Variants in *MTNR1B* influence fasting glucose levels

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Abstract

To identify previously unknown genetic loci associated with fasting glucose concentrations, we examined the leading association signals in ten genome-wide association scans involving a total of 36,610 individuals of European descent. Variants in the gene encoding melatonin receptor 1B (*MTNR1B*) were consistently associated with fasting glucose across all ten studies. The strongest signal was observed at rs10830963, where each G allele (frequency 0.30 in HapMap CEU) was associated with an increase of 0.07 (95% CI = 0.06-0.08) mmol/l in fasting glucose levels ($P = 3.2 = \times 10^{-50}$) and reduced beta-cell function as measured by homeostasis model assessment (HOMA-B, $P = 1.1 \times 10^{-15}$). The same allele was associated with an increased risk of type 2 diabetes (odds ratio = 1.09 (1.05-1.12), per G allele $P = 3.3 \times 10^{-7}$) in a meta-analysis of 13 case-control studies totaling 18,236 cases and 64,453 controls. Our analyses also confirm previous associations of fasting glucose with variants at the *G6PC2* (rs560887, $P = 1.1 \times 10^{-57}$) and *GCK* (rs4607517, $P = 1.0 \times 10^{-25}$) loci.

Blood and plasma fasting glucose levels are tightly regulated within a narrow physiologic range by a feedback mechanism that targets a particular fasting glucose set point for each individual[1,2]. Disruption of normal glucose homeostasis and substantial elevations of fasting glucose are hallmarks of type 2 diabetes (T2D) and typically result from sustained reduction in pancreatic beta-cell function and insulin secretion.

However, even within healthy, nondiabetic populations there is substantial variation in fasting glucose levels. Approximately one-third of this variation is genetic[3], but little of this

heritability has been explained. There is growing evidence to suggest that common variants contributing to variation in fasting glucose are largely distinct from those associated with major disruptions of beta-cell function that predispose to T2D. Common sequence variants in the *GCK* (gluco-kinase) promoter4-6, and around genes encoding the islet-specific glucose-6-phosphatase (*G6PC2*)5,6 and the glucokinase regulatory protein (*GCKR*)7-9, have each been associated with individual variation in fasting glucose levels, but have, at best, weak effects on T2D risk8,10. Furthermore, although there are now over 15 genetic loci strongly associated with the risk of T2D7,10-14, none shows compelling evidence for association with fasting glucose in the two genome-wide association scans (GWAS) so far reported5,6.

MAGIC (the Meta-Analyses of Glucose and Insulin-related traits Consortium) represents a collaborative effort to combine data from multiple GWAS to identify additional loci that affect glycemic and metabolic traits. Our genetic studies of fasting glucose levels were originally organized as four distinct consortia: (i) European Network for Genetic and Genomic Epidemiology (ENGAGE), combining data from deCODE, Northern Finland Birth Cohort 1966 (NFBC1966), Netherlands Twins Register/Netherlands Study of Depression and Anxiety (NTR/NESDA) and the Rotterdam Study; (ii) Genetics of Energy Metabolism (GEM), a meta-analysis of the Lausanne (CoLaus) and TwinsUK scans; (iii) DFS, involving the Diabetes Genetics Initiative (DGI), Finland-United States Investigation of NIDDM Genetics (FUSION) and SardiNIA scans; and (iv) the Framingham Heart Study (FHS). Details of the ten component studies ($n = 1,233$-6,479) are provided in Supplementary Table 1 online.

