BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2019, VOL. 76, NO. 3, 153–155
https://doi.org/10.1080/09674845.2019.1595870

BIOMEDICAL SCIENCE IN BRIEF

No association of SNP 313A→G in *GSTP1* with nephropathy, hypertension and dyslipidemia in type 2 diabetes mellitus.

S Abbas[a], ST Raza[a], S S. Mir[b], Z Siddiqi[c] and F Mahdi[a]

[a]Department of Biochemistry, Medicine Era's Lucknow Medical College and Hospital, Lucknow, India; [b]Department of Bioengineering, Integral University, Lucknow, India; [c]Medicine Era's Lucknow Medical College and Hospital, Lucknow, India

ARTICLE HISTORY Received 3 January 2019; Accepted 4 March 2019
KEYWORDS Glutathione S-transferase P1; gene polymorphism; T2DM with Dyslipidemia; T2DM with Hypertension; T2DM with Nephropathy

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycaemia resulting from the combination of resistance to insulin action, excessive or inappropriate glucagon secretion, and inadequate insulin secretion. Prevalence is increasing globally and has reached epidemic proportions in many countries. The number of people living with diabetes is expected to be 592 million by 2035 [1]. Chronic hyperglycaemia without proper management can lead to various short-term and long-term complications, which may be the main cause of mortality and morbidity. Currently, integrated diabetes care programs focus on diabetes-related comorbidities such as cardiovascular diseases, retinopathy, nephropathy and diabetic foot [2].

Diabetes is the leading cause of end-stage renal disease, chronic haemodialysis and renal transplantation worldwide. Diabetes-associated nephropathy is a progressive disorder of the microvasculature of the kidney. Microalbuminuria is common among adolescents with T2DM, occurring in 14–22% [3], and hypertension is more prevalent in patients with diabetes than in the non-diabetic population. At least 67% of persons with T2DM either have uncontrolled hypertension or are being treated for elevated blood pressure [4]. Hypertriglyceridemia and low HDL-cholesterol are more common in the diabetic population.

Several studies, such as [5], have identified polymorphisms in certain genes that have roles in the complications of diabetes. Hyperglycaemia induces overproduction of reactive oxygen species (ROS) resulting from oxidative stress, one of several mechanisms that contribute in the pathogenesis of T2DM and its related vascular complications through an imbalance between pro-oxidants and antioxidant defence systems. Glutathione S transferase (GST), an endogenous antioxidant, catalyses the conjugation of glutathione to a wide range of exogenous and endogenous hydrophobic electrophiles and so represents a protective mechanism against oxidative stress [6]. GST enzymes participate in destroying free radicals and ROS and act as a defence system [7]. A single nucleotide substitution (SNP, 313A→G) in *GSTP1* replacing isoleucine with valine substantially reduces enzymatic activity. Oxidative stresses due to decreased enzyme activity contribute to the destruction of β-cell, an important factor in the development of T2DM [8]. We hypothesized a link between this *GSTP1* SNP and the three major clinical complications of diabetes.

We recruited 370 T2DM subjects, of whom 122 had Nephropathy (DN), 119 had Hypertension (DH), 129 had Dyslipidemia (DD), and 120 controls from the Diabetic clinic of the Department of Medicine at Era's Lucknow Medical College & Hospital, Lucknow. Clinical variables including age, sex, blood pressure, Hb A1C (%), lipid profile, etc., were collected. Written informed consent was taken from all participants. Patients with overnight fasting plasma glucose of more than 6.99 mmol/L on two consecutive events were included in T2DM category. T2DM cases with 24-h urine albumin excretion rate of 30–300 mg/day (microalbuminuria) and >300 mg/day (macroalbuminuria) were included as diabetic nephropathy cases. T2DM cases with a mean systolic blood pressure (SBP) of >140 mmHg and mean diastolic blood pressure (DBP) of >90 mmHg or taking antihypertensive medications were included as diabetic hypertensive cases. T2DM cases with one or more abnormal lipid values (total cholesterol [TC], LDL and triglycerides [TG]) or HDL-cholesterol, alone or in combination were included in diabetic dyslipidemia cases. Control samples were defined as those with fasting blood sugar level below 6.99 mmol/l without family history of diabetes and its complication and none of them were receiving any medications at the time of participation. Patients suffering from type 1 diabetes, gestational diabetes, maturity-onset diabetes of the young and proved cases of coronary artery diseases and stroke were excluded. The project (Ref no.ELMC/R-Cell/EC/2014/100) was approved by the Ethics

CONTACT ST Raza tasleem24@gmail.com Department of Biochemistry, Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India

Committee of the Era's Lucknow Medical College and Hospital, Lucknow, India.

Serum creatinine levels, fasting blood sugar, serum cholesterol, serum triglyceride, high-density lipoprotein (HDL) and cholesterol were assessed by XL-300 Transasia Auto Analyzer (Transasia, Mannheim, Germany). Low-density lipoprotein (LDL) levels were calculated by using Friedewald's formula. HbA1C was measured using semiautoanalyzer (Transasia, Mannheim, Germany). HbA1c estimation was by Gen X hemoglobin A1c-Direct kit (Proton Biologicals India Pvt. Ltd, Bengaluru, India). The kit for calculation of results applied IFCC calibrated values by using the following equation NGSP = (0.0915 X IFCC) + 2.15 expected values. (NGSP units in % while IFCC units were in mmol/molHb). All the assays were performed following the standard manufacturer's protocols.

