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ARTICLE

# The diabetogenic *VPS13C/C2CD4A/C2CD4B* rs7172432 variant impairs glucose-stimulated insulin response in 5,722 non-diabetic Danish individuals

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**Abstract**
*Aims/hypothesis* A genome-wide association study in the Japanese population reported two genome-wide significant loci associated with type 2 diabetes of which the *VPS13C/C2CD4A/C2CD4B* locus was replicated in Europeans. We looked for potential associations between the diabetogenic *VPS13C/C2CD4A/C2CD4B* rs7172432 variant and diabetes-related intermediary traits.
*Methods* We genotyped the rs7172432 variant in the population-based Inter99 cohort ($n$=6,784) and analysed quantitative diabetes-related traits in 5,722 non-diabetic participants who all were examined by an OGTT.
*Results* The diabetes-associated A allele was associated with 0.60 cm higher waist circumference ($p$=0.004), 0.037 mmol/l higher fasting plasma glucose ($p=4\times10^{-5}$) and 0.11 mmol/l higher plasma glucose at 30 min during an OGTT ($p=4\times10^{-4}$). In analyses adjusted for concomitant insulin sensitivity levels the diabetogenic allele was associated with a lower acute glucose-stimulated insulin response (GSIR) as estimated by 30 min serum insulin ($\beta=-0.039$, $p=2\times10^{-7}$),

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insulinogenic index ($\beta$=−0.057, $p=1\times10^{-8}$) and BIGTT-acute insulin release ($\beta$=−0.041, $p=9\times10^{-9}$). As rs7172432 is situated in a region previously associated with glycaemic traits, we tested linkage disequilibrium (LD) with the reported regional lead single-nucleotide polymorphisms for fasting (rs11071657) and 2 h plasma glucose (rs17271305), and performed conditional analyses of rs7172432. Rs7172432 showed moderate LD with rs11071657 and rs17271305 ($R^2<$ 0.34) and we found strong association by almost unchanged effect sizes of rs7172432 with plasma glucose and estimates of GSIR in analyses conditional on rs11071657 and rs17271305.

*Conclusions/interpretation* The diabetogenic *VPS13C/C2CD4A/C2CD4B* rs7172432 A allele associates with GSIR in non-diabetic individuals from the general population, suggesting an impaired beta cell function as an intermediary diabetes-related trait.

**Keywords** Genetic epidemiology · Genetics · Insulin response · Type 2 diabetes

**Abbreviations**

| | |
|---|---|
| AIR | Acute insulin response |
| GSIR | Glucose-stimulated insulin response |
| GWAS | Genome-wide association study |
| IR | Insulin resistance |
| LD | Linkage disequilibrium |
| $S_I$ | Sensitivity index |
| SNP | Single-nucleotide polymorphism |

## Introduction

Since 2007 the number of common validated type 2 diabetes genes has increased considerably, owing to the results of genome-wide association studies (GWAS). Mostly these studies have been performed in European populations, and although many of the identified gene variants have subsequently been replicated in other ethnicities, the overlap of type 2 diabetes genes in various ethnicities is undetermined. In 2008 the results of two GWAS were published, highlighting *KCNQ1* as a strong type 2 diabetes susceptibility gene in the Japanese population [1, 2]; however these studies were of limited size with regard to both sample size and number of single-nucleotide polymorphisms (SNPs) genotyped. Recently, a three-stage GWAS of 459,359 SNPs in the Japanese population was reported, showing novel genome-wide significant associations of variants in two loci in combined analyses of up to ~27,000 Japanese samples [3]. Association with type 2 diabetes was subsequently extended to European populations of up to 15,000 Danish and French samples for the *VPS13C/C2CD4A/C2CD4B* locus, showing comparable type 2 diabetes risk increments of 10%–13% in both East Asian and European populations [3].

