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Insights into the Genetic Susceptibility to Type 2 Diabetes from Genome-Wide Association Studies of Glycaemic Traits

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Abstract Over the past 8 years, the genetics of complex traits have benefited from an unprecedented advancement in the identification of common variant loci for diseases such as type 2 diabetes (T2D). The ability to undertake genome-wide association studies in large population-based samples for quantitative glycaemic traits has permitted us to explore the hypothesis that models arising from studies in non-diabetic individuals may reflect mechanisms involved in the pathogenesis of diabetes. Amongst 88 T2D risk and 72 glycaemic trait loci, only 29 are shared and show disproportionate magnitudes of phenotypic effects. Important mechanistic insights have been gained regarding the physiological role of T2D loci in disease predisposition through the elucidation of their contribution to glycaemic trait variability. Further investigation is warranted to define causal variants within these loci, including functional characterisation of associated variants, to dissect their role in disease mechanisms and to enable clinical translation.

Keywords 2-h post-prandial glucose · Association · β-cell function · Birth weight · Body mass index · Candidate gene study · Cardiometabolic traits · Effect size · Fasting glucose · Fasting insulin · Genome-wide association study · Glycated haemoglobin (HbA_{1c}) · Homeostasis model assessment of β-cell function (HOMA-B) · Homeostasis model assessment of insulin resistance (HOMA-IR) · Insulin resistance · Insulin secretion · Maturity-onset diabetes of the young (MODY) · Meta-analysis · Oral glucose tolerance test (OGTT) · Proinsulin · Risk prediction · Single nucleotide polymorphism (SNP) · Thrifty gene hypothesis · Type 2 diabetes

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Introduction

Type 2 diabetes (T2D) accounts for 90 % of diabetes cases worldwide, with a global prevalence of approximately 340 million affected individuals; a number that is expected to rise to 530 million by 2035 [1]. Two critical processes leading to T2D development are β-cell dysfunction and insulin resistance in peripheral tissues including fat, muscle, liver and elsewhere [2]. Beginning long before the clinical diagnosis of T2D, these processes are hallmarks of prediabetes, whose progressive deterioration reaches a point when β cells are no longer able to meet the increased insulin demands from peripheral tissues and herald a shift towards the development of diabetes. Trajectories to T2D vary significantly, with some individuals developing impaired glucose tolerance (IGT), characterised by increased glucose levels 2 h after an oral glucose tolerance test (OGTT), while maintaining normal or mildly elevated fasting glucose (FG, Table 1). Other non-diabetic individuals develop fasting hyperglycaemia, which is also related to faster progression to the clinical T2D endpoint, but is characterised by a different pathophysiologic mechanism [3]. Insulin resistance is typically associated with obesity or other metabolic abnormalities resulting in reduced binding of insulin to its receptor on the cell membrane, but may also result from impairments in insulin signal transduction [2, 4]. Uncorrected FG in subjects with T2D is related to microvascular and long-term macrovascular complications [5–8]. Evaluation of glycaemic control takes into

Table 1 Definition of glycaemic traits and their use in T2D diagnosis

Glycaemic trait	Definition
Fasting plasma glucose (FG)	Is a measure of the amount of glucose in a blood sample after having not eaten anything for at least 8 h (fasting). It is used as one of the diagnostic criteria for diabetes. According to the ADA 2013 guidelines, a level of 5.6 to 6.9 mmol/l (100–125 mg/dl) [95] is indicative of impaired fasting glucose (IFG), a feature of prediabetes, and a level of 7.0 mmol/l (126 mg/dl) or higher is indicative of diabetes [1, 9].
Random plasma glucose (RG)	Is a measure of the level of glucose in the blood without prior fasting. It can be used as a diagnostic criterion for T2D, when RG levels are higher than 11.1 mmol/l (200 mg/dl) in the presence of diabetic symptoms [1, 9].
1- and 2-h glucose from the oral glucose tolerance test (OGTT)	FG alone fails to diagnose approximately 30 % of cases of diabetes; for this reason, the World Health Organisation recommends also retaining the OGTT as a diagnostic test. After giving patients a liquid containing a predefined quantity of glucose (usually 75 g) to drink, glucose concentration in blood is measured at 30-min time intervals. The measurement taken after 1 h (1-h post-prandial glucose) can be used to monitor glucose metabolism. Two-hour post-prandial glucose level (2hGlu) is used as a diagnostic measurement and is considered as indicative of diabetes if higher than 11.1 mmol/l (200 mg/dl) [1, 9].
Corrected insulin response to glucose (CIR)	Is measured at 30 min during an OGTT and is an indication of postprandial insulin secretion. It takes into account the dose-response relationship between glucose and insulin after oral glucose loading, representing the resulting β-cell response. It is only recently that this measure has been analysed in genetic studies of association for glycaemic traits [64••].
Glycated haemoglobin (HbA_{1c})	Is a form of glycated haemoglobin that is formed in a non-enzymatic glycation pathway by haemoglobin's exposure to plasma glucose. It is indicative of the average plasma concentration of glucose over prolonged periods of time, as it is influenced by average glycaemia over a 2- to 3-month period. As an HbA_{1c} test is indicative of the body's long-term blood sugar control, it is useful in monitoring diabetic control and is also considered a diagnostic test for diabetes (if ≥6.5 %) by the American Diabetes Association [95], but not the WHO [9].
Fasting proinsulin (FP)	Is the pro-hormone precursor of mature insulin and C-peptide, made in the β cells of the islets of Langerhans, which are specialised pancreatic regions. Higher circulating levels of proinsulin are associated with impaired β-cell function, raised glucose levels, and insulin resistance and T2D and appear to indicate an advanced stage of β-cell exhaustion. Consequently, fasting proinsulin may be used as a marker to inform therapeutic decisions in T2D. A normal proinsulin level is 2 to 6 pmol/l.
Insulin	Is a hormone secreted by the pancreas in response to carbohydrate consumption, which facilitates transport of sugars from the bloodstream into the cells, where they are either stored or used to generate ATP. Insulin resistance occurs when insulin does not work optimally to drive glucose into cells and tissues. Exogenous doses of insulin are essential for the treatment of type 1 diabetes and also sometimes used in patients with T2D, either alone or in combination with other medications [1]. Measuring fasting insulin (FI) in the blood is helpful in the diagnosis of insulin resistance. Insulin excess is defined as insulin levels equal to or greater than 15 μIU/ml (micro International Units per millilitre).
Homeostasis model assessment of β-cell function (HOMA-B) and of insulin resistance (HOMA-IR)	Is a method that permits assessment of β-cell function and insulin resistance (IR) from basal FG and insulin or C-peptide concentrations. It is based on the balance between hepatic glucose output and insulin secretion that is regulated by a feedback loop between the liver and β cells. The original equations used to estimate HOMA-B and HOMA-IR were [(20×FI)/(FG×3.5)] and [(FI×FG)/22.5], respectively [96]. In 1996, an updated and more accurate computer model was developed, which is able to determine insulin sensitivity and β-cell function (%B) even from C-peptide concentrations [97]. HOMA-B and HOMA-IR have been widely studied (they appear in more than 500 publications) and are used in clinics to characterise the pathophysiology in the presence of abnormal glucose tolerance [98].

consideration a variety of measurements, including glycated haemoglobin (HbA_{1c}) [1, 9]. The altered states of related physiological measurements, e.g. fasting insulin (FI) and proinsulin levels, random glucose, β-cell function and insulin resistance, are also often taken into account to characterise the glucose homeostasis of an individual (Table 1). Glycaemic

metabolic control is the primary target of most T2D treatments (e.g. metformin, sulfonylurea) [4].

Large-scale genetic association studies have proven to be a powerful tool to unravel the aetiology of T2D, a complex disease with a considerable heritable component [10]. The dramatic increase in the availability of genetic data in the past 8 years, with the development of genotyping arrays and the advent of genome-wide association (GWA) studies, together with the collaborative efforts of researchers working in this field, has resulted in the identification of 88 genetic loci associated with T2D (Online Resource 1) [11•, 12•, 13••, 14, 15, 16•, 17, 18•, 19•, 20–24, 25•, 26, 27•, 28, 29•]. However, despite these advances, the overall effect attributed to these loci is low and their contribution is of little clinical usefulness compared to evaluation of classical risk factors such as body mass index (BMI), age and family history [30–34]. Moreover, the cumulative effect of these loci currently explains only approximately 10 % of the estimated heritability of T2D [19•]. The discovery of DNA variants acting on physiological quantitative phenotypes related to disease endpoints may help in the identification of additional factors and mechanisms contributing to the missing heritability of diseases in whose predisposition and pathogenesis they are involved [35]. To date, a number of large association studies have been conducted based on the hypothesis that models arising from genetic analysis of glycaemic quantitative traits may reflect mechanisms involved in the pathogenesis of diabetes (Table 2).

