ARTICLE

Type 2 diabetes risk allele near *CENTD2* is associated with decreased glucose-stimulated insulin release

T. Nielsen · T. Sparsø · N. Grarup · T. Jørgensen ·
C. Pisinger · D. R. Witte · T. Hansen · O. Pedersen ·
**Diabetes Genetics Replication and Meta-analysis
(DIAGRAM) Consortium**

Abstract

Aims/hypothesis By combining multiple genome-wide association (GWA) studies and comprehensive replication efforts, 12 novel type 2 diabetes associated loci have recently been discovered. Here we evaluate the effect of lead variants of these loci on estimates of insulin release and insulin resistance derived from an oral glucose tolerance test.

Methods We examined 12 lead variants in or near *HMGA2*, *CENTD2* (also known as *ARAP1*), *KLF14*, *PRC1*, *TP53INP1*, *ZBED3*, *ZFAND6*, *CHCHD9*, *DUSP9*, *KCNQ1*, *BCL11A* and *HNF1A* in 5,722 middle-aged people from the population-based Inter99 sample.

Results Carriers of the major diabetogenic allele of rs1552224 in *CENTD2* had increased 30-min plasma glucose values (2.0%, $p=2\times10^{-5}$) as well as 4.2% reduced insulin release 30 min after an oral glucose load ($p=0.001$). Risk allele carriers also had decreased BIGTT-acute insulin release (AIR), which is a surrogate measure of insulin release where sex, BMI, plasma glucose and serum insulin are integrated (5.3%, $p=8\times10^{-7}$). In addition, a decreased corrected insulin response (CIR; 9.9%, $p=3\times10^{-8}$) was observed. For rs5945326 near *DUSP9* on the X-chromosome we stratified according to sex. Male carriers of the risk allele showed nominally decreased BIGTT-AIR (2.6%, $p=0.01$). No associations with intermediate meta-

T. Nielsen (✉) · T. Sparsø · N. Grarup · O. Pedersen
Marie Krogh Center for Metabolic Research, Section of Metabolic Genetics, Faculty of Health Sciences, University of Copenhagen,
Universitetsparken 1,
2100 Copenhagen, Denmark
e-mail: trine.nielsen@sund.ku.dk

T. Nielsen · T. Sparsø · N. Grarup · T. Hansen · O. Pedersen
Hagedorn Research Institute,
Copenhagen, Denmark

T. Jørgensen · C. Pisinger
Research Centre for Prevention and Health,
Glostrup University Hospital,
Glostrup, Denmark

T. Jørgensen
Faculty of Health Sciences, University of Copenhagen,
Copenhagen, Denmark

D. R. Witte
Steno Diabetes Center,
Gentofte, Denmark

T. Hansen
Faculty of Health Sciences, University of Southern Denmark,
Odense, Denmark

O. Pedersen
Institute of Biomedical Science, Faculty of Health Sciences,
University of Copenhagen,
Copenhagen, Denmark

O. Pedersen
Faculty of Health Sciences, University of Aarhus,
Aarhus, Denmark

bolic traits were found in women. For the remaining ten lead variants no consistent associations were demonstrated. *Conclusions/interpretation* Of the lead variants from 12 novel type 2 diabetes associated loci, *CENTD2* significantly associated with increased plasma glucose values and decreased glucose-stimulated insulin release, suggesting that the diabetogenic effect of this locus is mediated through an impaired pancreatic beta cell function.

Keywords Association study · Beta cell function · Genetic epidemiology · Type 2 diabetes

Abbreviations

AIR	Acute insulin release
CIR	Corrected insulin response
DI	Disposition index
DIAGRAM	Diabetes Genetics Replication and Meta-analysis Consortium
GWA	Genome-wide association
HOMA-IR	Homeostasis model assessment of insulin resistance
S_I	Sensitivity index
SNP	Single nucleotide polymorphism

Introduction

Since 2007, the list of common validated type 2 diabetes genes has grown substantially due to the results of genome-wide association (GWA) studies. The Diabetes Genetics Replication and Meta-analysis (DIAGRAM) consortium have, in 2008 and 2010, reported results from large meta-analyses [1, 2]. In the latest extended analysis (referred to as DIAGRAM+) 12 novel type 2 diabetes genes were discovered [2]; however, these novel loci affect disease risk modestly with odds ratios between 1.06 and 1.14 [2]. Only rs5945326, near *DUSP9* on the X chromosome, seems to have an effect size comparable to some of the loci discovered in the earlier studies (combined odds ratio 1.27).

