

A common genetic variant in *WFS1* determines impaired glucagon-like peptide-1-induced insulin secretion

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Abstract

Aims/hypothesis *WFS1* type 2 diabetes risk variants appear to be associated with impaired beta cell function, although it is unclear whether insulin secretion is affected directly or secondarily via alteration of insulin sensitivity. We aimed to investigate the effect of a common *WFS1* single-nucleotide polymorphism on several aspects of insulin secretion.

Methods A total of 1,578 non-diabetic individuals (534 men and 1,044 women, aged 40 ± 13 years, BMI 28.9 ± 8.2 kg/m² [mean \pm SD]) at increased risk of type 2 diabetes were genotyped for rs10010131 within the *WFS1* gene. All participants underwent an OGTT (and a subset additionally an IVGTT [$n=319$]) and a hyperglycaemic clamp combined with glucagon-like peptide-1 (GLP-1) and arginine stimuli ($n=102$).

Results rs10010131 was associated with reduced OGTT-derived insulin secretion ($p=0.03$). 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 infusion combined with a hyperglycaemic clamp showed a significant reduction of the insulin secretion rate during the first and second phases of GLP-1-induced insulin secretion in carriers of the risk allele (reduction of 36% and 26%, respectively; $p=0.007$ and $p=0.04$, respectively).

Conclusions/interpretation A common genetic variant in *WFS1* specifically impairs GLP-1-induced insulin secretion independently of insulin sensitivity. This defect might explain the impaired insulin secretion in carriers of the risk allele and confer the increased risk of type 2 diabetes.

Keywords Endoplasmic reticulum · GLP-1 · Incretin · Pancreatic beta cell · Single-nucleotide polymorphism · Wolfram syndrome · Wolframin

Abbreviations

GLP-1	Glucagon-like peptide-1
GWA	Genome-wide association
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
ISR	Insulin secretion rate
MAF	Minor allele frequency
NGT	Normal glucose tolerance
SNP	Single-nucleotide polymorphism
WFS	Wolfram syndrome

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Introduction

Recent genome-wide association (GWA) studies have not only confirmed the importance of established candidate gene loci for type 2 diabetes, such as *PPARG*, *KCNJ11* and *TCF7L2*, but have also identified novel type 2 diabetes risk variants in several genes, i.e. *SLC30A8*, *HHEX*, *CDKAL1*,

IGF2BP2 and *CDKN2A/B*, none of which was in the list of functional candidates [1–4]. Thorough metabolic characterisation of genotyped cohorts, comprising quantification of insulin sensitivity and insulin secretion by well-accepted scientific methods, revealed that the novel variants are all associated with insulin-secretory defects, but show little if any relationship to insulin resistance [5–12]. A very recent GWA study confirmed with *WFS1* a type 2 diabetes susceptibility gene region [13], which had been identified earlier by candidate-gene approaches [14–16]. Similarly to the other novel gene variants that are associated with type 2 diabetes, two previous studies suggested that *WFS1* risk alleles for type 2 diabetes might be associated with impaired pancreatic beta cell function as assessed by OGTT-derived indices of insulin secretion [17, 18]. However, it is unclear whether common genetic variants in *WFS1* affect insulin secretion directly or secondarily via alteration of insulin sensitivity.

WFS1 encodes an 890 amino acid-containing transmembrane polypeptide that is ubiquitously found, with high expression in pancreatic islets and specific neurons, and shows predominantly subcellular localisation to the endoplasmic reticulum [19]. Mutations in *WFS1* result in Wolfram syndrome (WFS; OMIM 222300), an autosomal recessive neurodegenerative disorder. According to its clinical presentation with diabetes insipidus, young-onset non-immune insulin-dependent diabetes mellitus, optic atrophy and deafness, WFS is also referred to as the DIDMOAD syndrome [20]. Mice conditionally lacking *Wfs1* showed progressive beta cell loss and impaired insulin secretion [21]. Reduction in beta cell survival resulted from enhanced endoplasmic reticulum stress and apoptosis [22, 23].

