ORIGINAL RESEARCH

The Human Genetic Variants CYP2J2 rs2280275 and EPHX2 rs751141 and Risk of Diabetic Nephropathy in Egyptian Type 2 Diabetic Patients

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Background: Diabetic nephropathy (DN), the primary driver of end-stage kidney disease, is a problem with serious consequences for society's health. Single nucleotide polymorphisms (SNPs) can define differences in susceptibility to DN and aid in development of personalized treatment. Giving the importance of epoxyeicosatrienoic acids (EETs) in kidney health, we aimed to study the association between two SNPs in the genes controlling synthesis and degradation of EETs *(CYP2J2* rs2280275 and *EPHX2* rs751141 respectively) and susceptibility of type 2 diabetes mellitus (T2DM) patients to develop DN.

Patients and Methods: Two hundred subjects were enrolled and categorized into three groups: group I (80 T2DM patients with DN), group II (60 T2DM patients without DN) and group III (60 healthy controls). Urea, creatinine, albumin/creatinine ratio (ACR), and eGFR were measured for all participants. Genotyping of *CYP2J2* rs2280275 and *EPHX2* rs751141 was done by real time PCR.

Results: There was no significant difference between the studied groups regarding *CYP2J2* rs2280275. In contrast, *EPHX2* rs751141 was associated with increased risk of DN under a dominant model (GG vs GA+AA: OR=0.375; 95% CI (0.19–0.75), P=0.006) in unadjusted model and after adjustment for age and sex (OR=0.440; 95% CI (0.21–0.92), P=0.029), recessive model (AA vs GG+GA: OR=0.195; 95% CI (0.05–0.74), P=0.017) and additive model (GA vs GG+AA): OR=0.195; 95% CI (0.05–0.74), P=0.017).

Conclusion: *CYP2J2* rs2280275 was not associated with DN predisposition. However, *EPHX2* rs751141 could be a genetic marker for development and progression of DN among Egyptian T2DM patients.

Keywords: diabetic nephropathy, *CYP2J2*, *EPHX2*, real time PCR

Introduction

Diabetic nephropathy (DN), one of the microvascular complications of diabetes mellitus (DM), is the primary single driver of end-stage kidney disease.[1] It is characterized by accumulation of advanced glycation end products and growth factors, aside from hemodynamic and hormonal disturbances, which contribute to proteinuria, hypertension, and progressive deterioration of glomerular filtration.[2] Not all diabetics develop DN, suggesting that individual genetic differences may be implicated in incidence and progression of DN. However, the total genetic profile remains elusive.[3]

Epoxyeicosatrienoic acids (EETs) have multiple biological activities in the cardiovascular and renal systems.[4] Renal EETs have anti-inflammatory,

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anti-apoptotic, proangiogenic, fibrinolytic and anti-hypertensive roles.[5] Therefore, EETs are often termed renoprotective agents.[6] EETs are formed from arachidonic acid by the action of cytochrome P450 (CYP) epoxygenase enzymes. The main EETs producers are CYP2C and CYP2J subfamilies[7] which show higher expression in the proximal tubules of the kidney.[8] CYP2J subfamily contains only one member termed CYP2J2.[9] Experimentally, over expression of CYP2J2 has been considered a safeguarding factor against kidney damage.[10] Genetic variations of *CYP2J2* gene affect its expression throughout the population.[11] One of them is the *CYP2J2* rs2280275 single nucleotide polymorphism (SNP) situated in the intronic region of *CYP2J2* gene. This SNP is in linkage disequilibrium with the promoter SNP rs890293 which results in reduced levels of CYP2J2 epoxygenase metabolites in vivo.[11,12]

Previous studies had documented the association of *CYP2J2* rs2280275 with cardiovascular diseases such as coronary heart diseases[13] and hypertension.[14]

Up to the present time, no studies have been conducted to analyze the implication of *CYP2J2* rs2280275 in susceptibility to DN.

The bioavailability of EETs depends not only on the rate of synthesis but also on the fact that they are quickly hydrolyzed to less active metabolites by soluble epoxide hydrolase (sEH) enzyme.[15] sEH is a cytosolic enzyme that is expressed in many tissues, including kidney and liver.[7] In many experimental studies, it was found that the inhibition of sEH had a renoprotective effect.[16,17] sEH is encoded by *EPHX2* gene.[18] A common SNP in the EPHX2 gene is the functional rs751141 G/A polymorphism. The variant Aallele of that SNP results in an sEH enzyme with 25–58% inadequate catalytic activity.[19]

The role of *EPHX2* polymorphisms in cardiovascular diseases has gained attention in many studies.[20] However, a limited number of studies have examined this SNP in kidney disorders,[6,21,22] with only one study performed in diabetic kidney disease.[23]

Considering the value of EETs in the kidney, we speculated that sequence variations within *CYP2J2* and *EPHX2* might have an impact on DN predisposition. In view of this, we designed this study to investigate the association of *CYP2J2* rs2280275 and *EPHX2* rs751141 with DN in Egyptian T2DM patients in relation to kidney function parameters.

Materials and Methods
Participants and Data Collection

This study was completed by collaboration between Medical Biochemistry and Molecular Biology, Internal Medicine and Clinical Pathology Departments; Faculty of Medicine, Menoufia University, Egypt in the period from July 2018 to June 2019. This study included 140 T2DM patients (80 patients with DN [group I] and 60 patients without DN [group II]) and 60 apparently healthy control subjects [group III]. Patients were selected from the nephrology unit at the Internal Medicine Department, Menoufia University Hospital with history of DN present for more than 3 months (group I) with a decreased eGFR< 60 mL/min/1.73 m^2 and albumin/creatinine ratio (ACR) > 30 mg/g. Diagnosis of T2DM was based on the recommendations of the American Diabetes Association; fasting blood glucose ≥ 126 mg/dL or 2 h blood glucose after overload with 75 g of glucose ≥ 200 mg/dL in oral glucose tolerance test or glycated hemoglobin (HbA1c) ≥ 6.5% in patients with classic symptoms of hyperglycemia (American Diabetes Association, 2015).[24] Group II included 60 T2DM patients without DN with ACR < 30 mg/g. Hypertensive patients were known to have long-standing hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg in at least two separate measurements or regular use of antihypertensive medication).[25] Exclusion criteria for both groups included other primary or secondary renal diseases, malignancy, hepatic or cardiac failure and severe infections.

