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Analysis of Candidate Genes on Chromosome 20q12-13.1 Reveals Evidence for BMI Mediated Association of *PREX1* with Type 2 Diabetes in European Americans

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Abstract

Chromosome 20q12-q13.1 has been linked to type 2 diabetes (T2D) in multiple populations. We examined the influence of genes in this region on T2D and BMI in two European American case-control populations. SNPs were genotyped in 300 diabetic patients and 310 controls. A subset of 72 SNPs were further genotyped in 470 cases and 442 controls. All genes examined showed evidence of association with T2D in the initial sample (additive P-value [P_a] =0.00090–0.045). SNPs near *PREX1* were also associated in the second case-control population (P_a=0.017–0.042). The combined analysis resulted in the same SNPs, among others, associated with T2D (P_a=0.0013–0.041). Stratification analysis by T2D status showed that association with BMI was observed solely in cases (P_a=0.0018–0.041). Mediation testing revealed 30–40% of the effects of these SNPs on T2D were significantly mediated by BMI. SNPs near *PREX1* may contribute to T2D susceptibility mediated through effects of adiposity in European Americans

Keywords

association; type 2 diabetes; genetics; adiposity; mediation analysis

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1. Introduction

The 20q12-13.1 region is one of the most consistently replicated areas of linkage to type 2 diabetes mellitus (T2DM) in family studies in both Caucasian [1–6] and Asian [7,8] populations. These results suggest that one or more genes contribute to T2DM susceptibility in this region. Efforts to identify T2DM genes have had mixed results. For example, evaluation of hepatocyte nuclear factor 4-alpha (*HNF4ά*), a maturity onset diabetes of the young gene, has shown evidence of association with T2DM [9–11] as well as lack of evidence [12]. The same is true for other candidates in the area including *PTPN1* and *SLC2A10* [13–16]. It is possible that population and genetic heterogeneity are an explanation for these seemingly contradictory results. Additionally, reports have shown significant changes in both linkage signals and p-values when accounting for the effects of BMI [7,8]. Alternatively, these inconsistencies may also suggest the true T2DM susceptibility gene(s) has not been identified or additional loci remain unidentified. In a prior SNP survey of a 6 MB region of 20q12-13.1 in our laboratory three genes: nuclear receptor coactivator 5 (*NCOA5*), cadherin-like 22 (*CDH22*), and phosphatidylinositol 3, 4, 5-triphosphate-dependent RAC exchanger 1 (*PREX1*) [17], with suggestive evidence of association with T2DM were identified warranting further investigation. In this study we built on our previous investigation by targeting these regions for higher density genotyping compared to our initial screen and evaluating these SNPs in an additional sample of cases and controls. Additionally, we conducted more targeted intensive analyses in an attempt to more clearly understand the effects of genotyped SNPs on T2DM risk and measures of adiposity.

Our objective was to examine the influence of *NCOA5*, *CDH22*, and *PREX1* on T2DM and BMI in two European American (EA) case-control populations by examining 95 common SNPs in these genes.

2. Results

Ninety five SNPs were genotyped to investigate evidence of association with T2DM in the regions containing the *NCOA5*, *CDH22*, and *PREX1* genes in European Americans. *NCOA5*, *CDH22*, and *PREX1* were identified as possible T2DM susceptibility genes in a SNP survey covering 6 MB in the 20q12-q13 region [17]. Here these genes were targeted for higher density SNP genotyping and genotyping in an additional sample of cases and controls. Fifty SNPs are from chr20:44,069,142–44,365,336 (UCSC Genome Browser, March 2006 assembly; [33]) encompassing the genes matrix metalloproteinase 9 preproprotein (*MMP9*), solute carrier family 12 member 5 (*SLC12A5*), nuclear receptor coactivator 5 (*NCOA5*), CD40 antigen isoform 1 precursor (*CD40*), and cadherin 22 precursor (*CDH22*). The 45 remaining SNPs were located in chr20:46,441,248–46,757,117 (UCSC; [33]) and encompassed exons 7–40 of the phosphatidylinositol-3,4,5-trisphosphate-dependent RAC exchanger 1 (*PREX1*) gene (*PREX1* is transcribed in the anti-sense direction) and an approximately 230kb area directly 5′ of the *PREX1* genic region. All SNPs were genotyped in the T2DM-ESRD samples while a subset of 72 SNPs were genotyped in the Diabetes Heart Study (DHS) population. Population characteristics are shown in Table 1. Briefly, the T2DM-ESRD population cases and controls were approximately of the same age. Controls of the T2DM-ESRD population had no BMI data available. In the DHS population, controls were slightly older than cases but showed lower levels of adiposity as measured through BMI.

2.1 Association analysis in the T2DM-ESRD population

Initially 95 SNPs were genotyped in a sample of 300 T2DM-ESRD cases and 310 non-diabetic controls to scan these loci for evidence of association with T2DM. Results of the single SNP association analyses with T2DM for the 72 SNPs that were also genotyped in the DHS

population are shown in Table 2. Results of additional SNPs genotyped in the T2DM-ESRD population but were not genotyped in the DHS population are shown in Supplementary Table 1. Genotype frequencies and counts for replicated SNPs are provided in Supplementary Table 2. In the *NCOA5-CDH22* region evidence of association with T2DM was observed with 10 SNPs (additive P [P_a]=0.00090–0.045). Two of these SNPs, rs2297200 and rs9074, were located in *SLC12A5* with P_a-values of 0.019 and 0.0083, respectively. Three associated SNPs (rs1537028, rs2903908, and rs1406826) were located in *NCOA5* (P_a=0.020, 0.024, and 0.017 respectively) while another, rs2868764 (P_a=0.045), was within 3.2kb 3′ of the gene. In *CDH22*, three SNPs (rs1010310, rs2425786, and rs6074069) showed evidence of association with T2DM with P_a-values of 0.00090, 0.011, and 0.022 respectively. rs6032664, located in an intragenic region, was also nominally associated under the additive model (P_a=0.031) but deviated from Hardy-Weinberg proportions in the population (Supplementary Table 2).

In the *PREX1* region 11 SNPs showed evidence of association with T2DM (P_a=0.0012–0.031). Three of these SNPs (rs2664570, rs2073072, and rs2294910) were located within the *PREX1* gene region (P_a=0.026, 0.031, and 0.013 respectively). The remaining eight associated SNPs were located 3′ of *PREX1* with respect to gene transcription; *PREX1* is coded on the reverse strand to the conventional physical sequence map forward direction. A cluster of five of these SNPs was positioned in an area of moderate to high linkage disequilibrium and had P_a-values ranging from 0.010–0.027 (Table 2). The most significant association with T2DM in the *PREX1* region was seen with rs4810813 (P=0.0012) but this marker was inconsistent with Hardy-Weinberg equilibrium in controls (P=0.026). Two other SNPs, rs6090875 and rs3924220, were associated with T2DM (P_a=0.030 and 0.0029 respectively) and both were in high LD with each other (D =0.867, r^2=0.741).

2.2 Association analysis in the DHS population

A subset of 72 SNPs were also genotyped in 470 samples from T2DM-affected individuals from the DHS and an independent set of 442 controls. Results of the association analyses for the SNPs genotyped in the DHS population are shown in Table 2. Genotype frequencies and counts for each SNP are provided in Supplementary Table 3. In this cohort none of the SNPs in the *NCOA5-CDH22* region or the *PREX1* genic region were significantly associated with T2DM. However, similar to the T2DM-ESRD population, multiple SNPs were associated with T2DM in the area 3′ of *PREX1* (Table 2). Three SNPs (rs7263053, rs1321006, and rs926692) showed evidence of association with T2DM in participants of the DHS (P_a=0.024, 0.020, and 0.041 respectively). Within this region rs999151, rs4809696, and 12481537 also showed nominal evidence with T2DM with P_a-values of 0.023, 0.017, and 0.042 respectively.

2.3 Association analysis of the combined population

To more thoroughly investigate the effects of the genes we analyzed the T2DM-ESRD and DHS populations as a single sample resulting in 770 diabetic cases and 752 healthy controls. Linkage disequilibrium structure plots of the *NCOA5-CDH22* and *PREX1* regions for the SNPs genotyped in this population are shown in Supplementary Figures 1 and 2 respectively. Results of the single SNP association analyses are shown in Table 2. Genotype frequencies and counts for each SNP are provided in Supplementary Table 4. In the *NCOA5-CDH22* region a single SNP (*CDH22* SNP rs1010310) was nominally associated with T2DM (P_a=0.019). All other SNPs in this region failed to show evidence of association (Table 2). In the *PREX1* region nine SNPs showed evidence of association with T2DM (P_a-values ranging from 0.0013 to 0.041). None of these SNPs were located within the *PREX1* gene sequence. Consistent with analyses of the individual populations, the associated SNPs in the combined sample were clustered in the 3′ region of *PREX1* transcription. Six of these SNPs resided in a region of high LD and generally showed stronger evidence of association compared to the evaluation of the T2DM-ESRD or DHS populations separately (Supplementary Figure 1 and Table 2). Two other SNPs

(rs6019212 and rs12481537) in high LD with the six SNP block (D >0.70 for all inter-SNP combinations, data not shown) were associated with T2DM as well (P_a=0.013 and 0.034 respectively). rs4810813 also showed modest evidence of association with T2DM (P_a=0.013) and was not in high LD with any other associated SNP.

