Common and rare exonic \textit{MUC5B} variants associated with type 2 diabetes in Han Chinese

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Abstract

Genome-wide association studies have identified over one hundred common genetic risk variants associated with type 2 diabetes (T2D). However, most of the heritability of T2D has not been accounted for. In this study, we investigated the contribution of rare and common variants to T2D susceptibility by analyzing exome array data in 1,908 Han Chinese genotyped with Affymetrix Axiom® Exome Genotyping Arrays. Based on the joint common and rare variants analysis of 57,704 autosomal SNPs within 12,244 genes using Sequence Kernel Association Tests (SKAT), we identified significant associations between T2D and 25 variants (9 rare and 16 common) in \textit{MUC5B}, \textit{p}-value $1.01 \times 10^{-14}$. This finding was replicated ($p = 0.0463$) in an independent sample that included 10,401 unrelated individuals. Sixty-six of 1,553 possible haplotypes based on 25 SNPs within \textit{MUC5B} showed significant association with T2D (Bonferroni corrected \textit{p} values $< 3.2 \times 10^{-5}$). The expression level of \textit{MUC5B} is significantly higher in pancreatic tissues of persons with T2D compared to those without T2D (\textit{p}-value $= 5 \times 10^{-5}$). Our findings suggest that dysregulated \textit{MUC5B} expression may be involved in the pathogenesis of T2D. As a strong candidate gene for T2D, \textit{MUC5B} may play an important role in the mechanisms underlying T2D etiology and its complications.

Introduction

Type 2 Diabetes (T2D) is a growing global health problem. Currently, about 415 million people worldwide have diabetes. By 2040, the number of people living with diabetes is expected to increase to 642 million, with two-thirds of all cases occurring in low to middle-income countries\cite{1}. In China, the prevalence of T2D increased exponentially over the past three decades. In 1980, the prevalence of T2D in China was less than 1%; this estimate increased to about 12% in 2010\cite{2}. By 2013, there were about 114 million people with diabetes and about 500 million people with prediabetes in China. This rapid increase, which is unlike the transition that...
Health (NIH) in March of 2008. In this regard, the initial IRB approval was from Howard University (IRB-06-MED-38, 1840 Seventh Street, NW, suite 309, Washington, DC 20001, phone: 202-865-8597), and IRB of Suzhou Central Hospital, Suzhou, China (address: 60 Longmen St, Suzhou, China, 441300, Phone: +86-722-324-1340). From 2008 to now, the CADM study has been reviewed annually and approved by the IRB of the National Human Genome Research Institute at the National Institutes of Health (Protocol# 09-HG-N071, Building 10 CRC, Room 6-3330, Bethesda, MD 20892, nhgrlrboffice@nih.gov).

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occurred in Western countries, coincided with economic growth, urbanization, changes in lifestyle and demographic characteristics in China.

In addition to the well-recognized influence of lifestyle factors on the risk of T2D, genetic factors play a major role in susceptibility to T2D. The successful application of genome wide association studies (GWAS) has provided some insight into the genetic basis of T2D. Until recently, it was generally assumed that common diseases such as T2D were caused by common variants[3]. Given that GWAS provided genotypic information on common variants, it appeared to be the ideal technique to identify variants. To date, over 100 common genetic risk variants with small effect sizes have been identified from GWAS and shown to be associated with T2D. However, the joint effects of these variants accounts for less than 10% of the heritability for T2D[4]. In this study, we examined the association of rare variants with T2D among a population of unrelated Chinese adults. Given that susceptibility to T2D likely involves the contribution of both common and rare variants, we conducted joint analysis of common and rare variants of about 58,000 autosomal SNPs.

Materials and methods

Study population

The China America Diabetes Mellitus (CADM) study is a large-scale genetic epidemiology study designed to investigate the genetic and environmental determinants of metabolic disorders including T2D, dyslipidemia, kidney disease, and hypertension. In CADM, ~2000 unrelated participants with written informed consent were enrolled from Suizhou, China, of whom 1908 were genotyped and included in these analyses. Suizhou, a historic city, is located in the Hubei province, central China and has a population of over 2 million, most of whom are Han Chinese (99.2%). Ethical approval for the study was obtained from the Institutional Review Boards of Howard University, the National Institutes of Health, and IRB of Suizhou Central Hospital, Suizhou, China. All enrolled participants provided written informed consent during the clinic visit before commencement of data collection by interview and collection of biospecimens. Details of the study protocol were clearly explained to each participants and potential participants had the opportunity to ask questions before signing the consent documents.

Phenotype definitions

During a clinic examination, interviewers collected demographic information from the participants. All enrolled individuals self-identified as Han Chinese. Weight was measured in light clothes on an electronic scale to the nearest 0.1 kg, and height was measured with a stadiometer to the nearest 0.1 cm. Body mass index (BMI) was computed as weight (kg) divided by the square of height (m²). Blood samples were obtained from all participants after an overnight fast. T2D diagnosis was based on any of the following criteria established by the American Diabetes Association Expert Committee: fasting plasma glucose concentration ≥ 126 mg/dl (7.0 mmol/l), 2-hour post load value in the oral glucose tolerance test ≥ 200 mg/dl (11.1 mmol/l) on more than one occasion, history of T2D or on prescribed medication for diabetes. Cases were defined as individuals diagnosed with T2D, while controls were individuals without T2D. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, or use of blood pressure medication.

DNA sample preparation, genotyping, and quality control

DNA was extracted from buffy coat samples using a chemagenic DNA Isolation Kit (PerkinElmer Chemagen Technologie GmbH, Baesweiler, Germany) following the manufacturer’s
instructions. Samples were genotyped using Affymetrix Axiom® Exome Genotyping Arrays. This array is primarily designed to detect coding variation and contains over 300,000 markers, including non-synonymous and synonymous SNPs as well as variants in splice and stop codons, and 30,000 single-base and complex indels. Genotypes were called using Axiom GT1 algorithm as implemented in Affymetrix genotyping console 4.1.3, which is a new genotyping procedure developed specifically for use with Affymetrix Axiom® Genome-wide human arrays.

All arrays passed plate quality control following the manufacturer’s recommendations. The genotyping concordance rate (evaluated using 16 SNPs that were blind-genotyped twice) was 99.64%. The concordance rate for 10 individuals that were typed twice on the entire array was 98.64%. Of the 290,890 markers on the array, 178,943 were monomorphic, 23,756 had genotyping call rate less than 0.95 and 1,458 markers failed HWE (p value < 10\(^{-6}\)). Of the remaining 86,733 markers, 85,009 were autosomal; 27,305 of the autosomal markers were removed for having minor allele counts less than 5. In all, 57,704 autosomal markers were carried forward for analysis in this study. Of these markers, 12,329 (21.37%) had a minor allele frequency (MAF) < 0.01, and 45,375 (78.63%) had MAF ≥ 0.01 (with 32,638 with MAF ≥ 0.05). A variant was classified as “common” if MAF ≥ \(\frac{1}{\sqrt{n}}\) and “rare” if MAF ≤ \(\frac{1}{\sqrt{n}}\) (n is number of individuals) = 0.0162\(^5\). Based on hg19 genome build 37 (GRCh37), the 57,704 markers were located within 12,244 gene regions.

