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Distribution of Glyoxalase-I Polymorphism among Zuni Indians: The Zuni Kidney Project

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Abstract

The Zuni Indians are experiencing simultaneous epidemics of type 2 diabetes mellitus (T2DM) and renal disease (Scavini et al., 2003 and Shah et al., 2003). Methylglyoxal, a highly reactive, cytotoxic, cross-linking endogenous aldehyde involved in modification of biological macromolecules, is elevated among patients with T2DM. Glyoxalase-I (Glo1) is the initial enzyme involved in the detoxification of methylglyoxal. Glo1 is a dimeric enzyme with three isoforms, Glo1-1, Glo2-1, and Glo2-2, resulting from a point mutation (A→C) at position 332 of the cDNA. The present study was conducted to explore the hypothesis that specific polymorphisms of Glo1 gene are associated with diabetes and/or albuminuria in Zuni Indians.

We studied four groups of Zuni Indians stratified by diabetes status and albuminuria assessed by urinary albumin:creatinine ratio (UACR): Group I: normal controls; Group II: T2DM and UACR <0.03; Group III: T2DM and UACR ≥0.03; and Group IV: non-diabetic participants with UACR ≥ 0.03. Genomic DNA was used as template for PCR amplification of the Glo1 gene. Products were digested to yield 110bp band (homozygous, CC), 54bp and 45bp bands (homozygous, AA), or all three bands (heterozygous, CA). Data on age, gender, UACR, serum creatinine, HbA1c, serum glucose, systolic and diastolic blood pressures and the duration of T2DM among participants in groups II and III were analyzed using analysis of variance. A generalized linear model logistic regression analysis was used to assess the relationships between specific Glo1 polymorphisms to T2DM and UACR.

All three Glo1 genotypes were present among the Zuni Indians. There were no significant differences in the distributions of Glo1 genotypes among the study groups (Chi-square, P=0.5590). The prevalence of the Glo1 A allele was higher among diabetic (Groups II and III combined) versus non-diabetic participants (Groups I and IV combined) (Chi-square (p=0.0233). There was an association (Odds Ratio = 2.9, 95% CI 1.3–7.2) between the Glo1 A allele and T2DM.

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Introduction

The chronic complications of type 2 diabetes mellitus (T2DM), including retinopathy, nephropathy, neuropathy, and vasculopathy, present a major public health challenge. However, patients with T2DM do not uniformly develop these complications (Sale et al., 2004; and Rotimi et al., 2004). Moreover, both the age at onset and the rate of progression of diabetic complications vary significantly among different racial and ethnic groups. Therefore, several investigators have postulated that genetic factors may modulate susceptibility to and/or progression of diabetic complications (Klemm and Paschke, 2000; McCarthy, 2003; Silander et al., 2004; Sale et al., 2004; and Rotimi et al., 2004)

The Zuni Indians live in a rural portion of New Mexico in the United States. There has been little immigration or emigration and the population have remained relatively endogamous (Personal communication -Office of the Zuni Tribal Census: Tribal Census 2000). The Zuni Indians are currently experiencing epidemics of T2DM and renal disease (Stidley et al., 2002 and 2003; Shah et al., 2003). The majority of diabetes among the Zuni Indians is T2DM (Long TP, 1978; Carter et al., 1989). The Zuni Kidney Project (ZKP) is a community based participatory research project which as been funded by the National Institutes of Health (NIH) since 1996. The mission of the ZKP is to reduce the burden of renal disease. The ZKP is currently conducting a study entitled the Genetics of Kidney Disease in Zuni Indians (GKDZI) designed to identify susceptibility genes for T2DM, diabetic nephropathy and non-diabetic renal disease.

The ubiquitous glyoxalase system is composed of two enzymes glyoxalase I and glyoxalase II. It is one of several defense systems designed to protect against production of advanced glycation end products (AGEs), which are important in the pathogenesis of diabetic complications (Friedman, 1999; Chen et al., 2004). Production of methylglyoxal (MG), a reactive, cytotoxic cross-linking aldehyde and a precursor to AGEs, is increased among patients with DM (Thornalley PJ, 1991, 1996 and 2003). Glyoxalase-dependent conversion of MG to D-lactate protects against production of AGEs from MG (Ratliff et al., 1996; Vander Jagt and Hunsaker, 2003). Glyoxalase I catalyzes the conversion of MG to D-S-lactoylglutathione by a glutathione-dependent reaction, and glyoxalase II catalyzes the hydrolysis D-lactoylglutathione into D-lactate.

