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Genome-wide interaction with the insulin secretion locus *MTNR1B* reveals *CMIP* as a novel type 2 diabetes susceptibility gene in African Americans

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Duality of Interests

There are no conflicts of interest relevant to this article.

Author Contributions

JMK wrote the manuscript and researched and analyzed the data. JNH, CG, MG, and NDP researched data, contributed to data analysis, and reviewed and edited the manuscript. JSP, MF, JGW, AC, LJRT, JIR, SSR, LEW, and BIF reviewed and edited the manuscript. BIF recruited and phenotyped WFSM participants. MCYN assisted with data analysis, designed the study, and reviewed and edited the manuscript. DWB contributed to manuscript writing and study design, contributed to the discussion, and reviewed and edited the manuscript. DWB is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Abstract

Although type 2 diabetes (T2D) results from metabolic defects in insulin secretion and insulin sensitivity, most of the genetic risk loci identified to date relates to insulin secretion. We reported that T2D loci influencing insulin sensitivity may be identified through interactions with insulin secretion loci, thereby leading to T2D. Here, we hypothesize that joint testing of variant main effects and interaction effects with an insulin secretion locus increases power to identify genetic interactions leading to T2D. We tested this hypothesis with an intronic *MTNR1B* SNP, rs10830963, which is associated with acute insulin response to glucose (AIR_g), a dynamic measure of insulin secretion. rs10830963 was tested for interaction and joint (main + interaction) effects with genome-wide data in African Americans (2,452 cases and 3,772 controls) from five cohorts. Genome-wide genotype data (Affymetrix HumanGenome 6.0 array) was imputed to a 1000 Genomes Project reference panel. T2D risk was modeled using logistic regression with rs10830963 dosage, age, sex, and principal component as predictors. Joint effects were captured using the Kraft 2 degree-of-freedom test. Genome-wide significant ($P<5\times10^{-8}$) interaction with *MTNR1B* and joint effects were detected for *CMIP* intronic SNP rs17197883 ($P_{interaction}=1.43\times10^{-8}$; $P_{joint}=4.70\times10^{-8}$). *CMIP* variants have been nominally associated with T2D, fasting glucose, and adiponectin in individuals of East Asian ancestry, with high density lipoprotein (HDL), and with waist-to-hip ratio (WHR) adjusted for body mass index (BMI) in Europeans. These data support the hypothesis that additional genetic factors contributing to T2D risk, including insulin sensitivity loci, can be identified through interactions with insulin secretion loci.

Keywords

Gene-gene interactions; insulin resistance; insulin sensitivity

Introduction

Type 2 diabetes (T2D), a disease twice as prevalent in African Americans compared to European Americans, is characterized by elevated plasma glucose resulting from loss of glucose homeostasis through beta cell dysfunction impacting insulin secretion and impaired insulin sensitivity(“2014 Statistics Report | Data & Statistics | Diabetes | CDC,” n.d.). Genome-wide association studies (GWAS) have identified over 120 T2D loci with the biological basis for most of these loci assumed to be related with beta cell dysfunction(Prasad & Groop, 2015).

We hypothesized that T2D risk loci, particularly those loci affecting insulin sensitivity, could be identified by interaction analyses with insulin secretion loci(Keaton et al., 2016). This hypothesis was based on physiological observations that strongly suggest non-additive interaction between insulin secretion deficits and insulin resistance resulting in T2D(Kahn et al., 1993; Lillioja et al., 1993). To test this hypothesis, a single nucleotide polymorphism (SNP) associated with acute insulin response to glucose (AIR_g) in samples from African

American in the Insulin Resistance Atherosclerosis Family Study (IRASFS) were analyzed for genome-wide interactions contributing T2D risk in a pooled cohort of African Americans.

Interaction analysis based solely on significance of interaction terms presents power challenges for identifying interacting loci. In this report, we extend genome-wide interaction analysis by incorporating both main and interaction effects in a two degree-of-freedom joint test to search for variants with marginal effects as well as interacting with the intronic insulin secretion variant rs10830963 in *MTNR1B*, a genetic variant powerfully associated with AIR_g (P=1.20×10^{-5} in a study of 492 African American individuals examining association with 247,870 variants from an exome microarray) and fasting glucose (P=9.29×10^{-15} in 20,209 African Americans), to increase T2D risk in African Americans(Keaton et al., 2016, Liu et al, 2016). This approach is more powerful than 1 degree-of-freedom tests when both marginal and interaction effects exist (Manning et al, 2011). Considering the higher prevalence rate of T2D, insulin resistance, and obesity with strong genetic predisposition, African Americans are optimal for the study of genetic interactions that contribute to T2D risk.

Research Design and Methods

Study Populations

A pooled cohort of African Americans was created from five cohorts. Participants included African American participants from the Atherosclerosis Risk in Communities study (ARIC; n=820 T2D cases, 371 controls), the Coronary Artery Risk Development in Young Adults study (CARDIA; n=94 T2D cases, 652 controls), the Jackson Heart Study (JHS; n=244 T2D cases, 1,089 controls), the Multi-Ethnic Study of Atherosclerosis (MESA; n=404 T2D cases, 773 controls), and the Wake Forest School of Medicine study (WFSM; n=890 T2D cases, 887controls) cohorts(Bild et al., 2002; Friedman et al., 1988; McDonough et al., 2011; Palmer et al., 2012; Taylor et al., 2005; The ARIC Investigators, 1989). Inclusion and exclusion criteria for T2D cases and controls have previously been described(Ng et al., 2013). Briefly, T2D was diagnosed according to the American Diabetes Association criteria with at least one of the following: fasting glucose ≥126 mg/dL, 2-h oral glucose tolerance test glucose ≥200 mg/dL, random glucose ≥200 mg/dL, use of oral hypoglycemic agents and/or insulin, or physician diagnosed diabetes. Subjects diagnosed before 25 years of age were excluded. Normal glucose tolerance was defined as fasting glucose <100 mg/dL and 2-h oral glucose tolerance test glucose <140 mg/dL (if available) without reported use of diabetes medications or clinically diagnosed diabetes. Controls <25 years of age were excluded.

Primary associations with AIR_g in IRASFS African Americans

Glucose homeostasis traits were measured by the frequently sampled intravenous glucose tolerance test (FSIGT)(Henkin et al., 2003). Briefly, a 50% glucose solution (0.3g/kg) and regular human insulin (0.03units/kg) were injected intravenously at 0 and 20 minutes, respectively. Blood was collected at −5, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 minutes for measurement of plasma glucose and insulin. AIR_g was calculated as the increase in

insulin at 2–8 minutes above the basal (fasting) insulin level after the bolus glucose injection at 0–1 minute.

