

Genomic Loci Evaluation with Albuminuria in New-Onset Insulin Dependent Diabetic Patients

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Abstract

Background: Extensive data supports the genetic underpinnings of diabetes and recent studies implicate several genetic loci associated with renal insufficiency and albuminuria. Moreover, albuminuria in diabetic patients is an important risk marker for atherosclerotic disease as well as cardiomyopathy. The purpose of this study was to identify genetic determinants of albuminuria in a diabetic patient population at the time of insulin initiation.

Methods: Study population included type 2 diabetic subjects at the time of initiation of insulin in an observational cohort who donated saliva samples for DNA extraction. Urine albumin to creatinine ratio (UACR) and HbA1c levels at baseline were collected and analyzed for association with genotype (N=128).

Results: Although none of the SNPs met statistical significance after Bonferroni adjustment, two regions showed near-significant associations with UACR; one on chromosome 1 ($p=8.66 \text{ E-}07$) and one on chromosome 12 ($p=9.82 \text{ E-}07$).

Conclusions: In this small study of newly insulin-dependent diabetics we identified two genomic regions possibly associated with albuminuria. These loci are good candidates for further investigation into the moderators of cardiovascular disease in diabetics. Larger confirmatory studies are needed.

Keywords: Genetics; Loci; Albuminuria; Diabetes

Introduction

Cardiovascular disease (CVD) accounts for the vast majority of morbidity and mortality in the diabetic patient population [1]. In 1999 diabetes was recognized as a coronary heart disease equivalent [2] and more recently it has been well recognized that the presence of diabetes increases the risk of heart failure by two to four fold [3]. Moreover, among diabetic and cardiovascular patients, renal dysfunction is common, and associated with a worse prognosis [4,5]. In this setting, albuminuria is not only a marker of renal disease but is also an important risk factor for CVD progression [6] and more recently it has been recognized as a risk factor for the development of heart failure [7]. While tight glycemic control reduces micro vascular complications, similar benefits in terms of CVD are not clear making early genetic susceptibility identification potentially clinically beneficial [8]. Thus, identifying the contributing factors which underlie CVD progression in patients with diabetes remains an important question in order to better anticipate risk and develop effective interventions.

Genetic factors may help explain some of the variability in diabetic CVD development, and genomic studies in this setting could help identify potential pathways to intervene upon. Genome wide association studies (GWAS) have already identified genetic loci related to reduce glomerular filtration rates (GFR) among patients with diabetes [9] but parallel efforts to examine diabetic CVD development or its risk factors are less common. For example GWAS have identified 16 loci for the estimated glomerular filtration rate (eGFR) in European populations and seven eGFR-associated single nucleotide polymorphisms (SNPs) from persons of African ancestry from the CKDGen Consortium and the CARE Renal Consortium, respectively [10,11].

Significant genetic loci of *UMOD*, *GCKR*, and *SHROOM3* in renal

complications among diabetic patients have been previously described in various population groups [12,13]. Since diabetes is an important risk factor for atherosclerosis and CVD, there is great importance of early identification and control. Microalbuminuria, a marker of renal disease, in the diabetic patient population is a poor prognostic indicator for CVD [14]. Therefore, the use and association of albuminuria as a target for further genetic investigations remains. Further, in the diabetic population, the use of insulin marks a severity disease where insulin resistance is high and disease is generally poorly controlled [15].