Although previous studies suggest that progressive loss of insulin secretion might be an important component of the phenotype which predisposes carriers of the *WFS1* variant to develop type 2 diabetes [17, 18], the pathogenic mechanism of impaired insulin secretion due to genetic variation in the *WFS1* locus is not completely established. The highly developed endoplasmic reticulum structure of beta cells is an important factor in beta cell function, comprising production and regulated secretion of insulin to control blood glucose levels [24]. A growing body of evidence suggests that incretin hormones, such as glucagon-like peptide-1 (GLP-1), not only enhance insulin secretion, but also directly upregulate insulin biosynthesis in the beta cell, while preventing beta cell apoptosis [25]. A recent study showed that GLP-1 receptor-mediated signalling directly modulates the endoplasmic reticulum stress response leading to promotion of beta cell adaptation and survival [26, 27]. Given the well-established association of genetic variation in the *WFS1* locus with risk of type 2 diabetes, which might result from an impairment of beta cell function [17, 18], the aim of the present study was to

investigate the influence of a common *WFS1* single-nucleotide polymorphism (SNP) on insulin secretion kinetics to i.v. administered glucose during an IVGTT and a hyperglycaemic clamp. In addition, we particularly investigated its influence on GLP-1-induced insulin secretion using a combined hyperglycaemic clamp with additional GLP-1 infusion and an arginine bolus [28].

In light of the strong effects of *TCF7L2* gene polymorphisms on different aspects of insulin secretion [29, 30], the association between common genetic variation within *WFS1* and insulin secretion was evaluated on the *TCF7L2* genetic background.

Methods

Participants We studied 1,578 non-diabetic participants who were at increased risk of type 2 diabetes because of a family history of diabetes (first-degree relatives of type 2 diabetic patients), history of gestational diabetes, overweight, impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) by an OGTT (Table 1). Participants were recruited from an ongoing study on the pathophysiology of type 2 diabetes [31]. A subset of 319 participants was studied by an IVGTT combined with a euglycaemic–hyperinsulinaemic clamp to determine insulin secretion capacity and insulin sensitivity in one test [32] (Table 2). Additionally, 102 participants were studied by a hyperglycaemic clamp, which was continued with an additional GLP-1 and arginine administration [28, 33]. Informed written consent for all studies was obtained from all participants, and the local ethics committee approved the protocols.

Genotyping Individuals were genotyped for rs10010131 in the *WFS1* gene and for rs7903146 in the *TCF7L2* gene. In a recent meta-analysis, rs10010131 in intron 4 of the *WFS1* gene has convincingly been found to be associated with risk of type 2 diabetes [16]. rs10010131 is in high linkage disequilibrium with the other reported high-risk variants in *WFS1* [13, 15–18] (Electronic supplementary material [ESM] Fig. 1). rs7903146 in intron 3 of the *TCF7L2* gene was found to be the most significantly associated SNP with an increased risk of diabetes among persons with IGT [29, 34]. Genotyping was done using the TaqMan assay (Applied Biosystems, Forster City, CA, USA). 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.

Table 1 Anthropometric and metabolic data from 1,578 participants who underwent an OGTT

Variable	rs10010131 genotype			p value
	G/G	G/A	A/A	
n	586	749	243	–
IFG/IGT/(IFG+IGT)	64/53/42	80/73/63	20/26/18	0.91
Sex (women/men)	391/195	494/255	159/84	0.93
Age (years)	39±13	39±13	40±14	0.86
BMI (kg/m ²)	29.0±8.5	29.1±8.4	27.8±6.9	0.10
Plasma glucose (mmol/l)				
Fasting	5.10±0.56	5.13±0.55	5.05±0.50	0.21
2 h	6.26±1.61	6.30±1.68	6.17±1.69	0.74
Plasma insulin (pmol/l)				
Fasting	62.1±46.8	65.8±58.7	61.3±48.0	0.71
2 h	419±387	446±474	426±537	0.8
Insulin sensitivity _{OGTT} (arbitrary units)	16.2±10.5	16.1±10.8	17.1±11.1	0.94
AUC C-peptide/AUC glucose (pmol/mmol)	311±101	326±112	324±106	0.03
Insulinogenic index (pmol/mmol)	131±417	147±234	168±356	0.08

Raw data (means ± SD) are shown. For statistical analysis, non-normally distributed data were log_e transformed. p values were obtained using χ^2 test or multivariate regression analysis. Anthropometric data were adjusted for sex and age. Indices of insulin sensitivity were adjusted for sex, age and BMI. Indices of insulin secretion were adjusted for sex, age, BMI and insulin sensitivity

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. 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/m²) of short-acting human insulin for 120 min. A variable infusion of 20% glucose was started to maintain the plasma glucose concentration at fasting level. Blood samples for the measurement of plasma glucose were obtained at 5 min intervals throughout the clamp.