This study was conducted in accordance with the Declaration of Helsinki.

An informed written consent was obtained from each participant approved by the Ethical Committee of Medical Research, Faculty of Medicine, Menoufia University.

Thorough medical and demographic histories, family history of DM, duration of DM, BMI [calculated as weight (kg)/height (m^2)], smoking, hypertension, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), 2 h postprandial glucose (2h PPG), glycated hemoglobin (HbA1c), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TGs), serum urea, serum creatinine, albumin/creatinine (ACR) and eGFR were obtained for all participants.

Sampling and Biochemical Measurements

Two types of samples were taken from each participant; blood and urine samples.

Blood Sampling

Ten milliliters of venous blood were withdrawn after overnight fasting. Two milliliters of blood were placed in a sodium fluoride containing tube for measurement of blood glucose profile (FBG and 2 h PPG) by glucose oxidase method (Spinreact diagnostics kit, Girona, Spain)[26] and HbA1c by quantitative colorimetric measurement kits as a percentage of total hemoglobin supplied by Techo Diagnostics, Lakeview Ave, Anaheim, CA, USA.[27] Four milliliters of blood were placed in a plain tube, allowed to clot at room temperature, and centrifuged for 15 min at 4000 rpm. The separated sera were used for measurement of lipid panel (TC, HDL-C, TGs) using the standard enzymatic colorimetric kits (Spinreact diagnostics kit, Spain)[28] and renal function tests (serum urea and creatinine) using the standard enzymatic colorimetric kits (DIAMOND diagnostics kits, Germany). Friedewal formula was used to calculate LDL-C.[29] The eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) formula from serum creatinine.[30] Four milliliters of blood were placed in EDTA tube for DNA extraction and genotyping of *CYP2J2* rs2280275 and *EPHX2* rs751141.

Urine Sampling

Fifteen milliliters of fresh morning urine samples were collected from every subject in sterile containers for the measurement of creatinine and albumin for calculation of ACR. Urinary albumin was estimated by a solid-phase enzyme-linked immunosorbent assay kit (DRG International Inc., USA; cat # EIA-2361).

Molecular Analysis and Genotyping Assay

DNA extraction from whole blood was performed by GeneJETGenomic DNA Purification Kit (Thermo Scientific, Lithuania) and quantified by a spectrophotometer.

Genotyping of CYP2J2 Rs2280275 and EPHX2 Rs751141

Genotyping of *CYP2J2* Rs2280275 and *EPHX2* Rs751141 was done by real-time PCR with allele discrimination using TaqMan® SNP Genotyping assay kit on Applied Biosystems 7500 real-time PCR with software version 2.0.1 (Applied Biosystems, Foster City, CA). During TaqMan SNP Genotyping, DNA polymerase from the TaqMan Master Mix amplifies target DNA using sequence specific primers. The fluorescence signals for allelic discrimination were provided by two TaqManfluorogenic probes labeled with VIC/FAM fluorescent dyes. The probe sequences were;

CCTGCTCTGGCCCAGGCAGGTTACC[A/G]GGTCCTA GCTATGGACATGAAAGGC for *EPHX2* rs751141, and TCTCTTTCCTCCAATGTCTAGTACA[C/T]GGCACCCC ACTCCCCATGGTAGGTT for *CYP2J2* rs2280275 (Applied Biosystems, USA). The total volume of PCR amplification (20 μL) consisted of: 10 μL of 2x TaqMan Genotyping Master Mix (Thermo Scientific), 1.25 μL of 20x SNP assay, 3.75 μL of nuclease free water, and 5 μL of template DNA. DNase-free water (negative control) was included in each assay run. The PCR cycling conditions were initial denaturation for 10 min at 95°C, followed by 50 cycles with denaturation of 15 sec at 94° C and 60 sec at 60°C for annealing and extension. The Applied Biosystems 7500 real-time PCR analyzer measures the fluorescence signal (Rn) from each well of the reading plate. The plotted fluorescence signals indicate which alleles are in each sample and then convert allele calls to genotypes.

Statistics

The data were analyzed by SPSS version 20 (SPSS Inc. Released 2011. IBM SPSS statistics for windows, version 20.0, IBM Corp, Armonk, NY). The Kolmogorov–Smirnov test was used to verify the normality of distribution of variables. Quantitative data were described using mean, standard deviation (SD), and median. Comparisons between groups for categorical variables were assessed using Chi-squared test (Fisher or Monte Carlo). Student's *t*-test was used to compare two groups for normally distributed quantitative variables while ANOVA was used for comparing the four studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. Kruskal Wallis test was used to compare different groups for abnormally distributed quantitative variables and followed by Post Hoc test (Dunn's for multiple comparisons test) for pairwise comparison. Mann Whitney test was used to compare between two groups for not normally distributed quantitative variables. Odds ratio (OR) was done using Logistic regression analysis and used to calculate the ratio of the odds and 95% Confidence Interval of an event occurring in one risk group to the odds of it occurring in the non-risk group. Significance of the obtained results was judged at the 5% level.

