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Genome-Wide Association Study of the Modified Stumvoll Insulin Sensitivity Index Identifies *BCL2* and *FAM19A2* as Novel Insulin Sensitivity Loci

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Genome-wide association studies (GWAS) have found few common variants that influence fasting measures of insulin sensitivity. We hypothesized that a GWAS of an integrated assessment of fasting and dynamic measures of insulin sensitivity would detect novel common variants. We performed a GWAS of the modified Stumvoll Insulin Sensitivity Index (ISI) within the Meta-Analyses of Glucose and Insulin-Related

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Traits Consortium. Discovery for genetic association was performed in 16,753 individuals, and replication was attempted for the 23 most significant novel loci in 13,354 independent individuals. Association with ISI was tested in models adjusted for age, sex, and BMI and in a model analyzing the combined influence of the genotype effect adjusted for BMI and the interaction effect between the genotype and BMI on ISI (model 3). In model 3, three variants reached genome-wide significance: rs13422522 (*NYAP2*; $P = 8.87 \times 10^{-11}$), rs12454712 (*BCL2*; $P = 2.7 \times 10^{-8}$), and rs10506418 (*FAM19A2*; $P = 1.9 \times 10^{-8}$). The association at *NYAP2* was eliminated by conditioning on the known *IRS1* insulin sensitivity locus; the *BCL2* and *FAM19A2* associations

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were independent of known cardiometabolic loci. In conclusion, we identified two novel loci and replicated known variants associated with insulin sensitivity. Further studies are needed to clarify the causal variant and function at the *BCL2* and *FAM19A2* loci.

Genome-wide association studies (GWAS) have identified common genetic variants associated with type 2 diabetes (1), a disease marked by a reduction in β-cell function and insulin sensitivity (2). While both β-cell function and insulin sensitivity traits are partly heritable, GWAS have demonstrated relatively few single nucleotide polymorphisms (SNPs) associated with insulin sensitivity (3).

Traits used to estimate insulin sensitivity from fasting measurements in prior large GWAS, including fasting insulin and the HOMA–insulin resistance (HOMA-IR), demonstrate approximately half the heritability of traits that incorporate both fasting and dynamic assessments of insulin sensitivity following a glucose load (4). Moreover, there is only modest genetic correlation between HOMA-IR and measures of insulin sensitivity by euglycemic clamp, which is considered the gold standard measure of peripheral insulin sensitivity (5,6). Thus, an alternative approach to discover new common genetic variants associated with insulin sensitivity is to perform GWAS using a dynamic measure of whole-body insulin sensitivity. As an example, a recent GWAS identified a novel insulin sensitivity locus at *NAT2* using euglycemic clamp and insulin suppression test techniques in 2,764 subjects, with replication in another 2,860 individuals (7). However, these direct, whole-body measures of insulin sensitivity are time- and resource-intensive interventions, which limits the feasible sample size of such experiments. Indices derived from an oral glucose tolerance test that integrate fasting and dynamic measures of insulin sensitivity reasonably approximate euglycemic clamp measures and can be applied in existing large cohorts with glycemic traits, potentially increasing the statistical power to detect novel variant associations.

We tested the hypothesis that a well-powered GWAS would detect common genetic variants for the modified Stumvoll Insulin Sensitivity Index (ISI). Insulin sensitivity assessed by the euglycemic-hyperinsulinemic clamp (average glucose infusion rate/average plasma insulin concentration [M/I]) has a stronger correlation with the ISI than with HOMA-IR (r = 0.79 vs. 0.59, respectively) (8). In addition, the ISI is well correlated (r = 0.69) with M/I, even when calculated using only fasting insulin values and glucose and insulin values 120 min after a 75-g oral glucose load (9); this modified version is widely available in existing cohorts, providing a larger sample size for association analyses than the sample size that would be available if indices requiring additional time points were used. We further hypothesized that a subset of these common genetic variants would influence the ISI independently or through their effect on BMI. Thus we tested the association of the modified ISI in statistical models without adjusting for BMI, in statistical models adjusting for BMI, and in a validated model (10,11) analyzing the combined influence of the genotype effect adjusted for BMI and the interaction effect between the genotype and BMI on ISI.

RESEARCH DESIGN AND METHODS

Cohort Descriptions

The cohorts participating in the Meta-Analyses of Glucose and Insulin-related Traits Consortium (MAGIC) contributed a total of 30,107 individuals to the analyses. Detailed information on the study cohorts and methods is provided in Supplementary Table 1. All participants were of white European ancestry from the United States or Europe and did not have diabetes. All studies were approved by local research ethic committees, and all participants gave informed consent.

Modified Stumvoll ISI

Missing trait data were not imputed, and outliers were not excluded from analyses. The ISI was calculated as previously described (9), according to the following formula:

$$\begin{aligned} 0.156 &- \left(0.0000459 \times \text{insulin}_{2\text{h[pmol/L]}}\right) \\ &- \left(0.000321 \times \text{insulin}_{\text{fasting[pmol/L]}}\right) \\ &- \left(0.0054 \times \text{glucose}_{2\text{h[mmol/L]}}\right) \end{aligned}$$

Discovery Effort: GWAS

Cohorts that were able to contribute genome-wide genotyping results during the course of the project were included in the discovery effort. These were the Framingham Heart Study (FHS), Sorbs, the Finland–United States Investigation of NIDDM (FUSION), the Cardiovascular Health Study (CHS), Ludwigshafen Risk and Cardiovascular Health (LURIC) study, the Uppsala Longitudinal Study of Adult Men (ULSAM), and Metabolic Syndrome in Men (METSIM) study. For the discovery GWAS, all samples with call rates <95% were excluded, and SNPs departing from Hardy-Weinberg equilibrium (at $P < 10^{-6}$), genotype rate <95%, or minor allele frequency <1% were excluded. Poorly imputed SNPs were excluded if R^2 <0.3 or proper-info was <0.4.