Hyperglycaemic clamp Exact details of the clamping procedures have been described previously [28, 35]. In brief, hyperglycaemic clamps 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

Table 2 Anthropometric and metabolic data of 319 participants who underwent a combined IVGTT and hyperinsulinaemic–euglycaemic clamp

Variable	rs10010131 genotype			p value
	G/G	G/A	A/A	
n	115	158	46	–
IFG/IGT/(IFG+IGT)	14/12/7	16/21/17	4/8/5	0.58
Sex (women/men)	67/48	87/71	26/20	0.87
Age (years)	46±10	45±11	42±12	0.10
BMI (kg/m ²)	29.5±6.3	29.2±5.1	30.0±6.3	0.68
Insulin sensitivity _{clamp} ($\mu\text{mol kg}^{-1} \text{min}^{-1} [\text{pmol/l}]^{-1}$)	0.069±0.039	0.070±0.040	0.063±0.051	0.40
Insulin secretion indices				
Sum of C-peptide levels _{IVGTT} (pmol/l) ^a	8,036±2,779	8,664±4,531	8,569±3,205	0.80
Sum of insulin levels _{IVGTT} (pmol/l) ^a	2,046±1,201	2,167±2,122	2,155±1,415	0.39

Raw data (means ± SD) are shown. For statistical analysis, non-normally distributed data were log_e transformed. p values were obtained using χ^2 test or multivariate regression analysis. Anthropometric data were adjusted for sex and age. Indices of insulin sensitivity were adjusted for sex, age and BMI. Indices of insulin secretion were adjusted for sex, age, BMI and insulin sensitivity

^aC-peptide and insulin levels were measured during the first 10 min after intravenous glucose administration in the IVGTT

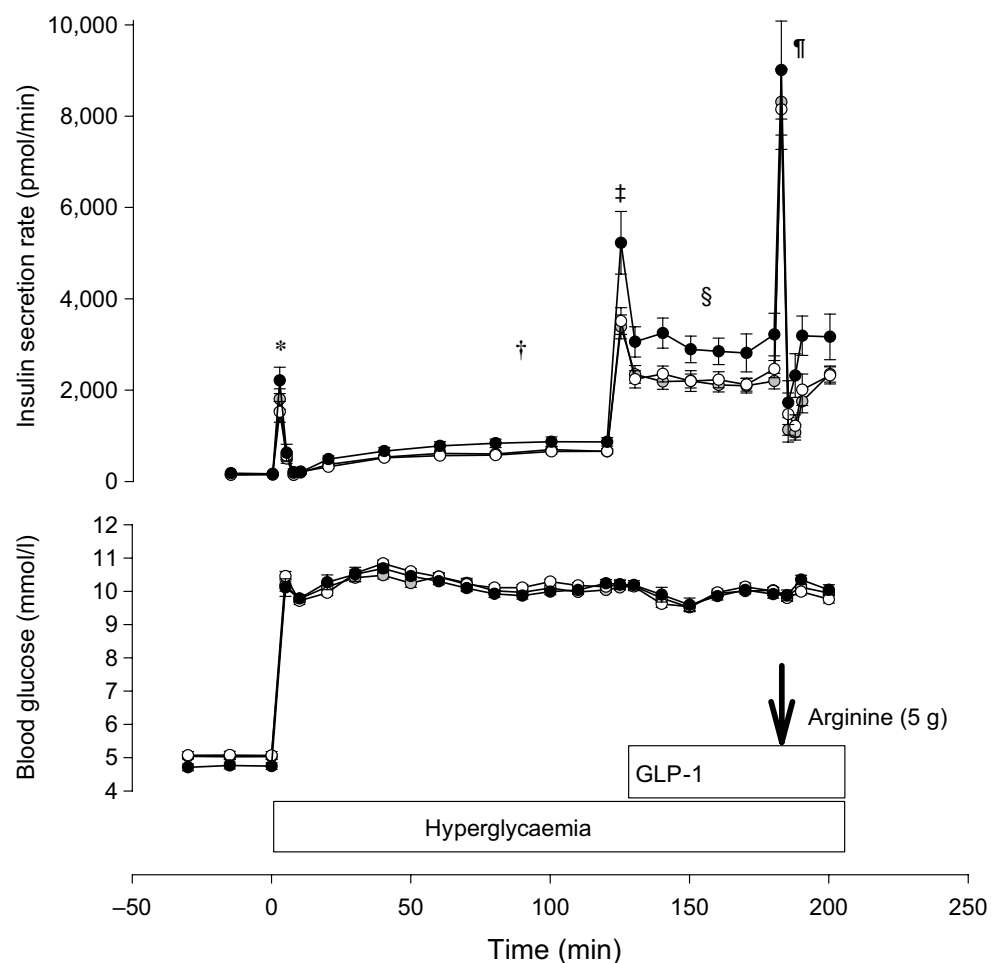
glucose levels were measured at the appropriate intervals to maintain a constant plasma glucose during the clamp. Blood samples for insulin were drawn at 2.5 min intervals during the first 10 min of the clamp and at 10–20 min intervals during the remainder.

