

# Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (*TCF7L2*) gene polymorphisms

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## Abstract

**Aims/hypothesis** Polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) gene are associated with type 2 diabetes and reduced insulin secretion. The transcription factor *TCF7L2* is an essential factor for glucagon-like peptide-1 (GLP-1)

secretion from intestinal L cells. We studied whether a defect in the enteroinsular axis contributes to impaired insulin secretion in carriers of *TCF7L2* polymorphisms.

**Methods** We genotyped 1,110 non-diabetic German participants for five single nucleotide polymorphisms in *TCF7L2*. All participants underwent an OGTT; GLP-1 secretion was measured in 155 participants. In 210 participants, an IVGTT combined with a hyperinsulinaemic–euglycaemic clamp was performed. In another 160 participants from the Netherlands and 73 from Germany, a hyperglycaemic clamp (10 mmol/l) was performed. In 73 German participants this clamp was combined with a GLP-1 infusion and an arginine bolus.

**Results** The OGTT data confirmed that variants in *TCF7L2* are associated with reduced insulin secretion. In contrast, insulin secretion induced by an i.v. glucose challenge in the IVGTT and hyperglycaemic clamp was not different between the genotypes. GLP-1 concentrations during the OGTT were not influenced by the *TCF7L2* variants. However, GLP-1-infusion combined with a hyperglycaemic clamp showed a significant reduction in GLP-1-induced insulin secretion in carriers of the risk allele in two variants (rs7903146, rs12255372,  $p < 0.02$ ).

**Conclusions/interpretation** Variants of *TCF7L2* specifically impair GLP-1-induced insulin secretion. This seems to be rather the result of a functional defect in the GLP-1 signalling in beta cells than a reduction in GLP-1 secretion. This defect might explain the impaired insulin secretion in carriers of the risk alleles and confers the increased risk of type 2 diabetes.

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## Abbreviations

GLP-1	glucagon-like peptide-1
SNP	single nucleotide polymorphism
TCF7L2	transcription factor 7-like 2

## Introduction

Genome-wide scans for diabetes susceptibility genes have been performed in various populations. In early 2006, it was reported that variants in the transcription factor 7-like 2 (*TCF7L2*) gene were strongly associated with an increased risk of type 2 diabetes in an Icelandic, a Danish and a US population [1]. The estimated population attributable risk of type 2 diabetes of this variation lies between 10 and 25%, which is in the range of the peroxisome proliferator-activated receptor  $\gamma$  gene (*PPAR $\gamma$* ) Pro12Ala (25% [2]) and the potassium inwardly-rectifying channel, subfamily J, member 11 gene (*KCNJ11*) Glu23Lys (15% [3]) polymorphisms.

In the initial report of Grant et al. [1], five single nucleotide polymorphisms (SNPs) within introns 3 and 4 of the *TCF7L2* gene (rs12255372, rs7903146, rs7901695, rs11196205, rs7895340) were identified to associate with an increased risk of type 2 diabetes. This finding initiated a series of reports in various populations confirming the effect of these polymorphisms within the *TCF7L2* gene on the type 2 diabetes risk [4–12]. Recently, the T allele of rs7903146 was identified as the variant that most strongly determines the risk of type 2 diabetes [13]. In carriers of the risk alleles for SNPs within *TCF7L2* several studies additionally showed an impaired insulin secretion estimated from an OGTT or IVGTT [6, 7, 9, 10]. These studies indicate that progressive loss of insulin secretion might be the essential component of the phenotype which predisposes carriers of the *TCF7L2* variant to develop type 2 diabetes. The pathogenic mechanism of the impaired insulin secretion due to polymorphisms in the *TCF7L2* gene is not yet clear. It could be the consequence of a reduction in beta cell mass or could reflect a dysfunction of pancreatic beta cells. Furthermore, a defect in incretin-induced stimulus secretion coupling could mediate a reduction of insulin secretion in carriers of the *TCF7L2* polymorphisms. The latter hypothesis is supported by the molecular mode of action of the transcription factor TCF7L2. TCF7L2 is an essential component of the wingless-type MMTV integration site family, member 1 (*WNT*) signalling pathway, which is crucial for the regulation of the glucagon gene (*GCG*) expression and the secretion of its product GLP-1 by the intestinal endocrine L cells [14]. Therefore, an alteration in the *WNT* signalling pathway through polymorphisms in the *TCF7L2* gene might result in an altered GLP-1 response, which in turn could lead to a lower postprandial insulin secretion.