The genomic region enclosing *VPS13C/C2CD4A/C2CD4B* has previously been reported to influence fasting plasma glucose (lead SNP rs11071657) [4] and plasma glucose 2 h after an oral glucose load (lead SNP rs17271305) [5]; yet the involved lead SNPs are in low or moderate linkage disequilibrium (LD). We previously reported a diminished insulin release in carriers of the rs11071657 glucose-raising allele [6]; however, this was not found in a meta-analysis of more than 15,000 individuals with OGTT data [7]. The rs17271305 variant has not shown any association with insulin release [5, 7].

The objective of the current study was to determine the intermediary mechanism responsible for risk of type 2 diabetes for the *VPS13C/C2CD4A/C2CD4B* lead rs7172432 SNP by testing association with type 2 diabetes-related phenotypes in 5,722 non-diabetic individuals of the population-based Inter99 cohort.

## Methods

*Participants* The Inter99 cohort is a randomised, non-pharmacological intervention study for the prevention of ischaemic heart disease, conducted on 6,784 randomly ascertained participants aged 30 to 60 years at the Research Centre for Prevention and Health in Glostrup, Copenhagen (ClinicalTrials.gov NCT00289237) [8]. An OGTT was performed with measurement of plasma glucose and serum insulin at fasting, and 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 type 2 diabetes ($n$=253) or previously diagnosed diabetes ($n$=119) according to WHO 1999 criteria. The analysis of quantitative diabetes-related phenotypes was performed in 5,722 non-diabetic participants. Detailed characteristics of Inter99 have been published previously [6].

Written informed 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 Declaration II. Biochemical and anthropometric measures were obtained as previously detailed [6].

*Derived estimates of insulin response and insulin sensitivity* Acute insulin response (AIR) was reported as the insulinogenic index calculated as [serum insulin $(\text{pmol/l})_{30\,\text{min}}$ − serum insulin $(\text{pmol/l})_{0\,\text{min}}$]/plasma glucose $(\text{mmol/l})_{30\,\text{min}}$, and as BIGTT-AIR. Insulin sensitivity was estimated by the HOMA of insulin resistance (IR) calculated as serum insulin

(pmol/l)$_{0\,min}$×plasma glucose (mmol/l)$_{0\,min}$/135, sensitivity index ($S_I$) Matsuda [9] and BIGTT-$S_I$. The BIGTT indices, which apply information on sex and BMI, combined with plasma glucose and serum insulin during an OGTT, to provide indices for AIR and $S_I$, were calculated as described by Hansen et al. [10].

*Genotyping* The rs7172432 variant was genotyped by the KASPar SNP Genotyping system (KBioscience, Hoddesdon, UK). The success rate was 96.6 with a mismatch rate of 0.3 in 596 pairs genotyped in duplicate. The distribution of genotypes was in Hardy–Weinberg equilibrium in Inter99 ($p$=0.9).

*Statistical analysis* General linear statistical methodology was used to test quantitative traits in relation to genotype, applying additive models. Values of serum insulin and derived indices (except BIGTT-$S_I$) were logarithmically transformed before analysis. A $p$ value of less than 0.05 was considered statistically significant. The exact models are described in the results and tables. All analyses were performed using RGui, version 2.10.0 (www.r-project.org). Estimates of statistical power in the Inter99 cohort have previously been reported [6].

## Results

First we analysed the diabetes-associated rs7172432 in relation to metabolic intermediary traits in Inter99 (Table 1). In a model adjusted for age and sex the diabetes-associated A allele associated with a per-allele 0.60 cm higher waist circumference ($p$=0.004), a per-allele 0.037 mmol/l higher fasting plasma glucose ($p=4\times10^{-5}$) and 0.11 mmol/l higher plasma glucose 30 min after an oral glucose load ($p=4\times10^{-4}$). The diabetogenic allele was also associated with a lower acute glucose-stimulated insulin response (GSIR), as estimated by 30 min serum insulin ($\beta$=−0.032, $p$=0.003), insulinogenic index ($\beta$=−0.052, $p=1\times10^{-5}$) and BIGTT-AIR ($\beta$=−0.038, $p=5\times10^{-6}$). To obtain association estimates with GSIR independent of adiposity, we adjusted analyses for BMI and found similar association with plasma glucose levels, whereas the association with GSIR was substantiated (Table 1). Similar results were obtained when adjusting for waist circumference