Results
Demographic, Clinical and Biochemical Composition of the Participants

These results were demonstrated in Table 1. The three studied groups were matched regarding age (p=0.598), with mean age of 58.3 ± 8.5 years in DN patients. The

Table I Demographic, Clinical, and Biochemical Parameters

	Group I (n = 80)	Group II (n = 60)	Group III (n=60)	Test of Sig.	p
Sex n(%)				χ^2=17.962*	<0.001*
Male	62 (77.5%)	28(46.7%)	29 (48.3%)		
Female	18 (22.5%)	32(53.3%)	31 (51.7%)		
Age (years)				F=0.515	0.598
Mean ± SD	58.3±8.5	57.1±9.3	58.4± 7.4		
Family history of DM n (%)	70 (87.5%)	45 (75%)	7 (11.7%)	χ^2=89.940*	<0.001*
Duration of DM (years)				t=1.806	0.074
Mean ± SD	13.4 ± 3.9	15 ± 5.7	–		
BMI (kg/m^2)				F=28.781*	<0.001*
Mean ± SD	25.3 ± 2.1	25.7 ± 1.7	23.3 ± 1.9		
Sig. bet. grps.	p_1=0.328,p_2<0.001*,p_3<0.001*				
Current smokers (%)	34 (42.5%)	30 (50%)	15 (25%)	χ^2= 8.348*	0.015*
History of hypertension (%)	45 (56.3%)	17 (28.3%)	–	χ^2=10.830*	0.001*
SBP (mmHg)				F=21.445*	<0.001*
Mean ± SD	127.9 ± 8.5	122.2 ± 10.9	118.1 ± 7		
Sig. bet. grps.	p_1=0.001*,p_2<0.001*,p_3=0.030*				
DBP (mmHg)				F=4.709*	0.010*
Mean ± SD	79.3 ± 6.5	76.7 ± 7.7	76.3 ± 5		
Sig. bet. grps.	p_1=0.046*,p_2=0.017*,p_3=0.939				
FBG (mg/dl)				F=94.853*	<0.001*
Mean ± SD	196.1 ± 69.3	203.5 ± 62.5	80.8 ± 8.7		
Sig. bet. grps.	p_1=0.720,p_2<0.001*,p_3<0.001*				
2 h PPG (mg/dl)				F=285.846*	<0.001*
Mean ± SD	311.9 ± 77.2	286.7 ± 54	90.8 ± 9.3		
Sig. bet. grps.	p_1=0.028*,p_2<0.001*,p_3<0.001*				
HbAIc (%)				F=102.004*	<0.001*
Mean ± SD	8.5 ± 1.9	8.8 ± 2.3	4.8 ± 0.3		
Sig. bet. grps.	p_1=0.644,p_2<0.001*,p_3<0.001*				
	Group I (n = 80)	**Group II (n = 60)**	**Group III (n=60)**	**Test of Sig.**	**P**
Total cholesterol (mg/dl)				F=154.058*	<0.001*
Mean ± SD.	256.4 ± 18	205.7 ± 39.2	170.7 ± 29.1		
Sig. bet. grps	p_1<0.001*,p_2<0.001*,p_3<0.001*				
HDL-C (mg/dl)				F=0.660	0.518
Mean ± SD.	49.2 ± 10.3	47.8 ± 10	47.3 ± 9.6		
LDL-C (mg/dl)				F=57.257*	<0.001*
Mean ± SD.	136.1 ± 30.9	129.2 ± 25.3	93.4 ± 8.1		
Sig. bet. grps	p_1=0.223,p_2<0.001*,p_3<0.001*				

(*Continued*)

Table 1 (Continued).

	Group I (n = 80)	Group II (n = 60)	Group III (n=60)	Test of Sig.	p
Triglycerides (mg/dl) Mean ± SD.	143.6 ± 45.7	119.7 ± 25.6	89.7 ± 6	F=47.729*	<0.001*
Sig. bet. grps	p_1<0.001*,p_2<0.001*,p_3<0.001*				
Serum creatinine (mg/dl) Mean ± SD.	2.27 ± 0.47	0.90 ± 0.14	0.83 ± 0.18	F=464.784*	<0.001*
Sig. bet. grps	p_1<0.001*,p_2<0.001*,p_3=0.379				
Serum urea (mg/dl) Mean ± SD.	68.1 ± 15.2	23.6 ± 3.1	22.4 ± 3.6	F=494.171*	<0.001*
Sig. bet. grps	p_1<0.001*,p_2<0.001*,p_3=0.807				
ACR (mg/g) Median (IQR)	825.0(400.0–869.5)	20.0(17.5–22.0)	18.0(15. 0–21.0)	H=144.85*	<0.001*
Sig. bet. grps	p_1<0.001*,p_2<0.001*,p_3=0.247				
eGFR (mL/min/1.73m^2) Mean ± SD.	57.31 ± 8.75	89.70 ± 12.81	90.28 ± 16.03	F=163.470*	<0.001*
Sig. bet. grps	p_1<0.001*,p_2<0.001*,p_3=0.965				

Notes: Group I: Diabetic nephropathy, Group II: Type 2 diabetics without diabetic nephropathy, Group III: Control, χ^2: Chi squared test, F: ANOVA test, H: Kruskal Wallis test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) *Statistically significant at p ≤ 0.05.
Abbreviations: DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; 2 h PPG, 2 hour postprandial glucose; HbA1c, glycated hemoglobin, p_1, p value for comparing between group I and group II; p_2, p value for comparing between group I and group III; p_3, p value for comparing between group II and group III.

