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RESEARCH ARTICLE

Some genetic variations are associated with susceptibility to diabetic nephropathy in type 1 diabetes.

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Abstract

The increased prevalence of diabetes mellitus has caused a rise in the occurrence of its chronic complications, such as diabetic nephropathy (DN), which is associated with elevated morbidity and mortality. Studies have demonstrated that besides the known environmental risk factors, diabetic nephropathy has a major genetic component. Great efforts have been made to identify these main genes, but results are still inconsistent. The aim of this study was to investigate if some common single nucleotide polymorphisms associated with diabetic nephropathy in type 1 diabetes and to evaluate the effect of these single nucleotide polymorphisms on less severe stage of diabetic nephropathy, defined as microalbuminuria. In our study we did not found any association between the studied genes and diabetic nephropathy so further studies must be done in larger populations with different phenotypes of renal impairment to evaluate if these genes are associated with the development of diabetic nephropathy.

Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycemia resulting from defects in both insulin secretion and action. Depending on the intensity and time of exposure to hyperglycemia, structural lesions can occur in the vascular endothelium and neuronal tissue, leading to the onset of diabetic chronic complications and, ultimately, causing dysfunction and failure of several organs and tissues. These complications can be divided into microangiopathic and macroangiopathic, and they are the most common causes of morbidity and mortality in diabetic patients (American Diabetes Association,2009).Type 1 Diabetes (T1D) is mostly an autoimmune disease in which the immune system targets and destroys the insulin-producing β -cells found in the islets of Langerhans in the pancreas. Without insulin, individuals develop the clinical syndrome of T1D. T1D is characterized by autoantibody production and progressive infiltration of immune cells into the islets of the pancreas followed by the destruction of the islet cells. During the onset of T1D, cells from both the innate and adaptive immune systems infiltrate islet lesions to produce insulitis (Michael etal,2008).Diabetic nephropathy (DN) is characterized by physiopathological changes resulting from diabetes mellitus, which begins with glomerular hyperfiltration and kidney hypertrophy, and tend to progress to proteinuria and progressive glomerular filtration rate reduction. Hyperglycemia, elevated blood pressure levels and genetic predisposition are major risk factors for DN. In addition, elevated levels of serum lipids, smoking, and the amount and source of dietary protein also appear to be risk factors for developing DN(Gross etal,2005).Albuminuria can play a pathophysiological role in the progression of chronic kidney disease (Remuzzi etal,2006). Moreover, individuals with type 1 diabetes and even modest amounts of albuminuria have an increased risk of premature mortality (Groop etal,2009).ENPP1 (ecto-nucleotide pyrophosphatase/phosphodiesterase 1) is one of the five cell-membrane proteins containing an active extracellular site which catalyzes the release of nucleoside-

5-phosphatase from nucleotides and their products. These proteins consist of a short terminal NH2 intracellular domain, a single transmembrane domain, two somatomedin-B-like domains, and one COOH-terminal nuclease-like domain (Denise etal, 2011). ENPP1 is a 230-260 kDa homodimer, and its reduced form has a molecular size of 115-135 kDa, depending on the cell type. Human ENPP1 has 873 amino acids. It is known that ENPP1 is expressed in several tissues, including skeletal tissue, adipose tissue, and liver and kidneys. However, the physiological functions of ENPP1 in these tissues have not yet been fully described. It is also expressed, in smaller amounts, in pancreatic islets, brain, heart, placenta, lungs, epididymis, salivary glands, chondrocytes, lymphocytes, and fibroblasts (Goldfine etal, 2008). Increased ENPP1 expression inhibits the tyrosine kinase activity of the insulin receptor in several cells (Maddux and Goldfine, 2000), and causes insulin resistance (Frittitta etal, 1997).The gene that encodes ENPP1 has 25 exons and is located on chromosome 6q22-23 (Denise etal, 2011). This gene is regulated by glucocorticoids, agents that lead to an increase in cAMP, protein kinase C activator, phorbol myristate acetate, growth factors such as fibroblast growth factor, and cytokines, including IL-1β and TNF-α (Goldfine etal, 2008). Oxidative stress is currently recognized as a major pathogenic factor of cellular damage caused by hyperglycemia, being considered the final common pathway through which hyperglycemia-related pathways (polyol,advanced glycation end products, protein kinase C and hexosamine) can trigger the chronic complications of diabetes (Brownlee,2001). NOX/NADPH oxidase catalyze NADPH-dependent reduction of O2 to $O2^{\bullet-}$, generating reactive oxygen species (ROS). NOX1, NOX2 and NOX4 are isoforms expressed in the kidney (Gill and Wilcox, 2006) whose activation requires association with subunit p22phox, encoded by the CYBA gene. The p22 phox subunit is the final transporter in the chain transferring electrons from NADPH to molecular oxygen, and is encoded by the α subunit of the cytochrome b 245 *(CYBA)* gene, which is located on chromosome 16q24. Several polymorphisms in the *CYBA* gene have been described, some of which have been associated with increased (San jose etal,2004) or decreased NOX activity (Guzik etal,2000), as well as reduced ROS generation (Bedard etal,2009). Glutathione (GSH) is one of the main endogenous antioxidants whose plasmatic concentrations were reported to be reduced in diabetes patients (Samiec etal, 1998). GSH synthesis requires the glutamate-cysteine ligase enzyme (γ-GCL) in the first and rate-limiting step for its production. This enzyme is a heterodimer composed by a heavy catalytic subunit, encoded by the GCLC gene, as well as a light regulatory subunit (Koide etal, 2003).The aim of this study was to investigate if some common SNPs associated with diabetic nephropathy in type 1 diabetes and to evaluate the effect of these SNPs on less severe stage of diabetic nephropathy, defined as microalbuminuria.

