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Pilot Genome Wide Association Search Identifies Potential loci for Risk of Erectile Dysfunction in Type 1 Diabetes Using the DCCT/EDIC Study Cohort

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Abstract

Purpose—To identify genetic predictors of diabetes-associated ED using genome wide and candidate gene approaches in a cohort of men with type I diabetes.

Methods—We examined 528 white men with T1D (125 with ED) from the DCCT and its observational follow up EDIC Study. ED was defined from a single item of the IIEF. An Illumina Human1M BeadChip was used for genotyping. 867,125 single nucleotide polymorphisms (SNPs) were subjected to analysis. Whole genome and candidate gene approaches tested the hypothesis that genetic polymorphisms may predispose men with T1D to ED. Univariate and multivariate models were used controlling for age, HbA1c, diabetes duration, and prior randomization to intensive or conventional insulin therapy during DCCT. A stratified false discovery rate was used to perform the candidate gene approach.

Results—Two SNPs located on chromosome 3 in one genomic loci were associated with ED with $p < 1 \times 10^{-6}$. rs9810233 had a p-value of 7×10^{-7} and rs1920201 had a p-value of 9×10^{-7} . The nearest gene to these two SNPs is ALCAM. The genetic association results at these loci were similar in univariate and multivariate analysis. No candidate genes met criteria for statistical significance.

Conclusions—Two SNPs, rs9810233 and rs1920101, which are 25 kb apart, are both associated with ED, albeit not meeting the standard GWAS significance criteria of $p < 5 \times 10^{-8}$. Other studies with larger sample sizes will be required to determine whether ALCAM represents a novel gene in the pathogenesis of diabetes associated ED.

MESH Key Words

Erectile Dysfunction; Diabetes; Genetics

Introduction

More than 40% of men over age 40 report erectile dysfunction (ED).¹ Currently, if all men in the U.S. with ED received treatment the cost would exceed \$10 billion.² While early detection and prevention therapies have the potential to significantly decrease the costs associated with ED, little is known about the genetic modulators of ED. A twin study in middle aged male veterans that included men with diabetes found that about one third of the variance in the ED phenotype could be explained by additive genetic factors.³ The G894T polymorphism (rs1799983) in endothelial nitric oxide synthase (eNOS or NOS3)) and G-protein GNB3 C825T (rs5443) have been jointly associated with ED, hypertension and coronary artery disease but results are inconsistent.^{4–6} The Angiotensin Converting Enzyme (ACE) DD genotype is significantly more frequent in men with ED than those without, and Angiotensin II when injected into the corpora cavernosa in mice can induce detumescence.^{4, 7}

Prior studies of the genetic contributors to ED have been limited by reliance on model in-vitro systems to generate *a priori* hypotheses that made identification of novel pathways unlikely and the failure to control adequately for ethnicity. On the other hand, the success of Genome Wide Association Study (GWAS) studies in identifying novel biologic mechanisms for diseases such as diabetes and prostate cancer suggests a role for a whole-genome approach for deciphering the genetic underpinnings of diseases such as ED.^{8, 9} Consequently, the aim of this study was to identify specific genetic markers for ED in a high-risk cohort of patients with type 1 diabetes using both a whole genome approach and a candidate gene analysis.¹⁰

Materials & Methods

Overview

Our study population consisted of men enrolled in the Diabetes Control and Complications Trial (DCCT) and its observational follow up the Epidemiology of Diabetes Interventions and Complications (EDIC) study, which consists of over 1300 men and women with type 1 diabetes who have been followed since 1983.¹¹ The DCCT (1983–93) was a randomized controlled clinical trial that examined the effects of near-normal glycemic control on the development and progression of diabetic complications. The presence of ED was self-reported at year 10 of EDIC (calendar year 2003) with a single item of the International Index of Erectile Function (IIEF)¹², obtained as part of an ancillary study of urological complications of type 1 diabetes (UroEDIC).¹³ We performed a whole genome as well as a candidate gene approach to identify genetic predictors of ED in 528 white men with type 1 diabetes who met both phenotypic and genotypic quality control metrics.

Study Subjects

DCCT randomly assigned 761 male patients with type 1 diabetes to intensive or conventional therapy, treating them for a mean of 6.5 years between 1983 and 1993. (Figure 1)¹⁴ The primary prevention cohort consisted of 378 male subjects with no detectable retinopathy by 7-field fundus photography, a urinary albumin excretion rate <40 mg per 24 hours, and diabetes duration 1 to 5 years at study entry. The secondary intervention cohort consisted of 383 male subjects who had mild to moderate non-proliferative retinopathy, urinary albumin excretion rate 200 mg per 24 hours, and diabetes duration of 1 to 15 years.

