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New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk

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Abstract

Circulating glucose levels are tightly regulated. To identify novel glycaemic loci, we performed meta-analyses of 21 genome-wide associations studies informative for fasting glucose (FG), fasting insulin (FI) and indices of β -cell function (HOMA-B) and insulin resistance (HOMA-IR) in up to 46,186 non-diabetic participants. Follow-up of 25 loci in up to 76,558 additional subjects identified 16 loci associated with FG/HOMA-B and two associated with FI/HOMA-IR. These include nine new FG loci (in or near *ADCY5*, *MADD*, *ADRA2A*, *CRY2*, *FADS1*, *GLIS3*, *SLC2A2*, *PROX1* and *FAM148B*) and one influencing FI/HOMA-IR (near *IGF1*). We also demonstrated association of *ADCY5*, *PROX1*, *GCK*, *GCKR* and *DGKB/TMEM195* with type 2 diabetes (T2D). Within these loci, likely biological candidate genes influence signal transduction, cell proliferation, development, glucose-sensing and circadian regulation. Our results demonstrate that genetic studies of glycaemic traits can identify T2D risk loci, as well as loci that elevate FG modestly, but do not cause overt diabetes.

Impaired β -cell function and insulin resistance are key determinants of type 2 diabetes (T2D). Hyperglycemia in the fasting state is one of the criteria that define the disease¹, can predict hard clinical endpoints in non-diabetic individuals^{2,3} and, when corrected in patients with T2D, may help prevent microvascular^{4,5} and long-term macrovascular^{6,7} complications. To date, there are nearly 20 published loci reproducibly associated with T2D⁸, with most of them also associated with decreased insulin secretion⁹ due to defective β -cell function or mass. Association studies for diabetes-related quantitative traits in non-diabetic participants have also identified loci influencing fasting glucose (FG) levels, whose effects appear to be mediated by impairment of the glucose-sensing machinery in β -cells^{10–17}.

We recently formed the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) to conduct large-scale meta-analyses of genome-wide data for continuous

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diabetes-related traits in non-diabetic participants¹⁵. We aimed to identify additional loci that influence glycemic traits in persons free of diabetes, and investigate their impact on related metabolic phenotypes. We were also interested in understanding variation in the physiological range and evaluating the extent to which the same variants influence pathological FG variation and T2D risk. The initial MAGIC collaboration identified the FG/T2D-associated locus *MTNR1B*¹⁵, which was also reported by others^{16,17}; this finding demonstrated that studies of continuous glycemic phenotypes in non-diabetic individuals can complement the genetic analyses of diabetes as a dichotomous trait, and improve our understanding of the mechanisms involved in β -cell function and glucose homeostasis. Here, we extend our previous approach by performing meta-analyses of ~2.5M directly genotyped or imputed autosomal SNPs from 21 genome-wide association studies (GWAS). These 21 cohorts include up to 46,186 non-diabetic participants of European descent informative for FG, and 20 GWAS including up to 38,238 non-diabetic individuals informative for fasting insulin (FI), as well as the surrogate estimates of β -cell function (HOMA-B) and insulin resistance (HOMA-IR) derived from fasting variables by homeostasis model assessment¹⁸. Follow-up of 25 lead SNPs in up to 76,558 additional individuals of European ancestry identified nine novel genome-wide significant associations (empirically determined as $P < 5 \times 10^{-8}$)¹⁹ with FG and one with FI/HOMA-IR. Five of these novel loci also demonstrated genome-wide significant evidence for association between the glucose-raising allele and T2D risk in up to 40,655 cases and 87,022 non-diabetic controls.

The wealth of novel FG and HOMA-B loci contrast with the sole FI/HOMA-IR novel finding and suggests a different genetic architecture for β -cell function and insulin resistance. Furthermore, our data support the hypothesis that not all loci that influence glycemia within the physiological range are also associated with pathological levels of glucose and T2D risk.

RESULTS

Genome wide association meta-analysis of glycemic traits (Stage 1)

We conducted a two-stage association study in individuals of European descent (Online Methods, Supplementary Figure 1, Supplementary Tables 1a and b). Because we sought to identify variants that influence FG in the normal population, hyperglycemia in the diabetic range exerts deleterious effects on β -cell function^{20,21}, and treatment can confound glucose and insulin measurements, we excluded individuals with known diabetes, on anti-diabetic treatment, or with FG ≥ 7 mmol/L. We combined data from 21 Stage 1 discovery GWAS for FG (N=46,186) and 20 GWAS for FI (N=38,238), HOMA-B (N=36,466) and HOMA-IR (N=37,037), and analyzed associations for ~2.5M autosomal SNPs directly genotyped and imputed^{22,23} from HapMap CEU sample data assuming an additive genetic effect for each of the four traits.

Inverse variance weighted meta-analyses revealed 12 independent loci associated with FG and/or HOMA-B at genome-wide significance levels (Table 1, Supplementary Table 2, Supplementary Figure 2a–b). These included five novel associations for loci in or near *ADCY5*, *MADD*, *ADRA2A*, *CRY2* and *FADS1* (Table 1, Figure 1a–j); four previously reported FG-associated loci in or near *GCK*, *GCKR*, *G6PC2*, and *MTNR1B*; the recently

reported *DGKB/TMEM19524*; and two loci in T2D susceptibility genes *TCF7L2* (rs4506565, $r^2=0.92$ with the previously reported SNP rs7903146) and *SLC30A8* (rs11558471, $r^2=0.96$ with the previously reported SNP rs13266634). Seven additional loci had reproducible evidence for association with FG and/or HOMA-B across studies at the arbitrary summary threshold of $P < 2 \times 10^{-5}$ chosen to prioritize SNPs for follow-up (Table 1, Supplementary Table 2). After excluding SNPs within the four previously genome-wide significant FG loci *GCK*, *GCKR*, *G6PC2* and *MTNR1B*, we still observed an excess of small *P*-values compared to a distribution expected under the null hypothesis (Figure 2a–b), suggesting that some of these additional loci are likely to represent novel FG and/or HOMA-B loci that merit additional investigation.

Stage 1 analyses of FI and HOMA-IR revealed no loci that reached genome-wide significance, but there were six loci with consistent evidence for association across study samples at $P < 2 \times 10^{-5}$ (Table 1, Supplementary Table 2, Supplementary Figure 2c–d). Comparison of the observed *P*-values with the distribution expected under the null hypothesis demonstrated an excess of small *P*-values which warrant further investigation (Figure 2c–d).

Replication studies (Stage 2) and global (Stage 1 + Stage 2) meta-analysis for 25 loci

We carried forward to Stage 2 all independent loci with association with any of the four traits at $P < 2 \times 10^{-5}$, except for SNPs in the known T2D genes *TCF7L2* and *SLC30A8* for which no further validation was sought (Table 1, Supplementary Table 2). We also included the nominally associated top SNP from a strong biological candidate (*IRSI*, $P=10^{-4}$ for HOMA-IR) and a locus with *P* values that approached genome-wide significance in several Stage 1 discovery cohorts (*PLXDC2/NEBL*), even though their overall Stage 1 *P*-values were $> 2 \times 10^{-5}$ (Table 1, Supplementary Table 2). In total, 25 loci were chosen for replication.

We directly genotyped 25 variants in 26 additional Stage 2 studies with up to 63,850 non-diabetic participants of European ancestry for FG, and 25 studies and up to 52,892 participants for FI, HOMA-IR and HOMA-B (Supplementary Table 1b, Online Methods). We also obtained *in silico* replication data for 12,708 additional individuals from seven studies for FG (9,372 participants and five studies for FI, HOMA-IR and HOMA-B), for a total of up to 76,558 individuals for FG and 62,264 for FI, HOMA-IR and HOMA-B in Stage 2 association analyses.

Our combined Stage 1 and 2 meta-analysis, including a total of up to 122,743 participants for FG (98,372 for FI, HOMA-IR and HOMA-B) established genome-wide significant associations for nine novel loci for FG and/or HOMA-B (*ADCY5*, *MADD*, *CRY2*, *ADRA2A*, *FADS1*, *PROX1*, *SLC2A2*, *GLIS3*, *FAM148B*) and one for FI and HOMA-IR (*IGF1*) (Table 1 and Figure 1a–j). We hereby replicate the recently reported loci *DGKB/TMEM195* (FG)24 and *GCKR* (FG, FI and HOMA-IR)11,12,25 at levels that exceed genome-wide significance. Loci that had previously achieved genome-wide significant associations with FG (*G6PC2*, *MTNR1B* and *GCK*) were also confirmed (Table 1).