SNP genotyping, imputation, and quality control

Genotyping and quality control for the IRASFS samples were performed using the Illumina Infinium HumanExome BeadChip v1.0 as previously described(Hellwege et al., 2014). The exome chip contained 247,870 variants (92% protein coding). In addition, the chip included 64 SNPs associated with T2D from previous GWAS in Europeans, many of which have been implicated in insulin secretion (exome chip design: http://genome.sph.umich.edu/wiki/Exome_Chip_Design). Sample and autosomal SNP call rates were ≥99%, and SNPs with poor cluster separation (<0.35) were excluded.

For the ARIC, CARDIA, JHS, MESA, and WFSM cohorts, samples were genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0. Genotyping and quality control were completed by the National Heart, Lung, and Blood Institute's (NHLBI's) Candidate Gene Association Resource (CARe) at the Broad Institute for all cohorts excluding WFSM(Lettre et al., 2011). Genotyping for the WFSM study was performed at the Center for Inherited Disease Research (CIDR). In a pooled analysis of all studies, pre-phasing was performed using SHAPEIT2 and imputation was performed using IMPUTEv2 to obtain missing genotypes and replace genotypes inconsistent with reference haplotypes(Howie, Donnelly, & Marchini, 2009; O'Connell et al., 2014). SNPs with call rate ≥95%, minor allele frequency (MAF) ≥1%, and Hardy-Weinberg Equilibrium $P<1\times10^{-4}$ that passed study-specific quality control were used for imputation(Hester et al., 2012; Lettre et al., 2011). The 1000 Genomes Project cosmopolitan reference panel (Phase I Integrated Release Version 3, March 2012) was used as reference(1000 Genomes Project Consortium et al., 2012). A total of 9,085,034 autosomal SNPs with MAF ≥5% and imputation quality (INFO) ≥0.5 were included in subsequent data analyses.

To account for the effect of population structure on genetic association in these African American samples, principal components analysis (PCA) was computed for all samples collectively using genotyped SNPs that passed quality control standards after exclusion of regions of high linkage disequilibrium (LD) and inversions. To adjust for population substructure, the first PC (PC1) was used as a covariate in all analyses.

Potential relatedness was assessed in the combined analysis of WFSM, ARIC, CARDIA, JHS, and MESA using identity-by-descent (IBD) performed in PLINK(Purcell et al., 2007). A total of 1065 duplicates (pi-hat >0.9) and first-degree relatives were removed according to sample call rate and phenotype to retain only unique unrelated subjects for analysis. Samples reflecting low call rate, gender mismatch, or population outliers were also excluded. After cleaning, a total of 2,452 T2D cases and 3,772 controls remained for analysis.

Analytic Methods

Primary inferences of association with insulin secretion were derived from African American participants (n=492 individuals from 42 families) in the Insulin Resistance Atherosclerosis Family Study (IRASFS), a metabolically well-characterized cohort(Henkin et al., 2003).

Logistic regression with T2D as the outcome was modeled including genetic main and gene × gene interaction effects as well as rs10830963 dosage, age, sex, and principal component covariates for all samples. One additional model was computed to additionally adjust for body mass index (BMI).

$$Y = \beta_0 + \beta_1 S + \beta_2 G + \beta_3 S \times G + \beta_4 C$$

Y is the log odds of T2D, S is the dosage for the *MTNR1B* SNP rs10830963, G is the dosage of the imputed variant, and C is the vector of all remaining covariates. Both the rs10830963 and imputed variant dosages were additively coded with values between 0 and 2. The ProbABEL package from the GenABEL suite of programs (http://www.genabel.org/) was used to calculate the genetic main effect β_2, the interaction effect β_3, and the corresponding robust standard errors and covariance used to construct the 2×2 covariance matrix for the Kraft test(Kraft, Yen, Stram, Morrison, & Gauderman, 2007). Hypothesis testing included a Wald test statistic following a chi-squared distribution with 1 degree-of-freedom under the null H_0:$\beta_3 = 0$, as well as the Kraft test statistic following a chi-squared distribution with 2 degrees-of-freedom under the null H_0:$\beta_2 = 0$, $\beta_3 = 0$. Test statistics and corresponding p-values were calculated in the statistical computing environment R(R Core Team, 2015).

Results

Study characteristics

A genome-wide interaction analysis with intronic insulin secretion SNP (rs10830963 in *MTNR1B*) was performed to detect interactions affecting T2D risk. The combined analysis included African American subjects from the ARIC, CARDIA, JHS, MESA, and WFSM studies. Characteristics of study participants by cohort are presented in Table 1. The percentage of male subjects was modestly increased (+2.6%) among control subjects compared to T2D cases across studies. The percentage of male subjects was highest (47.0%) among MESA T2D cases and lowest (19.1%) among CARDIA T2D cases. On average, T2D cases were 10.2 years older than controls across studies. The average older age for cases across all studies is driven by the average older age for cases in the WFSM study (15.1 years). WFSM includes cases with both T2D and end-stage renal disease (ESRD) with age at T2D diagnosis preceding age at ESRD diagnosis by an average of 16.4 years(McDonough et al., 2011). Healthy controls (i.e. no T2D or ESRD diagnosis) from WFSM were on average 9.5 years older than the age of T2D diagnosis of cases(McDonough et al., 2011). The ascertainment method of selecting cases with a long duration of T2D before ESRD diagnosis and selecting controls 10 years older than the age of T2D diagnosis in cases was employed to capture the largest possible sample to specifically examine the genetic determinants of T2D-ESRD and resulted in a larger age difference between cases and controls than observed in other cohorts included in this study. The average age of controls in this study was 51.1 years (Table 1) and controls <25 years of age were excluded. Thus, misclassification of controls with undiagnosed T2D cases is unlikely. MESA T2D cases had the highest mean age (67.6 years) while CARDIA controls had the lowest mean age (38.2 years), reflecting the differences in ascertainment ages between the cohorts. Average BMI

was 1.7 kg/m^2 higher in T2D cases compared to controls across studies. Highest mean BMI (34.5 kg/m^2) was reported in JHS T2D cases and lowest mean BMI (27.6 kg/m^2) was reported in ARIC controls. A detailed description of each study is provided in the Supplementary Methods.

