Contribution of *GSTM1*, *GSTT1*, and *MTHFR* polymorphisms to end-stage renal disease of unknown etiology in Mexicans

B. E. Gutiérrez-Amavizca[1,2], R. Orozco-Castellanos[3], R. Ortíz-Orozco[1], J. Padilla-Gutiérrez[4], Y. Valle[4], N. Gutiérrez-Gutiérrez[3], G. García-García[3], M. Gallegos-Arreola[5], L. E. Figuera[1,2]

[1]Division of Genetics, Biomedical Research Center of the West, IMSS, Guadalajara, [2]PhD in Human Genetics, University Center of Health Sciences, University of Guadalajara, Guadalajara,[3]Nephrology Service, Hospital Civil de Guadalajara "Fray Antonio Alcalde", Guadalajara, [4]Department of Clinics, University Center of Health Sciences, University of Guadalajara, Guadalajara, [5]Division of Molecular Medicine, Biomedical Research Center of the West, IMSS, Guadalajara, Jalisco, México

ABSTRACT

Oxidative stress is increased in chronic kidney disease, owing to an imbalance between the oxidative and antioxidant pathways as well as a state of persistent hyperhomocysteinemia. The enzymes glutathione S-transferases (GSTs) and methylenetetrahydrofolate reductase (*MTHFR*) are implicated in the regulation of these pathways. This study investigates the association between polymorphisms in the Glutathione S-transferase Mu 1 (*GSTM*1), glutathione S-transferase theta 1 (*GSTT*1), and *MTHFR* genes and end-stage renal disease (ESRD) of unknown etiology in patients in Mexico. A Case-control study included 110 ESRD patients and 125 healthy individuals. *GSTM*1 and *GSTT*1 genotypes were determined using the multiplex polymerase chain reaction (PCR). The *MTHFR* C677T polymorphism was studied using a PCR/restriction fragment length polymorphism method. In ESRD patients, *GSTM*1 and *GSTT*1 null genotype frequencies were 61% and 7% respectively. *GSTM*1 genotype frequencies differed significantly between groups, showing that homozygous deletion of the *GSTM*1 gene was associated with susceptibility to ESRD of unknown etiology ($P = 0.007$, odds ratios = 2.05, 95% confidence interval 1.21-3.45). The *MTHFR* C677T polymorphism genotype and allele distributions were similar in both groups ($P > 0.05$), and the CT genotype was the most common genotype in both groups (45.5% and 46.6%). Our findings suggest that the *GSTM*1 null polymorphism appears to be associated with the ESRD of unknown etiology in patients in Mexico.

Key words: End-stage renal disease, glutathione S-transferases, methylenetetrahydrofolate reductase, polymorphism

Introduction

Chronic kidney disease (CKD) is a global public health problem. The causes of CKD are heterogeneous, ranging from infectious diseases and metabolic multisystemic disease to congenital and genetic disorders. This variety of possible etiologies makes it difficult to identify the mechanisms involved in its pathogenesis.[1] In many cases, the etiology remains unknown.

The annual incidence of end-stage renal disease (ESRD) in Mexico is 346 cases per million people (pmp), with prevalence of 929 pmp.[2] An estimated 8.5% of the Mexican population have CKD,[3] and close to 60,000 individuals are on dialysis.[4]

Oxidative stress plays a key role in the pathogenesis and progression of CKD. The balance between the oxidative and antioxidant pathways is regulated by many factors. The glutathione S-transferases (GSTs) are a group of enzymes that participate in the biotransformation and detoxification of xenobiotics and endogenous substances; they catalyze the conjugation of glutathione with electrophilic xenobiotics. The GST superfamily is encoded by 16 genes, and at least seven different types of GST have been identified thus far.[5]

Address for correspondence:
Dr. Luis E. Figuera, Sierra Mojada 800, Zipcode 44340, Guadalajara, Jalisco, Mexico.
E-mail: luisfiguera@yahoo.com

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Glutathione S-transferase Mu 1 (*GSTM*1) (Online Mendelian Inheritance in Man, OMIM 138350) and glutathione S-transferase theta 1 (*GSTT*1) (OMIM 600436) are highly expressed in the human kidney, and they are located on chromosomes 1p13.3 and 22q11, respectively. Null genotypes of both genes, homozygous deletions, lead to a lack of expression of their respective enzymes. Therefore, the null polymorphisms of *GSTM*1 and *GSTT*1 result in a decreased antioxidant defense,[6] high accumulation of reactive oxygen metabolites, and consequent loss of renal function.[7]