Combined hyperglycaemic clamp This hyperglycaemic clamp combined with GLP-1 and arginine administration was performed as previously described [28]. After 120 min of the hyperglycaemic clamp at 10 mmol/l, a bolus of GLP-1 (4.5 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⁻¹ min⁻¹) 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 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.

Measurement of insulin secretion rate (ISR) Samples for RIA C-peptide measurement (Byk-Sangtec, Dietzenbach, Germany) were taken at –30, –15, 0, 2.5, 5, 7.5, 10, 20, 40, 60, 80, 100, 120, 125, 130, 140, 150, 160, 170, 180, 182.5, 185, 187.5, 190 and 200 min. Standard kinetic variables for C-peptide (rate constants, volume of distribution) adjusted for age, sex, BMI and body surface area were used [36] and assumed to remain unchanged throughout the experiment. These variables were used to calculate the ISR for time intervals indicated above from the plasma C-peptide concentrations by deconvolution as described previously [36, 37]. We also calculated insulin secretion based on plasma levels of insulin as previously described [30]. We decided to present ISRs derived from C-peptide data as they are less influenced by potentially different clearance rates.

Analytical procedures Blood glucose was determined using a bedside glucose analyser (glucose-oxidase method; Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma insulin and C-peptide concentrations were measured by a microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany) and an RIA (Byk-Sangtec).

Fig. 1 Associations between the genotypes of rs10010131 polymorphism in the *WFS1* gene and insulin secretion during a hyperglycaemic clamp in 102 German participants (black circles, AA; grey circles, GA; white circles GG). Values are means \pm SEM. Arrow: administration of 5 g arginine. * $p=0.34$ for differences between the genotypes for the first phase of glucose-induced insulin secretion; † $p=0.09$ for differences between the genotypes for the second phase of glucose-induced insulin secretion values; ‡ $p=0.007$ for differences between the genotypes for the first phase of GLP-1-induced insulin secretion; § $p=0.04$ for differences between the genotypes for the second phase of glucose-induced insulin secretion; ¶ $p=0.84$ for differences between the genotypes for the acute insulin secretory response to arginine (for calculations see the Methods). Insulin secretion is adjusted for insulin sensitivity



Calculations Insulin secretion in the OGTT was assessed by calculating the AUC for C-peptide divided by the AUC for glucose. AUCs were determined by the trapezoidal method. Insulin secretion was also assessed with the insulinogenic index by calculating (insulin at 30 min–insulin at 0 min)/(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 [38]. Insulin secretion during the IVGTT was assessed as the sum of C-peptide levels and of insulin levels, respectively, 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 the ISR was defined as the sum of the deconvoluted C-peptide levels during the first 10 min of the clamp. The second phase of insulin secretion was defined as the mean of ISR values during the last 40 min (80–120 min, normal glucose tolerance [NGT] group) 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 difference between the 125 and 120 min insulin levels and the second-phase GLP-1-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 (difference between the mean of 182.5 and 185 min)–(180 min ISR levels) [28]. The insulin sensitivity index was determined by relating the glucose infusion rate to the plasma insulin concentration during the last 40 min (NGT group).