Individuals were excluded from the DCCT if they had hypertension (systolic ≥ 140 or diastolic ≥ 90 mm Hg), or were taking antihypertensive medications symptomatic ischemic heart disease, or symptomatic peripheral neuropathy requiring treatment.

Of the 761 men enrolled, 746 completed DCCT, of whom 720 (97%) elected to participate in the first of annual examinations of the Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study in 1994. In the 10th year of EDIC, of the 713 men still active in the trial, 591 (83%) men agreed to participate in UroEDIC, an ancillary study of urological symptoms and sexual function. Of these, 571 (80%) men provided data on erectile function and comprise the study cohort for the current analyses. Figure 1 shows the flow of participants through the study. The institutional review board of each participating center approved the study, and a Certificate of Confidentiality was issued by the Federal Government.

DCCT interventions and ED measurements

Intensive glycemic therapy during DCCT consisted of insulin administered three or more times per day by injection or by continuous subcutaneous infusion with an external pump, with dose adjustments based on at least four self-monitored glucose measurements per day. Daily glucose goals were 70 to 120 mg/dl (3.9 to 6.7 mmol/l) before meals and peak levels of less than 180 mg/dl (10.0 mmol/l) after meals. The goal for HbA1c was $<6.05\%$. Intensively treated subjects also received frequent dietary counseling. Conventional therapy consisted of one or two daily injections of insulin and had no glucose goals beyond those needed to prevent symptoms of hyperglycemia and hypoglycemia.

Participants were asked the sixth question from the Erectile Function domain of the International Index of Erectile Function (IIEF)^{1, 15}: “Over the past 4 weeks, how would you rate your confidence that you get and keep your erection?” If the participant answered ‘Very Low’ (1) or ‘Low’ (2) they were considered to have erectile dysfunction. If they answered ‘Moderate’ (3), ‘High’ (4), or ‘Very High’ (5), they were considered to have no erectile dysfunction.

Genotyping and quality control

Genotyping was performed for 571 white male DCCT/EDIC subjects with the Illumina Human1M Beadchip (Illumina Inc., San Diego, CA, USA), of which 528 met quality control measures and had ED data available. Quality control was performed according to previously established metrics from other GWAS papers from EDIC.¹⁶ Genotypes were called using BeadStudio using all individuals at once. Data from three probands was excluded because of discrepancies between reported sex and genotype data and no individuals were removed because of low genotype call rate (minimum call rate threshold was 0.988).

Genotypes from 24 duplicate samples had an agreement rate of 99.9995%. Sample contamination was assessed by calculation of the mean heterozygosity across the genome for each individual, and using a range of 0.25–0.32, none were removed. To detect cryptically related individuals and/or sample mix-ups, identical-by-state estimates between all pairs of individuals were performed, and two probands were removed. A total of 841,342 SNPs with a minor allele frequency $>1\%$ were subsequently analyzed statistically.

Autosomal SNPs showing significant association with sex ($P < 10^{-8}$) or deviating from the Hardy-Weinberg equilibrium ($P < 10^{-8}$) were excluded from the analysis. To reduce the possibility of population stratification, we limited the analysis to individuals who self-identified as white and excluded individuals who were determined to be admixed between Caucasian and other ethnic groups through population genetic approaches, seeding with genotype data from the three major populations genotyped in HapMap Phase II.¹⁷ This

resulted in the exclusion of 3 patients from the study as their racial admixture was determined to be non-caucasian.

Statistical analysis

Initial descriptive analysis examined the distribution of basic demographic variables such as age, marital status, smoking status and markers of diabetes control and treatment in those with and without ED. Analyses employed hemoglobin A1c at DCCT eligibility, mean HbA1c from DCCT/EDIC) as well as the age of the participants at the time of the IIEF evaluation. The impact of medications on ED was not investigated. Population structure was assessed to ensure that all samples were of European ancestry. In our GWAS analysis we used logistic regression to examine the association of the 840,354 SNPs with ED using PLINK (v1.07) with genotypic and additive models. Two models were run for each SNP: one not adjusting for any covariates, and a multivariate model adjusting for explanatory covariates.

Based on our previous work¹, which showed that the only *a priori* predictors of developing ED for DCCT/EDIC patients were age, HbA1c at eligibility, and randomization to intensive glycemic therapy (in the secondary prevention cohort only), we initially began with a simple model (M1) that consisted of all the significant exposure variables from the previous study. Our next model, Model 2 (M2) included the terms of our previous model as well as other biological predictors of ED in type 1 diabetes: duration of diabetes, baseline HbA1c and mean HbA1c from DCCT/EDIC.