This article will review recent insights into the pathogenesis of T2D gained from the study of genetic variability of glycaemic traits and will focus on three main areas: what is known to date about the effects of glycaemic trait variants on T2D risk; how the mechanisms responsible for the maintenance of normal glucose homeostasis relate to those involved in the development of T2D; and what is the relationship between the effects of external factors and the processes leading from normal glucose homeostasis to T2D pathogenesis.

From Candidate Gene Analyses to First Steps in GWA Studies of Glycaemic Traits

Despite several attempts in the past decade to identify the genetic variants that contribute to variability in quantitative glycaemic traits through linkage studies, there was very limited success [36–38]. The only association with FG established before the GWA study era was at the *GCK* locus [39, 40•]. Encoding glucokinase (hexokinase 4), *GCK* is predominantly expressed in pancreatic β cells and the liver. Glucokinase is one of the principal regulators of FG concentration, catalysing the first step in glycolysis, which is the phosphorylation of glucose at the sixth carbon position. It also initiates the β-cell insulin secretory cascade. Through several linkage studies, it has been demonstrated that a subset of cases of maturity-onset diabetes of the young (MODY2), a particular subtype of diabetes characterised by onset before 25 years of age and caused by defects in insulin production or secretion, results from rare mutations in the *GCK* gene [41–44]. Based on this premise, Weedon and colleagues performed a candidate tagging SNP association study of FG in 19,806 subjects from six population-based studies. The rs1799884 variant, 46 bases upstream *GCK* gene, was associated with FG at a genome-wide level of significance ($P<5\times10^{-8}$) [39, 40•].

The picture has changed significantly with the advent of hypothesis-free genome-wide scans, aided by the new array-based SNP genotyping platforms which have enabled concurrent typing of initially hundreds of thousands, and more recently millions of SNPs at high accuracy and low cost, as well as the availability of catalogues of human genome-sequence variation (the HapMap Project, [45]) which permitted imputation of non-directly genotyped variants. In 2007, several GWA studies for T2D were published, providing a first picture of the landscape of T2D genetic susceptibility [46•, 47•, 48•, 49•]. These studies highlighted that the newly discovered variants contribute modestly to overall variance in diabetes risk and suggested that large-scale studies of glycaemic phenotypes may help in the identification of additional loci for T2D susceptibility [46•, 50•, 51•]. In fact, before the publication of the first glycaemic trait GWA studies, the Diabetes Genetics Initiative (DGI) T2D GWA study had demonstrated an indicative association at the glucokinase regulator (*GCKR*) locus (rs780094) with FG, insulin resistance and T2D as well as an association at the same variant with serum triglyceride levels [46•]. The product of *GCKR*, glucokinase regulatory protein (GKRP), regulates the first step of glycolysis, acting on GCK activity, and is therefore a highly plausible candidate involved in T2D pathogenesis. Association of the *GCKR* locus with both glycaemic traits and T2D susceptibility has since been confirmed in subsequent studies [13••, 52•]. This finding prompted further interest in well-powered GWA studies for glycaemic traits to detect reliable genetic associations which may be relevant to the pathogenesis of T2D.

At the beginning of 2008, two GWA studies for FG reported significant associations at two variants (rs560887 and rs563694, linkage disequilibrium [LD] $r^2=0.73$) located near the gene glucose-6-phosphatase catalytic subunit 2 (*G6PC2*) [50•, 51•]. The SNP rs560887 is in the third intron of the gene and was also associated with HbA_{1c} and homeostasis model assessment of β-cell function (HOMA-B), but not with FI or BMI [50•]. This supported the idea that *G6PC2* acts through an impact on β-cell function rather than on obesity-mediated insulin resistance. Indeed, the protein encoded by this gene catalyses the terminal step in the gluconeogenesis pathway

Table 2 GWA scans and meta-analyses for glycaemic traits

Phenotype(s)	Year	Sample size (follow-up or replication)	Novel loci for specific trait identified	GWA scan or meta-analysis	Reference
FG	2006	19,806	*GCK*	Weedon et al. (*AJHG*)	[40•][bf]
FG/HbA_{1c}/HOMA-B	2008	654 (9,353)	*G6PC2*	Bouatia-Naji et al. (*Science*)	[50•][f]
FG	2008	5,088 (18,436)	*G6PC2/ABCB11*	Chen et al. (*J Clin Invest*)	[51•]
FG	2009	2,151	*MTNR1B*	Bouatia-Naji et al. (*Nat Genet*)	[57••][f]
FG/HOMA-B	2009	36,610	*MTNR1B*	Prokopenko et al. (*Nat Genet*)	[54••][a]
FG/CIR	2009	7 different cohorts	*MTNR1B*	Lyssenko (*Nat Genet*)	[56••]
FG/FI/HOMA-B/HOMA-IR	2010	46,186 (766,558)	*ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1, C2CD4B, IGF1*	Dupuis et al. (*Nat Genet*)	[13••][a]
2hGlu	2010	15,234 (30,620)	*GIPR, VPS13C, ADCY5, GCKR, TCF7L2*	Saxena et al. (*Nat Genet*)	[58••][a]
HbA_{1c}	2010	46,368	*FN3K*, *HFE*, *TMPRSS6*, *ANK1*, *SPTA1, ATP11A/TUBGCP3*	Soranzo et al. (*Diabetes*)	[60••][a]
FP	2011	10,701 (16,378)	*LARP6, SGSM2/SRR, ARAP1, MADD, TCF7L2, VPS13C/ C2CD4A/B, SLC30A8, PCSK1, DDX31, SNX7*	Strawbridge et al. (*Diabetes*)	[61••][a]
FG/FGadjBMI/FI/FIadjBMI/ HOMA-B/HOMA-IR	2012	58,074 FG; 51,750 FI (38,422 FG; 33,823 FI)	*COBLL1/GRB14, IRS1, PPP1R3B, PDGFC, UHRF1BP1, LYPLAL1, ARAP1, FOXA2, DPYSL5, PCSK1, PDX1, OR4S1*	Manning et al. (*Nat Genet*)	[52•][a]
FG/FGadjBMI/FI/FIadjBMI/ 2hGlu/2hGluadjBMI	2012	133,010 FG; 108,557 FI; 42,854 2hGlu	*CDKN2B, PCSK1, PDX1, PPP1R3B, GRB10, ARAP1, FOXA2, IKBKAP, DNLZ, WARS, GIPR, CDKAL1, P2RX2, TOP1, IGF2BP2, KL, AMT, RREB1, ZBED3, GLS2, FTO, TET2, TCF7L2, GRB14, HIP1, LYPLAL1, RSPO3, PEPD, ARL15, IRS1, YSK4, IRS1, PPARG, ANKRD55-MAP3K1, PDGFC, FAM13A, UHRF1BP1, ERAP2*	Scott et al. (*Nat Genet*)	[63••][a]
CIR and other insulin secretion traits	2014	26,037	*GRB10, MTNR1B, HHEX/IDE/ KIF11, CDKAL1, GIPR, C2CD4A, GCK, ANK1*	Prokopenko et al. (*PLOS Genet*)	[64••][a]
Insulinogenic index and 32,33 split proinsulin	2014	11,268 and 2568	*HHEX/IDE, MTNR1B* and *ARAP1*	Dimas et al. (*Diabetes*)	[65••][a]
Studies in other ethnicities					
FG	2009	7,474	*MTNR1B*	Chambers et al. (*Diabetes*)	[69][c]
HbA_{1c}/1hGlu	2012	4,275 (3,782)	*CDKN1L*	Ryu & Lee (*Hum Mut*)	[70][d]
FI/HOMA-IR	2012	927 (570)	*SC4MOL, TCERG1L*	Chen et al. (*Hum Mol Gen*)	[71][ef]
1hGlu	2013	7,696 (6,536)	*MYL2, C12orf51, OAS1*	Go et al. (*J Hum Genet*)	[72][df]