As a prelude to more extensive data-sharing, the four consortia initially exchanged the identities of between 10 and 20 SNPs prominently associated with fasting glucose in their individual, interim, meta-analyses ($n = 6,479$-12,389; Supplementary Table 2 online). Comparison of these signals revealed three loci with consistent effects on fasting glucose detected in multiple studies. Two of these represented the previously reported signals in *G6PC2* and *GCK*. In addition, all four groups independently generated evidence for an association between fasting glucose and SNPs around the *MTNR1B*(melatonin receptor 1B) locus (ENGAGE: rs1387153, $P = 2.2$ 10^{-17}; GEM: rs10830963, $P = 7.4 \times 10^{-11}$; DFS: rs10830963, $P = 2.5 \times 10^{-7}$ FHS: rs11020107, $P = 5.8 \times 10^{-4}$, for the most strongly associated SNP exchanged from each analysis). The association signals at all three loci were confirmed on formal meta-analysis including results from all ten studies, after exclusion of individuals with known diabetes (rs560887 (*G6PC2*), $P = 1.1 \times 10^{-57}$; rs4607517), (*GCK*), $P = 1.0 \times 1.0^{-25}$; rs10830963 (*MTNR1B*), $P = P3.2 \times 10^{-50}$; Table 1, Fig. 1, Supplementary Fig. 1, Supplementary Table 3 and **Supplementary Methods** online). Subsequent efforts to harmonize additional aspects of data analysis strategies (including the additional exclusion, where necessary, of individuals with fasting glucose measures 7mmol/l) had only a marginal impact on estimates of significance and effect size (Supplementary Table 4 online).

We attempted to refine the location of the *MTNR1B* association signal by extending the meta-analysis to all SNPs (genotyped and imputed from the HapMap) within the 1-Mb region flanking the gene ($n = 35,812$; 981 SNPs). In all, 30 genotyped and imputed SNPs showed compelling evidence for association with fasting glucose ($P < 10^{-8}$). The strongest signal was detected at rs10830963: the minor (G) allele (frequency 0.30 in HapMap CEU15) at this SNP was associated with a per-allele increase of 0.07 (95% CI = 0.06-0.08) evidence for mmol/l in fasting glucose ($P = 3.2 \times 10^{-50}$). Consistent association at rs10830963 was observed in all ten component GWAS, irrespective of whether this SNP was genotyped or imputed, and of the genotyping platform (Table 1 and Supplementary Table 1). Repeat meta-analysis within the region after conditioning on rs10830963 revealed no additional independent signals of association (**Supplementary Note** online).

The strength of the association between rs10830963 and fasting glucose was unchanged after adjustment for body mass index (Supplementary Table 4). Analyses of fasting insulin levels as well as indices of beta-cell function (HOMA-B) and insulin sensitivity (HOMA-IR) estimated by the homeostasis model assessment16 were possible in ~24,000 participants from the ten studies. These established that the glucose-raising allele at rs10830963 was associated with reduced beta-cell function ($P = 1.1 \times 10^{-15}$), with no appreciable effect on fasting insulin or insulin sensitivity (Supplementary Table 5 and **Supplementary Note** online).

To determine the impact of variants within *MTNR1B* on T2D risk, we carried out a large-scale meta-analysis of 13 T2D case-control samples (18,236 T2D cases, 64,453 controls; corresponding to an effective sample size of 21,179 unrelated cases and 21,179 unrelated controls). We combined data from the deCODE13, Rotterdam17, KORA18, FUSION stage 2 (ref. 11) and METSIM10 studies and from several case-control samples from the UK10 with publicly available data from the DIAGRAM consortium (which itself aggregates GWA data from the WTCCC, DGI and FUSION scans)10 (**Supplementary Note**). We found strong evidence that the minor G allele of rs10830963 was associated with increased risk of T2D (odds ratio = 1.09 (1.05-1.12), $P = 3.3 \times 10^{-7}$; Fig. 2 and Supplementary Table 6 online). The possibility that the fasting glucose association might reflect the inclusion within the cross-sectional study samples of subjects with undiagnosed T2D can be discounted given that exclusion of those with either known diabetes, or a fasting glucose ⩾7mmol/l had little impact on the strength of the association signal (Table 1 and Supplementary Table 4). Although the association with T2D does not, despite large-scale replication efforts, reach the 5×10^{-8} threshold consistent with 'genome-wide significance'15, it seems highly probable, given the strong impact of this variant on beta-cell function (Supplementary Table 5), that this is a genuine effect.