Genomic DNA was isolated from whole blood using DNA extraction kit (MACHEREY-NAGEL, Germany) following manufacturers protocol. The DNA concentration was determined by a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific) and samples were stored at −20°C until use. PCR was employed for genotyping of the *GSTP1* SNP. Reactions were performed in a total volume of 20 µL, including 50 ng template DNA, 1X buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl), 0.25 µM primers, 2.0 mM $MgCl_2$, 0.25 mM dNTPs, and 0.5 U *Taq* DNA polymerase. PCR conditions were initial denaturation at 95°C for 5 min, 30 cycles of incubation at 94°C (30 s), 55°C (30 s), and 72°C (30 s). A final polymerization step of 72°C for 5 min was carried out to complete the elongation processes. After the confirmation of an amplified fragment of the expected size (176 bp) on an agarose gel, the PCR products were digested with 5 U of restriction enzyme, *Alw261* (Fermentas, UK) in a total volume of 25 µl. DNA fragments were submitted to electrophoresis through 3% agarose gel stained with ethidium bromide (10 mg/ml). The products generated were 176 bp for genotypes: A/A (Ile/Ile), 176, 91 and 85 bp for genotypes: A/G (Ile/Val) and 91, 85 bp for genotypes: G/G (Val/Val) (Figure 1).

Data are presented as mean (SD) for continuous variables and proportion/percentages for categorical variables. The genotyping data were compared between cases and controls using chi-square test. All statistical tests were performed using SPSS (Statistical Package for the Social Sciences) version 17 software.

The 370 patients were 122 DN cases (57 male and 65 female), 119 DH cases (58 male and 61 female), 129 DD cases (59 male and 70 female) ($p = 0.626$). The mean (SD) ages were 51.9 (10.7) in DN, 50.6 (11.3) in DH, 50.04 (11.39) in DD and 49.6 (10.2) in control groups, respectively ($p = 0.227$). *GSTP1* genotype distributions in DN, DH, DD cases ($p = 0.65, 0.43, 0.13$) and controls ($p = 0.52$) were in Hardy-Weinberg equilibrium. The mean HBA1c level was 8.1 (1.6) % in DN, 8.1 (1.5) % in DH, 7.5 (1.7) % in DD and 5.6 (0.4) % in control, respectively ($p = 0.045$).

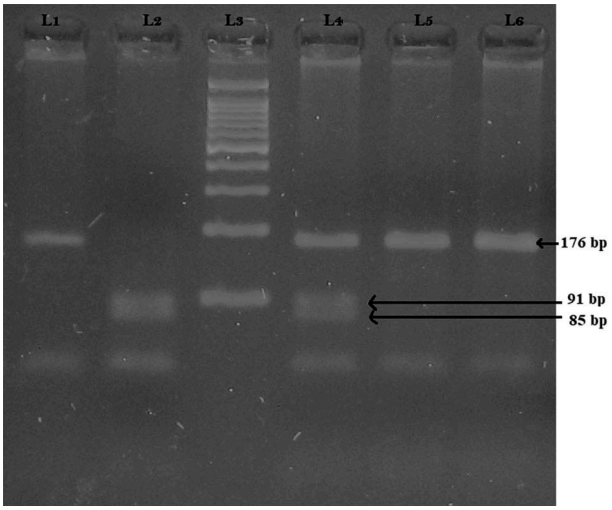

Figure 1. The 3% Agarose gel picture of *Alw261* digested products of *GSTP1 Ile105Val* gene. Lane 1 shows undigested PCR product corresponding to a band of 176 bp, Lane 2 shows VV genotype corresponding to two bands of 91 and 85 bp, Lane 4 shows the IV genotype corresponding to band sizes of 176 bp, 91 and 85 bp, Lane 5 and 6 show II genotype corresponding to a single band of 176 bp, whereas Lane 3 shows a 100 bp ladder.

Median (IQR) serum creatinine was higher in DN cases at 167 (134–186) µmol/l compared to the controls 81 (78–97.2) µmol/l ($p = 0.001$). The mean urine albumin level was 14.9 (6.7–24.7) mmol/l in DN cases and 1.2 (1.05–1.33) mmol/l in control. SBP and DBP in DH cases were 147 (17) mmHg and 91 (11) mmHg ($p < 0.001$ to controls). Mean serum cholesterol and HDL levels were 11.2 (2.8) mmol/l and 2.2 (0.4) mmol/l in DD (both $p < 0.001$ to controls). Significant difference was observed when the genotype and allele frequencies of DN compared to DD cases ($p = 0.018$), while no significant differences were observed on comparing the genotype and allele frequencies of DN, DH and DD cases with healthy controls (Table 1).