[a] Meta-analyses published by MAGIC consortium

[b] Candidate gene study

[c] Study in Indian Asian individuals

[d] Study in Korean individuals

[e] Study in African individuals

[f] All studies were carried out in individuals with FG of <7 mmol/l, with the exception of [f] studies, where a more conservative cutoff of FG <6.1 mmol/l was used

FG fasting glucose, *FI* fasting insulin, *FP* fasting proinsulin, *FGadjBMI* fasting glucose adjusted for BMI, *FIadjBMI* fasting insulin adjusted for BMI, *2hGlu* 2-h post-prandial glucose, *1hGlu* 1-h post-prandial glucose, HbA_{1c} glycated haemoglobin, *HOMA-B* homeostasis model assessment of β-cell function, *HOMA-IR* homeostasis model assessment of insulin resistance, *CIR* corrected insulin response to glucose

and is preferentially expressed in pancreatic islets [50•]. However, neither of the two studies found significant association of any variant in the *G6PC2* region with T2D, suggesting that variation at this locus might alter the glucose set point level for induced insulin secretion in pancreatic β cells, but additional factors may be required to lead to the development of T2D [50•, 51•]. Another interesting observation is that G6PC2 acts in the same glucose phosphorylation pathway as GCK and GKRP, suggesting that a complex interaction may exist between these three proteins that contributes to the regulation of glucose levels. Despite the small effect of the *G6PC2* locus variants on FG concentration (~0.065 mmol/l per trait-increasing allele), these studies provided the first evidence that GWA studies on glycaemic phenotypes could help in the identification of candidate loci providing an improved understanding of the physiological and pathophysiological processes of glucose metabolism, and began a wave of subsequent GWA studies.

First Glycaemic Trait Meta-analysis Establishes a Novel T2D Locus

At the beginning of 2008, a collaborative effort of four GWA study consortia, including the European Network of Genomic And Genetic Epidemiology (ENGAGE); the Framingham Heart Study (FHS) [53]; the DFS collaboration combining Diabetes Genetics Initiative (DGI), Finland-United States Investigation of NIDDM Genetics (FUSION) and National Institute on Aging (NIA) SardiNIA studies; and the Genetic of Energy Metabolism (GEM), was established [54••] as the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC, http://www.magicinvestigators.org/). MAGIC aims at consolidating the efforts of many groups working on glycaemic trait genetics, in order to understand the variation of these traits within the physiological range, and investigating the impact of glycaemic trait loci on T2D risk and other cardiometabolic traits. Initial data were available for 35,812 non-diabetic individuals from ten studies of individuals of European descent. The first MAGIC effort started as an exchange of identities of between 10 to 20 SNPs prominently associated with FG in the interim meta-analyses of four groups [54••]. It enabled confirmation of associations at the *GCK* and *G6PC2* loci and the identification of a significant signal (rs10830963) at the melatonin receptor 1B (*MTNR1B*) locus for FG and HOMA-B. Association with T2D initially fell just short of strict significance thresholds at the genome-wide level, but was subsequently confirmed [13••, 54••, 55•, 56••]. Simultaneously, another FG- and T2D-associated variant (rs1387153, LD r^2=0.6) at *MTNR1B* was reported in an independent GWA study [57••].

Expanding the Power of Meta-analysis Through Increasing Sample Size and Phenotypes Studied

To extend the previous approach, a new, larger, GWA meta-analysis (21 studies, up to 46,186 non-diabetic individuals) was performed by the MAGIC investigators, expanding the number of glycaemic trait loci to 16 [13••], including nine newly discovered loci for FG and HOMA-B (*ADCY5*, *MADD*, *ADRA2A*, *CRY2*, *FADS1*, *GLIS3*, *SLC2A2*, *PROX1* and *C2CD4B*) and one new locus (*IGF1*) for FI and homeostasis model assessment of insulin resistance (HOMA-IR). In a large-scale analysis of the FG/FI-associated loci for their role in diabetes susceptibility, novel effects on T2D at five of the loci (*ADCY5*, *GCK*, *GCKR*, *DGKB*, *PROX1*) were reported at genome-wide significance, thus providing support to the observation that the overlap between the genetic variation influencing glucose homeostasis and risk of T2D is only partial (Fig. 1a) [13••].

Following this study, MAGIC published three further GWA meta-analyses on other glycaemic traits. A study focusing on 2-h postprandial glucose (2hGlu) levels (15,234 non-diabetic individuals in discovery and up to 30,620 in replication) identified five associated loci (*GIPR*, *VPS13C*, *ADCY5*, *GCKR*, *TCF7L2*), including the novel locus *GIPR* containing the gene encoding the GIP receptor for the insulin-response stimulating hormone GIP (glucose-dependent insulinotropic polypeptide) in pancreatic islet β cells [58••]. Moreover, *GIPR* was associated with insulin response as measured by the OGTT, but showed no significant association with insulin response from the intravenous glucose tolerance test (IVGTT). In fact, GIP is not expected to influence the insulin response to an intravenous glucose load, since it manifests its stimulating activity only after an oral glucose challenge [58••]. The *GIPR* locus was only weakly associated with T2D [58••]. Later, a meta-analysis by the Diabetes Genetics Replication and Meta-analysis (DIAGRAM, http://www.diagram-consortium.org/) consortium established association with T2D at *GIPR* with larger effects in women at rs8108269, which is only weakly correlated with 2hGlu rs10423928 (HapMap CEU r^2=0.07) [19•]. These findings advocate further investigation of the complex genetic architecture at *GIPR* locus.

Genome-wide meta-analyses of HbA_{1c} in non-diabetic individuals initially identified the hexokinase 1 (*HK1*) locus in the 14,618 participants of the Women's Genome Health Study and also showed associations at *GCK*, *G6PC2* and *SLC30A8* [59]. Ten loci for HbA_{1c} were reported in GWA meta-analysis of 46,368 individuals by the MAGIC investigators, of which six were novel (*FN3K*, *HFE*, *TMPRSS6*, *ANK1*, *SPTA1* and *ATP11A*/*TUBGCP3*) and four were already established (*HK1*, *MTNR1B*, *GCK* and *G6PC2*) [60••]. Remarkably, only three of the ten reported HbA_{1c} signals (*G6PC2*, *GCK* and

MTNR1B) were also related to hyperglycaemia, with only the latter two involved in T2D susceptibility. Variants in the *ANK1* locus were subsequently associated with T2D, though only weak LD with the HbA_{1c} signal was observed [19•]. This study highlighted that six loci influence HbA_{1c} via non-glycaemic erythrocyte and iron biology pathways (Fig. 1a) [60••].