**Table 1** Quantitative metabolic traits in non-diabetic participants of the Inter99 cohort stratified according to the *VPS13C*/*C2CD4A*/*C2CD4B* rs7172432 genotype

| Variable | GG | GA | AA | Model 1 | Model 2 |
|---|---|---|---|---|---|
| *N* (men/women) | 1,062 (516/546) | 2,712 (1,313/1,399) | 1,718 (859/859) | | |
| Age (years) | 46±8 | 46±8 | 46±8 | | |
| Adiposity measures | | | | | |
| BMI (kg/m$^2$) | 25.7±4.3 | 26.1±4.4 | 26.1±4.4 | 0.17 (0.083), 0.05 | |
| Waist (cm) | 85.0±12.7 | 85.9±12.8 | 86.4±13.1 | 0.60 (0.21), 0.004 | 0.23 (0.10), 0.02 |
| Oral glucose tolerance test | | | | | |
| Fasting plasma glucose (mmol/l) | 5.4±0.5 | 5.5±0.5 | 5.5±0.5 | 0.037 (0.009), $4\times10^{-5}$ | 0.032 (0.0087), $2\times10^{-4}$ |
| 30 min plasma glucose (mmol/l) | 8.4±1.7 | 8.6±1.7 | 8.6±1.7 | 0.11 (0.031), $4\times10^{-4}$ | 0.096 (0.030), 0.001 |
| 120 min plasma glucose (mmol/l) | 6.0±1.6 | 6.0±1.5 | 5.9±1.5 | –0.025 (0.029), 0.4 | −0.040 (0.028), 0.2 |
| Fasting serum insulin (pmol/l) | 33 (24–49) | 34 (24–50) | 34 (24–50) | 0.0075 (0.011), 0.5 | −0.0012 (0.0098), 0.9 |
| 30 min serum insulin (pmol/l) | 254 (179–364) | 250 (182–354) | 237 (172–348) | −0.032 (0.011), 0.003 | −0.040 (0.0099), $5\times10^{-5}$ |
| 120 min serum insulin (pmol/l) | 140 (91–224) | 155 (97–248) | 159 (97–256) | 0.048 (0.015), 0.002 | 0.037 (0.014), 0.009 |
| Insulinogenic index | 26.1 (18.0–39.1) | 25.3 (17.5–37.4) | 23.5 (16.4–35.1) | −0.052 (0.012), $1\times10^{-5}$ | −0.057 (0.011), $4\times10^{-7}$ |
| BIGTT-AIR | 1,700 (1,360–2,200) | 1,650 (1,300–2,080) | 1,570 (1,260–2,020) | –0.038 (0.0082), $5\times10^{-6}$ | |
| HOMA-IR | 1.30 (0.93–2.0) | 1.37 (0.942–2.1) | 1.39 (0.937–2.09) | 0.014 (0.012), 0.2 | 0.0048 (0.010), 0.6 |
| BIGTT-$S_I$ | 10.0±4.0 | 9.0±4.0 | 9.0±4.0 | –0.13 (0.08), 0.1 | |
| $S_I$ Matsuda | 8.05 (5.57–11.2) | 7.78 (5.29–11) | 7.76 (5.27–11.3) | −0.011 (0.011), 0.3 | –0.0018 (0.0096), 0.9 |

For each genotype data are mean±SD or median (interquartile range)

For models 1 and 2 data are estimate (SE) and $p$ value

Estimates, SE and $p$ values are based on additive models for the diabetes-associated major A allele