A few studies have demonstrated the association of genetic polymorphisms of GST with the development of CKD, mostly in diabetes, without any conclusive results.[8-10] However, patients with ESRD have total serum homocysteine three times higher than the general population.[11] Hyperhomocysteinemia is common among hemodialysis patients and may result from genetic defects in enzymes involved in homocysteine metabolism.[12] Recent studies have associated the methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms with the diabetic nephropathy, and hyperhomocysteinemia with microalbuminuria.[13] Therefore, the presence of the *MTHFR* C677T (rs1801133) polymorphism might influence the clinical course and progression of CKD. This study investigates the association between *GSTM*1, *GSTT*1, and *MTHFR* polymorphisms in the context of ESRD of unknown etiology in Mexican patients.

Subjects and Methods

Study design

This was a case-control study designed to investigate the association of GST and *MTHFR* polymorphisms in ESRD patients. All patients and healthy subjects provided written informed consent to participate in this study, in adherence with the Declaration of Helsinki and the Mexican regulations for health and research. This study was approved by the Internal Review Board of the Hospital Civil "Fray Antonio Alcalde" in Guadalajara, Jalisco, Mexico.

Patients

We recruited 110 patients with ESRD of unknown etiology who were referred to the Hospital Civil of Guadalajara "Fray Antonio Alcalde". ESRD of unknown etiology was defined as ESRD with a glomerular filtration rate of <15 mL/min/1.73 m^2 or dialysis persisting for at least 3 months with no identifiable cause and not associated with any known risk factors (e.g., type 2 diabetes mellitus, essential hypertension, glomerulonephritis, infections, drugs, etc.,). A complete medical history was obtained. All patients had been on dialysis for at least 3 months. The reference group included 125 healthy individuals from Guadalajara, Jalisco, Mexico.

Deoxyribonucleic acid (DNA) extraction and genotyping

Genomic DNA was extracted from 10 mL of peripheral venous blood, collected in EDTA, according to the Miller method.[14] *GSTM*1 primers sequences were: 5′-TATGCAGCTGGGCATGATCT-3′ and 5′-TCAATGACAGCACTCAGAAAACT-3′. *GSTT*1 primers sequences were 5′-CTGACCTCGTAGCCATCACG-3′ and 5′-ACCCAGGGCATCAGCTTCT-3′.

*GSTM*1 and *GSTT*1 genotypes were determined using multiplex polymerase chain reaction (PCR). Three sets of primers were used to amplify a 412-bp fragment of the *GSTT*1 gene, a 460-bp fragment of the *GSTM*1 gene, and a 268-bp fragment of the β-globin gene as an amplification control. The PCR amplification of 20 ng of genomic DNA was performed in a total volume of 10 μL, containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.8 pM each primer, and 0.02 U Taq polymerase. PCR conditions required denaturation for 4 min at 94°C; followed by 30 cycles of 30 s at 94°C (denaturation), 1 min at 61°C (annealing), and 1 min at 72°C (elongation); with a final elongation step of 10 min at 72°C. PCR controls (known genotype) were included in every batch of PCR samples. In addition, null genotypes were assayed twice, as an additional quality control. The primer sequences and thermal conditions employed to amplify the *MTHFR* polymorphism were published previously by Gallegos-Arreola *et al.*[15] The PCR products were analyzed using electrophoresis in a 6% polyacrilamyde gel (29:1), followed by silver staining.

Statistical analysis

Allele frequencies were determined by counting, and the distribution of genotypes in both groups was compared using the χ^2 test or Fisher's exact test. Statistical estimates were assayed using the SPSS v. 20.0 software. Hardy-Weinberg equilibrium was tested using the χ^2 test. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to estimate the associations between genotypes and disease. P <0.05 was considered statistically significant.

Results

The mean age of patients was 29.24 ± 15.48 (range 15-90) years and 74.5% were younger than 30 years; 76% were male. The mean age at diagnosis was 27.56 ± 15.50 (range 5-90) years. All patients were on dialysis for a mean duration of 2.11 ± 2.09 (range 1-12) years. About 30%

were on continuous ambulatory peritoneal dialysis, 38% were on automated peritoneal dialysis, and 32% were on hemodialysis. The demographic characteristics of patients with ESRD are displayed in Table 1. Demographic and laboratory characteristics of the reference group were within normal parameters.

