Variants at *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* loci are associated with reduced glucose-stimulated beta cell function in middle-aged Danish people

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Abstract

Aims/hypothesis A meta-analysis of 21 genome-wide association studies identified 11 novel genetic loci implicated in fasting glucose homeostasis. We aimed to evaluate the impact of these variants on insulin release and insulin sensitivity estimated from OGTTs.

Methods Eleven variants in or near *DGKB/TMEM195*, *ADCY5*, *MADD*, *ADRA2A*, *FADS1*, *CRY2*, *SLC2A2*, *GLIS3*, *PROX1*, *C2CD4B* and *IGF1* were genotyped in 6,784 middle-aged participants of the population-based Inter99 cohort. Association studies of quantitative estimates of insulin release and insulin sensitivity were performed in

5,722 non-diabetic Danish participants on whom an OGTT was performed.

Results Assuming an additive genetic model, carriers of the alleles increasing fasting glucose in *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* showed decreased glucose-stimulated insulin release as assessed by the BIGTT-acute insulin response index (2.7–3.5%; $p < 0.005$ for all) and by corrected insulin response (2.8–5.9%; $p < 0.03$ for all). In addition, the *PROX1* glucose-raising allele showed a 2.9% decreased corrected insulin response ($p = 0.03$), while the hyperglycaemic allele of variants in or near *ADRA2A*, *FADS1*, *CRY2* and *C2CD4B* were associated with a 2.6% to

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9.3% decrease in one or both of two different OGTT-based disposition indices ($p < 0.02$ for all). After correction for multiple testing, variants in the *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* loci were associated with estimates of beta cell function.

Conclusions/interpretation We found that the lead variants at the *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* loci were associated with decreased glucose-stimulated insulin response. This association underlines the importance of pancreatic beta cell dysfunction in the genetic predisposition to hyperglycaemia and type 2 diabetes.

Keywords Association study · Beta cell dysfunction · Beta cell function · Genetic epidemiology · Glucose homeostasis · Glucose-stimulated insulin release · Type 2 diabetes

Abbreviations

AIR	Acute insulin release
CIR	Corrected insulin response
DI	Disposition index
GWAS	Genome-wide association study
HOMA-B	HOMA of beta cell function
HOMA-IR	HOMA of insulin resistance
S_I	Sensitivity index
SNP	Single nucleotide polymorphism

Introduction

For years it has been well known that genetic factors are crucially important for the development of type 2 diabetes. So far, genome-wide association studies (GWAS) and subsequent meta-analysis have identified 23 validated risk loci influencing risk of type 2 diabetes [1–3]. While many common gene variants have shown an influence on estimates of beta cell function, only few variants have an impact on insulin resistance [2, 4–7].

Besides evidence from association analysis of dichotomous phenotypes such as type 2 diabetes, novel knowledge of the genetic complexity of glucose regulation and homeostasis has come from GWAS of quantitative glycaemic traits. Hyperglycaemia in the fasting state is a major criterion in the definition of type 2 diabetes [8]. Type 2 diabetes is a risk factor for development of cardiovascular disease, but increased fasting glucose at non-diabetic levels also increases risk of cardiovascular events [9]. Several loci have been shown to influence this variable [10–17]. Recently, the international Meta-Analysis of Glucose and Insulin-related Trait Consortium (MAGIC) collaboration engaged in a large meta-analysis of data from 21 GWAS and identified novel loci influencing fasting glucose homeostasis. The initial discovery stage included 46,186

individuals. After replication in up to 76,558 individuals, 16 loci, ten of them novel, were found to be associated with fasting plasma glucose and one novel locus with fasting serum insulin at a genome-wide significant level [18]. Interestingly, variants at *GCK*, *GCKR*, *DGKB/TMEM195*, *ADCY5* and *PROX1* loci showed novel association with type 2 diabetes at a genome-wide significance level in case-control studies of up to 127,677 individuals [18]. These five loci are thus part of the growing list of common type 2 diabetes susceptibility loci [18]. Also, several variants showed robust association with the HOMA index of beta cell function (HOMA-B), a surrogate measure of beta cell function in the fasting state.