Statistical analysis Raw data are usually given as means \pm SD. For statistical analysis, non-normally distributed variables were logarithmically transformed (\log_e) to approximate normality. Distribution was tested for normality using the Shapiro–Wilk W test. Differences in anthropometrics and metabolic characteristics between genotypes were tested using χ^2 tests and multiple regression analysis. In these models the trait was the dependent variable, whereas age, sex, BMI, insulin sensitivity and genotype were the independent variables. Insulin sensitivity was included as a covariate in the analysis of insulin secretion indices, as insulin secretion is highly associated with insulin sensitivity [39]. $p < 0.05$ was considered to be statistically significant. The statistical software package JMP 7.0 (SAS Institute, Cary, NC, USA) was used.

The power in the different cohorts undergoing an OGTT, an IVGTT and a hyperglycaemic clamp is shown in Table 3. Power calculation was performed in the dominant model ($1-\beta > 0.8$; $\alpha = 0.05$) by two-tailed tests as well as by one-

tailed tests (the latter may be justified as the risk allele of the *WFS1* SNP is known) using G*power software, available at www.psych.uni-duesseldorf.de/aap/projects/gpower (accessed 24 January 2009).

Results

Genetic variation in the *WFS1* gene In our cohort, the minor allele frequency (MAF) for rs10010131 (A allele) was 0.391, whereas the MAF reported in HapMap was 0.267. While the observed MAF was in agreement with previous studies in Swedish (0.43 [16]), and Danish (0.42 [18]) populations, the difference between the observed MAF and the MAF published by HapMap might be because of genuine differences between our German population and HapMap's cohort of Utah residents with northern and western European ancestry. The analysed polymorphism was in Hardy–Weinberg equilibrium ($p = 1.0$).

OGTT: glucose tolerance, insulin sensitivity and insulin secretion As shown in Table 1, rs10010131 was not associated with the percentage of individuals with IFG and IGT, indices of glucose tolerance during OGTT, such as fasting glucose, fasting insulin, 2 h glucose and 2 h insulin, and insulin sensitivity estimated by the index of Matsuda and DeFronzo after adjustment for sex, age and BMI [38]. However, insulin secretion assessed as the ratio AUC C-peptide/AUC glucose during the OGTT was significantly reduced in participants with the risk allele after adjustment for relevant covariates ($p = 0.03$). Furthermore, we detected a trend for lower insulin secretion in risk allele carriers as assessed by the insulinogenic index ($p = 0.08$).

Combined IVGTT and hyperinsulinaemic–euglycaemic clamp: glucose-induced insulin secretion and insulin sensitivity As shown in Table 2, rs10010131 was not associated with C-peptide and insulin values during the IVGTT adjusted for relevant covariates. Insulin sensitivity

Table 3 Power in the different cohorts undergoing an OGTT, an IVGTT and a hyperglycaemic clamp

Cohort	<i>n</i>	Effect size <i>d</i> (%)	
		One-tailed test	Two-tailed test
OGTT	1,578	13	15
IVGTT	319	29	33
Hyperglycaemic clamp	102	53	59

Power calculation was performed in the dominant model ($1-\beta > 0.8$; $\alpha = 0.05$) using G*power software available at www.psych.uni-duesseldorf.de/aap/projects/gpower (accessed 24 January 2009)

measured with the clamp technique was not affected by the rs10010131 genotypes after adjustment for sex, age and BMI.

Hyperglycaemic clamp: glucose-, GLP-1- and arginine-induced ISR and insulin sensitivity As shown in Fig. 1, ISRs (measured from the deconvoluted C-peptide concentrations) were significantly different between the three genotypes in the first and second phase of GLP-1-induced insulin secretion ($p=0.007$ and $p=0.04$, respectively). Homozygous minor allele carriers showed the highest GLP-1-induced ISR. It is worth noting that the associations of rs10010131 with ISR during the first and second phase of GLP-1-induced insulin secretion, as well as with AUC C-peptide/AUC glucose during the OGTT, remained significant after exclusion of related individuals ($p=0.007$, $p=0.04$ and $p=0.0183$, respectively). In contrast, no significant differences in the ISR were found during either the first and second phase of the hyperglycaemic clamp ($p=0.34$ and $p=0.09$, respectively), or during arginine-induced insulin secretion ($p=0.84$; Table 4). Insulin levels during the clamp study showed essentially the same results (data not shown).

Effect of gene–gene interaction on insulin secretion Recently, we have also described a diminished insulin secretion response to GLP-1 in carriers of the *TCF7L2* gene polymorphism rs7903146 [30]. Therefore, we tested the alleles of rs10010131 in *WFS1* and of rs7903146 in *TCF7L2* for interactions on insulin secretion. Analysis of covariance did not reveal an interaction between *WFS1* and *TCF7L2* genotypes on the AUC C-peptide/AUC glucose ratio ($p=0.92$).