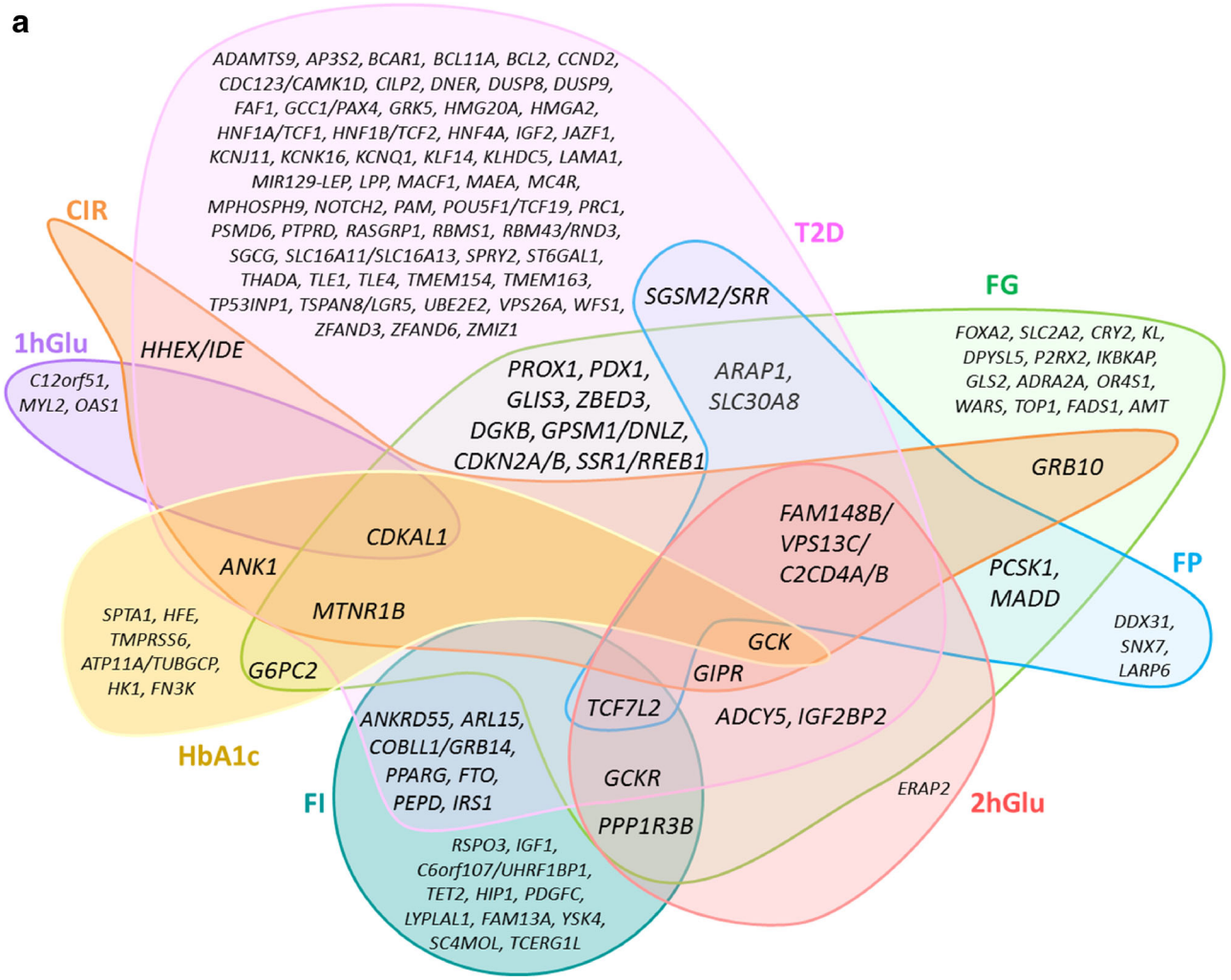
The increased circulating fasting proinsulin (FP) levels, induced in response to metabolic demands and not adequately processed by β cells into insulin, predict future T2D. Meta-analysis of GWA studies by MAGIC for FP levels adjusted for FI in order to identify loci associated specifically with relative levels of FP as opposed to overall insulin production identified ten loci, of which four demonstrated that both proinsulin-raising (for *TCF7L2*, *SLC30A8* and *VPS13C*/*C2CD4A*/*B*) and lowering alleles (for *ARAP1*) influence T2D risk through a decrease in insulin secretion caused by an impairment of β-cell function in the proinsulin conversion process, either distal or proximal, respectively [61••]. A lipid-associated locus at *MADD* [62] contributes to hyperglycaemia but not to T2D susceptibility, whereas three remaining proinsulin loci (*LARP6*, *SNX7*, *DDX31*) do not lead either to higher T2D risk nor to higher glycaemia, suggesting that it is a specific impairment in β-cell proinsulin processing rather than a mere elevation in proinsulin levels that contribute to pathological hyperglycaemia [61••].

Cost-Effective Large-Scale Follow-up Using Custom Metabochip Array

By 2009, it had become evident that existing GWA meta-analyses could provide, on the one hand, an excellent background for the identification of additional associated genetic loci and, on the other hand, deeper characterisation of established loci through fine mapping to detect the potential causal variants. Collaboration between six GWA study consortia on metabolic and atherosclerotic/cardiovascular diseases and traits supported the design of CardioMetabochip (Metabochip), an Illumina iSelect genotyping array [27•]. It permitted the follow-up of ~66,000 putative signals for cardiometabolic phenotypes and fine-mapping of 257 established loci (approximately 120,000 SNPs). Through the use of Metabochip genotyping as a cost-effective large-scale in silico follow-up of discovery GWA meta-analyses [13••], MAGIC was able to extend dramatically the number of collaborating studies and combine data from 133,010 individuals for FG, 108,557 for FI and 42,854 for 2hGlu [63••]. The follow-up strategy using Metabochip resulted in the discovery of 41 glycaemic associations not previously described: 20 loci were described for FG, 17 for FI, and four for 2hGlu (Table 3) [63••]. This brought the number of glycaemic trait loci to 53, including 36/19/9 for FG/ FI/ 2hGlu, respectively. All FG-raising alleles were related to increased T2D risk, and 20 (>60 %) were at least nominally associated ($P<0.05$) with T2D. Similarly, 13 of the 19 FI loci had nominal association with T2D, and all (except for *TCF7L2*) FI/insulin resistance increasing alleles were associated with higher T2D risk and showed an impaired lipid profile (Fig. 1b) [63••]. Subsequent GWA meta-analyses for glycaemic traits used Metabochip extensively for follow-up purposes.

In parallel, Manning and colleagues from MAGIC presented a joint meta-analysis approach for genetic association with fasting glycaemic phenotypes to evaluate the role of obesity in the development of insulin resistance [52•]. The method implemented by the authors simultaneously tested the main genetic effects on glycaemic traits, adjusted and unadjusted for BMI (as an index of adiposity), and potential interaction between each genetic variant and BMI, with the aim of discovering novel loci preferentially involved in insulin resistance pathways [52•]. Six loci not previously known to be associated with FI levels were discovered as well as seven

Fig. 1 Multi-phenotype effects of glycaemic trait loci: (**a**) Overlap ▶ between established glycaemic trait and T2D loci. (**b**) Overlap between established glycaemic and other cardiometabolic trait loci. (**c**) Heatmap of the effects of T2D and glycaemic loci on T2D and glycaemic traits from published GWA meta-analyses (FG [52•], FI [52•], FIadjBMI [52•], FP [61••], 2hGlu [58••], HOMA-B [52•] and HOMA-IR [52•], HbA_{1c} [60••], CIR [64••], T2D [55•]). **Legend**. We considered established associated variants in a context of genetic loci, where each locus represents a region of less than 300 kb containing one or more SNPs associated with T2D, glycaemic traits or both and with an LD value of $r^2 \geq 0.02$. A secondary signal in the same locus lies within 300 kb from an originally established signal, but has an LD value of $r^2 < 0.02$ with the primary associated top variant. In Fig. 1c, we have reported the strength and direction of associations from the discovery meta-analyses of GWA studies; therefore, the sample sizes were usually smaller than those reported after the replication or within combined analyses. Hence, for some loci, established associations, reported in the literature, were not genome-wide significant in the discovery GWA meta-analyses used for the heatmap: we thus listed all established associations as exceeding the genome-wide significance threshold ($P=5\times10^{-8}$), secondary signals within three T2D loci are also reported. Of the 131 established T2D (of which six with secondary signals) and/or glycaemic trait loci, 11 (*DUSP9*, *TCERG1L*, *MYL2, IGF2, PAM, RBM43/RND3, OAS1, C12orf51, SGCG, HNF1B, SLC16A13/SLC16A11*) loci and two (*PAM*, *CCND2*) secondary signals are not presented in the figure, because they contained missing data for five or more phenotypes, 29 glycaemic loci are not shown since they have not reached at least nominally significant ($P<0.05$) association with T2D, and vice versa, 18 T2D loci and one secondary signal are not reported since no nominally significant association with glycaemic traits was observed. For an extended picture including all loci and secondary signals, see Online Resource 1. Phenotypes: *FG* fasting glucose, *FI* fasting insulin, *FP* fasting proinsulin, *FIadjBMI* fasting insulin adjusted for BMI, *2hGlu* two-hour post-prandial glucose, *1hGlu* one-hour post-prandial glucose, *HbA_{1c}* glycated haemoglobin, *HOMA-B* homeostasis model assessment of β-cell function, *HOMA-IR* homeostasis model assessment of insulin resistance, *CIR* corrected insulin response to glucose, *adjBMI* adjusted for BMI. Available data from the latest publications of GWA meta-analyses with Metabochip follow-up by Morris et al. [19•] for T2D and Scott et al. [63••] for glycaemic traits were not used in this figure, since a large number of SNPs (≈30 %) were not genotyped directly on the array