MTNR1B interactions

The variant rs10830963 (*MTNR1B*) was selected to test for genome-wide gene-gene interactions based on the prior association with AIR_g (P=1.20×10^{-5}) and fasting glucose (P=9.39×10^{-15}) in African Americans (Keaton et al., 2016, Liu et al,. 2016). In this study, the AIRg and fasting glucose-lowering allele of rs10830963, G, had a frequency of 7.06% and was not associated with T2D in single variant association analysis (odds ratio [OR]=1.09; P=0.76). Genome-wide interactions with rs10830963 were tested by logistic regression modeled with T2D as the outcome and including genetic main and gene-gene interaction effects as well as rs10830963 dosage, age, sex, and principal component covariates in all samples. A secondary model adjusting for all prior covariates plus BMI was computed. Both interaction and joint effects were analyzed. Interaction and joint tests, with and without adjustment for BMI, were interrogated for genomic inflation through estimation of lambda values. Lambda values ranged from 1.019 to 1.062. The quantile-quantile plots with corresponding lambda values for each hypothesis test are presented in Supplementary Figure 1 and show adequate control for inflation. The most significant results (P_{JOINT} <5×10^{-6}) of this analysis are presented in Table 2. The effect allele, other allele, and effect allele frequency for variants in Table 2 are presented in Supplementary Table 1.

Figure 1 shows a comparison of main effect P-values from a single-SNP association model adjusted for age, sex, and principal component covariates (X-axis) and joint effect P-values from the interaction model (Y-axis). The line of identity in this figure represents equal power for the univariate association model SNP main effect hypothesis test and the interaction model joint hypothesis test. Similarly, Figure 2 shows the same comparison for 20 SNPs indexing established T2D loci which exhibit trans-ethnic transferability (locus-wide P<0.05, SNP P<1×10^{-3}) in the Meta-analysis of T2D in African Americans (MEDIA) consortium(Ng et al., 2013).

The most significant joint association was with *TCF7L2* intronic SNP rs7903146 (P_{JOINT}=5.47×10^{-11}), with another *TCF7L2* intronic SNP, rs34872471, attaining genome-wide significance (P_{JOINT}=3.36×10^{-8}). Neither rs7903146 (P_{INTXN}=0.31) nor rs34872471 (P_{INTXN}=0.99) exhibited a significant association with the interaction effect alone. A novel association was observed with the *CMIP* intronic SNP rs17197883 (P_{JOINT}=4.70×10^{-8}, P_{INTXN}=1.43×10^{-8}). In this study, the rs17197883 T2D effect allele, C, had a frequency of 10.90% and was not associated with T2D in single variant association analysis of pooled samples (OR=1.07; P=0.19). However, when samples were stratified by rs10830963 (*MTNR1B*) AIR_g-lowering allele carriers versus non-carriers, we observed a suggestively significant opposite effect in both groups. rs17197883 exhibited a T2D risk effect in carriers (OR=2.29, P=7.64×10^{-6}) and a protective effect in non-carriers (OR=0.78, P=8.00×10^{-4}), suggesting an antagonistic pattern of interaction (Table 3). In general, observed associations were robust to adjustment for BMI (Table 2).

Additional SNPs in loci (*e.g.*, *WRB*) exhibited a nominal association ($P_{JOINT}<5\times10^{-6}$) in joint tests of marginal and interaction effects. The majority of these loci were well-supported with multiple SNPs showing evidence of nominally significant association as shown in Figure 3. The association of these SNPs is driven, for the most part, by the interaction effect. The most significantly associated SNP in prior interaction analysis with the *MTNR1B* SNP rs10830963 in HapMap imputed data, rs7277627 at the *LCA5L* locus ($P_{INTXN}=1.65\times10^{-6}$), replicated in this study ($P_{JOINT}=5.46\times10^{-6}$)(Keaton et al., 2016). However, the use of a denser imputation panel (i.e., 1000 Genomes Project) provided the identification the SNP at the *LCA5L* locus, rs2223028, that exhibited stronger evidence of association ($P_{JOINT}=4.44\times10^{-6}$).

Discussion

A genome-wide analysis of interaction and joint effects with *MTNR1B* intronic insulin secretion SNP rs10830963 resulting in risk of T2D revealed significant associations at two loci, *TCF7L2* and *CMIP*. The strongest joint association was with *TCF7L2* intronic SNP rs7903146. Association with rs7903146 has been replicated in numerous African American studies suggesting that it is a key genetic determinant in development of T2D(Lewis et al., 2008; Long et al., 2012; Moore et al., 2008; Palmer et al., 2012; Saxena et al., 2012; Waters et al., 2010). Palmer *et al.* reported that rs7903146 is likely the causal variant contributing T2D susceptibility at the *TCF7L2* locus using a resequencing approach(Palmer et al., 2011). However, rs7903146 was not associated with fasting glucose in African Americans (Liu et al., 2016). The lack of interaction effect in the current study suggests that the joint signal is driven by marginal (i.e., additive) genetic effects.

The strongest novel association was with *CMIP* intronic SNP rs17197883 ($P_{JOINT}=4.70\times10^{-8}$, $P_{INTXN}=1.43\times10^{-8}$). Variants at this locus have previously been associated with HDL cholesterol (rs56823429, $P=2\times10^{-8}$), adiponectin levels (rs12051272, $P=6\times10^{-48}$), and WHR adjusted for BMI (rs2925979, $P=7\times10^{-13}$) in populations of European (EUR) descent and adiponectin levels (rs2925979, $P=2\times10^{-10}$), fasting glucose (rs16955379, $P=0.03$), and T2D (rs16955379, $P=3\times10^{-7}$) in populations of East Asian (EAS) descent(Cho et al., 2012; Dastani et al., 2012; Global Lipids Genetics Consortium et al., 2013; Sakai et al., 2013; Shungin et al., 2015; Surakka et al., 2015; Wu et al., 2014). All of the previously described variants at the *CMIP* locus are in weak LD with rs17197883 ($r^2<0.2$). There is no surrogate SNPs in high LD ($r^2 > 0.6$ in 1000 Genomes AFR population) with rs17197883, In addition, rs17197883 did not reside in a regulatory region and did not regulate nearby gene expression by HaploReg analysis. Interestingly, the frequency of the rs10830963 AIRg-lowering allele (G) is much higher in these populations compared to African Americans (AfA) (EAS=42%, EUR=29%, AfA=7%). Thus, associations of SNPs at the *CMIP* locus with T2D and biomarkers related to insulin resistance in individuals of EUR and EAS descent may reflect an underlying interaction with rs10830963 that is partially unmasked in these populations. In IRASFS, neither previously reported SNPs in the *CMIP* region nor the interacting SNP rs17197883 showed evidence of association with measures of insulin sensitivity, adiponectin levels, WHR adjusted for BMI, nor fasting glucose(Gao et al., 2015; Hellwege et al., 2014, 2017). However, 2 previously reported SNPs at the *CMIP* locus, rs2925979 ($P=0.02$) and rs56823429 ($P=0.009$), and the

interacting SNP rs17197883 (P=0.002) were nominally associated with HDL cholesterol in IRASFS Hispanics(Hellwege et al., 2017). The interacting *CMIP* variant, rs17197883, was not associated with T2D in the current study in a model adjusted for age, sex, and PC1 (P=0.19).