We further conducted a global meta-analysis of cohort results adjusted for body mass index (BMI), to test whether these diabetes-related quantitative trait associations may be mediated by associations with adiposity. The adjustment for BMI did not materially affect the strength of the associations with any of the traits (data not shown).

Effect size estimates for genome-wide significant loci

We restricted our effect size estimates (Table 2, Supplementary Table 2) to the Stage 2 replication samples (up to $N=76,558$) to avoid inflation introduced by the discovery cohorts (“winner’s curse”²⁶). The previously identified loci *G6PC2*, *MTNR1B* and *GCK* showed the largest effects on FG (0.075, 0.067 and 0.062 mmol/L per allele, respectively), with the remaining loci showing smaller effects (0.008 to 0.030 mmol/L per allele, Table 2). The proportion of variance in FG explained by the 14 FG loci with replication data (i.e. all FG loci except for *TCF7L2* and *SLC30A8*) ranged from 3.2–4.4% in six replication studies providing this information. Because results from our largest unselected community-based cohort (Framingham) were on the lower bound of these estimates (3.2%), we felt reassured that the winner’s curse is not a major concern in this instance, and selected it to estimate the proportion of heritability explained and the genotype score. With a heritability estimate of 30.4% in Framingham, these 14 loci explain a substantial proportion (~10%) of the inherited variation in FG. If these same loci harbor additional independent variants (e.g. those due to low frequency alleles not captured by this analysis) that also influence FG²⁷, this estimate of the heritability attributable to these loci is likely to be conservative.

We estimated the combined impact of the 16 loci associated with FG (the 14 loci included in the genotype score plus *TCF7L2* and *SLC30A8*) in some of the largest cohorts (Framingham, NFBC 1966 and ARIC) by constructing a genotype score equal to the sum of the expected number of risk alleles at each SNP weighted by their effect sizes (see Online Methods). FG levels were higher in individuals with higher genotype scores (Figure 3), with mean differences of ~0.4 mmol/L (5.93 vs 5.51 mmol/L in NFBC 1966; 5.36 vs 5.03 mmol/L in Framingham; 5.70 vs 5.29 mmol/L in ARIC) comparing individuals with a score of 23 or higher (5.6% of the sample) to those with a genotype score of 12 or lower (2.9% of the sample). The 0.4 mmol/L (7.2 mg/dl) difference between the two tails of the distribution of risk score in the population (top 5.6% vs bottom 2.9%) is of clinical relevance, as it represents a shift of approximately 25 centile points in the distribution of FG. Prospective evidence has shown that a difference of this magnitude in FG is associated with a relative risk of 1.54–1.73 for future T2D, accounting for other risk factors²⁸. The impact of individual SNPs on FG in the combined discovery and replication samples is shown in Supplementary Figure 3a–p.

We also analyzed data from 1,602 white European children aged 5.9–17.2 from two studies. Though directionally consistent with observations in adults, some effect size estimates were of smaller magnitude (data not shown). As in adults, the largest effect sizes were observed for risk alleles in *GCK* ($\beta=0.085$; $P=1.2\times 10^{-5}$; $N=1,602$), *G6PC2* ($\beta=0.062$; $P=1.9\times 10^{-4}$; $N=1,582$) and *MTNR1B* ($\beta=0.033$; $P=0.058$; $N=1,309$).

Impact of reproducibly associated loci on additional glycemic traits

We sought to investigate all 17 loci associated with FG/HOMA-B or FI/HOMA-IR at genome-wide significance for their effects on other continuous glycemic traits. While most of the 16 loci associated with FG are also strongly associated with HOMA-B (Tables 1 and 2), the associations between FG loci and FI were at best weak; *GCKR* is the only locus reaching genome-wide significant associations for both FG/HOMA-B and FI/HOMA-IR, with the glucose-raising C allele being associated with increased FI (global $P=3.6\times 10^{-20}$) and HOMA-IR (global $P=3.0\times 10^{-24}$). These patterns are consistent with the gross trait correlations obtained in Framingham for FG and HOMA-B ($r=-0.43$) and for FG and FI ($r=0.25$).

Impairment of glucose homeostasis may be characterized by elevated FG or FI, elevated 2-hour glucose or 2-hour insulin post-oral glucose tolerance test (OGTT), or elevated glycated hemoglobin (HbA_{1c}). We tested associations of each of the 17 loci in a subset of MAGIC cohorts with GWAS data informative for these traits. Since HbA_{1c} is a measure of average glycemia over the preceding 2–3 months, we hypothesized that if an association with additional traits was present it should be directionally consistent. The three loci with the largest effect sizes on FG (*G6PC2*, *MTNR1B* and *GCK*) all showed genome-wide significant and directionally consistent associations with HbA_{1c}, with *DGKB/TMEM195*, *ADCY5*, *SLC2A2*, *PROX1*, *SLC30A8* and *TCF7L2* showing nominal ($P<0.05$) evidence of directionally consistent association (Table 2). The FG-raising alleles at *TCF7L2*, *SLC30A8*, *GCK* and *ADCY5* were associated ($P<0.0002$) with increased 2-hour glucose (Table 2); a parallel MAGIC project reports the genome-wide significant association with 2-hour glucose of another *ADCY5* SNP in strong linkage disequilibrium (LD) with our lead SNP ($r^2=0.82$)²⁹. Consistent with previous reports that the FG-raising allele is associated with greater insulin release during OGTT^{11,12,30}, the FG-raising allele in *GCKR* was associated with lower 2-hour glucose.

Testing of these loci for association with T2D as a dichotomous trait in up to 40,655 cases and 87,022 non-diabetic controls demonstrated that the FG-raising alleles at seven loci (*ADCY5*, *PROX1*, *GCK*, *GCKR* and *DGKB/TMEM195* and the known T2D genes *TCF7L2* and *SLC30A8*), are robustly associated ($P<5\times 10^{-8}$) with increased risk of T2D (Table 2); the association of a highly correlated SNP in *ADCY5* with T2D in partially overlapping samples is reported by our companion manuscript²⁹. We found less significant T2D associations ($P<5\times 10^{-3}$) for variants in or near *CRY2*, *FADS1*, *GLIS3* and *FAM148B* (Table 2). These data clearly show that loci with very similar FG effect sizes may have very different T2D risk effects (see for example *ADCY5* and *MADD* in Table 2).

Given that several alleles associated with higher FG levels were also associated with increased T2D risk and that the T2D genes *TCF7L2* and *SLC30A8* showed association with FG, we systematically investigated association of all established T2D loci with the same four fasting diabetes-related quantitative traits. We found directionally consistent nominal associations ($P<0.05$) of T2D risk alleles with higher FG for 11 of 18 established T2D loci, including *MTNR1B* (Supplementary Table 3). These data demonstrate that a large T2D effect size does not always translate to an equivalently large FG effect in non-diabetic

persons, as clearly highlighted when contrasting the remarkably small effects of *TCF7L2* on FG compared to *MTNR1B* (Table 2).

Impact of reproducibly associated loci on additional metabolic traits

Next, we used available GWAS results for additional metabolic phenotypes (BMI from GIANT31, blood pressure from Global BPGen32, and lipids from ENGAGE33), to assess the impact of the newly discovered glycemic loci on these traits. None of the novel loci had significant ($P < 0.01$) associations with BMI or blood pressure (Table 3). Interestingly, the *FADS1* glucose-raising allele was associated with increased total cholesterol ($P = 2.5 \times 10^{-6}$), low-density lipoprotein cholesterol ($P = 8.5 \times 10^{-6}$) and high-density lipoprotein cholesterol ($P = 2.9 \times 10^{-5}$), but lower triglyceride levels ($P = 1.9 \times 10^{-6}$) (Table 3); a consistent association of this locus with lipid levels has been previously reported³⁴. The FG-associated variant in *MADD* was not associated with lipid levels and is not in LD ($r^2 < 0.1$) with a previously reported high-density lipoprotein cholesterol SNP (rs7395662)³³, suggesting two independent signals within the same locus, one affecting lipid levels and the other FG levels (Table 3).

Potential functional roles of novel associated loci

We investigated the likely functional role of genes mapping closest to the lead SNPs using several sources of data, including human disease databases, evidence from animal models and bioinformatic analyses (see Box, Online Methods and Supplementary Table 4). The newly discovered and established glycemic loci represent various biological functions: signal transduction (*DGKB/TMEM195*, *ADCY5*, *FADS1*, *ADRA2A*, *SLC2A2*, *GCK*, *GCKR*, *G6PC2*, *IGF1*); cell proliferation and development (*GLIS3*, *MADD*, *PROX1*); glucose transport and sensing (*SLC2A2*, *GCK*, *GCKR*, *G6PC2*); and circadian rhythm regulation (*MTNR1B*, *CRY2*). All of these pathways represent further avenues for physiological characterization and possible therapeutic intervention. However, we note that other genes could be causal (Box and Supplementary Table 4) and further experimental evidence will be needed to link unequivocally specific genes with phenotypes.

