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Association of XbaI *GLUT*1 Polymorphism with Susceptibility to Type 2 Diabetes Mellitus and Diabetic Nephropathy

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Abstract

Objectives: Diabetic nephropathy (DN) is one of the chronic microangiopathic complications of type 2 diabetes (T2DM) and has become the most frequent cause of end-stage renal disease. The XbaI polymorphism in the glucose transporter (GLUT1) has been suggested in the development of DN. We examined the association between XbaI polymorphism of GLUT1 and susceptibility to T2DM and development of DN. Methods: The study included 227 T2DM patients divided into 107 without DN (DM − DN) and 120 with DN (DM + DN), in addition to 100 apparently healthy controls. Genotyping was done by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Results: The GLUT1 XbaI T allele was associated with increased susceptibility to T2DM, when comparing the healthy controls to the whole diabetic group, odds ratio (OR) = 1.899, 95% confidence interval (CI) (1.149 - 3.136), *p* = 0.011. This association was also significant between healthy controls and DM − DN OR = 1.997 (1.079 - 3.699), *p* = 0.026 as well as between healthy controls and DM + DN OR = 1.818 (1.016 - 3.253), *p* = 0.042. However there was no significant association of XbaI polymorphism with DN when comparing DM − DN to DM + DN OR = 0.910 (0.474 - 1.747), *p* = 0.777. Conclusion: XbaI T allele is associated with increased susceptibility to T2DM, but not to development of DN. Further studies are needed to replicate such findings.

Keywords

Type 2 Diabetes Mellitus, Diabetic Nephropathy, Glucose Transporter 1, XbaI, Polymorphism

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1. Introduction

Diabetic nephropathy (DN) is a major long term chronic microangiopathic complications of diabetes mellitus, leading to end-stage renal disease [1]. It is defined by occurrence of albuminuria and/or proteinuria in a diabetic patient with no evidence of nondiabetic renal conditions [2]. It is characterized also by elevated arterial blood pressure, decline in glomerular filtration rate (GFR), and high risk of cardiovascular morbidity and mortality [3].

Glucose transporters (GLUTs) encompass a family of facilitative transporters classified into three classes [4]. GLUT1 is a member of class 1 and it is highly expressed in the glomeruli, mesangial, endothelial cells and podocytes [5]. *GLUT*1 gene (*SLC*2*A*1) (rs841853) is located on chromosome 1p34.2, and it contains 10 exons and 9 introns [6] [7]. It has a central role in the pathogenesis of DN because over expression of GLUT-1 in glomerular mesangial cells is expected to augment the basal glucose uptake [8], and activate cellular pathways involved in cellular growth and in accumulation of the extracellular matrix [9]. The expression and activity of mesangial GLUT1 in diabetic patients comprise great individual variability attributed mostly to genetic causes. From this perspective it becomes clear why only a certain group of diabetics are predisposed to the development of DN, and could also clarify the reason for the poor correlation between glycemic control and progression of nephropathy in a subset of diabetics [10]. Thus investigating the genetic susceptibility to the development of DN may shed light on the pathogenesis of renal involvement in diabetes mellitus. Several single nucleotide polymorphisms (SNP) of *GLUT*1 gene have been examined in relation to DN, of which is XbaI G > T, located within intron 2, which represents transversion of guanine (G) to thymine (T) [9].

The conflicting data from worldwide studies done concerning XbaI G > T *GLUT*1 polymorphism in relation to DN [1] [9], and the fact that there were no studies in Egyptian population concerning this issue, made it noteworthy to study this polymorphism.

2. Subjects and Methods

2.1. Selection of Study Participants

We performed a case-control study of 227 diabetic patients attending the Teaching Hospital of Medical Research Institute, Alexandria University. All patients enrolled in this study were diagnosed as type 2 diabetes mellitus (DM2) according to the criteria based on the American Diabetes Association criteria (ADA) [11]. One hundred healthy subjects were also recruited from the same population. The study was explained to all participants and written informed consents were required. The experimental design was approved by the local ethical committee, and all participants gave their informed consent. All diabetics were receiving oral hypoglycemic agents.

Based on the guidelines of the ADA, albumin was measured in a spot urine sample collected as the first urine in the morning to identify nephropathy. Accordingly, diabetics were classified into non diabetic nephropathy patients (DM − DN) who were the normoalbuminuric with normal urinary albumin excretion (<30 mg/gm urine creatinine), while diabetic nephropathy patients (DM + DN) were those with persistently increased urinary albumin excretion (≥30 mg/gm urine creatinine) based on the consensus of at least two consecutive overnight samples collected over a 3 - 6 month period [11].

