Decreased insulin secretion and increased risk of type 2 diabetes associated with allelic variations of the *WFS1* gene: the Data from Epidemiological Study on the Insulin Resistance Syndrome (DESIR) prospective study

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Abstract

Aims/hypothesis We investigated associations of allelic variations in the *WFS1* gene with insulin secretion and risk of type 2 diabetes in a general population prospective study. **Methods** We studied 5,110 unrelated French men and women who participated in the prospective Data from Epidemiological Study on the Insulin Resistance Syndrome

(DESIR) study. Additional cross-sectional analyses were performed on 4,472 French individuals with type 2 diabetes and 3,065 controls. Three single nucleotide polymorphisms (SNPs) were genotyped: rs10010131, rs1801213/rs7672995 and rs734312.

Results We observed statistically significant associations between the major alleles of the three variants and prevalent

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type 2 diabetes in the DESIR cohort at baseline. Cox analyses showed an association between the G-allele of rs10010131 and incident type 2 diabetes (HR 1.34, 95% CI 1.08–1.70, $p=0.007$). Similar results were observed for the G-allele of rs1801213 and the A-allele of rs734312. The GGA haplotype was associated with an increased risk of diabetes as compared with the ACG haplotype (HR 1.26, 95% CI 1.04–1.42, $p=0.02$). We also observed statistically significant associations of the three SNPs with plasma glucose, HbA_{1c} levels and insulin secretion at baseline and throughout the study in individuals with type 2 diabetes or at risk of developing diabetes. However, no association was observed in those who remained normoglycaemic at the end of the follow-up. Associations between the three variants and type 2 diabetes were replicated in cross-sectional studies of type 2 diabetic patients in comparison with a non-diabetic control group.

Conclusions/interpretation The most frequent haplotype at the haplotype block containing the *WFS1* gene modulated insulin secretion and was associated with an increased risk of type 2 diabetes.

Keywords Endoplasmic reticulum stress · Genetic epidemiology · Population studies · Wolframin

Abbreviations

ATF6 α	Activating transcription factor 6 α
DESIR	Data from Epidemiological Study on the Insulin Resistance Syndrome
DIABHYCAR	The Diabetes, Hypertension, Cardiovascular Events, and Ramipril study
ER	Endoplasmic reticulum
FPG	Fasting plasma glucose
GLP-1	Glucagon-like peptide 1
IFG	Impaired fasting glucose
HOMA%B	HOMA index of beta cell function
HOMA%S	HOMA index of insulin sensitivity
NCH	Necker and Cochin Hospitals
NFG	Normal fasting glucose
SNP	Single nucleotide polymorphism
WFS1	Wolframin

Introduction

Wolframin (WFS1) is a transmembrane protein of the endoplasmic reticulum (ER), found ubiquitously but with higher levels in pancreatic beta cells and specific neurons of the central nervous system [1]. WFS1 is a component of the unfolded protein response following ER stress [2]. It controls a negative regulatory feedback loop of the ER stress signalling network, acting as an ER stress signalling

suppressor [3]. ER stress signalling is tightly regulated in pancreatic beta cells to produce appropriate amounts of insulin in response to the frequent fluctuations of blood glucose levels [4, 5]. WFS1 is upregulated during insulin secretion and plays an important role in maintaining ER homeostasis and cell integrity in pancreatic beta cells [2]. Inactivation of the wolframin gene (*Wfs1*) in beta cells of rodents causes ER stress and beta cell death by accelerated apoptosis [2, 3, 6].

Mutations in *WFS1* are responsible for Wolfram syndrome [7, 8], a rare recessive disorder characterised by diabetes mellitus, optic atrophy and other neurological and endocrine abnormalities [9]. An increased prevalence of diabetes mellitus was reported in first-degree relatives of patients with Wolfram syndrome [10], suggesting a possible effect of *WFS1* mutation heterozygosis. More recently, common variants of *WFS1* were shown to be associated with type 2 diabetes in populations of European descent [11–13]. Associations of these variants with insulin secretion in response to oral or intravenous glucose was observed in some [14, 15], but not all studies [16–18]. In the present study, we investigated associations of allelic variations in the *WFS1* gene with insulin secretion, insulin sensitivity and the risk of hyperglycaemia (impaired fasting glucose [IFG]) and of type 2 diabetes in a 9 year population-based prospective cohort. We tested the hypothesis that discrepancies in the associations between *WFS1* variants and insulin secretion in previous reports could be related to differences in glycaemic and/or diabetes risk status of studied populations. For the purpose of replication, associations between *WFS1* variants and diabetes were also investigated in three independent type 2 diabetes cross-sectional cohorts.