To test this hypothesis we analysed the associations of the above-described *TCF7L2* polymorphisms with basal GLP-1 secretion and glucose-induced GLP-1 response during an OGTT in non-diabetic participants. We further studied the influence of the *TCF7L2* SNPs on insulin secretion kinetics to i.v. administered glucose during an IVGTT and a hyperglycaemic clamp. In addition, we particularly investigated the influence on GLP-1-induced insulin secretion using a combined hyperglycaemic clamp with additional GLP-1 infusion and arginine bolus [15].

## Methods

**Participants** We studied 1,110 non-diabetic participants by an OGTT (Table 1). Secretion patterns of GLP-1 were obtained in a subgroup of 155 participants. The anthropometric characteristics of this subgroup are shown in Table 2. All 1,110 participants were also genotyped for the following five SNPs in the *TCF7L2* gene: rs7903146, rs12255372, rs7901695, rs11196205 and rs7895340.

A subset of 210 participants were studied by an IVGTT combined with a euglycaemic–hyperinsulinaemic clamp to determine insulin secretion capacity and insulin sensitivity in one test [16] (Table 3).

Additionally, 233 participants were studied by a hyperglycaemic clamp [17]. The participants were selected from three independent studies in the Netherlands and Germany. The German and the Dutch cohort from Utrecht consisted of NGT and IGT participants, the Dutch cohort from Hoorn consisted of IGT participants only (Table 4). All participants in our studies underwent a standard OGTT to define their glucose tolerance status. Details of the study groups have been described previously [17–19]. In the German subgroup ( $n=73$ ), the hyperglycaemic clamp was continued with an additional GLP-1 and arginine administration [15, 20–22].

The participants were not related to each other. Informed written consent for all studies was obtained from all participants, and the local ethics committee approved the protocols.

**Genotyping** Detection of the *TCF7L2* polymorphisms described by Grant et al. [1] was done using the TaqMan assay (Applied Biosystems, Forster City, CA, USA) in the German population. The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 7000, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems). As a quality standard, we randomly included six positive (two homozygous wild-type allele carriers, two heterozygous and two homozygous risk allele carriers) and two negative (all components excluding DNA) sequenced controls in each TaqMan reader plate. Because all controls were correctly identified, we assumed

that the genotyping error rate of this method did not exceed 0.3% [23].

In the Dutch population, only rs7903146 was determined using an ABI PRISM 7900HT sequence detector (Applied Biosystems).

**OGTT** At 08:00 hours, participants ingested a solution containing 75 g glucose. Venous blood samples were obtained at 0, 30, 60, 90 and 120 min for determination of plasma glucose, insulin and C-peptide concentrations and 0, 30 and 120 min for determination of GLP-1 concentrations. The participants did not take any medication known to affect glucose tolerance or insulin sensitivity. Tests were performed after an overnight fast of 12 h.

**Combined IVGTT and hyperinsulinaemic–euglycaemic clamp** After an overnight fast and after baseline samples had been obtained, 0.3 g/kg bodyweight of a 20% (vol./vol.) glucose solution was given at time 0. Blood samples for the measurement of plasma glucose, plasma insulin and C-peptide were obtained at 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 min. After 60 min, a priming dose of insulin was given followed by an infusion (40 mU/m<sup>2</sup>) of short-acting human insulin for 120 min. A variable infusion of 20% glucose was started to maintain the plasma glucose concentration at 5.5 mmol/l. Blood samples for the measurement of plasma glucose were obtained at 5 min intervals throughout the clamp.