Due to the sparse knowledge concerning the diabetes-causing mechanisms of the newly identified variants, the aim was to explore the intermediate phenotypes that may explain the observed disease association. Hence, we characterised the influence of these variants on surrogate measures of beta cell function and insulin sensitivity derived from an OGTT in a random population of middle-aged Danes.

Methods

Study population All analyses were conducted in non-diabetic people from the Inter99 Study sample [3]. Clinical characteristics and a description of the Inter99 Study population are shown in Electronic supplementary material (ESM) Study population and ESM Table 1. Informed written consent was obtained prior to investigation. The study was conducted in accordance with the principles of the Helsinki Declaration II.

Derived estimates of insulin release and insulin sensitivity from an OGTT The insulinogenic index, BIGTT-acute insulin response (AIR) and the corrected insulin response (CIR) were reported as indices of oral glucose-stimulated insulin release. The insulinogenic index was calculated as follows: (serum insulin$_{30min}$ − serum insulin$_{0min}$[pmol/l])/plasma glucose$_{30min}$[mmol/l]. CIR was calculated as: ([serum insulin$_{30min}$ {pmol/l}/6.945] × 100)/(plasma glucose$_{30min}$ {mmol/l} × [plasma glucose$_{30min}$ {mmol/l} − 3.89]) [4]. The BIGTT indices integrate information on sex and BMI, combined with plasma glucose and serum insulin, during an OGTT and were calculated as reported [5]. The surrogate measures of insulin sensitivity, BIGTT sensitivity index (S_I) and the homeostasis model assessment of insulin resistance (HOMA-IR) ([plasma glucose$_{0 min}$ (mmol/l) × serum insulin$_{0 min}$ (pmol/l)]/22.5), respectively, were calculated as reported [5, 6]. Two different disposition indices (DIs) were calculated based on the OGTT; for DI 1 we multiplied BIGTT-AIR with BIGTT-S_I and for DI 2 we divided CIR by HOMA-IR.

Genotyping All variants were genotyped by KASPar SNP Genotyping (KBiosciences, Hoddesdon, UK). All success rates were above 95% and all error rates below 0.85% in 591 duplicates. All variants were in Hardy–Weinberg equilibrium in the Inter99 Study ($p > 0.005$).

Statistical analysis The effects of 12 lead variants on quantitative traits related to type 2 diabetes were analysed using linear regression models under an additive model. Values for plasma glucose, serum insulin and derived indices (except BIGTT-S_I), were logarithmically (log$_e$) transformed before analyses. To investigate the interaction between sex and *DUSP9* rs5945326 we included an interaction term (sex × genotype) in the linear model in addition to the main effects. Correction for multiple testing was performed by Bonferroni (correcting for 12 SNPs), and a p value below 0.004 was considered significant. A p value between 0.05 and 0.004 was considered nominally significant. All statistical analyses were performed with the statistical programming language R (version 2.10.1) (www.r-project.org).

Statistical power calculations Statistical power for quantitative traits was estimated using simulations. An additive genetic model was used for both the simulation of the data

Table 1 Anthropometrics and quantitative metabolic traits during an OGTT in the population-based sample of 5,529 middle-aged Danes stratified according to rs1552224 at *CENTD2*

rs1552224	TT (carriers of non-risk allele)	TG (carriers of one risk allele)	GG (carriers of two risk alleles)	Per-risk-allele effect size (95% CI)	p_{add}	p_{adj}
n (men/women)	172 (84/88)	1,619 (807/812)	3,738 (1,823/1,915)			
Age (years)	45±7	46±8	46±8			
BMI (kg/m^2)	26.1±4.5	26.1±4.5	26.0±4.3	−0.077 (−0.29, 0.13)	0.5	
Plasma glucose (mmol/l)						
Fasting	5.4±0.5	5.4±0.5	5.5±0.5	0.57 (0.16, 0.98)	0.007	
30 min post OGTT	8.3±1.7	8.4±1.6	8.6±1.7	1.97 (1.07, 2.88)	2×10^{-5}	
120 min post OGTT	5.80±1.5	6.0±1.5	6.0±1.6	0.38 (−0.85, 1.61)	0.6	
Serum insulin (pmol/l)						
Fasting	35 (24–51)	34 (24–51)	33 (23–49)	−0.82 (−3.31, 1.66)	0.5	
30 min post OGTT	250 (201–342)	258 (183–375)	240 (173–287)	−4.19 (−6.70, −1.67)	0.001	
120 min post OGTT	145 (84–233)	152 (97–250)	154 (93–244)	−0.29 (−3.90, 3.32)	0.9	
Insulinogenic index	27 (18–40)	27 (18–40)	24 (17–35)	−6.92 (−9.78, −4.07)	2×10^{-6}	2×10^{-6}
HOMA-IR	8.0 (5.7–13.1)	8.2 (5.7–12.4)	8.0 (5.5–12.2)	−0.26 (−2.86, 2.35)	0.9	
BIGTT-AIR	1,745 (1,380–2,261)	1,696 (1,344–2,182)	1,600 (1,280–2,035)	−5.27 (−7.36, −3.18)	8×10^{-7}	3×10^{-7}
BIGTT-S$_I$	9.37±3.75	9.40±4.03	9.48±3.93	0.072 (−0.31, 0.27)	0.5	
CIR	111.1 (69.1–186.4)	102.6 (65.1–175.6)	92.8 (57.8–149.3)	−9.88 (−13.37, 6.40)	3×10^{-8}	9×10^{-8}
DI 1	15,970 (10,980–20,240)	15,600 (11,130–16,220)	14,890 (10,700–19,720)	−3.83 (−6.33, −1.34)	0.003	
DI 2	13.2 (7.4–23.3)	12.5 (6.9–22.0)	11.0 (6.3–19.9)	−9.15 (−13.12, −5.15)	8×10^{-6}	

