Genetic variation in *TCF7L2* rs7903146 and history of GDM negatively and independently impact on diabetes-associated metabolic traits

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Abstract

Aims: Gestational diabetes (GDM) is recognized as a major risk factor for the development of type 2 diabetes (T2DM) later in life. Risk allele carriers at *TCF7L2* rs7903146 have increased susceptibility for both GDM and T2DM. We hypothesized that carrying *TCF7L2* risk alleles would further aggravate the negative impact of a positive history for GDM on metabolic traits related to T2DM later in life.

Methods: 210 women with a confirmed history of gestational diabetes and 810 controls without evidence for GDM underwent standardized 75 g oral glucose tolerance tests (OGTT). Liver fat was quantified in a subset of subjects (n=444) using magnetic resonance spectroscopy.

Results: 504 women were homozygous or heterozygous risk allele carriers. The risk allele carriers had a higher risk for GDM (p=0.0076, OR 1.52, 95% CI 1.11-2.06). Multivariable regression analysis demonstrated that both a history of GDM, or carrying a *TCF7L2* risk allele resulted in lower insulin secretion, impaired proinsulin processing and higher fasting and 2-hour glucose levels. Liver fat content was not associated with either a history of GDM or a *TCF7L2* risk genotype. There was no significant interaction (all p>0.05) between history of GDM and *TCF7L2* risk alleles on all diabetes-associated metabolic traits tested.

Conclusion: The *TCF7L2* rs7903146 polymorphism is a risk factor for gestational diabetes. However, the additional presence of *TCF7L2* rs7903146 risk alleles does not further aggravate the negative impact of a history of gestational diabetes on metabolic traits related to T2DM.

Keywords

Gestational diabetes, type 2 diabetes, oral glucose tolerance test, TCF7L2, insulin sensitivity, liver fat

Introduction

To date, many risk factors for type 2 diabetes mellitus (T2DM) have been evaluated. Among these, gestational diabetes mellitus (GDM), defined as glucose intolerance first presenting during pregnancy, is recognized as one of the strongest [1, 2]. A pregnancy is a stress test for the glucose metabolism of the mother, and GDM develops when increased demands of insulin secretion due to decreased insulin sensitivity are not met. Although glucose tolerance is usually restored after delivery, women with a history of GDM have a sevenfold increased risk to develop diabetes compared to women who had a normoglycemic pregnancy [3]. Up to 50% of women with prior GDM will develop T2DM within 10 years after delivery [4]. Episodes of reduced insulin sensitivity are present during GDM [5] and contribute to the development of β-cell dysfunction [6]. GDM and associated metabolic alterations may therefore be characterized as an additional metabolic risk factor for the development of T2DM.

The transcription factor 7-like 2 (TCF7L2) is a member of the Wnt signaling pathway. Together with β-catenin it is involved in β-cell development during embryogenesis. Additionally, a role in protection of mature β-cells has been described [7]. In the adult human organism, risk genotypes in *TCF7L2*, i.e. those associated with T2DM, are linked to impaired glucose-stimulated insulin secretion [8, 9], incretin-induced insulin secretion [10–12] and proinsulin conversion [13, 14]. Furthermore, a potential role as a negative regulator of hepatic gluconeogenesis has been proposed [7, 15]. There are several single nucleotide polymorphisms in *TCF7L2* that have been linked to T2DM [16]. The T allele of the rs7903146 polymorphism in *TCF7L2* is the strongest and most broadly validated genetic marker associated with T2DM [17], conferring a per-allele odds ratio of up to 1.46 [18]. Similarly, the *TCF7L2* rs7903146 polymorphism has been shown to be a risk factor for the development of GDM [19].

For a better prediction of the risk for T2DM in later life in women with a history of GDM, the determination of additional risk factors would be helpful. Such a genetic risk factor might be *TCF7L2.* In the present study, we hypothesized that carrying *TCF7L2* risk alleles would further aggravate the negative impact of a positive history of GDM on metabolic traits related to T2DM later in life. Such metabolic traits are impaired insulin secretion, insulin resistance, non-alcoholic fatty liver disease (NAFLD) and several other pathogenic mechanisms leading to hyperglycemia [20–22].

To test the hypothesis formulated above, we performed a metabolism-gene interaction study in women with and without a history of GDM which were carefully phenotyped for insulin secretion, sensitivity and clearance as well as proinsulin processing and liver fat content. The history of GDM is seen as an episode of metabolic stress representing a temporary prodiabetogenic milieu. The presence or absence of risk alleles in *TCF7L2* is the possibly interacting genetic factor which we tested for its impact on aggravating the metabolic alterations associated with GDM.

Material and Methods

Subjects

For the analysis we combined subjects from two cohorts. We selected women with at least one offspring and available data on history of GDM from the Tuebingen Family (TUEF) study [23] and the PPS-Diab (prediction, prevention and subclassification of type 2 diabetes) study [24]. Both studies are prospective, observational, single-center studies conducted in Tuebingen and Munich, Germany, respectively. Both centers are partners in the German Center for Diabetes Research (DZD), Neuherberg, Germany. The TUEF study’s objective is to characterize subjects at high risk for type 2 diabetes. Inclusion criteria are a positive family history of Type 2 Diabetes mellitus or a BMI >=27 kg/m2 or an existing prediabetes with either impaired fasting glucose (between 5.56 mmol/L and 7.0 mmol/L plasma glucose) or impaired glucose tolerance (between 7.78 mmol/L and 11.11 mmol/L plasma glucose after 2 h 75 g OGTT). Exclusion criteria are a diagnosed Type 2 Diabetes mellitus and the use of glucose lowering drugs. Each female participant of the TUEF study is asked via a questionnaire whether they had a diagnosed GDM in any of her pregnancies. Participants of the PPS-Diab study are women who had GDM confirmed by universal screening with a 75 g glucose OGTT in their last pregnancy and control subjects after a normoglycemic pregnancy. The cutoff values for GDM were 5.1/10.0/8.5 mmol/L plasma glucose following the International Association of the Diabetes and Pregnancy Study (IADPSG) recommendations. The participants of PPS-Diab were recruited 3 to 18 months after delivery. Exclusion criteria for this study were alcohol or substance abuse and chronic diseases requiring medication (except for hypothyroidism and mild hypertension). Each subject gave oral and written informed consent. The study protocols were approved by the local ethics committees.

