

GENETICS OF DIABETES: ASSESSMENT OF A GENETIC VARIANT IN THE GLUCOSE TRANSPORTER 2 (SLC2A2) GENE AND SUSCEPTIBILITY TO DIABETIC NEPHROPATHY IN PIMA INDIANS

Individuals with diabetes have blood glucose levels that are too high due to peripheral insulin resistance and/or insufficient insulin secretion.¹ Pima Indians have very high rates of diabetes and diabetic nephropathy (or kidney disease resulting from diabetes) compared to other populations.¹ Both of the diseases have a strong genetic component. The goal of this study was to identify genetic determinants of diabetic nephropathy in Pima Indians.

SLC2A2 is an integral plasma membrane glycoprotein found in liver, beta cells, intestine and kidney epithelium, where it mediates facilitated bidirectional glucose transport. SLC2A2 variants contribute to renal hyperfiltration in Fanconi-Bickel patients, suggesting that gene impairment may lead to kidney dysfunction. SLC2A2 normally has a low affinity for glucose, but hyperglycemic conditions may provide enough substrate to drive glucose transport in the kidney. The SLC2A2 gene is located on chromosome 3q26, a region previously linked to diabetic nephropathy in this Native American population. For these reasons, we hypothesized that genetic variants in SLC2A2 may contribute to diabetic nephropathy susceptibility in Pima Indians. We addressed this hypothesis by identifying all genetic variation in the SLC2A2 coding sequence and genotyping these variants in a population of Pima Indians with diabetic kidney disease and unaffected diabetic controls. The techniques that used to achieve these goals included DNA amplification using the Polymerase Chain Reaction or PCR, DNA sequencing, and SNP genotyping.

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INTRODUCTION

Diabetes is a disease which results in a loss of glucose homeostasis due to lack of sufficient insulin action or secretion.¹ Diabetes is a heterogeneous disorder because it results from different origins and produces varying clinical symptoms among individuals. Type 1 diabetes is an auto-immune disease in which the body's immune system has destroyed the beta cells within the pancreas. Once the beta cells are destroyed, the body can no longer produce insulin.

In type 2 diabetes mellitus (T2DM), the pancreas does not make enough insulin or the body's cells become resistant to insulin. T2DM is a progressive disease and is most common in adults over the age of 40, whereas type 1 diabetes is more frequently diagnosed at a younger age. Some characteristics of T2DM are: 1) it occurs in people who are overweight; 2) it can be a "silent disease"; 3) it usually runs in families; and 4) it can be caused by a combination of heredity, insulin resistance, and deficiency of the insulin-producing beta cells of the pancreas.¹ T2DM is increasing within populations because of an escalated number of aging individuals and a greater prevalence of obesity and sedentary lifestyles. The risk of developing T2DM increases with: 1) family history of diabetes; 2) high cholesterol or hypertension; 3) high birth weight (9 pounds or larger); or 4) of Native American, Hispanic, African or Asian ancestry.²

T2DM is also associated with significant morbidity and mortality. For example, diabetic nephropathy, or kid-

ney disease resulting from diabetes, is a serious microvascular complication of T2DM, which may result in a loss of kidney function. There is evidence for a genetic effect for both T2DM and its complications because certain ethnic groups are more susceptible to developing the diseases and parents who have diabetes are more likely to give birth to children who will be diabetic.

Native Americans have a greater prevalence of T2DM and diabetic nephropathy, and there are significant genetic determinants underlying these diseases.³ Our hypothesis was that there are genetic components which are responsible for causing the Pima Indians to be more susceptible to developing diabetic nephropathy. Therefore, we conducted genomic screening in the Pima Indians, a Native American tribe indigenous to the southwestern United States. In Pimas, genetic susceptibility in the relatively homogenous population is present. They are primarily affected only with T2DM; type 1 diabetes is very rare.³ Recently, a genome-wide scan in Pima Indians was conducted and linkage was found to diabetic nephropathy on chromosome 3q.

The gene encoding glucose transporter 2 is located on chromosome 3q26. The goal of this study was to assess the role of variants in the SLC2A2 gene in the development of diabetic nephropathy in Pima Indians. Genetic variation is common and frequent among individuals and is represented in part by "mutations" known as single nucleotide polymorphisms (SNPs). Recently, SNPs have been used to map complex diseases with a genetic basis.

METHODS

Subjects

The participants selected for genetic screening are full heritage Pima/Tohono O'odham. A list of 215 individuals was chosen from the Gila River study for case-control comparisons of diabetic nephropathy. There are 107 cases with end-stage renal disease (ESRD) and 108 controls with long duration diabetes but no ESRD. None of the individuals were first degree relatives. Controls were selected from categories to have similar age, sex, and duration of diabetes.