Genotype and allele frequencies of *GSTM*1, *GSTT*1, and *MTHFR* polymorphisms are shown in Table 2. Genotype distributions in the reference group were in agreement with the Hardy-Weinberg equilibrium. *GSTM*1 genotype frequencies differed significantly between groups; we observed that the homozygous deletion of *GSTM*1 is a risk factor for ESRD of unknown etiology ($P = 0.007$, OR = 2.05, 95% CI = 1.21-3.45). Genotypic differences were not observed regarding the *GSTT*1 polymorphism ($P = 0.35$). The CT genotype of the *MTHFR* polymorphism was commonly present in both ESRD patients and healthy participants (45.5% vs. 46.6%, respectively). Genotype and allele distributions did not differ significantly between groups ($P > 0.05$). The association between genotype combination and risk of ESRD of unknown etiology was significant among *GSTM*1/*GSTT*1 null carriers ($P = 0.01$, OR = 1.94, 95% CI = 1.14-3.32). *GSTM*1 and *GSTT*1 genotype and allele frequencies were also compared with other populations, confirming their genetic variability among populations [Figure 1].

Discussion

CKD patients are exposed to oxidative stress as a consequence of an increase in reactive oxygen species or a decrease in antioxidant defense, which in turn leads to the progressive deterioration of kidney function.[16] The GSTs constitute a superfamily of enzymes that prevent oxidative stress damage. GST polymorphisms may influence responses to damage induced by oxidative stress, and therefore may be involved in the development and progression of CKD. *GSTM*1 and *GSTT*1 null polymorphisms are caused by the deletion of the gene, and have been studied the most.[17]

Recent studies have demonstrated increased expression of GSTs in epithelial cells of the proximal tubule during the early stage of diabetes, likely in response to oxidative stress triggered by hyperglycemia or other toxic effects of glucose.[18] It has also been reported that the *GSTM*1+ genotype is associated with better survival in elderly peritoneal dialysis patients in the Chinese population.[19] Lin *et al.*, reported that the *GSTM*1 null genotype approximately doubled the risk for all-cause mortality among hemodialysis patients; patients without *GSTM*1 activity are more susceptible to oxidative stress and are at greater risk for death compared with those who possess *GSTM*1 activity. In addition, Lin *et al.,* observed that, among maintenance hemodialysis patients, the *GSTM*1 null genotype was associated with a significantly lower antioxidant capacity than the *GSTM*+ genotype.[20]

The null/low polymorphisms of the *GSTM*1 and *GSTT*1 genes have been associated with the risk of developing ESRD in North Indian patients.[17] In addition, Singh *et al.*, reported that patients with transplant therapy demonstrated an increasing trend toward carrying the *GSTM*1 null genotype (51.3%) versus healthy controls (40.4%) with a risk of about 1.5-fold ($P = 0.035$), and patients with a variant genotype of *GSTM*1 were at the higher risk of transplant rejection.[21]

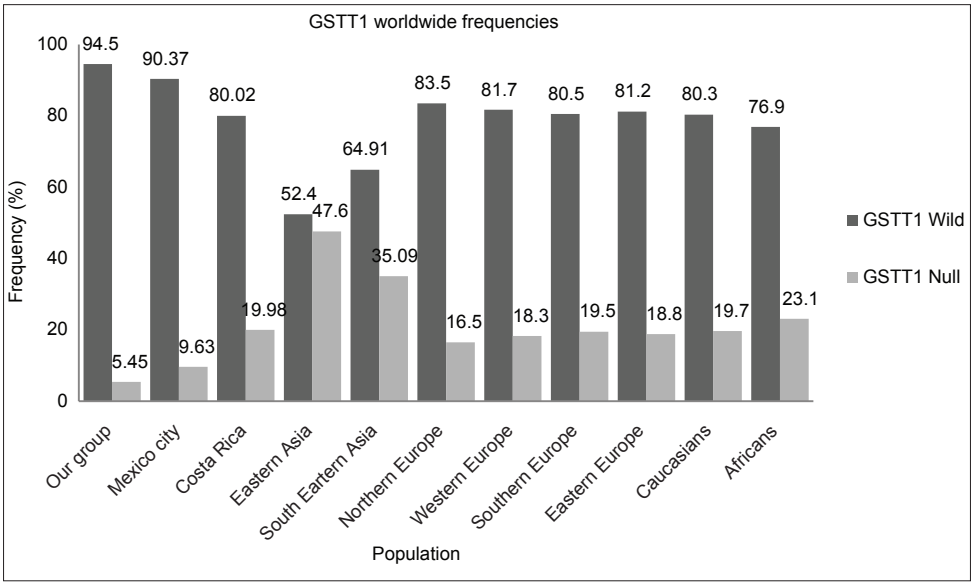

Figure 1: Glutathione S-transferase Mu 1 worldwide allele frequencies (wild and null), including 10 populations in addition to our group