OGTT

Subjects underwent standardized 5 point oral glucose tolerance tests (OGTT) with 75 g of glucose after an overnight fasting period of at least 10 hours. Venous blood samples were obtained at the time points 0, 30, 60, 90 and 120 minutes for the measurement of glucose, insulin, proinsulin and C-peptide.

Laboratory measurements and calculations

Plasma glucose was measured using a bedside glucose analyzer in TUEF (glucose oxidase method, Yellow Springs Instruments, Yellow Springs, OH, USA) and using Na-Fluoride vails and central laboratory measurement in PPS-Diab. Plasma insulin and C-peptide was determined by an immunoassay on ADVIA Centaur XP Immunoassay System (Siemens Healthcare Diagnostics, Erlangen, Germany) in TUEF and with chemiluminescent immunoassay technology on DiaSorin LIAISON systems (DiaSorin, Saluggia, Italy) in PPS-Diab. The insulin assays were validated against each other by measuring a set of reference samples at both sites. Proinsulin was measured with an enzyme immunoassay (IBL International, Hamburg, Germany) on a BEP III System (Siemens Healthcare GmbH, Erlangen, Germany).

Measures of insulin sensitivity and insulin secretion, insulin clearance and proinsulin processing were calculated from glucose, and insulin, proinsulin and C-peptide concentrations in the fasted state (minute 0) and during the OGTT (minutes 30, 60, 90 and 120). Insulin sensitivity was calculated with the composite whole-body insulin sensitivity index (ISI) proposed by Matsuda and DeFronzo [25]. Insulin secretion was assessed with the insulinogenic index (IGI) at 30 min: Δinsulin0-30/Δglucose0-30. Proinsulin to insulin conversion (proinsulin processing) was calculated by dividing AUC proinsulin by AUC insulin [13]. The insulin clearance was assessed by dividing AUC C-peptide by AUC insulin as described previously [26].

DNA was isolated (NucleoSpin, Macherey&Nagel, Düren, Germany), and genotyping of rs7903146 was carried out on the MassARRAY platform from Sequenom (Sequenom, San Diego, CA, USA). Genotyping reaction was amplified and fluorescence detected on an ABI Real Time PCR System 7500 (Applied Biosystems).

Body composition

Bioimpedance analysis was conducted with a BIA101 analyzer and body fat was calculated using Cyprus Version 2.7 (both from RJL Systems, Clinton Township, MI USA).

Liver fat

In a subset of subjects (n=444, TUEF cohort) liver fat was quantified by localised 1H-MR spectroscopy using a 1.5 T MR scanner (Magnetom Sonata, Siemens Healthcare, Erlangen, Germany). The cutoff value for nonalcoholic fatty liver disease (NAFLD) was 5.56% [27].

Statistical analysis

Statistical analyses were performed with SAS JMP version 11.0.0. Since we analyzed data from two different study sites, all multivariable linear regression models were adjusted for study site. The binary variable representing study site was used as a covariate in linear regression models to account for potential confounding due.to differences between study centers. Variables were tested for normal distribution using the Shapiro-Wilk W test and, if needed, transformed to their natural logarithm to approximate normal distribution. Multivariable linear regressions were performed to evaluate the effect of TCF7L2 genotype and the history of GDM and the interaction of both on diabetes-associated metabolic traits. Genotypes were coded using a dominant inheritance model. A p-value < 0.05 was considered statistically significant.

Results

In total, data of 1020 women were analyzed, with 139 participants from the PPS-Diab study and 881 participants from the TUEF study. 516 (50.6%) of the women were homozygous carriers of the major allele at *TCF7L2* rs7903146 and 504 (49.4%) were homo- or heterozygous risk allele carriers. 810 (79.4%) of the women had no history of gestational diabetes during previous pregnancies, whereas 210 (20.6%) had a history of previous GDM. The percentage of women with a history of GDM was higher in the group of risk allele carriers (p=0.0076, OR 1.52, 95%CI (1.11-2.06)). In table 1, the cohort characteristics are shown. In supplementary table S1 the characteristics of the TUEF and the PPS-Diab cohort are shown separately. In supplementary table S2 the subject characteristics for women without history of GDM and with history of GDM are presented.

We analyzed the effect of the genotype and the history of GDM status as well as their interaction on glycemia, insulin sensitivity, insulin secretion, insulin clearance, proinsulin processing and liver fat content with multivariable linear regression models adjusted for age, BMI and study site (table 2). The results of the univariate analyses are presented in supplementary table S3.The models fitting GDM history and genotype on IGI and liver fat were each additionally adjusted for ISI-Matsuda. The complete models for each trait are shown in the supplementary Tables S4-S17.

History of GDM resulted in higher fasting and 2-hour glucose and lower insulin secretion and sensitivity and clearance during OGTT in the multivariable models (all p < 0.005, table 2, figure 1). Proinsulin processing was improved in women with a history of GDM (p < 0.04). Liver fat was not different between women with history of GDM and controls.