Methods

Participants in prospective study: the DESIR cohort The Data from Epidemiological Study on the Insulin Resistance syndrome (DESIR) study was a 9 year prospective study conducted in 5,212 men and women from the French general population [19, 20]. Participants were recruited in ten healthcare centres in the western central part of France on the occasion of periodic health check-ups offered by Social Security. The study protocol included clinical and biological evaluations at inclusion and at visits after 3, 6 and 9 years of follow-up. Glycaemic status was classified in three groups, defined on the basis of fasting plasma glucose (FPG) and hypoglycaemic medication. Normal fasting glucose (NFG) was defined as FPG <6.1 mmol/l and IFG as FPG between 6.1 and 6.99 mmol/l, in the absence of treatment with a hypoglycaemic agent for both classes. Type 2 diabetes was defined as FPG ≥ 7.0 mmol/l or as

FPG <7.0 mmol/l in the presence of treatment with a diabetes drug and a previous diagnosis of diabetes. The research protocol was approved by the ethics committee of Bicêtre Hospital and all participants signed an informed consent form. At baseline, 4,634 individuals (88.9%) had NFG, 342 (6.6%) IFG and 134 (2.6%) diabetes. The glycaemic status of 102 participants (1.9%) could not be ascertained at baseline and they were excluded from the analyses. NFG individuals lost during follow-up and whose final glycaemic status could not be determined ($n=1,003$) were excluded from the analyses of incidence. For the purpose of this investigation, we have considered the incidence of type 2 diabetes and the incidence of hyperglycaemic events (NFG to IFG, NFG to diabetes or IFG to diabetes change) during follow-up. The incidence of type 2 diabetes was 5.1% (203 cases out of 3,973 NFG or IFG cases at baseline), while the incidence of hyperglycaemic events was 16.7% (663 cases out of 3,973 NFG or IFG cases at baseline).

Participants in cross-sectional studies: DIABHYCAR, DIA-BHYCAR Gene and NCH cohorts The Diabetes, Hypertension, Cardiovascular Events, and Ramipril (DIABHYCAR) study was a 6 year double-blind multicentre multinational clinical trial conducted in 4,912 men and women who had type 2 diabetes, were aged 50 years or older at baseline and were selected on the basis of persistent micro- or macroalbuminuria without renal failure. The trial tested in a parallel design and vs placebo whether a low dose of ramipril that was able to reduce urinary albumin concentration would also reduce cardiovascular and/or renal events such as myocardial infarction, stroke, acute heart failure, end-stage renal failure and cardiovascular death. The study design, methods and results of the trial (which were negative regarding the drug effect) have been published previously [21, 22]. DNA banking was undertaken for the 3,137 participants recruited in France. The DIABHYCAR Gene cohort was recruited concomitantly to DIABHYCAR. It included 607 men and women with type 2 diabetes, aged 50 years or older at baseline and not presenting with micro- or macroalbuminuria. The study protocols of the two cohorts were approved by the Angers University Hospital Ethics Committee. The Necker and Cochin Hospitals (NCH) cohort included type 2 diabetic men and women consecutively recruited at the diabetes departments of NCH in Paris [23]. The present investigation included 728 individuals of European descent. Clinical and biological data were obtained from medical records. The study protocol was approved by the Ethics Committee of Hôpital Necker. For the purpose of these cross-sectional studies, the three cohorts of type 2 diabetic patients were compared with a control group of 3,065 participants from the DESIR cohort who had remained normoglycaemic at the 9-year

follow-up (DESIR controls). This group from the French general population has been extensively used as a control group in genetic studies [24, 25].

DNA studies The *WFS1* gene spans ~33.4 kbp on chromosome 4p16 and is contained in a single haplotype block. Three single nucleotide polymorphisms (SNPs) were genotyped using assay by design kits from Applied Biosystems (Life Technologies, Carlsbad, CA, USA): rs10010131 (G>A, intron 4), rs1801213/rs7672995 (G>C, R228R, exon 6) and rs734312 (A>G, H611R, exon 8). The SNPs were chosen in HapMap on the basis of capturing information on ~80% of the allelic variation of SNPs with minor allele frequency $\geq 5\%$ at $r^2 > 0.8$ in the haplotype block containing *WFS1*.