Each SNP was tested for association with ISI in three different additive genetic models: model 1 was adjusted for age and sex; model 2 was adjusted for age, sex, and BMI; and model 3 analyzed the combined influence of the genotype effect adjusted for BMI and the interaction effect between the genotype and BMI on ISI (10,11). The associations in model 3 result from a test with two degrees of freedom. When no interaction is present, the additional degree of freedom results in a modest loss of statistical power. When interaction is present, however, the statistical power of the model is greater (11). To adjust for differences in insulin measurement between

cohorts, effect estimates were normalized to the SD of the ISI in each cohort (Supplementary Table 1). A robust estimate of the standard error was calculated in the interaction analysis using ProbAbel, QUICKtest, or generalized estimating equations using the R geepack package. An inverse-variance meta-analysis using METAL was performed on the β coefficient/SD from each cohort.

Following meta-analysis, SNPs with total sample size less than 8,500 (approximately half of the maximum sample size) or with heterogeneity *P* values $\leq 10^{-6}$ (a value chosen to take into account multiple hypothesis testing but below the level of strict Bonferroni correction) in the meta-analysis of the discovery cohorts were removed. Genomic correction of cohort-specific association statistics (i.e., correction for each individual study) was performed. In total, up to 2.4 million SNPs were meta-analyzed for association with ISI in the discovery effort.

Selection of SNPs for Replication

Candidate SNPs for replication were identified by their association *P* value $\leq 10^{-7}$ in one or more of the analysis models. For gene loci with multiple replication candidates, the SNP with the lowest *P* value and any other SNP in low linkage disequilibrium (LD; $r^2 < 0.5$) with the index SNP in Europeans were retained. Using these filters, 23 unique candidate SNPs from 23 loci were identified for replication. The SNP Annotation and Proxy Search site was used to find up to three proxies in high LD ($r^2 > 0.8$) in Europeans for each candidate SNP.

Replication Effort

Cohorts that did not contribute to the discovery effort but were able to contribute association results during the course of the project were included in the replication effort. These were the European Network on Functional Genomics of Type 2 Diabetes (EUGENE2) study, Amish Studies, the Relationship between Insulin Sensitivity and Cardiovascular Risk Study (RISC), the Tübingen Family Study for Type 2 Diabetes (Tübingen), Inter99 Study, the Segovia Study, the Pizarra Study, the Botnia Study, the 1936 Birth Cohort, and the Ely Study. Genotype data were obtained using in silico data from preexisting GWAS or de novo genotyping. In replication cohorts, SNPs with a minor allele count (MAC) <20 were excluded. Additional details of the replication cohort effort are provided in Supplementary Table 1.

Combined Meta-analysis

We required the absence of heterogeneity in the combined analysis of discovery and replication cohorts ($P > 10^{-6}$) as well as nominal significance ($P < 0.05$) in the replication effort and genome-wide significance ($P < 5 \times 10^{-8}$) in the combined meta-analysis for statistical evidence of association between a novel SNP and the ISI. To assess the effect of removing lower-frequency SNPs in model 3, a sensitivity analysis was performed using the MAC <20 filter on a cohort-wise basis in both the discovery and replication cohorts.

Assessment for Association of Known Insulin Sensitivity Loci With ISI

The associations of published insulin sensitivity loci were tested for association with the ISI in the discovery cohorts. Loci associated with fasting insulin without (12) and with adjustment for BMI (3,12), with fasting insulin using the approach in model 3 (10), and with direct measures of insulin sensitivity were included in these analyses (7). The published results for associations with fasting insulin with or without BMI adjustment (*N* = ~50,000–100,000) (3,12) or exploiting potential BMI-by-gene interaction (model 3; *N* = ~80,000) (10) used the same statistical approach as in the current study but were derived in a sample size approximately three to six times larger than that of the current study discovery cohort (*N* = ~16,000). The sample sizes of the published fasting insulin analyses were much greater because only fasting insulin and BMI phenotypes were required for cohort participation. To analyze the association with fasting insulin and ISI in a comparable sample, we also examined the subset of discovery cohorts that contributed to the current assessment of ISI and prior assessments of fasting insulin: FHS, Sorbs, FUSION, and CHS. In models 2 and 3, only data from FHS, Sorbs, and FUSION were analyzed because participant-level BMI data were not available in CHS. A binomial sign test was used to determine whether the expected direction of the effect for these published loci with ISI occurred more often than by chance.