Carrying at least one risk allele of *TCF7L2* rs7903146 resulted in higher fasting and 2-hour glucose and lower insulin secretion as well as impaired proinsulin processing in the multivariable models (all p < 0.04, table 2, figure 1). Insulin sensitivity and clearance during OGTT were higher in the risk allele carriers (p < 0.002, table 2, figure 1). Liver fat content was not affected by the presence or absence of the risk allele.

There was no significant interaction between history of GDM and the *TCF7L2* rs7903146 risk allele on fasting and 2-h-glucose, insulin sensitivity, insulin secretion, insulin clearance, proinsulin processing, and liver fat (all p > 0.05, see table 2, figure 1).

Discussion

In this study, we aimed to gain further information on potential interactions between GDM history and *TCF7L2* rs7903146 genotypes on glycemia, insulin sensitivity, insulin secretion, insulin clearance, proinsulin processing, and liver fat. We showed that both a prior GDM and *TCF7L2* risk allele status are associated with alteration in all of the examined metabolic traits, except for liver fat. The associations of *TCF7L2* genotype and history of GDM with the different metabolic traits related to T2DM are summarized in Figure 1. However, a genotype-environment interaction of GDM history and the *TCF7L2* risk allele could not be demonstrated on any of the metabolic phenotypes investigated.

Our findings on the association of the *TCF7L2* rs7903146 risk allele with metabolic traits are well in line with previously published results. Carriers of the risk allele have been shown to have impaired insulin secretion [8, 9, 28], and specifically incretin induced insulin secretion [10–12]. Probably as a result of impaired insulin secretion, fasting and 2-h glucose was slightly but significantly higher in risk-allele carriers in our study, after adjusting for age, BMI and study site. Furthermore risk allele carriers showed impaired proinsulin processing going along with impaired β-cell function which is in accordance with previously published results [13, 29–31]. Insulin sensitivity estimated by an OGTT derived index was increased in subjects carrying at least one *TCF7L2* rs7903146 risk allele which has been described in some [10, 32, 33] but not all [34–38] earlier studies. The increased insulin sensitivity in risk allele carriers found in healthy subjects of the present study may be due to a compensation of the TCF7L2 related reduced insulin secretion by increased insulin sensitivity [34].

To the best of our knowledge, no studies have investigated explicitly the role of the *TCF7L2* rs7903146 risk allele in insulin clearance. A lower insulin clearance is associated with glucose intolerance and predicts the progression to diabetes [35]. In our cohort, the risk allele was associated with a higher insulin clearance rate. This finding is not completely unexpected, since insulin clearance correlates with insulin sensitivity [36], and risk allele carriers have higher insulin sensitivity (table 2). Therefore, the same limitations should be taken into account as presented for the interpretation of increased insulin sensitivity in risk allele carriers. Clearly, these findings need further confirmation and replication.

Liver fat content was not affected by the *TCF7L2* polymorphism in our study, contrary to the proposition by Musso et al., who suggested that the *TCF7L2* rs7903146 risk allele predisposes to nonalcoholic fatty liver disease (NAFLD) [37]. Similarly, when analyzing only the subset of participants with a liver fat content >= 5.56%, defining NAFLD [27], we also did not find an association with the *TCF7L2* rs7903146 genotype (p=0.21, adjusted for BMI and age, supplementary table S18). We did neither find higher liver fat content nor a higher proportion of women with NAFLD (p=0.96, adjusted for BMI and age, supplementary table S19) among women with a history of GDM, which is in contrast to previous studies [38, 39]. However, in these studies liver fat was measured only by ultrasound [39], and the numbers of study participants were considerably smaller [38].

Our findings showing that a history of GDM is generally associated with an unfavorable metabolic status, specifically increased fasting and 2-h-plasma glucose levels, reduced insulin sensitivity, impaired insulin secretion and impaired insulin clearance, are in line with previous studies reviewed in [4]. However, the ratio of AUC proinsulin/AUC insulin was slightly lower in women with a GDM history indicating a higher rate of proinsulin processing. To date, we have no knowledge of studies confirming this rather unexpected finding. However, we may conclude that proinsulin processing is by all means not impaired in women with history of GDM.

Since a history of GDM and the *TCF7L2* rs7903146 polymorphism both constitute risk factors for diabetes, we hypothesized that these factors interact and augment each other in promoting T2DM associated metabolic traits. However, in our large dataset, we show for the first time that no such interactions exist. The lack of interaction could indicate that a history of GDM does not further amplify the influence of the *TCF7L2* rs7903146 risk allele on the diabetes risk and vice versa. How could this lack of interaction be explained?

On one hand, T2DM and GDM share common mechanisms, such as insufficient pancreatic β-cell function [40], inflammation [41], increased visceral adipose tissue [42] and genetic causes [43, 44]. The effect size of genetic polymorphisms and prior GDM on the incidence of T2DM are often similar [45]. In our analysis, the OR for GDM in risk allele carriers was 1.52 (95%CI 1.11-2.06) which is comparable with the pooled OR of 1.46 (95%CI 1.42–1.51) for T2DM reported by Cauchi et al. [18]. Therefore, our data suggest that the *TCF7L2* rs7903146 risk allele does not further increase T2DM risk in women with a history of GDM, since both forms of diabetes share in part common causes.