Computations and statistical analyses HOMA index of beta cell function (HOMA%B) and of insulin sensitivity (HOMA%S) [26], based on fasting levels of glucose and insulin, were computed using the Microsoft Excel spreadsheet implementation of the HOMA Calculator (v2.2) available at www.dtu.ox.ac.uk/Homacalculator/download.php (accessed 1 March 2006). Before genotype-related statistical analyses were performed, genotypes were verified to be in Hardy–Weinberg equilibrium in all groups of participants. Genotype associations with diabetes or hyperglycaemia were assessed by regression models. Cox proportional hazards survival regression analyses were used to examine the effect of explanatory variables on time-related survival (disease-free) rates in prospective analyses. Kaplan–Meier curves were used to plot survival (disease-free) rates over time according to genotype. Logistic regression analyses were used for cross-sectional analyses. Hazard ratios or odds ratios, respectively, with 95% CIs, were computed in these analyses for the risk allele. Adjustments for clinical and biological variables were carried out by including these variables as covariates in the regression model. The study had power >0.99 to detect associations of the SNPs with the incidence of diabetes in the DESIR study (for HR ≥ 1.2 and $\alpha=0.05$) and with the prevalence of diabetes in the cross-sectional study (for OR ≥ 1.3 and $\alpha=0.05$). It had power >0.80 (for $\alpha=0.05$) to detect differences in quantitative traits >10% between groups.

Comparisons of anthropometric, clinical and biological phenotypes between groups were assessed by ANOVA, analyses of covariance, contingency table χ^2 test and Fisher's exact test. For the comparisons of FPG, HbA_{1c}, insulin, HOMA%B and HOMA%S between genotypes, interaction between the glycaemic status at follow-up (NFG or IFG/diabetes) and genotype was assessed by including in the regression model a 'crossed' compound covariable (glycaemic status/genotype). Stratification by glycaemic status of genotype-related effects on phenotypic traits was

then performed by nesting the genotype variable within the glycaemic status variable in the regression analysis. This results in the computation of separate statistical effects for NFG and IFG/diabetes subgroups of individuals, adjusted for multiple comparisons due to the stratification by glycaemic status. For all analyses, data were log-transformed for the analyses when the normality of the distribution was rejected by the Shapiro–Wilk W test. Results are expressed as means $\pm$ SEM. $p<0.05$ was considered significant. Giving the strong linkage disequilibrium between the SNPs (see below), no p value correction for multiple SNP testing was performed, as it was considered that they were not independent analyses. Statistics were performed with JMP software (SAS Institute, Cary, NC, USA). Power calculations were performed with Quanto v1.2.4 (<http://hydra.usc.edu/gxe>; accessed 10 June 2009) and Logistic_Cox_Power (<http://hedwig.mgh.harvard.edu/biostatistics/node/19>; accessed 10 June 2009). Determination of linkage disequilibrium between SNPs and between haplotype frequencies, and computation of hazard and odd ratios associated with haplotypes were performed with THESIAS software v3.1 (<http://ecgene.net/genecanvas/uploads/THESIAS3.1/Documentation3.1.htm>; accessed 15 January 2010) [27].

Results

Prospective study: DESIR cohort Prevalence studies were conducted in 5,110 participants from the DESIR cohort (Table 1). The combined prevalence at baseline of IFG/diabetes according to genotype was: 10.3% (GG), 9.3% (GA) and 7.5% (AA) for rs10010131; 9.8% (GG), 9.3% (GC) and 7.5% (CC) for rs1801213; and 10.4% (AA), 9.4% (AG) and 7.8% (GG) for rs734312. Logistic regression

analyses showed associations of the major allele of the three variants with diabetes and with the combined prevalence of IFG/diabetes at baseline (Table 2). When we considered the prevalence of IFG only, associations were also observed for rs10010131 and rs734312, while only a trend towards an association was observed for rs1801213 (Table 2).

The main baseline characteristics of incident cases of type 2 diabetes or hyperglycaemic events are shown in Electronic supplementary material (ESM) Table 1. The incidence of type 2 diabetes according to genotype was: 5.5% (GG), 5.4% (GA) and 3.6% (AA) for rs10010131; 5.2% (GG), 5.4% (GC) and 3.3% (CC) for rs1801213; and 5.5% (AA), 5.6% (AG) and 4.0% (GG) for rs734312. This suggests a dominant effect of the major allele for all SNPs (Fig. 1). Cox proportional hazards survival regression analyses showed a statistically significant association between the G-allele of rs10010131 and the incidence of type 2 diabetes (HR 1.34, 95% CI 1.08–1.70, $p=0.007$ for a dominant GX vs AA model; Table 3). Similar results were observed for the G-allele of rs1801213 (HR 1.44, 95% CI 1.10–1.99, $p=0.007$) and for the A-allele of rs734312 (HR 1.22, 95% CI 1.02–1.48, $p=0.03$). Haplotype frequencies were determined for incident cases of diabetes and for participants who did not develop diabetes during the follow-up (NFG/IFG censored individuals) and Cox survival (disease-free) analyses were performed for the haplotypes. There was a strong linkage disequilibrium between the three SNPs ($0.74\leq D'\leq 0.93$), with haplotypes composed of the major (GGA) or minor (ACG) alleles of the variants representing $\sim 77\%$ of all haplotypes. GGA frequency was 51.0% in incident cases of type 2 diabetes and 47.6% in NFG/IFG censored individuals, while ACG frequencies were 26.6% and 29.2%, respectively. The GGA haplotype was significantly associated with an increased risk of diabetes compared with the ACG haplotype (HR