Model 1: adjusted for age and sex. Model 2: adjusted for age, sex and BMI. BIGTT-AIR and BIGTT-$S_I$ are only adjusted for age because sex and BMI are included in the calculation of the indices

**Table 2** Conditional analysis of *VPS13C*/*C2CD4A*/*C2CD4B* rs7172432 and rs11071657 on diabetes-related intermediary traits in 5,722 non-diabetic participants of the population-based Inter99 cohort

| Explanatory variables | Age, sex, log($S_I$ Matsuda), rs7172432[a] | Age, sex, log($S_I$ Matsuda), rs11071657[a] | Age, sex, log($S_I$ Matsuda), rs7172432[a], rs11071657 | Age, sex, log($S_I$ Matsuda), rs7172432, rs11071657[a] |
|---|---|---|---|---|
| Fasting plasma glucose (mmol/l) | 0.030 (0.0084), $3\times10^{-4}$ | 0.029 (0.0086), $8\times10^{-4}$ | 0.021 (0.010), 0.04 | 0.017 (0.010), 0.1 |
| 30 min plasma glucose (mmol/l) | 0.093 (0.029), 0.001 | 0.073 (0.03), 0.01 | 0.078 (0.035), 0.02 | 0.027 (0.035), 0.4 |
| 120 min plasma glucose (mmol/l) | −0.047 (0.027), 0.08 | −0.0048 (0.027), 0.9 | −0.061 (0.032), 0.06 | 0.025 (0.032), 0.4 |
| Fasting serum insulin (pmol/l) | −0.0022 (0.0047), 0.6 | −0.0087 (0.0048), 0.07 | 0.0019 (0.0056), 0.7 | −0.0082 (0.0057), 0.1 |
| 30 min serum insulin (pmol/l) | −0.039 (0.0074), $2\times10^{-7}$ | −0.017 (0.0076), 0.03 | −0.04 (0.0088), $6\times10^{-6}$ | 0.0029 (0.0090), 0.7 |
| 120 min serum insulin (pmol/l) | 0.038 (0.01), $2\times10^{-4}$ | 0.024 (0.01), 0.02 | 0.035 (0.012), 0.003 | 0.0054 (0.012), 0.7 |
| Insulinogenic index | −0.057 (0.01), $1\times10^{-8}$ | −0.029 (0.01), 0.004 | −0.056 (0.012), $2\times10^{-6}$ | −0.00085 (0.012), 0.9 |
| BIGTT-AIR | −0.041 (0.0071), $9\times10^{-9}$ | −0.030 (0.0073), $4\times10^{-5}$ | −0.033 (0.0084), $8\times10^{-5}$ | −0.013 (0.0086), 0.1 |

Data are per-allele estimate (SE), *p* value for diabetes-associated major rs7172432 A allele or glucose-raising rs11071657 A allele

All analyses were performed assuming an additive model. All traits except values of plasma glucose were logarithmically transformed before analysis. Initial findings in Inter99 on rs11071657 have been previously published [6]. Minutes are given following an OGTT

[a] The parameter for which the coefficient is given

instead of BMI (data not shown). As the individual insulin response is highly dependent on individual concomitant levels of insulin sensitivity, we made adjustments in the analysis of GSIR for insulin sensitivity estimated by Matsuda's index and found strong effects of rs7172432 on GSIR (30 min serum insulin: $\beta=-0.039$, $p=2\times10^{-7}$; insulinogenic index: $\beta=-0.057$, $p=1\times10^{-8}$; BIGTT-AIR: $\beta=-0.041$, $p=9\times10^{-9}$).