On the other hand, the *TCF7L2* rs7903146 risk allele and a history of GDM might constitute two completely different risk factor combinations. Even if tentatively additive, the impact of the *TCF7L2* polymorphism might be too weak to further increase the elevated T2DM risk for women who had a diabetic pregnancy [3]. Importantly, the association between *TCF7L2* rs7903146 and GDM was not strong enough to cause a relevant collinearity in our models (variance inflation factors < 1.36)

The strengths of our study are the large cohort size and the careful assessment of the metabolic status in all women. The major limitation of the study is that for most controls, a missing history of GDM cannot rule out unreported hyperglycemia during prior pregnancies. Therefore, an underreporting of GDM in the control group is possible. However, women in the control group had normal pregnancies with normal birth outcomes, which argues against a relevant rate of untreated GDM. We combined the two cohorts to yield a more balanced distribution of GDM cases and controls. In all models, statistical adjustment for study site was performed.

In summary, women carrying the *TCF7L2* rs7903146 risk alleles show an impaired insulin secretion and proinsulin conversion compared to women not carrying the risk alleles. Women with a history of GDM exhibit impaired insulin secretion, insulin resistance and impaired insulin clearance compared to women without a history of GDM. Importantly, there is no interaction between these variables indicating that the *TCF7L2* rs7903146 risk allele and a history of GDM each are independent risk factors for unfavorable metabolic changes linked to the development of T2DM. The additional presence of *TCF7L2* rs7903146 risk alleles does not further aggravate the negative impact of a history of gestational diabetes on metabolic traits related to T2DM.

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Conflict of interests

The authors declare that they have no conflict of interest.

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Contribution to authorship

LF was involved in the design of the study, data acquisition and analysis and interpretation of data and drafted the article. MS, RW, RL, HG were involved in data acquisition. HUH contributed to interpretation of the data and revised the article. AF, AL, RW and NS contributed to the conception and design of the study and to the analysis and interpretation of data and revised the article. All authors approved the final version of the article. AF and AL contributed equally to this work. AF is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Statement of human rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

The studies were approved by the Ethics Committees of both the Medical Faculty of the Eberhard Karls University of Tuebingen, Germany the Ludwig Maximilians University Munich, Germany.

Informed consent was obtained from all patients for being included in the study.

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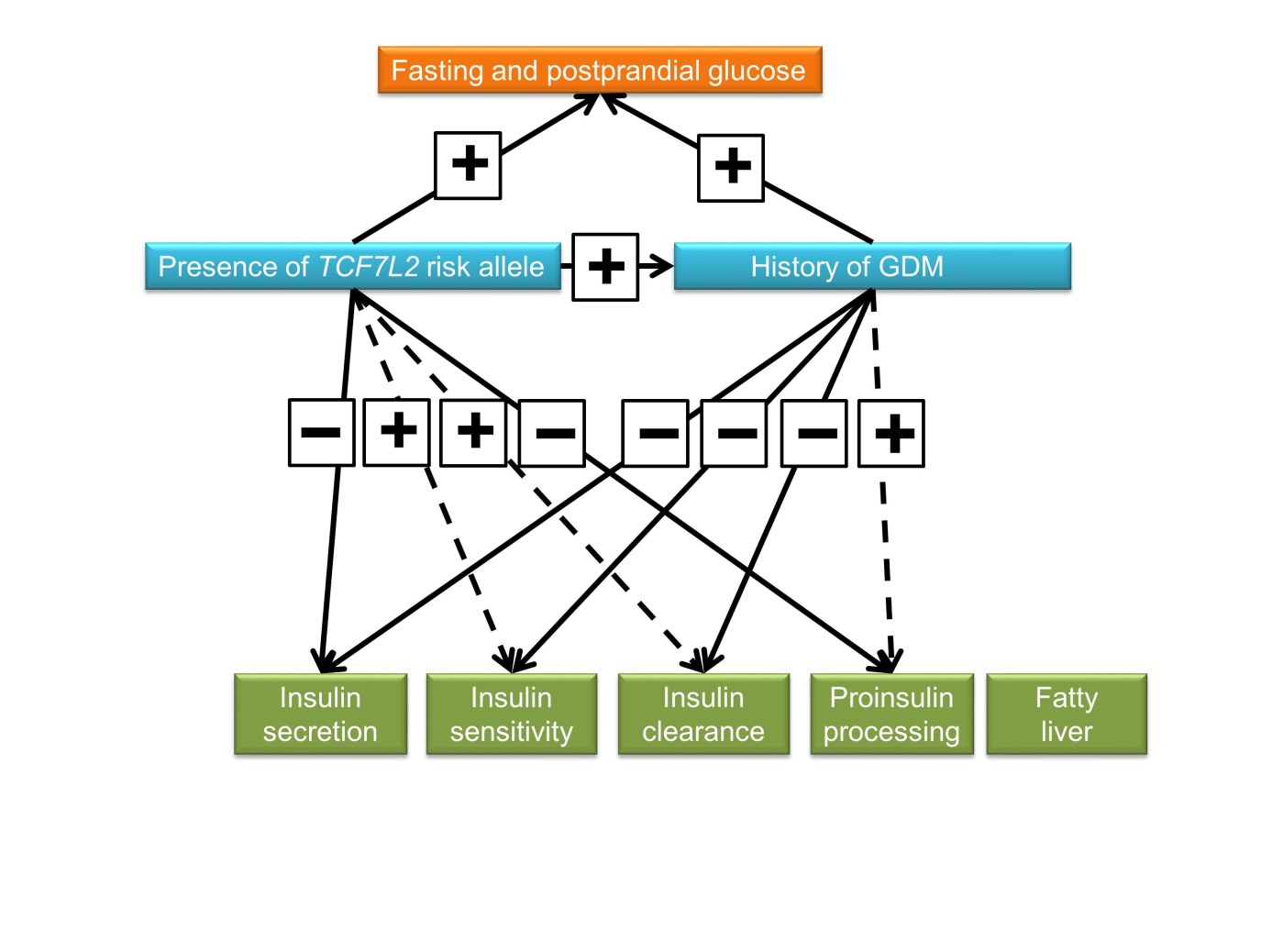
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**Fig. 1**

Negative (-) or positive (+) independent association of *TCF7L2* rs7903146 genotype or history of GDM with metabolic traits indicated by arrows. Associations which need further investigation are marked with dashed lines.