Table 1 Clinical and biological characteristics at baseline according to glycaemic status, the DESIR cohort

Characteristic	Glycaemic status			
	NFG	IFG	Type 2 diabetes	p value
n	4,634	342	134	
Age (years)	46.8 $\pm$ 0.1	51.9 $\pm$ 0.5	55.0 $\pm$ 0.7	<0.0001
Sex				<0.0001
Men (%)	47	73	71	
Women (%)	53	27	29	
BMI (kg/m ²)	24.5 $\pm$ 0.1	26.9 $\pm$ 0.2	28.9 $\pm$ 0.3	<0.0001
FPG (mmol/l)	5.20 $\pm$ 0.01	6.40 $\pm$ 0.03	8.40 $\pm$ 0.06	<0.0001
HbA _{1c} (%)	5.40 $\pm$ 0.01	5.83 $\pm$ 0.03	6.87 $\pm$ 0.04	<0.0001
Fasting insulin (pmol/l)	45 $\pm$ 1	65 $\pm$ 2	87 $\pm$ 2	<0.0001
HOMA%B (%)	77 $\pm$ 1	65 $\pm$ 1	54 $\pm$ 2	<0.0001
HOMA%S (%)	146 $\pm$ 1	100 $\pm$ 3	85 $\pm$ 5	<0.0001

Except where otherwise indicated, data are expressed as mean $\pm$ SEM

Comparisons are by ANOVA

Table 2 Genotype frequency according to glycaemic status at baseline, the DESIR cohort

Genotype by SNP	Glycaemic status			OR (95% CI) for comparison of genotype frequency by glycaemic status					
	NFG ^a	IFG ^b	DM ^c	IFG vs NFG	<i>p</i> value	DM vs NFG	<i>p</i> value	IFG/DM vs NFG	<i>p</i> value
rs10010131									
GG	0.369	0.385	0.466	1.39 (1.00–1.94)	0.05	1.98 (1.15–3.45)	0.01	1.50 (1.11–2.02)	0.008
GA	0.482	0.506	0.397						
AA	0.149	0.109	0.137						
rs1801213									
GG	0.472	0.485	0.530	1.38 (0.97–1.98)	0.07	1.47 (1.02–2.11)	0.04	1.43 (1.05–1.97)	0.02
GC	0.433	0.452	0.366						
CC	0.095	0.063	0.104						
rs734312									
AA	0.292	0.317	0.354	1.47 (1.06–2.04)	0.02	1.62 (1.0–2.72)	0.05	1.51 (1.13–2.01)	0.005
AG	0.494	0.515	0.446						
GG	0.214	0.168	0.200						

Values are ORs for the major alleles in a co-dominant model, adjusted for sex, and for age and BMI at baseline

^a*n*=4,634, ^b*n*=342, ^c*n*=134

DM, type 2 diabetes mellitus

1.26, 95% CI 1.04–1.42, *p*=0.02, comparison adjusted for sex, age and BMI).

The incidence of hyperglycaemic events according to genotype was: 17.1% (GG), 16.5% (GA) and 16.7% (AA) for rs10010131; 17.4% (GG), 16.5% (GC) and 15.3% (CC) for rs1801213; and 16.2% (AA), 17.7% (AG) and 15.9% (GG) for rs734312. Cox proportional hazards survival regression analyses showed a trend towards an association between the G-allele of rs1801213 and the incidence of hyperglycaemic events (HR 1.11, 95% CI 0.99–1.25, *p*=0.08 for a co-dominant additive model) (ESM Table 2), while no association was observed for the other variants.