While fasting levels of glucose and the derived HOMA-B index may depict important processes in the regulation of basal glycaemia, they are not informative with regard to complex dynamic postprandial glucose regulation [19, 20]. We therefore evaluated possible associations with estimates of insulin release and insulin sensitivity based on outcomes of OGTT, with a view to further describing the relationship between these variants and elements of glucose homeostasis. We investigated the 11 novel loci implicated in fasting glucose homeostasis in 5,722 non-diabetic individuals of the population-based Inter99 study on whom an OGTT had been performed. The remaining six loci have previously been investigated in this population [10, 12, 21–23].

Methods

Participants The Inter99 cohort is a population-based, randomised, non-pharmacological intervention study for the prevention of ischaemic heart disease, conducted on 6,784 middle-aged participants at the Research Centre for Prevention and Health in Glostrup, Copenhagen (ClinicalTrials.gov: NCT00289237) [24]. An OGTT was performed with measurement of plasma glucose and serum insulin at fasting, and at 30 and 120 min after glucose intake. Subsequently, 6,094 participants of Danish nationality and with available DNA were classified as having normal glucose tolerance ($n=4,525$), impaired fasting glycaemia ($n=504$), impaired glucose tolerance ($n=693$), screen-detected and treatment-naïve diabetes ($n=253$), or previously diagnosed diabetes ($n=119$) according to WHO 1999 criteria [8]. The analysis of quantitative diabetes-related phenotypes was performed in 5,722 non-diabetic participants. Detailed characteristics of Inter99 are presented in Table 1.

Informed written consent was obtained from all individuals before participation. The study was approved by the Ethical Committee of Copenhagen County and conducted in accordance with the principles of the Helsinki Declara-

Table 1 Quantitative metabolic traits in the population-based Inter99 cohort

Characteristics	Inter99 participants	
	Full cohort ^a	Non-diabetic individuals ^a
<i>n</i>	6,094	5,722
Men (<i>n</i>)	3,036	2,814
Women (<i>n</i>)	3,058	2,908
Age (years)	46±8	46±8
BMI (kg/m ²)	26.3±4.6	26±4.4
Waist circumference (cm)	86.7±13	85.9±13
Fasting plasma glucose (mmol/l)	5.6±1.1	5.5±0.5
30 min plasma glucose (mmol/l)	8.7±1.9	8.6±1.7
120 min plasma glucose (mmol/l)	6.2±2.2	6.0±1.5
Fasting serum insulin (pmol/l)	35 (24–52)	34 (23–49)
30 min serum insulin (pmol/l)	246 (175–355)	246 (176–355)
120 min serum insulin (pmol/l)	157 (96–257)	153 (94–246)
HbA _{1c} (%)	5.8 (5.6–6.1)	5.8 (5.5–6.1)
Corrected insulin response	92.9 (57–152)	96.1 (59.8–156)
BIGTT-AIR	1,630 (1,280–2,080)	1,640 (1,300–2,090)
HOMA-IR	1.21 (0.82–1.90)	1.16 (0.80–1.76)
BIGTT- <i>S</i> _I	9.18±4.10	9.44±3.90
DI 1	14,800 (10,300–19,800)	15,200 (10,900–20,000)
DI 2	74.8 (41.6–137)	79.2 (44.9–141)

Data are median (25–75% range) or mean±SD. DI 1 was calculated as BIGTT-AIR×BIGTT-*S*_I; DI 2 was calculated as CIR×1/HOMA-IR

^a With available DNA and glucose tolerance determined

tion II. Biochemical and anthropometric measures were obtained as previously detailed [25].

Indices of insulin release and insulin sensitivity Oral glucose-stimulated insulin release was reported as serum insulin at 30 min during the OGTT, the BIGTT-acute insulin response (AIR) index and the corrected insulin response (CIR). Insulin sensitivity was reported as the OGTT-derived BIGTT sensitivity index (*S*_I). CIR was calculated as: ([serum insulin_{30min}{pmol/l}/6.945] × 100)/(plasma glucose_{30min}{mmol/l} × [plasma glucose_{30min}{mmol/l} – 3.89]) [26]. The BIGTT indices apply information on sex and BMI, combined with plasma glucose and serum insulin during an OGTT, to provide indices for AIR and *S*_I, and were calculated as reported [27]. In order to construct OGTT-based disposition indices (DIs) we multiplied BIGTT-AIR with BIGTT-*S*_I (DI 1) and CIR with the reciprocal of HOMA of insulin resistance (HOMA-IR) (DI 2). The DI is an estimate of the ability of the pancreatic beta cell to respond appropriately to concomitant levels of insulin sensitivity.

