Diabetologia (2007) 50:972–979
DOI 10.1007/s00125-007-0623-2

ARTICLE

A variant in the transcription factor 7-like 2 (*TCF7L2*) gene is associated with an increased risk of gestational diabetes mellitus

N. Shaat · Å. Lernmark · E. Karlsson · S. Ivarsson · H. Parikh · K. Berntorp · L. Groop

Received: 24 October 2006 / Accepted: 21 January 2007 / Published online: 7 March 2007

Abstract
Aims/hypothesis Genetic and epidemiological studies suggest an association between gestational diabetes mellitus and type 2 diabetes. Both are polygenic multifactorial disorders characterised by beta cell dysfunction and insulin resistance. Our aim was to investigate whether common genetic variants that have previously been associated with type 2 diabetes or related phenotypes would also confer risk for gestational diabetes mellitus.
Materials and methods In 1,881 unrelated pregnant Scandinavian women (649 women with gestational diabetes mellitus, 1,232 non-diabetic control subjects) we genotyped the transcription factor 7-like 2 (*TCF7L2* rs7903146), adiponectin (*ADIPOQ* +276G>T), peroxisome-proliferator activated receptor, gamma 2 (*PPARG* Pro12Ala), PPARG-coactivator, 1 alpha (*PPARGC1A* Gly482Ser), forkhead box C2 (*FOXC2* −512C>T) and β3-adrenergic receptor (*ADRB3* Trp64Arg) polymorphisms using TaqMan allelic discrimination assay or RFLP.
Results The CC, CT and TT genotype frequencies of the *TCF7L2* rs7903146 variant differed significantly between women with gestational diabetes mellitus and control women (46.3, 43.6 and 10.1% vs 58.5, 35.3 and 6.2%, $p=3.7\times10^{-6}$, corrected p value [Pc] for multiple testing $P\text{c}=2.2\times10^{-5}$). The T-allele was associated with an increased risk of gestational diabetes mellitus (odds ratio 1.49 [95% CI 1.28–1.75], $p=4.9\times10^{-7}$ [$P\text{c}=2.8\times10^{-6}$]). Compared with wild-type CC-genotype carriers, heterozygous (CT-genotype) and homozygous (TT-genotype) carriers had a 1.6-fold (95% CI 1.26–1.93, $p=3.7\times10^{-5}$ [$P\text{c}=0.0002$]) and a 2.1-fold (95% CI 1.41–2.99, $p=0.0001$ [$P\text{c}=0.0008$]) increased risk of gestational diabetes mellitus, respectively. The other polymorphisms studied were not significantly associated with gestational diabetes mellitus (*ADIPOQ* +276G>T: 1.17 [1.01–1.36], $p=0.039$ [$P\text{c}=0.23$]; *PPARG* Pro12Ala: 1.06 [0.87–1.29], $p=0.53$; *PPARGC1A* Gly482Ser: 0.96 [0.83–1.10], $p=0.54$; *FOXC2* −512C>T: 1.01 [0.87–1.16], $p=0.94$; and *ADRB3* Trp64Arg: 1.22 [0.95–1.56], $p=0.12$).
Conclusions/interpretation The *TCF7L2* rs7903146 variant is associated with an increased risk of gestational diabetes mellitus in Scandinavian women.

Keywords Adiponectin · *ADRB3* · Association · *FOXC2* · GDM · Gestational diabetes mellitus · Polymorphism · *PPARG* · *PPARGC1A* · *TCF7L2*

Abbreviations
ADIPOQ adiponectin,
ADRB3 β3-adrenergic receptor

Electronic supplementary material The online version of this article (doi:10.1007/s00125-007-0623-2) contains supplementary material, which is available to authorised users.