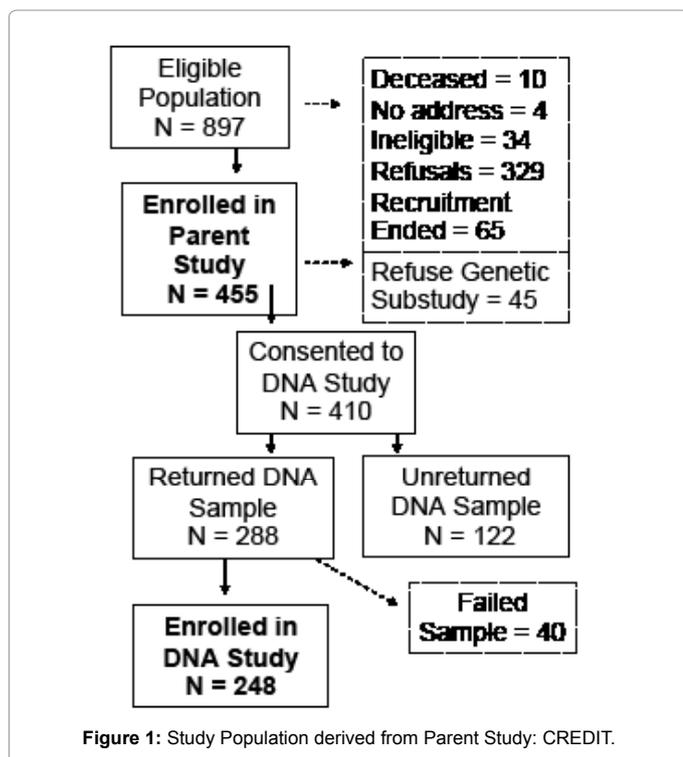
Identifying diabetics with a genetic predisposition to reno-vascular disease prior to the onset of chronic kidney disease (CKD) and CVD could lead to prevention of morbidity and reduce mortality among this population. This demonstrates the value of this strategy but additional data specific to albuminuria is needed. Common genetic loci have been studied in terms of association to albuminuria but no association has been found [16]. Despite this accumulating knowledge, the genetic and pathologic mechanisms that determine development and progression of CVD remain largely unknown [17].

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We sought to identify genetic determinants of albuminuria in diabetic patients recently initiated on insulin therapy. Acknowledging the great complexity of diabetes as a disease state, the use of insulin as a pre-requisite for inclusion criteria was aimed at evaluating a population with a similar disease process and severity. Our study team leveraged data collected as part of an ongoing observational study of diabetic patients who initiated insulin therapy (Long-Term International Non-Interventional Study in People with Type 2 Diabetes Treated with Insulin: CREDIT) (Figure 1), collecting blood samples for DNA isolation, obtaining hemoglobin A1c (HbA1c), and urine collection for albumin and creatinine measures from the electronic medical record (EMR).

Methods

The study was approved by the institutional review board of Henry Ford Hospital and all subjects provided written informed consent. The study was a single center retrospective cohort study of patients with diabetes who had first time initiation of insulin. Subjects were initially identified using EMR including claims-data indicating the initiation of insulin therapy within the previous 5 years and were recruited by telephone, then followed prospectively for up to two years as part of parent study involving recruitment of a cohort of Type 2 diabetic patients.

Patients who were identified as having type 2 DM (ICD 9 codes: 250.xx, 357.2x, 362.0x, 366.41), and who were treated with insulin for at least one month, were >40 years of age, and had a HbA1c measure within 3 months prior to starting insulin treatment were identified. Patients who initiated insulin between January 1, 2005 and October 31, 2009 were eligible. Those eligible were contacted via phone and once entered into the parent study were asked if they would also like to participate in the genetic sub-study. Among a total of 450 subjects recruited into the parent study, 248 consented to genetic analysis and provided a suitable sample for DNA extraction [18]. UACR and HbA1c

levels within 90 days of insulin initiation date were collected from the EMR and considered baseline.

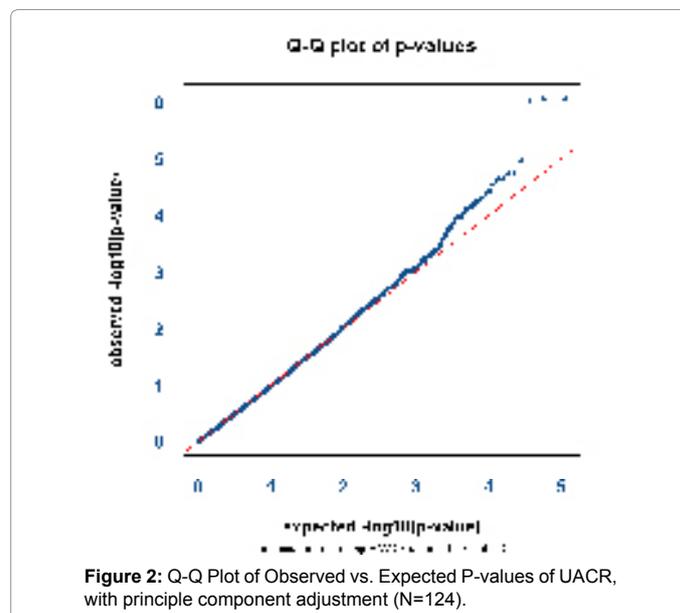
Genetic material was collected using saliva DNA isolation kits (DNA Genotek). After verbal consent was obtained, saliva kit and written consent form was mailed to the potential subject. Only samples that were accompanied by signed consent form were used in the study. Genotyping was accomplished using the CardioMetaboChip from Illumina® which types approximately 250,000 loci, many of which have previous associations with cardiac, metabolic, or anthropometric phenotypes. As quality control the SNPs with a call rate less than 90%, those with minor allele frequency of less than 1%, and samples with call rate less than 90% were excluded. This resulted in 248 evaluable samples.