Genotyping We genotyped 11 gene variants by KASPar SNP Genotyping system (KBiosciences, Hoddesdon, UK). All genotyping success rates were above 96% with a mismatch rate below 0.5% in 591 pairs genotyped in duplicate. The distribution of genotypes for all variants were in Hardy–Weinberg equilibrium in Inter99 ($p>0.05$),

except for rs21911349 and rs11920090 ($p=0.01$ and $p=0.008$, respectively).

Statistical analysis General linear statistical methodology was used to test quantitative traits in relation to genotype, applying additive models adjusting for the effect of age (BIGTT-*S*_I and BIGTT-AIR) or age and sex (all other traits). Values of serum insulin and derived indices (except BIGTT-*S*_I) were logarithmically transformed prior to analysis. The multivariate method, Hotelling's T^2 , was applied to test the simultaneous effect of genotype on BIGTT-*S*_I and BIGTT-AIR. A p value of less than 0.05 was considered significant. Correlation between quantitative metabolic traits independent of genotype was performed by Pearson correlation. Non-normally distributed traits were normalised by log-transformation. For the association of gene variants with fasting plasma glucose we calculated the proportion that could be accounted for by association with insulin release or insulin sensitivity by dividing the expected effect with the observed effect. The expected effect of gene variants on fasting plasma glucose, given the association with insulin release (BIGTT-AIR) or insulin sensitivity (BIGTT-*S*_I), was calculated by multiplying the single nucleotide polymorphism (SNP) effect on insulin release by the epidemiological effect between insulin release and fasting plasma glucose. This triangulation approach has been described previously [28]. The proportion of variance explained by genetic variants was assessed

by the partial R^2 from a linear regression model including main effects of all selected variants. Analyses were performed using RGui, version 2.10.0 (www.r-project.org).

Statistical power Statistical power was calculated as previously reported [25]. Depending on allele frequency (range 11–49%), we had 80% statistical power at a 5% significance level to detect an allele-dependent difference of 5.3% to 8.4% of a standard deviation corresponding to a 2.2% to 3.5% difference in BIGTT-AIR, a 3.8% to 6.0% difference in CIR and an absolute 0.21 to 0.33 difference in BIGTT- S_I .

Results

We genotyped the following 11 variants in the Danish population-based Inter99 cohort (Table 1): *DGKB/TMEM195* rs2191349, *ADCY5* rs11708067, *MADD* rs7944584, *ADRA2A* rs10885122, *FADS1* rs174550, *CRY2* rs11605924, *SLC2A2* rs11920090, *GLIS3* rs7034200, *PROX1* rs340874, *C2CD4B* rs11071657 and *IGF1* rs35767. Results in Inter99 for traits reflecting fasting glucose homeostasis (plasma glucose, serum insulin, HOMA-IR and HOMA-B) have been previously reported [18], but are presented for comparison in Table 2 and Electronic supplementary materials (ESM) Table 1. Inter99 association results for six known fasting glucose loci have been published previously [10, 12, 21–23]. In 5,722 non-diabetic Inter99 participants we tested the above-named 11 variants for association with quantitative OGTT-based estimates of beta cell function and insulin sensitivity (Table 2). Carriers of the *DGKB/TMEM195* rs2191349 glucose-raising T-allele showed decreased glucose-stimulated insulin release as assessed by serum insulin at 30 min (2.7% [95% CI 0.6–4.7], $p=0.01$), BIGTT-AIR (2.7% [1.1–4.2], $p=0.0009$) and CIR (2.8% [0.2–5.5%], $p=0.03$; Table 2). Likewise, carriers of the glucose-raising G-allele of *ADRA2A* rs10885122 showed a consistent decreased insulin response after oral glucose ingestion evaluated by CIR (5.9% [1.8–10%], $p=0.005$), BIGTT-AIR (3.5% [1.1–6.0%], $p=0.005$) and two different disposition indices (DI 1 4.2% [1.2–7.1%], $p=0.005$, DI 2 9.3% [4.6–14%], $p=0.0001$; Table 2). Also, carriers of the glucose-raising A-allele of *C2CD4B* rs11071657 had a 4.3% (1.6–7.0%, $p=0.002$) decreased insulin release as assessed by the CIR index, consistent with a 3.2% (1.5–4.8%, $p=0.0002$) decreased BIGTT-AIR index and a 3.1% (1.1–5.0%, $p=0.002$) decreased DI (Table 2). Similarly, carriers of the glucose-raising A-allele of *GLIS3* rs7034200 had a decreased glucose-stimulated insulin release assessed by BIGTT-AIR (3.1% [1.4–4.7%], $p=0.0002$). The *CRY2* rs11605924 glucose-raising A-allele was associated with a