N. Shaat (✉) · Å. Lernmark · E. Karlsson · H. Parikh · K. Berntorp · L. Groop
Department of Clinical Sciences/Diabetes & Endocrinology, Malmö University Hospital, Lund University, Malmö, Sweden
e-mail: nael.shaat@med.lu.se

Å. Lernmark
Robert H. Williams Laboratory, University of Washington, Seattle, WA, USA

S. Ivarsson
Department of Paediatrics, Malmö University Hospital, Lund University, Malmö, Sweden

L. Groop
Department of Medicine, Helsinki University Hospital, Helsinki, Finland

DBS	dried blood spots
FOXC2	forkhead box C2
GCK	glucokinase
KCNJ11	potassium inwardly rectifying channel subfamily J, member 11
MAF	minor allele frequency
OR	odds ratio
*P*c	corrected *p* value
PPAR	peroxisome-proliferator activated receptor
PPARG	peroxisome-proliferator activated receptor, gamma 2
PPARGC1A	PPARG-coactivator 1 alpha
SNP	single nucleotide polymorphism
TCF1	transcription factor 1, hepatic; LF-B1, hepatic nuclear factor (HNF1), albumin proximal factor
TCF7L2	transcription factor 7-like 2

Introduction

Genetic and epidemiological studies suggest an association between gestational diabetes mellitus and type 2 diabetes [1, 2]. The prevalence of gestational diabetes mellitus is increasing in parallel with the increased prevalence of type 2 diabetes [1, 2]. The former is a common metabolic disorder of pregnancy, affecting at least 2% of Scandinavian women and 5 to 10% of Asian, Hispanic/Mexican American, and Arabian women [2]. Gestational diabetes mellitus occurs when pancreatic beta cells are unable to compensate for increased insulin resistance during pregnancy [3]. Both insulin deficiency and insulin resistance are considered to be heritable with heritability estimates for insulin secretion of 0.75–0.84 and for insulin sensitivity of 0.53–0.55 [4].

Studies have consistently shown that women with a family history of diabetes have an increased risk of gestational diabetes mellitus [5, 6]. In addition, we and others have demonstrated that gestational diabetes mellitus shares some genetic risk factors with type 2 diabetes [2]. For example, potassium inwardly rectifying channel subfamily J, member 11 (*KCNJ11* E23K), glucokinase (*GCK* −30G>A) and transcription factor 1, hepatic; LF-B1, hepatic nuclear factor (HNF1), albumin proximal factor (TCF1, also known as hepatocyte nuclear factor 1-α [*HNF1A* I27L]) polymorphisms, all of which have been associated with type 2 diabetes [7–9], also increase the risk of gestational diabetes mellitus with a modest effect size [10, 11].

In the present study, therefore, we continued to study common genetic variants that have previously been associated with type 2 diabetes or related phenotypes such as the metabolic syndrome. In order to provide sufficient power to detect a modest odds ratio (OR≥1.3), we selected five variants with a minor allele frequency (MAF) of at least 15%. These polymorphisms were the transcription factor 7-like 2 (*TCF7L2* rs7903146) [12–19], adiponectin (*ADIPOQ* +276G>T) [20, 21], peroxisome-proliferator activated receptor (PPAR), gamma 2 (*PPARG* Pro12Ala) [22], PPARG-coactivator 1, alpha (*PPARGC1A* Gly482Ser) [23] and forkhead box C2 (*FOXC2* −512C>T) [24]. Despite its MAF ~10%, we also included the β3-adrenergic receptor (*ADRB3* Trp64Arg) polymorphism, as its putative association with gestational diabetes mellitus has been addressed in a number of small studies with inconsistent results [25–28]. These polymorphisms were genotyped in a case–control study of 1,881 unrelated pregnant Scandinavian women (649 with gestational diabetes mellitus, 1,232 non-diabetic control subjects).

Subjects and methods

Study subjects

Since 1995, all pregnant women in southern Sweden (Skåne) have been routinely offered a 75-g OGTT at 27–28 weeks of pregnancy, irrespective of family history of diabetes or any other risk factor for gestational diabetes mellitus. Women with previous gestational diabetes mellitus or a family history of diabetes are offered an additional 75-g OGTT at 12–13 weeks. The tests are performed in the local Maternity Health Care clinics, using HemoCue devices (HemoCue, Ängelholm, Sweden) for capillary whole-blood analysis. Gestational diabetes mellitus is defined, according to the proposal by the Diabetic Pregnancy Study Group of the European Association for the Study of Diabetes [29], as a 2-h capillary glucose concentration (double test) of at least 9 mmol/l. The compliance rate for OGTTs is approximately 90%.