Data are means±SD, median (interquartile range), or per-risk-allele effect size (95% CI)

Units of effect sizes are kg/m^2 (BMI), no units (BIGTT-S$_I$) or% (all other traits)

BIGTT indices integrate 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 [5]

Values of serum insulin and plasma glucose, as well as derived indices of insulinogenic index, HOMA-IR, BIGTT-AIR, CIR, DI 1 and DI 2 were logarithmically transformed before analysis

Calculated effect sizes and p values (p_{add}) were adjusted for age (BIGTT-S$_I$, BIGTT-AIR and DI 1) or age, sex and BMI (all other traits) assuming an additive model

For indices of insulin release, HOMA-IR has been added to the model as an independent variable (p_{adj})

and for testing the data using a linear model. We used the empirical variance of the observed traits (insulinogenic index and HOMA-IR) to simulate phenotypes from the normal distribution so that variance across genotypes is drawn from the estimated variance.

Since no prior studies have assessed the effect size of the OGTT-derived estimate of insulinogenic index and HOMA-IR on the 12 SNPs, we included the calculated effect sizes from the Inter99 Study population. Given a study sample of 5,000, *CENTD2* (also known as *ARAP1*) rs1552224 is the only novel SNP with statistical power above 80% to find the observed effect size for the insulinogenic index (ESM Table 2).

In addition, a complementary method to calculate statistical power was applied. We used the allele frequencies of the 12 novel type 2 diabetes genes and a sample size of 5,722 individuals to estimate the effect size for each allele for which we had 80% statistical power to detect an association in the given quantitative trait. We carried out 5,000 simulations and

applied a significance threshold of 0.004. We had 80% statistical power to detect an allelic difference of 5.4–9.7% in CIR, 2.8–5.3% in BIGTT-AIR and 0.27–0.51 absolute difference in BIGTT-S$_I$, respectively.

Results

In case–control settings, seven of the 12 variants that were reported to reach genome-wide significance in the DIA-GRAM+ meta-analysis were nominally associated with type 2 diabetes in the Danish population ($p<0.05$). These results are part of the DIAGRAM+ meta-analysis [2], but are shown in the ESM for comparison (ESM Table 3).

In the population-based Inter99 Study sample of 5,722 individuals, carriers of the major diabetogenic allele of rs1552224 near *CENTD2* had a 4.2% lower 30-min serum insulin release (95% CI 1.7–6.7, $p=0.001$) per risk allele

following an OGTT (Table 1). In addition, carriers of the same risk allele had nominally higher fasting and significantly higher 30-min plasma glucose levels (0.6% [95% CI 0.2–1.0, $p=0.007$] and 2.0% [95% CI 1.1–2.9, $p=2\times10^{-5}$], respectively). Surrogate measures of insulin release such as the insulinogenic index, BIGTT-AIR and CIR were significantly decreased ($p=2\times10^{-6}$, $p=8\times10^{-7}$ and $p=3\times10^{-8}$, respectively) for the major diabetogenic risk allele of CENTD2 rs1552224; these findings are consistent with a decrease in two estimates of OGTT-based disposition indices (DI 1 3.9% [95% CT 1.3–6.3, $p=0.003$]; DI 2 9.2% [95% CI 5.2–13.1, $p=8\times10^{-6}$]; Table 1). The results remained largely unchanged when we added HOMA-IR to the model as a covariate in the analyses of indices of insulin release (Table 1).