Conditional Analyses and Assessment of the Association of Top Findings With Direct Measures of Insulin Sensitivity

Findings that reached genome-wide significance were assessed for association with direct measures of insulin sensitivity in the Genetics of Insulin Sensitivity (GENESIS) consortium (7). Direct measures of insulin sensitivity were inverse normal transformed M value in cohorts with euglycemic insulin clamp assessments and inverse normal transformation of the steady state plasma glucose from cohorts with an insulin suppression test. These two traits are highly correlated ($r = -0.85$; $P < 0.001$) (13), and tests of association with the direct measure of insulin sensitivity showed no evidence of heterogeneity (*P* value for heterogeneity = 0.34 for the *BCL2* variant and 0.66 for the *FAM19A2* variant). Therefore, we did not perform separate tests of association in the smaller subsets of data with either the M value or the insulin suppression test phenotype. Statistical models were adjusted for age, sex, and BMI.

The top findings of the ISI analyses were also assessed in a MAGIC association analysis from Manning et al. (10) with fasting insulin using the approach in model 3. These ISI variants were only available in the discovery cohort from Manning et al. (*n* = 38,649 for rs12454712; *n* = 45,290 for rs10506418). We also performed association analyses with fasting insulin and ISI in a subset of the discovery cohort—FHS, Sorbs, and FUSION—to ascertain values in a comparable sample.

Approximate conditional analyses were performed to understand whether known loci contributed to the associations of novel findings with the ISI (14). These analyses were based on the summary-level statistics from the meta-analysis and the estimated LD using individual-level genotype data from the FHS discovery cohort. The software implementation for this approach does not incorporate the interaction term from model 3, and therefore conditional analyses were not performed in model 3.

RESULTS

The demographic characteristics of the participants included in the discovery and replication efforts are presented in Table 1. In total, the discovery, replication, and combined meta-analyses included up to 16,753, 13,354, and 30,107 participants, respectively.

Using a variance component approach implemented in SOLAR software (15), the heritability of the ISI (H2r ± SE) in related FHS participants (n = 2,833) was very similar without or with adjustment for BMI (34.6 ± 6.8%; P = 2.8×10^{-8} and 33.4 ± 6.8%; P = 1.0×10^{-6}, respectively). Within the ULSAM discovery cohort, the Spearman correlation between the ISI and M value from the euglycemic-hyperinsulinemic clamp was 0.71 (Fig. 1), consistent with reports from the literature (9); the Spearman correlation between ISI and fasting insulin was −0.49 (Fig. 1).

When tested in the full discovery cohort, 12 of 13 loci previously associated with fasting insulin in the literature (12) (P = 0.002 for binomial sign test) and 13 of 15 loci previously associated with fasting insulin after adjusting for BMI in the literature (3,12) (P = 0.004 for binomial sign test) showed the expected direction of effect with the ISI in the discovery cohorts (Supplementary Table 2). When these associations were examined in a subset of the current study discovery cohort (Supplementary Table 2), statistical significance was reduced, but effects at each loci remained in the expected direction (10 of 13 loci for ISI vs. fasting insulin without BMI adjustment, P = 0.03 for binomial sign test; 11 of 15 loci for ISI vs. fasting insulin with BMI adjustment, P = 0.04 for binomial sign test). Using a variant in LD with rs1208 (rs7815686; r^2 = 0.67), we also found the expected direction of effect with ISI in the discovery cohorts (n = 16,753) at the *NAT2* locus (model 1; β= −0.029; $P = 9 \times 10^{-3}$) (7).

The QQ plots for models 1, 2, and 3 are shown in Supplementary Figs. 1–3, respectively. Measures of genomic control were consistent with low inflation (model 1 λ_{GC} = 1.015; model 2 λ_{GC} = 1.006; model 3 λ_{GC} = 1.079). While genomic control was used to correct for each individual study, no additional corrections were applied to the meta-analysis results. The separate results of the discovery and replication results for model 1 (adjusting for age and sex), model 2 (adjusting for age, sex, and BMI), and model 3 (adjusting for age, sex, and BMI and analyzing the combined influence of the genotype effect adjusted for BMI and1the interaction effect between the genotype and BMI on ISI) are shown in Supplementary Table 3. Four SNPs selected from the discovery effort reached nominal significance ($P < 0.05$) in the replication analyses: rs13422522 (*NYAP2*) in models 1, 2, and 3; rs12454712 (*BCL2*) in models 2 and 3; rs10506418 (*FAM19A2*) in model 3; and rs6013915 (*PFDN4*) in model 3. Although the association with rs4548846 (*CDH13*) reached nominal significance in the replication effort for model 3, the association was in the opposite direction of effect, as in the discovery analyses; consequently, the association of this variant also had high heterogeneity in the combined meta-analysis.

We compared the β coefficients for the 22 SNPs identified in the discovery effort (rs4548846 [*CDH13*] was excluded given its high heterogeneity) with fasting insulin and ISI in a subset of the discovery cohort. Pearson correlations between the β for fasting insulin and the β for ISI were −0.494 in model 1, −0.797 in model 2, and −0.461 (for SNP effect) and −0482 (for interaction) in model 3.

The results of the combined discovery and replication cohort meta-analyses in each of the three models are shown in Table 2 and Supplementary Table 3. No association reached genome-wide significance in model 1. In model 2, rs13422522 (*NYAP2*; $P = 1.8 \times 10^{-11}$) and rs12454712 (*BCL2*; $P = 1.9 \times 10^{-8}$) achieved genome-wide significance. In model 3, rs13422522 (*NYAP2*; $P = 8.9 \times 10^{-11}$), rs12454712 (*BCL2*; $P = 2.7 \times 10^{-8}$), and rs10506418 (*FAM19A2*; $P = 1.9 \times 10^{-8}$) reached genome-wide significance. In model 3, rs6027072 (*ARHGAP40*; $P = 4.4 \times 10^{-9}$) also reached genome-wide significance but did not achieve nominal significance in the replication cohort, and rs6013915 (*PFND4*) had high heterogeneity in the combined meta-analysis of discovery and replication cohorts (P for heterogeneity = 6.03×10^{-7}); therefore associations with these SNPs were not included as trustworthy findings.

