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Testing of diabetes-associated *WFS1* polymorphisms in the Diabetes Prevention Program

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Abstract

Aims/hypothesis—Wolfram syndrome (diabetes insipidus, diabetes mellitus, optic atrophy and deafness) is caused by mutations in the *WFS1* gene. Recently, single nucleotide polymorphisms (SNPs) in *WFS1* have been reproducibly associated with type 2 diabetes. We therefore examined the effects of these variants on diabetes incidence and response to interventions in the Diabetes Prevention Program (DPP), in which a lifestyle intervention or metformin treatment was compared with placebo.

Methods—We genotyped the *WFS1* SNPs rs10010131, rs752 854 and rs734312 (H611R) in 3,548 DPP participants and performed Cox regression analysis using genotype, intervention and their interactions as predictors of diabetes incidence. We also evaluated the effect of these SNPs on insulin resistance and beta cell function at 1 year.

Results—Although none of the three SNPs was associated with diabetes incidence in the overall cohort, white homozygotes for the previously reported protective alleles appeared less likely to develop diabetes in the lifestyle arm. Examination of the publicly available Diabetes Genetics Initiative genome-wide association dataset revealed that rs10012946, which is in strong linkage

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disequilibrium with the three *WFS1* SNPs (r^2=0.88–1.0), was associated with type 2 diabetes (allelic odds ratio 0.85, 95% CI 0.75–0.97, p=0.026). In the DPP, we noted a trend towards increased insulin secretion in carriers of the protective variants, although for most SNPs this was seen as compensatory for the diminished insulin sensitivity.

Conclusions/interpretation—The previously reported protective effect of select *WFS1* alleles may be magnified by a lifestyle intervention. These variants appear to confer an improvement in beta cell function.

Keywords

Beta cell function; Diabetes prevention; Genetic association study; Single nucleotide polymorphism; Type 2 diabetes; Wolfram syndrome

Introduction

The search for common type 2 diabetes genes has followed one of two general strategies: a comprehensive scan of the entire genome, which is indifferent to biological function, or a specific test of association for selected candidate genes. The former, originally performed through linkage approaches, has only recently achieved the desired balance in polymorphism density, statistical power and affordable cost to be practicable via tests of association. Thus, investigators have traditionally compiled lists of candidate genes from various lines of available evidence. In this regard, monogenic syndromes of glucose intolerance transmitted in a Mendelian fashion provide theoretically attractive candidate genes: the expectation is that polymorphisms in those genes that have a less radical effect on function than the known index mutations may cause a less dramatic form of diabetes [1].

One such entity is Wolfram syndrome (OMIM no. 222300), which gives rise to diabetes insipidus, diabetes mellitus, optic atrophy and deafness. Onset occurs at 6–8 years of age and the outcome is often fatal. The clinical manifestations result from progressive degeneration of sensory neurons and pancreatic beta cells. The culprit mutations, transmitted in an autosomal recessive fashion, have been localised to the *WFS1* gene by positional cloning. *WFS1*, located on chromosome 4p16, encodes wolframin, a 100 kDa transmembrane protein, which is expressed in neurons and pancreatic beta cells and regulates calcium fluxes in the endoplasmic reticulum [2].

WFS1 was included in a list of 84 candidate genes recently evaluated for association with type 2 diabetes in a set of four white case–control populations [3]. A total of 1,536 single nucleotide polymorphisms (SNPs) were genotyped in a two-stage approach, with two of 18 SNPs originally associated with type 2 diabetes achieving replication in the second stage. The two SNPs, rs10010131 and rs6446482, were in strong linkage disequilibrium (LD) with each other (r^2=0.98) and both were located in *WFS1*. Fine-mapping of the region identified a correlated third intronic SNP (rs752854) as well as a missense SNP (rs734312), which codes for an R→H change at position 611 of wolframin (different from previously described Wolfram syndrome mutations). All four SNPs were strongly associated with type 2 diabetes in an expanded set of seven populations, comprising 9,533 patients and 11,389 control persons. The association was statistically robust ($p = 1.4 \times 10^{-7}$ for the best SNP, rs10010131) but modest (allelic odds ratio [OR] 0.90, 95% CI 0.86–0.93), with the minor allele conferring protection against type 2 diabetes.