We studied 1,881 unrelated pregnant Scandinavian women (649 with gestational diabetes mellitus, 1,232 pregnant non-diabetic control subjects). The diabetic women were recruited from Malmö or Lund University Hospitals between March 1996 and December 2003 (n=226) as well as from the women participating in the Diabetes Prediction in Skåne study, which is a prospective, longitudinal study for the prediction of type 1 diabetes in all newborns in southern Sweden during the period from September 2000 until August 2004 (n=423) [30]. All pregnant non-diabetic control subjects (n=1,232) were drawn from the same study [30]. Both study groups, i.e. women with gestational diabetes mellitus and control women, are considered to be genetically homogeneous since they have the same ethnic background (i.e. Scandinavian) and were recruited from the same place and during

the same time period. The characteristics of the majority of participants in the present study have been reported earlier [10]. Detailed phenotypic characteristics, including OGTT data, were available only for a small proportion of the women with gestational diabetes mellitus [31]. Informed voluntary consent was obtained from all study subjects. The study was approved by the ethics committee of Lund University.

Genetic analyses

Sample collection Total DNA was isolated from peripheral blood lymphocytes from 226 women with gestational diabetes mellitus. In the other subjects, blood samples were collected as dried blood spots (DBS) on filters (Grade 2992 filters; Schleicher and Schuell, Dassel, Germany).

Genotyping using DNA Genotyping of *TCF7L2* (rs 7903146), *ADIPOQ* +276G>T (rs1501299), *PPARGC1A* Gly482Ser (rs8192678), *FOXC2* −512C>T and *ADRB3* Trp64Arg (rs4994) was carried out using TaqMan allelic discrimination assay, whereas *PPARG* Pro12Ala (rs180 1282) polymorphism was genotyped by TaqMan assay or RFLP as previously described [31]. TaqMan assay was carried out on an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using 2 μl of DNA (5–10 ng) according to the manufacturer's instructions. Primers and probes were designed using Assays-by-Design (Applied Biosystems), except for *FOXC2* −512C>T, which was ordered from MWG Biotech Scandinavia (Risskov, Denmark). The primers and probes used are listed in Electronic supplementary material (ESM) Table 1.

Genotyping using DBS Using the primers listed in ESM Table 2, a template PCR was initially carried out to amplify the region of interest where the single nucleotide polymorphism (SNP) is located. The template PCR was followed by TaqMan allelic discrimination assay or RFLP. The primers used for the template PCRs were designed to be located outside the TaqMan primers and probes.

The template PCR was performed with an initial two cycles (4°C for 30 s, followed by 98°C for 3 min), followed by holding at 80°C while the PCR mix was added. Then the PCR was continued with initial denaturation (94°C for 5 min, except for *ADIPOQ* +276G>T at 96°C), followed by 45 cycles of denaturation (94°C for 30 s, except for *ADIPOQ* +276G>T at 96°C), annealing (30 s) and extension (72°C for 30–60 s), followed by final extension (72°C for 10 min). The reagents used for PCR amplification are listed in ESM Table 3.

The template PCR was followed by SNP genotyping, which was carried out using TaqMan allelic discrimination assay with 2 μl of the template PCR product according to the manufacturer's instructions. However, for some of the DBS samples, the *ADIPOQ* +276G>T polymorphism was genotyped by RFLP where 20 μl of the template PCR product was digested with Mva 1269I (Fermentas, St Leon-Rot, Germany) at 37°C for 4 h. PCR products were separated on 3% agarose gel (SeaKem, Rockland, ME, USA) and stained with ethidium bromide to visualise the fragments using ultra-violet light. The *PPARG* Pro12Ala polymorphism was also genotyped by RFLP for some of the DBS samples as previously described [31].

