser polymorphism and T2DM patients who developed nephropathy compared to T2DM patients without development of nephropathy. Furthermore, mutant AA genotype was significantly differing in DN, T2DM without nephropathy and controls. Our results were in agreement with Gayathri [7] who found that the Gly482Ser polymorphism of the PPARGC1A gene was significantly linked with diabetic nephropathy in Asian Indians. Meanwhile, several genetic association studies concerning this polymorphism were reported to be related with T2DM in different populations, including Danish [11], British [38], Caucasians [39], North Indian [40] and Han Chinese [6]. However, this relation with T2DM was not noticeable in French, Pima Indians and Austrians populations [41- 43]. The PPARGC1A gene is a multifunctional coregulator of cellular energy metabolism [44]. It has been verified that PPARGC1A mediates the expression of genes implicated in oxidative metabolism, adipogenesis and gluconeogenesis [45, 46] so that PPARGC1A plays a significant role in various aspects of glucose and fat metabolism and energy balance [47]. Our results could be explained on the basis that PPARGC1A has been shown to positively affect the expression of numerous ROS-detoxifying enzymes [48 - 50]. The pathogenesis of DN is multifactorial, the dominant being ROS-mediated renal injury [51]. Therefore, PPARGC1A, by controlling the removal of ROS by-products, would minimize the impact of ROS on cell physiology, suggesting that PPARGC1A exerts a beneficial effect in diabetic nephropathy.

On the other hand, our results failed to show any detectable changes in genotype distributions and allele frequencies in Thr394Thr polymorphism within PPARGC1A gene among the studies groups. The results of the current study were supported by Gayathri [7] who failed to show any significant difference concerning

to Thr394Thr polymorphism between diabetic nephropathy patients and type 2 diabetic patients without nephropathy. Meanwhile, in other studies concerning the Thr394Thr polymorphism of PPARGC1A gene in T2DM patients, Yan [52] reported that the Thr394Thr polymorphism was related to T2DM in Han Chinese population and Vimaleswaran [53] demonstrated that Thr394Thr PPARGC1A gene polymorphism is related to T2DM in Asian Indians.

One of the aims of the current study was to try to find combined genetic profiles that were associated with higher incidence of diabetic nephropathy. Our results suggested a synergistic effect between the ACE I/D, TCF7L2 rs7903146 C/T, PPARGC1A Gly482Ser G/A, and PPARGC1A Thr394Thr G/A polymorphisms and diabetic nephropathy. The DD/TT/AA/AA mutant genotypes were only reported in diabetic patients who developed nephropathy suggesting a risk for developing nephropathy in T2DM patients whereas the II/CC/GG/GG wild genotypes were more prevailed in diabetic without nephropathy patients than diabetic nephropathy patients suggesting a protective effect against nephropathy. Moreover, DD/CT/GA/GG genotypes were more prevailed in diabetic patients who developed nephropathy than diabetic patients without nephropathy while ID/CT/GA/GG genotypes were not reported in diabetic nephropathy patients suggesting a protective consequence against nephropathy. Hence, we will be able to assume that ACE I/D polymorphism might proceed synergistically with TCF7L2 rs7903146 C/T, PPARGC1A Gly482Ser G/A, and PPARGC1A Thr394Thr G/A polymorphisms to enhance nephropathy incidence within type 2 diabetic patients. The studies correlated to ACE-TCF7L2-Gly482Ser-Thr394Thr combinations is inadequate, there is extent for additional investigate the synergistic effects of these polymorphisms in diabetic nephropathy susceptibility.

The present study showed that, mutant DD genotype of I/D ACE polymorphism exhibited considerable correlation with high level of glycohemoglobin in DN patients. Since ACE activates production of angiotensin II, ACE polymorphism may play a significant role in elevation of glycohemoglobin level within diabetic nephropathy patients. The results of Kumar [35], in contrary to the current results, showed that ACE genotypes revealed no significant differences in glycohemoglobin level among DN patients with different genotypes. However the results of Jayapalan [54] showed that ACE genotypes were independently associated with glycohemoglobin level in diabetic nephropathy patients. According to the present study, there was significant difference between the Gly482Ser SNP in PPARGC1A gene and fasting plasma glucose level as well as urinary albumin concentration in diabetic nephropathy patients while, Vimaleswaran [53] reported that neither fasting plasma glucose nor urinary albumin concentrations were significantly different among Gly482Ser genotypes. On the other hand, the results of our study revealed that Thr394Thr polymorphism in PPARGC1A gene did not significantly affect fasting plasma glucose, glycohemoglobin, or urinary albumin concentration. Our results were in contrary to those noted by Vimaleswaran [53] who found that fasting plasma glucose was significantly difference among the different genotypes in the diabetic subjects. Moreover, current results showed lack of significant relation between rs7903146 of TCF7L2 gene and fasting glucose, glycohemoglobin levels and urinary albumin concentration.