This study was conducted to explore the impact of three methodological additions to an analysis model focused solely on the interaction term. First, after observing homogeneity in genetic effects across cohorts in a prior study(Keaton et al., 2016), we pooled (as opposed to meta-analyzed) samples from five African American T2D studies. Sung *et al.* suggested that results from pooled analysis and meta-analysis for main, interaction, and joint effects are largely consistent(Sung et al., 2014). Notably, we detected an interaction P-value reaching genome-wide significance (rs17197883, $P_{INTXN}=1.43\times10^{-8}$) in a pooled analysis, which is an order of magnitude smaller compared to the most significant P-value (rs16924460, $P_{INTXN}=1.70\times10^{-7}$) in our previous meta-analyzed study(Keaton et al., 2016). However, it is difficult to assess the impact of pooling samples due to additional methodological differences. For example, additional quality control identified some samples that were duplicated or had other quality control issues.

Second, we used genotype data imputed to a 1000 Genomes Project phase 1 reference panel(The 1000 Genomes Project Consortium, 2015). Compared to the HapMap reference panel previously used for imputation, the 1000 Genomes Project panel provides greater coverage of common variation and facilitates overall improvement of imputation quality(Wood et al., 2013). This expanded genotype data allowed for analysis of 9,085,034 autosomal SNPs with MAF >5% in our study cohorts, compared to 2,907,086 autosomal SNPs with a MAF >1% in the prior analysis(Keaton et al., 2016). The MAF threshold was increased in the current study to overcome the inherent power loss and potential for false positives for interaction analyses incorporating a low frequency exposure(Zhang, Lewinger, Conti, Morrison, & Gauderman, 2016).

Finally, in addition to hypothesis testing of the interaction term in our models, this analysis incorporates the Kraft 2 degree-of-freedom test to jointly analyze marginal and interaction effects(Kraft et al., 2007). This joint test allows for the detection of variants with both additive and non-additive effects contributing to T2D risk. The skew toward more significant joint effect P-values in Figure 1 suggests that genome-wide interaction analysis with a well-defined insulin secretion variant (*e.g.*, *MTNR1B* SNP rs10830963) incorporating a 2 degrees-of-freedom hypothesis test is a powerful approach for detection of novel T2D risk loci. In this figure, 3,435,184 out of 9,085,034 (37.8%) SNPs had a more significant joint effect P-value compared to the main effect P-value from an association model excluding the interaction term. The SNPs in *TCF7L2* fall below the line of identity on the main effect axis, while the *CMIP* SNP, plus many others fall above the line of identity, thus comparing power between the two tests. These results suggest that established T2D loci, originally identified by strong SNP main effects in a typical univariate association analysis and largely involved in insulin secretion biology themselves, are not likely candidates for interaction with insulin secretion loci. Alternatively, these established T2D loci, primarily discovered in European populations, do not exhibit strong SNP main effects in African American populations and may have a limited interaction effect to impact association with T2D.

To analyze performance of the joint test for known T2D loci, we compared main and joint effect *P*-values for the most significant African American SNP at 20 established T2D loci that exhibited trans-ethnic transferability in MEDIA(Ng et al., 2013). Figure 2 shows that SNPs from 3 loci, *IGF2-INS-TH*, *SLC11A2*, and *HMGA2*, had a more significant joint effect *P*-value compared to the main effect *P*-value. This result combined with the observation from Figure 1 suggests that the joint test is a powerful approach for detection of novel T2D loci, but a typical association model may be more powerful for established loci.

This study has limitations. To validate these findings, genome-wide interactions with rs10830963 associated with T2D must be replicated in additional studies, including studies of other ethnicities. Additionally, we did not account for gene-environment interactions which may account for T2D risk. Interactions with or stratified analysis of sex, age, BMI, and age at diagnosis may be appropriate in follow-up studies. It is also important to note that statistical interaction does not imply biological interaction, as the statistical interaction may be mediated through multiple biological factors. However, statistical interaction may revealed novel loci which may not have strong marginal effect.

In summary, the present findings demonstrate that analysis of physiologically defined genome-wide interactions with variants strongly associated with insulin secretion is a potentially powerful approach for discovery of novel T2D loci and for expanding the knowledgebase of disease etiology. A similar approach examining interactions with variants associated with key biomarkers may be of wider relevance in other complex human diseases. Results highlight the need for further study of genetic variation underlying T2D risk in African Americans as a means to improve our overall understanding of this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ARIC, CARDIA, JHS, and MESA.