Tables

Table 1 Cohort characteristics. Data are N (%) and means (SEM). Insulin sensitivity was calculated with the composite whole-body insulin sensitivity index (ISI) by Matsuda and DeFronzo 25. Insulin secretion was assessed with the insulinogenic index (IGI): Δinsulin0-30/Δglucose0-30. Proinsulin processing was calculated by dividing AUC proinsulin by AUC insulin. Insulin clearance was assessed by dividing AUC C-peptide by AUC insulin.

|  |  |
| --- | --- |
| **Parameter** | **Value** |
| N | 1020 |
| Age (years) | 46.17 (0.36) |
| BMI (kg/m2) | 31.86 (0.3) |
| Body fat (%) | 40.86 (0.35) |
| Liver fat (%) (N=444, TUEF cohort) | 6.16 (0.33) |
| Fasting plasma glucose (mmol/L) | 5.35 (0.02) |
| 2-h-plasma glucose (mmol/L) | 6.94 (0.06) |
| Insulin sensitivity (ISI Matsuda) | 9.71 (0.21) |
| Insulin secretion (IGI) | 182.9 (7.5) |
| Proinsulin processing (N=790, TUEF cohort) | 0.026 (0.0007) |
| Insulin clearance (N=842, TUEF cohort) | 4.40 (0.06) |
| No history of GDM/History of GDM | 810/210 (79.4/20.6%) |
| *TCF7L2* rs7903146 CC/XT | 516/504 (50.6%/49.4%) |

Table 2 Effect size and p-values of multivariate linear regression analysis. A negative beta estimate (effect size, Std Beta) indicates a lower value in the respective risk group (GDMhistory or *TCF7L2* rs7903146 risk allele carrier).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **GDMhist vs NGTpregnancy** | | ***TCF7L2* rs7903146 CC vs *TCF7L2* rs7903146 XT** | | **Interaction GDMhist × *TCF7L2* rs7903146** | |
| **Parameter** | **Std Beta** | **P** | **Std Beta** | **P** | **Std Beta** | **P** |
| Fasting plasma glucose | 0.091 | **0.0045 a** | 0.058 | **0.0364 a** | -0.046 | 0.2982 a |
| 2-h-plasma glucose | 0.146 | **<0.0001 a** | 0.059 | **0.0392 a** | 0.009 | 0.8512 a |
| IGI | -0.116 | **0.0006 b** | -0.067 | **0.0207 b** | 0.0502 | 0.2801 b |
| ISI | -0.135 | **<0.0001 a** | 0.059 | **0.012 a** | -0.003 | 0.935 a |
| Insulin clearance | -0.177 | **<0.0001 d** | 0.1 | **0.0014 d** | -0.0641 | 0.1902 d |
| Proinsulin processing | -0.076 | **0.0441 d** | 0.177 | **<0.0001 d** | 0.002 | 0.9706 d |
| Liver fat | 0.042 | 0.2684 c | 0.011 | 0.7521 c | -0.7521 | 0.6389 c |

a P-values are derived from multivariate linear regression analysis adjusted for age, BMI and study site. b P-values are derived from multivariate linear regression analysis adjusted for age, BMI, study site and ISI. c P-values are derived from multivariate linear regression analysis adjusted for age, BMI and ISI. d P-values are derived from multivariate linear regression analysis adjusted for age and BMI. Bold font indicates statistical significance.

**Electronic Supplementary Material**

**S1 Cohort characteristics for TUEF and PPS-Diab cohort.** Data are N (%) and means (SEM). Insulin sensitivity was calculated with the composite whole-body insulin sensitivity index (ISI) by Matsuda and DeFronzo25. Insulin secretion was assessed with the insulinogenic index (IGI): Δinsulin0-30/Δglucose0-30. Proinsulin processing was calculated by dividing AUC proinsulin by AUC insulin. Insulin clearance was assessed by dividing AUC C-peptide by AUC insulin.

|  |  |  |
| --- | --- | --- |
| **Parameter** | **TUEF cohort** | **PPS-Diab cohort** |
| N | 881 | 139 |
| Age (years) | 47.8 (0.39) | 35.9 (0.3) |
| BMI (kg/m2) | 32.8 (0.3) | 25.6 (0.5) |
| Body fat (%) | 42.3 (0.36) | 32.4 (0.7) |
| Liver fat (%) (N=444, TUEF cohort) | 6.16 (0.33) |  |
| Fasting plasma glucose (mmol/L) | 5.37 (0.02) | 5.15 (0.04) |
| 2-h-plasma glucose (mmol/L) | 7.05 (0.07) | 6.21 (0.13) |
| Insulin sensitivity (ISI Matsuda) | 10.3 (0.23) | 5.89 (0.3) |
| Insulin secretion (IGI) | 185.3 (7.5) | 167.7 (27.1) |
| Proinsulin processing (N=790, TUEF cohort) | 0.026 (0.0007) |  |
| Insulin clearance (N=842, TUEF cohort) | 4.40 (0.06) |  |
| No history of GDM/History of GDM | 763/118 (86.6/13.3%) | 47/92 (33.8/66.2%) |
| *TCF7L2* rs7903146 CC/XT | 462/419 (52.4%/47.6%) | 54/85 (38.9/61.1%) |