a
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CIR
T2D
FG
1hGlu
C12orf51, MYL2, OAS1
HHEX/IDE
SGSM2/SRR
FOXA2, SLC2A2, CRY2, KL, DPYSL5, P2RX2, IKBKAP, GLS2, ADRA2A, OR4S1, WARS, TOP1, FADS1, AMT
PROX1, PDX1, GLIS3, ZBED3, DGKB, GPSM1/DNLZ, CDKN2A/B, SSR1/RREB1
ARAP1, SLC30A8
GRB10
CDKAL1
FAM148B/ VPS13C/ C2CD4A/B
ANK1
PCSK1, MADD
FP
SPTA1, HFE, TMPRSS6, ATP11A/TUBGCP, HK1, FN3K
MTNR1B
GCK
DDX31, SNX7, LARP6
G6PC2
GIPR
TCF7L2
ADCY5, IGF2BP2
ANKRD55, ARL15, COBLL1/GRB14, PPARG, FTO, PEPD, IRS1
HbA1c
GCKR
ERAP2
FI
2hGlu
PPP1R3B
RSPO3, IGF1, C6orf107/UHRF1BP1, TET2, HIP1, PDGFC, LYPLAL1, FAM13A, YSK4, SC4MOL, TCERG1L
b
Glycaemic Traits
T2D
IGF1, C6orf107/UHRF1BP1, GRB10, G6PC2, ERAP2, YSK4, WARS, SLC2A2, CRY2, DDX31, DPYSL5, HK1, AMT, MADD, FOXA2, KL, HIP1, ADRA2A, LARP6, SPTA1, TMPRSS6, P2RX2, IKBKAP, ATP11A/TUBGCP3, PDGFC, SNX7, GLS2, FAM13A, OR4S1, FN3K, C12orf51, MYL2, OAS1, SC4MOL, TCERG1L
SGSM2/SRR, PROX1, ZBED3, MTNR1B, TCF7L2, SLC30A8, GLIS3, GCK, ADCY5, ANK1, ARAP1, CDKN2A/B, HHEX/IDE, DNLZ, SSR1/RREB1, DGKB, PDX1
AP3S2, BCAR1, BCL11A, BCL2, CCND2, CDC123/CAMK1D, DNER, DUSP8, DUSP9, FAF1, GCC1/PAX4, GRK5, HMG20A, HNF1B/TCF2, IGF2, KCNJ11, KCNK16, KLHDC5, LAMA1, MIR129-LEP, LPP, MACF1, MAEA, MPHOSPH9, NOTCH2, PAM, POU5F1/TCF19, PRC1, PSMD6, PTPRD, RASGRP1, RBMS1, RBM43/RND3, SGCG, SLC16A11/SLC16A13, ST6GAL1, THADA, TLE1, TLE4, TMEM154, TMEM163, TP53INP1, TSPAN8/LGR5, UBE2E2, VPS26A, WFS1, ZFAND3, ZFAND6, ZMIZ1
PPP1R3B, FADS1, TOP1
TET2
JAZF1, KCNQ1, HMGA2
IGF2BP2, FAM148B/VPS13C/ C2CD4A/B
ANKRD55, GCKR, ARL15, PEPD
HNF4A, CILP2, KLF14, HNF1A/TCF1
PCSK1
FTO, IRS1
Obesity
92
Lipids
PPARG, CDKAL1, GIPR
2
58
HFE
1
ADAMTS9, SPRY2
COBLL1/ GRB14
RSPO3
1
MC4R
LYPLAL1
Blood pressure and hypertension
Height
168
2
60

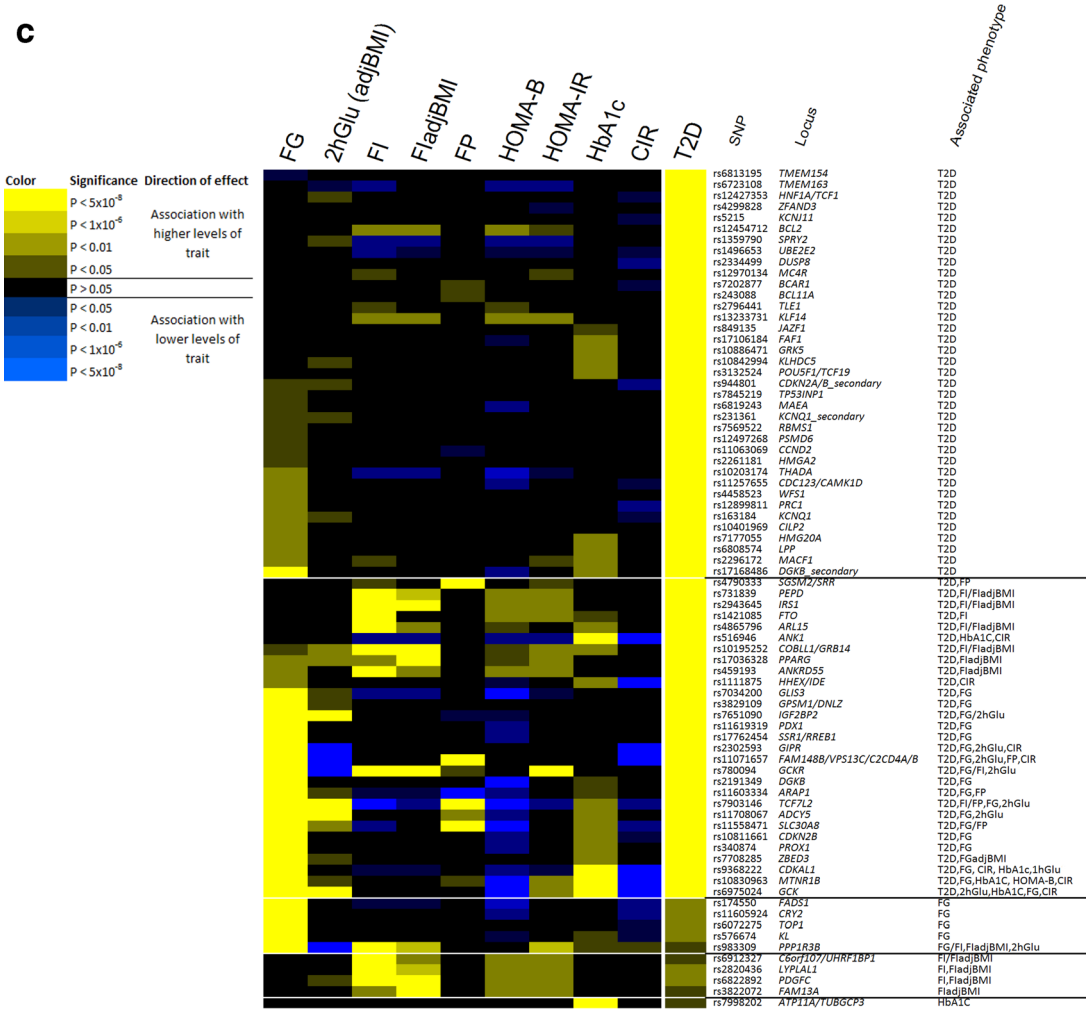

Fig. 1 (continued)

additional loci associated with FG levels. Most of the novel loci associated with higher insulin levels were also associated with lower high-density lipoprotein (HDL) cholesterol and higher triglycerides, suggesting that such a dyslipidemic profile may be a marker of insulin resistance (Fig. 1b). The association of FI corrected for BMI with the *COBLL1*/*GRB14* locus was a finding of particular interest, since several studies suggested that GRB14 may be a tissue-specific negative regulator of insulin receptor signalling through the regulation of adipose tissue distribution [52•].

Recently, two additional studies have examined associations with a wider range of glycaemic phenotypes which are specific indices of β-cell function, insulin secretion and action [64••, 65••]. The first meta-analysis performed a global search and established eight genome-wide significant loci for corrected insulin response (CIR), all of which were previously associated with T2D and/or other glycaemic traits (*MTNR1B*, *G6PC2*, *GCK*, *HHEX/IDE*, *CDKAL1*, *CDKN2A/2B*, *ANK1*, *C2CD4A/B*) (Fig. 1c) [64••]. The only novel locus, *GRB10* variant rs933360, was revealed to be implicated in a complex mechanism of glucose homeostasis regulation (including glucose concentration, insulin secretion and sensitivity) that potentially involves tissue-specific methylation and parental imprinting and leads to different effects depending on maternal or paternal inheritance [64••]. *GRB10* does not have a marked effect on T2D risk, although this may be because of potential bias resulting from this parent-of-origin effect. In a second study, which examined the effect of 37 known T2D loci on indices of insulin processing, secretion, sensitivity and clearance, Dimas and colleagues described associations at the *HHEX/IDE* and *MTNR1B* loci with defects in early insulin secretion through reduced insulinogenic index for the T2D risk allele, while the T2D risk allele at *ARAP1* was related to defects in the first steps of insulin production, through association with 32,33 split proinsulin [65••].