percentage of males was 77.5%, 46.7% and 48.3% in group I, group II and group III respectively (p < 0.001). There was a significant difference between the three studied groups regarding family history of DM (p < 0.001). When comparing both group I and group II with group III, significantly higher values of BMI (p=0.001), FBG (P=0.001), HbA1c (p=0.001) and LDL-C (p=0.001) were found in group I and group II. There was a higher incidence of hypertension in group I than in group II (p < 0.001). All variables such as smoking, SBP, 2 h PPG, total cholesterol and triglycerides differed significantly between the three groups. When comparing group I and group II, there was significantly higher DBP in group I (p=0.046), and no significant difference regarding duration of DM (p=0.074), BMI (p=0.328), FBG (p=0.720), HbA1c (p=0.644) and LDL-C (p=0.223). In group I, there was significantly higher DBP (p=0.017) when compared to group III, while DBP did not significantly differ between group II and group III (p=0.939). Regarding kidney function tests, there were significantly higher urea, creatinine, and ACR in group I compared to group II and group III (p=0.001), with no significant difference between group II

and group III (p=0.379, 0.807, 0.247 respectively). The eGFR showed significantly lower values in group I compared to group II (p=0.001) and group III (p=0.001), with no significant difference between group II and group III (p=0.965).

The Association of EPHX2 Rs751141 and CYP2J2 Rs2280275 with the Risk of DN

Both polymorphisms were in accordance with Hardy-Weinberg equilibrium (p>0.05). Talking about *EPHX2* rs751141 results, there were significant differences in genotype and allele distribution (p=0.005 and p < 0.001 respectively) between the three studied groups. In group I, there were significantly higher and lower frequencies of GG and AA genotypes respectively (Table 2). Based on the data, the *EPHX2* rs751141 was associated with an increased risk of DN under a dominant model (GG vs GA+AA: OR=0.375; 95% CI (0.19–0.75), P=0.006) in unadjusted model and after adjustment for age and sex (OR=0.440; 95% CI (0.21–0.92), P=0.029), recessive model (AA vs GG+GA: OR=0.195; 95% CI (0.05–0.74), P=0.017), and additive model (GA vs GG+AA):

Table 2 Prevalence of *EPHX2* (Rs751,141) and *CYP2J2* (Rs2,280,275) Genotypes and Alleles in the Studied Groups

	Group I (n = 80)	Group II (n = 60)	Group III (n = 60)	χ^x	^{MC}p
EPHX2 rs751141					
GG[®]	56(70%)	28(46.7%)	26(43.3%)	$\chi\chi\chi\chi\chi x^*$	0.005*
GA	21(26.3%)	22(36.7%)	23(38.3%)		
AA	3(3.8%)	10(16.7%)	11(18.3%)		
Sig. bet. grps.	χ^x_1=10.483*, p$_1$=0.005*, χ^x_χ=13.047*,p$_2$=0.001*, χ^x_χ=0.144,p$_3$=0.931				
Allele frequency					
G[®]	133(83.1%)	78(65%)	75(62.5%)	$\chi7\chi869^*$	<0.001*
A	27(16.9%)	42(35%)	45(37.5%)		
Sig. bet. grps.	χ^x_1=12.131, p$_1$<0.001*, χ^x_χ=15.270*,p$_2$<0.001*, χ^x_χ=0.162,p$_3$=0.687				
Dominant					
GG [®]	56(70%)	28(46.7%)	26(43.3%)	$\chi\chi\chi\chi\chi6^*$	0.002*
GA+AA	24(30%)	32(53.3)	34(56.7%)		
Recessive					
AA[®]	3(3.8%)	10(16.7%)	11(18.3%)	$8\chi67\chi^*$	0.013*
GG+GA	77(96.3%)	50(83.3%)	49(81.7%)		
Additive					
AA vs GA	3/21(14.3%)	10/22(45.5%)	11/23(47.8%)	3.369	0.186
GG vs GA	56/21(37.5%)	28/22(78.6%)	26/23(88.5%)	$6\chi\chi\chi x^*$	0.045*
GA vs GG+AA	3/77(3.9%)	10/50(20%)	11/49(22.4%)	$8\chi67\chi^*$	0.013*
	Group I (n = 80)	**Group II (n = 60)**	**Group III (n = 60)**	χ^x	**P**
CYP2J2 rs 2280275					
CC[®]	60(75%)	47(78.3%)	46(76.7%)	$\chi\chi4\chi\chi$	^{MC}p= 0.997
CT	17(21.3%)	11(18.3%)	12(20%)		
TT	3(3.8%)	2(3.3%)	2(3.3%)		
Sig. bet. grps.	χ^x_1=0.298, p$_1$=0.938, χ^x_χ=0.152,p$_2$=1.000, χ^x_χ=0.199,p$_3$=1.000				
Allele frequency					
C[®]	137(85.6%)	105(87.5%)	104(86.7%)	$\chi\chi\chi\chi\chi$	0.900
T	23(14.4%)	15(12.5%)	16(13.3%)		
Sig. bet. grps.	χ^x_1=0.206, p$_1$=0.650, χ^x_χ=0.062,p$_2$=0.803, χ^x_χ=0.037,p$_3$=0.847				
Dominant					
CC	60(75%)	47(78.3%)	46(76.7%)	0.213	0.899
CT+TT	20(25%)	13(21.7%)	14(23.3%)		
Recessive					
TT	3(3.8%)	2(3.3%)	2(3.3%)	0.194	1.000
CT+TT	77(96.3%)	58(96.7%)	58(96.7%)		
Additive					
CT	17(21.3%)	11(18.3%)	12(20%)	0.182	0.913
CC+TT	63(78.8%)	49(81.7%)	48(80%)		

Note: *Statistically significant at p ≤ 0.05
Abbreviations: [®], the reference genotype; MC, Monte Carlo; p$_1$, p value for comparing between group I and group II; p$_2$, p value for comparing betweengroup I and group III; p$_3$, p value for comparing between group II and group III; χ^x_1, Chi squared value for comparing between group I and group II; χ^x_χ, Chi squared value for comparing between group I and group III; χ^x_χ, Chi squared value for comparing between group II and group III.