Expression analyses

We measured expression of the genes mapping closest to our lead SNPs (in *DGKB/TMEM195*, *ADCY5*, *MADD*, its neighboring gene *SLC39A13* [a member of a family of zinc transporters mapping ~45 kb from the *MADD* lead SNP], *ADRA2A*, *FADS1*, *CRY2*, *SLC2A2*, *GLIS3*, *PROX1* and *FAM148B*) in human pancreas and other metabolically relevant tissues (Supplementary Figure 4a). While there was evidence of expression in human islets for nearly all genes tested (with the sole exception of *TMEM195*), we found that *DGKB* and *MADD* were most strongly expressed in brain, *SLC2A2*, *FADS1*, *TMEM195* and *PROX1* in liver and *ADCY5* in heart, while *SLC39A13*, *ADRA2A* and *CRY2* were broadly expressed. Strikingly, *FAM148B* was highly expressed in the whole pancreas with lower levels in isolated islets, suggesting that it is also present in exocrine cells. A duplicate experiment in a different laboratory obtained similar results (Supplementary Figure 4b). We further examined expression of these transcripts in flow-sorted human β -cells from two separate individuals and documented β -cell expression for all but *TMEM195*, with *SLC39A13*, *CRY2*,

GLIS3 and *PROX1* being particularly highly expressed in these cells (Supplementary Figure 4c). Expression levels in metabolically relevant tissues for *DGKB* (β cells) and *TMEM195* (liver) provide equally credible evidence for their respective candidacies as the causal gene at these loci. Furthermore, based on its relatively high expression levels in the β cell, *SLC39A13* (neighboring gene to *MADD*) constitutes an intriguing candidate gene that may merit further investigation.

Potential causal variants, eQTLs and copy number variants

Our results interrogate only a fraction of the common variants in any given genomic region; we therefore expect that for the majority of the loci here described the underlying causal variant has yet to be identified. Nevertheless for some there are intriguing possible SNP candidates: in *SLC2A2* the lead SNP (rs11920090) is in perfect LD ($r^2 = 1.0$) with rs5400 (Stage 1 discovery association $P=5.9\times 10^{-6}$), which codes for the amino acid substitution T110I, predicted to be “possibly damaging” by PolyPhen35 and PANTHER (Pdel= 0.92)36. In *GCKR* the lead SNP is in strong LD ($r^2=0.93$) with rs1260326, encoding P446L, a non-synonymous variant previously associated with the same traits30 and predicted by PolyPhen to be “probably damaging”. A recent functional study has demonstrated that this variant indirectly leads to increased GCK activity, resulting in the observed effects on FG and triglyceride levels37. Both *SLC2A2* T110I and *GCKR* P446L were predicted “tolerated” by SIFT38, highlighting the difficulties in obtaining consensus functional predictions from different informatic approaches.

We used publicly available eQTL datasets for liver39, cortex40 and Epstein-Barr virus-transformed lymphoblastoid cell lines41 to explore additional possible causal mechanisms testing for association between replicated loci and mRNA expression levels of nearby genes (Online Methods). The lead SNP in *FADS1*, rs174550, is in strong LD ($r^2=0.80$) and close proximity (130 bp) to rs174548, a SNP highly associated with *FADS1* mRNA expression levels in liver ($P=1.7\times 10^{-5}$) and with *FADS2* mRNA expression levels in lymphoblastoid cells ($P=3.1\times 10^{-4}$). SNP rs174548 has also been associated (up to $P=4.5\times 10^{-8}$) with a number of serum glycerophospholipid concentrations in a GWAS investigating metabolomic profiles42 and rs174550 also demonstrated strong associations ($P<5.2\times 10^{-7}$) with the same metabolites (data not shown). These results are substantiated by previous work associating SNPs in this region with the fatty acid composition of phospholipids43. The latter suggest the minor allele variant of rs174550 results in a reduced efficiency of the fatty acid delta-5 desaturase reaction42. Finally, bioinformatic analysis identifies a perfect proxy, rs174545 ($r^2=1$ with rs174550), whose glucose-raising allele abolishes a predicted miR-124 target site (see Online Methods). Taken together, these data support the hypothesis that not only fatty acid levels, but also their precise composition and degree of desaturation, may influence glucose homeostasis.

Although our study was not designed to explicitly investigate the impact of copy number variation on glycemic traits, we took advantage of existing data44 to investigate whether any of our lead SNPs are in LD with common, diallelic copy number polymorphisms (CNPs) mapping within a 1Mb window. Of the FG loci, only *DGKB/TMEM195* has a validated, common CNP affecting sequence within 1 Mb of the index SNP44. Despite the proximity of

this CNP to the associated SNP (~25 kb), the CNP is essentially uncorrelated with the index SNP ($r^2=0.01$ in HapMap CEU) and is therefore unlikely to explain the observed FG association.

DISCUSSION

In this meta-analysis of 21 Stage 1 discovery GWAS cohorts followed by targeted Stage 2 replication of 25 loci in 33 additional cohorts (totaling up to 122,743 non-diabetic participants), we report the novel genome-wide significant associations of SNPs in or near *ADCY5*, *MADD*, *ADRA2A*, *CRY2*, *FADS1*, *GLIS3*, *SLC2A2*, *PROX1* and *FAM148B* with FG and one SNP near *IGF1* with FI and HOMA-IR. We have also confirmed associations of variants in *GCK*, *GCKR*, *G6PC2* and *MTNR1B* with FG, and achieved genome-wide significance for the recently reported *DGKB/TMEM195* locus²⁴ and for variants in the known T2D-associated *TCF7L2* and *SLC30A8*. All of the FG-associated SNPs demonstrate consistent nominal associations with HOMA-B; and those in *GCK*, *G6PC2*, *MTNR1B*, *DGKB/TMEM195*, *ADCY5*, *FADS1* and *GLIS3* do so at genome-wide significant levels. As previously reported^{11,12,30}, *GCKR* is also associated with FI and HOMA-IR.

Importantly, in addition to the established T2D-associated loci *TCF7L2*, *SLC30A8* and *MTNR1B*, five of the loci that are associated with elevated FG levels in non-diabetic individuals (*ADCY5*, *GCK*, *GCKR*, *PROX1* and *DGKB/TMEM195*) also increase the risk of T2D in separate T2D case-control studies. However, this overlap is incomplete and highlights that the magnitude of the effect on FG is not predictive of the effect on T2D risk, as shown when comparing FG and T2D effect sizes for *MTNR1B* and *TCF7L2*, or for *ADCY5* and *MADD* (Table 2). The latter two loci have similar effect sizes on FG and similar allele frequencies, and yet the former is robustly associated with T2D risk (OR 1.12, $P=5.5\times 10^{-21}$) while the latter is not (OR 1.01, $P=0.3$) in the same samples. This suggests that not all loci associated with FG within the “physiological” range are also associated with “pathological” FG levels and T2D risk. Thus, variation in FG in healthy individuals is not necessarily an endophenotype for T2D, which posits the hypothesis that the mechanism by which glucose is raised, rather than a mere elevation in fasting glucose levels, is a key contributor to disease progression. On the other hand, we cannot rule out the existence of protective variants in loci where elevated FG does not progress to manifest T2D, or the effect of cohort selection in the detection of the loci with variable effects on FG and T2D risk. Nevertheless, this work shows that targeting quantitative traits in GWAS searches can help identify genetic determinants of overt disease.

With regard to insulin resistance, our analyses resulted in only one novel genome-wide significant locus associated with FI and HOMA-IR. The associated SNP rs35767 is 1.2 kb upstream of *IGF1*, raising the possibility that it may influence IGF1 expression levels (we have found no direct support for this notion in the limited eQTL data available). Although not reaching genome-wide-significance, we note that SNP rs4675095 in the insulin receptor substrate-1 gene (*IRS1*) was also associated with HOMA-IR ($P=4.6\times 10^{-3}$), which given *IRS1*'s excellent biological credentials will warrant further investigation. This SNP is not in LD with the widely studied missense SNP G972R (rs1801278) nor with the newly discovered T2D SNP rs294364145, whose C risk allele was only nominally associated with

increased FI ($P=0.02$) and HOMA-IR ($P=0.04$) in our discovery dataset. The previously reported associations of SNPs in *PANK1* with fasting insulin²⁴ did not receive strong support in our discovery cohorts ($P=0.04$ and 0.17 for rs11185790 and rs1075374, respectively).