Genotyping quality control Genotyping success rate was similar for women with gestational diabetes and for control subjects. In the former the success rates were: 90.1% (*TCF7L2* rs7903146); 98.8% (*ADIPOQ* +276G>T); 98.1% (*PPARG* Pro12Ala); 99.2% (*PPARGC1A* Gly482Ser); 97.8% (*FOXC2* −512C>T); and 98.5% (*ADRB3* Trp64Arg). For control subjects the success rates were: 90.2% (*TCF7L2* rs7903146); 99.4% (*ADIPOQ* +276G>T); 100% (*PPARG* Pro12Ala); 99.4% (*PPARGC1A* Gly482Ser); 97.7% (*FOXC2* −512C>T); and 99.6% (*ADRB3* Trp64Arg). Genotyping error rate was determined to be 0.4% using 1751 (15.5%) duplicate genotypes as well as 89 double samples (i.e. women with gestational diabetes mellitus who had both peripheral blood DNA and DBS or two DBS taken at different deliveries). All polymorphisms conformed to Hardy–Weinberg equilibrium (χ^2 test, $p>0.05$) in gestational diabetes mellitus and control groups. These quality control measures indicate that genotyping results of all SNPs are reliable for analyses.

Statistical analyses

ANOVA was used to test the significance of difference in continuous variables such as age between gestational diabetes mellitus and control groups. Age was presented as mean ± SEM. χ^2 analysis was used to test for difference in genotype and allele frequencies between gestational diabetes mellitus and control groups. Logistic regression analysis was used to calculate the age-adjusted and/or crude ORs and 95% CIs for the polymorphisms. Statistical analyses were performed using the Number Cruncher Statistical Systems (NCSS, Kaysville, UT, USA). Bonferroni correction was used to correct for multiple testing where significant p values were multiplied by 6 (i.e. the number of tested SNPs). A two-sided p value <0.05 was considered statistically significant.

Power calculations were performed using Genetic Power Calculator (available at http://ibgwww.colorado.edu/~pshaun/gpc/) [32]. The prevalence of gestational diabetes mellitus was assumed to be 2%. The present study has

>80% power, under a multiplicative model, to detect an effect size of 1.3 (as measured in terms of genotypic relative risk) when the frequency of the predisposing allele is 15% (for α=0.05). The study has at least 80% power to detect a genotypic relative risk of 1.22 (for α=0.05) when the predisposing allele frequency is >30%.

Results

Characteristics of the subjects

Women with gestational diabetes mellitus were slightly older than pregnant non-diabetic control women (32.3±0.2 vs 30.5±0.1, $p<1\times10^{-10}$). The genotype and allele frequency distributions of all polymorphisms studied are presented in Table 1.

TCF7L2 rs7903146

The CC, CT and TT genotype frequencies of the *TCF7L2* rs7903146 variant differed significantly between women with gestational diabetes mellitus and control subjects (46.3, 43.6 and 10.1% vs 58.5, 35.3 and 6.2%, $p=3.7\times10^{-6}$, corrected p value [Pc] for multiple testing Pc=2.2×10^{-5}). The T-allele was associated with an increased risk of gestational diabetes mellitus (OR 1.49 [95% CI 1.28–1.75], $p=4.9\times10^{-7}$ [Pc=2.8×10^{-6}]). Compared with wild-type CC-genotype carriers, heterozygous (CT-genotype) and homozygous (TT-genotype) carriers had a 1.56-fold (95% CI 1.26–1.93, $p=3.7\times10^{-5}$ [Pc=0.0002]) and a 2.05-fold (95% CI 1.41–2.99, p=0.0001 [Pc=0.0008]) increased risk of gestational diabetes mellitus, respectively. Age-adjusted risk of gestational diabetes mellitus for CT-genotype and TT-genotype carriers was 1.60 (95% CI 1.29–1.98, $p=2\times10^{-5}$ [Pc=0.0001]) and 2.08 (95% CI 1.42–3.05, p=0.0002 [Pc=0.001]), respectively. In addition, the effect size was found to change slightly (1.58 [1.27–1.95], $p=2.8\times10^{-5}$ [Pc=0.0002] for CT-genotype; 1.95 [1.33–2.86], p=0.0005 [Pc=0.003] for TT-genotype) when women who were positive for autoantibodies to GAD65 or to protein tyrosine phosphatase or both were removed from analyses. Data on antibody measurements were not available for all subjects.