Materials and Methods:

The study was performed on 134 patients with type 1 diabetes. All patients were selected from outpatient Clinics of National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt. Type 1 DM was diagnosed according to American diabetic association 2007). Diabetic nephropathy was defined as a positive microalbuminuria (Albumin Creatinine Ratio = 30-299 mg/gm). Demographic data was recorded for each subject using self-made questionnaire. Approval had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes. An informed consent was obtained from all patients that described the aim of the study and the procedures that would be required from them. The study included 2 groups of subjects, Group 1control group: type 1 diabetes with (ACR <30) (n=61), the mean age (14.05±4.33) years, Group 2: type 1 diabetes with (ACR >30) (n=73) the mean age (13.84±4.5) years. The following variables were also recorded: age, BMI, blood pressure, GFR, and ACR. BMI was calculated as weight (kg) divided by height squared meter (Kg/m2) according to Shiwaku et al., 2004). Blood pressure measurements were performed by trained technicians or nurses with a mercury sphygmomanometer and the first and fifth Korotkoff sounds were recorded to represent the systolic and diastolic pressure, two measurements were obtained and averaged. Hypertension was considered if the systolic blood pressure was G140mm Hg or diastolic blood pressure G90 mm Hg, or use of medication for hypertension. The GFR can be easily estimated using the newer prediction equation developed by Schwartz GJ, et al., 2009), Recently, it's found that it is more typical to compare the amount of albumin in the urine sample against its concentration of creatinine. This is termed the albumin-to-creatinine ratio "ACR" expressed in *'mg/gm* creatinine'. The use of ACR for these random samples might replace 24-hr urine collections, thus ACR may be a useful diagnostic tool than microalbumin (μalb) itself Justesen et al., 2006). ACR was estimated by dividing the μalb. concentration in *mg/L* over the urine creatinine concentration in *g/L*. ACR was thus expressed in *mg/gm* creatinine.Blood samples were collected after 12 h overnight fasting from diabetic patients into three types of vacutainer tubes first vacutainer tube without additive. Blood was centrifuged at 3000 rpm for 10 minutes. Serum was rapidly separated and subdivided into aliquots one of them to measure lipid profile, serum creatinine, serum urea, serum ALT, serum AST at once. Second part of collected blood was taken on 2 EDTA tubes for determination of HbA1c level and the second tube for DNA extraction. Hemolysed samples were excluded. Third part of collected blood was taken on fluoride for determination