In order to determine whether combinations of alleles from associated SNPs 3′ of *PREX1* led to enhanced T2DM risk we performed extensive haplotypic association analyses in the region. Based on the single SNP association results and the LD structure in the combined population multiple haplotypes were evaluated. From these analyses the most significantly associated haplotypes consisted of alleles from five contiguous SNPs: rs7263053, rs1321006, rs4809696, rs926693, and rs926692 (Global P=3.26×10^{-6}). Upon further investigation we observed several rare haplotypes in this region with frequencies less than 0.02. To evaluate the effect these uncommon haplotypes contributed to T2DM risk we repeated the analysis excluding haplotypes with frequencies less than 0.02. When evaluating only common haplotypes we still observed evidence of association with T2DM (Global P=0.0044) (Table 3). The most common haplotype in both cases and controls (frequency = 0.506 and 0.561 respectively) was associated with protection from T2DM with a haplotype-specific P-value of 0.0032 (OR=0.87) while the 2nd most common haplotype (frequency = 0.290 and 0.224 respectively) was associated with increased risk for T2DM with a haplotype-specific P-value of 5.93×10^{-4} and OR of 1.41 (Table 3).

To explore the possible effect of adiposity on T2DM risk in the combined population an association analysis was repeated adjusting for BMI using SNPADDMIX (Table 4). BMI data was available for 761 cases and 407 controls (Table 1). Evidence of association with T2DM in the region 3′ of *PREX1* was similar in this subset of individuals prior to BMI adjustment (data not shown). However, after BMI adjustment evidence of association with T2DM was substantially reduced. SNPs rs926693 and rs926692 remained marginally associated with T2DM after BMI adjustment (P_a=0.044 and 0.030 respectively) and six other SNPs in the region were trending towards significance (P_a-values ranging from 0.055 to 0.092).

Based on the BMI-adjustment results of the T2DM association analysis, SNPs were tested for association with BMI itself (Table 5). In the region 3′ of *PREX1* 10 SNPs were significantly associated with BMI in the combined population (P_a-values ranging from 0.0015 to 0.029): evidence of association comparable to that observed with T2DM (Table 5). The effect size of these SNPs ranged from 0.010 to 0.015 units of log transformed BMI for each copy of BMI-increasing allele. For the most associated SNP in the region, rs999151, individuals with CT and TT genotypes have a 1.16 kg/m^2 and 1.87 kg/m^2 increase in BMI when compared to CC carriers respectively.

In an effort to clarify the relationship between the SNPs 3′ of *PREX1*, BMI, and T2DM, we further evaluated association with BMI in the case and control populations separately. In the control population, which consisted of 407 individuals, no SNP in the region 3′ of *PREX1* showed evidence of association with BMI (Table 5). However, the case population, which consisted of 761 individuals (Table 5), multiple SNPs in the region 3′ of *PREX1* were associated with BMI including 12 adjacent genotyped SNPs showing evidence of association with P_a-value ranging from 0.0018 to 0.041.

The observation that SNPs in the region 3′ of *PREX1* were associated with BMI solely in the case stratum suggested BMI may mediate the effect of these SNPs on T2DM risk. Therefore, a formal mediation analysis was performed assuming the independent variable (SNP genotype) significantly affected the dependent variable (T2DM status). Only SNPs associated with T2DM in the subjects with BMI data (761 cases and 407 controls) were tested for mediation by BMI. Seven SNPs, all in the region 3′ of *PREX1*, were associated with T2DM in this population

(Table 6) and the mediation analysis revealed that 30–40% of the effects of six of these SNPs on T2DM were significantly mediated by BMI according to both the Sobel and Goodman tests of mediation (Table 6).

3. Discussion

Recently, several genome-wide association (GWA) and candidate gene studies have reported evidence for multiple type 2 diabetes mellitus susceptibility genes throughout the genome [34–38]. Even though these publications have provided a wealth of information for diabetes investigators, it is now clear that these genome-wide approaches account for 20% or less of the genetic risk for T2DM. This suggests that other more targeted approaches which encompass in depth analysis of data will be productive in identifying new loci. Historically, the 20q12-13.1 region of chromosome 20 is one of the most consistently replicated areas of linkage to T2DM in family studies [1–8]. Conflicting results from numerous candidate gene studies in the region leads to the question of whether or not the T2DM risk loci have indeed been identified. Based on prior data from a dense SNP map of chromosome 20q13.1 performed at our center [17] we have performed a detailed evaluation of the candidate genes *NCOA5*, *CDH22*, and *PREX1*.

In our T2DM-ESRD population consisting of 300 diabetic cases with ESRD and 310 healthy controls we observed evidence of association with T2DM in the *NCOA5*, *CDH22*, and *PREX1* gene regions. Seven SNPs genotyped between intron 21 of *SLC12A5* throughout all of the *NCOA5* sequence to approximately 30kb 3′ of *NCOA5* were associated with T2DM. *SLC12A5* is a potassium-chloride cotransporter that maintains intracellular chloride concentrations but is not expressed in tissues that seem directly involved in either T2DM or nephropathy [39]. *NCOA5* encodes a coregulator for the alpha and beta estrogen receptors [40]. Alpha estrogen receptor knockout mice are insulin resistant, obese, and have impaired glucose tolerance [41]. Preliminary functional studies in our laboratory have shown moderate levels of *NCOA5* in L6 and C2C12 skeletal muscle cell lines (data not shown). Three SNPs located from intron 3 to intron 6 of *CDH22* were associated with T2DM. *CDH22* encodes a calcium-dependent cell adhesion protein that is involved in morphogenesis and tissue formation and is expressed in diabetes-related tissues such as pancreatic beta cells and liver [42]. Eleven SNPs were associated with T2DM in the *PREX1* region. Three of these SNPs were located from intron 16 to the 3′ UTR of *PREX1* while most of the evidence of association with T2DM was observed 3′ of the gene sequence. *PREX1* encodes a guanine exchange factor for different RAC proteins in multiple cell types and is activated by the lipid second messenger phosphatidylinositol 3, 4, 5-triphosphate, generated by phosphoinositide 3-kinase (PI3K) and the βγ subunits of heterotrimeric G-proteins [43,44]. There is evidence that the PREX1 protein directly affects RAC1 and RAC2 activity [45]. RAC1 is a member of the of the Rho subfamily of small GTP-binding proteins and its activity has been implicated in cytoskeletal (actin) reorganization, glucose transporter 4 (GLUT4) translocation, and insulin secretion [46,47]. Therefore, it could be hypothesized that changes in the *PREX1* sequence, its expression, or its protein could influence the diabetes-related traits mediated by RAC1.

Despite the evidence of association with T2DM in our T2DM-ESRD population we failed to replicate observed associations near *NCOA5* and *CDH22* in the DHS sample population. This may reflect a lack of power given the small sample sizes of the T2DM-ESRD or DHS populations and the moderate effect sizes observed in the initial T2DM-ESRD population data. Recently however, association of *CDH22* with T2DM was observed in a genome-wide association study of a Scandinavian population [34]. In addition, genotyping of *NCOA5* SNPs in samples from the Insulin Resistance Atherosclerosis Study has provided suggestive evidence of association with SNPs located in *NCOA5* with various quantitative measures of glucose homeostasis (data not shown). The initial *NCOA5* and *CDH22* results were from cases with diabetes and ESRD. There is no biological information that supports a role for either

NCOA5 or *CDH22* with any form of nephropathy, although we cannot discount the effects of these genes based on this information alone.

SNPs that were associated with T2DM in the DHS population were those located 102–184kb 3′ of *PREX1*. More specifically, this area is part of a larger 800kb 'gene desert' region flanked by *SULF2* (sulfatase 2) and *PREX1* (45.9–46.7Mb, UCSC Genome Browser, March 2006 assembly). There are no annotated genes in the region that showed evidence of association and only 1 short mRNA found in testis [33]. In addition, none of the associated SNPs genotyped 3′ of *PREX1*, with the exception of rs4809696, are located in any LINE or SINE human repeats or long segmental duplications that may cause spurious evidence of association. rs4809696 is located in a 205bp L1 family member of the LINE superfamily (L1MDa) [33]. Not unlike previous investigations, this is another example of non-genic regions of DNA that are significantly associated with T2DM [35,37,48].

In the combined T2DM-ESRD and DHS population analysis we generally observed stronger evidence of association with T2DM with the SNPs 3′ of *PREX1* while no evidence was observed in *NCOA5* or *CDH22*. It is possible that the lack of association in *NCOA5* and *CDH22* in the combined population could be the result of heterogeneity between the DHS and T2DM-ESRD populations. However, we have no evidence that T2DM is expressed differently in the presence of ESRD. In fact, prior investigations in our laboratory examining other candidate genes have shown little to no differences in T2DM association results when comparing populations with or without ESRD. Detailed haplotype analyses in the region 3′ of *PREX1* showed both common and rare five-SNP haplotypes in this area were associated with altered risk for T2DM. If one takes into consideration that multiple SNPs were genotyped, correcting for multiple comparisons using a Bonferroni adjustment would result in no association P-values reaching the designated threshold (0.00053). However, due to the presence of LD among associated SNPs and the a priori nature of this study based on linkage results we believe that such a correction method is too stringent.