The *VPS13C*/*C2CD4A*/*C2CD4B* rs7172432 variant is positioned in a region previously associated with glycaemic traits and we therefore tested LD with the reported lead SNPs for genome-wide significant associations with fasting plasma glucose (rs11071657) and plasma glucose at 2 h during OGTT (rs17271305). Rs7172432 showed moderate LD with rs11071657 ($R^2=0.27$, $D'=0.49$) and rs17271305 ($R^2=0.34$, $D'=0.63$) in the population-based Inter99 sample. To evaluate the relationship between these variants and the association with glucose-related traits, we performed conditional analysis of the three lead SNPs. The rs7172432 variant showed strong association by virtually unchanged effect sizes with fasting plasma glucose and estimates of GSIR in analysis conditional on rs11071657 (Table 2) and rs17271305 (Table 3). By contrast, in analysis conditional on rs7172432 associations of rs11071657 with fasting glucose and GSIR, traits were strongly attenuated and non-significant (Table 2). The same was the case for the association of rs17271305 with GSIR conditional on rs7172432; however, the association with 2 h plasma glucose remained borderline significant after adjustment for rs7172432 (Table 3).

## Discussion

To date most of the large-scale GWAS of type 2 diabetes and traits related to glucose homeostasis have been

**Table 3** Conditional analysis of *VPS13C*/*C2CD4A*/*C2CD4B* rs7172432 and rs17271305 on diabetes-related intermediary traits in 5,722 non-diabetic participants of the population-based Inter99 cohort

| Explanatory variables | Age, sex, log($S_I$ Matsuda), rs7172432[a] | Age, sex, log($S_I$ Matsuda), rs17271305[a] | Age, sex, log($S_I$ Matsuda), rs7172432[a], rs17271305 | Age, sex, log($S_I$ Matsuda), rs7172432, rs17271305[a] |
|---|---|---|---|---|
| Fasting plasma glucose (mmol/l) | 0.03 (0.0084), $3\times10^{-4}$ | −0.014 (0.0084), 0.08 | 0.032 (0.01), 0.003 | 0.0038 (0.01), 0.7 |
| 30 min plasma glucose (mmol/l) | 0.093 (0.029), 0.001 | −0.064 (0.029), 0.03 | 0.088 (0.036), 0.01 | −0.0064 (0.036), 0.9 |
| 120 min plasma glucose (mmol/l) | −0.047 (0.027), 0.08 | 0.06 (0.027), 0.02 | −0.0023 (0.033), 0.9 | 0.07 (0.033), 0.04 |
| Fasting serum insulin (pmol/l) | −0.0022 (0.0047), 0.6 | 0.0018 (0.0047), 0.7 | −0.0036 (0.0058), 0.5 | −0.0015 (0.0059), 0.8 |
| 30 min serum insulin (pmol/l) | −0.039 (0.0074), $2\times10^{-7}$ | 0.014 (0.0074), 0.05 | −0.046 (0.0091), $6\times10^{-7}$ | −0.012 (0.0092), 0.2 |
| 120 min serum insulin (pmol/l) | 0.038 (0.01), $2\times10^{-4}$ | –0.018 (0.01), 0.07 | 0.047 (0.012), $1\times10^{-4}$ | 0.011 (0.012), 0.4 |
| Insulinogenic index | −0.057 (0.01), $1\times10^{-8}$ | 0.027 (0.01), 0.008 | −0.063 (0.012), $4\times10^{-7}$ | −0.0099 (0.012), 0.4 |
| BIGTT-AIR | −0.041 (0.0071), $9\times10^{-9}$ | 0.02 (0.0071), 0.006 | −0.044 (0.0088), $7\times10^{-7}$ | −0.0068 (0.0088), 0.4 |

Data are per-allele estimate (SE), *p* value for diabetes-associated major rs7172432 A allele or 2 h glucose-raising rs17271305 G allele

All analyses were performed assuming an additive model. All traits except values of plasma glucose were logarithmically transformed before analysis. Initial findings in Inter99 on rs17271305 have been previously published [5]. Minutes are given following an OGTT