**Hyperglycaemic clamp** Hyperglycaemic clamps were performed at 10 mmol/l in all participants. The Dutch NGT and IGT participants underwent a 3 h clamp. In the German NGT participants, the clamp lasted for 2 h followed by the GLP-1 and arginine stimulation (see below). After an overnight fast, the participants received an i.v. glucose bolus to acutely raise glucose levels to 10 mmol/l. Plasma glucose levels were measured at the appropriate intervals to maintain a constant plasma glucose during the clamp. Blood samples for insulin were drawn at 2.5 min intervals during the first 10 min of the clamp and at 10–20 min intervals during the remainder. Exact details of the clamping procedures in the different study groups have been described previously [15, 18, 19].

**Combined hyperglycaemic clamp** This hyperglycaemic clamp combined with GLP-1 and arginine administration was performed as previously described [15, 20–22]. After 120 min of hyperglycaemic clamp at 10 mmol/l, a bolus of GLP-1 (0.6 pmol/kg) was given (human GLP-1(7-36) amide; Poly Peptide, Wolfenbüttel, Germany) followed by a continuous GLP-1 infusion (1.5 pmol kg<sup>-1</sup> min<sup>-1</sup>) during the next 80 min. At 180 min, a bolus of 5 g arginine hydrochloride (Pharmacia & Upjohn, Erlangen, Germany) was injected over 45 s while the GLP-1 infusion was

continued. Blood for the measurement of glucose, insulin, proinsulin and C-peptide was obtained during the time-points shown in Fig. 1. This clamp allows measurement of different aspects of stimulus secretion coupling: first and second phases of glucose-induced insulin secretion, GLP-1-induced insulin secretion, and the response to additional arginine administration.

**Analytical procedures** Plasma glucose was determined as previously described [14, 17, 20, 21]. GLP-1 was determined during the OGTT at baseline, 30 and 120 min. GLP-1 immunoreactivity was determined using an RIA specific for the C-terminus of the peptide [24]. Samples were stored at –80°C. The assay measures the sum of the intact peptide plus the primary metabolite, GLP-1(9–36)amide using the polyclonal anti-GLP-1 antibody 89390 and synthetic GLP-1(7–36)amide as standard.

Plasma insulin and C-peptide concentrations in the German cohort were measured by a microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany) and an RIA (Byk-Santec, Dietzenbach, Germany). In the participants from the Netherlands, insulin was measured using an in-house competitive RIA employing a polyclonal anti-insulin antibody (Caris 46), <sup>125</sup>I-labelled insulin (IM 166; Amersham, Roosdaal, the Netherlands) as a tracer and regular insulin (Humulin; Eli Lilly, Indianapolis, IN, USA) as a standard.

**Calculations** Insulin secretion in the OGTT was assessed by calculating the AUC for C-peptide divided by the AUC for glucose (AUC<sub>CP</sub>/AUC<sub>GLC</sub>). AUCs were determined by the trapezoidal method. Furthermore, insulin secretion was calculated as insulin:glucose ratio by dividing (insulin at 30 min–insulin at 0 min) by (glucose at 30 min–glucose at 0 min). Insulin sensitivity during the OGTT was estimated from glucose and insulin values as proposed by Matsuda and DeFronzo [25].

Insulin secretion during the IVGTT was calculated as the sum of C-peptide levels during the first 10 min after glucose administration. Insulin sensitivity during the hyperinsulinaemic–euglycaemic clamp was calculated by dividing the average glucose infusion rate during the last 40 min of the clamp by the average plasma insulin concentration during the same time interval.

Insulin secretion during the hyperglycaemic clamp was calculated using insulin levels determined during the clamp. The first phase of insulin secretion was defined as the sum of the insulin levels during the first 10 min of the clamp. The second phase of insulin secretion was defined as the mean of the insulin values during the last 40 min (80–120 min, NGT group, Germany) or the last 30 min (150–180 min, NGT and IGT groups, the Netherlands) of the clamp.

In the combined hyperglycaemic clamp with GLP-1 and arginine administration, first-phase GLP-1-induced insulin secretion was defined as the mean of the 125 and 130 min insulin levels, second-phase GLP-induced insulin secretion (plateau) was defined as the mean of the 160–180 min insulin levels. The acute insulin response to arginine was calculated as the mean of 182.5 and 185 min minus 180 min insulin levels [21].