General exclusion criteria included: DM2 with less than 10 years duration, type 1 diabetes, secondary diabetes, smoking, pregnancy, heart failure, previously diagnosed nondiabetic kidney disease. During urine sample collection; acute fever, diabetic ketoacidosis, significant bacteriuria or hematuria, or patients who performed excessive exercise within 24 hours were excluded and repeated after condition resolution. Ultrasound examination was done to exclude other non-diabetic organic kidney disease.

2.2. Examination, Sampling and Biochemical Analysis

All subjects had a standardized physical examination and provided detailed history regarding diagnosis and complications of DM as well as the type of anti-diabetic treatment received. Anthropometric measurements (weight and height) and calculation of body mass index were done to all participants.

Following an overnight 12 hour fast, eight milliliters of whole venous blood were withdrawn from each participant; whole EDTA blood was used for genomic DNA extraction and serum was used for routine clinical chemistry (concentrations of urea, creatinine, uric acid, triglycerides, cholesterol and its high density fraction). In addition, urinary albumin and creatinine were measured from morning urine sample. Glycated hemoglobin

was determined using immunoturbidimetric assays. Analyses were conducted on the Olympus AU400 clinical chemistry analyzer (Beckman Coulter Inc., Brea CA, USA). Calculations of low density lipoprotein fraction using the Friedwald formula, estimated glomerular filtration rate (GFR) using the Cockroft and Gault formula, and urinary albumin to creatinine (UAC) ratio were done.

2.3. Genotyping of XbaI Polymorphism of the GLUT1 Gene

Genomic DNA was extracted using a commercially available kit (Qiagen). The concentration and purity of extracted DNA were determined by NanoDropTM 1000 spectrophotometer (Thermo Fisher Scientific) at 260 and 280 nm. The polymerase chain reactions were carried out in a Veriti thermal cycler (Applied Biosystems) according to the method described by Grabellus *et al.* (2010) [12]. The PCR reaction was carried out in a total volume of 25 µL, containing 10 µL of genomic DNA, 12.5 ml of DreamTaq™ Green PCR Master Mix, 20 pmol/l of each primer, 2.1 µL of nuclease free water. The primer sequences were; forward 5'-TGC AAC CCA TGA GCT AAC AA-3' and reverse 5'-GAA CCC AGC ACT CTG TAG CC-3' [12]. PCR program was initial denaturation 94˚C for 3 min followed by annealing and extension for 30 cycles of 45 s at 94˚C and 45 s at 56˚C and 72˚C for 45 s, with a final extension step of 7 min at 72˚C. The PCR products were digested with XbaI restriction enzyme (Roche Molecular Dagnostics, Germany) and incubated in 37˚C for 1 hour. In the mutant form, guanine (G) has been transverse to thymine (T) and abolished the recognition site. Hence, T allele which did not contain the XbaI restriction enzyme site remained undigested as 305 bp fragments, whereas G allele yields 232- and 73-bp fragments [12]. The resultant PCR products were visualized by electrophoresis on 3% agarose gel stained with ethidium bromide for visualization under UV light (**Figure 1**). In 5% of the samples genotyping was performed in duplicate and was fully concordant.

2.4. Statistical Analysis

Continuous results that satisfied a normal distribution were expressed as mean ± standard deviation. The Student's t-test, chi-square test, and Fisher exact test were used to assess the general characteristics between groups. The Hardy-Weinberg equilibrium was performed using chi square test by comparing the observed to the expected genotype frequencies. The association between variant alleles and their susceptibility to disease was assessed, and odds ratios (ORs) with 95% confidence intervals and chi-square tests were calculated. All statistical tests were two-tailed and p-values less than 0.05 were considered statistically significant. Statistical Program for Social Sciences (SPSS) version 20 was used for analysis of data.

3. Results

This case-control study included 100 healthy adults (56% males) with age 47 (34 - 66) years and 227 unrelated type 2 diabetics, stratified according to occurrence of diabetic nephropathy into; 107 diabetics without diabetic nephropathy (DM − DN) and 120 diabetics with diabetic nephropathy (DM + DN).