Hence, rs13422522 (*NYAP2*), rs12454712 (*BCL2*), and rs10506418 (*FAM19A2*) were the three SNPs that reached our a priori requirements for claiming statistical evidence. The association at rs13422522 (*NYAP2*) was in LD (r^2 = 0.7) with previously reported results at the known insulin sensitivity signal rs2943641 (*IRS1*) (10), and the association with the ISI in model 2 was greatly reduced by conditioning rs13422522 on rs2943641 in the discovery cohort (β = −0.066 ± 0.01; $P = 4.29 \times 10^{-8}$ to β = −0.025 ± 0.01; P = 0.01). Thus, this SNP was considered a reflection of the known *IRS1* signal and not an independent signal. The associations for rs12454712 (*BCL2*) and rs10506418 (*FAM19A2*) with the ISI were consistent across the discovery and replication cohorts (Supplementary Figs. 4 and 5, respectively). When stratifying by BMI, the effect of the minor allele (A) at rs10506418 (*FAM19A2*) on insulin sensitivity was negative at lower BMI and became positive and stronger with increasing BMI (Fig. 2), and the effect of the major allele (T) at rs12454712 (*BCL2*) on ISI was more negative with increasing BMI (Fig. 3).

The genomic inflation of models 1 and 2 was low and slightly higher in model 3. Because the same individuals were used in each model, inflation in model 3 was unlikely

Table 1—Cohort and participant demographics

Cohort	Participants (*n*)	Female (%)	Age (years)	BMI (kg/m^2)	Fasting glucose (mmol/L)	Fasting insulin (pmol/L)	Stumvoll ISI ([μmol * pmol]/[kg * min * L])
Discovery							
FHS	2,602	54	54.0 ± 9.9	26.8 ± 4.5	5.2 ± 0.5	28.6 ± 9.9	0.111 ± 0.012
Sorbs	802	60	46.3 ± 15.9	26.5 ± 6.4	5.5 ± 1.2	40.7 ± 26.5	0.105 ± 0.023
FUSION	462	63	66.5 ± 6.7	27.6 ± 4.2	5.1 ± 0.5	68.5 ± 36.0	0.087 ± 0.023
CHS	2,761	62	72.3 ± 5.3	26.0 ± 4.3	5.5 ± 0.5	93.3 ± 47.8	0.059 ± 0.038
LURIC	962	24	61.9 ± 27.1	27.1 ± 3.8	5.5 ± 0.6	61.6 ± 49.9	0.065 ± 0.038
ULSAM	962	0	71.0 ± 0.6	26.0 ± 3.2	5.4 ± 0.6	73.0 ± 40.1	0.074 ± 0.031
METSIM	7,388	0	57.0 ± 6.96	26.8 ± 3.8	5.7 ± 0.5	49.8 ± 35.3	0.093 ± 0.030
Replication							
EUGENE2	885	56	39.4 ± 9.2	26.5 ± 4.8	5.1 ± 0.5	49.0 ± 34.9	0.091 ± 0.028
Amish Studies	334	61	45 ± 12.7	27.4 ± 4.7	4.9 ± 0.5	63.4 ± 26.0	0.09 ± 0.02
RISC	921	56	44 ± 8.37	25.5 ± 4.0	5.1 ± 0.6	34.4 ± 18.7	0.106 ± 0.018
Tübingen	2,470	65	40.2 ± 13.2	30.9 ± 9.6	5.2 ± 0.6	83.4 ± 72.2	0.070 ± 0.049
Inter99	5,318	51	45.9 ± 7.9	26.1 ± 4.4	5.5 ± 0.5	41.1 ± 26.3	0.101 ± 0.021
Segovia	420	53	52.1 ± 11.4	26.7 ± 3.8	4.5 ± 0.6	71.2 ± 39.7	0.087 ± 0.025
Pizarra	640	66	43.6 ± 13.0	27.8 ± 4.9	5.4 ± 0.7	46.6 ± 34.2	0.101 ± 0.023
Botnia Study	1,235	52	58.3 ± 10.2	27.1 ± 3.9	5.4 ± 0.5	44.7 ± 28.7	0.099 ± 0.020
1936 Birth Cohort	576	54	60.5 ± 0.5	26.5 ± 4.0	5.2 ± 0.5	42.5 ± 23.7	0.098 ± 0.022
Ely Study	1,442	54	61.1 ± 9.2	27.3 ± 4.8	5.0 ± 0.6	57.1 ± 35.7	0.088 ± 0.031

Continuous results are shown as mean ± SD. Additional information for each cohort can be found in Supplementary Table 1.