The analyses we performed interrogate only a fraction of common sequence variants in a given region—it is likely that the causal variant for this locus is yet to be identified. The SNP with the strongest statistical evidence so far, rs10830963, maps within the single 11.5-kb intron of *MTNR1B* but does not seem to disrupt consensus transcription factor binding or cryptic alternative splice sites. The association signal is bounded by recombination hot spots defining a ~60-kb interval within which all our strongly associated SNPs lie and the causal variant is likely to reside. This interval contains the entire coding region of *MTNR1B*. The only other nearby genes (the coding regions of which lie well outside this 60-kb region) are *SLC36A4* and *FAT3*, neither of which are compelling candidates. *SLC36A4* encodes a proton/amino acid transmembrane transporter moderately similar to *Rattus norvegicus* lysosomal amino acid transporter 1, and *FAT3* encodes a cadherin family member which is the human homolog of the *Drosophila melanogaster* FAT tumor suppressor gene. Ultimately, detailed fine mapping and functional analyses will be required to define the causal allele(s) and to confirm that this effect is mediated through altered function or expression of *MTNR1B*.

The size of the MAGIC dataset also allowed us to examine the *G6PC2* and *GCK* regions in greater detail than had previously been possible. In the *G6PC2* region, rs560887, within intron 3 of the gene, remained the strongest signal whether or not imputed data were included ($P = 1.1 \times 10^{-57}$ across all ten studies; Supplementary Fig. 1 online). This is the same SNP reported in one recent paper5 and is in substantial linkage disequilibrium (LD; $r^2 = 0.72$ in HapMap CEU) with the lead SNP (rs563694) identified in another6. In the *GCK* region, rs4607517, which lies 6.6-kb upstream of the gene, was the most strongly associated SNP ($P = 1.0 \times 10^{-25}$; Supplementary Fig. 1 and Table 1). This SNP is also in strong LD (r^2 ⩾ 1 in HapMap CEU) with the *GCK* promoter SNP (rs1799884) that was featured in previous

reports4. Repeat meta-analysis after conditioning on the respective lead SNPs revealed no additional independent association signals at either locus (**Supplementary Note**).

As with the variant in *MTNR1B*, the magnitude of the fasting glucose associations for both these signals was unchanged after adjustment for BMI (Supplementary Table 4). Glucose-raising alleles at *GCK* and *G6PC2* were associated with reduced beta-cell function (rs4607517[A], $P = 9.8 \times 10^{-6}$; rs560887[C], $P = 1.2 \times 10^{-26}$; Supplementary Table 5 and **Supplementary Note**). However, in line with previous reports4,9, neither signal was strongly associated with T2D in the large-scale meta-analysis: in fact, the glucose-raising allele at *G6PC2* was weakly associated with reduced T2D risk (rs4607517[A], per-allele OR = 1.05 (1.00-1.10), $P = 0.031$; rs560887[C], 0.93 (0.89-0.97), $P = 0.0017$; Supplementary Table 6).

We found no influence of the noncoding lead SNPs rs10830963, rs560887 or rs4607517 on gene expression of *MTNR1B, SLC36A4, FAT3, G6PC2* or *GCK* in genome-wide expression QTL datasets from lymphocyte-derived cell lines19,20, cerebral cortex21 or liver22, and no evidence for epistatic effects among the three lead SNPs was observed (P for two-way interactions >0.19 in each of the seven studies including only unrelated individuals; interactions were not examined in the other three studies).

MTNR1B encodes one of two known human melatonin receptors23. Although this is the first study to implicate genetic variation in *MTNR1B* in the regulation of fasting glucose levels and predisposition to T2D, this relationship is biologically credible. As well as being highly expressed in the brain, retina and elsewhere24, *MTNR1B* is transcribed in human islets and rodent insulinoma cell lines25, and the translated receptor is thought to mediate the inhibitory effect of melatonin on insulin secretion26. Melatonin release is characterized by marked circadian variability and these inhibitory effects on insulin secretion may contribute to the entrainment of circadian patterns of insulin release27. There is substantial evidence in human and rodent studies linking disturbances of circadian rhythmicity to metabolic conditions including diabetes28,29, and overexpression of melatonin receptors has been observed in islets from individuals with T2D as compared to nondiabetic controls30. Taken together, these findings suggest that the association with raised fasting glucose and T2D may be driven by variants that augment expression and/or activity of islet melatonin receptors.