We assessed the impact of the SNPs on intermediate phenotypes such as FPG, HbA_{1c}, fasting insulin, and HOMA%B (insulin secretion) and HOMA%S (insulin sensitivity) indices. Analyses were performed with baseline values and with all available values collected throughout

the study (baseline plus values at 3, 6 and 9 year visits). Results at baseline for rs10010131 genotypes are shown in Table 4. These phenotypic analyses revealed a strong interaction between genotype and glycaemic status at the end of the study (NFG or IFG/diabetes). Genotype-related effects on intermediate traits were observed in the subset of the population presenting with IFG or diabetes at the end of the study, but not in individuals with NFG. In the former subset of the population, significantly higher FPG and HbA_{1c} levels and lower HOMA%B levels were observed in carriers of the GG risk genotype than in AA carriers. HOMA%S was not significantly different between groups. The exclusion of participants receiving sulfonylurea (*n*=15), metformin (*n*=15) or both (*n*=12) had no significant effect on the computations. Comparable results were observed when we considered the values of these variables collected throughout the study (ESM Table 3). Similar results were

Fig. 1 Kaplan–Meier survival (disease-free) curves for the DESIR cohort during follow-up according to genotype. **a** rs10010131, AA (dashed line), GA (dotted line), GG (solid line); **b**) rs1801213, CC

(dashed line), GC (dotted line), GG (solid line); and **c**) rs734312, GG (dashed line), AG (dotted line), AA (solid line)

Table 3 Genotype frequency according to incidence of diabetes during follow-up, the DESIR cohort

	Genotype by SNP	Incidence of diabetes		HR (95% CI) for incidence	<i>p</i> value
		No ^a	Yes ^b		
Hazard ratios (HR) for the major alleles in a dominant model (<i>MX</i> vs <i>mm</i>) determined by Cox proportional hazards survival regression analyses adjusted for sex, and for age and BMI at baseline. Time to event was defined as the number of years of follow-up until the diagnosis of type 2 diabetes or the duration of follow-up for right-censored participants without diabetes at the end of follow-up	rs10010131				
	GG	0.372	0.395	1.34 (1.08–1.70)	0.007
	GA	0.476	0.500		
	AA	0.152	0.105		
	rs1801213			1.44 (1.10–1.99)	0.007
	GG	0.474	0.482		
	GC	0.430	0.457		
	CC	0.096	0.061		
	rs734312			1.22 (1.02–1.48)	0.03
	AA	0.295	0.309		
	AG	0.489	0.527		
	GG	0.216	0.164		

^a *n*=3770, ^b *n*=203

also observed for the other two SNPs, both at baseline and throughout the study (data not shown).

Cross-sectional study: DIABHYCAR, DIABHYCAR Gene and NCH cohorts Clinical characteristics of participants from the type 2 diabetes cohorts and from the control cohort are shown in ESM Table 4. A logistic regression analysis was performed for each SNP with the cohort membership as the dependent variable, genotype as independent covariable, and sex, age and BMI as adjustment covariables. Odds ratio associated with genotype was computed for each type 2 diabetes cohort against the control cohort (ESM Table 5). Statistically significant associations of the major allele of the three variants with diabetes was confirmed in all type 2 diabetes cohorts individually, as well as with pooled data of the three cohorts. Linkage disequilibrium between the variants was computed and haplotype frequencies were determined for patients and for controls. A logistic regression analysis was

performed with glycaemic status as the dependent variable, the haplotypes as independent covariable, and sex, age and BMI as adjustment covariables (ESM Table 6). The GGA haplotype (major allele of the three variants) was significantly associated with diabetes compared with the ACG haplotype (minor alleles of the variants).

Discussion

We observed statistically significant associations of the major alleles of rs10010131, rs1801213 and rs734312 in the *WFS1* locus with an increased risk of type 2 diabetes in individuals with NFG or IFG in a 9 year prospective study in a French cohort issued from the general population. Only a trend towards an association with one of the variants (rs1801213) was observed when we considered the incidence of all hyperglycaemic events (NFG to IFG, NFG to

Table 4 Intermediate phenotypes at baseline according to rs10010131 genotype and glycaemic status at follow-up, the DESIR cohort

Glycaemic status	NFG			<i>p</i> value	IFG/type 2 diabetes			<i>p</i> value	Interaction <i>p</i> value ^a
	GG	GA	AA		GG	GA	AA		
Genotype									
Participants (<i>n</i>)	1,111	1,421	459		400	482	141		
FPG (mmol/l)	5.18±0.02	5.17±0.02	5.20±0.04	0.80	6.17±0.04	6.09±0.04	5.87±0.06	0.0005	0.002
HbA _{1c} (%)	5.40±0.01	5.39±0.01	5.41±0.02	0.64	5.82±0.03	5.71±0.02	5.61±0.04	<0.0001	<0.0001
Fasting insulin (pmol/l)	45±1	44±1	45±1	0.47	53±1	57±1	59±2	0.31	0.60
HOMA%B (%)	77±1	77±1	77±1	0.92	65±1	67±1	71±2	0.0002	0.0009
HOMA%S (%)	146±2	147±1	144±3	0.86	125±3	124±3	123±5	0.23	0.34

