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Glutathione S-Transferase Gene Polymorphisms Are Not Major Risks for Susceptibility to Posttransplantation Diabetes Mellitus in Taiwan Renal Transplant Recipients

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Glutathione *S*-transferase (GST) M1 null genotype has been reported playing a significant role in the diabetes mellitus (DM) susceptibility in Turkish population. We investigated whether the GSTM1, GSTA1, and GSTP1 gene polymorphisms are associated with posttransplantation diabetes mellitus (PTDM) in Taiwan. There were 283 renal transplant recipients (RTRs) enrolled. Polymerase chain reaction–restriction fragment length polymorphism was used for the measurement of GSTA1, M1, and P1 genetic polymorphisms. PTDM was diagnosed according to the American Diabetes Association guidelines. Eight-five patients (30%) were diagnosed with PTDM. The averaged posttransplant follow-up period was 77.9 ± 27.2 months. Duration from transplantat to diagnosis of PTDM ranged from 0.2 to 103.1 months (19.2 ± 26.3 months). There were significantly differences between non-DM and PTDM groups in age (50.6 ± 11.0 vs. 54.6 ± 9.36 years, $P = 0.005$), BMI (22.4 ± 3.6 vs. 24.3 ± 3.8, $P < 0.001$). The distributions of GSTA1, GSTP1, and GSTM1 genotypes alleles were not significantly different between PTDM and non-DM group. Patients carrying the different GSTA1, GSTP1, and GSTM1 genetic and allelic polymorphisms had no differences for the development of PTDM. These overall results suggested a lack of strong association with GSTA1, GSTP1, and GSTM1 genetic polymorphisms to the susceptibility of PTDM in Taiwanese RTRs. J. Clin. Lab. Anal. 25:432–435, 2011.

Key words: glutathione S-transferases; posttransplantation diabetes mellitus; polymorphism; renal transplantation

INTRODUCTION

The incidence of post-transplantation diabetes mellitus (PTDM) is continuously increasing, ranging from 2 to 53%, resulting in a significant impact on long-term patient and graft survival (1). Some risk factors of PTDM have been reported, including types of immunosuppressant therapy, ethnicity, older age, and body mass index (BMI) (2). Recently, oxidative stress is suggested to contribute to pathological processes in many diseases, including diabetes (3,4), and increased levels of oxidative stress have been identified in diabetes (5). Glutathione *S*-transferases (GST) have been suggested to play an important role against the damaging effects of oxidative stress, but with controversial results in the association with DM (6). In Turkish population, GSTM1 null genotype was reported to play a significant role in the pathogenesis of DM, but no relationship between GSTM1 and diabetic nephropathy has been reported in Japanese type 2 DM (7,8).

Recently, Kang et al. demonstrated that multiple single nucleotide polymorphisms, which included TCF7L2,

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SLC30A8, HHEX, CDKAL1, CDKN2A/B, and KCNQ1, were associated with the development of PTDM (9), and patients with variant genotype of GSTM1 and GSTP1 were at higher risk for rejection and delayed graft function (10). Because there are controversial roles of GST for the development of PTDM, the objective of this study was to evaluate whether the GSTA1, GSTP1, and GSTM1 gene polymorphisms were associated with the risk of developing PTDM.

MATERIALS AND METHODS

From April 1987 to January 2008, patients who received renal transplantation and had regular follow up were recruited at Chung Shan Medical University Hospital, Taichung, Taiwan. Those who were diagnosed with preexisting diabetes mellitus, received cyclosporine-based immunosuppression and refusing to sign informed consent were excluded. Two hundred and eighty-three transplant recipients (145 males and 138 females) were enrolled. The follow-up period lasted until April 2010. This study was approved by the institutional review board of our hospital.

Recipient age, gender, BMI, laboratory, clinical data, including HBV or HCV infection, and smoke were recorded. PTDM was diagnosed according to the American Diabetes Association guidelines (11). These criteria were: fasting blood glucose $\geq$126 mg/dl (7.0 mmol/l); or symptoms of diabetes plus plasma glucose concentration $\geq$200 mg/dl (11.1 mmol/l) at any time of day; or 2 hr postload glucose $\geq$200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test. The blood samples for determination of GSTA1, P1, and M1 genotypes were obtained from the monthly follow-up at our out-patient clinic if patients agreed to provide informed consents.

Genomic DNA Extraction

Genomic DNA was extracted from whole blood samples collected from study subjects by use of QIAamp DNA blood mini kits (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions. DNA was dissolved in TE buffer (10 mM Tris, pH 7.8; 1 mM EDTA) and then quantitated by a measurement of optical density (OD260). The final preparation was stored at –20% and was used to create templates in the polymerase chain reaction (PCR).