Statistical analysis
To minimize the potential effect of population structure, we adjusted all analyses by the first two principal components (PC1 and PC2) obtained from R package, SNPRelate \(^6\), which generates genetic covariance matrix followed by the extraction of eigenvalues and eigenvectors for the calculation of PCs. Single marker analysis for Common SNPs was implemented in PLINK \(^7\) under a genetic additive model, adjusting for sex, age, BMI, Hypertension, and first two PCs. A permutation procedure was used to generate significance levels empirically to deal with rare alleles and small sample size\(^7\). Simple label swapping of phenotype (T2D) was used for 100,000,000 permutation tests. The empirical permutation p value (Emp) was pointwise and was calculated by Emp = \(\frac{E+1}{N+1}\), where E is number of statistic values ≥ observed statistic value, and N is the total number of permutation.

Gene-base analyses of rare variants only and of joint common and rare variants were conducted using Sequence Kernel Association Test (SKAT)\(^5\), with models adjusted as in the common single marker analysis. The overall joint effect of rare and common variants by gene regions was tested by combining the test statistics directly using weighted-sum statistics, \(Q_{\theta,p1,p2} = (1-\theta)Q_{\text{rare}} + \theta Q_{\text{common}}\) with \(\theta = \frac{SD^2Q_{\text{rare}}}{SD^2Q_{\text{rare}} + SD^2Q_{\text{common}}}\), given (\(\theta,p1,p2\)). As rare variants are assumed to have larger effect sizes, different weight functions were used for rare and common variants as follows: \(\beta(MAF,\alpha = 1,\beta = 25)\) for rare, and \(\beta(MAF,\alpha = 0.5,\beta = 0.5)\) for common variants. Under null, the distribution of \(Q_{\theta,p1,p2}\) is a mixture of \(\chi_1^2\) distributions. These \(\chi_1^2\) distributions are independent and identically distributed chi-square random variables with 1-degree freedom. An asymptotic p value was then computed with Davies’ method or moment matching\(^5\). The genome-wide and suggestive significant threshold were established as \(\alpha = 2.5 \times 10^{-6}\) and \(\alpha = 2.5 \times 10^{-5}\) respectively\(^8\).

Haplotype phasing and analysis
Haplotype phasing of SNPs was performed with the BEAGLE program\(^9\), which uses the hidden Markov model (HMM) to find the most likely haplotype pair for each individual,
conditional on that individual’s genotypes. Haplotype phasing was conducted on the set of 25 SNPs T2D-associated MUC5B SNPs. Haplotypes were tested in a logistic regression model that included age, sex, BMI, hypertension status, and first two PCs as covariates. A total of 1,553 possible haplotypes across MUC5B were tested. Bonferroni correction was used to adjust for multiple tests \((0.05/\text{number of possible haplotype} = 3.2 \times 10^{-5})\).

**Replication analysis**

Replication analysis was performed in 10,401 African ancestry samples obtained from the Atherosclerosis Risk in Communities (ARIC, \(n = 3,137\)) [10], the Cleveland Family Study (CFS, \(n = 653\)) [11], the Howard University Family Study (HUFS, \(n = 1,976\)) [12], Jackson Heart Study (JHS, \(n = 2,187\)) [13], Multi-Ethnic Study of Atherosclerosis (MESA, \(n = 1,611\)) [14], and Africa America Diabetes Mellitus Study (AADM, \(n = 1802\)) [15]. Analysis was conducted using human genomic reference (hg19) coordinates. LiftOver (https://genome.ucsc.edu/cgi-bin/hgLiftOver) was used to convert genome coordinates and genome annotation between assemblies. Rare and common variants were defined as in the discovery study. The set of 25 rare and common SNPs associated with T2D in the discovery study were extracted from replication datasets. Sixteen (13 common and 3 rare variants) of 25 SNPs were available for joint common and rare variants analysis in SKAT. As in the discovery analysis, sex, age, BMI, first two principal components (PC1, and PC2) were included as covariates.

Replication of published GWAS findings was attempted using two strategies, 1) exact and local \(\text{(i.e., SNPs in Linkage disequilibrium \([LD]\) with the reported SNP[16])}\) for those gene regions that contained only common variants; and 2) a gene-level approach for gene regions containing both common and rare variants using SKAT. HapMap CHB reference data for Chinese ancestry populations was used for the identification of markers in LD with published variants. To adequately account for multiple testing, we estimated the effective degrees of freedom \((\text{df})\) for the spectrally-decomposed covariance matrix for the block of markers using this study’s (CADM) genotype data as previously described[17].

**Microarray analysis of human islets**

Data was extracted from publicly-available MIAME compliant gene expression data (GEO, accession number GSE25724; GDS3882; http://www.ncbi.nlm.nih.gov), using the R package, GEOquery. The original data was generated from the analysis of islets of Langerhans isolated from T2D and non-T2D organ donors[18]. RNA was biotinylated, fragmented, and hybridized onto Affymetrix Human Genome U133A Array chips. The expression data was scanned and log2 normalized, and the differential gene expression between T2D and non-T2D samples was assessed. Two-tailed tests were used, and \(p\) values lower than 0.01 were considered as differentially-expressed[18].

**Results**

Characteristics of study participants are displayed in Table 1. In this case-controls study of 1,908 individuals, about 50% of the cases and controls were female. Cases were older, heavier and, as expected, had significantly higher mean fasting blood glucose levels. Also, the cases had higher mean systolic and diastolic blood pressure and higher prevalence of hypertension compared to the controls \((63.7\% \text{ vs } 39.56\%, \text{respectively})\).

In the joint common and rare variant analysis \((12,244 \text{ genes})\), we observed a significant association between T2D and variants in the MUC5B gene \((\text{mucin 5B, oligomeric mucus/gel-forming, GeneID: 727897, 11p15.5})\) with \(p\)-value of \(1.01 \times 10^{-14}\) (Table 2; Fig 1 and QQ plot S1 Fig). This analysis included nine rare and sixteen common variants in MUC5B (Table 2).
Adjustment for smoking strengthened the association \( (p\text{-value} = 6.29 \times 10^{-15}) \). Replication analysis was conducted in 10,401 African ancestry individuals (S1 Table) using 16 available SNPs (3 rare and 13 common) of the 25 SNPs in the MUC5B gene (S2 Table). The MUC5B finding replicated in this large sample of individuals \( (p = 0.0463) \). In CADM, the frequency of the T allele in one of the rare variants (rs12282798, MAF = 0.0047) was 0.011 among cases and <0.001 among controls with an empirical p-value of \( 1.85 \times 10^{-4} \) (Table 3). Four common SNPs (rs201894106 allele T, rs199967813 allele A, rs192744525 allele A, and rs199285958 allele C) with allele frequencies <0.01 in cases, and >0.04 in controls, statistically significant difference (empirical p-value of \( 10^{-8} \)). The complete list of allele counts within MUC5B by T2D status and associated p-values obtained from permutation \( (n = 10^8) \) tests are presented in Table 3.