Unless otherwise stated, data are expressed as mean±SEM

Comparisons are analysis of covariance, adjusted for sex, age and BMI, and computed with log-transformed data

^a Indicates the statistical significance observed in the regression model for a crossed compound covariable 'glycaemic status–genotype'

diabetes or IFG to diabetes change) during follow-up. The variants were also associated with the prevalence of IFG and/or diabetes at baseline. Together, these results suggest that allelic variations of *WFS1* increase the risk of diabetes, but have a lesser impact on the risk of IFG in the DESIR cohort. Associations with diabetes were confirmed in cross-sectional studies of three cohorts of type 2 diabetic patients compared with normoglycaemic controls. The most frequent haplotype at the haplotype block containing the *WFS1* gene was associated with an increased risk of diabetes in the prospective study and with the prevalence of diabetes in cross-sectional studies.

Our results are in agreement with data from the literature. Sandhu and co-workers identified associations of the major alleles of rs10010131, rs6446482, rs752854 and rs734312 SNPs with *WFS1* and diabetes risk in 9,533 cases and 11,389 controls from UK and Ashkenazi populations [11]. Lyssenko and co-workers observed an association between the major allele of rs10010131 and an increased risk of diabetes in two prospective cohorts with a median follow-up of 23.5 years and including 16,061 Swedish and 2,770 Finnish participants [13]. An association between rs752854 and type 2 diabetes was also observed in a Swedish cross-sectional study of 1,296 cases and 1,412 controls, with the minor allele conferring protection against diabetes [12]. Other studies have yielded less conclusive results. Florez and co-workers examined the effects of the rs10010131, rs752854 and rs734312 variants on diabetes incidence and response to interventions in the Diabetes Prevention Program, in which a lifestyle intervention or metformin treatment was compared with placebo [28]. They found no statistically significant interactions between genotypes and Diabetes Prevention Program intervention for any of the SNPs. None showed a statistically significant effect on diabetes incidence in the entire cohort, although in the lifestyle arm homozygosity for the minor alleles was consistent with protection from diabetes. Only a trend towards association with diabetes was observed for the rs10010131 variant in a Chinese cross-sectional study of 1,849 type 2 diabetic patients and 1,785 controls [16]. Although it is clear that *WFS1* mutations cause diabetes [7, 8], the genetic basis of the association of these common *WFS1* variants with type 2 diabetes is still unclear. A recent fine-mapping study in UK, Swedish and Ashkenazi populations identified six novel, highly correlated SNPs showing strong and comparable associations with type 2 diabetes risk [29]. None had obvious functional properties that could predict deleterious effects on protein function. Given the strong linkage disequilibrium in the *WFS1* region, the authors suggested that further refinement of these associations for identification of the variants responsible for increased diabetes risk would require studies in ethnically diverse populations and/

or studies with much larger sample sizes (10,000 to 100,000 individuals) [29].

We also observed statistically significant associations of the risk alleles of the three SNPs with higher FPG and HbA_{1c} levels, and lower HOMA%B levels, both at baseline and throughout the study in participants who developed hyperglycaemia or type 2 diabetes. Interestingly, no allelic association with these intermediate phenotypes was observed in individuals who remained normoglycaemic at the end of the follow-up. The design of our study had some intrinsic limitations. Glucose tolerance status of participants was based on FPG only and not on an OGTT. Second, insulin secretion and insulin sensitivity were assessed by HOMA indices, which provide only an estimation of these variables. Nonetheless, we do not believe that these limitations have substantially biased our results, as they are in agreement with other observations. Sparso and co-workers observed a similar interaction between the glycaemic status and the genotype effect of rs734312 on insulin secretion in the Inter99 cohort, which involved 4,568 glucose-tolerant individuals and 1,471 individuals with treatment-naïve abnormal glucose regulation (IFG, impaired glucose tolerance or screen-detected diabetes) [14]. The risk allele was associated with a decrease in insulinogenic index and decreased 30 min serum insulin levels after an oral glucose load in individuals with abnormal glucose regulation, but not in participants with normal glucose tolerance. It seems that this interaction between glycaemic status and genotype might explain the heterogeneous results reported by previous studies of associations between *WFS1* variants and insulin secretion. Schafer and co-workers studied 1,578 non-diabetic German individuals at increased risk of type 2 diabetes, i.e. persons with a history of gestational diabetes, IFG, impaired glucose tolerance or family history of type 2 diabetes in first-degree relatives [15]. They found that the rs10010131 risk allele was associated with reduced insulin secretion during an OGTT. Interestingly, insulin secretion in response to an IVGTT or a hyperglycaemic clamp was not different between genotypes. However, when the hyperglycaemic clamp was combined with a glucagon-like peptide 1 (GLP-1) infusion, first- and second-phase insulin secretion were significantly decreased in carriers of the risk allele. A study from the Netherlands with similar size and power, but performed in participants with normal glucose tolerance, observed no significant genotype effect on insulin secretion in response to GLP-1 and glucose infusion during a hyperglycaemic clamp [17]. Other studies in large cohorts of non-diabetic Finnish [18] or Chinese [16] participants observed no association between rs10010131 and insulin secretion during an OGTT.