The gene encoding glyoxalase I (Glo1) has a common single nucleotide polymorphism (SNP) (reported in dbSNP as rs2736654) with either an A or C in position 332. This point mutation (A→C) results in alanine (Glo1-Ala) (C332) or glutamic acid (Glo1-Glu) (A332) in protein residue 111. The presence of the additional acidic charge from the 111Glu residue results in a conformational change of the protein and decreased enzyme activity (Pierluigi et al., 2005). There are three glyoxalase enzyme phenotypes, Glo1-1 (Glo1-Ala), Glo2-1 (Glo1-Ala/Glo1-Glu) and Glo2-2 (Glo1-Glu) (Kim et al., 1993, 1995; Ranganathan et al., 1993; Ridderstrom and Mannervik, 1996).

Several investigators have compared the distributions of Glo1 phenotypes and/or genotypes among diabetic and non-diabetic participants selected from different population samples (Gale et al., 2004; Junaid et al., 2004; Piskorska and Kopieczna 1998; Pierlugi et al., 2005; Sinohara et al., 1998; Subramanian et al., 1994). However, these previous studies have yielded conflicting results. The prevalence of the Glo1 A allele was increased among Australian patients with type 1 diabetes mellitus. In contrast, McCann et al observed no differences in the distributions of Glo1 A allele among Australians with T2DM versus normal controls (McCann et al., 1981). Moreover, studies conducted among Japanese (Tokunaga et al., 1982; Mimura et al., 1990) and South Indian population samples (Kirk et al., 1985; Allanic et al., 1985) found

no differences in the distributions of Glo1 genotypes among diabetic and non –diabetic subjects.

The present study was conducted to explore the hypothesis that polymorphisms of the Glo1 gene may be associated with T2DM and albuminuria among the Zuni Indians. Our study had two specific aims: (1) determine which Glo1 alleles are expressed in the Zuni Indians; (2) determine if the distributions of Glo1 alleles differ by diabetes status and/or the degree of albuminuria.

Materials and Methods

Study Population

We identified potential participants for the present study from people who previously took part in the population-based, cross-sectional survey conducted by the ZKP. Participants in the ZKP were stratified into four groups by diabetes status and degree of albuminuria assessed by urinary albumin:creatinine ratios (UACR) in a single spot urine sample. Participants were considered to have diabetes if they had ≥1 of the following criteria: (a) prior history of diabetes; (b) random serum glucose ≥ 200 mg/dl; and (c) $HbA1_c$ >7.0%. Participants were considered to have albuminuria if the UACR was ≥ 0.03. This classification scheme was used to define the four study groups: Group I: normal controls; Group II: participants with more than an eight year history of T2DM and a UACR <0.03; Group III: participants with T2DM and UACR ≥0.03; Group IV: non-diabetic participants with a UACR ≥0.03. A computer generated random numbering system was used to select participants from each of the four groups. A restriction on age (≥30 years) was placed on participants in group I in order to reduce the possibility of misclassification since these subjects may subsequently develop T2DM. No restrictions on age were placed on any of the other groups.

Molecular Biology Procedures

DNA isolation—Genomic DNA was extracted from PBMCs obtained from the participants and stored at −80°C. These samples were used as templates for PCR amplification of the Glo1 gene.

Primer Design—The nucleotide databases of Pubmed (National Library of Medicine) and Ebioinformatics (www.ebioinformatics.com) were used to examine the Glo1 genome. A twenty bp sense primer 42bp upstream of exon 3 (in the region of intron 2), and another 20bp antisense primer 41bp downstream from the Glo1 polymorphic site in exon 3 were designed using the Primer 3 program (www-genome.wi.mit.edu). The Glo1 genome region flanked by these primers (#4741 – #4870 bp in the genomic sequence) failed to show any restriction sites for BsaI/BsmaI other than the previously known site at the polymorphic position 332 of the cDNA sequence (www.biotools.umassmed.edu). The A to C substitution at this site removes the BsaI/BsmaI restriction site that is present in the ancestral gene. Primers were obtained from Integrated DNA technologies, Inc (IDT®).

Gene amplification, cleanup, digestion, and visualization—Patient genomic DNA samples were assigned a coded identification numbers prior to use for PCR to comply with applicable HIPPA regulations. PCR was performed using the Applied Biosystems Gene Amp® PCR system 9700. The reaction included a denaturation step at 95°C for 4 min, 35 cycles of 95°C at 1min, 56°c at 1min, and 72°C at 1 min, and an extension step at 72°C for 7 min. PCR products were run on 2% agarose gel (NuSIEVE® GTG agarose, FMC® Bioproducts) for visualization. Samples were then purified using Qiaquick® PCR purification kit by using the enclosed protocol. Sample DNA concentration was measured on a Beckman DUR 640 spectrophotometer. DNA was digested with Bsma1 endonuclease (New England Biolabs Inc.)

overnight at 37°C. One to three ul of digested samples were run on Agilent 2100 Bioanalyzer using the DNA 500 Assay kit, DNA Lab chip®, and the provided protocol of Agilent Technologies.