To better understand the relationship between the genotyped SNPs, adiposity, and T2DM, we repeated the association test accounting for BMI resulting in only two SNPs remaining marginally associated with T2DM in the region and six others trending towards significance with P_a-values ranging from 0.055 to 0.092. Subsequently, we tested whether these SNPs were associated with BMI itself and observed evidence of association comparable to that observed with T2DM. The test for association between BMI and these SNPs stratified by T2DM status identified 13 SNPs that were significantly associated with BMI solely in the case stratum suggesting that BMI may mediate the effect of these SNPs on T2DM. A formal mediation analysis revealed that approximately 30–40% of the effects six SNPs have on T2DM risk are significantly mediated by BMI. It is well known that obesity is a significant risk factor in the development of T2DM. In addition, there is strong evidence to suggest that various measures of obesity (i.e. BMI) and T2DM might be influenced by the same genetic and environmental factors. Therefore, it is sensible to imagine a causal pathway where the effect of these factors on T2DM is mediated by obesity. These results suggest that SNPs near the *PREX1* gene may contribute to diabetes susceptibility, which is mediated through effects of adiposity in European Americans.

These results, driven by extensive evidence in family studies, leads to the question of whether these associated SNPs are the basis for the prior evidence of linkage. The family sample in our study which lead us to focus on this region of chromosome 20 [1] is quite modest by current standards and has little power to address this issue. These results do, however, suggest a reappraisal of linkage in other study populations. This may be especially pertinent with the reemerging interest in family studies.

In conclusion, we have evaluated three candidate genes (*NCOA5*, *CDH22*, and *PREX1*) in the 20q12-13.13 T2DM-linked region on chromosome 20. Although we could not replicate these findings for *NCOA5* and *CDH22* in an independent population, recent reports and/or preliminary functional studies in our laboratory suggest there may be a role for these genes in T2DM risk. SNPs in a region 3′ of *PREX1* were also associated with T2DM in two independent populations. Takeuchi *et al*. recently described evidence of association with T2DM near *PREX1* [49]. Interestingly, the effect of these SNPs located 3′ of *PREX1* on T2DM risk are in some way mediated by measures of adiposity. Further evaluation of these genes is necessary to understand the biological impact these polymorphisms have on both T2DM and adiposity, but this study demonstrates the importance of targeted intensive analysis of genomic regions to locate T2DM genes.

4. Research Design and Methods

Subjects

Two independently recruited collections of T2DM patients and control subjects have been evaluated in this study. The first group consisted of 300 unrelated EA T2DM patients with end-stage renal disease (ESRD) and 310 randomly ascertained unrelated EA controls without known diabetes or renal disease. Collectively, this group is referred to here as the “T2DM-ESRD” population. Their recruitment has been previously described in detail [18–20]. Briefly, both cases and controls were recruited simultaneously. The T2DM-ESRD cases had a mean age at diagnosis of T2DM of 46.5 years, a mean duration of T2DM greater than 15 years, a mean BMI of 28.5 kg/m^2, and a mean HbA_{1c} of 8.6%. Controls had a mean age of 45.8 years and no BMI data available.

The second collection of T2DM-affected participants consisted of 470 probands from the Diabetes Heart Study (DHS) [21]. The DHS is a single-center family-based study of atherosclerosis in T2DM-enriched families. These participants (DHS cases) had a mean age of diagnosis of T2DM of 51.6 years old, mean BMI of 33.0 kg/m^2, a mean duration of T2DM of 11.2 years, and a mean HbA_{1C} of 7.5%. Unlike the T2DM-ESRD cases, DHS participants were recruited with an exclusion of diabetic nephropathy. As a control population for this group we genotyped DNA from 442 unrelated healthy self-declared EA subjects, who had a mean age of 57.3 years old and a mean BMI of 28.1kg/m^2. Collectively, this case-control sample is referred to as the “DHS” population. This study was approved by the Institutional Review Board of Wake Forest University School of Medicine and was in accordance with the principles of the Declaration of Helsinki

SNP Selection and Genotyping

All SNPs were selected from the dbSNP database prior to phase II of the HAPMAP project. SNPs were preferentially selected that were validated, had minor allele frequencies >0.1 in the Caucasian European sample (CEU) of the HAPMAP project, and were spaced evenly over the regions of interest. In the *NCOA5-CDH22* region 50 SNPs were selected for genotyping. Thirty four of these SNPs were genotyped in the HAPMAP project and corresponded to a mean r^2 >0.70 over the entire 296kb region compared to a mean r^2 <0.39 in our prior investigation. Similarly, in the *PREX1* region (~316kb) 45 SNPs were selected for genotyping, 33 of which were genotyped in the HAPMAP project and corresponding to a mean r^2 >0.60 compared to a mean r^2 <0.28 in our prior investigation [17].

Genotyping was performed using the Sequenom MassArray genotyping system (Sequenom Inc., San Diego, CA) [22]. Primer sequences are available upon request. SNP genotyping success rates in the combined population were >95.3% in cases and >94.4% in controls. Concordance between blind duplicate samples included in the genotyping was >98%.

Statistical Analyses

Each SNP was tested for departures from Hardy-Weinberg equilibrium (HWE) expectations by calculating a chi-square goodness of fit test. Structures of the haplotype blocks were ascertained using Haploview 3.2 [23] using the criteria outlined in Gabriel *et al*. [24].

Measures of linkage disequilibrium (LD) and association were calculated using the program SNPGWA [25]. For each pair of SNPs the LD statistics D′ and r^2 were computed. To test for an association between a SNP and T2DM the primary inference was based on the additive genetic model (Cochran-Armitage trend) and secondarily the overall 2-degree of freedom (genotypic) test. To test for departures from additivity a lack of fit test was calculated. All single-SNP association analyses were adjusted for age and sex, which did not significantly change the results. The haplotype frequencies in cases and controls were calculated using the expectation-maximization algorithm and compared using omnibus and haplotype-specific tests implemented in PLINK [26]. Tests of genotypic association as well as association under a priori genetic models adjusting for the effect of body mass index were calculated using SNPGWA. Quantitative trait association analyses were performed using a module of SNPGWA (QSNPGWA) [25]. BMI values were log-transformed to approximate normal distribution prior to analysis.

As part of this study we have carried out a Mendelian randomization analysis to test for the mediator effect of BMI on the association of the risk allele of a genetic locus with the outcome measure T2DM. Specifically, we tested if the effect of the allele on T2DM varies with the levels of BMI such that BMI may account for some of the correlation observed between the risk allele and T2DM [27–29]. This definition in itself is valid for both confounding and mediation effects. Statistical mediation has proven to be a valuable tool for understanding complex relationships between three or more variables, but this approach by itself is not sufficient to tease apart a confounding effect from a mediation effect.

Conventional mediation analyses assumes that both the mediator and outcome are continuous variables [30]. For example, three linear models are fitted: one for the direct effect of the independent variable (X) on the outcome of interest (Y), another for testing the same effects after adjusting for the mediator (Z), and finally, a model that tests for association between the independent variable (X) and the mediator (Z). The appropriate parameter estimates from each model are then combined into a statistical test for mediation. This approach was modified in our analysis since the outcome variable (T2DM) and independent variable (locus allele) were binary in nature. In this case, the estimated parameters needed to be standardized to account for the difference in scale observed between logistic and linear regressions. Here we have used the scaling methods described in Mackinnon *et al*., 1993 and the SAS macro provided by Jasti *et al*., 2008 to test whether obesity measured by BMI is mediator of the effect between SNPs within *PREX1* and T2DM [28,31]. Statistical significance was evaluated using large sample tests where the variance of the mediation parameter estimate is computed using both Sobel's [30] and Goodman's formulations [32].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Bowden DW, et al. Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. Diabetes 1997;46(5):882–6. [PubMed: 9133559]
2. Ji L, et al. New susceptibility locus for NIDDM is localized to human chromosome 20q. Diabetes 1997;46(5):876–81. [PubMed: 9133558]
3. Zouali H, et al. A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. Hum Mol Genet 1997;6(9):1401–8. [PubMed: 9285775]
4. Ghosh S, et al. Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. Proc Natl Acad Sci U S A 1999;96(5):2198–203. [PubMed: 10051618]
5. Klupa T, et al. Further evidence for a susceptibility locus for type 2 diabetes on chromosome 20q13.1-q13.2. Diabetes 2000;49(12):2212–6. [PubMed: 11118028]
6. Permutt MA, et al. A genome scan for type 2 diabetes susceptibility loci in a genetically isolated population. Diabetes 2001;50(3):681–5. [PubMed: 11246891]
7. Luo TH, et al. A genome-wide search for type II diabetes susceptibility genes in Chinese Hans. Diabetologia 2001;44(4):501–6. [PubMed: 11357482]
8. Mori Y, et al. Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. Diabetes 2002;51(4):1247–55. [PubMed: 11916952]
9. Love-Gregory LD, et al. A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 alpha gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an ashkenazi jewish population. Diabetes 2004;53(4):1134–40. [PubMed: 15047632]
10. Silander K, et al. Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. Diabetes 2004;53(4):1141–9. [PubMed: 15047633]
11. Weedon MN, et al. Common variants of the hepatocyte nuclear factor-4alpha P2 promoter are associated with type 2 diabetes in the U.K. population. Diabetes 2004;53(11):3002–6. [PubMed: 15504983]
12. Wanic K, et al. Polymorphisms in the gene encoding hepatocyte nuclear factor-4alpha and susceptibility to type 2 diabetes in a Polish population. Diabetes Metab 2006;32(1):86–8. [PubMed: 16523192]
13. Traurig M, et al. Protein tyrosine phosphatase 1B is not a major susceptibility gene for type 2 diabetes mellitus or obesity among Pima Indians. Diabetologia 2007;50(5):985–9. [PubMed: 17333110]
14. Bento JL, et al. Genetic analysis of the GLUT10 glucose transporter (SLC2A10) polymorphisms in Caucasian American type 2 diabetes. BMC Med Genet 2005;6:42. [PubMed: 16336637]
15. Florez JC, et al. Association testing of the protein tyrosine phosphatase 1B gene (PTPN1) with type 2 diabetes in 7,883 people. Diabetes 2005;54(6):1884–91. [PubMed: 15919813]
16. Spencer-Jones NJ, et al. Protein tyrosine phosphatase-1B gene PTPN1: selection of tagging single nucleotide polymorphisms and association with body fat, insulin sensitivity, and the metabolic syndrome in a normal female population. Diabetes 2005;54(11):3296–304. [PubMed: 16249458]
17. Bento JL, et al. Heterogeneity in gene loci associated with type 2 diabetes on human chromosome 20q13.1. Genomics 2008;92(4):226–34. [PubMed: 18602983]
18. Yu H, et al. Identification of human plasma kallikrein gene polymorphisms and evaluation of their role in end-stage renal disease. Hypertension 1998;31(4):906–11. [PubMed: 9535413]
19. Freedman BI, et al. Genetic analysis of nitric oxide and endothelin in end-stage renal disease. Nephrol Dial Transplant 2000;15(11):1794–800. [PubMed: 11071967]
20. Sale MM, et al. A genome-wide scan for type 2 diabetes in African-American families reveals evidence for a locus on chromosome 6q. Diabetes 2004;53(3):830–7. [PubMed: 14988270]
21. Wagenknecht LE, et al. Familial aggregation of coronary artery calcium in families with type 2 diabetes. Diabetes 2001;50(4):861–6. [PubMed: 11289053]
22. Buetow KH, et al. High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser

desorption/ionization time-of-flight mass spectrometry. Proc Natl Acad Sci U S A 2001;98(2):581–4. [PubMed: 11136232]

23. Barrett JC, et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21(2):263–5. [PubMed: 15297300]
24. Gabriel SB, et al. The structure of haplotype blocks in the human genome. Science 2002;296(5576): 2225–9. [PubMed: 12029063]
25. Matarin M, et al. A genome-wide genotyping study in patients with ischaemic stroke: initial analysis and data release. Lancet Neurol 2007;6(5):414–20. [PubMed: 17434096]
26. Purcell S, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81(3):559–75. [PubMed: 17701901]
27. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51(6):1173–82. [PubMed: 3806354]
28. MacKinnon DP, Dwyer JH. Estimating Mediated Effects in Prevention Studies. Evaluation Review 1993;17:144–158.
29. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. Annu Rev Psychol 2007;58:593–614. [PubMed: 16968208]
30. Sobel, M. Sociological methodology. San Francisco, CA: Jossey-Bass; 1982. Asymptotic intervals for indirect effects in structural equations models.
31. Jasti S, Dudley WN, Goldwater E. SAS macros for testing statistical mediation in data with binary mediators or outcomes. Nurs Res 2008;57(2):118–22. [PubMed: 18347484]
32. Goodman, L. Journal of the American Statistical Association. Vol. 55. 1960. On the exact variance of products; p. 708-713.
33. Kent WJ, et al. The human genome browser at UCSC. Genome Res 2002;12(6):996–1006. [PubMed: 12045153]
34. Saxena R, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316(5829):1331–6. [PubMed: 17463246]
35. Scott LJ, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316(5829):1341–5. [PubMed: 17463248]
36. Sladek R, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445(7130):881–5. [PubMed: 17293876]
37. Zeggini E, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316(5829):1336–41. [PubMed: 17463249]
38. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447(7145):661–78. [PubMed: 17554300]
39. Sallinen R, et al. Chromosomal localization of SLC12A5/Slc12a5, the human and mouse genes for the neuron-specific K(+)-Cl(−) cotransporter (KCC2) defines a new region of conserved homology. Cytogenet Cell Genet 2001;94(1–2):67–70. [PubMed: 11701957]
40. Sauve F, et al. CIA, a novel estrogen receptor coactivator with a bifunctional nuclear receptor interacting determinant. Mol Cell Biol 2001;21(1):343–53. [PubMed: 11113208]
41. Heine PA, et al. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci U S A 2000;97(23):12729–34. [PubMed: 11070086]
42. Sugimoto K, et al. Molecular cloning and characterization of a newly identified member of the cadherin family, PB-cadherin. J Biol Chem 1996;271(19):11548–56. [PubMed: 8626716]
43. Welch HC, et al. P-Rex1, a PtdIns(3,4,5)P3- and Gbetagamma-regulated guanine-nucleotide exchange factor for Rac. Cell 2002;108(6):809–21. [PubMed: 11955434]
44. Hill K, et al. Regulation of P-Rex1 by phosphatidylinositol (3,4,5)-trisphosphate and Gbetagamma subunits. J Biol Chem 2005;280(6):4166–73. [PubMed: 15545267]
45. Welch HC, et al. P-Rex1 regulates neutrophil function. Curr Biol 2005;15(20):1867–73. [PubMed: 16243035]
46. Khayat ZA, et al. Insulin-induced actin filament remodeling colocalizes actin with phosphatidylinositol 3-kinase and GLUT4 in L6 myotubes. J Cell Sci 2000;113(Pt 2):279–90. [PubMed: 10633079]

47. Kowluru A, Veluthakal R. Rho guanosine diphosphate-dissociation inhibitor plays a negative modulatory role in glucose-stimulated insulin secretion. Diabetes 2005;54(12):3523–9. [PubMed: 16306371]

48. Das SK, et al. Polymorphisms in the glucokinase-associated, dual-specificity phosphatase 12 (DUSP12) gene under chromosome 1q21 linkage peak are associated with type 2 diabetes. Diabetes 2006;55(9):2631–9. [PubMed: 16936214]

49. Takeuchi F, et al. Search of type 2 diabetes susceptibility gene on chromosome 20q. Biochem Biophys Res Commun 2007;357(4):1100–6. [PubMed: 17466274]

Table 1

Population Characteristics

	N	Mean Age ± Std. Deviation (Years)	Mean BMI ± Std. Deviation (Kg/m^2)	Mean HbA1C (%)
T2DM-ESRD Group				
Cases	300†	46.5 ± 12.8	28.5 ± 6.8	8.6
Controls	310	45.8 ± 10.6	-	-
Combined	610	46.1 ± 11.5	28.5 ± 6.8	-
DHS Group				
Cases	470	51.6 ± 9.3	33.0 ± 6.7	7.5
Controls	442*	57.3 ± 13.7	28.1 ± 5.2	-
Combined	912	54.4 ± 11.2	30.6 ± 6.5	-
Entire Population				
Cases	770†	49.6 ± 10.6	31.2 ± 7.1	7.9
Controls	752*	52.6 ± 12.1	28.1 ± 5.2	-
Combined	1522	51.1 ± 11.6	30.1 ± 6.7	-

*BMI data was available for 407 controls

†BMI data was available for 291 cases in the ESRD-T2DM group making a total of 761 cases with BMI data in the entire population

Table 2

Single-SNP additive test of association with T2DM in T2DM-ESRD, DHS, and combined populations