**S2 Characteristics for subjects without history of GDM and with history of GDM.** Data are N (%) and means (SEM). Insulin sensitivity was calculated with the composite whole-body insulin sensitivity index (ISI) by Matsuda and DeFronzo25. Insulin secretion was assessed with the insulinogenic index (IGI): Δinsulin0-30/Δglucose0-30. Proinsulin processing was calculated by dividing AUC proinsulin by AUC insulin. Insulin clearance was assessed by dividing AUC C-peptide by AUC insulin.

|  |  |  |
| --- | --- | --- |
| **Parameter** | **No history of GDM** | **History of GDM** |
| N | 810 | 210 |
| Age (years) | 48.4 (0.4) | 37.5 (0.4) |
| BMI (kg/m2) | 32.8 (0.3) | 28.2 (0.5) |
| Body fat (%) | 42 (0.4) | 36.5 (0.7) |
| Liver fat (%) (N=444, TUEF cohort) | 6.43 (0.37) | 5.13 (0.73) |
| Fasting plasma glucose (mmol/L) | 5.38 (0.02) | 5.23 (0.12) |
| 2-h-plasma glucose (mmol/L) | 6.98 (0.07) | 6.78 (0.13) |
| Insulin sensitivity (ISI Matsuda) | 10.1 (0.23) | 8.23 (0.4) |
| Insulin secretion (IGI) | 189.7 (9.2) | 156.7 (8.31) |
| Proinsulin processing (N=790, TUEF cohort) | 0.026 (0.001) | 0.021 (0.001) |
| Insulin clearance (N=842, TUEF cohort) | 4.46 (0.06) | 4.06 (0.16) |
| *TCF7L2* rs7903146 CC/XT | 427/383 (52.7%/47.3%) | 89/121 (42.3/57.6%) |

S3 Effect size and p-values of univariate analysis. A negative beta estimate (effect size, Std Beta) indicates a lower value in the respective risk group (GDMhistory or *TCF7L2* rs7903146 risk allele carrier). Fasting plasma glucose. 2-h-plasma glucose, IGI and ISI were adjusted for study site.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **GDMhist vs NGTpregnancy** | | ***TCF7L2* rs7903146 CC vs *TCF7L2* rs7903146 XT** | | **Interaction GDMhist × *TCF7L2* rs7903146** | |
| **Parameter** | **Std Beta** | **P** | **Std Beta** | **P** | **Std Beta** | **P** |
| Fasting plasma glucose | -0.05 | 0.16 | 0.052 | 0.09 | -0.03 | 0.61 |
| 2-h-plasma glucose | 0.04 | 0.3 | 0.052 | 0.09 | 0.03 | 0.55 |
| IGI | -0.03 | 0.37 | -0.106 | **0.0008** | 0.08 | 0.1 |
| ISI | -0.03 | 0.28 | 0.087 | **0.0045** | -0.006 | 0.25 |
| Insulin clearance TUEF cohort | -0.085 | **0.0137** | 0.12 | **0.0006** | -0.099 | 0.064 |
| Proinsulin processing TUEF cohort | -0.09 | **0.009** | 0.18 | **<0.0001** | -0.02 | 0.66 |
| Liver fat TUEF cohort | -0.08 | 0.1 | -0.05 | 0.263 | 0.05 | 0.46 |

Multivariate linear regression models adjusted for age, BMI and study site to evaluate the effect of *TCF7L2* genotype and the history of previous gestational diabetes and the interaction of both on fasting glucose (S4, S5), postprandial glucose (S6, S7), insulin secretion (S8, S9), insulin sensitivity, (S10,S11 ), insulin clearance (S12, S13), proinsulin processing (S14, S15),and liver fat (S16, S17, S18, S19). In table S18 and S19, only women with a liver fat content ≥ 5.56% were analyzed.

Tables with even number delineate the independent effect of TCF7L2 genotype and the history of previous gestational diabetes on the respective metabolic parameter.

Tables with even unnumber delineate additionally the Interaction of TCF7L2 genotype and the history of previous gestational diabetes on the respective metabolic parameter.

Study site is added in the model when parameters were obtained in both the Tübingen and Munich study center.

**S4: Effect of genotype and GDM history on fasting plasma glucose, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **fasting plasma glucose** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 0.5125592 | 0.067618 | 7.58 | <.0001 | 0 |
| log AGE | 0.1647371 | 0.013575 | 12.14 | <.0001 | 0.369896 |
| log BMI | 0.1542649 | 0.011749 | 13.13 | <.0001 | 0.378473 |
| GDM.history[GDM-NGT] | 0.0256088 | 0.008995 | 2.85 | 0.0045 | 0.091096 |
| dom rs7903146[1-0] | 0.0131327 | 0.006268 | 2.1 | 0.0364 | 0.057763 |
| study.site[1] | -0.01113 | 0.005357 | -2.08 | 0.038 | -0.06719 |

**S5: Effect of genotype and GDM history interaction on fasting plasma glucose, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **fasting plasma glucose** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 0.5098752 | 0.067664 | 7.54 | <.0001 | 0 |
| log AGE | 0.1644293 | 0.013578 | 12.11 | <.0001 | 0.369205 |
| log BMI | 0.1549668 | 0.011768 | 13.17 | <.0001 | 0.380195 |
| GDM.history[GDM-NGT] | 0.03453 | 0.012424 | 2.78 | 0.0055 | 0.122831 |
| dom rs7903146[1-0] | 0.0164333 | 0.007024 | 2.34 | 0.0195 | 0.072281 |
| study.site[1] | -0.011258 | 0.005358 | -2.1 | 0.0359 | -0.06796 |
| dom rs7903146[1-0]\*GDM.history[GDM-NGT] | -0.0162 | 0.015564 | -1.04 | 0.2982 | -0.04608 |