To test for the association between UACR and genotype, log10 transformation was first applied due to non-normal distribution. Following log transformation, linear mixed models that account for pairwise genotypic similarity of individuals, using efficient mixed-model association eXpedited (EMMAX), were used to test for association and population structure [19]. Models were adjusted for the first principal component to infer population structure and avoid spurious findings due to population stratification. Baseline HbA1c was also adjusted for utilizing first principle component in order to account for disease severity. The Q-Q plot (Figure 2) demonstrates the effectiveness of the adjustment for UACR with first principle component. Multiple comparisons were accounted for by Bonferroni correction with a critical P-value of 4.5×10^{-7} .

Results

After the DNA extraction, genotyping, quality control, and database merging were completed there were roughly 60 million individual genotypes, and 248 met all quality standards. Among those samples, 15 subjects had missing data on HbA1c and 109 subjects had missing data on UACR. All data including baseline (within 3 months of insulin initiation) UACR was available for 124 subjects. Demographic and baseline characteristics of the study population with complete data are presented in Table 1.

The primary results are depicted in Figure 3, a Manhattan plot



Characteristic	Value
Age at initial enrollment	64.9 ± 10.3
Gender	Male 51% Female 49%
Race	42.9%
African-American	54.5%
Caucasian	2.6%
Other	
BMI ≥ 30kg/m ²	68.9%
HbA1c (%)	8.0 ± 1.6
Hypertension	96.9%
Duration of DM (years)	10.7 ± 4.3
Hyperlipidemia	48.4%

Table 1: Study population characteristics (N=124).

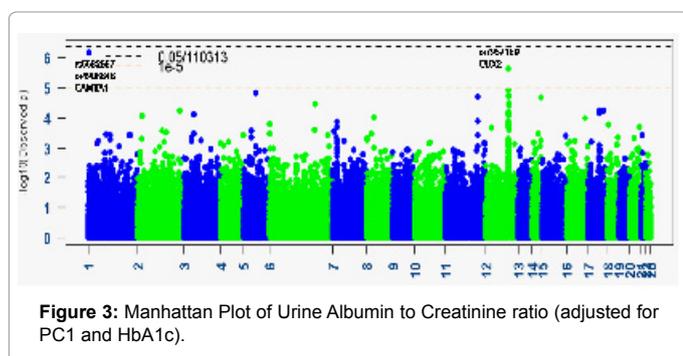


Figure 3: Manhattan Plot of Urine Albumin to Creatinine ratio (adjusted for PC1 and HbA1c).

CHR	SNP ID	parameter estimate	P value	Gene	Type
1	rs6682687	-0.4362	8.66E-07	CAMTA1	Intron
1	rs4908646	-0.4362	8.66E-07	CAMTA1	intron
12	rs7957169	-0.3459	9.82E-07	CUX2	Intron
12	rs10219736	-0.3313	2.36E-05	CUX2	near-gene
12	rs3803167	-0.3179	4.94E-05	CUX2	coding- synonymous
12	rs7398343	-0.2953	5.92E-05	CUX2	intron
12	rs2339718	-0.3118	7.22E-05	CUX2	intron
12	rs12423926	-0.3117	7.64E-05	CUX2	Intron

Table 2: SNPs with near-significant P-values associated with albuminuria.

of the genomic coordinate along the X-axis with associated P-value of UACR for each SNP displayed on the Y axis. Although none of the SNPs passed genome-wide significance after adjusting for multiple testing using the Bonferroni adjustment (Table 2), there were two regions with near-significant associations. The first, on chromosome 1, included two SNPs, rs6682687 and rs4908646 in the gene calmodulin-binding transcription activator 1 (CAMTA1) and showed a $p=8.6 \times 10^{-7}$. The other region was on chromosome 12, with the lowest $p=9.8 \times 10^{-7}$ for rs7957169. Interestingly, this site included a large number of nearby sequence variants with a range of p values in an association spike. This peak is quite broad, with its base being roughly 300kb (Figure 4).