Based on the 25 markers available in the MUC5B gene (~35kb), we evaluated all possible 1,553 haplotypes for association with T2D. A total of 66 haplotypes showed significant association with T2D status (Bonferroni corrected p value of < 3.2\( \times 10^{-5} \); S3 Table and Fig 2). Each of the 66 haplotypes contained at least one SNP that showed single marker association with T2D (Table 3). For example, we observed 85 copies (4.39%) of the haplotype “CTGCCC” (Fig 2, amino acid positions from 1310 to 2836) among the controls compared to 3 copies (0.16%) among the T2D cases with a highly significant protective odd ratio (OR) of 0.031 (p-value 6.93\( \times 10^{-8} \)). Also, there were 87 (4.49%) copies of the haplotype “AGAGC” (amino acid position from 5339 to 5732) among T2D cases compared to 3 (0.16%) copies among the controls (OR = 0.035, p-value 9.02\( \times 10^{-8} \)). The partial correlation between these two haplotypes (CTGCCC and AGAGC) is 0.90. We observed that 2 (0.11%) copies of both CTGCCC and AGAGC haplotypes were present among those with T2D; while 79 (3.99%) copies of both CTGCCC and AGAGC were present among those without T2D, p value of \( 1.55 \times 10^{-6} \) (OR and 95% C.I = 0.032 [0.008, 0.129]).

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**Table 1. Characteristics of the study participants by type 2 diabetes status.**

|            | T2D        | Non-T2D    | P-values ** |
|------------|------------|------------|-------------|
| N *        | 917 (48.06%) | 991 (51.94%) | 0.0902      |
| Female *   | 454 (49.51%) | 489 (49.34%) | <0.0001     |
| Age (yrs)  | 56.13 (9.80) | 51.65 (9.24) | <0.0001     |
| BMI (kg/m²)| 24.58 (3.10) | 23.95 (2.95) | <0.0001     |
| Systolic Blood Pressure (mmHg) | 137.4 (20.32) | 128.2 (18.61) | <0.0001     |
| Diastolic Blood Pressure (mmHg) | 86.17 (11.23) | 82.17 (12.29) | <0.0001     |
| Hypertension * | 584 (63.69%) | 392 (39.56%) | <0.0001     |
| Glucose (mg/dL) | 169.2 (62.20) | 89.32 (9.72) | <0.0001     |
| Current Smokers * | 194 (21.16%) | 208 (20.99%) | 0.9288      |
| Duration of T2D (yrs) | 4.66 (4.85) | NA         |

*numbers (percentage), other numbers are means and standard deviations

hypertension was defined as SBP \( \geq 140 \) mmHg or DBP \( \geq 90 \) mmHg or use of blood pressure medication.

** Significant level defined as \( \leq 0.05 \).

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**Table 2. Top results for the joint association analyses of common and rare exome variants with T2D in Han Chinese individuals.**

| Genes   | Regions | P-values * | # of SNPs | # of Rare | # of Common |
|---------|---------|------------|-----------|-----------|-------------|
| MUC5B   | 11p15.5 | 1.01E-14   | 25        | 9         | 16          |
| ABCC12  | 16q12.1 | 1.56E-5    | 3         | 2         | 1           |

* Genome-wide statistical significance threshold (p value < 2.5\( \times 10^{-5} \)) and suggestive genome wide significant threshold (p < 2.5\( \times 10^{-5} \)).

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Three variants (2 rare: rs200272726, rs34135219; and one common: rs7193955) in ABCC12 (ATP-binding cassette, sub-family C, member 12, GeneID: 94160, 16q12.1) had suggestive genome wide significant associations with T2D (Table 2). The MAF of rare variant rs200272726 (T allele), was 0.0141 for cases and 0.0020 for controls. The T allele of rs200272726 was significantly associated with T2D (empirical $p$-value = $8.01 \times 10^{-4}$). The G allele of common variant rs7193955 was associated with T2D (empirical $p$-value = 0.04143) and had MAF was 0.1334 for cases and 0.1681 for controls (Table 3).

Genome-wide association studies (GWAS) for T2D [19–42] have identified 76 loci associated with T2D in East Asians. Based on the joint analysis of common and rare variants using SKAT, we evaluated the 46 gene sets available in our dataset. Six of the 46 gene sets (CDKAL1, KCNJ11, KCNQ1, MPHOSPH9, PSMD6, and ZFAND6) were replicated in the combined rare and common variants analysis (Table 4). In our analysis, there are 31,901 SNPs with MAF $\geq 0.016$ (defined as common variants in SKAT). We replicated 2 (rs7754840, and rs4712524) of the 10 previously reported common CDKAL1 SNPs for T2D in 15 East Asian GWAS or GWAS meta-analysis studies[19–21, 25, 29, 30, 32–39]. Also, we replicated 2 (rs2237897, and rs2237892) of the 7 previously reported SNPs in KCNQ1 from 12 East Asian studies[19, 20, 24, 28–32, 36, 40–42]. Our local replication strategy (targeted SNP $\pm$ 250kb window) did not identify any significant association after adjustment for multiple comparisons.

Fig 1. Exome Array Association Results. The y axis represents the $-\log_{10}$ (p-value) and the x axis is variant positions by chromosome. Genome-wide and suggestive statistical significance thresholds are illustrated by the two dotted lines.

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Publicly available MIAME compliant gene expression data (GEO, accession number GSE25724; GDS3882; http://www.ncbi.nlm.nih.gov) generated from 13 pancreatic organ donors using the HG-U133A Affymetrix Chips was downloaded and evaluated for differential gene expression. Seven of the 13 donors did not have diabetes (mean age: 58 ± 17 years, gender: 4 males/3 females; mean BMI: 24.8 ± 2.5 kg/m²), and six had T2D (mean age: 71 ± 9 years; gender: 3 males/3 females; mean BMI: 26.0 ± 2.2 kg/m²). In a model that adjusted for sex, age and BMI, we observed significantly higher MUC5B expression in the group with T2D compared to those without diabetes (p-value = 0.00005; Fig 3).

**Discussion**

We identified both rare and common variants within the MUC5B gene that were associated with T2D in this study conducted among Han Chinese. These results were replicated in a large sample of over 10,000 African ancestry individuals. We also identified several haplotypes

Table 3. Allele Counts by type 2 diabetes status for variants in the MUC5B and ABCC12 genes.