Table 3 Established genetic variants associated with glycaemic traits at a genome-wide level of significance ($P \leq 5x10^{-8}$) and their effect on T2D risk

Associated phenotypes[a]	Chromosome: position (HG19)[b]	Most strongly associated SNP (followed by other published SNPs)[c]	Locus name[d]	Reference
Established T2D loci				
FG	1:214,159,256	rs340874	*PROX1*	[13••]
2hGlu, FG/FI	2:27,730,940	rs1260326, rs780094	*GCKR*	[13••, 58••]
FI/FIadjBMI, FI	2:165,513,091	rs10195252, rs7607980	*COBLL1/GRB14*	[52•, 63••]
FI/FIadjBMI	2:227,068,080	rs2943634, rs2943645, rs2972143	*IRS1*	[52•, 63••]
FIadjBMI	3:12,390,484	rs17036328	*PPARG*	[63••]
FG, 2hGlu	3:123,065,778	rs11708067, rs11717195	*ADCY5*	[13••, 58••]
FG/2hGlu	3:185,513,392	rs7651090	*IGF2BP2*	[63••]
FI/FIadjBMI	5:53,272,664	rs4865796	*ARL15*	[63••]
FIadjBMI	5:55,806,751	rs459193	*ANKRD55*	[63••]
FGadjBMI	5:76,425,867	rs7708285	*ZBED3*	[63••]
FG, CIR, HbA_{1c}/1hGlu	6:20,686,996	rs9368222, rs7756992, rs7747752[e]	*CDKAL1*	[63••, 64••, 70]
FG	6:7,213,200	rs17762454	*SSR1/RREB1*	[63••]
FG	7:15,064,309	rs2191349	*DGKB*	[13••]
FG/HbA_{1c}, 2hGlu, FG, CIR	7:44,229,068	rs1799884, rs6975024, rs4607517, rs3757840	*GCK*	[13••, 40•, 60••, 63••, 64••]
HbA_{1c}, CIR	8:41,549,194	rs6474359, rs4737009, rs12549902	*ANK1*	[60••, 64••]
FG/FP	8:118,185,733	rs11558471	*SLC30A8*	[13••, 61••]
FG	9:4,289,050	rs7034200	*GLIS3*	[13••]
FG	9:22,134,094	rs10811661	*CDKN2A/B*	[63••]
FG	9:139,256,766	rs3829109	*DNLZ*	[63••]
CIR	10:94,482,076	rs7923866	*HHEX/IDE*	[64••]
FG, FI/FP, 2hGlu	10:114,756,041	rs4506565, rs7903146, rs12243326	*TCF7L2*	[13••, 58••, 61••, 63••]
FG/FP	11:72,432,985	rs11603334	*ARAP1*	[52•, 61••, 63••]
FG/HbA_{1c}, FG/CIR, FG	11:92,673,828	rs1387153, rs10830963, rs2166706[f]	*MTNR1B*	[13••, 54••, 56••, 57••, 60••, 64••, 69]
FG	13:28,487,599	rs11619319, rs2293941	*PDX1*	[52•, 63••]
2hGlu, FP/CIR, FG	15:62,332,980	rs17271305, rs4502156, rs11071657	*FAM148B/VPS13C/C2CD4A/B*	[13••, 58••, 61••, 64••]
FI	16:53,800,954	rs1421085	*FTO*	[63••]
FP	17:2,262,703	rs4790333	*SGSM2/SRR*	[61••]
FI/FIadjBMI	19:33,899,065	rs731839	*PEPD*	[63••]
2hGlu, FG, CIR	19:46,182,304	rs10423928, rs2302593, rs11671664	*GIPR*	[58••, 63••]
Loci showing suggestive association ($P<0.05$) with T2D in [19]				
FI, FI, FIadjBMI	1:219,640,680	rs2820436, rs2785980, rs4846565	*LYPLAL1*	[52•, 63••]
FIadjBMI	4:89,741,269	rs3822072	*FAM13A*	[63••]
FI, FIadjBMI	4:157,720,124	rs4691380, rs6822892	*PDGFC*	[52•, 63••]
FG/FI, FG/FI, FIadjBMI, 2hGlu	8:9,177,732	rs983309, rs4841132, rs2126259, rs11782386	*PPP1R3B*	[52•, 63••]
HbA_{1c}	10:71,093,392	rs16926246	*HK1*	[60••]
FG	10:113,042,093	rs10885122	*ADRA2A*	[13••]
FG	11:45,873,091	rs11605924	*CRY2*	[13••]
FG	13:33,554,302	rs576674	*KL*	[63••]
FG	20:22,557,099	rs6113722, rs6048205	*FOXA2*	[52•, 63••]
FG	20:39,743,905	rs6072275	*TOP1*	[63••]
Other glycaemic trait loci				
FPadjFG	1:99,404,665	rs9727115	*SNX7*	[61••]
HbA_{1c}	1:158,585,415	rs2779116	*SPTA1*	[60••]
FG/FGadjBMI	2:27,152,874	rs1371614	*DPYSL5*	[52•]

Table 3 (continued)

Associated phenotypes[a]	Chromosome: position (HG19)[b]	Most strongly associated SNP (followed by other published SNPs)[c]	Locus name[d]	Reference
FI	2:135,755,629	rs1530559	*YSK4*	[63••]
FG/HbA_{1c}, HbA_{1c}	2:169,763,148	rs560887, rs552976	*G6PC2*	[13••, 50•, 60••]
FG	3:49,455,330	rs11715915	*AMT*	[63••]
FG	3:170,717,521	rs11920090	*SLC2A2*	[13••]
FIadjBMI, FI	4:106,071,064	rs974801, rs9884482	*TET2*	[63••]
FI	4:166,255,704	rs17046216	*SC4MOL*	[71]
FG, FG, FP	5:95,539,448	rs4869272, rs13179048, rs6235	*PCSK1*	[52•, 61••, 63••]
2hGlu	5:96,254,817	rs1019503	*ERAP2*	[63••]
HbA_{1c}	6:26,093,141	rs1800562	*HFE*	[60••]
FIadjBMI, FI	6:34,764,922	rs6912327, rs4646949	*C6orf107/UHRF1BP1*	[52•, 63••]
FI	6:127,452,935	rs2745353	*RSPO3*	[63••]
1hGlu	7:26,432,907	rs1229654	*MYL2*	[72]
FG, CIR	7:50,791,579	rs6943153, rs933360	*GRB10*	[63••, 64••]
FI	7:75,176,196	rs1167800	*HIP1*	[63••]
FG	9:111,680,359	rs16913693	*IKBKAP*	[63••]
FP	9:135,470,176	rs306549	*DDX31*	[61••]
FI	10:132,751,498	rs7077836	*TCERG1L*	[71]
FP, FP, FG	11:47,293,799	rs10501320, rs10838687, rs7944584	*MADD*	[13••, 61••]
FG/FGadjBMI	11:48,333,360	rs1483121	*OR4S1*	[52•]
FG	11:61,571,478	rs174550	*FADS1*	[13••]
FG/FGadjBMI	12:56,865,338	rs2657879	*GLS2*	[63••]
FI	12:102,875,569	rs35767	*IGF1*	[13••]
1hGlu	12:112,645,401	rs2074356	*C12orf51*	[72]
1hGlu	12:113,365,621	rs11066453	*OAS1*	[72]
FG	12:133,041,618	rs10747083	*P2RX2*	[63••]
HbA_{1c}	13:113,331,868	rs7998202	*ATP11A/TUBGCP3*	[60••]
FG	14:100,839,261	rs3783347	*WARS*	[63••]
FP	15:71,109,147	rs1549318	*LARP6*	[61••]
HbA_{1c}	17:80,685,533	rs1046896	*FN3K*	[60••]
HbA_{1c}	22:37,462,936	rs855791	*TMPRSS6*	[60••]

[a] Associated phenotypes follow rsID order and are divided by "," if reported in the literature as associated with different SNPs within the same locus, or by "/" if the same SNP was reported for more phenotypes. Phenotypes: *FG* fasting glucose, *FI* fasting insulin, *FP* fasting proinsulin, *FGadjBMI* fasting glucose adjusted for BMI, *FIadjBMI* fasting insulin adjusted for BMI, *2hGlu* two-hour post-prandial glucose, *1hGlu* one-hour post-prandial glucose, *HbA_{1c}* glycated haemoglobin, *CIR* corrected insulin response to glucose