Polymerase Chain Reaction–Restriction Fragment Length Polymorphism

The GSTP1, GSTA1, and GSTM1 gene polymorphism analysis (PCR–restriction fragment length polymorphism [PCR-RFLP]) and the primers were modified from previous studies (12). Briefly, PCR was performed in a 10 µl reaction containing 100 ng DNA template, 1.0 µl of 10 × PCR buffer (Invitrogen, Carlsbad, CA), 0.25 U of Taq DNA polymerase (Invitrogen), 0.2 mM deoxyribonucleotide triphosphates (dNTPs; Promega, Madison, WI), and 200 nM of each primer (MDBio Inc., Taipei, Taiwan). The PCR cycling started at 94% for 5 min, followed by 35 cycles of 94% for 1 min, 60% for 1 min, and 72% for 2 min, with a final step at 72% for 20 min to allow a complete extension of all PCR fragments.

Statistical Analysis

The Mann–Whitney *U* test was used in the comparison of continuous variables and chi-square analysis in the comparison of proportions. Results were presented as mean $\pm$ standard deviation or percentages. The alleles of GSTA1 and GSTP1 were calculated by directing counting. Hardy–Weinberg equilibrium (HWE) equilibrium was assessed using a goodness-of-fit chi-square test for GSTA1 and GSTP1. Odds ratio (OR) and 95% confidence interval were calculated to compare the differences in the distributions for GST A1, P1, and M1 genotypic frequencies of non-DM and PTDM groups. A *P*-value of less than 0.05 was considered statistically significant. The data were analyzed using Medcalc® version 11.2 statistical software.

RESULTS

There were 85 patients (30%) diagnosed to have PTDM in this study. The average posttransplant follow-up period was 77.9 $\pm$ 27.2 months and the duration from transplant to diagnosis of PTDM ranged from 0.2 to 103.1 months (19.2 $\pm$ 26.3 months). The clinical characteristics of our patients divided into non-DM and PTDM groups are shown in Table 1. There were

TABLE 1. Demographic Characteristics Between Non-DM and PTDM Groups

Variable	Non-DM	PTDM	*P*
Patient number	198	85	
Gender (male, %)	99 (50)	46 (54.1)	0.613
Age (year)	50.6 $\pm$ 11.0	54.6 $\pm$ 9.36	0.005
Biopsy-proven AR (*n*, %)	25 (12.6)	14 (16.5)	0.502
Biopsy-proven IF/TA (*n*, %)	17 (8.6)	9 (10.6)	0.756
Parental DM (*n*, %)	22 (11.1)	11 (12.9)	0.812
HBV (*n*, %)	24 (12.1)	13 (15.3)	0.594
HCV (*n*, %)	16 (8.1)	6 (7.1)	0.958
Smoking (*n*, %)	58 (29.7)	22 (26.2)	0.647
HLA (*n*, %)			
$\leq$3	99 (58.9)	40 (54.8)	0.649
>3	69 (41.1)	33 (45.2)	
Body mass index (kg/m^2)	22.4 $\pm$ 3.6	24.3 $\pm$ 3.8	<0.001
Duration of dialysis (month)	28.4 $\pm$ 33.3	21.4 $\pm$ 26.4	0.096

AR, acute rejection; IF/TA, interstitial fibrosis/tubular atrophy; DM, diabetes mellitus; HLA, human leukocyte antigen.

TABLE 2. Distribution of Genotypes and Alleles Between Non-DM and PTDM Groups

Variable	Non-DM	PTDM	*P*
GSTA1 (*n*, %)			
CC	157 (79.3)	67 (78.8)	0.886
CT	38 (19.2)	16 (18.8)	
TT	3 (1.5)	2 (2.4)	
C	352 (88.9)	150 (88.2)	0.936
T	44 (11.1)	20 (11.8)	
GSTP1 (*n*, %)			
AA	136 (68.7)	67 (78.8)	0.15
AG	59 (29.8)	16 (18.8)	
GG	3 (1.5)	2 (2.4)	
A	331 (83.6)	150 (88.2)	0.197
G	65 (16.4)	20 (11.8)	
GSTM1 (*n*, %)			
Present	77 (38.9)	28 (32.9)	0.415
Null	121 (61.1)	57 (67.1)	

TABLE 3. Risks of GSTA1, GSTP1, GSTM1 Genetic and Allelic Polymorphisms With Regards to Developing PTDM