ADIPOQ +276G>T

The T-allele of the +276G>T polymorphism was associated with a slightly increased risk of gestational diabetes mellitus (1.17 [1.01–1.36], p=0.039 [Pc=0.23]). In addition, GT-genotype carriers had an increased risk of gestational diabetes mellitus (1.27 [1.04–1.55], p=0.020 [Pc=0.12]) compared with GG-genotype carriers. A similar effect size (1.26 [1.04–1.53], p=0.018 [Pc=0.11]) was also observed under a dominant model (TT+GT vs GG).

Other polymorphisms

The other polymorphisms studied were not significantly associated with gestational diabetes mellitus (*PPARG* Pro12Ala: 1.06 [0.87–1.29], p=0.53; *PPARGC1A* Gly482Ser: 0.96 [0.83–1.10], p=0.54; *FOXC2* −512C>T: 1.01 [0.87–1.16], p=0.94; and *ADRB3* Trp64Arg: 1.22 [0.95–1.56], p=0.12).

Discussion

Gestational diabetes mellitus is a heterogeneous disorder where genetic and environmental factors interact to cause the disease [1, 2]. Studies have demonstrated that gestational diabetes mellitus shares some genetic and phenotypic features with other types of diabetes such as type 1, type 2 and MODY [1, 2]. However, different risk alleles/genes might be operative in different types of diabetes and gestational diabetes mellitus due to the heterogeneous nature of these disorders. During pregnancy beta cells undergo structural and functional changes in response to the increased insulin requirements. These changes include, among others, enhanced glucose-stimulated insulin secretion, increased insulin synthesis and increased beta cell proliferation and islet volume [33]. Genetic factors might therefore predispose to abnormal glucose tolerance during pregnancy by influencing one or more of these physiological processes.

TCF7L2 rs7903146

Our results provide evidence that *TCF7L2* is a major susceptibility gene for gestational diabetes mellitus in Scandinavian women. *TCF7L2*, which maps to chromosome 10q25.3, is a transcription factor belonging to the high mobility group-box transcription factors family [34]. It is involved in the wingless-type MMTV integration site family (WNT) signalling pathway, which is important for the development and growth regulatory mechanisms of the cell [35]. The *TCF7L2* rs7903146 variant was originally associated with type 2 diabetes in individuals from Iceland, Denmark and the USA [12]. This variant was reproducibly associated with type 2 diabetes in subsequent studies [13–19]. The mechanism by which it might lead to deterioration of glucose homeostasis is still unknown. However, recent findings suggest that it is associated with impaired insulin secretion [14, 15, 17]. TCF7L2 is abundantly produced in the gut and has been shown to bind to the promoter of proglucagon gene in vitro [36].

Table 1 Genotype and allele distributions and corresponding odds ratios for gestational diabetes mellitus