Assessment of the distribution of GWAS SNP p values was assessed with a Quantile-Quantile (QQ) plot derived from the unadjusted genotypic and trend models as well as the logistic regression models (M1 and M2). A Manhattan plot was also generated for the unadjusted results.

A candidate gene list was generated *a priori* from previously published work (PubMatrix)¹⁸, gene ontology classification and the results of microarray expression genechips that assayed gene expression in streptozotocin-induced diabetic rat cavernosa and human cavernosal endothelial cells. (Supplemental Table 1, url: <https://edic.bsc.gwu.edu/>).^{19, 20} These gene locations were used to define regions of 100 kilobases up and downstream of the location of these genes. A stratified False Discovery Rate (FDR) approach was used as previously described by Sun et al. and q-values were calculated.¹⁰

Results

UroEDIC

The UroEDIC ancillary study of DCCT/EDIC assessed male participants for ED in 2003 and found that 403 participants did not have ED and 125 were identified as having ED (Table 1). Those having ED were older (48.2 yrs vs. 43.5 yrs, $p<0.001$), and more likely to smoke (24.8% vs. 16.6%, $p=0.040$). Those with ED had higher hemoglobin A1c at DCCT baseline (9.0 vs. 8.6, $p=0.016$) and higher DCCT/EDIC mean hemoglobin A1c (8.6 vs. 8.0, $p<0.001$).

Multiple models were generated based on previous work and tested for trend and genotypic models, M1 and M2 (Table 2,3). The univariate trend model had the most statistically significant values.

Both the unadjusted trend model and the M1 model implicated a novel SNP associated with ED pathogenesis in men with type 1 diabetes, in or near Activated Leukocyte Cell Adhesion Molecule (ALCAM) on chromosome 3 (Table 3). The G allele of rs1920201 with minor allele frequency (MAF)=0.245 was associated with increased risk of ED in the univariate

trend model ($p=8.6e-07$). Similar borderline significant results were obtained for rs9810233, ~25 kb away, ($p=6.98e-07$). Further, the G risk allele was also implicated in the multivariate model for SNPs rs1920201 (OR=2.26, 95% CI 1.63–3.14, $p=9.4 \times 10^{-7}$ and SNP rs9810233 (OR=2.32, 95% CI 1.67–3.22, $p=5.9 \times 10^{-7}$). Although no SNPs achieved genome-wide significance ($p < 1 \times 10^{-8}$), the Manhattan Plot (Figure 2) revealed several signals of interest, such as ALCAM.

We also performed a candidate gene approach according to the *a priori* derived candidate genes highlighted in supplemental Table 1 (url: <https://edic.bsc.gwu.edu/>). There were no significant results (Table 4).

Discussion

The 528 men with type 1 diabetes in UroEDIC included in this study represent a well-defined cohort that allowed us to rigorously adjust for non-genetic risk factors for ED such as age and diabetes control. Moreover, the exclusion of all non-whites allowed us to disregard the impact of ethnicity.^{14, 21} Despite these advantages, the results of this small exploratory analysis with only 125 cases failed to reveal any robust new genetic associations with diabetic ED, although at least one SNP in linkage disequilibrium with ALCAM may be considered for further exploration in larger cohorts.

There were suggestive findings implicating the G allele of rs1920201 and rs9810233. Previous work in a cohort of 480 Danish families suggested that genetic variation in the exons of ALCAM may be involved in a mechanism whereby cytotoxic T lymphocyte antigen 4 modulates gene-gene interactions and confers genetic susceptibility of type 1 diabetes.²² The magnitude of the association for SNPs near ALCAM in our study was much larger than that typically found in GWAS studies for complex traits. This is suggestive of either involvement of these SNPs with diabetic ED or co-segregation with other factors that modulate ED in this region.

While the sample size in the current study was larger than in many previous investigations, a major limitation is its small size. Another potential source of error in the study is the variability in the ED measurements. The IIEF, a validated clinical instrument, is nevertheless a subjective form of reporting. Unfortunately, there are no validated quantitative clinical tests for ED and some subjects might have been unwilling or embarrassed to report ED, even though questionnaires were identified only by a study ID. Finally, the relatively young age and well controlled HbA1cs of this population may have led to incomplete penetrance of ED at the time of the study.