The insulin sensitivity index was determined by relating the glucose infusion rate to the plasma insulin concentration during the last 40 min (NGT, Germany) or 30 min of the clamp.

**Statistical analysis** Data are given as means $\pm$ SEM. Non-normally distributed parameters were logarithmically transformed to approximate linearity for statistical comparison. Distribution was tested for normality using the Shapiro–Wilk *W* test. Differences in anthropometrics and metabolic characteristics between genotypes were tested using ANOVA for the three genotype groups. The secretion indices were compared using multivariate regression models. In these models the trait was the dependent variable whereas age, sex, insulin sensitivity and genotype were the independent variables. Due to the relatively low number of participants who were homozygous for the rare alleles in the hyperglycaemic clamp group, a dominant model was used. A *p* value of <0.05 was considered to be statistically significant. The statistical software package JMP (SAS Institute, Cary, NC, USA) was used.

## Results

**Genetic variants in the *TCF7L2* gene** The SNPs described in the paper of Grant et al. [1] had an allelic frequency of the minor allele of 30% (rs7903146), 28% (rs12255372), 31% (rs7901695), 46% (rs11196205) and 45% (rs7895340) in our population. These polymorphisms were all in Hardy–Weinberg equilibrium and in linkage disequilibrium (Electronic supplementary material Table 1).

**OGTT: glucose tolerance, insulin secretion, insulin sensitivity and GLP-1 levels** The percentage of participants with IGT was significantly higher in carriers of the risk allele for rs7903146 (*p*=0.007), rs12255372 (*p*=0.02) and rs7901695 (*p*=0.02).

Insulin secretion assessed as  $AUC_{CP}/AUC_{Glc}$  during the OGTT was significantly reduced in participants with the risk alleles for rs7903146 and rs12255372 in the additive and dominant model both unadjusted and adjusted for relevant covariates. Participants with the risk allele for

rs7901695 had a significantly decreased insulin secretion in the additive model (*p*=0.02) (Table 1).

Insulin sensitivity, estimated by the index of Matsuda and DeFronzo [25], was significantly higher in participants carrying the risk alleles for rs7903146 and rs1255372 in *TCF7L2* (Table 1).

In a subgroup of 155 participants we measured GLP-1 concentrations at baseline, at 30 min and 120 min after oral ingestion of 75 g glucose. The anthropometric characteristics are shown in (Table 1). Neither basal GLP-1 plasma levels nor GLP-1 levels during the OGTT significantly differed between the genotypes in any of the above-described SNPs in the *TCF7L2* gene (Table 2).

**Combined IVGTT and hyperinsulinaemic–euglycaemic clamp: glucose-induced insulin secretion and insulin sensitivity** C-peptide and insulin values during the IVGTT were not significantly different between the genotypes in any of the above-described SNPs in the *TCF7L2* gene. Table 3 shows the results for rs7903146 in the additive and dominant model adjusted for relevant covariates. Including the glucose tolerance status as an additional co-variate in the model did not affect the results. Interestingly, a lower insulin secretion in this subgroup of participants could be demonstrated when insulin secretion was calculated from the OGTT as described above (Table 3). Insulin sensitivity measured with the clamp technique was not affected by any of the genotypes (all *p*>0.4).

**Hyperglycaemic clamp: glucose-, GLP-1- and arginine-induced insulin secretion and insulin sensitivity** First- and second-phase insulin secretion during the hyperglycaemic clamp were not significantly different between carriers and non-carriers of the risk allele for rs7903146 (Table 4) in any of the three populations from the Netherlands and from Germany. The other four SNPs in *TCF7L2* which were described above were determined in the German population only and were not associated with glucose-induced insulin secretion (data not shown) during the hyperglycaemic clamp.

The first phase of GLP-1-induced insulin secretion was significantly reduced in carriers of the risk alleles for rs7903146 (*p*=0.03) and rs12255372 (*p*=0.02). In addition, we found significant differences in the second phase of GLP-1-induced insulin secretion for rs7903146 (*p*=0.006), rs12255372 (*p*=0.005) and rs7901695 (*p*=0.02) with carriers of the risk alleles having a significantly lower second phase of GLP-1-induced insulin secretion. Table 4 and Fig. 1 show the results for SNP rs7903146.