Based on the present results of our study, ACE ID, TCF7L2 rs7903146 CT, and PPARGC1A Gly482Ser GA polymorphisms individually or in combination were reported to be associated with susceptibility to DN. Thus we suggested that these results might shed more light on the functional significance of ACE ID, TCF7L2 rs7903146, and PPARGC1A Gly482Ser polymorphisms and its contribution as a risk aspect for nephropathy in T2DM Egyptian patients. However, it is important to replicate the results in larger populations to decide whether to role ACE ID, TCF7L2 rs7903146, and PPARGC1A Gly482Ser polymorphisms measurement as a screening tool that might be useful for the prediction, prevention, and management of diabetic nephropathy. In conclusion, diabetics who are at high risk of developing nephropathy can be early identified in order to afford better clinical management.

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Conflict of interest statement

I (we) verify that there is no conflict of interest

with any financial organization regarding the material discussed in the manuscript.

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تأثير تعدد الأشكال الجينية مجتمعة لجينات PPARGC1A ، TCF7L2 ، ACE على الإعتلال الكلوى السكرى

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تهدف هذه الدراسة إلى تحقيق مدى ارتباط تطور مرض الاعتلال الكلوى السكري مع تعدد الأشكال الجينية منفردة أو مجتمعة لجينات TCF7L2 ،(ACE، و(PPARGC1A وذلك في المرضى المصريين المصابين بمرض السكري من النوع الثانى.

قد أجريت هذه الدراسة على 85 من المرضى المصابين بالسكري من النوع الثانى (45 مريض يعانون من الاعتلال الكلوي السكري و 40 مريض دون الاعتلال الكلوي) بالاضافة إلى مجموعة ضابطة وتشمل 45 من الأشخاص الأصحاء الغير مصابين بمرض السكري و أيضا الغير مصابين بالاعتلال الكلوي. فى هذه الدراسة تم تحديد تعدد الأشكال الجينية I/D في جين ACE و تعدد الأشكال الجينية rs7903146 في جين TCF7L2 وكذلك تعدد الأشكال الجينية Thr394Thr وGly482Ser في جين PPARGC1A.

أظهرت النتائج أن توزيع النمط الجيني المتحور DD في جين ACE وكذلك الفرد النظير للجين (اليل) D أعلى بكثير لدى مرضى السكري من النوع الثانى الذين يعانون من الاعتلال الكلوي السكري بالمقارنة بمرضى السكري دون اعتلال الكلى. فيما يتعلق بتعدد الأشكال الجينية rs7903146 CT لجين TCF7L2، ارتبط النمط الجيني المتحور TT و T أليل بشكل معنوى ذو دلالة احصائية مع مرضى الاعتلال الكلوي السكري بالمقارنة مع مرضى السكري من دون اعتلال الكلى. وفيما يتعلق بتعدد الأشكال الجينية Gly482Ser و Thr394Thr في جين PPARGC1A، أظهرت النتائج بوضوح وجود علاقة إيجابية ذات دلالة إحصائية بين أليل A المتحور من Gly482Ser والمرضى الذين يعانون من الاعتلال الكلوي السكري مقارنة بالمرضى المصابين بالسكري دون اعتلال الكلى. من ناحية أخرى، فشلت النتائج فى أن تظهر أية تغييرات يمكن اكتشافها في التعدد الجيني في Thr394Thr داخل جين PPARGC1A بين المجموعات التي تم دراستها.

في الختام، تم الكشف عن وجود علاقة بين التعدد فى الأشكال الجينية منفردة أو مجتمعة ACE ID، TCF7L2 rs7903146 CT، وPPARGC1A Gly482Ser GA مع التعرض لاعتلال الكلى في مرضى السكري من النوع الثاني.