We propose that the IIEF Erectile Function Domain or at the very least a single item question assessing ED be added into large cohort studies employing GWAS methodology such as the Wellcome Trust Sanger studies. A working group under the direction of the AUA or NIDDK could be formed to investigate genetics of benign urologic diseases such as ED through collaborative efforts to obtain the requisite sample sizes of 10–15,000 subjects needed to make definitive conclusions.

Conclusion

We performed a novel GWAS and candidate gene study for genetic predictors of ED in men with type I diabetes and identified 2 SNPs near ALCAM of potential interest. These SNPs should be explored in other cohorts to increase the sample size to validate findings of this pilot study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Figure 2.

Table 1

Demographics of erectile dysfunction in the UroEDIC genotyped cohort

CHARACTERISTIC	No Erectile Dysfunction (n=403)	Erectile Dysfunction (n=125)	p-value
Sociodemographic Attained Age(yr) *	43.5 (6.5)	48.2 (5.9)	0.00098
Diabetes Treatment and Control Intervention No. (%)			0.11
Intensive therapy	207 (51.3)	54 (43.2)	
Conventional therapy	196 (48.7)	71 (56.8)	
Cohort No.(%)			0.30
Primary	205 (50.9)	57 (45.6)	
Secondary	198 (49.1)	68 (54.4)	
HemoglobinA1c at DCCT baseline (%)	8.6 (1.5)	9.0 (1.5)	0.016
DCCT/EDIC mean Hemoglobin A1c	8.0 (1.3)	8.6 (1.4)	0.00076
Insulin dose (units/kg/day) *	0.66 (0.26)	0.63 (0.23)	0.40

* Assessed at EDIC year 10. All data are expressed as mean (SD) unless noted otherwise. P-values calculated based on chi-square for differences in proportions and linear regression for continuous measures.

Table 2

Adjusted logistic regression modeling of risk factors related to erectile dysfunction in the UroEDIC cohort

Factors	OR (95% CI)	SE	p-value [†]
Primary Prevention Cohort [*] (intensive vs. conventional)	1.20 (0.64, 2.24)	.38	0.57
Secondary Intervention Cohort [*] (intensive vs. conventional)	0.33 (0.17, 0.61)	.10	<0.001
Hemoglobin A1c at Eligibility (per HbA1c%)	1.30 (1.12, 1.50)	.14	<0.001
Diabetes Duration (per month)	1.00 (0.99, 1.01)	.0031	0.65
Age (per year)	1.13 (1.09, 1.17)	.021	<0.001
Primary v. Secondary Cohort	1.10 (0.60, 2.02)	.34	0.75

^{*} Logistic regression adjusted for DCCT intervention, HbA1c at eligibility, diabetes duration, age, primary vs. secondary cohort, interaction term for prevention cohort status and intensive vs. conventional glycemic control.

Table 3

Unadjusted and multivariate adjusted logistic regression of SNPs with erectile dysfunction for $P < 10^{-6}$

A. Unadjusted Results										
Chromosome	SNP	Position	A1	A2	MAF**	Missing count	Hardy-Weinberg equilibrium P	Location of SNP to the nearest gene	Nearest Gene	p-value *
3	rs9810233	106493580	G	A	0.270	1	0.725	flanking_5UTR	ALCAM	6.98E-07
3	rs1920201	106518787	G	T	0.245	5	0.653	flanking_5UTR	ALCAM	8.60E-07
2	rs836589	173201324	A	G	0.186	8	0.521	flanking_3UTR	PDK1	1.63E-06
3	rs1438552	106545285	G	A	0.247	2	0.603	flanking_5UTR	ALCAM	1.72E-06
6	rs6931865	144121547	A	G	0.336	1	0.901	intron	PHACTR2	3.57E-06
3	rs950146	36655103	C	A	0.491	5	0.698	flanking_3UTR	STAC	4.32E-06
3	rs9850224	142057977	A	G	0.010	1	1	flanking_5UTR	SLC25A36	4.80E-06
1	rs2990510	195287281	G	T	0.305	0	0.556	intron	F13B	5.89E-06
1	rs10754210	195278734	A	G	0.304	1	0.513	intron	F13B	5.89E-06
2	rs7566884	173189446	G	A	0.177	1	0.393	flanking_3UTR	PDK1	6.39E-06
1	rs4520444	195408611	T	G	0.296	5	0.842	intron	ZBTB41	7.77E-06
1	rs5998	195276421	G	A	0.454	0	0.696	coding	F13B	8.30E-06
1	rs7518773	195278259	G	A	0.453	2	0.615	intron	F13B	8.30E-06
1	rs1332668	195272359	T	C	0.455	1	0.655	flanking_3UTR	F13B	8.50E-06