2.6 to 3.8% decrease in two different disposition indices ($p<0.02$), while the diabetes-related *PROX1* rs340874 C-allele showed a 2.7% (0.6–4.8%, $p=0.01$) decreased serum insulin at 30 min during OGTT and 2.9% decreased CIR (0.3–5.5%, $p=0.03$). Also, association with a single estimate of glucose-induced insulin response was observed for *MADD* rs7944584 (DI 2 3.9% [0.4–7.4%], $p=0.03$), *FADS1* rs174550 (DI 1 –2.5% [–4.4 to –0.51%], $p=0.01$) and *SLC2A2* (CIR 4.3% [0.6–8.1%], $p=0.02$). Besides association of the *CRY2* variant ($p=0.02$), none of the examined variants was associated with the BIGTT- S_I estimate of insulin sensitivity. All results were virtually unchanged in models including BMI as an explanatory variable or when analysing a subset of participants with normal glucose tolerance (data not shown).

Given this complex pattern of association we determined the pairwise correlation between the investigated OGTT-derived traits and traits reflecting fasting glucose homeostasis in all non-diabetic participants of Inter99 (ESM Table 2). These analyses showed modest correlation between various OGTT-based indices of glucose-stimulated insulin release, accounting for some of the discrepancies in observed associations.

The relationship between concomitant insulin sensitivity and insulin release from the pancreatic beta cell is described by a hyperbola [29]. To illustrate the genotype-related relationship between estimates of insulin sensitivity and insulin release, we plotted two-dimensional standard error ellipses of the genotype-specific means of BIGTT- S_I and BIGTT-AIR (Fig. 1). Variants at *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* loci showed a distinct pattern consistent with a main effect on BIGTT-AIR, whereas the two-dimensional impact of variants in *CRY2*, *PROX1* and *FADS1* loci did not support an effect on glucose-stimulated insulin release. In this bivariate analysis the *CRY2* variant was associated with BIGTT-AIR and BIGTT- S_I , underlining the association with the BIGTT- S_I estimate of insulin sensitivity in univariate analysis (Table 2).

To assess the proportion of the association with fasting plasma glucose (Table 2) that could be attributed to insulin release or insulin sensitivity, we calculated the expected effect size for association between a SNP and fasting glucose given the SNP association with the BIGTT-AIR or BIGTT- S_I estimates of insulin release and sensitivity, and the SNP-independent association between BIGTT-AIR or BIGTT- S_I and fasting glucose. We then compared the expected effect with the observed effect on fasting glucose to assess the proportion of association possibly mediated by insulin release or sensitivity (ESM Table 3). These calculations were done for the seven variants associated with fasting plasma glucose in Inter99. For the four loci strongly associated with insulin release in Inter99 (*DGKB/*

Table 2 Association of novel fasting glucose homeostasis loci with estimates of glucose-stimulated insulin response and insulin sensitivity in 5,722 Danish non-diabetic individuals of the population-based Inter99 cohort