| GENES  | SNPs   | PPS   | Allele 1 | Allele 2 | MAF | HWE   | T2D | Non T2D |
|--------|--------|-------|----------|----------|-----|-------|------|---------|
| MUC5B  | rs2075853 | 1247458 | T | C | 0.3337 | 0.9418 | 630 | 1188 | 634 | 1336 | 0.9165 |
|        | rs80298911 | 1256409 | A | G | 0.0055* | 1.0000 | 12 | 1822 | 9 | 1959 | 1 |
|        | rs200226020 | 1261561 | T | C | 0.0042* | 1.0000 | 4 | 1828 | 12 | 1964 | 0.8113 |
|        | rs201894106 | 1262540 | T | C | 0.0284 | 0.1632 | 12 | 1812 | 96 | 1884 | 1e-08 |
|        | rs1541314 | 1263523 | A | G | 0.0625 | 0.6151 | 104 | 1724 | 134 | 1846 | 0.9905 |
|        | rs2943510 | 1263776 | T | C | 0.0661 | 1.0000 | 112 | 1718 | 140 | 1842 | 0.9833 |
|        | rs61997210 | 1264292 | T | C | 0.0045* | 1.0000 | 10 | 1814 | 7 | 1975 | 1 |
|        | rs12282798 | 1266617 | T | C | 0.0047* | 1.0000 | 18 | 1808 | 0 | 1982 | 0.000185 |
|        | rs55813014 | 1267325 | T | C | 0.3313 | 0.3922 | 585 | 1225 | 664 | 1296 | 0.9999 |
|        | rs58125533 | 1267475 | C | T | 0.1886 | 0.4358 | 368 | 1428 | 340 | 1618 | 0.1415 |
|        | rs117757264 | 1267670 | A | G | 0.0158* | 0.2768 | 24 | 1802 | 36 | 1944 | 0.9981 |
|        | rs34528873 | 1269215 | T | C | 0.0082* | 1.0000 | 14 | 1798 | 17 | 1961 | 1 |
|        | rs4963055 | 1269398 | C | T | 0.4015 | 0.4656 | 679 | 1069 | 797 | 1131 | 0.9671 |
|        | rs2943517 | 1271321 | C | G | 0.3322 | 0.1536 | 575 | 1197 | 662 | 1290 | 0.9987 |
|        | rs2943512 | 1272226 | A | C | 0.3296 | 0.0989 | 561 | 1175 | 654 | 1296 | 1 |
|        | rs202131299 | 1272527 | T | C | 0.0187 | 1.0000 | 35 | 1785 | 36 | 1946 | 1 |
|        | rs3021155 | 1272709 | A | G | 0.0590 | 0.0296 | 96 | 1732 | 128 | 1840 | 0.9429 |
|        | rs3021156 | 1272754 | G | A | 0.0633 | 0.6192 | 103 | 1729 | 137 | 1821 | 0.8796 |
|        | rs55693520 | 1272800 | T | C | 0.0026* | 1.0000 | 5 | 1829 | 5 | 1975 | 1 |
|        | rs3829224 | 1276327 | A | G | 0.2732 | 0.1588 | 481 | 1351 | 561 | 1421 | 0.982 |
|        | rs199967813 | 1276738 | A | G | 0.0260 | 0.1632 | 3 | 1827 | 96 | 1882 | 1e-08 |
|        | rs55741856 | 1277953 | A | G | 0.0029* | 1.0000 | 8 | 1826 | 3 | 1977 | 0.9425 |
|        | rs192744525 | 1280193 | A | G | 0.0256 | 0.4094 | 14 | 1788 | 82 | 1864 | 1e-08 |
|        | rs55856616 | 1280238 | A | G | 0.0029* | 1.0000 | 6 | 1824 | 5 | 1975 | 1 |
|        | rs199785958 | 1282744 | C | T | 0.0250 | 0.2651 | 3 | 1827 | 92 | 1884 | 1e-08 |
|        | rs200272726 | 48121966 | T | C | 0.0076* | 1.0000 | 24 | 1708 | 4 | 1944 | 0.0008088 |
|        | rs7193955 | 48122582 | G | A | 0.1316 | 0.7967 | 216 | 1612 | 285 | 1695 | 0.3939 |
|        | rs34135219 | 48145742 | A | T | 0.0047* | 1.0000 | 8 | 1818 | 10 | 1970 | 1 |

* Rare variants with MAF ≤ 0.0162.  
** Empirical p-values from 100,000,000 permutation analyses.

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within \textit{MUC5B} that showed significant associations with T2D. Notably, individuals with T2D had significantly higher expression levels of \textit{MUC5B} compared to those without T2D.

\textit{MUC5B} encodes a member of the mucin family of proteins. These proteins are highly glycosylated macromolecular components[43]. As indicated above, the expression of \textit{MUC5B} is increased among individuals with T2D compared to controls; however, the underlying

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Fig 2. Haplotypes association results across \textit{MUC5B}. The y axis represents $-\log_{10}$ (p values) and the x axis shows position within \textit{MUC5B}. Red dotted lines indicate the Bonferroni correction level ($-\log_{10} (0.05/1,553)$). Points above the line are odds ratio values $>1$, and below are odds ratio values $<1$. Green dotted lines indicate the positions of significantly associated SNPs in single SNP analyses. The “*” symbol by the SNP label indicates rare variants (MAF $\leq 0.0162$). The LD heat map presents pairwise $r^2$ values within \textit{MUC5B} based on the CADM study.

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Table 4. Replication of previous GWAS Findings in East Asian ancestry studies.

| PUBMED ID | Region | PPS  | Genes  | SNPs/Risk Allele  | Context | Freq. (Risk Allele) | Reported P | OR/Beta | 95%C.I. | p values | SKAT Exact SNP * |
|-----------|--------|------|--------|-------------------|---------|---------------------|------------|---------|---------|----------|-----------------|
| 24509480  | 6p23.3 | 20679478 | CDKAL1 | rs7756992/G intron | 0.260   | 2.00E-26            | 1.2        | [1.16–1.25] | 0.0347   |                    |
| 17460697  | 6p23.3 | 20679478 | CDKAL1 | rs7756992/G intron | 0.260   | 8.00E-09            | 1.2        | [1.13–1.27] | 0.0347   |                    |
| 23945395  | 6p23.3 | 20661019 | CDKAL1 | rs7754840/C intron | 0.420   | 2.00E-13            | 1.18       | [1.13–1.23] | 0.0347   | 0.0393          |
| 22961080  | 6p23.3 | 20661019 | CDKAL1 | rs7754840/C intron | 0.411   | 7.00E-10            | 1.35       | [1.23–1.48] | 0.0347   | 0.0393          |
| 17463246  | 6p23.3 | 20661019 | CDKAL1 | rs7754840/C intron | 0.310   | 4.00E-11            | 1.12       | [1.08–1.16] | 0.0347   | 0.0393          |
| 17463248  | 6p23.3 | 20661019 | CDKAL1 | rs7754840/C intron | 0.360   | 4.00E-11            | 1.12       | [1.08–1.16] | 0.0347   | 0.0393          |
| 22693455  | 6p23.3 | 20686342 | CDKAL1 | rs7766070/A intron | 0.270   | 7.00E-10            | 1.26       | [1.17–1.35] | 0.0347   |                    |
| 22693455  | 6p23.3 | 20686342 | CDKAL1 | rs7766070/A intron | 0.270   | 6.00E-11            | 1.21       | [1.14–1.28] | 0.0347   |                    |
| 21490949  | 6p23.3 | 20652486 | CDKAL1 | rs9295474/G intron | 0.360   | 9.00E-06            | 1.16       | [1.09–1.24] | 0.0347   |                    |
| 20581827  | 6p23.3 | 20687890 | CDKAL1 | rs10440833/A intron | 2.00E-22 | 1.25                |            | [1.20–1.31] | 0.0347   |                    |
| 19401414  | 6p23.3 | 20657333 | CDKAL1 | rs4712523/G intron | 0.410   | 7.00E-20            | 1.27       | [1.21–1.33] | 0.0347   |                    |
| 18711366  | 6p23.3 | 20657634 | CDKAL1 | rs4712524/G intron | 0.420   | 3.00E-10            | 1.22       | [1.15–1.31] | 0.0347   | 0.0459          |
| 18372903  | 6p23.3 | 20703721 | CDKAL1 | rs6931514/G intron | 1.00E-11 | 1.25                |            | [1.17–1.33] | 0.0347   |                    |
| 17463249  | 6p23.3 | 20660803 | CDKAL1 | rs10946398/C intron | 0.320   | 1.00E-08            | 1.16       | [1.10–1.22] | 0.0347   |                    |
| 17554300  | 6p23.3 | 20717024 | CDKAL1 | rs9465871/C intron | 0.180   | 3.00E-07            | 1.18       | [1.04–1.34] | 0.0347   |                    |
| 24509480  | 11p15.1| 17387083 | KCNJ11 | rs5215/C intron | 0.380   | 3.00E-11            | 1.08       | [1.04–1.12] | 0.0347   |                    |
| 18372903  | 11p15.1| 17387083 | KCNJ11 | rs5215/C intron | 4.00E-07 | 1.16                |            | [1.09–1.23] | 0.0474   |                    |
| 17463249  | 11p15.1| 17387083 | KCNJ11 | rs5215/C intron | 5.00E-11 | 1.14                |            | [1.10–1.19] | 0.0474   |                    |
| 19056611  | 11p15.1| 17388025 | KCNJ11 | rs5219/T intron | 5.00E-07 | 1.19                |            | [1.11–1.27] | 0.0474   |                    |
| 17463246  | 11p15.1| 17388025 | KCNJ11 | rs5219/T intron | 0.470   | 1.00E-07            | 1.15       | [1.09–1.21] | 0.0474   |                    |
| 17463248  | 11p15.1| 17388025 | KCNJ11 | rs5219/T intron | 0.460   | 7.00E-11            | 1.14       | [1.10–1.19] | 0.0474   |                    |
| 24509480  | 11p15.1| 2825839 | KCNQ1 | rs163184/G intron | 0.500   | 2.00E-14            | 1.09       | [1.04–1.13] | 0.0459   |                    |
| 24390345  | 11p15.1| 2837316 | KCNQ1 | rs2237897/C intron | 9.00E-15 | 1.31                |            | [1.22–1.41] | 0.0459   | 0.0047          |
| 18711366  | 11p15.1| 2837316 | KCNQ1 | rs2237897/C intron | 0.340   | 1.00E-16            | 1.33       | [1.24–1.41] | 0.0459   | 0.0047          |
| 24101674  | 11p15.1| 2810311 | KCNQ1 | rs8181588/A intron | 0.480   | 5.00E-09            | 1.3        |            | 0.0459   |                    |