In contrast to GLP-1-induced insulin secretion, arginine-induced insulin secretion was not affected by any of the analysed SNPs in *TCF7L2* (Table 4).

**Table 1** Anthropometric and metabolic data from all 1,110 participants who underwent an OGTT

	rs7903146			<i>p</i> value (ANOVA)	<i>p</i> value (CC vs XT)
	C/C	C/T	T/T		
Genotype	C/C	C/T	T/T		
<i>n</i>	546	474	90		
NGT/IGT	461/85	386/88	63/27	0.007	0.03
Sex (female/male)	346/200	315/159	50/40	0.13	0.64
Age (years)	39±1	40±1	41±1	0.39	0.67
BMI (kg/m <sup>2</sup> )	29.5±0.4	28.9±0.4	28.2±1.0	0.51	0.24
Plasma glucose (mmol/l)					
Fasting	5.1±0.02	5.1±0.02	5.2±0.07	0.25	0.39
2 h	6.1±0.07	6.2±0.07	6.7±0.2	0.06	0.17
Plasma insulin (pmol/l)					
Fasting	62.0±2.1	53.4±1.7	49.8±3.1	0.004	0.001
2 h	442±19	356±17	372±34	0.12	0.04
Insulin sensitivity <sub>OGTT</sub> (arbitrary units)	16.8±0.5	18.2±0.5	17.8±1.2	0.02/0.02 <sup>a</sup>	0.005/0.006 <sup>a</sup>
Insulin secretion <sub>OGTT</sub> (pmol/mmol)	319±5	301±5	292±10	0.003/0.04 <sup>b</sup>	0.0009/0.02 <sup>b</sup>
Insulin/glucose ratio (pmol/mmol)	143±5	127±5	124±13	0.003/0.03 <sup>b</sup>	0.001/0.01 <sup>b</sup>

Data are means±SEM

*p* values were obtained using  $\chi^2$  test or ANOVA

Analysis for rs12255372 and rs7901695 also revealed a significant difference in insulin secretion ( $p=0.003$  and  $p=0.05$ ), whereas for rs7895340 and rs11196205 no significant differences in insulin secretion were detected ( $p=0.53$  and  $0.45$ )

<sup>a</sup>*p* values are derived from multivariate linear regression models: adjusted for age, sex and BMI

<sup>b</sup>*p* values are derived from multivariate linear regression models: adjusted for age, sex, BMI and insulin sensitivity

**Table 2** Anthropometric and metabolic data of the subgroup of 155 participants with additional measurements of GLP-1

	rs7903146			<i>p</i> value (ANOVA)	<i>p</i> value (CC vs XT)
	C/C	C/T	T/T		
Genotype	C/C	C/T	T/T		
<i>n</i>	73	67	15		
NGT/IGT	64/9	48/19	10/5	0.03	0.009
Sex (female/male)	50/23	42/25	8/7	0.50	0.33
Age (years)	46±1	47±1	47±3	0.99	0.96
BMI (kg/m <sup>2</sup> )	29.8±0.7	30.3±0.7	27.1±1.1	0.15	0.88
Plasma glucose (mmol/l)					
Fasting	5.2±0.05	5.3±0.07	5.6±0.2	0.17	0.21
2 h	6.5±0.1	7.0±0.2	7.2±0.5	0.22	0.10
Insulin sensitivity <sub>OGTT</sub> (arbitrary units)	12.8±0.7	13.6±1.0	15.7±2.6	0.74/0.92 <sup>a</sup>	0.78/0.75 <sup>a</sup>
Insulin secretion <sub>OGTT</sub> (pmol/mmol)	304±12	287±13	235±19	0.01/0.02 <sup>b</sup>	0.02/0.01 <sup>b</sup>
GLP-1 (pmol/l)					
0 min	16.1±0.9	17.3±1.3	17.3±2.0	0.91	0.88
30 min	34.1±2.1	38.8±4.0	38.1±3.5	0.45	0.36
120 min	28.9±1.5	29.0±1.7	28.9±2.4	0.87	0.88
Fold increase 0–30 min	2.5±0.2	2.7±0.3	2.4±0.4	0.77	0.84