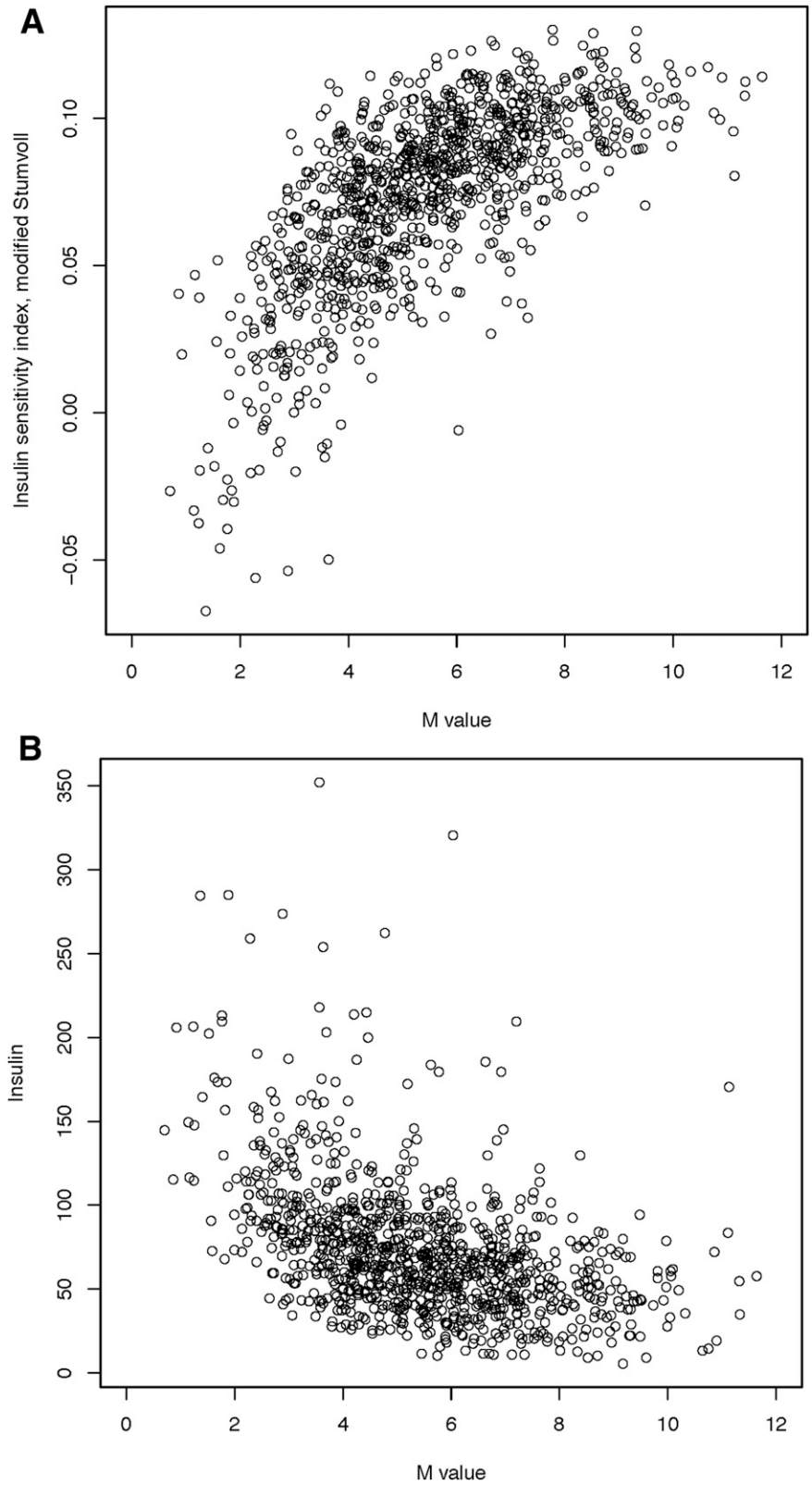

Figure 1—Correlation of ISI with the M value from the insulin clamp (*A*) and fasting insulin (*B*) in ULSAM. Insulin sensitivity was measured within the ULSAM discovery cohort (n = 1,025) using a hyperinsulinemic-euglycemic clamp (M value), the modified Stumvoll ISI, and fasting insulin. The ULSAM cohort contains only men, and individuals with known diabetes were excluded from these analyses. For the comparison of the M value with ISI, the Pearson correlation was 0.69 and the Spearman correlation was 0.71, which are consistent with prior published reports. For the comparison of the ISI with fasting insulin, the Pearson correlation was −0.45 and the Spearman correlation was −0.49.