(Continued)
mechanistic explanation driving the increased expression among diabetics has not been elucidated. Published studies suggest that the expression of MUC5B may be mediated through insulin-like growth factor-1 (IGF-1) and p38 mitogen-activated protein kinases (MAPK). MUC5B mRNA expression is induced by the action of IGF-1[44]. It has been reported that individuals with T2D, obesity, or both have increased levels of IGF-1[45–47] and that IGF-1 induced MUC5B expression is regulated by activation of p38 MAPK[44]. High levels of glucose have been shown to activate p38 MAPK signaling pathway in pancreatic β cells[48–50]. In animal studies, p38 has been shown to play an important role in diabetes-induced inflammation[51].

The lung is a target organ for T2D. Abnormal pulmonary function has been observed in individuals with T2D, the most consistent abnormalities include poor lung elasticity, reduced diffusion capacity due to impaired capillary blood volume, reduced absolute thoracic gas volumes, reduced lung volume and airflow resistance[52–54]. T2D may lead to abnormal pulmonary function through non-enzymatic glycosylation-induced alteration of the chest wall and bronchial tree collagen protein, which induces fibrous tissue formation, thickening of the basal lamina, increased protein catabolism, neuropathy of the phrenic nerve and diaphragmatic paralysis[54–57]. In healthy lungs, MUC5B is expressed in the goblet cells of bronchi and bronchioles. It has been found to be up-regulated in some human pulmonary diseases[58]. In a study of individuals with lung disease, a genome-wide linkage scan showed that a common promoter of MUC5B was associated with familial interstitial pneumonia and idiopathic pulmonary fibrosis; MUC5B was highly expressed among diseased individuals, compared to

| PUBMED ID | Region | PPS | Genes | SNPs/Risk Allele | Context | Freq. (Risk Allele) | Reported P | OR/Beta | 95%CI | p values | Exact SNP * p value |
|-----------|--------|-----|-------|-----------------|--------|---------------------|------------|--------|------|---------|------------------|
| 23945395  | 11p15.1| 2818521 | KCNQ1 | rs2237892/C intron | 0.610 | 4.00E-29 | 1.3 | [1.24–1.36] | 0.0459 | 0.0133 |
| 19401414  | 11p15.1| 2818521 | KCNQ1 | rs2237892/C intron | 0.590 | 1.00E-26 | 1.33 | [1.27–1.41] | 0.0459 | 0.0133 |
| 22961080  | 11p15.1| 2818521 | KCNQ1 | rs2237892/C intron | 0.657 | 1.00E-07 | 1.32 | [1.19–1.46] | 0.0459 | 0.0133 |
| 21573907  | 11p15.1| 2818521 | KCNQ1 | rs2237892/C intron | 4.00E-06 | 1.2 | [1.11–1.29] | 0.0459 | 0.0133 |
| 18711367  | 11p15.1| 2818521 | KCNQ1 | rs2237892/C intron | 0.610 | 2.00E-42 | 1.4 | [1.34–1.47] | 0.0459 | 0.0133 |
| 21799366  | 11p15.1| 2822986 | KCNQ1 | rs163182/C intron | 0.340 | 2.00E-17 | 1.28 | 0.0459 |
| 20581827  | 11p15.1| 2670241 | KCNQ1 | rs231362/G intron; ncRNA | 3.00E-13 | 1.08 | [1.06–1.10] | 0.0459 |
| 20174558  | 11p15.1| 2835964 | KCNQ1 | rs2237895/C intron | 0.330 | 1.00E-09 | 1.29 | [1.19–1.40] | 0.0459 |
| 24509480  | 12q24.31 | 123163006 | MPHOSPH9 | rs1727313/C ncRNA | 1.00E-08 | 1.06 | [1.04–1.08] | 0.0367 |
| 22195397  | 3p14.1 | 64062621 | PSMD6 | rs891577/c | 0.610 | 8.00E-11 | 1.09 | [1.06–1.12] | 0.0162 |
| 20581827  | 15q25.1 | 80139880 | ZFAND6 | rs11634937/G | 2.00E-09 | 1.06 | [1.04–1.06] | 0.0363 |

*Empty cells: information not available

Table 4. (Continued)

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controls[39]. A recent meta-analysis that included Asian populations showed a strong association between MUC5B (rs35705950 polymorphism) and risk of idiopathic pulmonary fibrosis [60]. The diabetes status of the individuals in the study was not stated.