[b] Position of the most significant variant

[c] Variant from the largest published meta-analysis listed first in the column "Reference"

[d] Nearby gene/genes

[e] Variant associated with HbA_{1c} and 1hGlu in a Korean cohort, from Ryu and Lee (Hum Mut 2012)

[f] Variant associated with FG in an Indian Asian cohort, from Chambers et al. (Diabetes 2009)

Glycaemic Trait GWA Studies in Non-European Cohorts

The prevalence of T2D differs between ethnic groups [66]. When examining the genetic component of susceptibility to common diseases, it is expected that common variants arose prior to the modern human exodus from Africa and therefore are represented amongst all populations. In contrast, lower-frequency alleles should be more recent, thus not widely represented and restricted to a particular or a limited number of populations. It is thus of great importance to undertake genetic studies of T2D and related continuous traits in other ethnicities, in order to discover new variants and to validate associations established in Europeans [67, 68]. To date, most of the discoveries for quantitative glycaemic traits have been made in samples of European origin from Europe or North America. Nevertheless, growing numbers of GWA studies in

samples from other parts of the world for diabetes-related traits have begun to appear [69–72]. Most of these studies are confirmatory for the associations of loci previously described in Europeans. In particular, *MTNR1B* was confirmed for FG in an Indian Asian sample and a Korean sample [69, 72]; the *CDKAL1* locus was instead strongly associated with 1-h postprandial glucose (1hGlu) in two independent Korean studies [70, 72], confirming its involvement in glucose metabolism. Likewise, *GCK*, *GCKR*, *G6PC2*, *IRS1* and *FTO* were confirmed in Indian Asian, Korean and African studies [69, 71, 72]. Recent non-European GWA studies have also reported association of two new loci encoding proteins involved in lipid metabolism, *SC4MOL* and *TCERG1L*, with FI and HOMA-IR in an African population [71] and association of the *MYL2*, *C12orf51* and *OAS1* loci with 1hGlu levels in a Korean study [72]. Furthermore, the increasing number of studies in non-European populations will enable combination of GWA studies across populations through trans-ethnic meta-analyses utilising the increased power of large sample sizes to identify causal variants shared across groups of differing ancestry.

GWAs of Glycaemic Traits and T2D: Are We Fishing from the Same Pond?

Since the first round of GWA studies for T2D that generated a number of new robust phenotype-genotype associations, it became clear that there is poor understanding of the functional role of many regional candidates [2]. Various research fields have undergone huge advancement since then and aimed to provide new insights into the mechanisms of disease. In GWA studies, the availability of large population cohorts with individuals unselected for diabetes status and with phenotypic information for the clinically relevant glycaemic traits allowed direct comparison between genetic effects (a) on glycaemic trait variability in the physiological range and (b) related to the pathological deterioration of glycaemic control in T2D subjects. Given the physiological link between processes regulating normal glucose homeostasis and those implicated in the pathogenesis of T2D, it was initially expected that the same loci would be associated with both these phenotypes. In fact, the first examples of FG loci discovered in large-scale studies, including *GCK* and *MTNR1B*, indeed affect both phenotypes [54••]. However, contradicting this hypothesis, *G6PC2* variants showed no effect on T2D risk [54••]. Since then, large-scale GWA meta-analyses for both glycaemic traits and T2D have increased dramatically the number of associated loci [13••, 63••]. Strikingly, we observe only 29 regions containing established overlapping loci reaching genome-wide significance ($P<5\times10^{-8}$) out of 88 T2D and 72 glycaemic trait associated loci (Table 3; Fig. 1a, c; Online Resource 1). Moreover, only 10 additional loci associated with FG/FI/HbA_{1c}/2hGlu show at least nominal association with T2D. Apart from *PPP1R3B* (locus associated with FG/FI/2hGlu, where 2hGlu-raising allele is associated with lower FG, FI and T2D risk [63••]), all other loci demonstrate an expected direction of the estimated effect on T2D susceptibility (Table 3) [19•]. These observations confirm that the pools of glycaemic trait and T2D loci are not identical and overlap only partially.

Shared loci, upon comparison, revealed additional complexity: the magnitudes of effects between described T2D and FG associations were demonstrated to be only weakly correlated, indicating that the mechanisms responsible for the pathogenesis of T2D and those influencing normal glucose homeostasis do not completely overlap [19•, 55•, 63••]. In fact, some loci (e.g. *TCF7L2* and *CDKN2A/B*) with the largest effects on T2D risk through their primary effect on β-cell function have only modest impact on FG variability in non-diabetic individuals, while other loci with the greatest contributions to FG levels, e.g. (*G6PC2*, *MADD* and *MTNR1B*, *GCK*), have no detectable effects on or show modest contribution to T2D susceptibility, respectively [13••, 63••]. One possible explanation of these disproportionate effects is that additional interactions with other factors may need to be accounted for to observe an effect on T2D. For example, for *G6PC2*, it has been hypothesised that the combined opposite activities of GCK and G6PC2 may be affected by genetic variation having an effect on pulsatile insulin secretion, which could interfere with normal insulin signalling and cause insulin resistance and subsequently T2D [73•]. Another reason for the observed disproportionate effects lies in the design of GWA studies, which do not test directly effects of the causal variants but look into associations with the tagging SNPs, leading to effects and correlations that appear weaker than in reality.

An equally complex scenario is observed for the overlap between genes causing monogenic forms of diabetes and glycaemic trait and T2D risk loci. It is known that common variants at several genes causative for monogenic forms of diabetes have an effect on T2D [19•, 55•]. Some of these genes (*PPARG*, *GLIS3*, *GCK*, *PDX1*) also contain variants associated with glycaemic traits [13••, 63••]. Interestingly, a variant at the *SLC2A2* locus, involved in permanent neonatal diabetes mellitus (PNDM) and MODY, shows association with FG [13••], but has not shown an effect on T2D in GWA studies to date.

Mechanistic Heterogeneity of T2D Loci on Physiological Traits in Non-diabetic Individuals

Large-scale discovery efforts in non-diabetic individuals have prompted investigation of the mechanistic role of T2D loci in

the pathogenesis of disease. While mechanistic effects of some compelling regional candidates are clear, *GCK* and *GCKR* being amongst the examples [74], inferences about most obvious gene candidates and their pathophysiological role for many association signals are hard to make. GWA studies of basal indices of glucose homeostasis (HOMA-B and HOMA-IR, Table 1) in normoglycaemic non-diabetic individuals have demonstrated that a large number of T2D loci contribute to the impairment of β-cell function and insulin secretion [13••, 19•, 54••, 55•, 63••]. Physiological characterisation of the effects of glycaemic and T2D loci on quantitative glycaemic traits also revealed a clear separation of hyperglycaemic loci (*MTNR1B* and *GCK*), with an effect on reduced insulin secretion and with suggested reduction in basal and stimulated β-cell secretory function and consequent fasting hyperglycaemia, from β-cell loci, showing an effect on insulin processing and secretion without detectable change in FG (*TCF7L2*, *SLC30A8*, *HHEX*/*IDE*, *CDKAL1*, *CDKN2A*/*2B*, *THADA*, *DGKB*, *PROX1*, *ADCY5*) [65••]. Most of the latter loci exert the strongest effects on T2D risk. Another earlier study of physiological glycaemic traits highlighted defects in both insulin processing and secretion for *GIPR* and *C2CD4A*/*B* [73•]. Furthermore, T2D risk variants at *ADCY5* and *CDKAL1* were recently associated with reduced birth weight [75•, 76•]. This observation confirms the foetal insulin hypothesis, which proposes that common genetic variation reducing insulin secretion may induce lower birth weight through the intrauterine action of insulin as a growth factor, but it can act even in adult life through altered glucose homeostasis and increased risk of developing T2D [77]. Similar effects, which however did not reach genome-wide significance level, were also seen for *HHEX*/*IDE* and *KCNQ1* [76•]. On the contrary, *GCK*, *MTNR1B* and *TCF7L2* T2D-risk alleles were suggestively associated with higher birth weight through the maternal genotype by affecting β-cell functionality and glucose homeostasis of the mother and thus increasing maternal glycaemia in pregnancy, resulting in changes in the intrauterine environment [39, 76•, 78]. T2D-risk alleles may alter β-cell function at different phases of the life course, and the observed phenotypic variability depends on the timing of their effects.