Notably, our large-scale meta-analyses produced more than a dozen robust associations with FG and only two with FI/HOMA-IR (*GCKR* and *IGF1*). Although the somewhat smaller sample size for insulin may have contributed to this discrepancy, a comparison of the similarly-powered HOMA-B and HOMA-IR analyses reveals associations with HOMA-B several orders of magnitude more significant than those seen with HOMA-IR (Figure 2). Because insulin itself is a component of the numerator in both measures, one cannot attribute this discrepancy to technical differences in insulin measurements across cohorts. Similarly, because the QQ plots are very similar for FI and HOMA-IR, we do not believe that the use of a mathematical formula (HOMA-IR) rather than a direct measurement (FI) has affected our analyses substantially. HOMA-B and HOMA-IR have comparable heritability estimates (0.26 and 0.27 in Framingham respectively) and their correlation is significant ($r=0.55$ in Framingham). Thus, not only there may be a difference in the identity of specific genetic determinants for each trait⁴⁶, but rather the genetic architecture may be distinct for each trait, with more modest effects, fewer loci, rarer variants or a stronger environmental modification underlying HOMA-IR. In addition, HOMA-IR (which is composed of fasting values) is an imperfect estimate of global insulin resistance, as it addresses mostly hepatic sensitivity to insulin and is partially affected by β -cell function: its heritability is lower than insulin sensitivity derived from the minimal model⁴⁷. Exploration of gene \times environment interactions and analysis of datasets that include 2-hour glucose and insulin values may reveal other genetic factors that increase insulin resistance in humans²⁹.

In conclusion, a large-scale meta-analysis of GWAS has identified ten novel loci associated with glycemic traits whose in-depth physiological investigation should further our understanding of glucose homeostasis in humans and may reveal novel pathways for diabetes therapeutics.

BOX: GENES NEAREST TO LOCI ASSOCIATED WITH FASTING DIABETES-RELATED QUANTITATIVE TRAITS

DGKB/TMEM195 – This locus was recently reported to be associated with FG²⁴; here we report genome-wide significant replication of that finding and evaluate the genes mapping closest to the lead SNP in further detail. *DGKB* encodes the β (1 of 10) isotype of the catalytic domain of diacylglycerol kinase, which regulates the intracellular concentration of the second messenger diacylglycerol. In rat pancreatic islets glucose increases diacylglycerol⁴⁹, which activates protein kinase C (PKC) and thus potentiates insulin secretion⁵⁰. *TMEM195* encodes transmembrane protein 195, an integral membrane phosphoprotein highly expressed in liver.

ADCY5 encodes adenylate cyclase 5, which catalyzes the generation of cAMP. Upon binding to its receptor in pancreatic β cells, glucagon-like peptide 1 (GLP-1) induces

cAMP-mediated activation of protein kinase A, transcription of the proinsulin gene and stimulation of insulin secretory processes⁵¹.

MADD encodes mitogen-activated protein kinase (MAPK) activating death domain, an adaptor protein that interacts with the tumor necrosis factor α receptor to activate MAPK. Both PKC and MAPK have been implicated in the proliferation of β cells induced by GLP-151, suggesting that *DGKB* and *MADD* may contribute to β -cell mass and insulin secretion in this manner as well. Also in this region, *SLC39A13* encodes a putative zinc transporter required for connective tissue development and BMP/TGF- β signaling⁵². *NR1H3* encodes the liver X receptor alpha (LXRA) protein, which contains the retinoid response element. Glucose stimulates the transcriptional activity of LXR, which acts as a molecular switch that integrates hepatic glucose metabolism and fatty acid synthesis⁵³.

ADRA2A encodes the α_{2A} adrenergic receptor, which is expressed in β cells and whose activation leads to an outward potassium current independent of the islet K_{ATP} channel, thus possibly modifying insulin release⁵⁴. Mice with null mutations display abnormal glucose homeostasis in addition to cardiac hypertrophy and abnormal heart rate and blood pressure.

FADS1 encodes fatty acid desaturase 1, which catalyzes the biosynthesis of highly unsaturated fatty acids from precursor essential polyunsaturated fatty acids. One such product is arachidonic acid; in rodent β cells, arachidonic acid liberated by phospholipase A_2 augments glucose-mediated insulin release⁵⁵. Two other members of the same family, *FADS2* and *FADS3*, also reside in this region. By directing fatty acids down this metabolic pathway, increased activity of these enzymes may lower circulating triglyceride concentrations.

CRY2 encodes cryptochrome 2, an integral component of the mammalian circadian pacemaker⁵⁶. Mice with null mutations in this gene present with abnormal circadian rhythmicity and several metabolic abnormalities including impaired glucose tolerance, increased insulin sensitivity, decreased body weight and adipose tissue, and abnormal heart rate. Together with *MTNR1B15–17* this is the second circadian gene associated with FG in humans, contributing further evidence to this emerging pathway regulating glucose homeostasis⁵⁷. In the same region, *MAPK8IP1* encodes the scaffolding protein JIP1. Cross-talk between JIP1 and JIP3 has been implicated in the regulation of ASK1-SEK1-JNK signaling during glucose deprivation⁵⁸. A missense mutation in this gene (S59N) segregates with diabetes in one family affected with a Mendelian form of the disease⁵⁹.

SLC2A2 encodes the GLUT2 transporter responsible for transport of glucose into β cells and triggering the glucose-mediated insulin secretion cascade. In humans, recessive mutations in this gene lead to Fanconi-Bickel Syndrome, a rare disorder characterized by hepatorenal glycogen accumulation, proximal renal tubular dysfunction and impaired utilization of glucose and galactose⁶⁰; mouse mutants also display hyperglycemia and abnormal glucose homeostasis⁶¹.

GLIS3 encodes the transcription factor GLIS family zinc finger 3 isoform, a Krüppel-like zinc finger protein that both activates and represses transcription and participates in β -cell

ontogeny^{62,63}. Functional mutations in this gene cause a syndrome of neonatal diabetes and congenital hypothyroidism⁶³. Polymorphisms within this gene have recently been associated with type 1 diabetes risk (t1dgc.org).

PROX1 encodes the prospero homeobox protein 1, a novel co-repressor of hepatocyte nuclear factor 4 α ⁶⁴ that plays a crucial role in β -cell development; mutations in its target gene *HNF4A* cause maturity-onset diabetes of the young, type 165.

FAM148B encodes the nuclear localized factor 2 (NLF2). It is expressed in endothelial cells and up-regulated by pro-inflammatory cytokines⁶⁶. As shown here, it has a high level of expression in the pancreas, although its putative molecular connection with glucose homeostasis is presently unclear.