Data are means±SEM

*p* values were obtained using  $\chi^2$  test or ANOVA

Analysis for rs7901695 also revealed a significant difference in insulin secretion ( $p=0.02$ ), whereas for rs7895340 and rs11196205 no significant differences in insulin secretion were detected ( $p=0.73$  and  $0.76$ )

<sup>a</sup>*p* values are derived from multivariate linear regression models: adjusted for age, sex and BMI

<sup>b</sup>*p* values are derived from multivariate linear regression models: adjusted for age, sex, BMI and insulin sensitivity



**Table 3** Anthropometric and metabolic data of 210 participants who underwent a combined IVGTT and hyperinsulinaemic–euglycaemic clamp

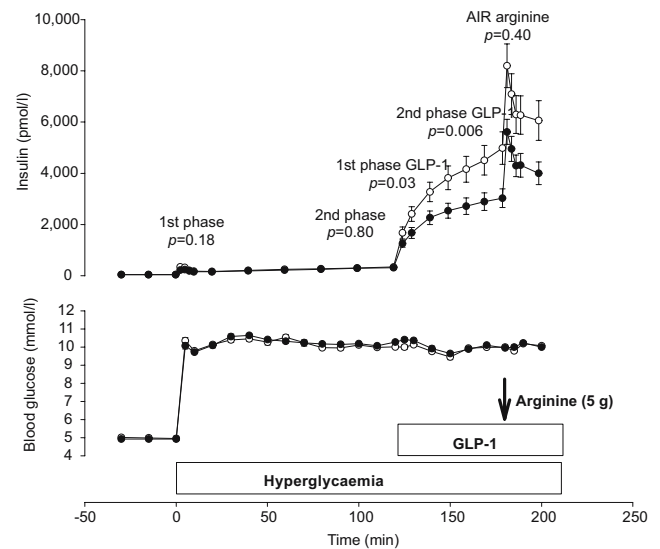
	rs7903146		<i>p</i> value (CC vs XT)
	C/C	X/T	
Genotype	C/C	X/T	
<i>n</i>	97	113	
NGT/IGT	80/17	81/32	0.06
Sex (female/male)	59/38	64/49	0.58
Age (years)	44±1	45±1	0.29
BMI (kg/m <sup>2</sup> )	29.0±0.5	28.8±0.5	0.82
Insulin sensitivity <sub>clamp</sub> (μmol kg <sup>-1</sup> min <sup>-1</sup> [pmol/l] <sup>-1</sup> )	0.07±0.01	0.07±0.01	0.77
Insulin secretion index <sup>a</sup>			
IVGTT C-peptide secretion (pmol/l)	7,904±306	7,679±323	0.40
IVGTT insulin secretion (pmol/l)	2,059±131	1,924±129	0.25
OGTT insulin secretion (pmol/mmol)	314±8	292±8	0.04

Data are means±SEM

*p* values were obtained using  $\chi^2$  test or unpaired *t* test or multivariate linear regression models

None of the four other SNPs in TCF7L2 (rs12255372, rs7901695, rs11196205 and rs7895340) affected significantly insulin secretion in the IVGTT (all *p*>0.2)

<sup>a</sup> Adjusted for age, sex and BMI, and insulin sensitivity



**Fig. 1** Associations between the genotypes of rs7903146 polymorphism in the *TCF7L2* gene with insulin secretion during a hyperglycaemic clamp in 73 German participants. *Open circles* CC; *closed circles* CT and TT. *AIR* Acute insulin response. *Arrow* Administration of 5 g arginine. The *p* values show the differences for first and second phases of glucose-induced insulin secretion, first and second phases of GLP-1-induced insulin secretion and acute insulin secretory response to arginine (*AIR*) (for calculation see ‘Methods’; insulin secretion is adjusted for insulin sensitivity)

**Table 4** Anthropometric and metabolic data of 233 participants who underwent a hyperglycaemic clamp and of 73 participants who underwent a modified hyperglycaemic clamp with additional GLP-1 and arginine administration