A fasting proinsulin locus was grouped separately suggesting a distinct underlying mechanism characterised by defects specifically in early steps of insulin production (*ARAP1*) with reduced basal and stimulated insulin secretion, an effect that runs counter to the usual epidemiological relationships [65••].

Finally, loci with effects on insulin sensitivity represent a much smaller proportion of T2D variants. Similarly, physiological characterisation of T2D loci grouped variants with primary effects on insulin sensitivity in basal and stimulated state (*IRS1*, *GCKR*, *PPARG*, *KLF14*); in addition to these, weak effects on insulin sensitivity have also been suggested for *HMGA2* [55•]. Insulin sensitivity indices showed consistently decreased effects for T2D risk alleles only for loci with known effects on insulin resistance at basal measures (HOMA-IR) [65••].

For a number of loci, the association with both lipids and T2D (*HNF4A*, *CILP2*, *KLF14*, *HNF1A*) and/or glycaemic traits (*MC4R*, *RSPO3*, *HFE*, *FADS1*, *TOP1*), in particular with FI (*GRB14*, *GCKR*, *FTO*, *PEPD*, *ANKRD55*, *IRS1*, *ARL15*, *PPP1R3B*), has been reported independently for each phenotype, underlying the close relationship between increased lipids/adiposity and increased insulin (Fig. 1b) [63••]. This picture is consistent with the first stages of diabetes, where high adiposity in peripheral tissues causes insulin resistance, which is complemented by an increase in β-cell insulin production.

The Link Between Circadian Rhythms and T2D

The levels of insulin production are subject to cyclic day-night variations. Circadian oscillations in the body are characteristic of nearly every hormone. Such daily hormonal profiles are the product of interaction between daylight exposure and many external and internal factors. The inverse correlation between the levels of the neuro-hormone melatonin, secreted by the pineal gland, and insulin has long been known. However, very few studies had investigated the relationship between the signalling of melatonin through its (G-protein coupled) receptors in pancreatic islets and metabolic disease [79], until powerful associations between rs10830963-G variant within the first intron of melatonin receptor 2 (*MTNR1B*) gene and higher FG levels and lower insulin secretion were discovered [54••, 56••, 57••, 69, 72], as well as a clear link to increased risk of T2D [13••, 54••, 55•]. Longitudinal studies demonstrated greater transition rates from normal glucose tolerance (NGT) to impaired fasting glucose (IFG) and increased risk of future T2D for the risk allele carriers [56••, 80].

MTNR1B encodes one of the two receptors for melatonin (melatonin receptor 2, MT2), which regulates the circadian rhythm of biological activities in peripheral tissues by translating photoperiodic information to the brain. In addition to genetic data, additional strands of evidence link melatonin regulation of circadian rhythm with glucose homeostasis; in fact, according to its function, *MTNR1B* is highly expressed in the retina, brain, and hypothalamus as well as in pancreatic islets and β cells in humans and mice [57••]. Moreover, studies have reported that the circadian rhythm in melatonin secretion is perturbed in T2D [79]. Functional studies have permitted retracing of the potential mechanism of interaction between melatonin and insulin secretion: the MT2 modulates inhibitory G protein-adenylyl cyclase, which is the predominant mode of action of GIP for raising intracellular cAMP. The expression of *MTNR1B* could be increased as a result of the absence of negative feedback regulatory events under

conditions of impaired MT2 signalling; cellular cAMP levels will be lower, and its potentiating effect on β-cell activity would be diminished, leading to impaired insulin secretion [81••, 82].

A large-scale exon re-sequencing study of the *MTNR1B* gene in 7632 Europeans, including 2186 individuals with T2D, has identified and functionally characterised 40 non-synonymous variants, of which 13 were partial- and loss-of-function and contributed to T2D risk (OR=5.67), while four led to complete loss of melatonin binding and increased odds of developing T2D (OR=3.88, 8153 individuals with T2D and 10,100 controls) [81••]. This investigation highlighted the value of targeted sequencing for the identification of potentially damaging variants and provided support for the melatonin receptor signalling impairment [81••].

The connection between increased melatonin, decreased insulin levels and augmented risk of T2D may have relevant therapeutic implications, for example, for the use of antagonists of melatonin receptors in β cells or through adopting alternative therapies in carriers of *MTNR1B* risk alleles, who may be less responsive to classical cAMP-activating treatments [56••].

In addition to *MTNR1B*, the FG-associated *CRY2* locus contains the cryptochrome circadian clock 2 gene, which prompted a pilot study of the genetic link between T2D and tagging SNPs in/near nine circadian genes [83]. No study-wise significant associations were identified, while suggestive association with *GRY2* FG-raising variant and T2D was observed, confirming its role in disease susceptibility.

Thrifty Gene Hypothesis

It has long been suggested that the high prevalence of metabolic disorders related to impaired glucose homeostasis may be a result of selective evolutionary advantage of T2D and obesity risk variants during periods of scarce food resources, which resulted in an increase in their frequency at the population level (thrifty gene hypothesis) [84, 85]. Given that food intake is known to act as a trigger for insulin release, it has also been hypothesised that a positive selection may have operated in particular on those loci associated with T2D through an influence on β-cell function [86•]. Some evidence of directional population differentiation and nominal positive selection at individual T2D risk loci, including *TCF7L2*, *THADA* and *NOTCH2*, has been reported [86•, 87–91]. The collective analysis of all T2D-associated variants, even when stratified by their impact on β-cell function or insulin resistance, has to date found no support for global or differential positive selection at T2D loci, thus offering little support for the thrifty gene hypothesis [86•, 87].

Role of Glycaemic Trait Genetic Variants in T2D Risk Prediction

Genetic studies of T2D and glycaemic traits have provided substantial insight into the biological factors underlying T2D pathogenesis, but have so far fallen short of clinical utility for T2D risk prediction and patient stratification. In longitudinal studies, the discriminatory capacity of genetic risk scores (GRS) to increase the area under the receiver-operating-characteristic curve compared to models including only clinical parameters in predictive models has provided little improvement in T2D prediction [92, 93]. Though, the discriminative capacity of GRSs showed modest but significant improvement in T2D reclassification rates in models including 65 T2D risk loci, and the inclusion of 36 FG loci modestly improved reclassification rates of incident and non-incident T2D and IFG. This latter finding suggests that the inclusion of risk loci associated with glycaemic traits may be beneficial for intermediate phenotypes [94]. Further investigations including increasing numbers of loci associated with glycaemic traits will be required to improve insight into the longitudinal impact of genetic variants associated with glycaemic traits on risk of T2D development as well as their impact on T2D development trajectories.

Conclusions

The relationships between quantitative traits and their cognate disease endpoints are complex. In this review, we have demonstrated that the study of genetic variation contributing to glycaemic trait variability in non-diabetic individuals can improve understanding of the pathophysiological processes leading to T2D, including relationships to other metabolic traits. Mechanistic insights into the processes underlying pathophysiologic heterogeneity of this disease are being provided through the elucidation of the respective roles of β-cell function, insulin secretion, processing and sensitivity, and glucose metabolism. The possibility to carry out large-scale GWA studies of glycaemic traits in Europeans followed by other ethnic groups has provided an excellent background for the forthcoming implementation of data from other next-generation technologies. In the very near future, this field will also benefit from the availability of extensive genome and exome sequencing data on a variety of ethnic groups. An effort undertaken by The Haplotype Reference Consortium will soon extend the reference set for genome-wide imputation to over 30,000 individuals, which will permit high-quality imputation of lower-frequency variants that currently cannot be evaluated using existing GWA arrays and the 1000 Genomes Project data (www.1000genomes.org). Data from the Exome chip genotyping array, which provides high coverage of putative functional exonic variants, has recently been

generated for hundreds of thousands individuals and is currently being evaluated. Better-powered fine mapping, identification of functionally relevant disease variants and extension of the variant set to other types of genetic variability, including copy number variation, are warranted for the dissection of glycaemic trait genetics. Taken together, these data will represent a powerful tool for the further dissection of glycaemic trait variability to improve the understanding of T2D pathogenesis and clinical translation of genetic findings.

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Compliance with Ethics Guidelines

Conflict of Interest Letizia Marullo, Julia S. El-Sayed Moustafa and Inga Prokopenko declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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