IGF1 encodes the insulin-like growth factor 1, the sole genome-wide significant locus associated with HOMA-IR in our study. Humans and mice null for *igf1* display abnormal glucose homeostasis, with insulin resistance, increased circulating insulin and insensitivity to growth hormone⁶⁷.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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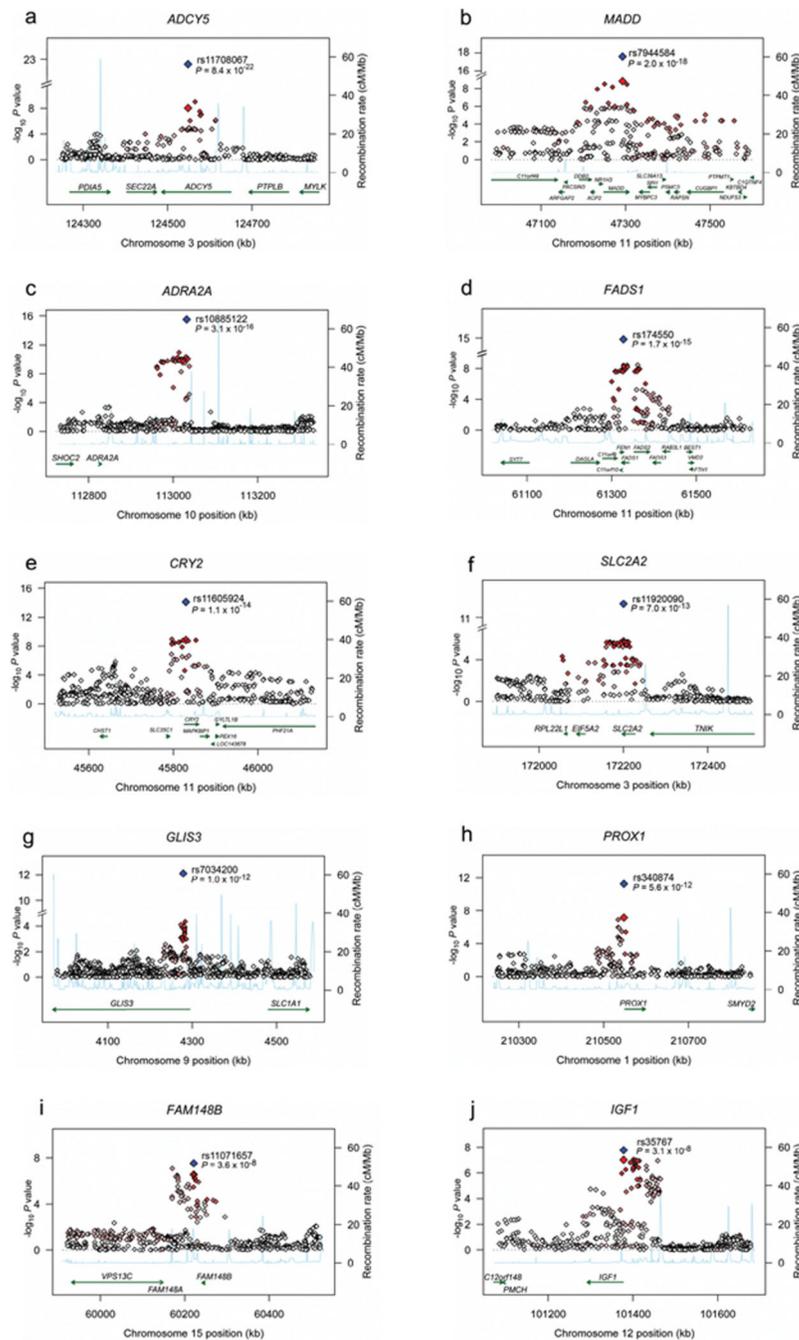


Figure 1. Regional plots of ten novel genome-wide significant associations. For each of the *ADCY5* (a), *MADD* (b), *ADRA2A* (c), *FADS1* (d), *CRY2* (e), *SLC2A2* (f), *GLIS3* (g), *PROX1* (h), *FAM148B* (i) and *IGF1* (j) regions, directly genotyped and imputed SNPs are plotted with their meta-analysis P values (as $-\log_{10}$ values) as a function of genomic position (NCBI Build 35). In each panel, the Stage 1 discovery SNP taken forward to Stage 2 replication is represented by a blue diamond (with global meta-analysis P value), with its Stage 1 discovery P value denoted by a red diamond. Estimated recombination rates (taken from

HapMap) are plotted to reflect the local LD structure around the associated SNPs and their correlated proxies (according to a white to red scale from $r^2 = 0$ to 1, based on pairwise r^2 values from HapMap CEU). Gene annotations were taken from the University of California Santa Cruz genome browser.

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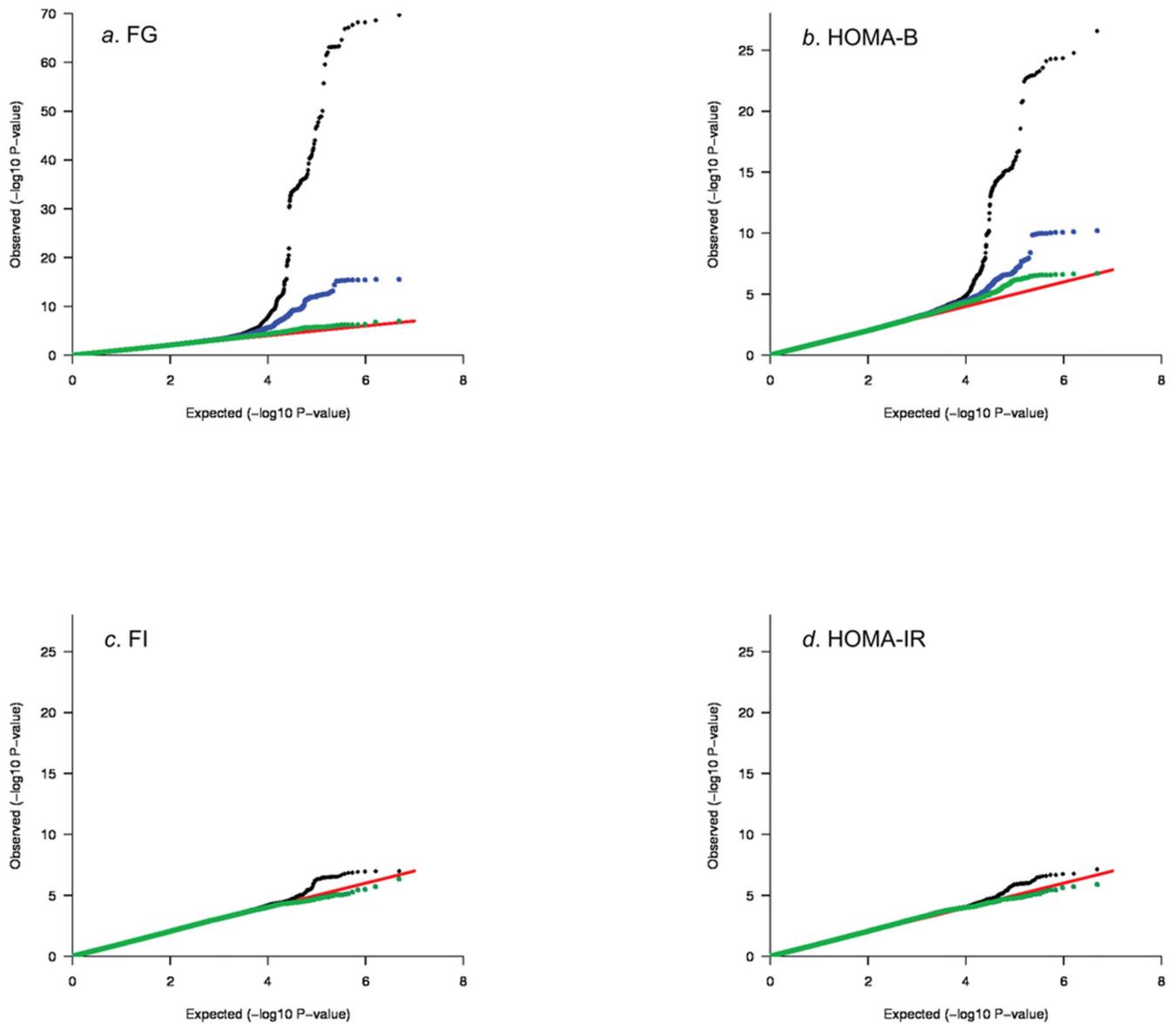


Figure 2.

Quantile-quantile (Q-Q) plots for fasting glucose (FG) (a), β -cell function by homeostasis model assessment (HOMA-B) (b), fasting insulin (FI) (c), and insulin resistance by homeostasis model assessment (HOMA-IR) (d). In each plot, the expected null distribution is plotted along the red diagonal, the entire distribution of observed P values is plotted in black, and a distribution that excludes the ten novel findings in Figure 1 is plotted in green. For FG and HOMA-B, the distribution that excludes the four genome-wide significant FG-associated loci (*GCK*, *GCKR*, *G6PC2* and *MTNR1B*) is plotted in blue. A comparison of the observed P values for each trait shows that FG/HOMA-B associations are much more likely to be detected than FI/HOMA-IR associations.