The MUC5B gene is composed of tandem repeats which are flanked by cysteine-rich subdomains (845 residues upstream and 700 residues downstream). The cysteine-rich subdomains were similar to the D-domains of human pro-Von Willebrand factor[61, 62]. Increased levels of von Willebrand factor, an indication of damage to endothelial cells, have been showed association with diabetes[63]. It also reported as a predictive markers for diabetic nephropathy and neuropathy, thus providing a clue that endothelial dysfunction precedes the onset of diabetic microangiopathy[63]. In previous studies of Sjögren’s syndrome, a chronic autoimmune disease in which the body’s white blood cells destroy the exocrine glands, a relationship between MUC5B, von Willebrand factor and diabetes was suggested[64, 65], indicating a potential role of MUC5B in cardiovascular complications of T2D. An NF-kappa-B binding site in the MUC5B promoter showed that activation of the NF-kappa-B signaling pathway upregulated MUC5B mRNA expression 2 fold[66]. NF-kappa-B signaling pathway plays an important role in immune and inflammatory response[67], supporting a potential role of MUC5B in T2D.
Conclusions
We identified rare and common variants in the *MUC5B* gene that are associated with T2D in Han Chinese. Our findings suggest that dysregulated *MUC5B* expression may be involved in the pathogenesis of T2D. As a strong candidate gene for T2D, *MUC5B* may play an important role in the mechanisms underlying T2D etiology and its complications.

Supporting information

**S1 Fig. QQ plots exome array association results.** The y axis represents observed $-\log_e(p)$ values, and the x axis is expected $-\log_e(p)$ values. (TIFF)

**S1 Table. Basic characteristics of the study participants by type 2 diabetes status in African ancestry replication study.** (XLSX)

**S2 Table. Description of SNPs in the discovery and replication analyses.** (XLSX)

**S3 Table. *MUC5B* haplotype frequencies and associations with T2D.** (XLSX)

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**Human Subjects:** Discovery datasets (CADM) and replication data (AADM and HUFS) were initially approved by the Institutional Review Boards (IRB) of the Howard University, then by the National Human Genome Institute (NHGRI) at NIH. CADM is also approved by IRB of Suizhou Central Hospital, Suizhou, China. Other replication datasets, with the exception of HUFS and AADM, were accessed through an approved request for controlled-access data from dbGaP (pmid 17898773) as follows: ARIC (phs000280.v2.p1, phs000090.v2.p1), CFS (phs000284.v1.p1), JHS (phs000286.v4.p1, phs000499.v2.p1), and MESA (phs000209.v13.p1, phs000420.v6.p3). Details of each of these studies has been previously described (ARIC [2646917], CFS [7881656], JHS [16320381], and MESA [12397006]). The ARIC study was approved by the Institutional Review Boards (IRB) of the University of North Carolina at Chapel Hill, Johns Hopkins University, University of Mississippi Medical Center, Wake Forest University, University of Minnesota, Brigham and Women’s Hospital, and Baylor College of Medicine. CFS was approved by the University Hospitals Case Medical Center. The Jackson Heart Study was approved by the IRB of the University of Mississippi Medical Center, Jackson State University, and Tougaloo College. MESA was approved by the IRB at each of the six field centers.

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References
1. Federation ID. IDF Diabetes Atlas update poster Brussels, Belgium 2015. Available from: http://www.diabetesatlas.org.
2. Ma RC, Lin X, Jia W. Causes of type 2 diabetes in China. Lancet Diabetes Endocrinol. 2014; 2(12):980–91. Epub 2014/09/15. https://doi.org/10.1016/S2213-8587(14)70145-7 PMID: 25218727
3. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009; 461(7265):747–53. Epub 2009/10/09. PubMed Central PMCID: PMC2831613. https://doi.org/10.1038/nature08494 PMID: 19812666
4. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 2012; 44(9):981–90. Epub 2012/08/14. PubMed Central PMCID: PMC3442244. https://doi.org/10.1038/ng.2383 PMID: 22885922
5. Ionita-Laza I, Lee S, Makarov V, Buxbaum JD, Lin X. Sequence kernel association tests for the combined effect of rare and common variants. Am J Hum Genet. 2013; 92(6):841–53. Epub 2013/05/21. PubMed Central PMCID: PMC3675243. https://doi.org/10.1016/j.ajhg.2013.04.015 PMID: 23684009
6. Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics. 2012; 28(24):3326–8. PubMed Central PMCID: PMCPMC3675243. https://doi.org/10.1093/bioinformatics/bts606 PMID: 23060615
7. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559–75. Epub 2007/08/19. PubMed Central PMCID: PMC1950838. https://doi.org/10.1086/519795 PMID: 17701901
8. Madsen BE, Browning SR. A groupwise association test for rare mutations using a weighted sum statistic. PLoS Genet. 2009; 5(2):e1000384. Epub 2009/02/14. PubMed Central PMCID: PMC2633048. https://doi.org/10.1371/journal.pgen.1000384 PMID: 19214210
9. Browning SR, Browning BL. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. Am J Hum Genet. 2007; 81(5):1084–97. Epub 2007/10/10. PubMed Central PMCID: PMC2265661. https://doi.org/10.1086/521987 PMID: 17924948
10. Yu B, Zheng Y, Alexander D, Manolio TA, Alonso A, Nettleton JA, et al. Genome-Wide Association Study of a Heart Failure Related Metabolomic Profile Among African Americans in the Atherosclerosis Risk in Communities (ARIC) Study. Genet Epidemiol. 2013; 37(8):840–5. https://doi.org/10.1002/gepi.21752 PMID: 23934736
11. Dean DA, Goldberger AL, Mueller R, Kim M, Rueschman M, Mobjely D, et al. Scaling Up Scientific Discovery in Sleep Medicine: The National Sleep Research Resource. Sleep. 2016; 39(5):1151–64. https://doi.org/10.5665/sleep.5774 PMID: 27070134
12. Adeyemo A, Gerry N, Chen G, Herbert A, Doumatay A, Huang H, et al. A genome-wide association study of hypertension and blood pressure in African Americans. PLoS Genet. 2009; 5(7):e1000564.
Takeuchi F, Serizawa M, Yamamoto K, Fujisawa T, Nakashima E, Ohnaka K, et al. Confirmation of multiple risk Loci and genetic impacts by a genome-wide association study of type 2 diabetes in the

MUCSB associated with T2D

PubMed Central PMCID: PMCPMC2702100. https://doi.org/10.1371/journal.pgen.1000564 PMID: 19609347

13. Fox ER, Sarpong DF, Cook JC, Samdarshi TE, Nagarajaroa HS, Liebson PR, et al. The relation of diabetes, impaired fasting blood glucose, and insulin resistance to left ventricular structure and function in African Americans: the Jackson Heart Study. Diabetes Care. 2011; 34(2):507–9. PubMed Central PMCID: PMCPMC3024377. https://doi.org/10.2337/dc10-0838 PMID: 21216853

14. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol. 2002; 156(9):871–81. PMID: 12397006

15. Adeyemo AA, Tekola-Ayele F, Doumatey AP, Bentley AR, Chen G, Huang H, et al. Evaluation of Genome Wide Association Study Associated Type 2 Diabetes Susceptibility Loci in Sub-Saharan Africans. Front Genet. 2015; 6:335. PubMed Central PMCID: PMCPMC4656823. https://doi.org/10.3389/fgene.2015.00335 PMID: 26635871

16. Ramos E, Chen G, Shriner D, Doumatey A, Gery NP, Herbert A, et al. Replication of genome-wide association studies (GWAS) loci for fasting plasma glucose in African-Americans. Diabetologia. 2011; 54(4):783–8. Epub 2010/12/29. PubMed Central PMCID: PMC3052446. https://doi.org/10.1007/s00125-010-2002-7 PMID: 21183353

17. CHRISTOPHER S. BRETHERON MW, DYMNIK OV VALENTIN P., WALLACE JOHN M., AND BLADE ILEANA. The Effective Number of Spatial Degrees of Freedom of a Time-Varying Field. JO U R N A L O F C L I M A T E. 1999; 12:20.