In order to better characterise the phenotypic effects of these variants, assess their impact on diabetes incidence, extend these observations to other populations and assess whether genotype at this locus impacts on the effectiveness of diabetes preventive interventions, we genotyped three of the *WFS1* SNPs in the Diabetes Prevention Program (DPP) [4].

Methods

The DPP

The DPP enrolled 3,234 US participants at high risk of developing diabetes (on the basis of overweight, increased fasting glucose and impaired glucose tolerance) and randomised them to placebo, metformin 850 mg twice daily or a lifestyle intervention aimed at ≥7% weight loss and ≥150 min of physical activity per week; a fourth arm of 585 participants initially randomised to troglitazone was terminated early because of concerns with hepatotoxicity [4]. The main endpoint was development of diabetes confirmed by OGTT. The trial was conducted at 27 centres, all of which obtained individual Institutional Review Board approval. The DPP showed that participants treated with metformin or with a lifestyle intervention were 31% or 58% less likely to develop diabetes after an average of 3 years of follow-up, respectively [4].

The 3,548 DPP participants presented here (2,994 who completed the trial in the placebo, metformin or lifestyle arms, plus 554 originally randomised to troglitazone) provided informed consent specific to genetic investigation. The distribution of self-reported ethnicities among participants in this genetic study was 56.4% white, 20.2% African American, 16.8% Hispanic, 4.3% Asian and 2.4% American Indian. The mean age was 51 years and mean BMI was 34.0 kg/m^2.

Quantitative glycaemic traits

The baseline and 1-year OGTTs were used to calculate measures of beta cell function and insulin sensitivity as previously described [5]. The insulinogenic index was calculated as: ([insulin at 30 min] − [insulin at 0 min])/([glucose at 30 min] − [glucose at 0 min]). The insulin sensitivity index (reciprocal of insulin resistance by the homeostasis model assessment of insulin resistance [HOMA-IR]) was calculated as described previously [5].

SNP selection and genotyping

We attempted to genotype the three SNPs shown to have statistically robust associations in the original report (rs10010131, rs6446482 and rs752854) [3], as well as the missense SNP rs734312 (R611H). Genotyping was initially performed by allele-specific primer extension of single-plex amplified products, with detection by matrix-assisted laser desorption ionisation-time of flight mass spectroscopy on a Sequenom platform (San Diego, CA, USA), as previously described [5]. After two separate genotyping attempts, rs10010131 and rs6446482 continued to fail Hardy–Weinberg equilibrium (HWE) in the white subpopulation when scored by the automatic Sequenom genotype-calling algorithm. Visual inspection of the traces revealed preferential heterozygote dropout for these two SNPs. Manual correction of genotypes achieved HWE for both SNPs, while computerised clustering did so for rs10010131 only. To confirm the genotypes assigned by the computerised clustering algorithm, we re-genotyped rs10010131 on a TaqMan platform (Applied Biosystems, Foster City, CA, USA): concordance between clustered Sequenom and TaqMan genotypes was 98.8%, with genotyping success rates of 98.9% on Sequenom and 99.7% on TaqMan (when genotypes were discordant between both platforms, a null genotype was assigned to that sample). Because of lingering concerns about genotype quality for SNP rs6446482 and its very strong LD with rs10010131 (r^2= 0.96 and 1.0 in HapMap Europeans and Africans, respectively), this SNP was not examined further.

Statistical analysis

The primary endpoint was time to onset of diabetes. We examined Cox regression models with genotype, intervention and genotype–intervention interactions as the independent variables predicting time to diabetes. We performed analyses based on three separate genotypic groups for each SNP as well as the additive genetic model. For the quantitative trait analyses, we used

general linear models to compare baseline and 1 year measures in the entire cohort according to genotype at each SNP. All analyses were repeated in white participants only. Because this study represented an attempt to replicate and further characterise a previously established finding, a p value of <0.05 was considered statistically significant.