Table 2—Meta-analysis results for variant association with the ISI

SNP	Chromosome	Locus	Allele (effect/ other)	Frequency of the effect allele	Model 1, β ± SE (*P* value)	Model 2, β ± SE (*P* value)	Model 3, β ± SE (main/interaction) (joint *P* value)	*N* (minimum/ maximum)
rs13422522	2	*NYAP2*	C/G	0.77	−0.04 ± 0.01 (1.6×10^{-5})	−0.06 ± 0.01 (1.2×10^{-11})	0.10 ± 0.06/−0.01 ± 0.002 (8.9×10^{-11})	30,057/30,078.3
rs4078023	16	*GP2*	T/G	0.98	−0.028 ± 0.04 (0.49)	−0.05 ± 0.04 (0.20)	0.80 ± 0.17/−0.03 ± 0.01 (3.2×10^{-7})	24,727/24,742
rs12372926	15	*ARRDC4*	T/C	0.41	−0.03 ± 0.01 (0.001)	−0.03 ± 0.01 (1.6×10^{-5})	0.11 ± 0.05/−0.005 ± 0.002 (4.2×10^{-4})	30,073/30,095
rs16924527	8	*TOX*	A/C	0.02	0.12 ± 0.04 (0.001‡)	0.07 ± 0.03 (0.02)	−0.08 ± 0.14/0.01 ± 0.01 (3.7×10^{-6}‡)	24,994/25,005
rs2828537	21	*MRPL39*	A/T	0.97	−0.04 ± 0.03 (0.12)	−0.03 ± 0.02 (0.16)	0.42 ± 0.10/−0.02 ± 0.004 (2.6×10^{-5})	29,733/29,753.9
rs3900087	4	*ADAMTS3*	T/C	0.98	−0.04 ± 0.05 (0.33)	−0.04 ± 0.04 (0.31)	0.74 ± 0.21/−0.03 ± 0.01 (4.7×10^{-4})	22,350/22,351
rs6027072	20	*ARHGAP40*	A/G	0.03	0.10 ± 0.02 (0.0001)	0.08 ± 0.02 (4.1×10^{-4})	−0.39 ± 0.12/0.02 ± 0.005 (4.4×10^{-9})	28,877/28,896
rs12454712	18	*BCL2*	T/C	0.58	−0.04 ± 0.01 (0.0003)	−0.05 ± 0.01 (1.9×10^{-8})	0.04 ± 0.05/−0.003 ± 0.002 (2.7×10^{-8})	25,973/26,761
rs10506418	12	*FAM19A2*	A/G	0.03	0.06 ± 0.03 (0.05)	0.06 ± 0.03 (0.01)	−0.62 ± 0.13/0.03 ± 0.005 (1.9×10^{-8})	26,011/26,024
rs1857095	1	*ELTD1*	T/C	0.98	−0.01 ± 0.03 (0.84)	−0.02 ± 0.03 (0.37)	0.08 ± 0.12/−0.0003 ± 0.005 (7.9×10^{-9}‡)	26,596/26,608.9
rs11594101	10	*NRG3*	A/G	0.98	0.02 ± 0.04 (0.57)	−0.002 ± 0.03 (0.94)	0.62 ± 0.14/−0.02 ± 0.005 (9.5×10^{-5})	27,885/27,904
rs12583553	13	*FGF9*	A/T	0.97	−0.04 ± 0.03 (0.19)	−0.05 ± 0.03 (0.04)	0.55 ± 0.12/−0.02 ± 0.005 (3.6×10^{-9}‡)	29,195/29,215
rs4548846	16	*CDH13*	T/C	0.02	0.02 ± 0.04 (0.72)	−0.002 ± 0.04 (0.96)	0.59 ± 0.18/−0.03 ± 0.01 (1.1×10^{-5}‡)	18,401/18,405.99
rs12522198	5	*FAM134B*	A/G	0.02	−0.03 ± 0.04 (0.48)	0.01 ± 0.04 (0.84)	0.79 ± 0.19/−0.03 ± 0.01 (1.6×10^{-4}‡)	19,798/20,589
rs10483182	22	*ISX*	A/G	0.01	0.06 ± 0.04 (0.17)	0.03 ± 0.04 (0.39)	−1.16 ± 0.18/0.05 ± 0.01 (7.8×10^{-12}‡)	20,399/20,409
rs10520638	15	*AGBL1*	T/C	0.01	0.004 ± 0.05 (0.93)	−0.01 ± 0.04 (0.77)	0.89 ± 0.19/−0.04 ± 0.01 (1.2×10^{-7}‡)	12,369/12,383
rs6013915	20	*PFDN4*	A/G	0.03	0.05 ± 0.03 (0.14)	0.06 ± 0.03 (0.05)	−0.84 ± 0.19/0.04 ± 0.01 (1.5×10^{-9}‡)	23,111/23,121.9
rs9658121	6	*PPARD*	A/G	0.02	−0.01 ± 0.04 (0.80)	0.02 ± 0.04 (0.63)	−0.40 ± 0.15/0.02 ± 0.01 (7.3×10^{-4}‡)	16,973/16,985
rs10508754	10	*KIAA1462*	A/G	0.08	−0.03 ± 0.02 (0.08)	−0.05 ± 0.02 (0.01)	0.14 ± 0.09/−0.01 ± 0.004 (0.07)	25,146/25,150
rs11627967	14	*NPAS3*	T/G	0.016	−0.02 ± 0.05 (0.69)	−0.03 ± 0.04 (0.44)	−0.94 ± 0.21/0.04 ± 0.01 (1.6×10^{-7}‡)	17,593/17,595.98
rs10495667	2	*VSNL1*	A/G	0.04	0.02 ± 0.02 (0.32)	0.01 ± 0.02 (0.69)	−0.51 ± 0.12/0.02 ± 0.005 (3.8×10^{-5})	27,332/27,345.9
rs13059110	3	*TXNDC6*	T/G	0.13	−0.05 ± 0.02 (0.0001)	−0.04 ± 0.01 (2.3×10^{-4})	0.03 ± 0.07/−0.002 ± 0.003 (0.01)	26,420/26,425
rs11790816	9	*SH3GL2*	T/C	0.02	0.01 ± 0.03 (0.63)	0.02 ± 0.03 (0.45)	−0.37 ± 0.14/0.02 ± 0.01 (0.001‡)	21,814/21,833.92

Model 1 is adjusted for age and sex; model 2 is adjusted for age, sex, and BMI; model 3 assesses the combined influence of the SNP effect adjusted for BMI and the interaction effect between the genotype and BMI on ISI. For models 1 and 2, the effect (β), SE, and *P* values for the SNP are shown. For model 3, the β and SE are provided for the SNP and the interaction; a *P* value is provided for the joint influence of the SNP and interaction effect. *N* = sample size for the combined analysis of discovery and replication cohorts. Effect sizes are presented as the SD per effect allele. ‡*P* value for heterogeneity in the combined analysis of discovery and replication cohorts ($P \leq 10^{-6}$).