Polymorphism (rs number)	Genotype or allele	GDM, *n* (%)	Control subjects, *n* (%)	GDM, allelic effect, additive models[a]	GDM, recessive model[a]	GDM, dominant model[a]
TCF7L2 IVS3C>T (rs7903146)	CC	271 (46.3)	650 (58.5)	1		
	CT	255 (43.6)	392 (35.3)	1.56 (1.26–1.93)[c]		
	TT	59 (10.1)	69 (6.2)[b]	2.05 (1.41–2.99)[d]	1.69 (1.18–2.44)[f]	1.63 (1.34–2.0)[g]
	T	373 (31.9)	530 (23.8)	1.49 (1.28–1.75)[e]		
ADIPOQ +276G>T (rs1501299)	GG	301 (46.9)	646 (52.7)	1		
	GT	285 (44.5)	482 (39.4)	1.27 (1.04–1.55)		
	TT	55 (8.6)	97 (7.9)	1.22 (0.85–1.74)	1.09 (0.77–1.54)	1.26 (1.04–1.53)
	T	395 (30.8)	676 (27.6)	1.17 (1.01–1.36)[h]		
PPARG Pro12Ala (rs1801282)	Pro/Pro	468 (73.5)	918 (74.5)	1		
	Pro/Ala	158 (24.8)	298 (24.2)	1.04 (0.83–1.30)		
	Ala/Ala	11 (1.7)	16 (1.3)	1.35 (0.62–2.93)	1.34 (0.62–2.89)	1.06 (0.85–1.31)
	Ala	180 (14.1)	330 (13.4)	1.06 (0.87–1.29)		
PPARGC1A Gly482Ser (rs8192678)	Gly/Gly	284 (44.1)	533 (43.5)	1		
	Gly/Ser	294 (45.7)	548 (44.8)	1.01 (0.82–1.23)		
	Ser/Ser	66 (10.2)	143 (11.7)	0.87 (0.63–1.20)	0.86 (0.63–1.18)	0.98 (0.81–1.19)
	Ser	426 (33.1)	834 (34.1)	0.96 (0.83–1.10)		
FOXC2 −512C>T	TT	244 (38.4)	456 (37.9)	1		
	CT	291 (45.8)	568 (47.2)	0.96 (0.78–1.18)		
	CC	100 (15.8)	180 (14.9)	1.04 (0.78–1.39)	1.06 (0.82–1.39)	0.98 (0.80–1.19)
	C	491 (38.7)	928 (38.5)	1.01 (0.87–1.16)		
ADRB3 Trp64Arg (rs4994)	Trp/Trp	534 (83.6)	1060 (86.4)	1		
	Trp/Arg	100 (15.6)	158 (12.9)	1.26 (0.96–1.65)		
	Arg/Arg	5 (0.8)	9 (0.7)	1.10 (0.37–3.31)	1.07 (0.36–3.20)	1.25 (0.96–1.63)
	Arg	110 (8.6)	176 (7.2)	1.22 (0.95–1.56)		

GDM gestational diabetes mellitus

[a] OR (95% CI)

[b] $p=3.7\times10^{-6}$ ($Pc=2.2\times10^{-5}$) for difference in genotype frequencies between women with and without GDM

[c] $p=3.7\times10^{-5}$ ($Pc=0.0002$) for comparison of CT vs CC between women with and without GDM

[d] $p=0.0001$ ($Pc=0.0008$) for comparison of TT vs CC between women with and without GDM

[e] $p=4.9\times10^{-7}$ ($Pc=2.8\times10^{-6}$) for difference in T-allele frequencies between women with and without GDM

[f] $p=0.004$ ($Pc=0.02$) for comparison of TT vs CT/CC (recessive model) between women with and without GDM

[g] $p=1.7\times10^{-6}$ ($Pc=1\times10^{-5}$) for comparison of TT/CT vs CC (dominant model) between women with and without GDM

[h] $p=0.039$ ($Pc=0.23$) for difference in T-allele frequencies between women with and without GDM

Hypothetically, *TCF7L2* might influence insulin secretion through regulation of the insulinotropic hormone, glucagon-like peptide-1, which is encoded by the proglucagon gene [37]. A recent report demonstrated that *TCF7L2* is expressed in pancreatic beta cells [13]. Thus, by affecting the WNT signalling, the variant could influence the beta cell proliferation (and hence insulin secretion) that normally takes place during pregnancy [33, 35]. The effect size for gestational diabetes mellitus conferred by this variant is similar to that reported in patients with type 2 diabetes [12]. Also, the allele frequency is comparable with that reported in Scandinavians [12, 15].

ADIPOQ +276G > T

ADIPOQ is a physiologically active polypeptide hormone derived from adipose tissue with insulin-sensitising properties [38]. Decreased plasma adiponectin during pregnancy has been associated with gestational diabetes mellitus [39]. In addition, reduced *ADIPOQ* mRNA levels in adipose tissue from women with gestational diabetes mellitus have been reported [39]. The common +276G>T variant is one of the most extensively studied variants within the *ADIPOQ* gene. It has been associated with type 2 diabetes, but different populations were found to have different at-

risk alleles [20, 21]. Thus, it is possible that another polymorphism in linkage disequilibrium with the +276G>T variant could confer the risk. In the present study, we found a nominal association between the T-allele of the +276G>T variant and gestational diabetes mellitus, but the significance disappeared after correcting for multiple testing. This variant might therefore predispose to gestational diabetes mellitus, but this needs to be further investigated in a larger sample and/or in other populations.