Genetic variation within *TCF7L2* has a prominent impact on insulin secretion [29]. To rule out that the effects of *WFS1* genetic variants on insulin secretion result only from an abnormal distribution of *TCF7L2* genotypes within the *WFS1* genotype groups, we analysed the *TCF7L2* genotype distribution in homozygous major allele carriers, heterozygotes and homozygous minor allele carriers of *WFS1*. However, *TCF7L2* genotype distribution was not different in the *WFS1* genotype subgroups (χ^2 test; $p=0.39$).

Discussion

In our study group, comprising 1,578 German non-diabetic participants at increased risk of 2 type diabetes, we found that the *WFS1* type 2 diabetes risk variant rs10010131 was associated with reduced insulin secretion during an OGTT independently of insulin sensitivity. Our findings are in agreement with previous studies showing nominal association of rs10010131 and rs734312, which is in high linkage disequilibrium with rs10010131, and OGTT-derived insulin sensitivity in individuals with IGT [17, 18]. In contrast, the i.v. glucose application during an IVGTT did not affect insulin secretion in carriers of the rs10010131 risk allele. The same results were obtained using i.v. glucose challenge during a hyperglycaemic clamp in a subgroup of our study population. The observed difference between an orally and i.v. administered glucose challenge indicates an impairment of the incretin-induced insulin secretion by a common genetic variant in the *WFS1* gene. In accord with this assumption, the acute (first phase) and prolonged (second phase) GLP-1-induced insulin secretion during a combined

Table 4 Anthropometric and metabolic data from 102 participants who underwent a modified hyperglycaemic clamp with additional GLP-1 and arginine administration

Variable	rs10010131 genotype			p value
	G/G	G/A	A/A	
n	34	46	22	–
NGT/IGT	22/12	36/10	15/7	0.38
Sex (women/men)	18/16	30/16	8/14	0.08
Age (years)	41±13	38±13	38±14	0.54
BMI (kg/m ²)	25.1±6.4	25.4±5.0	26.7±5.0	0.40
Plasma glucose (mmol/l)				
Fasting	5.3±0.1	5.0±0.1	5.0±0.1	0.19
2 h	6.9±0.4	6.2±0.3	6.3±0.5	0.43
Insulin sensitivity (μmol kg ⁻¹ min ⁻¹ [pmol/l] ⁻¹)	0.15±0.09	0.16±0.12	0.13±0.10	0.35
ISR (pmol min ⁻¹)				
First phase	2,086±223	2,303±267	2,841±329	0.34
Second phase	632±48	653±43	858±94	0.09
First phase GLP-1	2,850±265	2,730±237	4,360±628	0.007
Second phase GLP-1	2,270±158	2,135±150	2,962±378	0.04
Arginine ^a	5,679±735	6,115±636	5,791±888	0.84

Raw data are given as means ± SEM. For statistical analysis, non-normally distributed data were log_e transformed. p values were obtained using χ^2 test or multivariate regression analysis. Anthropometric data were adjusted for sex and age. Indices of insulin sensitivity were adjusted for sex, age and BMI. Insulin concentrations were adjusted for sex, age, BMI and insulin sensitivity. ISR was adjusted for age and insulin sensitivity

^a Arginine values were available in only 101 individuals

hyperglycaemic clamp was significantly impaired in carriers of the risk allele in *WFS1*. These data suggest that impaired insulin secretion in participants carrying the risk allele results from disturbances of the GLP-1 signalling chain. Recently, we have also described a diminished insulin secretion response to GLP-1 in carriers of *TCF7L2* gene polymorphisms [30]. Testing the interaction between the *WFS1* and the *TCF7L2* variants on insulin secretion, we did not find a significant association. Furthermore, it is worth noting that the association of the *WFS1* SNP with insulin secretion was not dependent on the *TCF7L2* genetic background.