OR=0.195; 95% CI (0.05–0.74), P=0.017) (Table 3). Regarding allele distribution, the significantly decreased frequency of the A allele in group I (16.9% Table 2) increased risk of DN (OR=0.377; 95% CI (0.22–0.66), P=0.001) which remained significant after adjustment for age and sex (OR=0.424; 95% CI (0.24–0.76), P=0.004)

Table 3 Risk Assessment of Diabetic Nephropathy Under the Three Genetic Models for *EPHX2* Rs751141 and *CYP2J2* Rs2280275

	Unadjusted		Adjusted[a]		Adjusted [b]	
	OR (95% CI)	P	OR (95% CI)	p	OR (95% CI)	p
***EPHX2*rs751141**						
Dominant (GG[®]vs GA+ AA)	0.375(0.19–0.75)	0.006*	0.440(0.21–0.92)	0.029*	0.681(0.16–2.82)	0.596
Recessive(AA vs GG+GA[®])	0.195(0.05–0.74)	0.017*	0.296 (0.07–1.21)	0.091	0.284(0.02–3.77)	0.340
Additive (GA vs GG + AA[®])	0.195(0.05–0.74)	0.017*	0.296 (0.07–1.21)	0.091	0.284(0.02–3.77)	0.340
GG[®] versus GA	0.477(0.23–1.01)	0.053	0.512(0.23–1.12)	0.095	0.832(0.18–3.87)	0.815
GG[®] versus AA	0.150(0.04–0.59)	0.007*	0.165(0.04–0.74)	0.019*	0.112(0.005–2.28)	0.154
Allele (G[®] versus A)	0.377(0.22–0.66)	0.001*	0.424(0.24–0.76)	0.004*	0.447(0.18–1.13)	0.090
***CYP2J2*rs2280275**						
Dominant (CC[®]vs CT+TT)	1.205(0.54–2.67)	0.646	1.160(0.50–2.68)	0.728	0.310(0.05–1.97)	0.215
Recessive(TT vs CT+TT [®])	1.130(0.18–6.98)	0.895	0.834(0.12–5.61)	0.852	1.700(0–67,518.5)	0.922
Additive (CT vs CC+TT [®])	1.202(0.52–2.80)	0.670	1.228(0.50–2.99)	0.651	0.302(0.05–1.92)	0.205
CC[®] versus CT	1.211(0.52–2.83)	0.659	1.221(0.50–2.99)	0.662	0.302(0.05–1.93)	0.206
CC[®] versus TT	1.180(0.19–7.32)	0.863	0.869(0.13–5.91)	0.886	1.358(0–309,284.7)	0.961
Allele (C[®] versus T)	1.175(0.59–2.36)	0.651	1.164(0.56–2.42)	0.684	0.450(0.10–2.14)	0.315

Notes: [a]Adjusted for age and sex, [b]adjusted for age, sex, BMI, duration of diabetes, hypertension, smoking, total cholesterol, and triglycerides levels. *Statistically significant at p ≤ 0.05.
Abbreviations: CI, confidence interval; OR, odds ratio; [®], the reference genotype.

(Table 3). The genotype GA decreased risk of DN (OR=0.477; 95% CI (0.23–1.01), P=0.053), while AA genotype caused more decreased risk (OR=0.150; 95% CI (0.04–0.59), P=0.007) and (OR=0.165; 95% CI (0.04–0.74), P = 0.019) after adjustment for age and sex (Table 3).

The results indicated that the *EPHX2* rs751141 might have a significant association with DN risk. However, in the current study, there was no significant association between *CYP2J2* rs2280275 and DN risk. The genotypes and allele frequencies of *CYP2J2* rs2280275 did not differ significantly between the three studied groups (p = 0.997 and p = 0.900 respectively). Genotype frequencies of *CYP2J2* rs2280275 CC, CT, and TT were 75%, 21.3%, and 3.8% in group I, 78.3%, 18.3%, and 3.3% in group II, and 76.7%, 20%, and 3.3% in group III respectively (Table 2).

Meanwhile, we statistically analyzed the association of *EPHX2* rs751141 genotypes and different parameters in DN group. Due to the small number of homozygous variant carriers (AA), both homozygous (AA) and heterozygous (GA) variants were gathered in one group for statistical reasons. (GA+AA) patients showed significantly lower values of BMI (p=0.031), serum creatinine (p < 0.001), ACR (p < 0.001) and a significantly higher eGFR (p < 0.001). However, no significant difference was observed between GG patients and (GA+AA) patients regarding age, sex, duration of DM, smoking, hypertension, SBP, DBP, FBG, 2h PPG, HbA1c, lipid panel and serum urea (Table 4).

Analysis of the relation of *EPHX2* rs751141 with clinical and biochemical parameters within T2DM without DN (group II), revealed significant differences regarding age (p=0.023), sex (p=0.041), duration of diabetes (p=0.012), and BMI (p=0.003), while other parameters showed non-significant differences (Table 5).

Regarding *CYP2J2* rs2280275, diabetic nephropathy patients with CC genotypes exhibited significantly higher values of creatinine (p=0.006), higher ACR (p=0.003) and lower eGFR (p<0.001) in comparison to patients with CT +TT genotypes (Table 6). Within T2DM without DN (group II), there were significant differences between CC patients and CT+TT patients regarding age (p=0.049), duration of diabetes (p=0.007), BMI (p=0.001), smoking (p=0.028) and total cholesterol (p=0.002) (Table 7).

Discussion

The restricted EETs' production in the vascular endothelial and renal epithelial cells lines up with the natural activities and commitment of EETs toward cardiovascular and renal health.[31,32] So, genetic variations in genes controlling synthesis and degradation of EETs may affect renal functions.