PPARG Pro12Ala

PPARG, which maps to chromosome 3p25, is a transcription factor with a pivotal role in adipocyte differentiation and function. The Ala allele of the Pro12Ala polymorphism has been consistently associated with reduced risk of type 2 diabetes [22, 40]. In vitro, the Ala allele leads to decreased PPARG activity and thereby to decreased transcription of a number of target genes, resulting in increased insulin sensitivity [41]. In the present study, and consistent with our previous finding in a smaller study of Scandinavian and Arabian women [31], we did not find an association between this variant and gestational diabetes mellitus. However, we were unable to rule out a smaller effect size (OR<1.3) of this variant on the risk of gestational diabetes mellitus.

PPARGC1A Gly482Ser

PPARGC1A is a transcriptional co-activator of several nuclear receptors including PPAR gamma and PPAR alpha, which play a role in the transcriptional control of mitochondrial fatty acid beta-oxidation enzymes [42]. *PPARGC1A*, located on chromosome 4p15.1, is expressed in various tissues including adipose tissue, skeletal muscle and pancreas [43]. The common Gly482Ser polymorphism in *PPARGC1A* has been associated with an increased risk of type 2 diabetes [23]. In addition, it influences maximum volume of oxygen uptake ($\dot{V}O_{2max}$) [44] and insulin secretion [45]. We found no effect of this variant on gestational diabetes mellitus in Scandinavian women, a finding which is in agreement with a recent small study in Austrian Europids [46].

FOXC2 −512C > T

FOXC2 is a key regulator of adipocyte metabolism [47]. The common −512C>T polymorphism located in the 5′ UTR of the *FOXC2* gene has been associated with enhanced insulin sensitivity and lower plasma triacylglycerol levels in female subjects from Scandinavia and in Pima Indian women [24, 48]. This sex-specific association with insulin resistance prompted us to investigate whether this polymorphism also influences the risk of gestational diabetes mellitus. Our results suggest that this polymorphism has no impact on the development of gestational diabetes mellitus in Scandinavian women.

ADRB3 Trp64Arg

We and others have previously reported a polymorphism (Trp64Arg) located in the first intracellular loop of the ADRB3 receptor, which has been associated with early-onset type 2 diabetes, abdominal obesity and features of the metabolic syndrome [49, 50]. Analysis of subsequent studies has shown an association of this polymorphism with features of the metabolic syndrome [40]. In addition, the Trp64Arg polymorphism has been associated with ‘mild’ gestational diabetes mellitus in Austrian [25], but not in Greek [26], Taiwanese [27] or Italian [28] women. However, it was associated with increased weight gain and increased glucose and insulin levels during pregnancy [25, 27]. The present study failed to find a role of this variant in the risk of gestational diabetes mellitus in Scandinavian women.

There are some limitations to the present study. First, we lack detailed phenotypic information on insulin secretion and action during pregnancy. Second, although the study is the largest of its kind, it is not sufficiently powered to detect a weak effect size (OR<1.3). Although insulin resistance is a key feature of gestational diabetes mellitus, genetic variants that affect insulin sensitivity do not seem to increase the risk of gestational diabetes mellitus in Scandinavian women. It could be hypothesised that, due to the massive insulin resistance during pregnancy, we were unable to detect a potential weak effect of the above-mentioned variants on insulin sensitivity.

In conclusion, the *TCF7L2* rs7903146 variant is associated with an increased risk of gestational diabetes mellitus. Given the influence of the variant on insulin secretion [14, 15, 17] and our previous findings of associations of *KCNJ11* E23K, *GCK* 30G>A and *TCF1* I27L [10, 11] variants with gestational diabetes mellitus, this finding supports the central role of impaired beta cell function in the pathogenesis of gestational diabetes mellitus [3].

Acknowledgements This work was supported by grants from the Swedish Research Council, the Söderberg foundation, the Lundberg Foundation, the Novo Nordisk Foundation and the Diabetes Association in Malmö. We are indebted to M. Svensson, A. Berglund and A. Nilsson for excellent technical assistance.

Duality of interest The authors declare that no conflict of interest exists in connection with this study.

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