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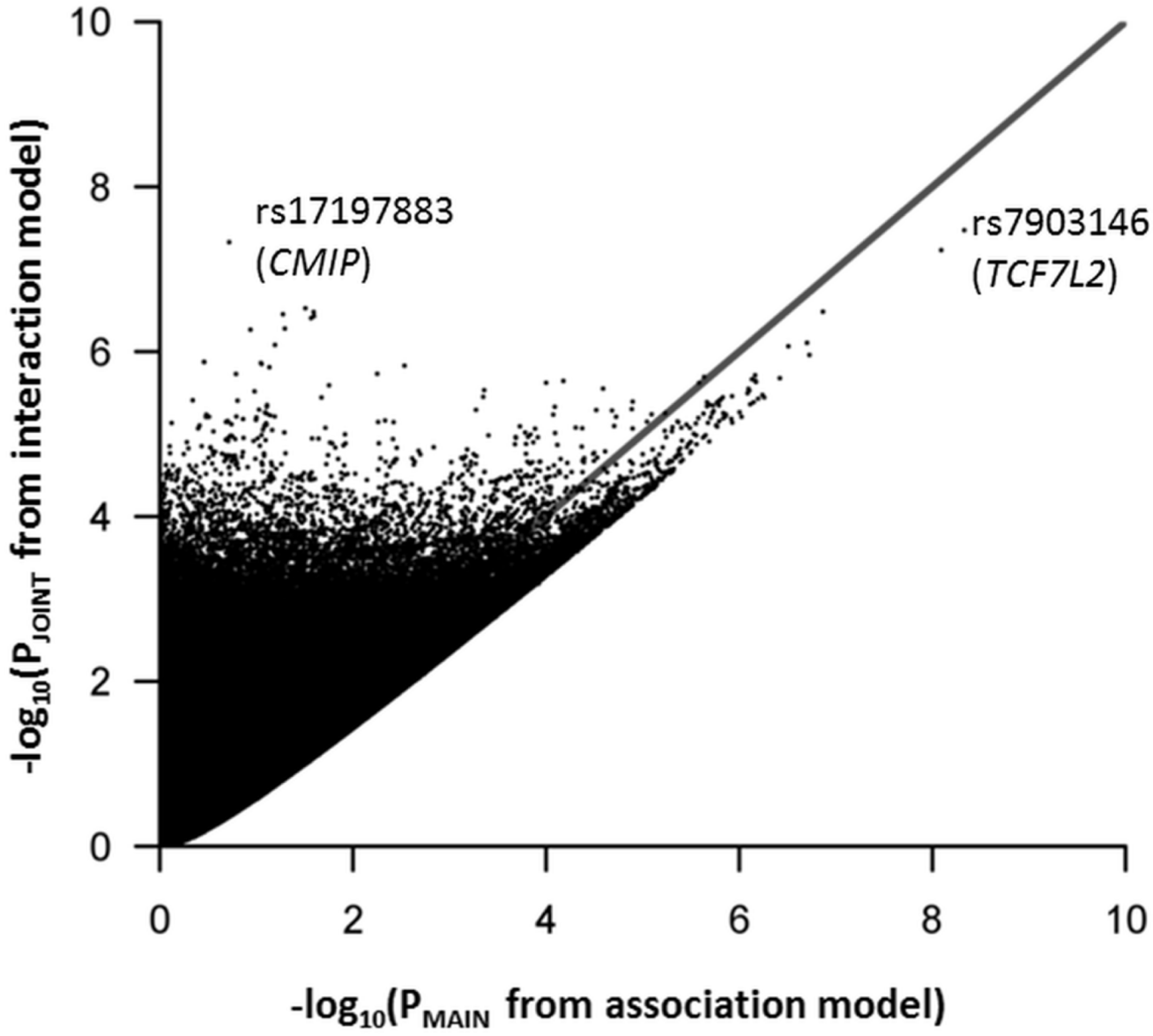

Figure 1.

A comparison of P-values from association and interaction models. The X-axis represents negative logarithm transformed *P*-values from the 1 degree-of freedom hypothesis test of SNP main effects from the univariate association model not including the interaction term, the Y-axis represents negative logarithm transformed *P*-values from the 2 degree-of freedom joint hypothesis test of SNP main and interaction effects from the interaction model, and the line of identity represents equal power between the 2 tests. Each point represents p-values for a genomic SNP depending upon the model.

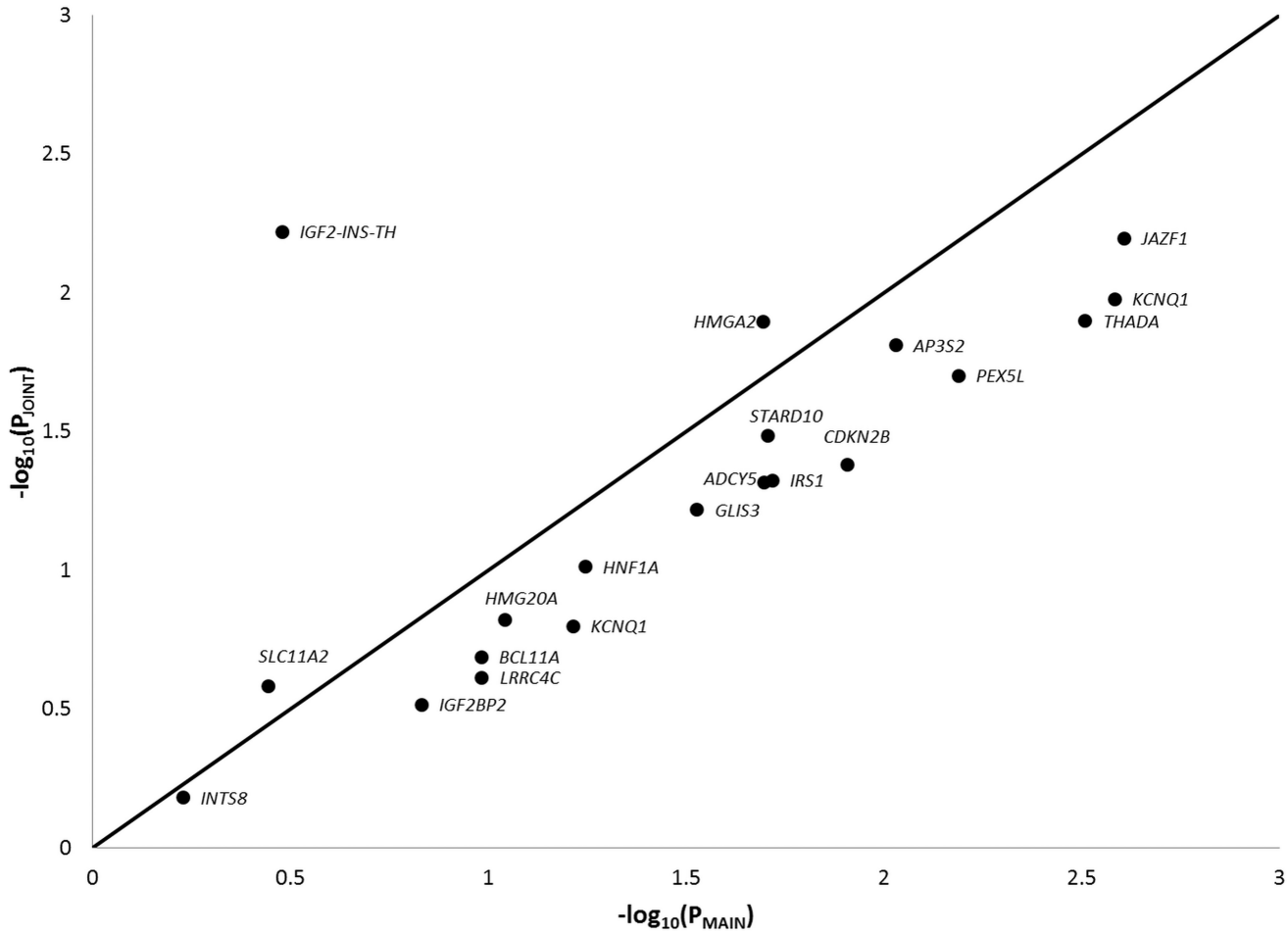

Figure 2.