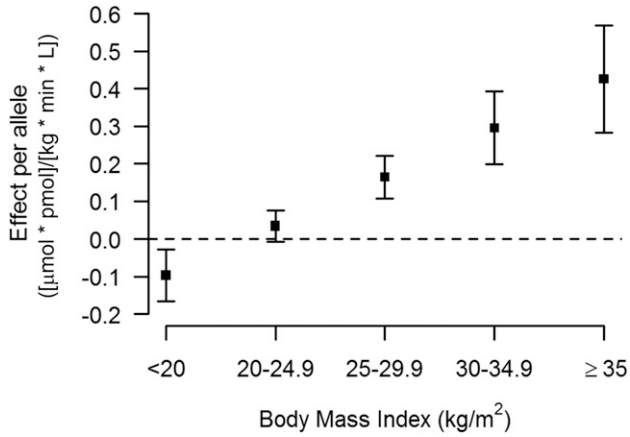

Figure 2—The effect of rs10506418 (*FAM19A2*) on insulin sensitivity by BMI category. The effect of the minor allele (A) at rs10506418 (*FAM19A2*) on the ISI is shown by BMI category. At a low BMI (<20 kg/m^2), the effect is negative. At each category of increasing BMI above 20 kg/m^2, the effect is positive and stronger.

to arise from population stratification. We performed an additional sensitivity analysis that applied the MAC < 20 filter to both the discovery and replication cohorts (Supplementary Table 4), which tended to reduce the statistical significance of associations with high heterogeneity and slightly reduced the statistical significance of the association at the *FAM19A2* locus in model 3 without markedly reducing the magnitude of effect or affecting heterogeneity ($\beta = -0.62 \pm 0.13$; $P = 1.9 \times 10^{-8}$; P for heterogeneity = 0.11 to $\beta = -0.58 \pm 0.13$; $P = 8.0 \times 10^{-7}$; P for heterogeneity = 0.07). The sample size for the *FAM19A2* locus association in model 3 was 462 individuals fewer when the MAC filter was applied in the discovery cohorts versus when the minor allele frequency filter was applied, and the resulting loss in power was likely responsible for the slight reduction in statistical significance.

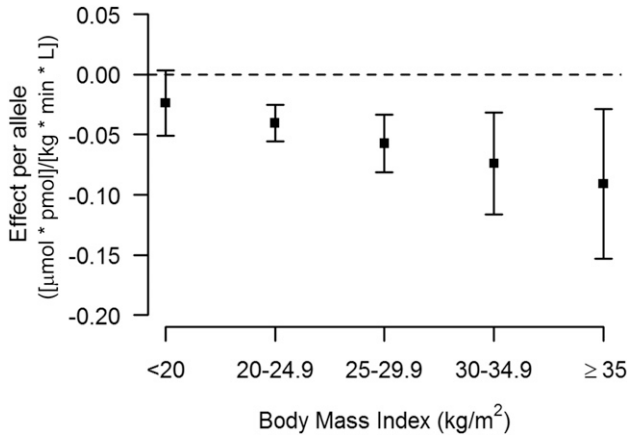

Figure 3—The effect of rs10506418 (*BCL2*) on insulin sensitivity by BMI category. The effect of the major allele (T) at rs10506418 (*BCL2*) on the ISI is shown by BMI category. At each category of increasing BMI, the effect is negative and stronger.

Conditioning the results at either variant with known signals at least 1 Mb away did not attenuate the association with the ISI in the discovery cohorts of model 2 (a full description is provided in Supplementary Table 5). The rs10506418 (*FAM19A2*) variant was not associated with fasting insulin using model 3 in a separate GWAS result (10) or with direct measures of insulin sensitivity in GENESIS. The major allele (T) of rs12454712 (*BCL2*), which was associated with lower insulin sensitivity in this study, was also associated with a trend toward higher fasting insulin in a separate GWAS result using model 3 (SNP effect -0.006 ± 0.003; interaction effect 0.001 ± 0.001; $P = 5.9 \times 10^{-5}$; N = 38,649) (10). Similar trends were observed when the variant was tested for association with ISI and fasting insulin in the same discovery cohort subset (Supplementary Table 5).

DISCUSSION

In a study of over 30,000 participants, we found novel, independent, genome-wide significant associations for the ISI at rs12454712 (*BCL2*) and rs10506418 (*FAM19A2*). Strengths of this study's design include a large sample size, individuals with glycemic and metabolic phenotyping, high-quality genomic data, and use of traditional and contemporary statistical models to account for the influence of BMI on insulin sensitivity. In addition, our approach targeted a phenotype not previously examined in GWAS: the modified Stumvoll ISI. By incorporating glucose and insulin measures before and after a glucose load, this phenotype captures information that fasting assessments such as HOMA-IR or insulin alone would not. Indeed, the correlation between ISI and the M value is higher than that between the M value and fasting insulin (16), which has been used in prior genetic studies of insulin sensitivity (10,12). At the same time, the use of measures obtained at only two time points (fasting and 120 min into an oral glucose tolerance test) permitted the assembly of the large sample size required to achieve adequate statistical power.