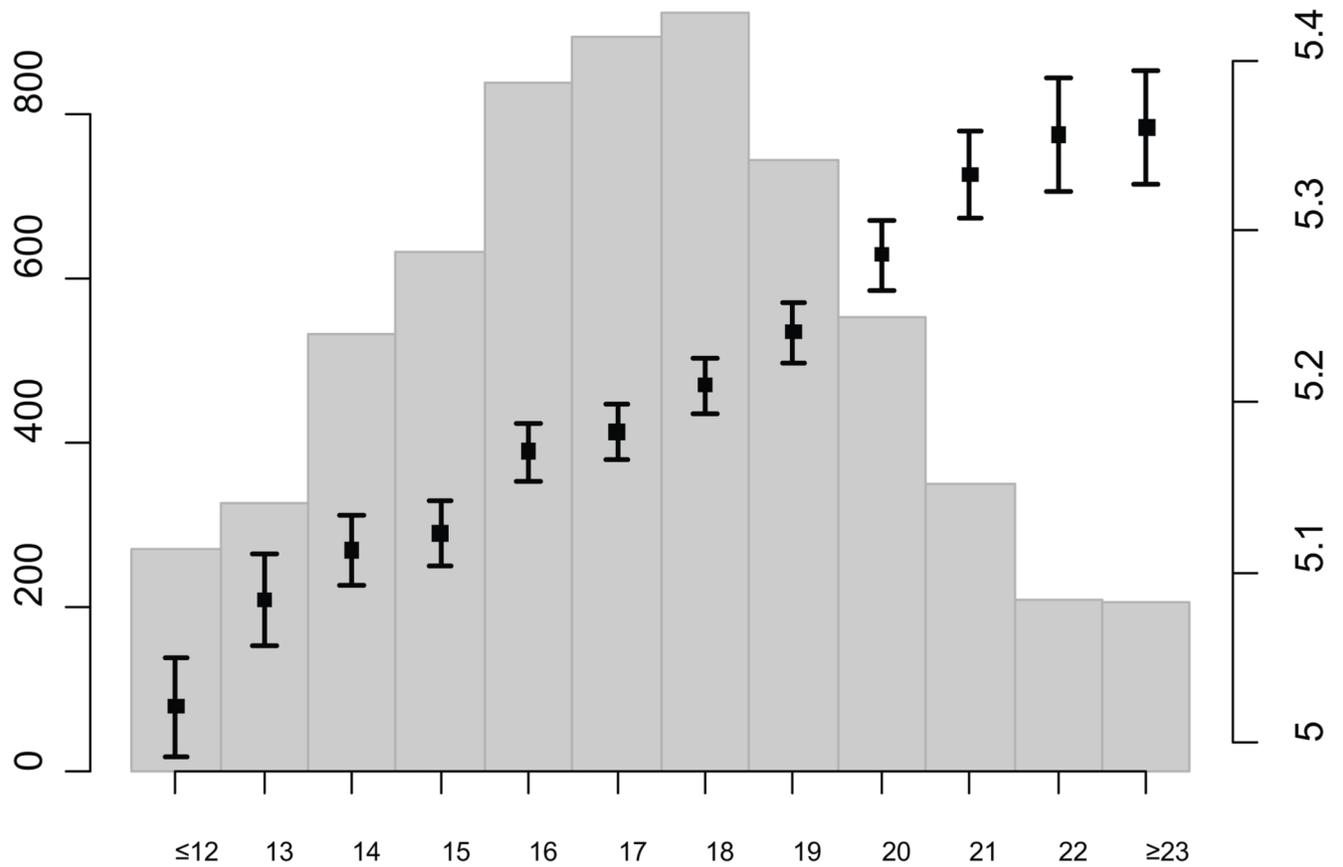


Figure 3.

Variation in levels of fasting glucose depending on the number of risk alleles at novel loci, weighted by effect size in an aggregate genotype score for the Framingham Heart Study.

The bar plots show the average and standard error of fasting glucose in mmol/L for each value of the genotype score based on the regression coefficient (right Y axis), and the histogram denotes the number of individuals in each genotype score category (left Y axis). Comparable results were obtained for the NFBC 1966 and ARIC cohorts. On average, the range spans ~0.4 mmol/L (~7.2 mg/dl) from low to high genotype score.

Table 1
SNPs associated with fasting glucose-related or insulin-related traits at genome-wide significance levels

SNP	Glucose/HOMA-B selected SNPs				Fasting glucose				HOMA-B			
	Nearest gene(s)	Alleles (effect/other)	Freq	Discovery P value	r ² estimate (P value)	Global P value	Joint analysis N	Discovery P value	r ² estimate (P value)	Global P value	Joint analysis N	
rs560887	<i>G6PC2</i>	C/T	0.70	4.4×10 ⁻⁷⁵	0.31 (0.18)	8.7×10 ⁻²¹⁸	119,169	2.0×10 ⁻²⁸	0.54 (0.01)	1.5×10 ⁻⁶⁶	94,839	
rs10830963	<i>MTNR1B</i>	G/C	0.30	1.2×10 ⁻⁶⁸	0.00 (1.00)	5.8×10 ⁻¹⁷⁵	112,844	1.8×10 ⁻²²	0.45 (0.03)	2.7×10 ⁻⁴³	90,364	
rs4607517	<i>GCK</i>	A/G	0.16	4.5×10 ⁻³⁶	0.19 (0.46)	6.5×10 ⁻⁹²	118,500	7.5×10 ⁻⁸	0.36 (0.12)	1.8×10 ⁻¹⁶	94,112	
rs2191349	<i>DGKB/TMEM195</i>	T/G	0.52	7.8×10 ⁻¹⁷	0.10 (0.68)	3.0×10 ⁻⁴⁴	122,743	5.4×10 ⁻¹¹	0.09 (0.71)	2.8×10 ⁻¹⁷	98,372	
rs780094	<i>GCKR</i>	C/T	0.62	2.5×10 ⁻¹²	0.00 (1.00)	5.6×10 ⁻³⁸	118,032	0.25	0.32 (0.18)	3.2×10 ⁻⁴	93,990	
rs11708067	<i>ADCY5</i>	A/G	0.78	8.7×10 ⁻⁹	0.04 (0.89)	7.1×10 ⁻²²	118,475	2.2×10 ⁻⁴	0.37 (0.10)	2.5×10 ⁻¹²	94,212	
rs7944584	<i>MADD</i>	A/T	0.75	1.5×10 ⁻⁹	0.00 (1.00)	2.0×10 ⁻¹⁸	118,741	1.1×10 ⁻⁴	0.16 (0.51)	3.5×10 ⁻⁵	94,408	
rs10885122	<i>ADRA2A</i>	G/T	0.87	8.4×10 ⁻¹¹	0.00 (1.00)	2.9×10 ⁻¹⁶	118,410	3.7×10 ⁻⁶	0.11 (0.66)	2.0×10 ⁻⁶	94,128	
rs174550	<i>FADS1</i>	T/C	0.64	1.5×10 ⁻⁸	0.00 (1.00)	1.7×10 ⁻¹⁵	118,908	4.5×10 ⁻⁵	0.01 (0.99)	5.2×10 ⁻¹³	94,536	
rs11605924	<i>CRY2</i>	A/C	0.49	1.5×10 ⁻⁹	0.00 (1.00)	1.0×10 ⁻¹⁴	116,479	5.2×10 ⁻⁶	0.03 (0.94)	3.2×10 ⁻⁵	92,326	
rs11920090	<i>SLC2A2</i>	T/A	0.87	1.9×10 ⁻⁶	0.00 (1.00)	8.1×10 ⁻¹³	119,024	1.4×10 ⁻⁴	0.36 (0.11)	4.5×10 ⁻⁶	94,629	
rs7034200	<i>GLIS3</i>	A/C	0.49	1.2×10 ⁻⁴	0.00 (1.00)	1.0×10 ⁻¹²	106,250	1.9×10 ⁻⁶	0.19 (0.46)	1.2×10 ⁻¹³	83,759	
rs340874	<i>PROX1</i>	C/T	0.52	7.1×10 ⁻⁸	0.00 (1.00)	6.6×10 ⁻¹²	116,882	3.7×10 ⁻⁵	0.00 (1.00)	5.3×10 ⁻⁶	92,942	
rs11071657	<i>FAM148B</i>	A/G	0.63	2.8×10 ⁻⁷	0.00 (1.00)	3.6×10 ⁻⁸	114,454	0.23	0.08 (0.73)	0.002	90,675	
rs11558471	<i>SLC30A8</i>	A/G	0.68	2.6×10 ⁻¹¹	-	-	45,996	1.4×10 ⁻⁶	-	-	36,283	
rs4506565	<i>TCF7L2</i>	T/A	0.31	1.2×10 ⁻⁸	-	-	46,181	1.4×10 ⁻⁶	-	-	36,461	

Insulin/HOMA-IR selected SNPs				Fasting insulin				HOMA-IR			
rs780094	<i>GCKR</i>	C/T	0.62	1.1×10 ⁻⁴	0.14 (0.57)	3.6×10 ⁻²⁰	96,126	9.9×10 ⁻⁷	0.25 (0.32)	3.0×10 ⁻²⁴	94,636
rs35767	<i>IGF1</i>	G/A	0.85	1.0×10 ⁻⁷	0.17 (0.50)	3.3×10 ⁻⁸	94,590	7.8×10 ⁻⁸	0.26 (0.28)	2.2×10 ⁻⁹	93,141

Directly genotyped and imputed SNPs were tested for association with fasting glucose, fasting insulin and homeostasis model assessment of β -cell function (HOMA-B) and insulin resistance (HOMA-IR). Twenty one discovery cohorts with genome-wide data were meta-analyzed (Stage 1 discovery) and 25 SNPs were promoted for replication of the same trait in a set of 33 additional cohorts with *in silico* (N=7) or *de novo* (N=26) genotype data (N=31 for fasting insulin, HOMA-B and HOMA-IR; for Stage 2 replication P-values and effect sizes see Table 2). A joint analysis was then performed (global). Heterogeneity in the discovery sample was assessed using the I² index⁴⁸. Replication was not attempted for SNPs in two known type 2 diabetes genes (*SLC30A8* and *TCF7L2*) which achieved genome-wide significance for FG in Stage 1. Freq denotes the allele frequency of the glucose-raising allele. N=sample size. Note that the previously reported *GCKR* SNP has associations with glucose-related and insulin-related traits.