of Plasma glucose level at once by glucose oxidase method according to Barham and Trinder; 1972). Serum total cholesterol was determined by the enzymatic method according to Allain et al., 1974). Triacylglycerol was assayed by peroxidase-coupled method according to Mc Gowan et al., 1983). HDL-c was measured by enzymatic method according to Finley et al., 1978), LDL-c was measuring according to Friedewald et al., 1972). Serum urea was assayed by the enzymatic method according to Tiffany et al., 1972). Serum creatinine was measured by colorimetric method according to Vasiliades, 1976).Serum Alanine Amino Transferase was measured by kinetic method according to Hafkenscheid and Dijit, 1979). Serum Aspartate Amino Transferase was measured by kinetic method according to Bowers and Mc Comb,1984).Sampling, reagent delivery, mixing , processing, calculating and printing were full automatically performed by the Dimension® RxL Max Integrated chemistry system (SEIMENS instruments Inc, USA), and HbA1c was assayed using ion-exchange high performance liquid chromatography (HPLC) with Bio-Rad Variant Hemoglobin Testing System (Bio-Rad Laboratories, USA) according to method of Lahousen et al., 2002). Fresh morning urine sample was collected from each subject into sterile container and used for determination of microalbumin by immuno-turbidimetric method according to Gentilini et al., 2005) and urinary creatinine was measured by colorimetric method according to Vasiliades, 1976). Estimation of albumin to creatinine ratio (ACR) according to Justesen et al., 2006). DNA Genotyping: Genomic DNA extracted from EDTA whole blood samples using the QiAmp DNA blood mini kit on fully automated (Qiacube instruments, Qiagen, Düsseldorf, Germany).Genotyping of CYBA (rs 4673), GCLC (rs 17883901), ENPP-1 (rs 1805101) variants was done by combining the TaqMan® Universal Master Mix II with the TaqMan® SNP genotyping assay into 96-well plate using the Applied Biosystems (Stratagene Mx 3000 P real- time PCR systems instruments -Germany) according to a standardized protocol. Resulting sequences were analyzed by the MxPRO software.

Statistical Analysis

Raw data were coded and transformed into coding sheets. Data were entered into SPSS system files (SPSS package version 22 ©2013) using personal computer. The following statistical measures were used for analysis of data. Descriptive statistics including frequency, distribution, mean, and standard deviation were used to describe different characteristics.Kolmogorov–Smirnov test was used to examine the normality of data distribution.Univariate analyses including: t test and Mann–Whitney test were used to test the significance of results of quantitative variables. Moreover, X^2 test was used to test for significance among qualitative variables.The chi-square test was used to compare allele and genotype frequencies between groups and to estimate the Hardy–Weinberg equilibrium. Genotypes frequency of SNP gene polymorphism was tested for being expressed in Hardy–Weinberg equilibrium using X^2 test.Odds ratio for renal affection were calculated with different SNP gene polymorphism for each of the studied genes. All P-values were two-tailed, and 95% confidence intervals (CIs) were calculated. The significance of the results was at the 5% level of significance (Norman and Streiner, 2000).

Results:

Demographic characteristics of the studied groups are represented in table (1) . No significant differences were found between the normoalbuminuric group (control) and microalbuminuric group (cases) with respect to age, gender, duration of diabetes, BMI ,SBP and DBP($P>0.05$) .

Table (1): Demographic characteristics of normoalbuminuric group (control) and microalbuminuric group (cases). (Mean±SD)

Parameters	Normoalbuminuric group (control)(N=61)	Microalbuminuric group (cases)(N=73)	P- value
Age (years)	14.05±4.32	13.84±4.54	0.782
Gender (F/M)	32/29	37/36	0.864
Duration of diabetes(years)	7.705±3.18	8.176±3.10	0.432
BMI(Kg/m^2)	21.79±4.98	21.21±5.27	0.516
SBP(mmHg)	117.62±10.47	115.41±10.79	0.233
DBP(mmHg)	75.82±8.12	75.75±8.02	0.962

Table (2): showed the biochemical characteristics of glycemic index in the normoalbuminuric group (control) and microalbuminuric group (cases), plasma fasting glucose and HbA1c showed significant increase in the microalbuminuric group (cases) compared to normoalbuminuric group (control) ($P<0.05$).

Table (2): Biochemical characteristics of glycemic index in normoalbuminuric group (control) and microalbuminuric group (cases). (Mean±SD)

Parameters	Normoalbuminuric group (control)(N=61)	Microalbuminuric group (cases)(N=73)	P- value
FBG(mg/dl)	199.08±108.73	247.72±94.39	0.007*
HbA1c (%)	9.83±2.2	11.2±1.42	0.0001***

Table (3): showed lipid profile in normoalbuminuric group (control) and microalbuminuric group (cases),serum cholesterol, Triacylglycerol were significantly higher (P<0.05) in the microalbuminuric group (cases) as compared to normoalbuminuric group (control),while HDL-c, LDL-c showed no significant differences between the two groups (P>0.05).