WFS1 protects cells from the damaging effects of hyperactivation of ER stress signalling [3]. ER stress is

caused by physiological and pathological stimuli that lead to accumulation of a large load of unfolded and misfolded proteins in the ER. For instance, physiological ER stress occurs in pancreatic beta cells during postprandial stimulation of insulin synthesis [30]. Activation of ER stress signalling is tightly regulated because hyperactivation or chronic activation of this signalling pathway can cause cell death by inducing apoptosis [31]. Recent results have shown that *WFS1* has an important function in the tight regulation of ER stress through its interaction with activating transcription factor 6 α (ATF6 α), a key transcription factor implicated in ER stress signalling [3]. *WFS1* recruits ATF6 α to the proteasome, where in the absence of ER stress it undergoes degradation. In situations of ER stress, ATF6 α is released from *WFS1* in the ER membrane and translocates to the nucleus, where it upregulates stress signalling targets. As *WFS1* is induced by ER stress, it causes eventual degradation of ATF6 α when ER homeostasis is established.

A possible reason why *WFS1* variants only associate with insulin secretion in individuals with abnormal glucose regulation is that these polymorphisms have only a minor impact on *WFS1* function in physiological situations. Thus, their effect on insulin secretion can be best appreciated in situations of chronic activation of ER stress signalling, such as in participants with decreased insulin sensitivity and chronic stimulation of insulin synthesis. Moreover, participants with abnormal glucose regulation may have other stressors of beta cell function, including unfavourable alleles in other pancreatic beta cell genes, as well as glucotoxicity, lipotoxicity or defects in the potentiation of insulin secretion by incretins (GLP-1, glucose-dependent insulinotropic polypeptide) [32], which could interact with susceptibility variants of *WFS1* and aggravate ER stress-mediated apoptosis. *WFS1* risk alleles have been shown to be associated with reduced insulin response to oral, but not to intravenous glucose [14, 15], and with reduced response to GLP-1 stimulation during hyperglycaemic clamps [15]. This suggests an incretin-related defect. GLP-1 not only enhances insulin secretion in response to glucose, but also directly modulates the ER stress response, preventing beta cell apoptosis and promoting beta cell adaptation and survival [32, 33]. At the present time, the mechanisms of the putative interaction of GLP-1 signalling and *WFS1* on ER function are still unclear.

In conclusion, allelic variations in the *WFS1* gene modulated insulin secretion in normoglycaemic French individuals at risk of developing type 2 diabetes and also increased the risk of type 2 diabetes in a 9 year prospective study. In our study, the most frequent haplotype at the haplotype block containing the *WFS1* gene was associated with an increased risk of diabetes. The genetic basis of these associations is still unclear. Further studies with much

larger sample sizes are required to identify the functional variants responsible for increased diabetes risk.

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

References

1. 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
2. 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 β -cells. *J Biol Chem* 280:39609–39615
3. Fonseca SG, Ishigaki S, Osowski CM et al (2010) Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. *J Clin Invest* 120:744–755
4. Lipson KL, Fonseca SG, Ishigaki S et al (2006) Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. *Cell Metab* 4:245–254
5. Fonseca SG, Lipson KL, Urano F (2007) Endoplasmic reticulum stress signaling in pancreatic beta-cells. *Antioxid Redox Signal* 9:2335–2344
6. 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
7. Inoue H, Tanizawa Y, Wasson J et al (1998) A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). *Nat Genet* 20:143–148
8. Strom TM, Hörtznagel K, Hofmann S et al (1998) Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (wolframin) coding for a predicted transmembrane protein. *Hum Mol Genet* 7:2021–2028
9. Barrett TG, Bunday SE, Macleod AF (1995) Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. *Lancet* 346:1458–1463
10. Fraser FC, Gunn T (1977) Diabetes mellitus, diabetes insipidus, and optic atrophy. An autosomal recessive syndrome? *J Med Genet* 14:190–193
11. Sandhu MS, Weedon MN, Fawcett KA et al (2007) Common variants in *WFS1* confer risk of type 2 diabetes. *Nat Genet* 39:951–953
12. 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