				ESRD-DHS Population		T2DM-ESRD Population		DHS Population		ESRD-DHS Population	
Gene	**SNP**	**Position (kb)**	**Major/Minor Alleles**	**Minor Allele Frequency Cases**	**Minor Allele Frequency Controls**	**P_a-value**	**OR (95% CI)**	**P_a- value**	**OR (95% CI)**	**P_a- value**	**OR (95% CI)**
	rs3918241	44069.142	T/A	0.148	0.137	0.2254	1.22 (0.88–1.68)	0.9176	1.01 (0.78–1.32)	0.3781	1.10 (0.89–1.34)
MMP9	rs20544	44078.417	T/C	0.417	0.410	0.3206	1.12 (0.89–1.41)	0.7632	0.97 (0.80–1.18)	0.6996	1.03 (0.89–1.19)
	rs6130998	44086.526	C/T	0.204	0.189	0.9004	1.02 (0.76–1.36)	0.2185	1.16 (0.92–1.46)	0.2928	1.10 (0.92–1.32)
	rs3848726	44100.003	G/T	0.374	0.392	0.2478	0.87 (0.68–1.10)	0.7187	0.96 (0.80–1.17)	0.3103	0.93 (0.80–1.07)
	rs2297198	44107.690	C/T	0.207	0.200	0.4246	1.12 (0.84–1.50)	0.9789	1.00 (0.79–1.27)	0.5887	1.05 (0.88–1.26)
SLC12A5	rs2297200	44117.933	T/A	0.328	0.326	**0.0193**	1.33 (1.04–1.70)	0.0830	0.84 (0.69–1.02)	0.9064	1.01 (0.87–1.17)
	rs2297201	44118.385	C/T	0.061	0.070	0.7937	1.06 (0.70–1.61)	0.1208	0.75 (0.51–1.09)	0.3262	0.87 (0.65–1.15)
	rs9074	44122.072	G/A	0.249	0.271	**0.0083**	0.70 (0.54–0.91)	0.6251	1.06 (0.85–1.31)	0.1656	0.89 (0.75–1.05)
	rs1537028	44124.757	T/G	0.331	0.328	**0.0196**	1.33 (1.04–1.71)	0.1181	0.85 (0.70–1.04)	0.8488	1.02 (0.87–1.18)
	rs2903908	44127.354	T/C	0.269	0.264	**0.0237**	1.35 (1.04–1.75)	0.1429	0.86 (0.69–1.06)	0.7582	1.03 (0.87–1.21)
NCOA5	rs3092502	44130.838	G/T	0.449	0.456	0.0699	0.81 (0.64–1.02)	0.3169	1.10 (0.91–1.33)	0.7052	0.97 (0.84–1.13)
	rs1406826	44140.771	A/G	0.449	0.463	**0.0168**	0.75 (0.59–0.95)	0.3368	1.10 (0.91–1.33)	0.4634	0.95 (0.82–1.10)
	rs1950174	44147.811	C/A	0.208	0.210	0.2038	0.83 (0.62–1.11)	0.4436	1.09 (0.87–1.36)	0.9015	0.99 (0.83–1.18)
	rs6065921	44147.917	A/G	0.191	0.187	0.7786	1.04 (0.78–1.40)	0.8922	1.02 (0.81–1.28)	0.7594	1.03 (0.86–1.24)
	rs2868764	44155.418	T/G	0.448	0.461	**0.0445**	0.79 (0.63–0.99)	0.4849	1.07 (0.89–1.30)	0.4446	0.95 (0.82–1.09)
	rs6032664	44172.826	T/A	0.235	0.250	**0.0306**	0.75 (0.58–0.97)	0.4497	1.09 (0.87–1.36)	0.3681	0.93 (0.79–1.09)
CD40	rs3746821	44188.518	G/T	0.111	0.108	0.1237	0.75 (0.53–1.08)	0.0987	1.30 (0.95–1.78)	0.8127	1.03 (0.81–1.30)
	rs1535043	44201.131	T/A	0.440	0.447	0.7283	1.04 (0.83–1.30)	0.4563	0.93 (0.78–1.12)	0.7281	0.98 (0.85–1.13)
	rs2064405	44210.912	G/T	0.105	0.100	0.3136	0.83 (0.58–1.19)	0.1478	1.27 (0.92–1.75)	0.6788	1.05 (0.83–1.34)
	rs2425754	44222.040	G/A	0.139	0.143	0.6208	0.92 (0.66–1.28)	0.9742	1.00 (0.76–1.31)	0.7052	0.96 (0.78–1.19)
CDH22	rs6131030	44241.394	G/A	0.390	0.377	0.4616	1.09 (0.86–1.38)	0.7946	1.03 (0.85–1.24)	0.5003	1.05 (0.91–1.22)
	rs6074061	44246.711	C/G	0.272	0.279	0.8967	0.98 (0.77–1.26)	0.6310	0.95 (0.77–1.17)	0.6340	0.96 (0.82–1.13)

				ESRD-DHS Population		T2DM-ESRD Population		DHS Population		ESRD-DHS Population	
Gene	**SNP**	**Position (kb)**	**Major/Minor Alleles**	**Minor Allele Frequency Cases**	**Minor Allele Frequency Controls**	**P_a-value**	**OR (95% CI)**	**P_a- value**	**OR (95% CI)**	**P_a- value**	**OR (95% CI)**
	rs1321001	44250.143	T/G	0.173	0.152	0.0510	1.33 (0.99–1.77)	0.6875	1.05 (0.82–1.36)	0.1253	1.16 (0.96–1.41)
	rs4383400	44253.272	T/C	0.066	0.052	0.0571	1.49 (0.98–2.26)	0.7317	1.07 (0.71–1.62)	0.1262	1.26 (0.94–1.69)
	rs2868767	44258.031	A/G	0.180	0.183	0.5940	1.08 (0.81–1.45)	0.4829	0.91 (0.71–1.17)	0.8279	0.98 (0.81–1.18)
	rs1010310	44268.451	C/A	0.292	0.331	**0.0009**	0.67 (0.52–0.86)	0.7090	0.96 (0.78–1.18)	**0.0191**	0.83 (0.71–0.97)
	rs2425785	44272.729	G/C	0.351	0.328	0.0646	1.26 (0.99–1.61)	0.8791	1.02 (0.84–1.23)	0.1872	1.11 (0.95–1.29)
	rs2425786	44277.882	T/C	0.330	0.313	**0.0108**	1.38 (1.08–1.77)	0.3977	0.91 (0.74–1.12)	0.3216	1.08 (0.92–1.27)
	rs761048	44284.465	G/C	0.074	0.082	0.3866	0.84 (0.57–1.25)	0.7534	0.95 (0.66–1.35)	0.3871	0.89 (0.68–1.16)
	rs6074069	44288.133	G/A	0.219	0.206	**0.0221**	1.38 (1.04–1.84)	0.5068	0.93 (0.73–1.17)	0.3780	1.08 (0.91–1.30)
	rs2050113	44293.878	A/C	0.075	0.081	0.7841	0.95 (0.64–1.42)	0.5401	0.90 (0.63–1.27)	0.5065	0.91 (0.70–1.19)
	rs966567	44300.632	G/C	0.247	0.231	0.0532	1.30 (0.99–1.70)	0.9129	0.99 (0.79–1.23)	0.2859	1.10 (0.93–1.30)
	rs3904166	44306.828	C/A	0.243	0.227	0.0705	1.29 (0.98–1.69)	0.9227	0.99 (0.80–1.23)	0.2982	1.09 (0.92–1.30)
	rs3848729	44308.103	G/T	0.361	0.356	0.8748	0.98 (0.77–1.25)	0.6653	1.04 (0.86–1.26)	0.7897	1.02 (0.88–1.18)
	rs2425807	44314.952	A/G	0.428	0.439	0.9567	1.01 (0.79–1.27)	0.3878	0.92 (0.77–1.11)	0.5451	0.96 (0.83–1.11)
	rs2425809	44321.008	C/T	0.447	0.454	0.8441	0.98 (0.77–1.23)	0.6887	0.96 (0.80–1.16)	0.6829	0.97 (0.84–1.12)
	rs2425817	44328.693	G/A	0.445	0.458	0.4385	0.92 (0.73–1.15)	0.7526	0.97 (0.80–1.17)	0.4904	0.95 (0.82–1.10)
	rs2425855	44344.506	T/A	0.464	0.458	0.4536	1.10 (0.87–1.39)	0.8583	0.98 (0.82–1.18)	0.7499	1.02 (0.89–1.18)
	rs6032754	44352.166	T/C	0.263	0.260	0.3428	1.13 (0.88–1.46)	0.5904	0.94 (0.76–1.17)	0.8339	1.02 (0.86–1.20)
	rs6017767	44365.336	C/G	0.475	0.494	0.1061	0.83 (0.66–1.04)	0.9768	1.00 (0.82–1.20)	0.2887	0.92 (0.80–1.07)
	rs6095091	46441.248	C/T	0.261	0.260	0.6237	1.07 (0.82–1.39)	0.7561	0.97 (0.79–1.19)	0.9504	1.01 (0.85–1.18)
	rs6066724	46451.845	G/A	0.289	0.286	0.8435	0.97 (0.76–1.25)	0.6920	1.04 (0.85–1.28)	0.8523	1.01 (0.87–1.19)
	rs2008645	46458.897	G/A	0.418	0.424	0.6372	0.95 (0.75–1.19)	0.9372	0.99 (0.83–1.19)	0.7427	0.98 (0.85–1.13)
	rs4809688	46461.222	A/G	0.433	0.447	0.3206	0.89 (0.71–1.12)	0.8746	0.98 (0.82–1.19)	0.4587	0.95 (0.82–1.09)
Replicated region 3′ of *PREX1*	rs999151	46488.676	C/T	0.302	0.269	0.5953	1.07 (0.83–1.39)	**0.0297**	1.26 (1.02–1.56)	**0.0411**	1.18 (1.00–1.40)
	rs7263053	46496.933	C/T	0.288	0.237	**0.0263**	1.36 (1.04–1.78)	**0.0244**	1.28 (1.03–1.58)	**0.0013**	1.31 (1.11–1.55)
	rs1321006	46499.668	G/A	0.374	0.318	**0.0194**	1.34 (1.05–1.72)	**0.0199**	1.26 (1.03–1.54)	**0.0013**	1.29 (1.11–1.51)
	rs4809696	46507.214	T/C	0.369	0.318	0.0534	1.28 (1.00–1.63)	**0.0174**	1.27 (1.04–1.55)	**0.0030**	1.27 (1.09–1.48)
	rs926693	46512.364	A/G	0.491	0.434	**0.0103**	1.35 (1.08–1.70)	0.0569	1.20 (0.99–1.44)	**0.0022**	1.25 (1.09–1.45)
	rs926692	46515.682	A/C	0.494	0.435	**0.0103**	1.36 (1.08–1.72)	**0.0413**	1.22 (1.01–1.46)	**0.0016**	1.27 (1.09–1.46)
	rs12624650	46526.719	G/A	0.203	0.191	0.3767	1.13 (0.86–1.50)	0.7423	1.04 (0.83–1.30)	0.4024	1.08 (0.90–1.28)