Table (3): Serum lipid profile in normoalbuminuric group (control) and microalbuminuric group (cases). (Mean±SD)

Parameters	Normoalbuminuric group (control)(N=61)	Microalbuminuric group (cases)(N=73)	P- value
Cholesterol (mg/dl)	176.54±32.7	204±35.76	0.0001***
Triacylglycerol(mg/dl)	90.31±54.34	133.01±50.12	0.0001***
HDL-c(mg/dl)	58.75±12.62	56.59±14.5	0.363
LDL-c(mg/dl)	104.61±26.78	111.12±32.48	0.213

Table (4): showed the biochemical characteristics of the kidney function in the normoalbuminuric group (control) and microalbuminuric group (cases), blood urea, creatinine and GFR showed no significant difference between normoalbuminuric group (control) and microalbuminuric group (cases) (P>0.05) while there is an increase in the ACR in microalbuminuric group (cases) when compared to normoalbuminuric group (control)(P<0.05).

Table (4): Biochemical characteristics of kidney function in normoalbuminuric group (control) and microalbuminuric group (cases). (Mean±SD).

Parameters	Normoalbuminuric group (control)(N=61)	Microalbuminuric group (cases)(N=73)	P- value
Blood urea(mg/dl)	21.59±5.41	21.01±4.91	0.52
Creatinine(mg/dl)	0.605±0.084	0.60±0.1	0.97
GFR(ml/min/1.73m^2)	112.64±21.35	106±20.3	0.68
ACR(mg/gm)	12.8±5.74	66.22±39.78	0.0001***

Table(5) Regarding CYBA gene polymorphism rs=4673:The genotype distribution of the CYBA gene polymorphism in normoalbuminuric group (controls) and microalbuminuric group (cases) was as follows: The homozygous genotype (TT) was observed as17 of 61 controls with (27.9%) and 16 of 73 of the cases with (21.9%), the wild type (CC) was observed as3 of 61 controls with (4.9%) and 12 of 73 of the cases with (16.4%) while the heterozygous genotype (CT) was found as 41 of 61 controls with (67.2%) and 45 of 73 of the cases with (61.6%) with (p-value >0.05). Cases as well as Controls were deviated from Hardy–Weinberg equilibrium (*P* = 0.04 and 0.001, respectively). Presence of the mutant variant "T" in individuals as follow (95.1 % in controls as compared to cases with 83.6%) which increased risk by 0.26 for developing DN (X^2 = 4.44, OR = 0.26 with 95%CI =0.07-0.98, P- value =0.04*). Regarding ENPP-1 gene polymorphism rs=1805101:The genotype distribution of the ENPP-1 gene polymorphism in normoalbuminuric group (controls) and microalbuminuric group (cases) was as follows: The homozygous genotype (CC) was observed as 4 of 61 controls with (6.6%) and 4 of 73 of the cases with (5.5%), the wild type (AA) was observed as19 of 61 controls with (31.1%) and 31 of 73 of the cases with (42.5%) while the heterozygous genotype (AC) was found as 38 of 61 controls with (62.3%) and 38 of 73 of the cases with (52.1%) with (p-value >0.05). Cases were found to be in Hardy–Weinberg equilibrium but not the controls (*P* = 0.07 and 0.01, respectively). Presence of the mutant variant "C" in individuals as follow (68.9 % in controls as compared to cases with 57.5%) which increased risk by 0.61 for developing DN (X^2 = 1.82, OR = 0.61 with 95%CI =0.30-1.25, P- value =0.18). Regarding GCLC gene polymorphism rs=17883901:The genotype distribution of the GCLC gene polymorphism in normoalbuminuric group (controls) and microalbuminuric group (cases) was as follows: The homozygous genotype (TT) was observed as 30 of 61 controls with (49.2%) and 29 of 73 of the cases with (39.7%), the wild type (CC) was observed as 6 of 61 controls with (9.8%) and 5 of 73 of the cases with (6.8%) while the heterozygous genotype (CT) was found as 25 of 61 controls with (41.0%) and 39 of 73 of the cases with (53.4%) with (p-value >0.05). Cases & controls were found to be in Hardy–Weinberg equilibrium (*P* = 0.09 and 0.81, respectively).Presence of the mutant variant "T" in individuals as follow (90.2 % in controls as compared to cases

with 93.2%) which increased risk by 1.48 for developing DN (X^2 = 0.39, OR = 1.48 with 95%CI =0.43-5.12, P-value =0.53).