The present study observed significant association of *EPHX2* rs751141 with DN among T2DM patients. We found significantly higher frequencies of GG genotype

Table 4 Association of *EPHX2* (Rs751141) Genotypes with Different Parameters in DN Patients

	EPHX2 rs751141		Test of Sig.	p
	GG (n= 56)	**GA+AA (n= 24)**		
Sex n (%)				
Male	44(78.6%)	18(75%)	χ^2= 0.123	0.726
Female	12(21.4%)	6(25%)		
Age (years)				
Mean± SD.	58.9 ± 7.5	57 ± 10.6	t=0.804	0.427
Family history of DM n (%)	48(85.7%)	20(83.3%)	χ^2=89.940*	<0.001*
Duration of DM (years)				
Mean ± SD.	13.6 ± 3.6	13 ± 4.5	t=0.598	0.552
BMI (kg/m^2)				
Mean ± SD.	25.6 ± 2.1	24.5 ± 1.8	t=2.191*	0.031*
Current smokers (%)	26(46.4%)	8(33.3%)		
History of hypertension (%)	29(51.8%)	16(66.7%)	χ^2=1.512	0.219
SBP (mmHg)				
Mean ± SD.	127.7 ± 8	128.3 ± 9.6	t=0.266	0.791
DBP (mmHg)				
Mean ± SD.	79.5 ± 6.3	78.8 ±7	t=0.495	0.622
FBG (mg/dl)				
Mean ±SD.	196.4 ± 66.6	195.4 ± 76.8	t=0.063	0.950
2 h PPG (mg/dl)				
Mean ± SD.	314.8 ± 72.3	305.2 ± 88.7	t=0.471	0.640
HbA1c (%)				
Mean ± SD.	8.6 ± 1.7	8.4 ± 2.2	t=0.344	0.732
Total cholesterol (mg/dl)				
Mean ± SD.	256 ± 17.5	257.3 ± 19.6	t=0.305	0.761
HDL-C (mg/dl)				
Mean ± SD.	49.4 ± 10.8	48.6 ± 9.3	t=0.326	0.745
LDL-C (mg/dl)				
Mean ± SD.	134.7 ± 32.6	139.4 ± 26.8	t=0.616	0.539
Triglycerides (mg/dl)				
Mean ± SD.	149.1 ± 48.6	130.8 ± 35.6	t=1.656	0.102
Serum creatinine (mg/dl)				
Mean ± SD.	2.5 ± 0.4	1.8 ± 0.2	t=10.283	<0.001*
Serum urea (mg/dl)				
Mean ± SD.	69 ± 15	66.1 ± 15.7	t=0.791	0.431
ACR (mg/g)				
Median (IQR)	852.5(821–911)	346.5(302–391)	U=0.0*	<0.001*
eGFR (mL/min/1.73m^2)				

Notes: Mean ± SD. 52.2 ± 4.5 69.1 ± 2.5 t=21.445* <0.001*, *statistically significant at p ≤ 0.05.
Abbreviations: U, Mann Whitney test; t, Student's *t*-test; χ^2, Chi squared test.

Table 5 Association of *EPHX2* Rs751141 Genotypes with Different Parameters in T2DM Patients Without Nephropathy

	EPHX2 rs 751141		Test of Sig.	p
	GG (n= 28)	GA+AA (n= 32)		
Sex Male Female	17(60.7%) 11(39.3%)	11(34.4%) 21(65.6%)	χ^2= 4.163*	0.041*
Age (years) Mean ± SD.	54.1 ± 8.9	59.6 ± 9.1	t=2.338*	0.023*
Family history of DM	18(64.3%)	27(84.4%)	χ^2=3.214	0.073
Duration of DM (years) Mean ± SD.	13.0 ± 4.9	16.7 ± 5.9	t=2.583*	0.012*
BMI (kg/m^2) Mean ± SD.	25.0 ± 1.6	26.3 ± 1.6	t=3.150*	0.003*
Current smokers (%) **History of hypertension (%)**	11(39.3%) 9(32.1%)	19(59.4%) 8(25%)	χ^2= 2.411 χ^2=0.375	0.121 0.540
SBP (mmHg) Mean ± SD.	121.5 ± 12.7	122.8 ± 9.2	t=0.442	0.660
DBP (mmHg) Mean ± SD.	75.1 ± 8.4	78.0 ± 6.8	t=1.439	0.155
FBG (mg/dl) Mean ± SD.	206.5 ± 60.5	200.8 ± 65.1	t=0.349	0.728
2hr PPG (mg/dl) Mean ± SD.	292.4 ± 60.1	281.6 ± 48.6	t=0.767	0.446
HbA1c (%) Mean ± SD.	9.1 ± 2.4	8.5 ± 2.2	t=0.901	0.371
Total cholesterol (mg/dl) Mean ± SD.	200.1 ± 41.7	210.5 ± 36.8	t=1.022	0.311
HDL-C (mg/dl) Mean ± SD.	49.4 ± 9.6	46.4 ± 10.2	t=1.187	0.240
LDL-C (mg/dl) Mean ± SD.	123.2 ± 25.7	134.4 ± 24.2	t=1.740	0.087
Triglycerides (mg/dl) Mean ± SD.	116.6 ± 27.2	122.4 ± 24.2	t=0.874	0.386
Serum creatinine (mg/dl) Mean ± SD.	0.9 ± 0.1	0.9 ± 0.2	t=0.515	0.609
Serum urea (mg/dl) Mean ± SD.	23.9 ± 3.5	23.2 ± 2.8	t=0.872	0.387
ACR (mg/g) Median (IQR)	19.5(17.5–22.5)	20(17.5–21.5)	U=408.5	0.557
eGFR (mL/min/1.73m2) Mean ± SD.	88.6 ± 15.5	90.7 ± 10.1	t=0.598	0.553

Note: *Statistically significant at p ≤ 0.05.
Abbreviations: U, Mann Whitney test; t, Student's *t*-test; χ^2, Chi squared test.