The virtual gels obtained were then evaluated for presence of bands (110bp, 54bp, 45bp) for the homozygous or heterozygous polymorphism. Once genotype assignment was performed, it was verified independently by 2 other laboratory personnel.

Statistical Analysis

Statistical analyses were performed using Statistical Analysis System ™ (SAS) (SAS Institute Inc, Cary, NC). Differences in demographic and clinical laboratory parameters between the four study groups were assessed using analysis of variance (ANOVA). Chi-square tests were used to determine if the distributions of Glo1 genotypes differed by gender, diabetes status and/or UACR. ANOVA was used to assess the relationships between Glo1 polymorphism and albuminuria and HbA1c. Data are shown as mean and standard deviation (SD). Statistical significance was defined as $p<0.05$.

Results

Demographics

The demographic characteristics of the four study groups are shown (Table 1). Participants in Group IV were younger than those in the Groups I, II and III ($p< 0.0001$). The ages of participants in groups I, II and III were similar (NS). The proportions of females among groups I, II and III were higher than in Group IV.

Clinical parameters

The clinical laboratory values are shown (Table 2). As anticipated from the experimental design the UACR values were higher among participants in groups III and IV compared to Groups I and II ($p<0.0001$). UACR was highest among participants in Group III ($p<0.001$). Similarly, as anticipated, HbAlc levels were higher among participants in Groups II and III compared to Groups I and IV ($p<0.001$). Serum glucose was higher among the two groups consisting of diabetic participants. Serum creatinine values were similar among all groups (NS). The duration of DM was similar among participants in Groups II and III (NS). Systolic and diastolic blood pressures were similar across all groups.

Distribution and relationship of alleles to albuminuria and diabetes

The distribution of alleles by study groups is shown (Table 3). There were no significant differences in the distributions of Glo1 genotypes by study groups (Chi-square, $p=0.0868$). The prevalence of the Glo1 A homozygous allele was higher among participants with T2DM (28.6%) compared to those without T2DM (23.5%). Among all participants with T2DM combined (Table 4), 90% had at least one copy of the Glo1 A allele compared to 76% of the non-diabetic participants ($p=0.02$). There was an association (Odds Ratio = 2.9, 95% CI 1.3–7.2) between the Glo1 A allele and T2DM.

ANOVA revealed no significant differences in either mean UACR levels ($p=0.559$) or mean HbA1c levels ($p=0.107$) by Glo1 A allele groups for the study population as a whole. Frequency (percent) of Glo1 genotypes, stratified by UACR are presented in table 5. There were no significant differences in serum creatinine levels, systolic and diastolic blood pressure, respectively, between four groups.

Discussion

Previous investigators have reported heterogeneity in the distribution of Glo1 genotypes among selected populations (Allanic et al., 1985; McCann et al., 1981; Mimura et al., 1990; Miyata et al., 2001; Tokunga et al., 1982; Gale et al, 2004; Junaid et al, 2004; Piskorska and Kopieczna 1998; Subramanian et al, 1994). Subramanian *et al* observed an association (Relative Risk = 4.06) of the Glo2-1 genotype with T2DM among a South Indian population (Subramanian et al., 1994), In contrast, Kirk et al observed no differences in the distributions of Glo1 genotypes between people with and without T2DM in another South Indian population sample (Kirk et al., 1985).

To our knowledge the present study is the first to examine the distributions of Glo1 genotypes among an American Indian population. Our study, conducted in a relatively endogamous population demonstrates several important new findings which may further our understanding of the epidemiology of T2DM among American Indians. First, we demonstrated that the Glo1 AA, AC and CC genotypes were each present among the relatively endogamous Zuni population. Second, we demonstrated that the presence of ≥ 1 copy of the Glo1 A allele was associated with T2DM among the Zuni Indians. Third, we were unable to demonstrate an association between any of three Glo1 alleles and albuminuria or HbA1c levels.

The present study has several important strengths. First, the population studied was relatively endogamous. Second, diabetes status and UACR were assessed in accord with current methodology accepted for epidemiologic studies. Third, genetic studies designed to identify susceptibility genes for diabetes mellitus and renal phenotypes are relatively uncommon among American Indians.

Unfortunately, the present study also has several important limitations. First, this was not a population based study since the participants were classified into groups and then randomly selected from each group. Second, the small sample size severely limited our statistical power which may have accounted for our inability to detect an association between a given Glo1 genotype and albuminuria. Third, an age restriction was not placed on participants in Group IV. This may have led to misclassification since some participants in Group IV may be at significant risk for developing T2DM. Even though the potential miss-classification of diabetics in the non diabetic group (group I and group IV combined), they in fact showed a difference in the Glo1 A allele prevalence. Fourth, AGEs were not measured thus we were unable to test associations between GloI genotypes and circulating AGE levels. Fifth, patients with elevated serum creatinine concentrations were not included in the study precluding us from testing for associations between Glo1 genotypes and advanced stages of chronic kidney disease.