				ESRD-DHS Population		T2DM-ESRD Population		DHS Population		ESRD-DHS Population	
Gene	**SNP**	**Position (kb)**	**Major/Minor Alleles**	**Minor Allele Frequency Cases**	**Minor Allele Frequency Controls**	**P_a-value**	**OR (95% CI)**	**P_a- value**	**OR (95% CI)**	**P_a- value**	**OR (95% CI)**
	rs6019212	46527.953	G/T	0.479	0.433	**0.0268**	1.29 (1.03–1.63)	0.1467	1.15 (0.95–1.38)	**0.0131**	1.20 (1.04–1.38)
	rs6019216	46530.327	G/A	0.285	0.252	0.0921	1.24 (0.97–1.58)	0.2481	1.13 (0.92–1.38)	0.0532	1.17 (1.00–1.37)
	rs6122689	46533.130	C/T	0.184	0.159	0.1303	1.26 (0.94–1.71)	0.2602	1.16 (0.90–1.49)	0.0656	1.20 (0.99–1.45)
	rs12481537	46572.852	A/G	0.492	0.453	0.3588	1.11 (0.89–1.39)	**0.0418**	1.21 (1.01–1.47)	**0.0343**	1.17 (1.01–1.35)
	rs2904081	46593.440	C/G	0.310	0.283	0.2670	1.16 (0.89–1.52)	0.2387	1.13 (0.92–1.38)	0.0965	1.14 (0.97–1.34)
	rs4810813	46601.875	T/C	0.325	0.283	**0.0012**	1.54 (1.17–2.02)	0.4499	1.08 (0.88–1.32)	**0.0134**	1.22 (1.04–1.44)
	rs6019300	46634.916	A/G	0.470	0.449	0.7097	0.96 (0.76–1.20)	0.0531	1.22 (1.00–1.49)	0.2437	1.09 (0.94–1.27)
	rs6090875	46641.633	T/C	0.242	0.227	**0.0295**	1.34 (1.02–1.76)	0.6288	0.94 (0.75–1.18)	0.3237	1.09 (0.92–1.30)
	rs3924220	46642.853	T/C	0.244	0.224	**0.0029**	1.51 (1.15–1.98)	0.4404	0.92 (0.73–1.14)	0.1966	1.12 (0.94–1.33)
	rs4810837	46662.565	T/G	0.409	0.400	0.1565	1.19 (0.94–1.50)	0.5477	0.95 (0.79–1.14)	0.6365	1.04 (0.90–1.20)
	rs6012501	46672.841	G/C	0.434	0.420	0.0868	1.23 (0.97–1.56)	0.7426	0.97 (0.81–1.16)	0.4274	1.06 (0.92–1.23)
PREX1	rs2664570	46674.765	C/T	0.209	0.196	**0.0256**	1.39 (1.04–1.86)	0.5165	0.93 (0.73–1.17)	0.3775	1.09 (0.91–1.30)
	rs3935549	46686.895	C/T	0.488	0.499	0.5336	0.93 (0.75–1.16)	0.6918	0.96 (0.80–1.16)	0.4874	0.95 (0.83–1.09)
	rs2073072	46703.010	C/G	0.202	0.194	**0.0311**	1.39 (1.03–1.88)	0.3391	0.89 (0.71–1.13)	0.5696	1.06 (0.88–1.27)
	rs2294910	46709.789	A/G	0.204	0.190	**0.0126**	1.47 (1.09–1.98)	0.4515	0.92 (0.73–1.16)	0.3406	1.09 (0.91–1.32)
	rs6095228	46713.283	G/A	0.077	0.087	0.4748	0.85 (0.55–1.34)	0.5192	0.91 (0.69–1.21)	0.3743	0.90 (0.71–1.14)
	rs6012506	46719.044	A/G	0.477	0.465	0.2798	1.14 (0.90–1.43)	0.9665	1.00 (0.83–1.20)	0.5190	1.05 (0.91–1.21)
	rs6095239	46730.946	G/A	0.332	0.325	0.4730	1.09 (0.86–1.39)	0.9791	1.00 (0.81–1.22)	0.6752	1.03 (0.88–1.21)
	rs3746819	46741.342	A/G	0.085	0.088	0.3016	0.80 (0.52–1.24)	0.7886	1.04 (0.76–1.43)	0.7091	0.95 (0.74–1.23)
	rs4809718	46744.395	T/A	0.213	0.219	0.1877	0.84 (0.64–1.09)	0.5525	1.07 (0.85–1.35)	0.6849	0.96 (0.81–1.15)
	rs736659	46757.117	G/A	0.218	0.220	0.3608	0.88 (0.68–1.15)	0.5412	1.07 (0.86–1.35)	0.8823	0.99 (0.83–1.17)

Bold: P-values <0.05 and corresponding OR

Table 3

Haplotype analysis of five-SNP haplotypes 3′ of *PREX1* in the combined population

Haplotype (rs7263053, rs1321006, rs4809696, rs926693, and rs926692)	Case Frequency (%)	Control Frequency (%)	Empirical Hap-specific P-value	OR (95% CI)	Global Simulated P-value
CGTAT	0.506	0.561	**0.003**	0.87 (0.75–0.99)	
TACGG	0.290	0.224	**5.93×10^{-4}**	1.41 (1.19–1.67)	**0.004**
CGTGG	0.125	0.130	0.660	0.99 (0.79–1.23)	
CACGG	0.081	0.077	0.685	1.09 (0.84–1.41)	

Haplotypes with frequencies <0.02 were excluded from this analysis. **Bold**: P-values <0.05

Table 4

Single-SNP tests of association with T2DM adjusted for BMI (ESRD-DHS population)

Gene	SNP	Position (kb)	Major/Minor Alleles	Minor Allele Frequency Cases	Minor Allele Frequency Controls	P_a-value	OR (95% CI)
	rs3918241	44069.142	T/A	0.149	0.146	0.5409	1.08 (0.84–1.39)
MMP9	rs20544	44078.417	T/C	0.417	0.425	0.8752	1.01 (0.85–1.22)
	rs6130998	44086.526	C/T	0.205	0.196	0.5769	1.07 (0.85–1.33)
SLC12A5	rs3848726	44100.003	G/T	0.374	0.390	0.3707	0.92 (0.77–1.10)
	rs2297198	44107.690	C/T	0.209	0.207	0.8507	1.02 (0.82–1.28)
	rs2297200	44117.933	T/A	0.330	0.342	0.6478	0.96 (0.80–1.15)
	rs2297201	44118.385	C/T	0.061	0.066	0.5736	0.90 (0.63–1.29)
	rs9074	44122.072	G/A	0.248	0.246	0.8036	1.03 (0.84–1.26)
NCOA5	rs1537028	44124.757	T/G	0.333	0.338	0.8882	0.99 (0.82–1.19)
	rs2903908	44127.354	T/C	0.270	0.286	0.4415	0.93 (0.76–1.13)
	rs3092502	44130.838	G/T	0.448	0.438	0.4665	1.07 (0.89–1.28)
	rs1406826	44140.771	A/G	0.449	0.442	0.5833	1.05 (0.88–1.26)
	rs1950174	44147.811	C/A	0.208	0.217	0.3365	0.90 (0.73–1.12)
	rs6065921	44147.917	A/G	0.190	0.195	0.9420	0.99 (0.79–1.24)
	rs2868764	44155.418	T/G	0.447	0.443	0.6388	1.04 (0.87–1.25)
	rs6032664	44172.826	T/A	0.235	0.223	0.4161	1.09 (0.89–1.34)
CD40	rs3746821	44188.518	G/T	0.110	0.091	0.0992	1.29 (0.95–1.75)
	rs1535043	44201.131	T/A	0.440	0.463	0.3283	0.92 (0.77–1.09)
	rs2064405	44210.912	G/T	0.104	0.087	0.1208	1.28 (0.94–1.74)
	rs2425754	44222.040	G/A	0.137	0.142	0.6543	0.94 (0.73–1.22)
	rs6131030	44241.394	G/A	0.390	0.376	0.4270	1.08 (0.90–1.29)
CDH22	rs6074061	44246.711	C/G	0.272	0.279	0.9198	0.99 (0.81–1.20)
	rs1321001	44250.143	T/G	0.174	0.147	0.1149	1.21 (0.95–1.55)
	rs4383400	44253.272	T/C	0.067	0.048	0.1573	1.31 (0.90–1.91)
	rs2868767	44258.031	A/G	0.180	0.181	0.9527	0.99 (0.79–1.25)