**S6: Effect of genotype and GDM history on 2 h plasma glucose, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **2 h plasma glucose** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -0.426805 | 0.168456 | -2.53 | 0.0114 | 0 |
| log AGE | 0.2937319 | 0.03382 | 8.69 | <.0001 | 0.274554 |
| log BMI | 0.3408685 | 0.029271 | 11.65 | <.0001 | 0.348133 |
| GDM.history[GDM-NGT] | 0.0985655 | 0.022408 | 4.4 | <.0001 | 0.145957 |
| dom rs7903146[1-0] | 0.0322403 | 0.015614 | 2.06 | 0.0392 | 0.059032 |
| study.site[1] | 0.0095406 | 0.013345 | 0.71 | 0.4748 | 0.023974 |

**S7: Effect of genotype and GDM history interaction on 2 h plasma glucose, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **2 h plasma glucose** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -0.425599 | 0.168659 | -2.52 | 0.0118 | 0 |
| log AGE | 0.2938701 | 0.033844 | 8.68 | <.0001 | 0.274684 |
| log BMI | 0.3405532 | 0.029333 | 11.61 | <.0001 | 0.347811 |
| GDM.history[GDM-NGT] | 0.0945578 | 0.030968 | 3.05 | 0.0023 | 0.140022 |
| dom rs7903146[1-0] | 0.0307576 | 0.017507 | 1.76 | 0.0792 | 0.056317 |
| study.site[1] | 0.0095982 | 0.013355 | 0.72 | 0.4725 | 0.024119 |
| dom rs7903146[1-0]\*GDM.history[GDM-NGT] | 0.0072778 | 0.038794 | 0.19 | 0.8512 | 0.008618 |

**S8: Effect of genotype and GDM history on insulin secretion (IGI), complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable:  Insulin secretion (**IGI)** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 8.074447 | 0.563231 | 14.34 | <.0001 | 0 |
| log AGE | -0.784213 | 0.090915 | -8.63 | <.0001 | -0.27865 |
| log BMI | 0.120152 | 0.100527 | 1.2 | 0.2323 | 0.046648 |
| log ISI | -0.304527 | 0.039565 | -7.7 | <.0001 | -0.29616 |
| GDM.history[GDM-NGT] | -0.206629 | 0.060177 | -3.43 | 0.0006 | -0.11615 |
| dom rs7903146[1-0] | -0.096445 | 0.041622 | -2.32 | 0.0207 | -0.06706 |
| study.site[1] | 0.1948303 | 0.03945 | 4.94 | <.0001 | 0.185653 |

**S9: Effect of genotype and GDM history interaction on insulin secretion (IGI), complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable:  Insulin secretion (**IGI)** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 8.0931796 | 0.563451 | 14.36 | <.0001 | 0 |
| log AGE | -0.782083 | 0.090929 | -8.6 | <.0001 | -0.2779 |
| log BMI | 0.1151418 | 0.100625 | 1.14 | 0.2528 | 0.044703 |
| log ISI | -0.304459 | 0.039562 | -7.7 | <.0001 | -0.29609 |
| GDM.history[GDM-NGT] | -0.268155 | 0.082841 | -3.24 | 0.0012 | -0.15074 |
| dom rs7903146[1-0] | -0.119093 | 0.046598 | -2.56 | 0.0107 | -0.08281 |
| study.site[1] | 0.1958777 | 0.039459 | 4.96 | <.0001 | 0.186651 |
| dom rs7903146[1-0]\*GDM.history[GDM-NGT] | 0.1114465 | 0.103136 | 1.08 | 0.2801 | 0.0502 |

**S10: Effect of genotype and GDM history on insulin sensitivity (ISI), complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable:  Insulin sensitivity (ISI) | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 8.6483058 | 0.354893 | 24.37 | <.0001 | 0 |
| log AGE | -0.371194 | 0.07125 | -5.21 | <.0001 | -0.13555 |
| log BMI | -1.608727 | 0.061667 | -26.09 | <.0001 | -0.64191 |
| GDM.history[GDM-NGT] | -0.233561 | 0.047209 | -4.95 | <.0001 | -0.13513 |
| dom rs7903146[1-0] | 0.0827809 | 0.032895 | 2.52 | 0.012 | 0.059218 |
| study.site[1] | 0.4354872 | 0.028115 | 15.49 | <.0001 | 0.427549 |

**S11: Effect of genotype and GDM history interaction on insulin sensitivity (ISI), complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable:  Insulin sensitivity (ISI) | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 8.6472015 | 0.355325 | 24.34 | <.0001 | 0 |
| log AGE | -0.37132 | 0.071302 | -5.21 | <.0001 | -0.1356 |
| log BMI | -1.608438 | 0.061798 | -26.03 | <.0001 | -0.6418 |
| GDM.history[GDM-NGT] | -0.229891 | 0.065242 | -3.52 | 0.0004 | -0.133 |
| dom rs7903146[1-0] | 0.0841389 | 0.036884 | 2.28 | 0.0227 | 0.06019 |
| study.site[1] | 0.4354344 | 0.028137 | 15.48 | <.0001 | 0.427497 |
| dom rs7903146[1-0]\*GDM.history[GDM-NGT] | -0.006666 | 0.081731 | -0.08 | 0.935 | -0.00308 |