18. Dominguez V, Raimondi C, Somanath S, Bugliani M, Loder MK, Edling CE, et al. Class II phosphoinositide 3-kinase regulates exocytosis of insulin granules in pancreatic beta cells. J Biol Chem. 2011; 286(6):4216–25. Epub 2010/12/04. PubMed Central PMCID: PMCPMC3039383. https://doi.org/10.1074/jbc.M110029205 PMID: 21127054

19. Haraj K, Fujita H, Johnson TA, Yamauchi T, Yasuda K, Horikoshi M, et al. Genome-wide association study identifies three novel loci for type 2 diabetes. Hum Mol Genet. 2014; 23(1):239–46. Epub 2013/08/16. https://doi.org/10.1093/hmg/ddq399 PMID: 23943995

20. Li H, Gian W, Lu L, Dong X, Han X, Hu C, et al. A genome-wide association study identifies GRK5 and RASGRPR1 as type 2 diabetes loci in Chinese Hans. Diabetes. 2013; 62(1):291–8. Epub 2012/09/11. PubMed Central PMCID: PMC3526061. https://doi.org/10.2337/db12-0454 PMID: 22961080

21. Perry JR, Voight BF, Yengo L, Amin N, Dupuis J, Ganser M, et al. Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. PLoS Genet. 2012; 8(5):e1002741. Epub 2012/06/14. PubMed Central PMCID: PMC3364960. https://doi.org/10.1371/journal.pgen.1002741 PMID: 22693455

22. Imamura M, Maeda S, Yamauchi T, Haraj K, Yasuda K, Morizono T, et al. A single-nucleotide polymorphism in ANK1 is associated with susceptibility to type 2 diabetes in Japanese populations. Hum Mol Genet. 2012; 21(13):3042–9. Epub 2012/03/30. https://doi.org/10.1093/hmg/dds113 PMID: 22456796

23. Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in East Asians. Nat Genet. 2012; 44(1):67–72. Epub 2011/12/14. PubMed Central PMCID: PMC3582398.

24. Cuil B, Zhu X, Xu M, Guo T, Zhu D, Chen G, et al. A genome-wide association study confirms previously reported loci for type 2 diabetes in Han Chinese. PLoS One. 2011; 6(7):e22353. Epub 2011/07/30. PubMed Central PMCID: PMC3142153. https://doi.org/10.1371/journal.pone.0022353 PMID: 21799836

25. Sim X, Ong RT, Suro C, Tay WT, Liu J, Ng DP, et al. Transferability of type 2 diabetes implicated loci in multi-ethnic cohorts from Southeast Asia. PLoS Genet. 2011; 7(4):e1001363. Epub 2011/04/15. PubMed Central PMCID: PMC3072366. https://doi.org/10.1371/journal.pgen.1001363 PMID: 21490949

26. Shu XO, Long J, Cai Q, Qi L, Xiang YB, Cho YS, et al. Identification of new genetic risk variants for type 2 diabetes. PLoS Genet. 2010; 6(9):e1001127. Epub 2010/09/24. PubMed Central PMCID: PMC2940731. https://doi.org/10.1371/journal.pgen.1001127 PMID: 20862305

27. Yamauchi T, Haraj K, Maeda S, Yasuda K, Takahashi A, Horikoshi M, et al. A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. Nat Genet. 2010; 42(10):864–8. Epub 2010/09/08. https://doi.org/10.1038/ng.660 PMID: 20818381

28. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, Chang CT, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. PLoS Genet. 2010; 6(2):e1000847. Epub 2010/02/23. PubMed Central PMCID: PMC2824763. https://doi.org/10.1371/journal.pgen.1000847 PMID: 20174558

29. Takeuchi F, Serizawa M, Yamamoto K, Fujisawa T, Nakashima E, Ohnaka K, et al. Confirmation of multiple risk Loci and genetic impacts by a genome-wide association study of type 2 diabetes in the
30. Unoki H, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, Andersen G, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet. 2008; 40(9):1098–102. Epub 2008/08/20. https://doi.org/10.1038/ng.208 PMID: 18711366

31. Yasuda K, Miyake K, Horikawa Y, Hara K, Osawa H, Furuta H, et al. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat Genet. 2008; 40(9):1092–7. Epub 2008/08/20. https://doi.org/10.1038/ng.207 PMID: 19401414

32. Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet. 2014; 46(3):234–44. Epub 2014/02/11. PubMed Central PMCID: PMC3969612. https://doi.org/10.1038/ng.2897 PMID: 24509480

33. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benedictsson R, Jonsdottir T, Walters GB, et al. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet. 2007; 39(6):770–5. Epub 2007/04/27. https://doi.org/10.1038/ng2043 PMID: 17460697

34. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007; 316(5829):1331–6. Epub 2007/04/28. https://doi.org/10.1126/science.1142358 PMID: 17463246

35. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007; 316(5829):1341–5. Epub 2007/06/29. PubMed Central PMCID: PMC3214617. https://doi.org/10.1126/science.1142382 PMID: 17463248

36. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. 2010; 42(7):579–89. Epub 2010/06/29. PubMed Central PMCID: PMC214617. https://doi.org/10.1038/ng.609 PMID: 20581827

37. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet. 2008; 40(5):638–45. Epub 2008/04/01. PubMed Central PMCID: PMC2672416. https://doi.org/10.1038/ng.120 PMID: 18372903

38. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447(7145):661–78. Epub 2007/06/08. PubMed Central PMCID: PMC3772310. https://doi.org/10.1038/nature05911 PMID: 17554300

39. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447(7145):661–78. Epub 2007/06/08. PubMed Central PMCID: PMC2719288. https://doi.org/10.1038/nature05911 PMID: 17554300

40. Hanson RL, Muller YL, Kobes S, Guo T, Bian L, Ossowski V, et al. A genome-wide association study in American Indians implicates DNER as a susceptibility locus for type 2 diabetes. Diabetes. 2014; 63(1):369–76. Epub 2013/10/09. PubMed Central PMCID: PMC3868040. https://doi.org/10.2337/db13-0416 PMID: 24101674

41. Williams AL, Jacobs SB, Moreno-Macias H, Huerta-Chagoya A, Churchhouse C, Marquez-Luna C, et al. Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. Nature. 2014; 506(7486):97–101. Epub 2014/01/07. PubMed Central PMCID: PMC4127086. https://doi.org/10.1038/nature12828 PMID: 24390345

42. Parra EJ, Below JE, Korthika S, Valladares A, Barta JL, Cox NJ, et al. Genome-wide association study of type 2 diabetes in a sample from Mexico City and a meta-analysis of a Mexican-American sample from Starr County, Texas. Diabetologia. 2011; 54(8):2038–46. Epub 2011/05/17. PubMed Central PMCID: PMC3381860. https://doi.org/10.1007/s00125-011-2172-y PMID: 21573907

43. Desseyn JL, Guyonnet-Duperat V, Porchet N, Aubert JP, Laine A. Human mucin gene MUC5B, the 10.7-kb large central exon encodes various alternate subdomains resulting in a super-repeat. Structural evidence for a 11p15.5 gene family. J Biol Chem. 1997; 272(6):3168–78. Epub 1997/02/07. PMID: 9013550