Although duration of diabetes was higher in (DM + DN) when compared to (DM − DN) but this did not reach the level of statistical significance ($p = 0.348$). HbA1c was significantly higher in (DM + DN) when compared to (DM − DN). As expected, the studied renal parameters were higher in (DM + DN) when compared to (DM − DN). The lipid profile showed no significant difference among groups, except for higher triglyceride level in (DM + DN) when compared to (DM − DN) (**Table 1**).

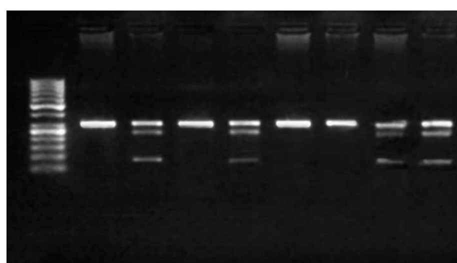

Figure 1. Agarose gel electrophoresis showing patterns of XbaI polymorphism of the GLUT1 gene. Lane 1: 50 bp ladder; lanes 2, 4, 6, 7 denote TT genotype; lanes 3, 5, 8, 9 denote GT genotype.

Table 1. Demographic and biochemical parameters of the studied sample.

Variable	Whole diabetics	DM − DN	DM + DN	*p* value
Number (%)	227 (100)	107 (47.1)	120 (52.9)	-
Gender (% men)	110 (48.2)	49 (45.8)	61 (50.8)	0.448
Age (years)	51.0 (32.0 - 66.0)	52.0 (38.0 - 65.0)	51.0 (32.0 - 66.0)	0.850
Duration of diabetes (years)	12.0 (10.0 - 22.0)	11.0 (10.0 - 20.0)	14.0 (11.0 - 22.0)	0.348
BMI (Kg/m^2)	31.2 (21.5 - 39.5)	32.0 (21.5 - 39.5)	31.0 (23.4 - 39.1)	0.951
HbA_{1C} (%)	8.65 ± 1.83	8.19 ± 1.53	9.07 ± 0.97	<0.001*
UAC (mg/gm)	46.0 (4.6 - 1749.0)	14.5 (4.6 - 29.0)	172.7 (43.3 - 1749.2)	<0.001*
Serum creatinine (mg/dl)	1.0 (0.6 - 5.7)	0.9 (0.7 - 1.3)	1.1 (0.6 - 5.7)	<0.001*
eGFR (ml/min)	85.7 (14.6 - 155.0)	91.5 (62.5 - 155.0)	72.0 (14.6 - 132.7)	<0.001*
Uric acid (mg/dl)	4.7 (3.2 - 10.2)	4.5 (3.2 - 6.7)	5.6 (3.7 - 10.2)	<0.001*
Total cholesterol (mg/dl)	219.0 (130.0 - 286.0)	219.0 (168.0 - 269.0)	221.5 (130.0 - 286.0)	0.661
Triglycerides (mg/dl)	168.0 (68.0 - 272.0)	158.0 (78.0 - 266.0)	171.0 (68.0 - 272.0)	0.029*
HDL-C (mg/dl)	43.0 (25.0 - 78.0)	45.0 (26.0 - 78.0)	43.0 (25.0 - 67.0)	0.275
LDL-C (mg/dl)	143.7 ± 29.2	143.3 ± 27.1	144.1 ± 31.1	0.830

DM − DN: diabetic patients without diabetic retinopathy, DM + DN: diabetic patients with diabetic retinopathy, BMI: body mass index, HbA1c: glycated hemoglobin, UAC: urinary albumin creatinine ratio, eGFR: estimated glomerular filtration rate, HDL-C, LDL-C: high and low density lipoprotein cholesterol.

All genotype groups obeyed the Hardy-Weinberg equilibrium; $p = 0.07$ in healthy control and $p = 0.145$ in whole diabetics group. We did not find any homozygous (GG) genotype in the studied population neither in healthy controls, nor in diabetics. TT genotype was significantly more frequent in whole diabetics (82.38%), DM − DN (83.2%) and DM + DN (81.7%) than in the healthy controls (69%). The difference in the genotype frequencies was also reflected in the allelic frequencies. XbaI polymorphism T allele was associated with increased susceptibility to diabetes, when comparing healthy controls to that of the whole diabetic group, odds ratio (OR) = 1.899, 95% confidence interval (CI) (1.149 - 3.136), $p = 0.011$. This significant difference was also present between healthy controls and DM – DN, OR = 1.997 (1.079 - 3.699), $p = 0.026$ as well as between healthy controls and DM + DN OR = 1.818 (1.016 - 3.253), $p = 0.042$. However, there was no association of XbaI polymorphism between DM − DN and DM + DN, OR = 0.910 (0.474 - 1.747), $p = 0.777$ (**Table 2**). We examined the studied parameters according to genotypes of XbaI polymorphism, but no significant difference was noted (**Table 3**).

