Investigation of PAXIP1L as a Candidate Gene for Diabetic Nephropathy

Student Researcher: Morganna Bradley
Mentor: Johanna Wolford, PhD

Type 2 diabetes occurs when the body either does not produce enough insulin or cannot use insulin efficiently. Uncontrolled diabetes causes damage to tissues in the kidneys, which can lead to a condition known as diabetic nephropathy. The gene encoding the PAX2 transcription activation domain-interacting protein, PAXIP1L, is located on chromosome 7q36 in a region that has previously been linked to diabetic nephropathy in Pima Indians and Caucasians. PAXIP1L is highly expressed in the human kidney; disruption of this gene may lead to renal hypoplasia and kidney dysfunction. This study investigated PAXIP1L as a candidate gene for diabetic nephropathy in the Pima Indians because this population has high rates of diabetes and diabetic kidney disease compared to other populations. Approximately 15 kb of PAXIP1L was sequenced in 36 Pima Indians with diabetes with or without nephropathy to identify variants within the gene. All polymorphisms were genotyped in 107 cases with diabetic end-stage renal disease (60 females/47 males; mean duration of diabetes = 20.4 ± 7.1 yrs) and 108 diabetic controls (74 females/34 males; mean duration of diabetes = 20.7 ± 5.5 yrs) and no evidence of macroalbuminuria (urinary albumin-creatinine ratio <300 mg/g). Allele distributions were compared between the two groups.

From the Translational Genomics Research Institute; Phoenix, Arizona.

Introduction

Type 2 diabetes, characterized by elevated blood glucose levels, can cause severe damage to the tissues in the kidneys if not adequately controlled and can lead to diabetic nephropathy. However, even individuals who have good glucose control develop diabetic nephropathy, indicating that other factors play a role in the development of diabetic nephropathy. In Pima Indians, there is evidence that susceptibility to diabetic nephropathy is strongly influenced by genetic factors.[1–2] The chromosome 7q36 region has been previously linked to diabetic nephropathy in Pima Indians and Caucasians. The candidate gene studied, PAXIP1L, is located in this region. PAXIP1L is highly expressed in the human kidney, where it combines with the p8 protein to modulate PAX2 transcription factor function. Disruption of this complex eventually leads to renal hypoplasia and kidney dysfunction. The gene was sequenced in 36 Pimas: 18 patients with end-stage renal disease resulting from diabetes (ESRD) and 18 patients with diabetes with no evidence of kidney dysfunction. All genetic variation within the exons and exon-intron boundaries were identified.

Methods

SNP Detection

The PAXIP1L genomic sequence was taken from BAC RP11-5C23 (NCBI Accession AC093726). All exons and exon-intron boundaries were sequenced using genomic DNA of the 18 ESRD cases and 18 diabetic controls. If the true sample allele frequency is 0.05, the power of this sample is 97% to detect such an allele [power = $1 - (1 - p_1)^{2n}$, where p_1 is the frequency of the minor allele and n is the number of individuals typed].

DNA was amplified in a final reaction volume of 10 μl using 60 ng genomic DNA, 10× standard PCR buffer, 0.8 μM dNTPs, 0.4 μM 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 minute, 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. Amplicons were bidirectionally sequenced by the TGen sequencing core using the BigDye Terminator v3.1 on the 3730 XL sequence analysis system (Applied Biosystems). 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.

Results

The average size of PCR amplicons used for sequencing was 740 base pairs. As shown in Table 1, a total of 25 variants were identified including 23 SNPs and two insertion/deletion polymorphisms. Nineteen of the variants were found in the dbSNP database and six were novel. Six SNPs were located in coding sequence, including two (SNP 43306 and rs3501) that produced amino acid substitutions.

The extent of genotypic concor-

Table 1. Name, location and variant, function and minor allele frequency of the polymorphisms

Name of SNP	Location	Variant	Function	Minor Allele Frequency
rs12539951	intron 1	C/G		0.14
rs306281	intron 3	G/A		0.28
rs306282	intron 3	C/T		0.28
rs3835185	intron 6	A+/−		0.14
57193*	intron 6	G/A		0.04
43406*	exon 8	G/A	Val-Val	0.06
43306*	exon 8	T/C	Thr-Ile	0.47
43009*	exon 8	G/A	Leu-Leu	0.31
rs2272176	intron 8	T/C		0.15
rs893942	intron 8	T/C		0.31
rs748809	intron 9	G/A		0.31
rs748810	intron 9	A/T		0.46
rs748811	intron 9	G/A		0.14
rs2272175	intron 10	T/C		0.15
rs2293264	intron 10	A/T		0.46
rs2293263	intron 10	T/C		0.31
rs2293261	intron 11	G/A		0.15
rs2293260	intron 11	A/T		0.4
rs2272174	intron 13	G/A		0.46
rs11325562	intron 14	[G+/−]		0.46
rs3817480	intron 15	G/A		0.3
28726*	exon 17	G/A	Val-Val	0.04
28693*	exon 17	G/A	Thr-Thr	0.01
rs3501	exon 19	T/C	Val-Met	0.46
rs930436	intron 21	T/C		0.48

* Accession AC093726.

dance, or the correlation of alleles at different SNP loci, was assessed for all identified SNPs. Strong evidence for genotypic concordance was found among variants in this gene, such that 20 polymorphisms segregated into five distinct clusters. The remaining five polymorphisms did yield alleles that corresponded with alleles at other loci and thus, represented unique SNPs.

CONCLUSION AND IMPLICATIONS

Because of its chromosomal location, its high expression in human kidney, and its putative role in the regulation of PAX2 transcription, PAXIP1L represents a biologically plausible candidate gene for potential effects on diabetic nephropathy susceptibility. We identified 25 polymorphisms, including two which result in amino acid substitutions, one a threonine to isoleucine shift in exon 8, the other a valine to methionine in exon 19. It is possible that these amino acid substitutions will have effects on the function of the protein; functional studies will be necessary to address this possibility.

ACKNOWLEDGMENTS

The author would like to thank Johanna Wolford, PhD who served as the project's mentor and Kim Yeatts for her assistance.

REFERENCES

1. Imperatore G, Knowler WC, Pettitt DJ, Kobes S, Bennett PH, Hanson RL. Segregation analysis of diabetic nephropathy in Pima Indians. *Diabetes.* 2000;49(6):1049–1056.
2. National Center for Chronic Disease Prevention and Health Promotion. *Trends in Diabetes Prevalence Among American Indian and Alaska Native Children, Adolescents, and Young Adults—1990–1998.* Atlanta, GA; Centers for Disease Control and Prevention. May 2004. Available at: www.cdc.gov/diabetes/pubs/factsheets/aian.htm