Of the remaining 11 SNPs, 10 demonstrated no convincing association with any quantitative traits related to insulin release or insulin sensitivity (ESM Table 4). DUSP9 rs5945326 was analysed further because of its location on the X-chromosome. When we stratified according to sex, we found nominal associations with indices of insulin release in men ($n=2,694$). Male risk allele carriers had nominally lower BIGTT-AIR, (2.6% [95% CI 0.6–4.5, $p=0.01$]) and, when adding HOMA-IR as a covariate, the association with BIGTT-AIR (2.8% [95% CI 1.0–4.6, $p=0.002$]) was strengthened. A tendency towards a decrease in CIR and the two disposition indices was also observed ($p<0.06$; ESM Table 5). We did not find associations with any quantitative trait in women (ESM Table 5). We also examined for interaction between sex and DUSP9 genotype, and found indications of different association patterns for traits related to beta cell dysfunction, indicating stronger effects of the genotype in men (ESM Table 5).

Discussion

We report the association testing of 12 recently discovered type 2 diabetes risk variants with intermediary diabetes-related phenotypes in middle-aged individuals. Our results suggest an impairment of pancreatic beta cell function for diabetes risk alleles of the lead variant (rs1552224) at CENTD2. In addition, we provide suggestive evidence that rs5945326, near DUSP9 on the X-chromosome, is mediating its effect through reduced beta cell function in men. As for the remaining ten type 2 diabetes susceptibility loci, no convincing associations were established to the surrogate measurements of beta cell function or insulin sensitivity in this Danish population-based study.

The DIAGRAM+ consortium provided a new understanding of the potential physiological roles of the 12 new type 2 diabetes susceptibility loci by running quantitative trait studies of fasting plasma glucose and fasting serum

insulin levels [2]. The majority of SNPs showed higher fasting glucose for type 2 diabetes risk alleles. In addition, the lead variant of KLF14 showed an increased fasting serum insulin level, suggesting a primary insulin desensitising effect, whereas the diabetes associated variants at CENTD2 associated with reduced fasting serum insulin concentrations indicating reduced beta cell function. However, in the DIAGRAM+ Study, only two of these 12 new loci showed significant association to surrogate measures of insulin release and insulin sensitivity, as the risk allele at CENTD2 associated with a lower HOMA-B, and the risk allele at KLF14 associated with a higher HOMA-IR [2]. Although we recognise HOMA-B as a widely used measure of beta cell capacity, it is important to emphasise that this surrogate measure reflects insulin release under non-stimulated conditions [7], whereas, in the present report, we applied OGTT-derived surrogate measures to estimate the glucose-stimulated insulin response; a method which has been shown to be superior to the HOMA-B model in estimating the crucial first phase insulin release [7]. For the CENTD2 locus, DIAGRAM+ and the present study both report that CENTD2 mediates its effect through a reduced beta cell function, whereas we failed to reproduce the effect of KLF14 on estimates of insulin sensitivity. In the Inter99 Study sample we only have a statistical power of 16% to find a similar effect size of HOMA-IR for KLF14 as reported in the DIAGRAM+ paper [2], which might explain the missing associations in the present study.

Our studies of middle-aged men from the Inter99 cohort indicate that rs5945326, near DUSP9, may increase risk of type 2 diabetes through an impairment of glucose-stimulated insulin release. Yet, the evidence for these findings is sparse. In DIAGRAM+, the X-chromosome was only investigated with regard to fasting glucose and insulin in a subset of the full study population, and no associations with these traits were reported, nor was any sex specific association demonstrated. A future meta-analysis of association signals, GWAS, and additional fine mapping of the DUSP9 locus with emphasis on this possible sex specific association will be of interest.

In contrast to CENTD2, where there is no reported biological data to support an effect on beta cell function, DUSP9 (which codes for mitogen-activated dual specificity protein kinase phosphastase-4) has been identified as a gene involved in insulin signalling and in the pathogenesis of insulin resistance [8–10].

For the remaining ten loci, with odds ratios of 1.06–1.14, we found no convincing association to any quantitative traits related to type 2 diabetes in the Inter99 Study. By combining data in large meta-analyses, as in the DIAGRAM consortium, these new, interesting type 2 diabetes susceptibility loci were discovered. However, investigations of intermediary phenotypes with low effect sizes require

large numbers of individuals. To illustrate this we performed statistical power analyses in different scenarios (see ESM Table 2), demonstrating the need for combining efforts in further meta-analyses when searching for the diabetes intermediary phenotype.

In conclusion, we report data that support and extend the findings of an impaired beta cell function in carriers of the major diabetogenic allele of *CENTD2* rs1552224. The situation regarding the remaining 11 loci calls for further investigation in large-scale association studies in well-characterised study populations.

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