DNA Amplification

The glucose transporter 2 genomic sequence was obtained from BAC RP11-654K19 (NCBI Accession AC061708). The SLC2A2 exons, exon-intron boundaries, and 1200 bp of 5' flanking sequence were screened by direct sequencing using genomic DNA obtained from 36 Pima Indians: 18 ESRD cases and 18 diabetic controls. DNA was amplified in a final reaction volume of 10 μ L using 60ng genomic DNA, 10 \times standard PCR buffer, 0.8 μ M dNTPs, 0.4 μ M oligonucleotide primers, and 0.5 U DNA polymerase mix (AmpliTaq Gold; Applied Biosystems; Foster City, CA). PCR cycling conditions consisted of an initial denaturation at 96°C for 7 minutes, followed by 35 cycles of 96°C for 20 seconds, 57°C for 30 seconds, and 72°C for 45 seconds, ending with a final elongation step at 72°C for 5 minutes.

DNA Sequencing

PCR product concentration was estimated by gel electrophoresis and depending on yield, 2.5–5 μ l of PCR product was treated at 37°C for 15 min/80°C for 15 min with 1–2 μ l of ExoSAP-IT[®] (USB; Cleveland, OH) to remove unconsumed dNTPs and oligonucleotide primers. Amplicons were bidirectionally sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosys-

tems) and 35 cycles of 96°C for 10 s, 55°C for 10 s, and 60°C for 4 m. After column purification, sequences were resolved on the AB 3730XL sequence analysis system (Applied Biosystems).

SNP Genotyping

SNPs were genotyped using the technique of allelic discrimination (AD-PCR). AD-PCR primers and probes were designed from published sequence using the Primer Express 2.0 software (Applied Biosystems). In some cases, Assays-on-Demand SNP Genotyping Products (Applied Biosystems), which are pre-designed assays containing optimized primer and probe sequences and concentrations, were available for SNPs detected by direct sequencing, and these were utilized for genotyping. Both approaches follow the same protocol. Sixty nanograms of each sample of genomic DNA was combined with the Taqman Universal PCR Master Mix (No AmpErase UNG at 1 \times concentration), SNP assay mix at 1 \times concentration, 0.25 U AmpliTaq Gold, and 0.3 μ g RNase A. PCR cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by two-step thermocycling for 40–55 cycles of 95°C/15 s and 60°C/60 s. After amplification, fluorescence will be measured on the ABI 7000 Sequence Detector using the allelic discrimination software Sequence Detection System Software Version 2.0 (Applied Biosystems). Genotypes are derived from FAM:VIC ratios, following normalization to a ROX reference signal.

Data Analysis

Sequencing chromatograms were analyzed using Sequencher software (Gene Codes Corporation; Ann Arbor, MI) and polymorphisms (single nucleotide polymorphisms [SNPs] and insertion/deletions) were identified by visual inspection. Minor allele frequency was determined by allele counting. Genotypic concordance was determined by assessing the pairwise correlation of alleles at

one locus with alleles at a second locus. The statistical evidence for association in cases and controls was assessed by analysis of contingency tables and the strength of the association between genotypes and affection status was assessed by the odds ratio, calculated by logistic regression. Logistic regression analysis was used to control for potentially confounding variables.

RESULTS

We screened approximately 6 Kb of the glucose transporter 2 gene corresponding to all exons, exon-intron boundaries, and promoter sequence. We identified three polymorphisms corresponding to a C/T variant in intron 4B, an A/G variant in intron 3, and a A/G variant in intron 1. The C/T variant was found to be rs8192675 in the national SNP database. The A/G variant (intron 3) was found to be rs7356034 and the A/G variant (intron 1) was found to be rs5396 in the national SNP database. All three of these SNPs were in 100% genotypic concordance. Thus, only one of the three SNPs, rs8192675, was genotyped. The association between rs8192675 and ESRD prevalence was assessed in a case-control study group. Associations between alleles at the SNP were evaluated by logistic regression, controlling for effects of age, sex, and duration of diabetes. Table 1 shows the results of the analysis conducted under an additive model, which assumes an additive effect contingent on the number of 'risk' alleles. No evidence for association between this SNP and ESRD prevalence was found under this model ($P=.90$).

Genotypic comparisons were assessed in 107 diabetic Pima individuals with end-stage renal disease (ESRD) and 108 diabetic control individuals with diabetes duration > 10 years. The frequency of the more common allele is listed in the second column. The most

Table 1. Association of rs8192675 with ESRD in Native Americans

SNP	Freq	GT	N (%ESRD)	OR (95% CI)	P-value
rs8192675	0.79 [T/C]	TT	135 (0.50)	0.97 (0.58, 1.62)	0.90
		TC	67 (0.49)		
		CC	6 (0.50)		

common genotype (GT) is listed first with the number and percentage of individuals with each genotype given. Odds ratios (OR) were calculated under an analytical model assuming an additive allele effect (eg, expressed per difference in number of alleles) with adjustment for age, sex, and duration of diabetes.

CONCLUSION

After analyzing the results, three SNPs were detected. We did not find evidence for association between this variant and ESRD in Native Americans and conclude that this gene does not likely contribute significantly to diabetic nephropathy risk in this population.

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