For power calculations of diabetes incidence within each treatment arm, we assumed HWE within each ethnic group, a homogeneous genetic effect across ethnic groups and an additive genetic model; for the overall cohort, we further assumed no interaction of genotype with intervention [6]. These calculations show that the overall DPP cohort has 54% power to detect the previously reported effect size of ~0.9 for a SNP of 40% frequency; the placebo arm has only 31% power.

Results

There were no statistically significant interactions between genotype and DPP intervention for any of the three *WFS1* SNPs. None showed a statistically significant effect on diabetes incidence in the full cohort, although in the lifestyle arm hazard ratios (HRs) for participants carrying two copies of the minor allele were consistent with protection from diabetes, with 95% CI overlapping the point estimates previously reported in cross-sectional case–control samples (Table 1). This apparent protection achieved nominal statistical significance for white minor allele homozygotes at SNP rs752854 (HR 0.30, 95% CI 0.09–0.99, p=0.048). Analyses in white participants under the additive model showed comparable HR in the lifestyle arm (0.72–0.88) but did not reach nominal significance (p= 0.07–0.42).

Several SNPs showed reciprocal effects on insulin secretion and insulin sensitivity. For example, at baseline minor allele homozygotes at SNP rs734312 had a higher insulinogenic index (p=0.02), but this could be interpreted as an appropriate compensatory response to their nominally lower insulin sensitivity (p=0.04; Table 2). After 1 year of lifestyle intervention, a similar phenomenon was noted for the same SNP in the full cohort (Table 3) and for all three SNPs in white participants only (Electronic supplementary material [ESM] Tables 1, 2).

This seemingly compensatory effect was uncoupled for SNP rs734312 in the metformin arm: minor allele homozygotes showed a higher insulinogenic index at 1 year than their heterozygous or major allele homozygous counterparts, despite similar levels of insulin sensitivity (Table 3).

Finally, we examined publicly available genome-wide datasets for SNPs in this region. In the Diabetes Genetics Initiative [7] (http://www.broad.mit.edu/diabetes, last accessed in November 2007), SNP rs10012946, which is in strong LD with the three *WFS1* SNPs (r^2=0.88–1.0), was associated with type 2 diabetes (allelic OR 0.85, 95% CI 0.75–0.97, p=0.026). The diabetic samples for the UK Wellcome Trust Case Control Consortium [8] (http://www.wtccc.org.uk, last accessed in November 2007) had already been studied in the original report that explored this gene [3]; not surprisingly, results for SNP rs10012946 were consistent with those reported for the four *WFS1* SNPs analysed previously (allelic OR 0.93, 95% CI 0.85–1.01, p=0.08).

Discussion

Well-powered replication attempts and, more recently, genome-wide association scans have generated a growing list of reproducible diabetes genes (reviewed in [9]). A recent report that achieved similar levels of statistical evidence for SNPs in *WFS1* [3], coupled with consistent results from independent [7] and overlapping [8] genome-wide association scans, as well as the data presented here and in an accompanying report in this issue [10], confirm that *WFS1* should join that expanding list as a genuinely novel type 2 diabetes gene.

The results we have obtained in the DPP, while consistent with the previous report, only achieved marginal statistical significance. This could be due, as suggested by our power calculations, to lack of power (particularly when analyses were restricted to a single ethnic group or treatment arm). Other likely factors include: (1) the ethnic heterogeneity in our cohort; (2) its starting point as a subgroup with altered glycaemic physiology at baseline; and (3) a clinical trial design in which the intervention arms were specifically intended to diminish the number of incident events. Nevertheless, consistent genetic effects were detected in the lifestyle arm.