Our findings bring the number of common variant loci influencing fasting glucose levels to four, three of which were detected in the present study. Variants in *GCKR* have a smaller effect size than the others7,9, and the present study design (based on exchange of a limited number of prominent signals between component groups) was not well-powered to detect these. However, subsequent meta-analysis of *GCKR* variants across all ten study samples confirms the association with fasting glucose (rs780094, $P = 8.5\ 10^{-9}$; Supplementary Table 4). The total variance in fasting glucose now attributable to these four signals is 1.5%, indicating that additional loci remain to be found3. In comparison with *GCK* and *G6PC2*, variants in *MTNR1B* seem to have a more marked effect on risk of T2D, the effect size being comparable in magnitude (OR = 1.09 (1.05-1.12)) to several other T2D-susceptibility genes recently identified in GWAS10. Thus, although the physiological regulation of fasting glucose set point and the pathological decline in beta-cell function that characterizes common forms of T2D generally seem to involve different processes, the *MTNR1B* finding suggests that this is not always the case. Not only can the study of diabetes-related quantitative traits provide an important path to the identification of additional T2D susceptibility loci, but there may also be opportunities for useful therapeutic overlap.

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Supplementary Material

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Acknowledgments

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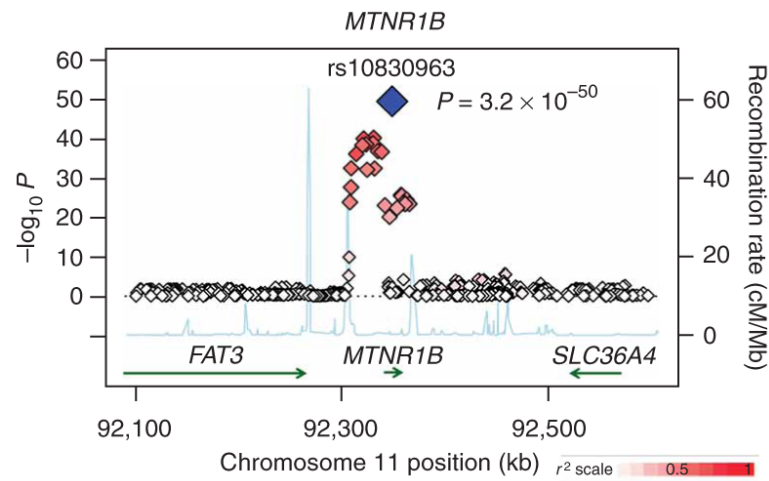
Figure 1.
Regional plot of fasting glucose association results for the *MTNR1B* locus across ten MAGIC GWAS. Meta-analysis $-\log_{10} P$ values are plotted as a function of genomic position (NCBI build 35). The SNP with the strongest signal (rs10830963) is denoted by a blue diamond. Estimated recombination rates (from HapMap) are plotted to reflect the local linkage disequilibrium structure around associated SNPs and proxies (according to a white-to-red scale from $r^2 = 0$ to $r^2 = 1$ and based on pairwise r^2 values from HapMap CEU). Gene annotations were taken from the University of California Santa Cruz genome browser.
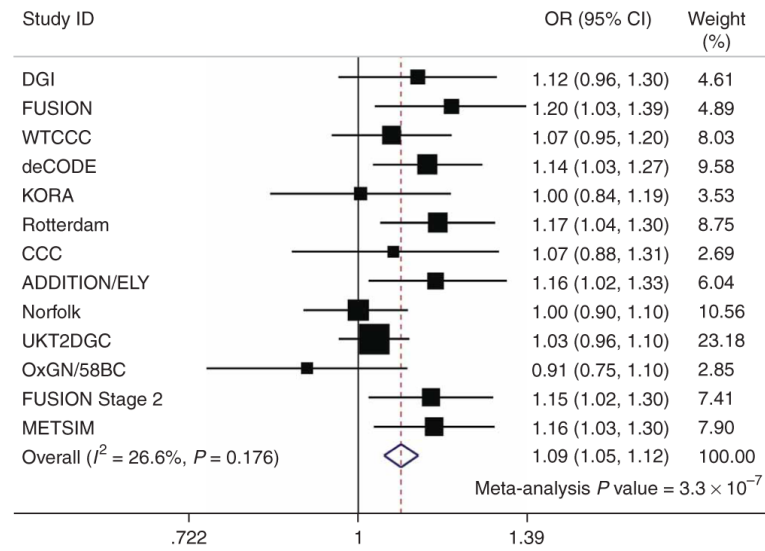
Figure 2.
Association of rs10830963 with type 2 diabetes (T2D) in 13 case-control studies.