				95% confidence interval	
Variable	*N* (%)	*P*	OR	Lower	Upper
GSTA1					
CT[a)]	16 (18.8)	0.968	0.987	0.515	1.891
TT[a)]	2 (2.2)	0.629	1.562	0.255	9.564
CT/TT[a)]	18 (21.2)	0.929	1.029	0.551	1.919
C	150 (88.2)		1		
T	20 (11.8)	0.822	1.067	0.608	1.871
GSTP1					
AG[b)]	16 (18.8)	0.061	0.55	0.295	1.029
GG[b)]	2 (2.4)	0.744	1.353	0.221	8.293
AG/GG[b)]	18 (21.2)	0.085	0.589	0.323	1.075
A	150 (88.2)		1		
G	20 (11.8)	0.158	0.679	0.397	1.162
GSTM1					
Present	28 (32.9)		1		
Null	57 (67.1)	0.343	1.295	0.759	2.212

PTDM, posttransplantation diabetes mellitus; CI, confidence interval; GST, glutathione S-transferase; OR, odds ratio.
[a)]Means compared with GSTA1 CC genotype.
[b)]Means compared with GSTP1 AA genotype.

significant differences between transplant recipients with non-DM and PTDM in age (50.6 ± 11.0 vs. 54.6 ± 9.36 years, $P = 0.005$), BMI (22.4 ± 3.6 vs. 24.3 ± 3.8, $P < 0.001$), but comparable in recipient age, gender, parental DM, duration receiving dialysis, smoke, HBV, HCV, biopsy-proven AR, and biopsy-proven IF/TA.

The alleles of GSTA1 and GSTP1 are in agreement with the HWE ($\chi^2 = 0.671$, $P = 0.412$; $\chi^2 = 0.415$, $P = 0.52$, respectively). The distribution of GSTA1 CC, CT, and TT genotype were 79.1, 19.1, and 1.8%. The distribution of GSTP1 AA, AG, and GG genotype were 71.7, 26.5, and 1.8%. The distribution of GSTM1 present and null genotype was 37.1 and 62.9%. The distributions of GSTA1, GSTP1, and GSTM1 genotypes alleles were not significantly different between non-DM and PTDM group (Table 2). Additionally, the OR of non-DM and PTDM recipients carrying the variant genotype and allele of GSTA1, GSTP1, and GSTM1 were comparable (Table 3).

DISCUSSION

In this study, RTRs diagnosed with PTDM was older and had higher BMI, which were similar with the previous studies (13). PTDM is a well-known complication following solid organ transplantation with 4–25% (1,14), the percentage of the development of PTDM in this study was 30% with the possible reason that we enrolled only recipients who received tacrolimus-based immunosuppressive regimen. The risk factors predisposed to the development of PTDM included nonmodifiable and modifiable ones: the nonmodifiable risk factors were reported as race, genetic background, aged, family history of diabetes, and previous glucose intolerance; the modifiable included obesity, hepatitis C virus infection, cytomegalovirus infections, and immunosuppressive drugs (9,15).

The GST supergene family of phase II drug metabolizing enzymes comprises at least six families of genes (mu, pi, theta, alpha, kappa, and zeta) encoding enzymes involved in the detoxification of a variety of potentially mutagenic compounds and free radicals with reduced glutathione (16). Increasing evidences suggested that enzymes of the GSTA1, GSTP1, and GSTM1 are important in the protection of cells from the toxic products of oxidative stress-mediated reactions (17,18). Oxidative stress and the GST gene polymorphisms had been supposed to be a relevant pathophysiological factor for delayed graft function and for renal allograft rejection development of RTRs (10,19,20).

Studies showed that diminished expression of GSTs may result in a reduction in the capacity of defense against the oxidative stress (17,18).) The oxidative stress had been reported to be associated with hyperglycemia with a significant decline in blood glutathione content at the recent onset of diabetes ($P < 0.0001$) (21). Yalin et al. compared the distribution of GSTM1, GSTT1, and GSTP1 gene variants in DM and healthy control and found that GSTM1 alone was associated with the development of DM (OR 3.7; 95% CI: 2.05–6.7). The combined analysis of these three GST genotypes showed higher risk for enhanced susceptibility to DM (OR 5.7, 95% CI: 1.51–31.07) (7).