Table 2
Association of novel SNPs with glycemic traits in MAGIC and type 2 diabetes replication meta-analyses

SNP	Nearest gene(s)	Alleles (effect/other)	Fasting glucose (mmol/L)	HOMA-B	Fasting insulin (pmol/L)	HOMA-IR	HbA _{1c} (%)	2-hr glucose (mmol/L)	2-hr insulin (pmol/L)	Type 2 diabetes ^a
rs560887	<i>G6PC2</i>	C/T	Effect* 0.075 (0.003)	-0.042 (0.004)	-0.007 (0.004)	0.006 (0.004)	0.032 (0.004)	0.017 (0.020)	-0.031 (0.013)	0.97 (0.95-0.99)
			P-value 8.5×10^{-122}	7.6×10^{-29}	0.11	0.16	1.0×10^{-17}	0.41	0.01	0.012
rs10830963	<i>MTNR1B</i>	G/C	Effect* 0.067 (0.003)	-0.034 (0.004)	-0.006 (0.004)	0.004 (0.004)	0.024 (0.004)	0.056 (0.022)	0.034 (0.015)	1.09 (1.06-1.12)
			P-value 1.1×10^{-102}	1.1×10^{-22}	0.14	0.37	3.0×10^{-9}	0.01	0.02	8.0×10^{-13}
rs4607517	<i>GCK</i>	A/G	Effect* 0.062 (0.004)	-0.025 (0.005)	0.004 (0.006)	0.015 (0.006)	0.041 (0.005)	0.097 (0.026)	-0.012 (0.015)	1.07 (1.05-1.10)
			P-value 1.2×10^{-44}	1.2×10^{-6}	0.46	0.01	6.3×10^{-19}	2.0×10^{-4}	0.42	5.0×10^{-8}
rs2191349	<i>DGKB/TMEM195</i>	T/G	Effect* 0.030 (0.003)	-0.017 (0.003)	-0.002 (0.003)	0.002 (0.004)	0.008 (0.003)	0.000 (0.019)	-0.006 (0.012)	1.06 (1.04-1.08)
			P-value 5.3×10^{-29}	6.4×10^{-8}	0.48	0.61	0.01	0.98	0.60	1.1×10^{-8}
rs780094	<i>GCKR</i>	C/T	Effect* 0.029 (0.003)	0.014 (0.003)	0.032 (0.004)	0.035 (0.004)	0.004 (0.004)	-0.091 (0.019)	0.000 (0.011)	1.06 (1.04-1.08)
			P-value 1.7×10^{-24}	1.4×10^{-5}	3.6×10^{-19}	5.0×10^{-20}	0.32	1.4×10^{-6}	1.00	1.3×10^{-9}
rs11708067	<i>ADCY5</i>	A/G	Effect* 0.027 (0.003)	-0.023 (0.004)	-0.011 (0.004)	0.006 (0.005)	0.015 (0.004)	0.094 (0.023)	0.008 (0.015)	1.12 (1.09-1.15)
			P-value 1.7×10^{-14}	3.6×10^{-8}	0.01	0.16	5.1×10^{-4}	6.6×10^{-5}	0.60	9.9×10^{-21}
rs7944584	<i>MADD</i>	A/T	Effect* 0.021 (0.003)	-0.007 (0.004)	0.002 (0.004)	0.005 (0.004)	0.001 (0.004)	-0.017 (0.022)	-0.019 (0.013)	1.01 (0.99-1.03)
			P-value 5.1×10^{-11}	0.07	0.60	0.26	0.84	0.44	0.15	0.30
rs10885122	<i>ADRA2A</i>	G/T	Effect* 0.022 (0.004)	-0.010 (0.005)	0.001 (0.005)	0.004 (0.005)	0.007 (0.005)	0.004 (0.030)	-0.051 (0.019)	1.04 (1.01-1.07)
			P-value 9.7×10^{-8}	0.03	0.90	0.47	0.21	0.89	0.007	0.020
rs174550	<i>FADS1</i>	T/C	Effect* 0.017 (0.003)	-0.020 (0.003)	-0.011 (0.004)	0.008 (0.004)	0.007 (0.004)	0.013 (0.019)	-0.003 (0.012)	1.04 (1.02-1.06)
			P-value 8.3×10^{-9}	5.3×10^{-10}	2.7×10^{-3}	0.03	0.053	0.49	0.82	2.3×10^{-4}
rs11605924	<i>CRY2</i>	A/C	Effect* 0.015 (0.003)	-0.005 (0.003)	0.001 (0.004)	0.003 (0.004)	0.001 (0.003)	0.023 (0.018)	0.006 (0.011)	1.04 (1.02-1.06)
			P-value 8.1×10^{-8}	0.13	0.73	0.34	0.72	0.20	0.62	1.7×10^{-4}
rs11920090	<i>SLC2A2</i>	T/A	Effect* 0.020 (0.004)	-0.012 (0.005)	0.002 (0.005)	0.005 (0.005)	0.017 (0.005)	0.015 (0.027)	-0.022 (0.016)	1.01 (0.99-1.04)
			P-value 3.3×10^{-6}	0.02	0.77	0.37	5.8×10^{-4}	0.58	0.19	0.34
rs7034200	<i>GLIS3</i>	A/C	Effect* 0.018 (0.003)	-0.020 (0.004)	-0.014 (0.004)	0.011 (0.004)	0.003 (0.003)	0.037 (0.018)	0.010 (0.011)	1.03 (1.01-1.05)
			P-value 1.2×10^{-9}	8.9×10^{-9}	2.7×10^{-4}	4.6×10^{-3}	0.32	0.04	0.36	1.3×10^{-3}

SNP	Nearest gene(s)	Alleles (effect/other)	Fasting glucose (mmol/L)	HOMA-B	Fasting insulin (pmol/L)	HOMA-IR	HbA _{1c} (%)	2-hr glucose (mmol/L)	2-hr insulin (pmol/L)	Type 2 diabetes [^]
rs340874	<i>PROXI</i>	C/T	Effect* 0.013 (0.003) P-value 6.6×10^{-6}	-0.008 (0.003) 0.02	-0.002 (0.004) 0.68	0.001 (0.004) 0.74	0.009 (0.004) 9.5×10^{-3}	0.030 (0.020) 0.13	-0.007 (0.012) 0.56	1.07 (1.05–1.09) 7.2×10^{-10}
rs11071657	<i>FAM148B</i>	A/G	Effect* 0.008 (0.003) P-value 0.01	-0.013 (0.004) 8.1×10^{-4}	-0.009 (0.004) 0.03	0.008 (0.004) 0.07	0.001 (0.004) 0.79	-0.065 (0.020) 0.001	-0.006 (0.013) 0.65	1.03 (1.01–1.05) 2.9×10^{-3}
rs13266634	<i>SLC30A8</i>	C/T	Effect* 0.027 (0.004) P-value 5.5×10^{-10}	-0.016 (0.004) 2.4×10^{-5}	-0.004 (0.005) 0.44	0.0002 (0.005) 0.97	0.016 (0.004) 3.3×10^{-5}	0.093 (0.022) 2.0×10^{-5}	-0.011 (0.015) 0.47	1.15 (1.10–1.21) [#] 1.5×10^{-8}
rs7903146	<i>TCF7L2</i>	T/C	Effect* 0.023 (0.004) P-value 2.8×10^{-8}	-0.02 (0.004) 1.4×10^{-7}	-0.012 (0.004) 0.004	0.010 (0.005) 0.03	0.013 (0.003) 1.8×10^{-4}	0.118 (0.021) 2.6×10^{-8}	0.010 (0.013) 0.42	1.40 (1.34–1.46) [#] 2.2×10^{-51}
rs35767	<i>IGF1</i>	G/A	Effect* 0.012 (0.005) P-value 0.01	0.009 (0.005) 0.09	0.010 (0.006) 0.10	0.013 (0.006) 0.04	0.010 (0.005) 0.050	0.027 (0.025) 0.28	0.015 (0.016) 0.33	1.04 (1.01–1.07) 6.6×10^{-3}
Sample size for each trait			45,049–76,558	35,435–61,907	37,199–62,264	35,901–62,001	33,718–44,856	15,221–15,234	7,051–7,062	40,655 cases/87,022 controls

* Per-allele effect (SE) for quantitative traits was estimated from Stage 2 replication samples for fasting glucose, homeostasis model assessment of β -cell function (HOMA-B), fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR), and from discovery meta-analyses of MAGIC GWAS for glycated hemoglobin (HbA_{1c}), 2-hour glucose after an oral glucose tolerance test (BMI-adjusted) and 2-hour insulin (BMI-adjusted). For the first four traits, the regression coefficients are obtained from the replication cohorts so as to avoid an overestimate of the effect size caused by the “winner’s curse”. Results from replication samples were unavailable for rs7903146 and rs13266634, thus discovery meta-analysis results are shown for both SNPs for fasting glucose (N=45,049–45,051), HOMA-B (N=35,435–35,437), fasting insulin (N=37,199–37,201) and HOMA-IR (N=35,901–35,903).