In summary, the AA, AC and CC Glo1 genotypes were each present among the Zuni Indians. The Glo1 A allele was associated with T2DM. However, we were unable to detect an association between any of the three alleles and albuminuria. In conclusion, additional studies are needed to assess the relationship between Glo1 alleles and diabetes, renal disease and related intermediate phenotypes.

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Table 1

General characteristics of the Study Groups

	Group I	Group II	Group III	Group IV	P value
Participants	51	56	47	58	
Mean age, year	48.8 (9.4)	49.7 (13.5)	50.3 (14.4)	32.9 (14.9)*	<0.0001
Age range, year	40–73	21–81	21–80	6–84	
Female (%)	25 (49)	38 (68)	28 (60)	21 (36)	
Duration of diabetes, years		9.1(7.6)	10.2 (10.5)		0.581

Group I: normals; Group II type 2 diabetes mellitus (T2DM) with urinary albumin:creatinine ratio (UACR) <0.03; Group III T2DM with UACR ≥ 0.03; Group IV: non-diabetic with UACR ≥ 0.03; data presented as mean (SD);

* p<0.0001

Table 2

Laboratory and clinical parameters in the study groups

	Group I	Group II	Group III	Group IV	P value
UACR	7.8 (6.1)	10.9 (7.8)	683.6 (995.9)*	377.7 (717.5)*	<0.0001
Serum creatinine, mg/dl	0.9 (0.2)	0.8 (0.2)	0.9 (0.4)	0.9 (0.4)	0.207
$HbA1_c$, %	5.6 (0.6)	7.9 (2.1)*	9.4 (2.5)*	5.4 (0.5)	<0.0001
Serum glucose, mg/dl	109 (21.3)	191 (11.1)*	237 (12.0)*	100 (18.2)	<0.0001
Systolic BP, mmHg	126 (14.8)	142 (13.9)	132 (13.2)	129 (22.5)	0.6796
Diastolic BP, mmHg	82 (10.2)	80 (11.3)	81 (11.0)	86 (17.2)	0.0598

Group I: normals; Group II type 2 diabetes mellitus (T2DM) with urinary albumin:creatinine ratio (UACR) <0.03; Group III T2DM with UACR ≥ 0.03; Group IV: non-diabetic with UACR ≥ 0.03; data presented as mean (SD); Data presented as mean (SD);

*p<0.0001; $HbA1_C$ -Hemoglobin $A1_C$

Table 3

Frequency (percent) of glyoxylase I genotypes stratified by study group

Genotype	AA	CA	CC	Total (%)
Group I	12 (24)	25 (49)	14 (27)	51 (24)
Group II	16 (29)	37 (66)	3 (5)	56 (26)
Group III	10 (21)	30 (64)	7 (15)	47 (22)
Group IV	11 (19)	35 (60)	12 (21)	58 (27)
Total	49 (23)	127 (60)	36 (17)	212 (100)

Group I: normals; Group II type 2 diabetes mellitus (T2DM) with urinary albumin:creatinine ratio (UACR) <0.03; Group III T2DM with UACR ≥ 0.03; Group IV: non-diabetic with UACR ≥ 0.03; Data are expressed as counts (%). There were no significant differences in Glo1 allele distribution between study groups (Chi-square, P=0.0868).

Table 4

Frequency (percent) of glyoxylase I genotypes by diabetes status

Genotype	AA	CA	CC	Total (%)
Non-diabetic participants (groups I and IV combined)	23 (21.1)	60 (55.1)	26 (23.9)	109 (51.4)
Participants with type 2 diabetes mellitus (groups II and III combined)	26 (25.2)*	67 (65.1)*	10 (9.7)	103 (48.6)
Total	49 (23.1)	127 (59.9)	36 (17.0)	212 (100.0)

* The prevalence of allele A was higher among participants with type 2 diabetes mellitus than among non-diabetic participants; Chi square, p=0.0233.

Table 5

Frequency (percent) of glyoxylase I genotypes, stratified by urinary albumin:creatinine ratio (UACR)

Genotype	AA	CA	CC	Total (%)
UACR<0.03 (%)	28 (26.17)	62 (57.94)	17 (15.89)	107 (50.47)
UACR ≥ 0.03 (%)	21 (20.00)	65 (61.90)	19 (18.10)	105 (49.53)
Total (%)	49 (23.11)	127 (59.91)	36 (16.98)	212 (100.00)

Data are expressed as counts (%). There were no significant differences in the Glo1 distribution between participants with normal and increased UACR (Chi-square, p=0.5590).