A comparison of P-values from association and interaction models for established T2D loci exhibiting transferability in African Americans. In this figure, the X-axis represents negative logarithm transformed *P*-values from the 1 degree-of freedom hypothesis test of SNP main effects from the univariate association model not including the interaction term, the Y-axis represents negative logarithm transformed *P*-values from the 2 degree-of freedom joint hypothesis test of SNP main and interaction effects from the interaction model, and the line of identity represents equal power between the 2 tests. Each point represents p-values for a trans-ethnic transferrable SNP depending upon the model.

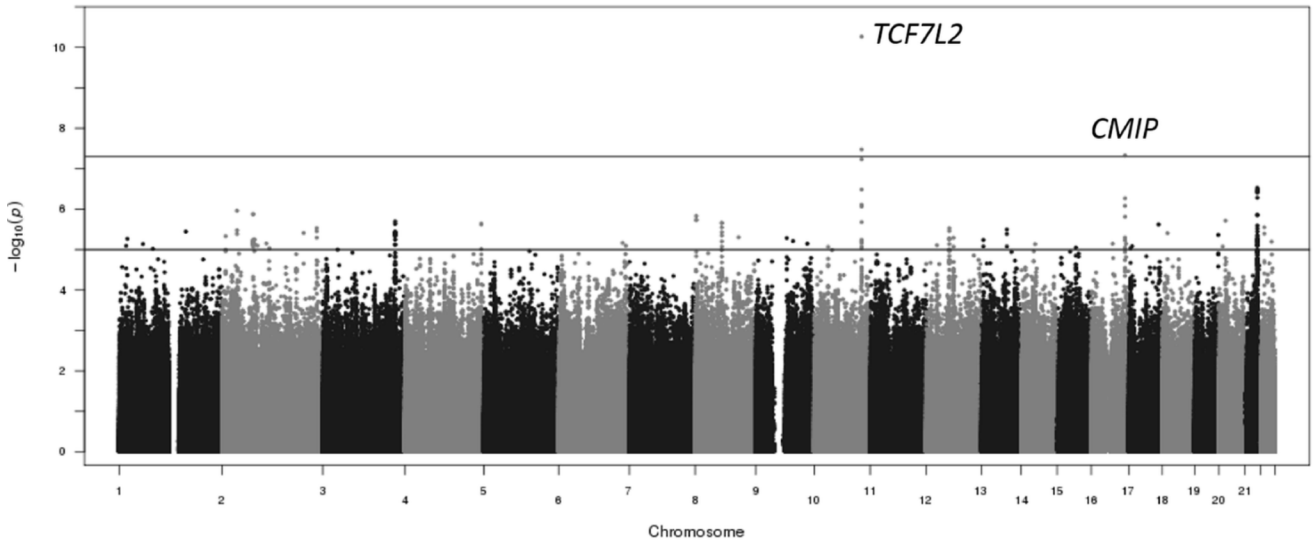

Figure 3.

Manhattan plot of results from the 2 degree-of-freedom test. Top line denotes genome-wide significance at $P < 5\times10^{-8}$. Second line denotes suggestive significance at $P < 5\times10^{-6}$

Table 1

Descriptive characteristics of African American cases with type 2 diabetes and controls.

	ARIC		CARDIA		JHS		MESA		WFSM		Combined	
Characteristic[a]	**T2D cases**	**Controls**	**T2D cases**	**Controls**	**T2D cases**	**Controls**	**T2D cases**	**Controls**	**T2D cases**	**Controls**	**T2D cases**	**Controls**
N	820	371	94	652	244	1089	404	773	890	887	2452	3772
Male (%)	36.0	32.1	19.1	38.3	36.1	40.8	47.0	42.8	38.4	43.9	38.0	40.6
Age (years)[b]	61.4±5.9	59.4±6.4	40.5±3.8	38.2±4.4	55.6±10.7	49.1±10.7	67.6±9.3	65.3±10.3	62.2±10.1	47.1±11.7	61.3±10.1	51.1±13.3
BMI (kg/m^2)[b]	32.1±6.7	27.6±5.8	33.8±8.1	29.6±6.8	34.5±7.3	31.1±7.3	31.7±6.2	28.7±5.8	29.6±7.1	30.1±7.1	31.5±7.0	29.8±6.8

[a] Data are shown as count, percentage, or mean ± SD.

[b] Age and BMI are shown for the last available visit for the prospective studies including ARIC, CARDIA, and MESA (Exam 4); and the baseline visit for JHS and WFSM.

Table 2

Top signals ($P_{JOINT}<5\times10^{-6}$) from the 2 degree-of-freedom test with the *MTNR1B* SNP rs10830963 interaction effect and the marginal SNP effect regressed on T2D risk in pooled analysis of ARIC, CARDIA, JHS, MESA, and WFSM African American cases with type 2 diabetes and controls.