Gene	SNP	Position (kb)	Major/Minor Alleles	Minor Allele Frequency Cases	Minor Allele Frequency Controls	P_a-value	OR (95% CI)
	rs1010310	44268.451	C/A	0.294	0.318	0.1971	0.88 (0.72–1.07)
	rs2425785	44272.729	G/C	0.351	0.344	0.5299	1.06 (0.88–1.27)
	rs2425786	44277.882	T/C	0.330	0.312	0.4619	1.07 (0.89–1.30)
	rs761048	44284.465	G/C	0.074	0.069	0.9006	0.98 (0.69–1.38)
	rs6074069	44288.133	G/A	0.219	0.207	0.4121	1.09 (0.88–1.36)
	rs2050113	44293.878	A/C	0.075	0.075	0.6678	0.93 (0.66–1.30)
	rs966567	44300.632	G/C	0.248	0.231	0.3345	1.11 (0.90–1.36)
	rs3904166	44306.828	C/A	0.244	0.226	0.3125	1.11 (0.91–1.36)
	rs3848729	44308.103	G/T	0.362	0.358	0.7501	0.97 (0.81–1.16)
	rs2425807	44314.952	A/G	0.429	0.456	0.1471	0.88 (0.73–1.05)
	rs2425809	44321.008	C/T	0.448	0.467	0.2672	0.90 (0.75–1.08)
	rs2425817	44328.693	G/A	0.446	0.466	0.2638	0.90 (0.76–1.08)
	rs2425855	44344.506	T/A	0.465	0.473	0.6489	0.96 (0.81–1.14)
	rs6032754	44352.166	T/C	0.264	0.265	0.8399	1.02 (0.84–1.24)
	rs6017767	44365.336	C/G	0.473	0.491	0.2973	0.91 (0.76–1.09)
	rs6095091	46441.248	C/T	0.261	0.264	0.4615	0.93 (0.76–1.13)
	rs6066724	46451.845	G/A	0.289	0.290	0.4338	0.92 (0.76–1.13)
	rs2008645	46458.897	G/A	0.418	0.432	0.2778	0.91 (0.76–1.08)
	rs4809688	46461.222	A/G	0.432	0.449	0.2889	0.91 (0.76–1.08)
Replicated Region 3′ of *PREX1*	rs999151	46488.676	C/T	0.300	0.273	0.5161	1.07 (0.87–1.31)
	rs7263053	46496.933	C/T	0.285	0.251	0.2228	1.13 (0.93–1.39)
	rs1321006	46499.668	G/A	0.372	0.323	0.0804	1.18 (0.98–1.43)
	rs4809696	46507.214	T/C	0.368	0.321	0.0921	1.18 (0.97–1.42)
	rs926693	46512.364	A/G	0.491	0.434	**0.0442**	**1.20 (1.00–1.43)**
	rs926692	46515.682	A/C	0.493	0.433	**0.0296**	**1.22 (1.02–1.46)**
	rs12624650	46526.719	G/A	0.204	0.197	0.9284	1.01 (0.82–1.25)
	rs6019212	46527.953	G/T	0.479	0.428	0.0812	1.17 (0.98–1.39)
	rs6019216	46530.327	G/A	0.286	0.244	0.0668	1.20 (0.99–1.46)
	rs6122689	46533.130	C/T	0.183	0.162	0.3881	1.11 (0.88–1.41)
	rs12481537	46572.852	A/G	0.492	0.439	0.0551	1.19 (1.00–1.42)

Gene	SNP	Position (kb)	Major/Minor Alleles	Minor Allele Frequency Cases	Minor Allele Frequency Controls	P_a-value	OR (95% CI)
	rs2904081	46593.440	C/G	0.310	0.291	0.3480	1.10 (0.90–1.33)
	rs4810813	46601.875	T/C	0.325	0.308	0.4122	1.08 (0.90–1.31)
	rs6019300	46634.916	A/G	0.469	0.430	0.0682	1.19 (0.99–1.44)
	rs6090875	46641.633	T/C	0.242	0.240	0.9232	1.01 (0.82–1.25)
	rs3924220	46642.853	T/C	0.243	0.244	0.9131	0.99 (0.80–1.22)
	rs4810837	46662.565	T/G	0.407	0.437	0.1841	0.89 (0.74–1.06)
	rs6012501	46672.841	G/C	0.433	0.451	0.4630	0.94 (0.79–1.12)
PREX1	rs2664570	46674.765	C/T	0.208	0.212	0.9822	1.00 (0.81–1.25)
	rs3935549	46686.895	C/T	0.489	0.489	0.9594	1.00 (0.84–1.19)
	rs2073072	46703.010	C/G	0.202	0.210	0.6025	0.94 (0.76–1.18)
	rs2294910	46709.789	A/G	0.203	0.208	0.7711	0.97 (0.78–1.21)
	rs6095228	46713.283	G/A	0.078	0.104	0.0803	0.79 (0.60–1.03)
	rs6012506	46719.044	A/G	0.475	0.480	0.8122	0.98 (0.82–1.17)
	rs6095239	46730.946	G/A	0.333	0.329	0.8153	1.02 (0.84–1.24)
	rs3746819	46741.342	A/G	0.085	0.099	0.3775	0.87 (0.65–1.18)
	rs4809718	46744.395	T/A	0.211	0.211	0.9583	0.99 (0.80–1.23)
	rs736659	46757.117	G/A	0.216	0.211	0.7848	1.03 (0.83–1.28)

Bold: P-values <0.05 and corresponding OR

Table 5

Single-SNP tests of association with BMI (ESRD-DHS population)

				Combined Population			Cases Only		Controls Only	
Gene	**SNP**	**Position (kb)**	**Major/Minor Alleles**	**Minor Allele Frequency**	**Additive P-value**	**β ± SE**	**Additive P-value**	**β ± SE**	**Additive P-value**	**β ± SE**
	rs3918241	44069.142	T/A	0.148	0.0628	−0.010 ± 0.006	0.0763	−0.012 ± 0.007	0.4191	−0.007 ± 0.008
MMP9	rs20544	44078.417	T/C	0.420	**0.0105**	−0.009 ± 0.004	**0.0121**	−0.012 ± 0.005	0.4975	−0.003 ± 0.006
	rs6130998	44086.526	C/T	0.202	0.9588	0.000 ± 0.005	0.9190	−0.001 ± 0.006	0.8569	0.000 ± 0.007
SLC12A5	rs3848726	44100.003	G/T	0.379	0.5163	0.002 ± 0.004	0.5365	0.003 ± 0.005	0.5379	0.003 ± 0.006
	rs2297198	44107.690	C/T	0.208	0.5791	−0.002 ± 0.005	0.4020	−0.005 ± 0.006	0.7411	0.004 ± 0.007
	rs2297200	44117.933	T/A	0.334	0.6902	−0.002 ± 0.004	0.9803	−0.002 ± 0.005	0.5892	−0.003 ± 0.006
	rs2297201	44118.385	C/T	0.062	0.5738	0.003 ± 0.008	0.2019	0.011 ± 0.010	0.3183	−0.011 ± 0.012
	rs9074	44122.072	G/A	0.247	0.4453	−0.002 ± 0.005	0.4894	−0.003 ± 0.006	0.6769	−0.002 ± 0.007
NCOA5	rs1537028	44124.757	T/G	0.335	0.7710	−0.002 ± 0.004	0.9846	−0.002 ± 0.005	0.6425	−0.002 ± 0.006
	rs2903908	44127.354	T/C	0.276	0.7295	−0.002 ± 0.004	0.7881	−0.003 ± 0.006	0.8927	0.002 ± 0.006
	rs3092502	44130.838	G/T	0.445	0.2287	−0.004 ± 0.004	0.1583	−0.006 ± 0.005	0.8456	−0.002 ± 0.006
	rs1406826	44140.771	A/G	0.447	0.2736	−0.004 ± 0.004	0.1743	−0.006 ± 0.005	0.9784	0.000 ± 0.006
	rs1950174	44147.811	C/A	0.211	0.1169	0.008 ± 0.005	0.1293	0.010 ± 0.006	0.4001	0.006 ± 0.007
	rs6065921	44147.917	A/G	0.192	0.5812	−0.004 ± 0.005	0.5225	−0.004 ± 0.006	0.9178	−0.001 ± 0.007
	rs2868764	44155.418	T/G	0.445	0.2133	−0.004 ± 0.004	0.1621	−0.006 ± 0.005	0.8534	−0.001 ± 0.006
	rs6032664	44172.826	T/A	0.230	0.3123	−0.003 ± 0.005	0.1764	−0.006 ± 0.006	0.9680	0.001 ± 0.007
CD40	rs3746821	44188.518	G/T	0.104	0.3864	−0.005 ± 0.006	0.1216	−0.012 ± 0.008	0.6696	0.004 ± 0.010
	rs1535043	44201.131	T/A	0.448	0.7317	−0.001 ± 0.004	0.6996	−0.002 ± 0.005	0.6441	0.003 ± 0.006
	rs2064405	44210.912	G/T	0.098	0.4413	−0.005 ± 0.007	0.1759	−0.012 ± 0.008	0.7779	0.003 ± 0.010
	rs2425754	44222.040	G/A	0.139	0.8272	0.001 ± 0.006	0.5980	0.004 ± 0.007	0.7508	−0.001 ± 0.008
CDH22	rs6131030	44241.394	G/A	0.385	0.7158	−0.002 ± 0.004	0.3584	−0.004 ± 0.005	0.5555	0.002 ± 0.006
	rs6074061	44246.711	C/G	0.275	0.2439	−0.005 ± 0.004	0.1045	−0.008 ± 0.006	0.5423	0.002 ± 0.006
	rs1321001	44250.143	T/G	0.164	0.6618	0.002 ± 0.005	0.5954	0.003 ± 0.006	0.3787	−0.005 ± 0.008