B. Multivariate Results *														
Chrom	SNP	Position	A1	A2	MAF#	missing	Hardy-Weinberg equilibrium P	Location of SNP to nearest gene	Nearest Gene	Model	OR	95% CI	p-value	
3	rs9810233	106493580	G	A	0.270	1	0.725	flanking_5UTR	ALCAM	M1	2.32	1.67	3.22	5.93E-07
3	rs1920201	106518787	G	T	0.245	5	0.653	flanking_5UTR	ALCAM	M1	2.26	1.63	3.14	9.39E-07
6	rs6931865	144121547	A	G	0.336	1	0.901	intron	PHACTR2	M1	2.02	1.50	2.74	4.85E-06
3	rs950146	36655103	C	A	0.491	5	0.698	flanking_3UTR	STAC	M1	0.48	0.35	0.66	4.77E-06
2	rs836589	173201324	A	G	0.186	8	0.521	flanking_3UTR	PDK1	M1	2.27	1.58	3.25	8.29E-06
3	rs950146	36655103	C	A	0.491	5	0.698	flanking_3UTR	STAC	M2	0.46	0.33	0.64	6.52E-06
6	rs6931865	144121547	A	G	0.336	1	0.901	intron	PHACTR2	M2	2.48	1.73	3.54	6.89E-07

* Fishers exact test.

** Minor Allele Frequency A1 is the minor allele.

* All models run with additive tests for significance in PLINK with covariates.

** Adjusting for cohort, treatment, cohort*treatment. (M1)

*** Adjusting for cohort, treatment, cohort*treatment, diabetes duration, age, initial HgbA1c at eligibility. (M2)

Minor Allele Frequency

Table 4

Candidate Gene Results for Multivariate M2* model with Q Scores

Candidate Gene	Chrom	SNP	Position	A1	A2	MAF**	Function	p-value	Q-Score
S100 Ca2-binding protein A4	1	rs2274739	151878757	C	T	0.453	intron(dbSNP)	0.0065	0.6837
S100 Ca2-binding protein A4	1	rs12564925	151880169	G	A	0.454	intron(dbSNP)	0.0081	0.6837
Ceruloplasmin	3	rs7634024	150475920	G	A	0.102	intergenic(GVS)	0.0131	0.6837
Ceruloplasmin	3	rs13098532	150373682	G	T	0.012	mrna-utr(dbSNP)	0.0157	0.6837
Lysyl Oxidase	5	rs890750	121463478	C	A	0.197	coding- nonsynonymous (dbSNP)	0.0435	0.9756
Lysyl Oxidase	5	rs2731659	121498469	A	G	0.204	intron(dbSNP)	0.0524	1.0000
eNOS/CD36	7	rs1404313	79992399	G	A	0.429	intergenic(GVS)	0.0065	0.6837
eNOS/CD36	7	rs2366735	79994250	G	A	0.422	intergenic(GVS)	0.0066	0.6837
Lipoprotein Lipase	8	rs2410622	19899053	T	C	0.155	intergenic(GVS)	0.0294	0.9027
Lipoprotein Lipase	8	rs1561747	19777848	G	T	0.435	intergenic(GVS)	0.0401	0.9756
Kruppel-like factor 5	13	rs11544631	72437467	G	A	0.026	coding-nonsynonymous (dbSNP)	0.0237	0.8174
Kruppel-like factor 5	13	rs9530145	72543301	T	C	0.154	intron(dbSNP)	0.0334	0.9558
Gremlin 1	15	rs3743103	30812919	G	A	0.408	mrna-utr(dbSNP)	0.0398	0.9756
Gremlin 1	15	rs1258749	30873773	C	T	0.173	intron(dbSNP)	0.0421	0.9756
ACE	17	rs17687734	58838777	T	C	0.124	intron(dbSNP)	0.0307	0.9027
ACE	17	rs4968772	58835351	G	A	0.254	intron(dbSNP)	0.0778	1.0000
O-linked GlcNAc transferase	23	rs5937104	70789692	NA	NA	0.139	intron(GVS)	0.0746	1.0000
O-linked GlcNAc transferase	23	rs3736670	70693455	NA	NA	0.114	intron(dbSNP)	0.0932	1.0000

* All models run with additive tests for significance in PLINK with covariates. Adjusting for cohort, treatment, cohort*treatment, diabetes duration, age, initial HgbA1c at eligibility. (M2)

** Minor Allele Frequency