SNP[a] (Nearest Gene)	Chr	Position[b]	SNP Function	MAF[c]	β_{MAIN} (SE_{MAIN})[d]	β_{INTXN} (SE_{INTXN})[e]	P_{INTXN}[f]	P_{JOINT}[f]	$P_{INTXN\_ADJ\_BMI}$[g]	$P_{JOINT\_ADJ\_BMI}$[g]
rs7903146 (*TCF7L2*)	10	114758349	intronic	0.30	0.29 (0.05)	0.14 (0.14)	3.09E-01	5.47E-11	6.23E-01	1.44E-10
rs34872471 (*TCF7L2*)	10	114754071	intronic	0.35	0.25 (0.05)	0.00 (0.13)	9.94E-01	3.36E-08	6.35E-01	3.15E-08
rs17197883 (*CMIP*)	16	81523013	intronic	0.11	−0.24 (0.08)	1.12 (0.20)	1.43E-08	4.70E-08	2.53E-07	8.91E-07
rs35198068 (*TCF7L2*)	10	114754784	intronic	0.36	0.25 (0.05)	0.00 (0.13)	9.77E-01	5.88E-08	5.99E-01	5.72E-08
rs2836995 (*WRB*)	21	40748801	intergenic	0.49	0.00 (0.04)	−0.58 (0.11)	3.49E-07	2.97E-07	2.70E-06	8.43E-07
rs11819613 (*TCF7L2*)	10	114746717	intronic	0.11	0.36 (0.07)	−0.24 (0.20)	2.30E-01	3.28E-07	1.00E-01	8.46E-07
rs4818031 (*WRB*)	21	40747523	intergenic	0.37	−0.01 (0.05)	−0.60 (0.12)	5.23E-07	3.31E-07	2.83E-06	5.24E-07
rs4818032 (*WRB*)	21	40747583	intergenic	0.36	0.00 (0.05)	−0.61 (0.12)	3.03E-07	3.52E-07	1.34E-06	5.97E-07
rs2836994 (*WRB*)	21	40748006	intergenic	0.48	−0.01 (0.04)	−0.58 (0.12)	5.61E-07	3.72E-07	5.64E-06	1.09E-06
rs4818033 (*WRB*)	21	40747624	intergenic	0.48	−0.01 (0.04)	−0.58 (0.12)	5.61E-07	3.92E-07	5.60E-06	1.15E-06
rs13230 (*WRB*)	21	40769290	untranslated-3'	0.45	−0.01 (0.04)	0.60 (0.12)	3.68E-07	5.25E-07	8.07E-07	1.39E-06
rs56140527 (*CMIP*)	16	81515361	intronic	0.12	−0.23 (0.07)	0.94 (0.18)	2.64E-07	5.41E-07	1.57E-06	4.01E-06
rs7069007 (*TCF7L2*)	10	114756285	intronic	0.11	0.34 (0.07)	−0.16 (0.19)	3.99E-01	7.81E-07	1.95E-01	1.84E-06
rs56155262 (*CMIP*)	16	81511653	intronic	0.12	−0.25 (0.07)	0.90 (0.18)	6.73E-07	8.29E-07	3.31E-06	5.52E-06
rs61329995 (*TCF7L2*)	10	114748105	intronic	0.11	0.35 (0.07)	−0.21 (0.20)	2.78E-01	8.61E-07	1.25E-01	2.18E-06
rs79029870 (---)	2	35998316	intergenic	0.11	0.34 (0.07)	0.10 (0.20)	6.28E-01	1.10E-06	5.43E-01	1.34E-06
rs116123815 (*C2orf65*)	2	74873329	intronic	0.05	0.13 (0.10)	−1.49 (0.29)	2.05E-07	1.33E-06	2.22E-06	1.23E-05
rs116472028 (*C2orf65*)	2	74872566	intronic	0.05	0.13 (0.10)	−1.49 (0.29)	2.05E-07	1.33E-06	2.22E-06	1.23E-05
rs73364034 (*WRB*)	21	40744651	intergenic	0.36	0.01 (0.05)	−0.59 (0.12)	8.20E-07	1.37E-06	2.83E-06	1.89E-06
rs8131718 (*WRB*)	21	40766181	intronic	0.45	−0.01 (0.04)	0.59 (0.12)	6.77E-07	1.41E-06	1.61E-06	3.97E-06
rs73659516 (*AK128880*)	8	2587387	intergenic	0.14	−0.08 (0.07)	−0.90 (0.20)	1.16E-05	1.48E-06	7.05E-06	7.63E-07
rs10438632 (*CMIP*)	16	81510920	intronic	0.12	−0.24 (0.07)	0.88 (0.18)	1.20E-06	1.55E-06	5.38E-06	9.35E-06
rs73659517 (*AK128880*)	8	2587464	intergenic	0.16	−0.07 (0.07)	−0.88 (0.20)	8.99E-06	1.86E-06	7.20E-06	1.20E-06
rs2924879 (*CSMD1*)	8	2702216	intergenic	0.39	0.02 (0.05)	−0.59 (0.12)	6.73E-07	1.87E-06	4.43E-06	1.33E-05
rs6043626 (*MACROD2*)	20	15916192	intronic	0.45	0.18 (0.04)	0.17 (0.12)	1.42E-01	1.93E-06	3.30E-01	9.55E-06