Although many diseases, including diabetic nephropathy, coronary artery disease risk, and even the posttransplant allograft outcomes, were associated with

the GST gene variants, the relationship of GST gene polymorphisms with PTDM had not been examined previously (4,8,10,19,20). To our knowledge, this is the first study to investigate the association of GST gene polymorphisms with the development of PTDM, although the results lacked association. Additionally, because of the limited number of patients in this study, further investigation with larger sample size may allow us to clarify the effects of GST genetic polymorphism on the development of PTDM.

In conclusion, the overall results suggested that GSTA1, GSTP1, and GSTM1 polymorphisms were not major risk factors for the susceptibility to PTDM in Taiwan RTRs.

REFERENCES

1. Montori VM, Basu A, Erwin PJ, Velosa JA, Gabriel SE, Kudva YC. Posttransplantation diabetes: A systematic review of the literature. Diabetes Care 2002;25:583–592.
2. Reisaeter AV, Hartmann A. Risk factors and incidence of posttransplant diabetes mellitus. Transplant Proc 2001;33:8S–18S.
3. Tiwari AK, Prasad P, B KT, et al. Oxidative stress pathway genes and chronic renal insufficiency in Asian Indians with Type 2 diabetes. J Diabetes Complicat 2009;23:102–111.
4. Manfredi S, Calvi D, del Fiandra M, Botto N, Biagini A, Andreassi MG. Glutathione *S*-transferase T1- and M1-null genotypes and coronary artery disease risk in patients with Type 2 diabetes mellitus. Pharmacogenomics 2009;10:29–34.
5. Lapshina EA, Sudnikovich EJ, Maksimchik JZ, et al. Antioxidative enzyme and glutathione *S*-transferase activities in diabetic rats exposed to long-term ASA treatment. Life Sci 2006;79:1804–1811.
6. Awasthi YC, Sharma R, Singhal SS. Human glutathione *S*-transferases. Int J Biochem 1994;26:295–308.
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. Fujita H, Narita T, Meguro H, et al. No association of glutathione *S*-transferase M1 gene polymorphism with diabetic nephropathy in Japanese type 2 diabetic patients. Ren Fail 2000;22:479–486.
9. Kang ES, Kim MS, Kim CH, et al. Association of common type 2 diabetes risk gene variants and posttransplantation diabetes mellitus in renal allograft recipients in Korea. Transplantation 2009;88:693–698.
10. Singh R, Manchanda PK, Kesarwani P, Srivastava A, Mittal RD. Influence of genetic polymorphisms in GSTM1, GSTM3, GSTT1 and GSTP1 on allograft outcome in renal transplant recipients. Clin Transplant 2009;23:490–498.
11. American Diabees Association. Clinical Practice Recommendations. Diabetes Care 2005;28 Suppl 1:S1–S79.
12. Chen MK, Tsai HT, Chung TT, et al. Glutathione *S*-transferase P1 and alpha gene variants; role in susceptibility and tumor size development of oral cancer. Head Neck 2010;32:1079–1087.
13. Pham PT, Pham PC, Lipshutz GS, Wilkinson AH. New onset diabetes mellitus after solid organ transplantation. Endocrinol Metab Clin North Am 2007;36:873–890.
14. Davidson J, Wilkinson A, Dantal J, et al. New-onset diabetes after transplantation: 2003 International consensus guidelines. Proceedings of an international expert panel meeting. Barcelona, Spain, 19 February 2003. Transplantation 2003;75: SS3–SS24.
15. Balla A, Chobanian M. New-onset diabetes after transplantation: A review of recent literature. Curr Opin Organ Transplant 2009;14:375–379.
16. Hayes JD, Strange RC. Glutathione *S*-transferase polymorphisms and their biological consequences. Pharmacology 2000;61:154–166.
17. Zhong SL, Zhou SF, Chen X, et al. Relationship between genotype and enzyme activity of glutathione *S*-transferases M1 and P1 in Chinese. Eur J Pharm Sci 2006;28:77–85.
18. Ping J, Wang H, Huang M, Liu ZS. Genetic analysis of glutathione *S*-transferase A1 polymorphism in the Chinese population and the influence of genotype on enzymatic properties. Toxicol Sci 2006;89:438–443.
19. Pagliuso RG, Abbud-Filho M, Alvarenga MP, et al. Role of glutathione *S*-transferase polymorphisms and chronic allograft dysfunction. Transplant Proc 2008;40:743–745.
20. Zahmatkesh M, Kadkhodaee M, Mahdavi-Mazdeh M, et al. Oxidative stress status in renal transplant recipients. Exp Clin Transplant 2010;8:38–44.
21. Dominguez C, Ruiz E, Gussinye M, Carrascosa A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. Diabetes Care 1998;21:1736–1742.