The increased prevalence of T2DM is a major problem worldwide. Oxidative stress is one of several mechanisms that contribute to the pathogenesis and its complications. Oxidative and nitrosative stresses

Table 1. The genotypes frequency of GSTP1 Ile105Val gene polymorphism among cases and controls.

Genotype			*p* Value
	Control	**T2DM + Hypertension**	
I V	41(34.2%)	52(42.6%)	0.098
V V	4(3.3%)	9(7.4%)	
I I	75(62.5%)	61(50.0%)	
	Control	**T2DM + Hypertension**	
I V	41(34.2%)	40(33.6%)	
V V	4(3.3%)	8(6.7%)	0.484
I I	75(62.5%)	71(59.7%)	
	Control	**T2DM + Dyslipidemia**	
I I	41(34.2%)	37(28.7%)	
V V	4(3.3%)	5(3.9%)	0.644
V V	75(62.5%)	87(67.4%)	

I: Ile = isoleucine; V: val = valine.

contribute to the destruction of insulin-producing beta cells, an important aetiological factor in the development and progression of T2DM. Glutathione S Transferases are endogenous antioxidants to protect cells from oxidative stress. They catalyse the conjugation of glutathione to a wide range of electrophiles and represents a protective mechanism against oxidative stress [6].

We examined the association of the *GSTP1* SNP and T2DM groups and controls. Epidemiological studies show that allelic variants of *GSTP1 Ile105Val* gene polymorphism, which encode the P1 isoenzyme, create differences in individual susceptibility to several inflammatory diseases. However, the *GSTP1* II, IV, VV genotypes were not linked with nephropathy, hypertension or dyslipidemia in our diabetic population.

Oxidative stress is a causative agent in the ethiopathogenicity of diabetic nephropathy. Since *GSTP1* Val alleles are associated with a reduction of enzymatic activity, a hypothesis can be stated where inadequate GST enzymes levels in the presence of increased oxidative stress can predispose to microvascular complications such as diabetic nephropathy in T2DM. No link between the *GSTP1* and diabetic nephropathy was found in the overall or subgroups analysis in previous studies [6], in agreement with our study where no significant association was found with diabetic nephropathy ($p = 0.269$).

Recently several hypotheses shown the association of oxidative stress in the pathogenesis of hypertension [10,11]. Dyslipidemia observed to be one of the major vascular risk factors in T2DM. In the current study, we further investigated the association of the *GSTP1* genotypes with dyslipidemia in T2DM cases. There was no association between the genotypes and dyslipidemia in diabetic patients was observed ($p = 0.822$). These data are consistent with the previous reports which demonstrated that there was no association between *GSTP1* genotypes and blood lipids in T2DM patients [12].

This work represents an advance in biomedical science because it shows that *GSTP1* is not associated with nephropathy, hypertension or dyslipidemia in type 2 diabetes mellitus.

Acknowledgements

The study was supported by an intramural grant from the Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India. We thank the Research & Development cell Integral University for providing manuscript communication no. (IU/R&D/2017-MCN00076).

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Guariguata L, Whiting DR, Hambleton I, et al. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014;103:137–149.
[2] Valent F, Tillati S, Zanier L. Prevalence and comorbidities of known diabetes. J Diabetes Invest. 2013;4:355–359.
[3] Pinhas-Hamiel O, Zeitler P. Acute and chronic complications of type 2 diabetes mellitus in children and adolescents. Lancet. 2007;369:1823.
[4] Suh DC, Kim CM, Choi IS, et al. Trends in blood pressure control and treatment among type 2 diabetes with comorbid hypertension in the United States: 1988–2004. J Hypertens. 2009;27:1908–1916.
[5] Abbas SA, Raza ST, Mir SS, et al. Role of variants rs5030717 and rs5030718 of TLR4 gene in the risk prediction of diabetic nephropathy and co-morbidities (hypertension and dyslipidemia) in type 2 diabetes Mellitus. Br J Biomed Sci. 2018;74:163–168.
[6] Wang L, Zhang Q. Genetic polymorphisms of GSTT1, GSTM1 and NQO1 genes and diabetes mellitus risk in Chinese population. Biochem Biophys Res Commun. 2006;341:310–313.
[7] Yalin S, Hatungil R, Tamer L, et al. Glutathione S-transferase gene polymorphisms in Turkish patients with diabetes mellitus. Cell Biochem Funct. 2007;25:509–513.
[8] Butler AE, Janson J, Bonner S, et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003;52:102–110.
[9] Orlewski J, Orlewska E. Effects of genetic polymorphisms of glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) on the risk of diabetic nephropathy: a meta-analysis. Pol Arch Med Wewn. 2015;125:649–658.
[10] Montezano AC, Touyz RM. Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies. Ann Med. 2012;44:2–16.
[11] Rodrigo R, González J, Paoletto F. The role of oxidative stress in the pathophysiology of hypertension. Hypertens Res. 2011;34:431–440.
[12] Ramprasath TP, Mrugan S, Prabakaran AD, et al. Potential risk modification of GSTT1, GSTM1 and GSTP1 (glutathione S-transferases) variants and their association to CAD in patients with type 2 diabetes mellitus. Biochem Biophys Commun. 2011;407:49–53.