44. Bae CH, Kim JS, Song SY, Kim YW, Park SY, Kim YD. Insulin-like growth factor-1 induces MUCB and MUC5B expression via ERK1 and p38 MAPK in human airway epithelial cells. Biochem Biophys Res Commun. 2013; 430(2):683–8. Epub 2012/12/06. https://doi.org/10.1016/j.bbrc.2012.11.091 PMID: 23211593

45. Clauson PG, Brismar K, Hall K, Linnarsson R, Grill V. Insulin-like growth factor-I and insulin-like growth factor binding protein-1 in a representative population of type 2 diabetic patients in Sweden. Scand J Clin Lab Invest. 1998; 58(4):353–60. Epub 1998/09/19. PMID: 9741824
46. Frystyk J, Skjaerbaek C, Vestbo E, Fisker S, Orskov H. Circulating levels of free insulin-like growth factors in obese subjects: the impact of type 2 diabetes. Diabetes Metab Res Rev. 1999; 15(5):314–22. Epub 1999/12/10. PMID: 10585616

47. Nam SY, Lee EJ, Kim KR, Cha BS, Song YD, Lim SK, et al. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. Int J Obes Relat Metab Disord. 1997; 21(5):355–9. Epub 1997/05/01. PMID: 9152736

48. Lal AS, Clifton AD, Rouse J, Segal AW, Cohen P. Activation of the neutrophil NADPH oxidase is inhibited by SB 203580, a specific inhibitor of SAPK2/p38. Biochem Biophys Res Commun. 1999; 259(2):465–70. Epub 1999/06/11. https://doi.org/10.1006/bbrc.1999.0759 PMID: 10362531

49. Bao W, Behm DJ, Nerurkar SS, Ao Z, Bentley R, Mirabile RC, et al. Effects of p38 MAPK Inhibitor on angiotensin II-dependent hypertension, organ damage, and superoxide anion production. J Cardiovasc Pharmacol. 2007; 49(6):362–8. Epub 2007/06/20. https://doi.org/10.1097/FJC.0b013e318046f34a PMID: 17577100

50. Yoo BK, Choi JW, Shin CY, Jeon SJ, Park SJ, Cheong JH, et al. Activation of p38 MAPK induced peroxynitrite generation in LPS plus IFN-gamma-stimulated rat primary astrocytes via activation of iNOS and NADPH oxidase. Neurochem Int. 2008; 52(6):1188–97. Epub 2008/02/22. https://doi.org/10.1016/j.neuint.2007.12.009 PMID: 18289732

51. Du Y, Tang J, Li G, Berti-Mattera L, Lee CA, Bartkowski D, et al. Effects of p38 MAPK inhibition on early stages of diabetic retinopathy and sensory nerve function. Invest Ophthalmol Vis Sci. 2010; 51(4):2158–64. Epub 2010/01/15. PubMed Central PMCID: PMC2868413. https://doi.org/10.1167/iovs.09-3674 PMID: 20071676

52. Goldman MD. Lung dysfunction in diabetes. Diabetes Care. 2003; 26(6):1915–8. Epub 2003/05/27. PMID: 12766133

53. Nicolaie T, Zavoianu C, Nuta P. Pulmonary involvement in diabetes mellitus. Rom J Intern Med. 2003; 41(4):365–74. Epub 2004/11/06. PMID: 15526520

54. Sandler M. Is the Lung a Target Organ in Diabetes-Mellitus. Arch Intern Med. 1990; 150(7):1385–8. PMID: 2196023

55. Kaparianos A, Argyropoulou E, Sampsonas F, Karkoulias K, Tsiamita M, Spriopoulos K. Pulmonary complications in diabetes mellitus. Chron Respir Dis. 2008; 16(4):365–74. Epub 2004/01/01. PMID: 14807380

56. Zhu QQ, Zhang XL, Zhang SM, Tang SW, Min HY, Yi L, et al. Association Between the MUC5B Promoter Polymorphism rs35705950 and Idiopathic Pulmonary Fibrosis: A Meta-analysis and Trial Sequential Analysis in Caucasian and Asian Populations. Medicine (Baltimore). 2015; 94(43):e1901. Epub 2015/10/30.

57. Mathai SK, Newton CA, Schwartz DA, Garcia CK. Pulmonary fibrosis in the era of stratified medicine. Thorax. 2016.

58. Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, Loyd JE, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011; 364(16):1503–12. Epub 2011/04/22. PubMed Central PMCID: PMC3379886. https://doi.org/10.1056/NEJMoa1013660 PMID: 21506741

59. Zhu QQ, Zhang XL, Zhang SM, Tang SW, Min HY, Yi L, et al. Association Between the MUC5B Promoter Polymorphism rs35705950 and Idiopathic Pulmonary Fibrosis: A Meta-analysis and Trial Sequential Analysis in Caucasian and Asian Populations. Medicine (Baltimore). 2015; 94(43):e1901. Epub 2015/10/30.

60. Gum JR, Hicks JW, Toribara NW, Rothe EM, Lagace RE, Kim YS. The Human Muc2 Intestinal Mucin Has Cysteine-Rich Subdomains Located Both Upstream and Downstream of Its Central Repetitive Region. Journal of Biological Chemistry. 1992; 267(30):21375–83. PMID: 1400449

61. Mancuso DJ, Tuley EA, Westfield LA, Worrall NK, Shelton-Inloes BB, Sorace JM, et al. Structure of the gene for human von Willebrand factor. J Biol Chem. 1989; 264(33):19514–27. Epub 1989/11/25. PMID: 2584162

62. Kessler L, Wiesel ML, Attali P, Mossard JM, Cazenave JP, Pinet M. Von Willebrand factor in diabetic angiopathy. Diabetes Metab. 1998; 24(4):327–36. Epub 1998/11/07. PMID: 9805643

63. da Costa SR, Wu K, Veigh MM, Pidgeon M, Ding C, Schechter JE, et al. Male NOD mouse external lacrimal glands exhibit profound changes in the exocytotic pathway early in postnatal development. Exp Eye Res. 2006; 82(1):33–45. Epub 2005/07/12. PubMed Central PMCID: PMC1351294. https://doi.org/10.1016/j.exer.2005.04.019 PMID: 16005870

64. Bahamondes V, Albornoz A, Aguiera S, Alliende C, Molina C, Castro I, et al. Changes in Rab3D expression and distribution in the acini of Sjøgren’s syndrome patients are associated with loss of cell polarity
and secretory dysfunction. Arthritis Rheum. 2011; 63(10):3126–35. Epub 2011/06/28. https://doi.org/10.1002/art.30500 PMID: 21702009

66. Moehle C, Ackermann N, Langmann T, Aslanidis C, Kel A, Kel-Margoulis O, et al. Aberrant intestinal expression and allelic variants of mucin genes associated with inflammatory bowel disease. J Mol Med (Berl). 2006; 84(12):1055–66. Epub 2006/10/24.

67. Smahi A, Courtois G, Rabia SH, Doffinger R, Bodemer C, Munnich A, et al. The NF-kappaB signalling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes. Hum Mol Genet. 2002; 11(20):2371–5. Epub 2002/09/28. PMID: 12351572