[^] Replication genotyping was undertaken in 27 independent type 2 diabetes (T2D) case/control samples for all except the *TCF7L2* and *SLC30A8* signals.

[#] Association with T2D for SNPs in *TCF7L2* and *SLC30A8* loci was estimated from the DIAGRAM+ meta-analysis for a total of 8,130 cases/58,987 controls. For these loci we have included data on the most commonly associated SNPs with T2D in previously published data.

Table 3

Association of novel SNPs with related metabolic traits in other GWAS datasets

SNP	Nearest gene	Alleles (effect/other)	BMI (kg/m ²)	Diastolic blood pressure (mm Hg)	Systolic blood pressure (mm Hg)	Hypertension	HDL	LDL	Total cholesterol	Triglycerides
rs560887	<i>G6PC2</i>	C/T	Effect* P-value	-0.146 (0.091) 0.12	-0.105 (0.135) 0.46	-0.023 (0.028) 0.41	-0.004 (0.004) 0.32	0.01 (0.011) 0.35	0.019 (0.011) 0.10	0.004 (0.005) 0.52
rs10830963	<i>MTNR1B</i>	G/C	Effect* P-value	0.002 (0.010) 0.86	0.034 (0.098) 0.56	-0.003 (0.030) 0.91	0.005 (0.004) 0.26	-0.015 (0.013) 0.25	0.002 (0.014) 0.88	-0.004 (0.007) 0.58
rs4607517	<i>GCK</i>	A/G	Effect* P-value	0.004 (0.011) 0.75	-0.136 (0.111) 0.23	-0.013 (0.033) 0.70	-0.006 (0.005) 0.21	0.012 (0.014) 0.38	-0.002 (0.015) 0.87	0.013 (0.007) 0.054
rs2191349	<i>DGKB/TMEM195</i>	T/G	Effect* P-value	0.001 (0.009) 0.95	-0.075 (0.082) 0.37	0.007 (0.025) 0.79	0.002 (0.003) 0.64	0.009 (0.01) 0.40	0.015 (0.011) 0.18	0.004 (0.005) 0.44
rs780094	<i>GCKR</i>	C/T	Effect* P-value	0.012 (0.009) 0.17	0.052 (0.084) 0.55	0.006 (0.124) 0.96	0.009 (0.003) 8.7×10 ⁻³	0.007 (0.01) 0.51	-0.019 (0.011) 0.08	-0.055 (0.005) 9.6×10 ⁻²⁷
rs11708067	<i>ADCY5</i>	A/G	Effect* P-value	-0.010 (0.011) 0.35	-0.056 (0.104) 0.60	0.028 (0.031) 0.37	0.0004 (0.004) 0.92	-0.014 (0.013) 0.26	-0.013 (0.013) 0.32	-0.003 (0.006) 0.62
rs7944584	<i>MADD</i>	A/T	Effect* P-value	0.023 (0.010) 0.02	-0.208 (0.093) 0.03	-0.038 (0.028) 0.18	0.007 (0.004) 0.06	-0.013 (0.012) 0.27	-0.016 (0.012) 0.18	-0.007 (0.006) 0.26
rs10885122	<i>ADRA2A</i>	G/T	Effect* P-value	-0.021 (0.014) 0.14	-0.079 (0.131) 0.56	0.073 (0.039) 0.07	0.01 (0.007) 0.15	-0.019 (0.02) 0.34	-0.02 (0.021) 0.33	-0.02 (0.01) 0.04
rs174550	<i>FADS1</i>	T/C	Effect* P-value	0.003 (0.009) 0.73	-0.208 (0.086) 0.02	0.013 (0.026) 0.62	0.014 (0.003) 2.9×10 ⁻⁵	0.046 (0.010) 8.5×10 ⁻⁶	0.052 (0.011) 2.5×10 ⁻⁶	-0.025 (0.005) 1.9×10 ⁻⁶
rs11605924	<i>CRY2</i>	A/C	Effect* P-value	0.011 (0.009) 0.21	0.123 (0.082) 0.15	0.004 (0.025) 0.87	0.005 (0.004) 0.13	0.005 (0.011) 0.62	0.008 (0.011) 0.46	-0.009 (0.005) 0.10
rs11920090	<i>SLC2A2</i>	T/A	Effect* P-value	0.010 (0.012) 0.42	-0.034 (0.117) 0.78	-0.030 (0.036) 0.41	0.003 (0.005) 0.60	-0.004 (0.014) 0.81	-0.009 (0.015) 0.57	-0.015 (0.007) 0.04
rs7034200	<i>GLIS3</i>	A/C	Effect* P-value	-0.002 (0.009) 0.86	0.093 (0.082) 0.27	0.006 (0.025) 0.80	0.0002 (0.003) 0.94	0.015 (0.01) 0.15	0.028 (0.011) 8.3×10 ⁻³	0.005 (0.005) 0.37
rs340874	<i>PROX1</i>	C/T	Effect* P-value	-0.007 (0.009) 0.113 (0.085)	0.093 (0.127) 0.029 (0.026)	0.009 (0.01) 0.007 (0.003)	0.009 (0.01) 0.003 (0.011)	0.009 (0.01) 0.003 (0.011)	0.003 (0.011) 0.007 (0.005)	0.007 (0.005) 0.007 (0.005)

SNP	Nearest gene	Alleles (effect/other)	BMI (kg/m ²)	Diastolic blood pressure (mm Hg)	Systolic blood pressure (mm Hg)	Hypertension	HDL	LDL	Total cholesterol	Triglycerides
			<i>P</i> -value	0.46	0.20	0.48	0.04	0.39	0.81	0.19
rs11071657	<i>FAM148B</i>	A/G	Effect*	-0.006 (0.010)	0.132 (0.091)	-0.007 (0.135)	-0.004 (0.004)	0.012 (0.011)	0.002 (0.011)	0.006 (0.005)
			<i>P</i> -value	0.54	0.16	0.96	0.22	0.28	0.86	0.30
rs13266634	<i>SLC30A8</i>	C/T	Effect*	-0.026 (0.011)	-0.081 (0.094)	-0.072 (0.139)	0.003 (0.004)	0.016 (0.011)	0.013 (0.011)	0.005 (0.005)
			<i>P</i> -value	0.01	0.40	0.62	0.47	0.13	0.24	0.33
rs7903146	<i>TCF7L2</i>	T/C	Effect*	-0.033 (0.009)	0.026 (0.091)	0.025 (0.137)	0.005 (0.004)	0.007 (0.012)	0.007 (0.012)	-0.006 (0.006)
			<i>P</i> -value	4.4×10^{-4}	0.78	0.86	0.22	0.53	0.55	0.31
rs35767	<i>IGF1</i>	G/A	Effect*	0.003 (0.012)	-0.102 (0.113)	-0.078 (0.167)	0.003 (0.005)	-0.009 (0.015)	-0.012 (0.015)	-0.002 (0.007)
			<i>P</i> -value	0.81	0.38	0.65	0.556	0.519	0.425	0.839
			N	28,225-32,530	28,591-34,130	28,557-34,135	21,045	17,521	17,529	21,104
						8145-9553 cases				
						8175-9749 controls				

* Per-allele effect (SE). Results for BMI, blood pressure traits and lipid levels were kindly provided by the GIANT 31, GlobalBPGen 32 and ENGAGE 33 consortia respectively.