Nearest gene/SNP	Effect alleles	FPG ^c		S-insulin at 30 min ^d		BIGTT-AIR		CIR		BIGTT-S _i		DI 1 ^e		DI 2 ^f	
		Alleles ^a	EAF ^b	Effect	p value	Effect	p value	Effect	p value	Effect	p value	Effect	p value	Effect	p value
DGKB/TMEM195															
rs2191349	T/G	0.51	0.028 (0.0088)	0.002	−0.027 (0.011)	0.01	−0.027 (0.008)	0.0009	−0.028 (0.013)	0.03	0.09 (0.078)	0.2	−0.012 (0.0095)	0.2	−0.011 (0.015)
ADCY5															
rs11708067	A/G	0.75	0.027 (0.01)	0.008	0.0097 (0.012)	0.4	0.00043 (0.0094)	1.0	−0.0023 (0.015)	0.9	−0.022 (0.091)	0.8	−0.00089 (0.011)	0.9	0.008 (0.018)
MADD															
rs7944584	A/T	0.18	0.012 (0.010)	0.2	0.014 (0.012)	0.3	0.0026 (0.0094)	0.8	0.025 (0.016)	0.1	0.05 (0.091)	0.6	0.0068 (0.011)	0.5	0.039 (0.018)
ADRA2A															
rs10885122	G/T	0.89	0.036 (0.014)	0.01	−0.026 (0.017)	0.1	−0.035 (0.013)	0.005	−0.059 (0.021)	0.005	−0.063 (0.12)	0.6	−0.042 (0.015)	0.005	−0.093 (0.024)
FADS1															
rs174550	T/C	0.66	0.021 (0.0094)	0.03	0.0018 (0.011)	0.9	−0.0033 (0.0085)	0.7	−0.0096 (0.014)	0.5	−0.12 (0.083)	0.1	−0.025 (0.01)	0.01	−0.024 (0.016)
CRY2															
rs11605924	A/C	0.49	0.025 (0.0090)	0.006	0.0025 (0.011)	0.8	−0.0019 (0.0082)	0.8	−0.019 (0.013)	0.2	−0.19 (0.079)	0.02	−0.026 (0.0096)	0.007	−0.038 (0.016)
SLC2A2															
rs11920090	T/A	0.86	0.0077 (0.013)	0.5	0.025 (0.015)	0.1	0.018 (0.012)	0.1	0.043 (0.019)	0.02	−0.075 (0.11)	0.5	0.0085 (0.014)	0.5	0.036 (0.022)
GLIS3															
rs7034200	A/C	0.48	0.020 (0.0091)	0.03	−0.0097 (0.011)	0.4	−0.031 (0.0084)	0.0002	−0.031 (0.014)	0.02	0.11 (0.081)	0.2	−0.018 (0.0099)	0.06	−0.0039 (0.016)
PROX1															
rs340874	C/T	0.54	−6.9×10 ^{−5} (0.009)	1	−0.027 (0.011)	0.01	−0.011 (0.0081)	0.2	−0.029 (0.013)	0.03	0.12 (0.079)	0.1	0.0074 (0.0096)	0.4	−0.0052 (0.016)
C2CD4B															
rs11071657	A/G	0.62	0.025 (0.0092)	0.006	−0.021 (0.011)	0.06	−0.032 (0.0085)	0.0002	−0.043 (0.014)	0.002	−0.017 (0.082)	0.8	−0.031 (0.0099)	0.002	−0.037 (0.016)
IGF1															
rs35767	G/A	0.85	−0.0025 (0.013)	0.8	0.002 (0.015)	0.9	0.0037 (0.012)	0.7	0.0021 (0.019)	0.9	−0.078 (0.11)	0.5	−0.00018 (0.014)	1	−0.0064 (0.022)

Data are per-allele effect size (beta values of the regression model; SE) and *p* values of an additive genetic effect adjusted for age and sex or age (BIGTT-AIR and BIGTT-S_i). Units of effect sizes are mmol/l (plasma glucose), no unit (BIGTT-S_i) or % (all other traits)

Effect alleles are those known to increase fasting plasma glucose or fasting serum insulin (rs35767) [18]

All traits, except BIGTT-S_i, were logarithmically transformed before analysis

^a Effect allele/other allele; ^b effect allele frequency

^c Fasting plasma glucose in mmol/l; all data previously published in a meta-analysis by the MAGIC investigators [18]

^d Serum insulin at 30 min

^e BIGTT-AIR × BIGTT-S_i; ^f CIR/HOMA-IR

EAF, effect allele frequency; FPG, fasting plasma glucose

Fig. 1 Two-dimensional SEM of BIGTT- S_1 and logarithm of BIGTT-AIR stratified according to the genotype of (a) *DGKB/TMEM195* rs2191349, (b) *ADRA2A* rs10885122, (c) *FADS1* rs174550, (d) *CRY2* rs11605924, (e) *GLIS3* rs7034200, (f) *PROX1* rs340874 and (g) *C2CD4B* rs11071657 in 5,722 non-diabetic participants of the Inter99 cohort. SE ellipses around the mean of each genotype level were constructed assuming bivariate normal distribution. The multivariate method, Hotelling T^2 , was applied to test the simultaneous effect of genotype on the two traits of insulin release and insulin sensitivity assuming an additive model with age as a covariate. Continuous line, SE for homozygous reference allele carriers; dashed line, SE for heterozygous carriers; dotted line, SE for homozygous glucose-raising allele carriers. **a** $p=0.005$; **b** $p=0.001$; **c** $p=0.2$; **d** $p=0.03$; **e** $p=0.002$; **f** $p=0.2$; **g** $p=5 \times 10^{-4}$