	Germany			The Netherlands (Utrecht)			The Netherlands (Hoom)		
	rs7903146	<i>p</i> value		rs7903146	<i>p</i> value		rs7903146	<i>p</i> value	
Genotype	C/C	X/T		C/C	X/T		C/C	X/T	
Sex (female/male)	15/20	24/14	0.08	5/31	22/11	<0.01	17/11	30/33	0.27
NGT/IGT	30/5	27/11	0.13	31/5	26/7	0.53	0/28	0/66	–
Age (years)	38±2	40±2	0.66	47±1	47±1	0.94	56±1	58±1	0.43
BMI (kg/m <sup>2</sup> )	26.1±0.9	25.1±0.6	0.41	25.6±0.7	26.2±0.7	0.49	29.2±0.8	27.9±0.4	0.13
Plasma glucose (mmol/l)									
Fasting	5.1±0.1	5.0±0.1	0.72	4.6±0.1	4.8±0.1	0.18	6.6±0.1	6.6±0.1	0.75
2 h glucose	5.9±0.3	6.5±0.3	0.21	5.7±0.3	5.6±0.3	0.74	9.5±0.1	9.5±0.1	0.84
Insulin sensitivity (μmol kg <sup>-1</sup> min <sup>-1</sup> [pmol/l] <sup>-1</sup> )	0.15±0.02	0.15±0.01	0.90	0.17±0.02	0.18±0.02	0.60	0.11±0.01	0.14±0.01	0.21
Insulin secretion index (pmol/l) <sup>a</sup>									
First phase (sum 0–10 min)	1,067±158	815±105	0.18	963±75	891±96	0.76	699±70	679±72	0.68
Second phase	308±47	291±41	0.80	389±42	376±47	0.71	304±45	303±38	0.18
First phase GLP-1	2,049±258	1,466±175	0.03	n.a.	n.a.		n.a.	n.a.	
Second phase GLP-1	4,567±568	2,881±341	0.006	n.a.	n.a.		n.a.	n.a.	
Arginine	2,680±226	2,252±181	0.40	n.a.	n.a.		n.a.	n.a.	

Data are means±SE

*p* values were obtained using  $\chi^2$  test, unpaired *t* test or multivariate linear regression models

Carriers of the risk allele for SNP rs12255372 showed reduced first phase (*p*=0.02) and second phase (*p*=0.005) GLP-1-induced insulin secretion

Carriers of the risk allele for SNP rs7901695 showed reduced second phase (*p*=0.02) GLP-1-induced insulin secretion

*n.a.* Not available

<sup>a</sup> Adjusted for age, sex and BMI, and insulin sensitivity

## Discussion

The variants in *TCF7L2* described by Grant et al. [1] that mediate an increased risk of type 2 diabetes have been found to be associated with reduced insulin secretion [6, 7, 9, 10]. We could confirm this reduced insulin secretion in our study in a group of 1,110 German non-diabetic participants using C-peptide levels during an OGTT. Unexpectedly, the i.v. application of glucose during an IVGTT did not affect insulin secretion in carriers of the risk alleles for SNPs in *TCF7L2*. The same results were obtained using i.v. glucose challenge during a hyperglycaemic clamp in subgroups of the German population and a population from the Netherlands. The observed different results between an orally and i.v. administered glucose challenge generate the hypothesis that incretin-induced insulin secretion is affected by the variants in the *TCF7L2* gene. Possible mechanisms explaining these findings include impaired incretin production or incretin signalling.

To address this issue we first measured basal GLP-1 concentrations and GLP-1 response during an OGTT. None of the tested variants that have been shown in the literature to exert a reproducible effect on type 2 diabetes were associated with basal GLP-1 levels or with GLP-1 response during the OGTT.

Furthermore, we combined the hyperglycaemic clamp with additional GLP-1 infusion to measure GLP-1-induced insulin secretion. Both acute (so called first phase) and prolonged (so called second phase) GLP-1-induced insulin secretion during the hyperglycaemic clamp were significantly impaired in carriers of the risk alleles in *TCF7L2*. These data suggest that participants carrying the risk alleles have an impaired insulin secretion due to an impairment of the GLP-1 signalling chain.