Common genetic variation in *TCF7L2* might directly impair pancreatic beta cell function, growth and differentiation through alteration of the WNT signalling pathway [40]. In contrast, the underlying molecular mechanism for the effects of *WFS1* gene variation on GLP-1-induced insulin secretion has not been established. In pancreatic beta cells, the endoplasmic reticulum is a key site for insulin biosynthesis and the folding of newly synthesised proinsulin [24]. Endoplasmic reticulum homeostasis depends on a complex mechanism, known as the unfolded protein response, which modulates the capacity and quality of the endoplasmic reticulum protein-folding machinery to prevent the accumulation of unfolded or misfolded proteins [41]. The protein coded for by *WFS1* has recently been identified as a component of the unfolded protein response with an important function in maintaining homeostasis of the endoplasmic reticulum in pancreatic beta cells [42]. Therefore, polymorphisms in the *WFS1* gene might impair beta cell function and response to GLP-1 through alteration of the endoplasmic reticulum homeostasis. 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 [25].

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 *WFS1* SNP rs10010131. The arginine bolus in the combined hyperglycaemic clamp produces a maximal challenge for the secretory capacity of the beta cell and can be possibly considered as a surrogate for beta cell mass [28, 35]. rs10010131 did not affect this maximal insulin secretion, indicating that this variant in *WFS1* might not influence beta cell mass, at least in the prediabetic state. In addition, impaired beta cell function might also include the efficiency of the conversion from proinsulin to insulin [7]. However, there was no evidence for this abnormality related to the *WFS1* variant during the hyperglycaemic clamp (data not shown).

The present study has certain limitations that need to be taken into account. First, we performed a relatively large number of statistical tests (three phenotypes), which might

increase the risk of a statistical type I error. However, even after Bonferroni correction for multiple comparisons (corrected α level; $p < 0.0167$) the association between SNP rs10013110 and the first phase of GLP-1-induced insulin secretion remained significant. Second, the negative findings for the IVGTT might be because of a type II error. The IVGTT study was sufficiently powered ($1 - \beta > 0.8$) to detect effect sizes as small as 0.33. Thus, small effects may have been missed.

In summary, our data show that a common genetic variant in the *WFS1* gene is associated with impaired GLP-1-induced insulin secretion, suggesting a state of relative incretin resistance.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

- Sladek R, Rocheleau G, Rung J et al (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881–885
- Saxena R, Voight BF, Lyssenko V et al (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331–1336
- Zeggini E, Scott LJ, Saxena R et al (2008) Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 40:638–645
- Scott LJ, Mohlke KL, Bonnycastle LL et al (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345
- Grarup N, Rose CS, Andersson EA et al (2007) Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10, 705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes* 56:3105–3111
- Staiger H, Machicao F, Stefan N et al (2007) Polymorphisms within novel risk loci for type 2 diabetes determine beta-cell function. *PLoS ONE* 2:e832
- Kirchhoff K, Machicao F, Haupt A et al (2008) Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia* 51:597–601
- Boesgaard TW, Zilinskaite J, Vantinen M et al (2008) The common SLC30A8 Arg325Trp variant is associated with reduced first-phase insulin release in 846 non-diabetic offspring of type 2 diabetes patients—the EUGENE2 study. *Diabetologia* 51:816–820
- Pascoe L, Tura A, Patel SK et al (2007) Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. *Diabetes* 56:3101–3104