4. Discussion

Diabetic nephropathy (DN) occurs in 20% - 40% of diabetics and is the single leading cause of end stage renal disease. It imposes a high social and economic burden. Persistent albuminuria in the range of 30 - 299 mg/ 24 h has been shown to be an early stage of DN in type 1 diabetes and a marker for development of nephropathy in type 2 diabetes. In addition, DN is a well-established marker of increased cardiovascular risk [11]. Apparently, hyperglycemia can no longer be the sole etiological factor in development of DN. This is supported by familial clustering of renal complications and ethnic variations. Therefore, genetic causes are considered a major contributor of occurrence and progression of DN [10]. The *GLUT*1 is a plausible candidate gene in diabetes research,

Table 2. Genotype and allele frequency of XbaI polymorphism of *GLUT*1 in the studied population.

Genotype/allele	Healthy (n = 100)	Whole diabetics (n = 227)	DM − DN (n = 107)	DM + DN (n = 120)	*p* value	OR, 95% CI
GG	0	0 (0)	0 (0)	0 (0)		
GT	31	40 (17.62)	18 (16.8)	22 (18.3)	0.007*	2.100 (1.219 - 3.610)
TT	69	187 (82.38)	89 (83.2)	98 (81.7)	0.017#	2.221 (1.148 - 4.300)
					0.029≈	2.000 (1.069 - 3.747)
					0.766¶	0.901 (0.454 - 1.789)
Allele						
G	31 (15.5)	40 (8.8)	18 (8.4)	22 (9.2)		
T	169 (84.5)	414 (91.2)	196 (91.6)	218 (90.8)	0.011*	1.899 (1.149 - 3.136)
					0.026#	1.997 (1.079 - 3.699)
					0.042≈	1.818 (1.016 - 3.253)
					0.777¶	0.910 (0.474 - 1.747)

DM − DN: diabetic patients without diabetic retinopathy, DM + DN: diabetic patients with diabetic retinopathy. OR: odds ratio, CI: confidence interval. *Comparison between healthy control and whole diabetics; #Comparison between healthy control and DM − DN; ≈Comparison between healthy control and DM + DN; ¶Comparison between DM − DN and DM + DN.

Table 3. Demographic data and biochemical parameters according to genotypes of XbaI polymorphism in type 2 diabetics.

Item	TT	GT	*p* value
Number (%)	187 (82.4%)	40 (17.6%)	-
Gender (% men)	88 (47.1%)	22 (55.0%)	0.362
Age (years)	51 (38 - 66)	52 (32 - 66)	0.550
Duration of diabetes (years)	12.0 (10.0 - 22.0)	14.0 (10.0 - 22.0)	0.390
BMI	31.0 (21.50 - 39.10)	32.0 (22.0 - 39.50)	0.716
Fasting plasma glucose (mg/dl)	169 (78 - 346)	167 (79 - 327)	0.330
HbA_{1C} (%)	8.63 ± 1.81	8.77 ± 1.93	0.652
UAC ratio (mg/gm)	46.0 (4.6 - 1749.2)	49.2 (5.3 - 954.5)	0.622
Serum creatinine (mg/dl)	1.0 (0.6 - 5.7)	1.0 (0.6 - 5.7)	0.723
eGFR (ml/min)	85.7 (14.6 - 155.0)	89.5 (14.6 - 136.5)	0.716
Uric acid (mg/dl)	4.7 (3.2 - 10.2)	4.7 (4.1 - 8.9)	0.358
Total cholesterol (mg/dl)	219 (134 - 286)	224 (130 - 265)	0.466
Triglycerides (mg/dl)	168 (78 - 272)	188 (68 - 266)	0.350
HDL-C (mg/dl)	43 (25 - 67)	43 (26 - 78)	0.955
LDL-C (mg/dl)	143.7 ± 28.9	143.9 ± 31.2	0.977

BMI: body mass index, HbA1c: glycated hemoglobin, UAC: urinary albumin creatinine ratio, eGFR: estimated glomerular filtration rate, HDL-C, LDL-C: high and low density lipoprotein cholesterol.

because GLUT1 is the main facilitative glucose transporter. In this case control study, we found that the T allele of XbaI *GLUT*1 gene polymorphism was associated with increased risk of susceptibility to T2DM, but not to development of DN.