Although the reciprocal effects of these SNPs on measures of insulin secretion and insulin sensitivity precluded us from drawing strong conclusions as to their mechanism(s) of action, the few settings in which the protective allele increased insulin secretion in the absence of decreased insulin sensitivity suggest that these variants act on the pancreatic beta cell; such a model is consistent with what is known about the pattern of expression of wolframin and the pathophysiology of Wolfram syndrome. A more detailed characterisation may require more sensitive measures of insulin secretion and sensitivity, although given the modest impact of these variants, a very large sample will be required.

Given the modest OR reported for most novel diabetes-associated variants identified in recent genome-wide association studies [9] and the number of samples required to detect true effects in case–control designs [7,8], studies seeking to confirm or extend these observations will need to account for possible type II error. This may be even more pertinent for population-based studies (particularly if short in duration or ethnically heterogeneous), as well as for clinical trials powered to demonstrate a significant impact of an intervention, but not necessarily an interaction with a genetic variant of weak effect.

In conclusion, we present evidence that supports the role of common variants in *WFS1* as modest contributors to diabetes risk and suggest that they may do so by conferring an impairment in insulin secretion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DPP

Diabetes Prevention Program

HOMA-IR
homeostasis model assessment of insulin resistance

HR
hazard ratio

HWE
Hardy–Weinberg equilibrium

LD
linkage disequilibrium

OR
odds ratio

SNP
single nucleotide polymorphism

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Table 1

Diabetes incidence by genotype at three *WFS1* SNPs in the DPP

SNPs	Alleles (M/m)	MAF	LRT *p* value	Genotype	All arms		Placebo		Metformin		Lifestyle	
					HR (95% CI)	Cox *p* value	HR (95% CI)	Cox *p* value	HR (95% CI)	Cox *p* value	HR (95% CI)	Cox *p* value
All participants (*n*=2,994)[a]												
rs10010131	G/A		0.38	Mm vs MM	1.05 (0.89–1.25)	0.57	0.97 (0.75–1.26)	0.83	1.17 (0.86–1.58)	0.32	1.06 (0.75–1.50)	0.72
				mm vs MM	1.05 (0.81–1.35)	0.73	1.15 (0.78–1.71)	0.48	1.21 (0.80–1.83)	0.37	0.66 (0.35–1.22)	0.18
rs752854	A/G		0.24	Mm vs MM	1.08 (0.91–1.27)	0.39	1.09 (0.84–1.40)	0.51	1.11 (0.82–1.50)	0.49	1.01 (0.72–1.42)	0.95
				mm vs MM	0.98 (0.72–1.33)	0.90	1.14 (0.73–1.79)	0.56	1.18 (0.73–1.89)	0.50	0.41 (0.17–1.02)	0.056
rs734312	A/G		0.13	Mm vs MM	1.11 (0.91–1.36)	0.30	0.98 (0.72–1.33)	0.90	1.29 (0.90–1.86)	0.17	1.14 (0.78–1.68)	0.49
				mm vs MM	1.15 (0.93–1.42)	0.21	1.19 (0.87–1.63)	0.28	1.39 (0.95–2.02)	0.09	0.77 (0.49–1.23)	0.27
White participants only (*n*=1,674)[b]												
rs10010131	G/A	0.39	0.28	Mm vs MM	0.98 (0.78–1.24)	0.88	0.99 (0.69–1.44)	0.98	1.02 (0.67–1.55)	0.92	0.91 (0.58–1.43)	0.67
				mm vs MM	1.10 (0.80–1.51)	0.56	1.40 (0.87–2.26)	0.17	1.21 (0.72–2.03)	0.46	0.53 (0.23–1.19)	0.12
rs752854	A/G	0.32	0.10	Mm vs MM	1.02 (0.81–1.28)	0.87	1.02 (0.71–1.45)	0.93	1.16 (0.78–1.74)	0.46	0.87 (0.56–1.36)	0.54
				mm vs MM	1.04 (0.73–1.47)	0.83	1.37 (0.83–2.26)	0.22	1.22 (0.70–2.16)	0.48	0.30 (0.09–0.99)	0.048
rs734312	A/G	0.45	0.65	Mm vs MM	1.03 (0.81–1.32)	0.79	0.96 (0.65–1.41)	0.84	1.11 (0.71–1.72)	0.66	1.07 (0.66–1.71)	0.79
				mm vs MM	1.04 (0.76–1.40)	0.83	1.09 (0.69–1.74)	0.71	1.20 (0.72–2.00)	0.49	0.69 (0.34–1.39)	0.30