The frequencies of important functional mutations and alleles result in broad ethnic variation. The frequencies reported for homozygous *GSTM*1 deletions are approximately 50% in Caucasian, 21.7% in Nigerian, 43% in French, and 58.3% in Chinese populations.[22] The *GSTT*1 null polymorphism is present in 13-26% of Asians and in 35%-52% of Caucasians.[23] The combined deletion frequency of both genes, *GSTM*1 and *GSTT*1, is 8% in Malaysia, 6% in North America, and 8% in Egypt.[24]

We compared the *GSTM*1 and *GSTT*1 frequencies obtained from this study with those reported elsewhere [Figures 1 and 2].[25,26] The *GSTT*1 comparison frequencies revealed that there are statistically significant differences among other populations ($P < 0.0001$), but not in Mexico City ($P = 0.05$). The *GSTM*1 frequencies in the Western Mexican population were statistically different from South-east Asian ($P = 0.004$), Northern European ($P = 0.0025$), Caucasian ($P = 0.032$), and African populations ($P < 0.0001$).

Regarding the genetic distribution of *GSTT* and *GSTM* among ESRD patients, there are a few studies reported mainly caused by diabetic nephropathy in Asian population.[8,10,17,19,20] The *GSTT* null frequency is quite low in our experimental population compared with North Indians[17] (7% vs. 58.7%, $P < 0.0001$); however, the *GSTM* null is more frequent in our group (60.6% vs. 46.7%, $P = 0.02$). Although these differences may be explained by the genetic structure of our population, it is important to consider that the etiology of ESRD may vary among these patient groups.

It is also important to highlight the limitations of this study, which detected only the presence or absence of the *GSTM*1 and *GSTT*1 gene; gene dosage effects could not be assessed. Another limitation is that the small population size. In addition, the enzyme activities of GST and *MTHFR* were not determined, and markers of oxidative stress, such as serum vitamin C, malondialdehyde, carbonyl, and reduced glutathione concentrations, were not measured, neither were homocysteine levels. We observed an association between ESRD of unknown etiology and the *GSTM*1 null deletion, but not *GSTT*1 or *MTHFR* C677T polymorphisms, in Mexican individuals in an attempt to associate the clinical variables with the genetic variants. However, additional controlled studies involving enzymes other than *GSTM*1 are required to elucidate whether

Table 1: Demographic characteristics of patients with ESRD of unknown etiology

	All patients (*n*=110)	*GSTM*1			*GSTT*1			*MTHFR* C677T			
		Present (*n*=43)	Null (*n*=67)	*P*	Present (*n*=101)	Null (*n*=9)	*P*	C/C (*n*=35)	C/T (*n*=50)	T/T (*n*=25)	*P*
Demographics											
Age, years	29.2±15.4	31.33±16.4	27.90±14.7	NS	29.58±15.8	26.25±9.9	NS	26.17±11.4	27.60±13.6	36.8±21.1	NS
Sex, F/M	26/84	12/31	14/53	-	25/76	1/7	-	10/25	13/37	3/22	-

Values represent the mean±standard deviation, F/M: Female/male, ESRD: End-stage renal disease, *GSTM*1: Glutathione S-transferase Mu 1, *GSTT*1: Glutathione S-transferase theta 1, *MTHFR*: Methylenetetrahydrofolate reductase, NS: Not significant