The genotypes reported in our study obeyed the Hardy-Weinberg equilibrium. The G and T allele frequencies in the healthy control group were 15.5% and 84.5% respectively. We did not find GG genotype in any of the

studied subgroups. This was quite different from other studies [9] [10] [13] [14]. Variation in XbaI polymorphism genotype frequencies in healthy population is noted among different ethnicities for example the frequency of T allele in British Caucosid [15] was more than twice that reported in Chinese (51% in former and 21% in latter) [10], taken into consideration that both studies did not deviate from Hardy-Weinberg equilibrium.

In this case-control study, the *GLUT*1 XbaI T allele was associated with increased susceptibility to T2DM, when comparing the healthy controls to the whole diabetic group, odds ratio (OR) = 1.899, 95% confidence interval (CI) (1.149 - 3.136), $p = 0.011$. This association was also significant between healthy controls and DM − DN OR = 1.997 (1.079 - 3.699), $p = 0.026$ as well as between healthy controls and DM + DN OR = 1.818 (1.016 - 3.253), $p = 0.042$. The XbaI polymorphism has been previously studied in association to risk of DM, but results were inconsistent. Significant association was reported in Japanese [16] [17] and Italians [18], but other studies could not confirm such association [19] [20]. The XbaI polymorphism has an intronic nature and it can hardly cause changes in the protein sequence. Thus, it can be assumed that the XbaI polymorphism is in linkage disequilibrium with another locus which does have significant functional implications at the protein level [9].

In the current study, *GLUT*1 XbaI polymorphism was not associated with DN. Over the past years, conflicting results were reported from different studies concerning the association of GLUT1 Xba polymorphism and DN in type 2 diabetic patients [9] [10] [13] [14] as well as in type 1 diabetic patients [15] [21] [22]. The non significant association of the XbaI polymorphism of *GLUT*1 gene with DN found in the present study was in agreement with studies done in T2DM in Caucasian Mediterranean population [23] and in Tunisians [14]. Also in type 1 DM no significant association of the XbaI polymorphism with DN was reported in Danish population [21]. On the other hand, a study by Liu *et al*. indicated that type 2 diabetic patients with XbaI (T) allele of the *GLUT*1 gene may be prone to DN in Chinese subjects [10]. A finding that was supported by later studies in different populations including; a study on Mediterranean Caucasian population which showed an association with T2DM and possibly a severe form of it that leads to the development of DN. It further showed a statistically significant association between the XbaI (T) carriage and the presence of arterial hypertension in type 2 DM patients. However when they considered hypertension as a confounding factor, the association between *GLUT*1 XbaI G > T polymorphism and DN was no longer significant [9]. Also, another study on European Americans found that those having homozygous XbaI T allele were associated with DN, and suggested that enhancer 2 (Enh2) SNP, and not XbaI, is the causative polymorphism associated with diabetic albuminuria [24]. There is great heterogeneity in genetic studies; ethnicity of the studied population, demographics including different age and gender distribution. And above all, non uniformity in selection criteria of cases, for example definition of DN can range from microalbuminuria in spot urine sample, proteinuria in 24 hr urine and/or impaired renal function, thus making comparability of results quite difficult [1].

It is worth mentioning that a meta-analysis including several studies upon different populations concluded that XbaI polymorphhism in *GLUT*1 gene may represent a genetic susceptibility to DN. However, it did not support the association between XbaI and the severity of DN [25]. Nevertheless, (Grzeszczak *et al*., 2001) study in Caucasians from Poland was not in agreement with all the aforementioned studies and it even suggested that the XbaI (T) allele protects against the development of DN, given that the frequency of the XbaI (GG) genotype increased with each stage of DN. They found that patients with microalbuminuria had a higher frequency of XbaI (GG) genotype than those with normoalbuminuria and in the group with proteinuria/chronic renal failure the frequency of XbaI, (GG) genotype was the highest [13].

5. Conclusion

In conclusion, the association between *GLUT*1 XbaI polymorphism and DN is still debatable up to date due to the contradictory reports from different populations. In the same context, the present study was only able to demonstrate an association between this polymorphism and susceptibility to T2DM but not DN. Further studies on larger sample size are needed to replicate such findings.

Disclosure

Authors have not conflict of interest to disclose.

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