10. Staiger H, Stancakova A, Zilinskaite J et al (2008) A candidate type 2 diabetes polymorphism near the HHEX locus affects acute glucose-stimulated insulin release in European populations: results from the EUGENE2 study. *Diabetes* 57:514–517
11. Palmer ND, Goodarzi MO, Langefeld CD et al (2008) Quantitative trait analysis of type 2 diabetes susceptibility loci identified from whole genome association studies in the Insulin Resistance Atherosclerosis Family Study. *Diabetes* 57:1093–1100
12. Stancakova A, Pihlajamaki J, Kuusisto J et al (2008) Single-nucleotide polymorphism rs7754840 of CDKAL1 is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. *J Clin Endocrinol Metab* 93:1924–1930
13. van Hoek M, Dehghan A, Witteman JC et al (2008) Predicting type 2 diabetes based on polymorphisms from genome-wide association studies: a population-based study. *Diabetes* 57:3122–3128
14. Minton JA, Hattersley AT, Owen K et al (2002) Association studies of genetic variation in the WFS1 gene and type 2 diabetes in U.K. populations. *Diabetes* 51:1287–1290
15. Sandhu MS, Weedon MN, Fawcett KA et al (2007) Common variants in WFS1 confer risk of type 2 diabetes. *Nat Genet* 39:951–953
16. Franks PW, Rolandsson O, Debenham SL et al (2008) Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. *Diabetologia* 51:458–463
17. Florez JC, Jablonski KA, McAteer J et al (2008) Testing of diabetes-associated WFS1 polymorphisms in the Diabetes Prevention Program. *Diabetologia* 51:451–457
18. Sparsø T, Andersen G, Albrechtsen A et al (2008) Impact of polymorphisms in WFS1 on prediabetic phenotypes in a population-based sample of middle-aged people with normal and abnormal glucose regulation. *Diabetologia* 51:1646–1652
19. Takeda K, Inoue H, Tanizawa Y et al (2001) WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. *Hum Mol Genet* 10:477–484
20. Barrett TG, Bunday SE (1997) Wolfram (DIDMOAD) syndrome. *J Med Genet* 34:838–841
21. Ishihara H, Takeda S, Tamura A et al (2004) Disruption of the WFS1 gene in mice causes progressive beta-cell loss and impaired stimulus-secretion coupling in insulin secretion. *Hum Mol Genet* 13:1159–1170
22. Riggs AC, Bernal-Mizrachi E, Ohsugi M et al (2005) Mice conditionally lacking the Wolfram gene in pancreatic islet beta cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. *Diabetologia* 48:2313–2321
23. Yamada T, Ishihara H, Tamura A et al (2006) WFS1-deficiency increases endoplasmic reticulum stress, impairs cell cycle progression and triggers the apoptotic pathway specifically in pancreatic beta-cells. *Hum Mol Genet* 15:1600–1609
24. Scheuner D, Kaufman RJ (2008) The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. *Endocr Rev* 29:317–333
25. Holst JJ, Vilsboll T, Deacon CF (2009) The incretin system and its role in type 2 diabetes mellitus. *Mol Cell Endocrinol* 297:127–136
26. Yusta B, Baggio LL, Estall JL et al (2006) GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. *Cell Metab* 4:391–406
27. Shu L, Sauter NS, Schulthess FT, Matveyenko AV, Oberholzer J, Maedler K (2008) Transcription factor 7-like 2 regulates beta-cell survival and function in human pancreatic islets. *Diabetes* 57:645–653
28. Fritsche A, Stefan N, Hardt E, Schutzenauer S, Häring H, Stumvoll M (2000) A novel hyperglycaemic clamp for characterization of islet function in humans: assessment of three different secretagogues, maximal insulin response and reproducibility. *Eur J Clin Invest* 30:411–418
29. Florez JC, Jablonski KA, Bayley N et al (2006) TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355:241–250
30. Schäfer SA, Tschritter O, Machicao F et al (2007) Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. *Diabetologia* 50:2443–2450
31. Stefan N, Machicao F, Staiger H et al (2005) Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. *Diabetologia* 48:2282–2291
32. Tripathy D, Wessman Y, Gullstrom M, Tuomi T, Groop L (2003) Importance of obtaining independent measures of insulin secretion and insulin sensitivity during the same test: results with the Botnia clamp. *Diabetes Care* 26:1395–1401
33. 't Hart LM, Fritsche A, Rietveld I et al (2004) Genetic factors and insulin secretion: gene variants in the IGF genes. *Diabetes* 53 (Suppl 1):S26–S30
34. Grant SF, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38:320–323
35. Fritsche A, Stefan N, Hardt E, Häring H, Stumvoll M (2000) Characterisation of beta-cell dysfunction of impaired glucose tolerance: evidence for impairment of incretin-induced insulin secretion. *Diabetologia* 43:852–858
36. Van Cauter E, Mestrez F, Sturis J, Polonsky KS (1992) Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377
37. Eaton RP, Allen RC, Schade DS, Erickson KM, Standefer J (1980) Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. *J Clin Endocrinol Metab* 51:520–528
38. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462–1470
39. Stumvoll M (2004) Control of glycaemia: from molecules to men. Minkowski Lecture 2003. *Diabetologia* 47:770–781
40. Jin T (2008) The WNT signalling pathway and diabetes mellitus. *Diabetologia* 51:1771–1780
41. Marciniak SJ, Ron D (2006) Endoplasmic reticulum stress signaling in disease. *Physiol Rev* 86:1133–1149
42. Fonseca SG, Fukuma M, Lipson KL et al (2005) WFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pancreatic beta-cells. *J Biol Chem* 280:39609–39615