Table 1

Association of rs10830963 (*MTNR1B*) with fasting glucose levels in ten studies within MAGIC and meta-analysis of best SNPs across all ten studies for three loci associated with fasting glucose (*MTNR1B*, *G6PC2* and *GCK*)

Study sample	N	G allele frequency	Mean mmol/l fasting glucose[a] per genotype (s.d.)			Per-allele effect, mmol/l (s.e.m.)	P value
			CC	CG	GG		
CoLaus	5,000	0.32	5.36 (0.71)	5.46 (0.80)	5.54 (0.81)	0.094 (0.016)	1.9×10^{-9}
deCODE	6,240	0.27	5.29 (0.71)	5.39 (0.71)	5.44 (0.71)	0.086 (0.016)	9.2×10^{-8}
DGI	1,455	0.31	5.29 (0.54)	5.32 (0.53)	5.39 (0.60)	0.042 (0.022)	0.054
Framingham[b]	6,479	0.28	5.16 (0.48)	5.21 (0.48)	5.26 (0.46)	0.050 (0.012)	2.2×10^{-13}
FUSION	1,233	0.33	5.28 (0.49)	5.33 (0.47)	5.40 (0.44)	0.057 (0.016)	5.8×10^{-4}
NFBC1966	4,245	0.34	5.63 (0.46)	5.70 (0.49)	5.80 (0.46)	0.079 (0.012)	1.7×10^{-11}
NTR/NESDA	3,166	0.27	5.22 (0.64)	5.26 (0.62)	5.38 (0.63)	0.062 (0.019)	1.2×10^{-3}
Rotterdam	2,058	0.28	5.58 (0.81)	5.75 (0.91)	5.83 (1.03)	0.145 (0.029)	7.9×10^{-7}
Sardinia	4,108	0.20	5.62 (0.89)	5.68 (0.89)	5.76 (0.89)	0.070 (0.019)	3.2×10^{-4}
TwinsUK[c]	1,828	0.30	4.58 (0.65)	4.67 (0.50)	4.74 (0.57)	0.084 (0.032)	7.9×10^{-3}
rs10830963 (*MTNR1B*)					Meta-analysis	0.072 (0.005)	3.2×10^{-50}
rs560887 (*G6PC2*)					Meta-analysis	0.064 (0.004)	1.1×10^{-57}
rs4607517 (*GCK*)					Meta-analysis	0.062 (0.007)	1.0×10^{-25}

Fasting glucose levels (mmol/l) are reported untransformed and unadjusted for covariates. Effect of the risk allele and s.e.m. were calculated using untransformed fasting glucose values. *P* values are reported for the additive genetic model with study-specific transformation of fasting glucose values, adjusted for sex and age.

[a] Fasting glucose levels in NFBC1966 and SardiNIA were measured in whole blood; in other samples measures were conducted on plasma samples. For these two studies, values in the table are corrected to plasma fasting glucose using a correction factor of 1.13.

[b] In Framingham study, mean fasting glucose values for the imputed SNPs are reported for proxies: rs560887 (proxy rs573225, $r^2 = 0.96$); rs4607517 (proxy rs1799884, $r^2 = 1$); rs10830963 (proxy rs7936247, $r^2 = 0.59$).

[c] In the TwinsUK study, mean fasting glucose values per genotype are estimated for a subset of unrelated individuals only.