				Combined Population			Cases Only		Controls Only	
Gene	**SNP**	**Position (kb)**	**Major/Minor Alleles**	**Minor Allele Frequency**	**Additive P-value**	**β ± SE**	**Additive P-value**	**β ± SE**	**Additive P-value**	**β ± SE**
	rs4383400	44253.272	T/C	0.060	0.1432	0.012 ± 0.008	0.4964	0.006 ± 0.009	0.2441	0.017 ± 0.013
	rs2868767	44258.031	A/G	0.181	0.8945	0.000 ± 0.005	0.6289	0.002 ± 0.006	0.5354	−0.004 ± 0.008
	rs1010310	44268.451	C/A	0.302	0.6530	0.001 ± 0.004	0.3653	0.004 ± 0.006	0.8965	0.000 ± 0.006
	rs2425785	44272.729	G/C	0.349	0.1778	−0.005 ± 0.004	0.0506	−0.010 ± 0.005	0.5873	0.003 ± 0.006
	rs2425786	44277.882	T/C	0.324	0.4132	0.004 ± 0.004	0.7151	−0.002 ± 0.005	**0.0376**	0.013 ± 0.006
	rs761048	44284.465	G/C	0.072	**0.0101**	−0.014 ± 0.003	0.0588	−0.017 ± 0.009	0.0681	0.021 ± 0.011
	rs6074069	44288.133	G/A	0.215	0.5557	−0.003 ± 0.005	0.1256	−0.010 ± 0.006	0.1892	0.009 ± 0.007
	rs2050113	44293.878	A/C	0.075	**0.0373**	0.015 ± 0.007	0.1416	0.013 ± 0.009	0.0792	0.019 ± 0.011
	rs966567	44300.632	G/C	0.242	0.7891	−0.001 ± 0.004	0.4096	−0.005 ± 0.006	0.5788	0.004 ± 0.007
	rs3904166	44306.828	C/A	0.237	0.7528	−0.001 ± 0.004	0.3851	−0.005 ± 0.006	0.6170	0.003 ± 0.007
	rs3848729	44308.103	G/T	0.360	**0.0150**	0.010 ± 0.004	**0.0108**	0.013 ± 0.005	0.6466	0.003 ± 0.006
	rs2425807	44314.952	A/G	0.439	0.3675	0.004 ± 0.004	0.0584	0.010 ± 0.005	0.3480	−0.005 ± 0.006
	rs2425809	44321.008	C/T	0.455	0.4152	0.003 ± 0.004	0.0575	0.010 ± 0.005	0.1974	−0.007 ± 0.006
	rs2425817	44328.693	G/A	0.453	0.4154	0.003 ± 0.004	0.0570	0.010 ± 0.005	0.1728	−0.007 ± 0.006
	rs2425855	44344.506	T/A	0.468	0.5482	0.002 ± 0.004	0.1053	0.009 ± 0.005	0.1133	−0.009 ± 0.006
	rs6032754	44352.166	T/C	0.265	0.2331	−0.005 ± 0.004	**0.0256**	−0.013 ± 0.006	0.1036	0.011 ± 0.006
	rs6017767	44365.336	C/G	0.479	0.4295	0.003 ± 0.004	0.2188	0.006 ± 0.005	0.8541	−0.001 ± 0.006
	rs6095091	46441.248	C/T	0.262	**0.0292**	0.011 ± 0.004	**0.0152**	0.015 ± 0.005	0.7758	0.003 ± 0.006
	rs6066724	46451.845	G/A	0.289	**0.0038**	0.013 ± 0.004	**0.0018**	0.018 ± 0.006	0.6074	0.004 ± 0.006
	rs2008645	46458.897	G/A	0.423	0.0931	0.007 ± 0.004	**0.0091**	0.014 ± 0.005	0.3998	−0.003 ± 0.006
	rs4809688	46461.222	A/G	0.438	0.2546	0.005 ± 0.004	**0.0350**	0.011 ± 0.005	0.2779	−0.005 ± 0.006
Replicated region 3′ of *PREX1*	rs999151	46488.676	C/T	0.291	**0.0015**	0.015 ± 0.004	**0.0022**	0.019 ± 0.006	0.6512	0.004 ± 0.006
	rs7263053	46496.933	C/T	0.273	**0.0109**	0.011 ± 0.004	**0.0144**	0.014 ± 0.006	0.9664	0.002 ± 0.007
	rs1321006	46499.668	G/A	0.355	**0.0038**	0.012 ± 0.004	**0.0089**	0.014 ± 0.005	0.8033	0.003 ± 0.006
	rs4809696	46507.214	T/C	0.352	**0.0038**	0.012 ± 0.004	**0.0109**	0.013 ± 0.005	0.6947	0.003 ± 0.006
	rs926693	46512.364	A/G	0.471	**0.0045**	0.011 ± 0.004	**0.0125**	0.012 ± 0.005	0.7415	0.003 ± 0.006
	rs926692	46515.682	A/C	0.472	**0.0053**	0.011 ± 0.004	**0.0127**	0.013 ± 0.005	0.8798	0.002 ± 0.006
	rs12624650	46526.719	G/A	0.201	0.1647	0.006 ± 0.005	**0.0406**	0.012 ± 0.006	0.3010	−0.007 ± 0.007
	rs6019212	46527.953	G/T	0.461	**0.0056**	0.011 ± 0.004	**0.0089**	0.013 ± 0.005	0.9358	0.002 ± 0.006

					Combined Population		Cases Only		Controls Only	
Gene	**SNP**	**Position (kb)**	**Major/Minor Alleles**	**Minor Allele Frequency**	**Additive P-value**	**β ± SE**	**Additive P-value**	**β ± SE**	**Additive P-value**	**β ± SE**
	rs6019216	46530.327	G/A	0.271	0.1533	0.005 ± 0.004	0.1067	0.008 ± 0.005	0.3320	−0.006 ± 0.006
	rs6122689	46533.130	C/T	0.176	0.0682	0.010 ± 0.005	0.2326	0.008 ± 0.006	0.2654	0.010 ± 0.008
	rs12481537	46572.852	A/G	0.474	**0.0159**	0.010 ± 0.004	0.0562	0.010 ± 0.005	0.6199	0.003 ± 0.006
	rs2904081	46593.440	C/G	0.303	0.8359	0.001 ± 0.004	0.8794	0.002 ± 0.005	0.5358	−0.004 ± 0.006
	rs4810813	46601.875	T/C	0.319	0.7201	0.000 ± 0.004	0.6625	0.000 ± 0.005	0.7271	−0.002 ± 0.006
	rs6019300	46634.916	A/G	0.456	0.9065	0.000 ± 0.004	0.9455	0.001 ± 0.005	0.2973	−0.007 ± 0.006
	rs6090875	46641.633	T/C	0.241	0.9511	0.000 ± 0.005	0.8617	0.001 ± 0.006	0.8113	−0.001 ± 0.007
	rs3924220	46642.853	T/C	0.244	0.8670	0.001 ± 0.005	0.7664	0.002 ± 0.006	0.8770	0.000 ± 0.007
	rs4810837	46662.565	T/G	0.418	0.7858	−0.001 ± 0.004	0.2829	0.006 ± 0.005	0.0621	−0.010 ± 0.006
	rs6012501	46672.841	G/C	0.440	0.6323	−0.002 ± 0.004	0.4876	0.004 ± 0.005	0.0593	−0.010 ± 0.005
PREX1	rs2664570	46674.765	C/T	0.210	0.3460	−0.004 ± 0.005	0.3561	−0.006 ± 0.006	0.8109	−0.001 ± 0.007
	rs3935549	46686.895	C/T	0.489	0.9600	−0.001 ± 0.004	0.2332	−0.008 ± 0.005	**0.0214**	0.013 ± 0.005
	rs2073072	46703.010	C/G	0.205	0.9508	−0.001± 0.005	0.7675	0.002 ± 0.006	0.8574	0.000 ± 0.007
	rs2294910	46709.789	A/G	0.205	0.8618	0.001 ± 0.005	0.7979	0.002 ± 0.006	0.9837	0.001 ± 0.007
	rs6095228	46713.283	G/A	0.087	0.4407	−0.005 ± 0.006	0.4604	0.008 ± 0.009	0.0763	−0.014 ± 0.008
	rs6012506	46719.044	A/G	0.477	0.9783	0.000 ± 0.004	0.2386	0.007 ± 0.005	**0.0357**	−0.012 ± 0.006
	rs6095239	46730.946	G/A	0.332	0.8347	−0.001 ± 0.004	0.6831	0.003 ± 0.006	0.2004	−0.008 ± 0.006
	rs3746819	46741.342	A/G	0.090	0.4284	−0.006 ± 0.007	0.5773	0.006 ± 0.009	**0.0314**	−0.021 ± 0.009
	rs4809718	46744.395	T/A	0.211	0.9615	0.001 ± 0.005	0.2891	0.008 ± 0.006	0.0649	−0.013 ± 0.007
	rs736659	46757.117	G/A	0.214	0.9392	0.001 ± 0.005	0.3171	0.008 ± 0.006	0.0654	−0.013 ± 0.007

Bold: P-values <0.05

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Table 6

Effect mediated by BMI on SNPs associated with T2DM 3′ of *PREX1*

	761 Cases and 407 Controls		Mediation Analysis		
SNP	**T2DM P_a-value**	**BMI P_a-value**	**Sobel P- value**	**Goodman P- value**	**Mediated Effect**
rs1321006	0.025	0.004	0.010	0.010	**0.39**
rs4809696	0.025	0.004	0.010	0.010	**0.40**
rs926693	0.010	0.004	0.010	0.010	**0.33**
rs926692	0.013	0.005	0.010	0.010	**0.30**
rs6019212	0.016	0.006	0.010	0.010	**0.38**
rs6019216	0.044	0.153	0.160	0.160	0.19
rs12481537	0.016	0.016	0.020	0.020	**0.30**

Bold: Mediation effects are valid based on Sobel and Goodman tests