TMEM195, *ADRA2A*, *GLIS3* and *C2CD4B*), the proportion of effect on fasting glucose that could be ascribed to association with BIGTT-AIR ranged from 34% to 57%. Also, the proportions of effect on fasting glucose at the *FADS1* and *CRY2* loci that were explained by association with insulin sensitivity (BIGTT- S_1) were 39% and 48%, respectively (ESM Table 3).

Discussion

Recent advances in the genetic exploration of traits related to fasting glucose homeostasis have revealed 17 loci associated with fasting plasma glucose or fasting serum insulin [18], of which six known loci have been previously reported [10, 12, 21–23]. In the current paper we aimed to evaluate the impact of variants at the 11 novel loci on OGTT-based insulin sensitivity and to estimate beta cell function by analysis of a range of measures of glucose-stimulated insulin release in non-diabetic middle-aged individuals. The study demonstrates that of ten novel loci associated with increased fasting plasma glucose at least four (*DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B*), and possibly one additional locus (*PROX1*), are associated with reduced insulin response to oral glucose. These loci thus join the growing list of common variants with a modest effect on pancreatic beta cell function. We found that the lead variants in or near *DGKB/TMEM195*, *ADRA2A*, *CRY2*, *GLIS3*, *PROX1* and *C2CD4B* were associated with two or more of five correlated estimates of insulin release, while variants in or near *FADS1*, *SLC2A2* and *MADD* were associated with a single insulin release measure. With the exception of probably sporadic associations with *SLC2A2* and *MADD*, all effects showed a decrease in glucose-stimulated insulin release in carriers of the allele that also increased fasting glucose in the previous combined report [18]. However, two-dimensional plots of insulin sensitivity in relation to insulin release did not support the impact of *CRY2* and *FADS1* variants on glucose-stimulated insulin release. In line with univariate analysis, two-dimensional data for *CRY2* pointed to a possible impact on insulin sensitivity. Yet, the MAGIC study did not show an effect on HOMA-IR in more than 35,000 individuals, underlining the uncertainty of the present association [18].

The *IGF1* variant previously associated with increased fasting insulin levels and increased HOMA-IR did not associate with an OGTT-based insulin S_1 in the present study, highlighting the difficulties in establishing genetic variants that influence insulin sensitivity. Besides lack of statistical power, these difficulties could be explained by the relatively poor phenotypic estimation of insulin resistance in most datasets. Also, insulin resistance variants are probably more prone to interaction with correlated phenotypes or environmental factors, such as obesity, physical activity or dietary intake, thus obscuring association and replication in studies of genetic main effects [6].

Association of *DGKB/TMEM195*, *ADRA2A*, *GLIS3*, *PROX1* and *C2CD4B* loci with glucose-stimulated insulin release is consistent with genes in these loci being expressed in the pancreas and human islet cells [18]. However, the more exact molecular mechanisms of action

cannot be inferred from the current data. Interestingly, *DGKB/TMEM195* rs2191341 and *PROX1* rs340874 were associated with type 2 diabetes at genome-wide significance ($p < 5 \times 10^{-8}$) in the MAGIC publication, while the other three variants showing an effect on pancreatic beta cell function had limited impact on risk of type 2 diabetes in data from the MAGIC investigators (OR 1.03–1.04, $p = 0.001$ – 0.02) [18]. In addition, when comparing the effect on insulin release of variants associated with type 2 diabetes in the MAGIC publication [18] with that of variants not associated with type 2 diabetes, we observed no clear distinction between these groups. Given these observations, there is no clear correlation between genetically impaired beta cell function in the general population and risk of type 2 diabetes. However, it is possible that lack of statistical power to obtain high confidence in association for these low-impact variants may have obscured the genuine coherence.