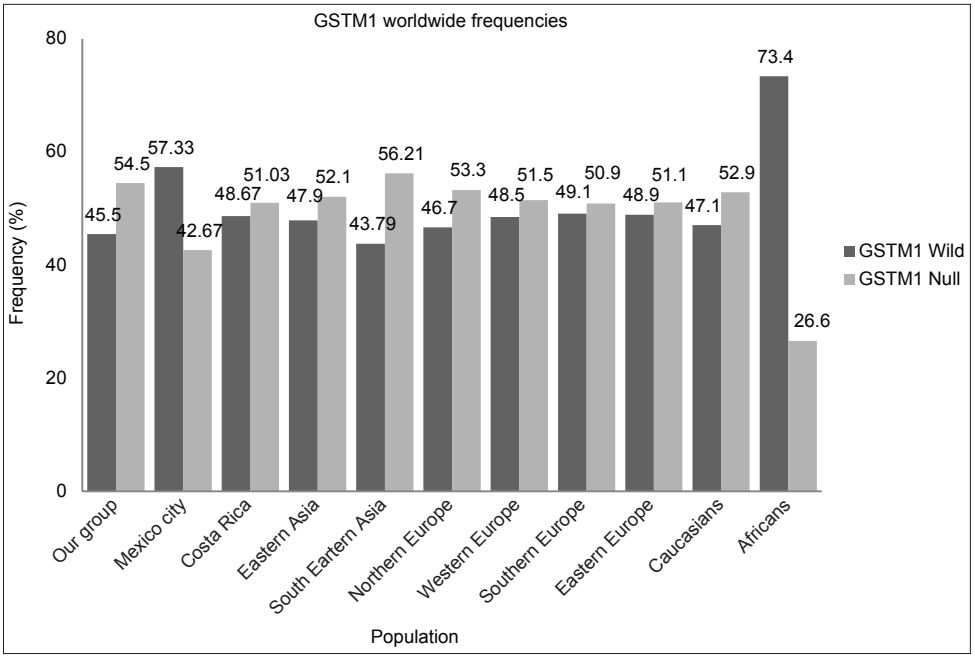

Figure 2: Glutathione S-transferase theta 1 worldwide allele frequencies (wild and null), including 10 populations in addition to our group

Table 2: Genotype and allele frequencies of *MTHFR* C677T, *GSTM*1, and *GSTT*1 polymorphisms in patients with ESRD of unknown etiology and healthy participants

Polymorphism	CKD (*n*=110)	Control (*n*≥125)	OR (95% CI)	*P*
MTHFR C677T				
Genotype				
C/C	35 (32)	59 (35)	1	
C/T	50 (45)	79 (46)	1.07 (0.62-1.85)	0.82
T/T	25 (23)	32 (19)	1.32 (0.67-2.57)	0.42
Allele				
C	120 (55)	197 (58)	1	
T	100 (45)	143 (42)	1.15 (0.82-1.62)	0.43
*GSTT*1 null				
Present	101 (93)	121 (97)	1	
Null	9 (7)	4 (3)	2.70 (0.80-9.01)	0.15*
*GSTM*1 null				
Present	43 (39)	71 (57)	1	
Null	67 (61)	54 (43)	2.05 (1.21-3.45)	0.007
Gene combination				
*GSTT*1/*GSTM*1	41 (37)	69 (55)	1	
*GSTT*1/*GSTM*1^{null}	60 (55)	52 (42)	1.94 (1.14-3.32)	0.01
*GSTT*1^{null}/*GSTM*1	2 (2)	2 (1.6)	1.68 (0.23-12.4)	0.63*
*GSTT*1^{null}/*GSTM*1^{null}	7 (6)	2 (1.6)	5.89 (1.17-29.6)	0.03*

All figures in parentheses are percentages, CKD: Chronic kidney disease, Control: Healthy subjects, ESRD: End-stage renal disease, *Fisher's exact test, *GSTM*1: Glutathione S-transferase Mu 1, *GSTT*1: Glutathione S-transferase theta 1, *MTHFR*: Methylenetetrahydrofolate reductase, OR: Odds ratio, CI: Confidence interval

genetic factors participate in the modulation of glomerular filtration rate.

Our results suggest that the presence of the *GSTM*1 null genotype, alone or in combination with the *GSTT*1 null genotype, might be associated with an increase in oxidative stress and susceptibility to ESRD of unknown etiology in the Mexican population, possibly because of the diminished expression of the *GSTM*1 enzyme, which results in a reduced ability to defend against oxidative stress. Changes in its function might contribute to the development of ESRD through the increase of oxidative stress or the production of free radicals, or might be associated with other factors, such as pollution, nutritional status, smoking, or gene-environment interactions. Additional studies are needed to understand the roles of these genes in the development of ESRD and to confirm these results. In conclusion, the *GSTM*1 null allele may be is a risk factor for ESRD of unknown etiology in Mexican individuals.

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