Several findings serve as positive controls for our results and demonstrate that the ISI is a robust measure of fasting and whole-body insulin sensitivity. First, we observe a strong correlation of ISI with direct measures of insulin sensitivity. Second, we show that the ISI can detect genetic influences on measures of fasting insulin sensitivity (3,10,12), generally ascribed to hepatic physiology, as well as on measures of whole-body insulin sensitivity, which also incorporates contributions from muscle and adipose tissue. Integrated measures of insulin sensitivity may have clinical relevance, since a reduction in peripheral insulin sensitivity may be an early contributor to the development of type 2 diabetes (17–19).

Consistent with prior genetic explorations of insulin sensitivity (10), the association of variants at the *BCL2* and *FAM19A2* loci became stronger and genome-wide significant after accounting for the effect of BMI on ISI. Notably, the ISI can be calculated with or without BMI in the formula, and the correlation of the ISI with M/I is

greater when BMI is included (r = 0.69 vs. 0.79) (8,9). We note that the effect of these loci on insulin sensitivity is modest, consistent with published findings on other common genetic variants for glycemic traits, such as glucose (12) and fasting insulin (3,10,12). Yet our findings are meaningful because they provide a more complete understanding of the contribution of common genetic variations to insulin sensitivity.

The existing literature bolsters our finding of *BCL2* as a novel candidate insulin sensitivity locus. The major allele (T) at rs12454712, which was associated with lower insulin sensitivity in our analysis, has been previously associated with type 2 diabetes in a multiethnic GWAS (odds ratio 1.09 [95% CI 1.05–1.11]; $P = 2.1 \times 10^{-8}$) (20) in analyses adjusted for BMI. Further, this same variant was recently associated with higher BMI-adjusted waist-to-hip ratio in women ($\beta = 0.035$; $P = 1.1 \times 10^{-9}$; N = 96,182) but not in men ($\beta = 0.007$; $P = 0.25$; N = 73,576) (21). All these findings suggest that the metabolically deleterious effects of the *BCL2* locus become more evident after adjusting for BMI. Last, we find that the statistical association of rs12454712 (*BCL2*) is stronger with the ISI than with fasting insulin (10). Notably, the published fasting insulin results were from a study much larger than ours. The ability of the ISI to detect a genome-wide significant finding in a smaller sample suggests that the *BCL2* locus may have a greater influence on insulin sensitivity when fasting and postprandial phenotypes are assessed together.

The mechanism by which *BCL2* influences insulin sensitivity remains unclear. The *BCL2* family of proteins regulate apoptosis through control of mitochondrial permeability (22). Mouse models suggest that inhibiting *Bcl2* improves glucose tolerance through effects on pancreatic β-cells (23). Conversely, pharmacological inhibition of the BCL2 protein causes hyperglycemia among a subset of patients with chronic lymphocytic leukemia (24), but the mechanism of this observation is unknown. By contrast, there is little direct published literature to support the role of FAM19A2 in insulin sensitivity. We found that the association of the minor allele (A) at the FAM19A2 locus with reduced insulin sensitivity was detected at BMI <30 kg/m^2. This may suggest the variant is more deleterious among individuals with lower levels of adiposity. While *BCL2* and *FAM19A2* are the closest genes to rs12454712 and rs10506418, respectively, we have not excluded other genes in the region (Supplementary Figs. 6 and 7). Additional in silico findings at the *BCL2* and *FAM19A2* variants are provided in Supplementary Table 5.

We recognize limitations to our study. First, analyses were performed exclusively in white individuals of European ancestry. Exploring these loci in other racial and ethnic groups is necessary. Second, we used an estimate of whole-body insulin sensitivity derived from measures of glucose and insulin after a glucose load, rather than direct measures of insulin sensitivity. The wide availability of the ISI provided increased statistical power of the association analyses relative to that of other indices that are better correlated with euglycemic measures of insulin sensitivity, such as the Matsuda Index (25). Assessment of our novel findings in the GENESIS consortium suggests that the ISI may be capturing different information on insulin sensitivity than that provided by the insulin clamp or the insulin suppression test, or that the power in the GENESIS analyses was limited to detect this association. Third, conditional analyses could not be performed in model 3, which would have been the best method of assessing the dependence of the signals at *BCL2* and *FAM19A2*. However, the LD for each variant with other known glucose and insulin loci in the region was low, and the nominally significant associations of the *BCL2* and *FAM19A2* variants with ISI were stable after conditioning in model 2, suggesting that analyses in model 3 would have probably confirmed secondary loci. Fourth, given our desire for the early dissemination of these results, no experimental attempts at determining the causal gene and mechanisms of action in our novel candidate insulin sensitivity loci were performed.

In conclusion, we identified two novel candidate insulin sensitivity loci through a GWAS of the modified Stumvoll ISI. Our results demonstrate that the ISI is a robust measure of fasting and whole-body measures of insulin sensitivity and suggest that genetic variation in the *FAM19A2* and *BCL2* loci influence insulin sensitivity. While further functional work is needed to clarify the causal genes and mechanisms of action of these loci, our work and the published literature provide support for genes in these loci having an effect on human glycemic metabolism.

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