Table (5): Association between CYBA, ENPP-1and GCLC gene polymorphism and the studied groups.

Gene name	CYBA			ENPP-1			GCLC		
rs	4673			1805101			17883901		
Genotype	CC	CT	TT	AA	AC	CC	CC	CT	TT
Genotype (n)(%) in normoalbuminuric group (control)	(3) (4.9%)	(41) (67.2%)	(17) (27.9%)	(19) (31.1%)	(38) (62.3%)	(4) (6.6%)	(6) (9.8%)	(25) (41.0%)	(30) (49.2%)
Genotype (n)(%) in microalbuminuric group (cases)	(12) (16.4%)	(45) (61.6%)	(16) (21.9%)	(31) (42.5%)	(38) (52.1%)	(4) (5.5%)	(5) (6.8%)	(39) (53.4%)	(29) (39.7%)
OR (95% CI)	0.26(0.07-0.98)			0.61(0.30-1.25)			1.48(0.43—5.12)		
P- value	0.1(P>0.05)			0.4(P>0.05)			0.53(P>0.05)		

Table (6): Considering the allelic frequencies distribution in the studied groups, regarding the CYBA gene (rs= 4673) allele (C) was present in 38.52% of the control group and 47.26 % of cases group, whereas allele (T) was present in 61.48% and 52.74%, respectively, in controls and cases with (P-value = 0.15, OR = 0.7 at 95% CI = 0.43-1.14). Regarding the ENPP-1 gene (rs= 1805101) allele (A) was present in 62.3% of the control group and 68.49 % of cases group, whereas allele (C) was present in 37.7% and 31.51%, respectively, in controls and cases with (P-value = 0.29, OR = 0.76 at 95% CI = 0.46-1.26). Regarding the GCLC gene (rs= 17883901) allele (C) was present in 30.33% of the control group and 33.56 % of cases group, whereas allele (T) was present in 69.67% and 66.44%, respectively, in controls and cases with (P-value = 0.57, OR = 0.86 at 95% CI = 0.51-1.44).

Table (6): Allelic frequency of CYBA, ENPP-1and GCLC gene polymorphism in normoalbuminuric group (control) and microalbuminuric group (cases).

Gene name	CYBA		ENPP-1		GCLC	
rs	4673		1805101		17883901	
Alleles	C	T	A	C	C	T
Allele (n) (%) in normoalbuminuric group (control)	(47) (38.52%)	(75) (61.48)	(76) (62.3%)	(46) (37.7%)	(37) (30.33%)	(85) (69.67%)
Allele (n) (%) in microalbuminuric group (cases)	(69) (47.26%)	(77) (52.74%)	(100) (68.49%)	(46) (31.51%)	(49) (33.56%)	(97) (66.44%)
OR (95% CI)	0.7(0.43-1.14)		0.76(0.46-1.26)		0.86(0.51-1.44)	
P- value	0.15 (P>0.05)		0.29 (P>0.05)		0.57(P>0.05)	

Discussion:

Diabetic nephropathy (DN) is a chronic disease characterized by proteinuria, glomerular hypertrophy, decreased glomerular filtration and renal fibrosis with loss of renal function (Sun et al., 2013).Many environmental factors have been established as contributing to the development of DN (Murussi, etal, 2003).It is known that factors such as hypertension, dyslipidemia and hyperglycemia play a role in the development of DN. In our study , Table (2) elucidates that fasting plasma glucose and HbA1c levels showed pronounced increase in diabetic patients with microalbuminuria compared with normoalbuminuric patients (controls) so these finding are agreement with previous studies which suggest that hyperglycemia is the driving force for the development of diabetic nephropathy (Sun et al., 2013). Abnormal lipid metabolism and renal accumulation of lipids have been proposed to affect the progression of diabetic nephropathy (Tovar etal, 2011). It was reported that lipid droplet accumulation in glomeruli is associated with an increase in hyperglycemia-induced renal damage, suggesting a pathophysiological role for lipid accumulation in mesangial cells in diabetic nephropathy (kiss etal, 2013). Many clinical and experimental studies suggest that serum cholesterol may play an important role in the development and progression of diabetic nephropathy (Caramori, 2006) this is in accordance with our finding which there is an increase in the total cholesterol and Triacylglycerol in diabetic patients with microalbuminuria compared with normoalbuminuric