Discussion

In this small study we have identified candidate genes for UACR/albuminuria in diabetics. Since albuminuria is an important marker of cardiovascular disease, specifically of atherosclerosis and heart failure, these are thus candidate genes for the progression of CVD in diabetics. There remains a critical public health need to better understand the

pathophysiology of diabetes in terms of cardiovascular complications and knowledge of the genetic underpinnings should further this goal. Acknowledging the complexity of disease states of DM, CVD and CKD confounded by the interplay of comorbid conditions compounds the difficulty in analysis. Our data is the first that we know of to identify genetic factors contributing to albuminuria timed to the onset of insulin use in diabetics. The use of insulin initiation as a marker of disease severity, with presumed insulin resistance prompting initiation, allows for a more focused population of study. These findings are consistent with and add to the existing data. Work by Ellis et al. [13] tested genetic loci previously associated with eGFR for association with albuminuria but did not find significant loci. Findings in Deshmukh et al. [9], with confirmation of the existence of eGFR-associated SNPs (UMOD, GCKR, and SHROOM3) elucidated the importance of correcting for albuminuria presence as it changed gene effect within the study; this suggests that further elucidation of a gene for albuminuria is warranted with consideration of eGFR. Larger studies aimed at a disease-focused population, such as diabetic patients with recent insulin initiation, is an avenue for further evaluation.

The two genomic loci that we found to be associated with albuminuria on chromosomes 1 and 12 did localize within specific gene regions. On chromosome 1, two SNPs of interest ($p=8.6 \times 10^{-7}$) were within introns of calmodulin-binding transcription activator 1 (CAMTA1, rs6682687 and rs4908646). These particular variants did not have any prior reported clinical associations that we could identify despite other SNPs within the CAMTA1 gene with GWAS associations to heart failure and coronary artery disease (rs12740374 and rs4908443, respectively) [20,21]. The chromosome 12 SNP of interest (rs7957169, $p=9.8 \times 10^{-7}$) was at the tip of broad peak of association, spanning roughly 300kb. The most prominent gene in the peak is the cut-like homeobox 2 (CUX2) gene. The CUX2 gene region has recently been associated with Type 1 diabetes in the 1000 Genomes Project using data from the WellcomeTrust Case Control Consortium [22]. To our knowledge this is its first association with albuminuria. In addition there are other nearby genes (Figure 4) that should also be considered. Additional studies are needed to confirm and extend these preliminary findings. Fortunately, there are ongoing, large meta-analyses of type 2 diabetes studies (using genotype imputation via the 1000 Genomes reference panel) underway which hopefully will shed further light on this important phenotype [23].

Our data should be interpreted in view of some limitations. This was a small study without a validation set, so these findings should be regarded as preliminary and require further confirmation. This was an observational cohort thus selection bias is always a concern; however

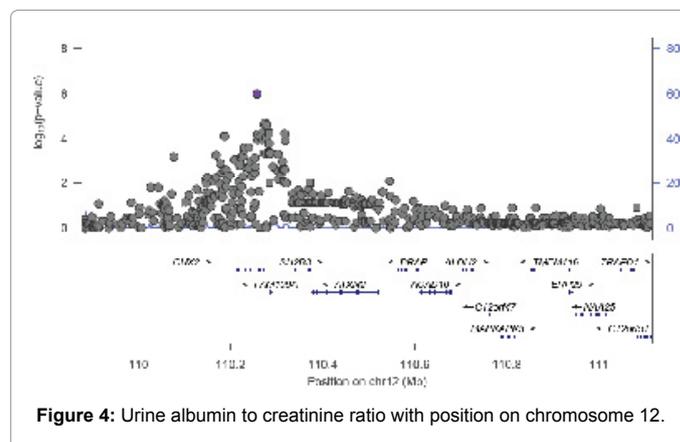


Figure 4: Urine albumin to creatinine ratio with position on chromosome 12.

the investigators put great effort into recruiting all potential subjects who met enrollment criteria. Moreover there could be selection bias on the part of the subjects since they could volunteer or not to participate in this portion of the study. However, we have previously shown that the genetic cohort was similar to the non-genetic cohort except for HbA1c, and we included adjustment for HbA1c in our analysis. We also did not stratify by self-identified race due to the modest cohort size, but feel that adjustment using principle components should have adequately accounted for this potential limitation.

In conclusion, using this modest sized cohort of diabetic patients newly initiated on insulin, we have identified two genomic regions as candidate loci for albuminuria and CVD disease progression. Additional investigation is warranted.

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