Table 6 Relation Between *CYP2J2* Rs2280275 and Different Parameters in DN

	CYP2J2rs2280275		Test of Sig.	p
	CC (n= 60)	**CT+TT (n= 20)**		
Sex n (%)				
Male	45(75%)	17(85%)	χ^2=0.860	0.538
Female	15(25%)	3(15%)		
Age (years)				
Mean ± SD.	59 ± 7.3	56.3 ± 11.3	t=1.004	0.325
Family history of DM n (%)	51(85%)	19(95%)	χ^2=1.371	0.437
Duration of DM (years)				
Mean ± SD.	13.7 ± 3.5	12.5 ± 4.9	t=1.262	0.211
BMI (kg/m^2)				
Mean ± SD.	25.4 ± 2	24.7 ± 2.1	t=1.457	0.149
Current smokers (%)	26(43.3%)	8(40%)	χ^2= 0.068	0.794
History of hypertension (%)	32(53.3%)	13(65%)	χ^2=0.830	0.362
SBP (mmHg)				
Mean ± SD.	128.2 ± 8.4	127 ± 8.7	t=0.554	0.581
DBP (mmHg)				
Mean ± SD.	79.6 ± 6.5	78.5 ± 6.5	t=0.635	0.527
FBG (mg/dl)				
Mean ± SD.	193.1 ± 65.8	205.4 ± 80	t=0.685	0.495
2hr PPG (mg/dl)				
Mean ± SD.	307.9 ± 73.8	324.1 ± 87.5	t=0.812	0.419
HbA1c (%)				
Mean ± SD.	8.4 ± 1.6	9 ± 2.4	t=1.176	0.732
Total cholesterol (mg/dl)				
Mean ± SD.	254.9 ± 18.3	261 ± 16.8	t=1.313	0.193
HDL-C (mg/dl)				
Mean ± SD.	50 ± 10.7	46.7 ± 9	t=1.260	0.211
LDL-C (mg/dl)				
Mean ± SD.	134.4 ± 32	141.2 ± 27.3	t=0.841	0.403
Triglycerides (mg/dl)				
Mean ± SD.	141.9 ± 46.5	148.8 ± 44	t=0.578	0.565
Serum creatinine (mg/dl)				
Mean ± SD.	2.4 ± 0.5	2 ± 0.4	t=2.826*	0.006*
Serum urea (mg/dl)				
Mean ± SD.	67.8 ± 15.3	69.2 ± 15	t=0.343	0.733
ACR (mg/g)				
Median (IQR)	839(793–886.5)	390(330–806)	U=328.5*	0.003*
eGFR (mL/min/1.73m^2)				
Mean ± SD.	54.8 ± 7.5	64.8 ± 7.9	t=5.095*	<0.001*

Note: *Statistically significant at p ≤ 0.05.
Abbreviations: χ^2, Chi squared test; t, Student's *t*-test; U, Mann Whitney test.

Table 7 Association of *CYP2J2* Rs2280275 with Different Parameters in T2DM Patients Without Nephropathy

	CYP2J2 rs2280275		Test of Sig.	p
	CC (n= 47)	CT+TT (n= 13)		
Sex Male Female	23(48.9%) 24(51.1%)	5(38.5%) 8(61.5%)	χ^2= 0.449	0.503
Age (years) Mean ± SD.	55.8 ± 9.6	61.5 ± 7.1	t=2.007*	0.049*
Family history of DM (%)	35(74.5%)	10(76.9%)	χ^2=0.033	1.000
Duration of DM (years) Mean ± SD.	13.9 ± 5.5	18.7 ± 5.2	t=2.814*	0.007*
BMI (kg/m^2) Mean ± SD.	25.4 ± 1.8	26.7 ± 0.9	t=3.598*	0.001*
Current smokers (%) **History of hypertension (%)**	20(42.6%) 14(29.8%)	10(76.9%) 3(23.1%)	χ^2= 4.812* χ^2=0.226	0.028* 0.740
SBP (mmHg) Mean ± SD.	122.4 ± 11.8	121.5 ± 6.9	t=0.337	0.738
DBP (mmHg) Mean ± SD.	76.0 ± 8.2	78.8 ± 4.6	t=1.594	0.120
FBG (mg/dl) Mean ± SD.	205.7 ± 61.5	195.7 ± 68.1	t=0.505	0.615
2hr PPG (mg/dl) Mean ± SD.	287.2 ± 56.7	284.8 ± 45.2	t=0.141	0.889
HbA1c (%) Mean ± SD.	8.9 ± 2.2	8.4 ± 2.6	t=0.732	0.467
Total cholesterol (mg/dl) Mean ± SD.	200.0 ± 41.5	226.2 ± 18.7	t=3.228*	0.002*
HDL-C (mg/dl) Mean ± SD.	48.7 ± 10.2	44.5 ± 8.9	t=1.341	0.185
LDL-C (mg/dl) Mean ± SD.	128.7 ± 24.7	131.1 ± 28.7	t=0.299	0.766
Triglycerides (mg/dl) Mean ± SD.	118.0 ± 25.4	125.9 ± 26.4	t=0.986	0.328
Serum creatinine (mg/dl) Mean ± SD.	0.9 ± 0.1	0.9 ± 0.1	t=1.613	0.112
Serum urea (mg/dl) Mean ± SD.	23.7 ± 3.4	23.0 ± 2.1	t=0.925	0.362
ACR (mg/g) Median (IQR)	20(17–22)	19(18–21)	U=294.50	0.843
eGFR (mL/min/1.73m2) Mean ± SD.	88.6 ± 13.4	93.5 ± 10.1	t=1.226	0.225

Note: *Statistically significant at p ≤ 0.05.
Abbreviations: U, Mann Whitney test; t, Student's *t*-test; χ^2, Chi squared test.

and G allele and significantly lower frequencies of AA genotype and A allele in DN patients in comparison to T2DM without DN patients. The presence of the A allele reduced the odds of DN (OR=0.377). Such differences may indicate that carriers of the risk-free A allele are less susceptible to developing DN. This significant association was present under the three genetic models (dominant, recessive and additive).