**S12: Effect of genotype and GDM history on insulin clearance, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **insulin clearance** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 3.7034062 | 0.255601 | 14.49 | <.0001 | 0 |
| log AGE | -0.069009 | 0.048889 | -1.41 | 0.1585 | -0.04656 |
| log BMI | -0.589329 | 0.043224 | -13.63 | <.0001 | -0.43023 |
| dom rs7903146[1-0] | 0.0752581 | 0.023461 | 3.21 | 0.0014 | 0.099689 |
| GDM.history[GDM-NGT] | -0.188558 | 0.035639 | -5.29 | <.0001 | -0.17657 |

**S13: Effect of genotype and GDM history interaction on insulin clearance, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **insulin clearance** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | 3.686307 | 0.255824 | 14.41 | <.0001 | 0 |
| log AGE | -0.069998 | 0.048874 | -1.43 | 0.1525 | -0.04722 |
| log BMI | -0.585013 | 0.043331 | -13.5 | <.0001 | -0.42708 |
| dom rs7903146[1-0] | 0.0880619 | 0.025403 | 3.47 | 0.0006 | 0.116649 |
| GDM.history[GDM-NGT] | -0.139803 | 0.051495 | -2.71 | 0.0068 | -0.13092 |
| dom rs7903146[1-0]\*GDM.history[GDM-NGT] | -0.087526 | 0.066754 | -1.31 | 0.1902 | -0.06407 |

**S14: Effect of genotype and GDM history on proinsulin processing, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **proinsulin processing** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -4.207464 | 0.56376 | -7.46 | <.0001 | 0 |
| log AGE | 0.2300884 | 0.107219 | 2.15 | 0.0322 | 0.079666 |
| log BMI | -0.196136 | 0.09409 | -2.08 | 0.0374 | -0.07425 |
| dom rs7903146[1-0] | 0.2600024 | 0.051321 | 5.07 | <.0001 | 0.176823 |
| GDM.history[GDM-NGT] | -0.160081 | 0.079407 | -2.02 | 0.0441 | -0.0757 |

**S15: Effect of genotype and GDM history interaction on proinsulin processing, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **proinsulin processing** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -4.206697 | 0.564503 | -7.45 | <.0001 | 0 |
| log AGE | 0.2301868 | 0.107321 | 2.14 | 0.0323 | 0.0797 |
| log BMI | -0.196361 | 0.094348 | -2.08 | 0.0377 | -0.07433 |
| dom rs7903146[1-0] | 0.259231 | 0.055456 | 4.67 | <.0001 | 0.176299 |
| GDM.history[GDM-NGT] | -0.163075 | 0.113626 | -1.44 | 0.1516 | -0.07711 |
| dom rs7903146[1-0]\*GDM.history[GDM-NGT] | 0.0054646 | 0.148293 | 0.04 | 0.9706 | 0.002 |

**S16: Effect of genotype and GDM history on liver fat, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **Liver fat** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -6.411187 | 1.137869 | -5.63 | <.0001 | 0 |
| log AGE | 0.9675952 | 0.188667 | 5.13 | <.0001 | 0.194916 |
| log BMI | 1.6397897 | 0.22288 | 7.36 | <.0001 | 0.301344 |
| log ISI | -0.758019 | 0.073846 | -10.26 | <.0001 | -0.4261 |
| GDM.history[GDM-NGT] | 0.1171395 | 0.1057 | 1.11 | 0.2684 | 0.041651 |
| dom rs7903146[1-0] | 0.0248338 | 0.078583 | 0.32 | 0.7521 | 0.010884 |

**S17: Effect of genotype and GDM history interaction on liver fat, complete model of multivariate linear regression analysis.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **Liver fat** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -6.384147 | 1.140339 | -5.6 | <.0001 | 0 |
| log AGE | 0.9678598 | 0.188836 | 5.13 | <.0001 | 0.194969 |
| log BMI | 1.6344684 | 0.223366 | 7.32 | <.0001 | 0.300366 |
| log ISI | -0.758617 | 0.073923 | -10.26 | <.0001 | -0.42643 |
| GDM.history[GDM-NGT] | 0.0699633 | 0.145908 | 0.48 | 0.6318 | 0.024877 |
| dom rs7903146[1-0] | 0.0060475 | 0.088246 | 0.07 | 0.9454 | 0.00265 |
| dom rs7903146[1-0]\*GDM.history[GDM-NGT] | 0.0904292 | 0.192609 | 0.47 | 0.6389 | 0.024858 |

**S18: Effect of *TCF7L2* rs7903146 on liver fat in women with NAFLD (liver fat >= 5.56%), n=151**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **Liver fat** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -0.585475 | 1.175827 | -0.5 | 0.6193 | 0 |
| log BMI | 0.5413652 | 0.236355 | 2.29 | 0.0234 | 0.184837 |
| log AGE | 0.319279 | 0.19696 | 1.62 | 0.1072 | 0.129744 |
| dom rs7903146[1-0] | -0.098677 | 0.077923 | -1.27 | 0.2074 | -0.10195 |

**S19: Effect of GDM history on liver fat in women with NAFLD (liver fat >= 5.56%), n=151**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dependent variable: **Liver fat** | Estimate | Std Error | t Ratio | Prob>|t| | Std Beta |
| Intercept | -0.766726 | 1.208485 | -0.63 | 0.5268 | 0 |
| log BMI | 0.5804186 | 0.235882 | 2.46 | 0.015 | 0.198171 |
| log AGE | 0.3195341 | 0.213489 | 1.5 | 0.1366 | 0.129847 |
| GDM.history[GDM-NGT] | -0.005745 | 0.116241 | -0.05 | 0.9607 | -0.00429 |