Our novel findings of glucose-stimulated insulin release are largely in agreement with the MAGIC paper [18], in which four of the ten fasting glucose variants examined here were also associated at genome-wide significance with basal beta cell function under non-stimulated conditions as estimated from the HOMA-B model. In our study of large-scale genetic physiology, we applied OGTT to estimate the glucose-stimulated insulin response, a method that is superior to the HOMA-B model in providing a reliable surrogate measure of first-phase insulin release [19, 27]. Yet the correlation between different OGTT-based estimates of insulin release was not complete, as shown by results in ESM Table 2. Interestingly, HOMA-B correlated strongly with HOMA-IR and fasting serum insulin, and modestly with the CIR and BIGTT-AIR estimates of insulin release in Inter99. Therefore it is essential to investigate stimulated insulin release in order to substantiate a beta cell dysfunction as the pathogenic mechanism potentially leading to increased fasting glucose and type 2 diabetes. The importance of this statement is stressed by the observation that *ADRA2A* rs10885122 was not associated with HOMA-B at genome-wide significance in the MAGIC paper and did not replicate strongly ($p = 0.03$) in ~60,000 individuals [18]; yet the current data provide strong evidence of a postprandial insulin release defect. The functional and genetic impact of *ADRA2A* on dynamic insulin secretion was also described in a recent paper showing reduced in vitro insulin secretion and impaired docking of insulin granule in carriers of variants in *ADRA2A* [30]. Despite the application of OGTT-based estimates of beta cell function, we cannot rule out the possibility that the lack of consistent association with beta cell function for variants at *ADCY5*, *MADD*, *FADS1*, *CRY2* and *SLC2A2* in the present study is due to lack of statistical power. Interestingly, in up to 61,907 replication samples of the MAGIC study [18], variants in *MADD*, *CRY2* and *SLC2A2* that had otherwise

been associated with HOMA-B did not convincingly replicate, a finding supported by the current data, which question the effect of these variants on pancreatic beta cell function. Also, the lack of consistent association of the *ADCY5* variant with orally stimulated insulin release in the present report is in accordance with results of another report by the MAGIC investigators involving ~19,000 OGTT-investigated individuals who were tested for the highly correlated ($r^2 = 0.85$) rs2877716 variant [31].

In Inter99 we had 80% statistical power to detect an effect size of 0.05 to 0.08 SD depending on allele frequency. For comparison, the effect on fasting plasma glucose in Inter99 ranged from 0 SD (*C2CD4B*) to 0.07 SD (*ADRA2A*), indicating that the Inter99 cohort is marginally underpowered to detect such low impacts. Also, we acknowledge that the present report applies a liberal statistical significance threshold. Performing conservative Bonferroni correction for 11 gene variants implies a corrected alpha of 0.0045, leaving associations between glucose-stimulated insulin response and variants at *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* loci significant at this threshold.

Interestingly, from a genetic viewpoint the major impact on risk of type 2 diabetes and fasting hyperglycaemia is through decreased function of the pancreatic beta cell. In triangulation analyses we estimated that for the four variants with an impact on beta cell function, around 30% to 60% of the effect on fasting plasma glucose could be accounted for by association with glucose-stimulated insulin release. These estimates suggest the existence of additional glucose-raising mechanisms that are independent of glucose-stimulated insulin release. Despite the high number of statistically highly associated variants discovered in the last few years, the explained proportion of the inter-individual variation reported in Inter99 for the combination of all 33 validated type 2 diabetes and/or fasting plasma glucose gene variants is only 5.8% for fasting plasma glucose and 4.2% to 5.7% for different estimates of glucose-stimulated insulin release in non-diabetic Danish people (data not shown). These numbers should be interpreted in relation to heritability estimates of 30% to 40% and 60% to 80% for fasting glucose and first-phase insulin release, respectively [32], indicating that 15% to 20% and 5% to 10%, respectively, of the genetic contribution to these traits has been accounted for. These estimates imply that a major part of the genetic contribution to these quantitative traits is yet to be unravelled.

In conclusion, in a large OGTT-investigated population-based cohort we found that the lead variants at the *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* loci were associated with decreased glucose-stimulated insulin response, underlining the importance of pancreatic beta cell dysfunction in genetic predisposition to hyperglycaemia and type 2 diabetes.

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Duality of interest K. Borch-Johnsen is director of the Steno Diabetes Center, a hospital integrated into the Danish National Health Care Service and owned by Novo Nordisk, in which K. Borch-Johnsen holds employee shares. All other authors declare that there is no duality of interest associated with this manuscript.

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