The *EPHX2* rs751141 polymorphism involves substitution of A instead of G in exon 8 of the gene encoding sEH. This nucleotide substitution leads to amino acid substitution from arginine to glutamine at codon 287 (R287Q). The A allele results in a form of sEH enzyme with lower EETs hydrolytic capability resulting in higher levels of EETs.[33] Yi et al, documented lower EETs level in GG patients in comparison to GA and AA patients that was associated with development of early neurological deterioration in Chinese patients with acute minor ischemic stroke.[20] A previous study revealed that lower levels of EETs in glomeruli of the kidney are involved in glomerular deterioration in the early stage of DN.[34] Now it turns out that the renoprotective actions of persistently higher EETs levels in diabetic patients with the rs751141 A allele might be one of the reasons of being less susceptible to DN.[19]

Endothelial impairment is a hallmark of cardiovascular and renal diseases.[21] EETs maintain endothelial health.[22] So, *EPHX2* rs751141 might be implicated in progression of DN. As previously shown, the A allele will lead to slow degradation of EETs[19,35] and consequently, endothelial protection and resistance to glomerular injury. In line with this, our DN patients with the A allele showed significantly lower serum creatinine, lower ACR and higher eGFR in comparison to levels in GG patients. This means that the A allele might counteract DN progression and preserve, to some extent, kidney function among these patients.

In the following paragraphs, we will list the results of several studies that unquestionably support the protective effect of the A allele of EPHX2 rs751141 in vascular associated diseases.

Ma et al, in a study performed on Chinese T2DM patients, recorded significantly different distribution of *EPHX2* rs751141 genotypes and alleles between DM patients with or without DN, with lower frequency of the A allele (20.94%) in DN patients. They also demonstrated that the AA genotype decreased risk of DN by 73% after adjustment for established DN risk factors.[23]

The study done by Lee et al, revealed a significant effect of *EPHX2* rs751141 on progression of IgA nephropathy (IgAN) in Korean population. They noticed better kidney survival in IgAN patients with the A allele, remarkable kidney vascular damage in patients with the G allele and higher in vivo activity of sEH in patients carrying the G allele.[22] They assumed that the A allele decreased sEH activity in IgAN patients affecting intrarenal blood pressure preserving kidney function. Moreover, they found that patients with GG genotype showed significantly lower eGFR and higher systolic blood pressure than other groups (GA/AA).

Our data additionally bolsters the results of past reports that the A allele of *EPHX2* rs751141 confers significantly lower risk of ischemic stroke.[19,36] Donelly et al attributed the reason behind better outcomes in the A allele patients with aneurysmal subarachnoid hemorrhage to the fact that the *EPHX2 287*Gln variant is a missense SNP accompanied by diminished EETs degradation and ischemic cell death.[37]

Consistently, several experimental studies indicated that genetic inhibition of sEH protects the kidney against diabetic injury, as evident by decreased creatinine, blood urea nitrogen and urinary albumin excretion.[5,16,38]

In contrast, Lee et al, in their study on 204 kidney transplant donor-recipient population, found no significant association between allograft dysfunction and *EPHX2* rs751141 GG genotype. The explanation given was the lack of prolonged follow-up.[21] Moreover, Gervasini et al, stated that *EPHX2* rs751141 did not affect serum creatinine and eGFR in Caucasian renal transplant recipients and deceased donors.[6]

CYP2J2 is one of the key enzymes of epoxygenase pathway of AA.[9] The anti-hypoxic, anti-inflammatory, xenobiotic detoxification ability, and EETs' generation qualify CYP2J2 protein as a strong candidate for vascular protective lipid metabolizingepoxygenase.[39]

A previous study confirmed the significant association of *CYP2J2* rs2280275 with coronary artery disease in a male Uygur population in China, indicating its protective role in vascular endothelium.[13] Our results did not support what they found, as this study revealed no significant difference in genotype and allele distributions of *CYP2J2*rs2280275 between DN patients, T2DM patients, and healthy subjects. Based on this evidence, *CYP2J2* rs2280275 is probably not involved in genetic susceptibility to DN. However, the relatively small sample size, ethnic and geographic elements

176

The Application of Clinical Genetics 2020:13

may explain these findings, so further larger studies are needed.

It is worth noting that our study is the first in terms of examining the *CYP2J2* rs2280275 in DN. Moreover, the significant association of *EPHX2* rs751141 with creatinine, ACR and eGFR is a novel finding. However, there are some limitations. First, the relatively small sample size may result in lack of association between *CYP2J2* rs2280275 and DN. Second, this study did not determine the effect of other functional SNPs in *CYP2J2*. Thus, further studies on a larger set of SNPs could be constructed. Finally, extensive research is required to elucidate the mechanism of the *EPHX2* rs751141 A allele to preserve kidney function.

Conclusion

No association was found between *CYP2J2* rs2280275 and DN susceptibility among Egyptian T2DM patients. In contrast, our study revealed that the *EPHX2* rs751141 might be considered as one of the genetic determinants of DN. In fact, it has been shown that the *EPHX2* rs751141 A allele is linked not only to protection against DN, but also to reduced disease progression.

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Disclosure

The authors declare that they have no conflicts of interest.

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