[a] The parameter for which the coefficient is given

performed in populations of European ancestry. However, recently a three-stage GWAS in Japanese individuals was reported [3]. The lead SNP in one of two novel Japanese type 2 diabetes susceptibility loci was also associated with type 2 diabetes in Europeans. Here we report a strong association with lower GSIR in risk allele carriers, indicating an impaired beta cell function as the intermediary phenotype increasing the risk of type 2 diabetes. The impact of rs7172432 on GSIR was evident from analysis of OGTT-derived estimates of stimulated insulin responses and was substantiated by adjusting for concomitant individual levels of insulin sensitivity. We observed a per-allele 3%–8% lower GSIR for the rs7172432 diabetes-risk allele, which in the Danish population is comparable to the effect sizes of the type 2 diabetes risk variants with the highest influence on GSIR such as *HHEX* rs1111875 and *MTNR1B* rs10830963 [11, 12]. We also show a 0.60 cm increased waist circumference in carriers of the diabetogenic allele, and from the current data we cannot exclude the possibility that rs7172432 has impacts on both central obesity and the pancreatic beta cell that together contribute to the increased risk of type 2 diabetes. This locus has not been found in recent large GWAS of measures of obesity, and obviously we cannot exclude a false positive finding. We have investigated only one SNP, but we analysed 13 traits, and although these traits are correlated, the association with waist circumference is merely borderline significant at a Bonferroni-corrected significance threshold of 0.004. This is in contrast to $p$ values of association with estimates of insulin release that are well below the Bonferroni-corrected significance threshold.

The genomic region in which rs7172432 is located has recently been shown to contain SNPs increasing fasting plasma glucose and plasma glucose 2 h after an oral glucose load in a non-diabetic population compiled by the Meta-Analysis of Glucose and Insulin-related Trait Consortium (MAGIC) [4, 5]. The rs17271305 variant associated with glucose at 2 h is situated in the neighbouring *VPS13C* gene, whereas the fasting glucose lead SNP (rs11071657) is located between the *C2CD4A* and *C2CD4B* genes. In conditional analyses we show that in 5,722 non-diabetic individuals of the Inter99 sample, the rs7172432 variant has an effect superior to rs11071657 on fasting plasma glucose and measures of GSIR; however, the association signals from rs7172432 and rs11071657 do not seem to be independent as analysis of rs11071657 conditional on rs7172432 attenuated this signal strongly. Yet, whether the rs7172432 plays a functional role in decreasing GSIR and increasing fasting glucose and risk of type 2 diabetes is unknown. rs7172432 is located between *C2CD4A* and *C2CD4B* and may lead to regulatory changes in gene expression or be in LD with an as-yet-undefined functional variant. Very little is known about the biological function of *VPS13C*, *C2CD4A* and *C2CD4B*, and the molecular relation to glucose homeostasis and beta cell function is unknown. Of interest, all three genes are expressed in the pancreas and in the beta cells [4, 5] and may therefore regulate insulin release or beta cell function. Further large-scale studies fine-mapping this region combined with functional studies may elucidate these matters. Yet this case highlights the value of performing genetic studies in the form of GWAS or fine-mapping efforts in various ethnic populations to locate functional elements in disease associations. In the near future this aspect will probably be very valuable with the emergence of novel second-generation dense SNP genotyping arrays. Here a more precise relationship between association signals from various ethnic populations can be achieved to narrow the region containing the causal variant.

In conclusion, we demonstrate that the recently described diabetogenic *VPS13C*/*C2CD4A*/*C2CD4B* rs7172432 A allele associates strongly with glucose-stimulated insulin release in non-diabetic individuals from the general Danish population, suggesting an impaired beta cell function as the intermediary diabetes-causing mechanism.

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**Duality of interest** T. Hansen and O. Pedersen are employed at the Hagedorn Research Institute, which is owned by Novo Nordisk. D. R. Witte is employed by Steno Diabetes Center, which is a research and teaching hospital facility in the Danish National Health Service and owned by Novo Nordisk. N. Grarup, D. R. Witte, T. Hansen and O. Pedersen hold personal shares in Novo Nordisk.

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