patients (controls). The development of diabetic nephropathy is a gradual process which mainly starts with progression from normal albuminuria to microalbuminuria (Batlle, 2003). Clinical hallmarks of diabetic nephropathy include a progressive increase in urinary albumin excretion which occurs in association with an increase in blood pressure, ultimately leading to end stage renal failure (Sara et al., 2006).this is agree with our study in which there is an increase in the A/C ratio in the diabetic patients with microalbuminuria compared with normoalbuminuric patients (controls) as seen in table (4).Considering that DN is a multifactorial disease, the mode of transmission is likely polygenic, and genetic interaction with other environmental factors and clinical data such as duration of diabetes, hypertension , hyperlipidemia and smoking would lead to the development of DN (Adler, etal,2000). NADPH oxidase is a key enzyme that catalyzes the production of superoxide, mediating the downstream generation and injurious effects of ROS. This enzyme is highly expressed in neutrophils and endothelial cells (Griendling, etal, 2000) and possesses several subunits, including the $p22^{phox}$ subunit, which serves as the final transporter transferring an electron from NADPH to molecular oxygen. This key electron transfer protein is encoded by the *CYBA* gene. Given its importance in mediating oxidative stress-mediated cellular injury in response to ischemic and toxic kidney injury, one might anticipate that polymorphisms disrupting the function or expression of the NOX p22 phox subunit would alter oxidative stress–mediated cellular responses in AKI. Several polymorphisms have been described in the *CYBA* gene that are variably linked to measures of oxidative stress, including the *CYBA* rs4673 (Tyr 72 His, exon 4) polymorphism (associated with reduced NOX activity) (Guzik, etal, 2000). The participation of NOX-derived ROS in DN has been previously demonstrated in experimental models of diabetes (Satoh, etal, 2005) while the contribution of genes coding proteins belonging to the NADPH system to genetic predisposition for DN has been little explored. The association of one CYBA SNP (+242 C → T [rs4673]) with overt DN was previously demonstrated in type 1 diabetes patients (Hodgkinson, etal, 2003).This is in accordance with our results as in table (5) in which there is no association between CYBA gene polymorphism and the diabetic patients with microalbuminuria group (cases) this may be due to the study should include a large sample to obtain very small P-values and also this study not include a patients with proteinuria. ENPP1 is by far the best-characterized ENPP. It was first discovered as a surface marker of antibody-secreting B-cells, hence the name plasma-cell differentiation antigen-1 or PC-1 (Bollen, etal, 2000). Now, however, it is known that ENPP1 is expressed in various tissues, including muscle, fat, liver, and kidney (Goding and Howard, 1998). The physiologic functions of ENPP1 in these tissues have not been described. However, it has been found that overexpression of ENPP1 inhibits insulin receptor tyrosine kinase activity in various cells (Maddux and Goldfine, 2000) and causes insulin resistance (Frittitta, etal, 1997). This pattern of expression is compatible with the localization of diabetic nephropathy disease process. In our result we found no association between ENPP-1 gene polymorphism and the studied groups, this in accordance with De Cosmo and cols (2000) who reported that ENPP-1 polymorphism has an effect on the rate of kidney function loss in Caucasians with type 1 DM and proteinuria. Also de Azevedo and cols. (2002), did not find an association between K121Q polymorphism, and the development of new cases of DN or diminished GFR. Jacobsen and cols. (2002) did not observe any association between the K121Q polymorphism and DN progression in Danish type 1 DM patients. This may be due to the molecular mechanism responsible for the association between the ENPP-1 and the development of advanced stages of DN may be only hypothesized, also may be depend on the ethnic group. Also from table (5) there is no association between GCLC gene polymorphism with the studied group may be due to that the treatment with insulin improves antioxidant defenses to prevent chronic diabetic complications.

Conclusions

From this study, we concluded that further studies must be done in larger populations with different phenotypes of renal impairment to evaluate if this genes are more involved with the development of DN, also the further studies must be done with a large number of patients with a decline in GFR than by albuminuria. So, replication study for this functional gene needs to be carried out.

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