SNP[a] (Nearest Gene)	Chr	Position[b]	SNP Function	MAF[c]	β_{MAIN} (SE_{MAIN})[d]	β_{INTXN} (SE_{INTXN})[e]	P_{INTXN}[f]	P_{JOINT}[f]	$P_{INTXN\_ADJ\_BMI}$[g]	$P_{JOINT\_ADJ\_BMI}$[g]
rs4319449 (*TCF7L2*)	10	114769406	intronic	0.09	0.38 (0.08)	−0.10 (0.22)	6.31E-01	2.10E-06	3.48E-01	4.13E-06
rs1369455 (*YTHDF3*)	8	64082963	intergenic	0.07	−0.37 (0.09)	−0.28 (0.22)	2.07E-01	2.17E-06	2.40E-01	2.25E-05
rs2043525 (*YTHDF3*)	8	64107847	intergenic	0.07	−0.37 (0.09)	−0.26 (0.22)	2.40E-01	2.25E-06	2.72E-01	2.37E-05
rs10866267 (*LOC728175*)	4	185239161	near-gene-5'	0.36	−0.12 (0.05)	−0.42 (0.13)	9.05E-04	2.27E-06	3.86E-03	6.87E-06
rs9842579 (*NAALADL2*)	3	174700592	intronic	0.19	0.29 (0.06)	−0.15 (0.17)	3.59E-01	2.33E-06	2.88E-01	5.99E-07
rs812665 (*LOC728175*)	4	185245310	intergenic	0.34	−0.11 (0.05)	−0.41 (0.12)	7.08E-04	2.39E-06	1.90E-03	6.24E-06
rs2836997 (*WRB*)	21	40751811	near-gene-5'	0.49	−0.02 (0.04)	−0.55 (0.12)	5.11E-06	2.56E-06	2.34E-05	4.12E-06
rs1807685 (*CRYBB2*)	22	25639858	intergenic	0.23	0.28 (0.06)	−0.40 (0.15)	9.40E-03	2.81E-06	5.68E-03	1.21E-06
rs115597883 (*SLC19A3*)	2	228559189	intronic	0.11	0.35 (0.07)	−0.69 (0.20)	7.17E-04	2.93E-06	2.93E-04	1.77E-06
rs2291268 (*MMP19*)	12	56236043	intergenic	0.32	−0.22 (0.05)	−0.15 (0.13)	2.53E-01	2.96E-06	3.37E-01	1.72E-05
rs1060180 (*WRB*)	21	40769017	untranslated-3'	0.46	−0.01 (0.04)	0.57 (0.12)	1.38E-06	3.03E-06	3.95E-06	9.47E-06
rs1360814 (---)	13	80403917	intergenic	0.18	0.24 (0.06)	0.15 (0.15)	3.13E-01	3.19E-06	4.28E-01	2.85E-06
rs17017402 (---)	2	36003758	intergenic	0.11	0.31 (0.07)	0.07 (0.19)	7.02E-01	3.35E-06	5.58E-01	3.49E-06
rs1681087 (*DNAJC14*)	12	56180764	intronic	0.26	0.21 (0.05)	0.17 (0.13)	1.85E-01	3.47E-06	2.48E-01	1.55E-05
rs114812021 (*SLC19A3*)	2	228559188	intronic	0.11	0.35 (0.07)	−0.68 (0.20)	8.43E-04	3.53E-06	3.44E-04	2.05E-06
rs9845886 (*NAALADL2*)	3	174704267	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.23E-01	3.58E-06	1.55E-01	3.96E-07
rs58538128 (*FCGR3A*)	1	161520858	near-gene-5'	0.14	0.35 (0.08)	0.08 (0.20)	7.04E-01	3.59E-06	7.93E-01	3.93E-05
rs2837021 (*LCA5L*)	21	40796623	intronic	0.36	−0.02 (0.05)	−0.56 (0.12)	5.26E-06	3.60E-06	3.02E-06	2.86E-06
rs4739079 (*YTHDF3*)	8	64200821	intergenic	0.09	0.35 (0.08)	0.08 (0.19)	6.62E-01	3.72E-06	8.58E-01	1.27E-05
rs59246912 (*NAALADL2*)	3	174704393	intronic	0.26	0.24 (0.05)	−0.16 (0.14)	2.27E-01	3.82E-06	1.57E-01	4.14E-07
rs9862955 (*NAALADL2*)	3	174703978	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.26E-01	3.88E-06	1.57E-01	4.22E-07
rs9862818 (*NAALADL2*)	3	174703904	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.26E-01	3.88E-06	1.57E-01	4.20E-07
rs16846841 (*HECW2*)	2	197063250	intergenic	0.20	0.06 (0.06)	−0.81 (0.16)	6.80E-07	3.89E-06	2.22E-06	1.09E-05
rs9862801 (*NAALADL2*)	3	174703876	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.26E-01	3.90E-06	1.57E-01	4.25E-07
rs7228666 (*SLMO1*)	18	12427118	intronic	0.23	−0.17 (0.05)	0.68 (0.14)	1.77E-06	3.93E-06	4.21E-06	8.64E-06
rs9821147 (*NAALADL2*)	3	174703737	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.26E-01	3.94E-06	1.57E-01	4.31E-07
rs13323013 (*NAALADL2*)	3	174703232	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.26E-01	4.07E-06	1.57E-01	4.50E-07
rs1335279 (---)	13	80399950	intergenic	0.21	0.21 (0.05)	0.23 (0.15)	1.21E-01	4.10E-06	1.73E-01	1.04E-05
rs56864167 (---)	2	35969614	intergenic	0.11	0.31 (0.07)	0.07 (0.19)	7.12E-01	4.10E-06	5.87E-01	4.52E-06

SNP[a] (Nearest Gene)	Chr	Position[b]	SNP Function	MAF[c]	β_{MAIN} (SE_{MAIN})[d]	β_{INTXN} (SE_{INTXN})[e]	P_{INTXN}[f]	P_{JOINT}[f]	$P_{INTXN\_ADJ\_BMI}$[g]	$P_{JOINT\_ADJ\_BMI}$[g]
rs9820271 (*NAALADL2*)	3	174703026	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.25E-01	4.13E-06	1.56E-01	4.59E-07
rs10412272 (*USP29*)	19	57597884	intergenic	0.05	0.45 (0.10)	0.14 (0.27)	5.87E-01	4.34E-06	7.59E-01	6.81E-06
rs2223028 (*LCA5L*)	21	40810373	intronic	0.34	0.01 (0.05)	−0.60 (0.13)	2.26E-06	4.44E-06	1.01E-06	2.16E-06
rs7016339 (*YTHDF3*)	8	64121730	intergenic	0.07	−0.36 (0.09)	−0.30 (0.23)	1.91E-01	4.48E-06	2.25E-01	4.53E-05
rs76240879 (*NAALADL2*)	3	174704464	intronic	0.26	0.24 (0.05)	−0.17 (0.14)	2.25E-01	4.50E-06	1.55E-01	4.49E-07
rs9810343 (*NAALADL2*)	3	174701501	intronic	0.26	0.24 (0.05)	−0.18 (0.14)	1.94E-01	4.60E-06	1.33E-01	5.23E-07
rs6735529 (*LINC00299*)	2	8614384	intergenic	0.05	0.23 (0.10)	0.78 (0.24)	1.41E-03	4.67E-06	5.54E-03	2.97E-05
rs16870669 (*RIMS2*)	8	104773461	intronic	0.11	0.22 (0.07)	0.47 (0.19)	1.41E-02	4.98E-06	2.82E-02	1.30E-06

[a] SNP interacting with rs10830963.

[b] NCBI build 37.

[c] Minor allele frequency.

[d] Marginal genetic effect from interaction models adjusted for age, gender, and PC1.

[e] Interaction effect from interaction models adjusted for age, gender, and PC1.

[f] Interaction and joint p-values from interaction models adjusted for age, gender, and PC1.

[g] Interaction and joint p-values from interaction models adjusted for age, gender, PC1, and BMI.

Table 3

Association of CMIP variant rs17197882 with T2D stratified by MTNR1B genotype

MTNR1B genotype	β (SE)[a]	OR (95%CI)[b]	P
CC	−0.25 (0.07)	0.78 (0.67–0.90)	8.00E-04
CG or GG	0.83 (0.18)	2.29 (1.59–3.28)	7.64E-06

[a]Effect and standard error from a simple T2D association model adjusted for age, sex, and PC1.

[b]Odds ratio and 95% confidence interval.