The putative molecular mechanism for this finding is not clear. As the transcription factor *TCF7L2* plays an essential role in the *WNT* signalling pathway [14], which on the one hand is required for a normal development of the pancreas and the pancreatic islets [26] and on the other hand is crucial for the regulation of GLP-1 expression and secretion in intestinal L cells [14]. In theory, polymorphisms in the *TCF7L2* gene might therefore through alteration of the *WNT* signalling pathway directly affect beta cell growth, beta cell differentiation and beta cell function. Furthermore insulin secretion might also be affected indirectly via the enteroinsular axis either through an impaired overall GLP-1 secretion or through a defective or dysfunctional GLP-1-induced insulin secretion. An impaired or dysfunctional GLP-1 effect might result first in a reduced postprandial insulin secretion and second, might influence stimulation of beta cell growth and beta cell differentiation.

Our data show that basal and glucose-induced GLP-1 secretion during the OGTT were not affected by any of the analysed SNPs, whereas the first and second phases of

GLP-1-induced insulin secretion were significantly reduced in carriers of the risk alleles in three of the five above-described SNPs in the *TCF7L2* gene, among them rs7903146, which is most strongly associated in the literature with type 2 diabetes [1, 6, 9, 12, 13]. The finding that basal and glucose-stimulated GLP-1 secretion were not influenced by the *TCF7L2* polymorphisms indicates that a reduced GLP-1 secretion is not likely to explain the impaired insulin secretion in carriers of the risk genotypes, although it can not fully exclude an effect of these SNPs on GLP-1 levels. First, by measuring total GLP-1 levels, we may have missed a subtle defect in GLP-1 secretion, which may have been detected by measuring the active form of GLP-1. Second, systemic GLP-1 levels may not adequately reflect the level of the active hormone acting in the gut wall on the autonomic nervous system [27]. Third, impaired *TCF7L2* activity might tissue-specifically reduce the GLP-1 levels in the brain, which are believed as well to be important for insulin secretion [28].

While we have no evidence for reduced GLP-1 secretion we have strong evidence for an impaired GLP-1-induced insulin secretion in carriers of the above-described polymorphisms in *TCF7L2*. The data show a reduced efficiency of GLP-1 to stimulate insulin secretion in pancreatic beta cells. Alterations of the GLP-1 signalling pathway in beta cells might thereby play a role. Recently the *TCF7L2* gene was also identified in human pancreas [14]. Therefore variations in this transcription factor could specifically impair the transcription of genes involved in the incretin signalling chain, resulting in resistance of the pancreatic beta cells to GLP-1.

In contrast to the observed reduction in the first and second phases of GLP-1-induced insulin secretion, the arginine-induced insulin secretion was not significantly affected by the analysed SNPs in the *TCF7L2* gene. The arginine bolus in the combined hyperglycaemic clamp produces a maximal challenge for the secretory capacity of the beta cell and can be considered as a surrogate for beta cell mass [15, 20]. The SNPs do not affect this maximal insulin secretion, suggesting that the variants in *TCF7L2* do not influence beta cell mass, at least in the prediabetic state. In addition, impaired beta cell function may also include the efficiency of the conversion from proinsulin to insulin [21]. However, there was no evidence for this abnormality related to the variants in the *TCF7L2* gene during the hyperglycaemic clamp (data not shown).

We also found that insulin sensitivity calculated from the OGTT using an established index [25] was significantly increased in participants carrying the risk alleles for all analysed SNPs in the *TCF7L2* gene. This was also described in the study of Florez et al. [9]. When we measured insulin sensitivity with the gold standard, the euglycaemic–hyperinsulinaemic clamp and the hypergly-

caemic–hyperinsulinaemic clamp, we found no association of the risk alleles with insulin sensitivity. Taken together, our data suggest that the variants in the *TCF7L2* gene do not have a strong effect on insulin sensitivity.

In summary, our data show that variations in the *TCF7L2* gene are associated with impaired GLP-1-induced insulin secretion. This might be consistent with a state of relative incretin resistance. Increasing the incretin levels by pharmacological tools may thus be a logical therapy to overcome impaired insulin secretion in carriers of the *TCF7L2* variants.

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