13. Lyssenko V, Jonsson A, Almgren P et al (2008) Clinical risk factors, DNA variants, and the development of type 2 diabetes. *N Engl J Med* 359:2220–2232
14. Sparso 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
15. Schafer SA, Mussig K, Staiger H et al (2009) A common genetic variant in WFS1 determines impaired glucagon-like peptide-1-induced insulin secretion. *Diabetologia* 52:1075–1082
16. Hu C, Zhang R, Wang C et al (2009) PPARG, KCNJ11, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-HHEX, IGF2BP2 and SLC30A8 are associated with type 2 diabetes in a Chinese population. *PLoS ONE* 4:e7643
17. Simonis-Bik AM, Nijpels G, van Haften TW et al (2009) Gene variants in the novel type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A, and MTNR1B affect different aspects of pancreatic beta-cell function. *Diabetes* 59:293–301
18. Stancakova A, Kuulasmaa T, Paananen J et al (2009) Association of 18 confirmed susceptibility loci for type 2 diabetes with indices of insulin release, proinsulin conversion, and insulin sensitivity in 5,327 nondiabetic Finnish men. *Diabetes* 58:2129–2136
19. The D.E.S.I.R. Study Group (1996) An epidemiologic survey from a network of French Health Examination Centres. D.E.S.I.R. (Epidemiologic Data on the Insulin Resistance Syndrome). *Rev Epidemiol Sante Publ* 44:373–375
20. Balkau B, Lange C, Fezeu L et al (2008) Predicting diabetes: clinical, biological, and genetic approaches: data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diab Care* 31:2056–2061
21. Lieve M, Marre M, Chatellier G et al (2000) The non-insulin-dependent diabetes, hypertension, microalbuminuria or proteinuria, cardiovascular events, and ramipril (DIABHYCAR) study: design, organization, and patient recruitment. *DIABHYCAR Study Group. Control Clin Trials* 21:383–396
22. Marre M, Lieve M, Chatellier G, Mann JF, Passa P, Menard J (2004) Effects of low dose ramipril on cardiovascular and renal outcomes in patients with type 2 diabetes and raised excretion of urinary albumin: randomised, double blind, placebo controlled trial (the DIABHYCAR study). *BMJ* 328:495
23. Reis AF, Dubois-Laforgue D, Bellanné-Chantelot C, Timsit J, Velho G (2004) A polymorphism in the promoter of UCP2 gene modulates lipid levels in patients with type 2 diabetes. *Mol Genet Metab* 82:339–344
24. Dina C, Meyre D, Gallina S et al (2007) Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 39:724–726
25. 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
26. Wallace TM, Levy JC, Matthews DR (2004) Use and abuse of HOMA modeling. *Diab Care* 27:1487–1495
27. Tregouet DA, Escolano S, Tired L, Mallet A, Golmard JL (2004) A new algorithm for haplotype-based association analysis: the Stochastic-EM algorithm. *Ann Hum Genet* 68:165–177
28. Florez JC, Jablonski KA, McAteer J et al (2008) Testing of diabetes-associated WFS1 polymorphisms in the Diabetes Prevention Program. *Diabetologia* 51:451–457
29. Fawcett KA, Wheeler E, Morris AP et al (2010) Detailed investigation of the role of common and low-frequency WFS1 variants in type 2 diabetes risk. *Diabetes* 59:741–746
30. Eizirik DL, Cardozo AK, Cnop M (2008) The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr Rev* 29:42–61
31. Cnop M, Ladriere L, Hekerman P et al (2007) Selective inhibition of eukaryotic translation initiation factor 2 alpha dephosphorylation potentiates fatty acid-induced endoplasmic reticulum stress and causes pancreatic beta-cell dysfunction and apoptosis. *J Biol Chem* 282:3989–3997
32. Holst JJ, Vilsboll T, Deacon CF (2009) The incretin system and its role in type 2 diabetes mellitus. *Mol Cell Endocrinol* 297:127–136
33. 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