Diabetes incidence was assessed by Cox proportional hazards in all DPP ethnic groups or in white participants only, minus participants randomised to the troglitazone arm

Because a likelihood ratio test (LRT) assessing genotype–intervention interactions was not statistically significant for any of the three SNPs, results are reported in the full cohort as well as stratified by treatment arm

M Major allele in white participants, *m* minor allele in white participants, *MAF* minor allele frequency in white participants

[a] Conversion to diabetes: all arms, *n*=640 (21%); placebo, *n*=285 (29%); metformin, *n*=209 (21%); lifestyle, *n*=146 (15%)

[b] Conversion to diabetes: all arms, *n*=353 (21%); placebo, *n*=150 (27%); metformin, *n*=118 (21%); lifestyle, *n*=85 (16%)

Table 2

Baseline measures of insulin sensitivity and beta cell function by *WFS1* SNP in all DPP participants

Variable	Genotype	rs10010131 (*n*=3,549)		rs752854 (*n*=3,535)		rs734312 (*n*=3,530)	
		Mean (95% CI)	*p* value	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value
Insulin sensitivity index	AA	0.157 (0.149–0.166)	0.07	0.166 (0.162–0.171)	0.44	0.170 (0.165–0.177)	0.04
	AG	0.163 (0.159–0.168)		0.162 (0.157–0.167)		0.164 (0.159–0.168)	
	GG	0.168 (0.163–0.173)		0.162 (0.152–0.172)		0.160 (0.155–0.165)	
Insulinogenic index	AA	142.9 (133.6–152.3)	0.76	146.4 (141.8–150.9)	0.68	141.9 (135.6–148.3)	0.02
	AG	144.1 (139.0–149.2)		143.1 (137.6–148.7)		141.6 (136.4–146.8)	
	GG	146.2 (141.2–151.3)		144.9 (133.3–156.6)		152.4 (146.3–158.6)	

The insulin sensitivity index (reciprocal of insulin resistance by HOMA-IR) and the insulinogenic index ([pmol/l]/[mmol/l]) were estimated in DPP participants at baseline

Log-transformed baseline measures were compared across genotypes by ANOVA (*F* test); untransformed means are presented

The previously reported protective alleles are A for rs10010131 and G for both rs752854 and rs734312

Table 3

Measures of insulin sensitivity and beta cell function by *WFS1* SNP and treatment arm in all participants of the Diabetes Prevention Program at 1 year

Variable	Genotype	Placebo		Metformin		Lifestyle		Troglitazone	
		Mean (95% CI)	*p* value	Mean (95% CI)**	*p* value	Mean (95% CI)**	*p* value	Mean (95% CI)**	*p* value
rs10010131[a]									
Insulin sensitivity index	AA	0.164 (0.149–0.182)	0.25	0.204 (0.188–0.222)	0.79	0.204 (0.184–0.226)	0.02	0.218 (0.185–0.257)	0.85
	AG	0.176 (0.167–0.184)		0.198 (0.189–0.208)		0.241 (0.228–0.255)		0.231 (0.211–0.253)	
	GG	0.166 (0.158–0.175)		0.198 (0.189–0.207)		0.230 (0.218–0.242)		0.228 (0.209–0.248)	
Insulinogenic index	AA	154.8 (132.2–178.3)	0.64	150.1 (134.0–166.7)	0.64	143.1 (124.0–162.9)	0.92	143.2 (116.0–171.8)	0.51
	AG	142.8 (132.4–153.5)		141.3 (131.7–151.1)		139.7 (129.3–150.3)		134.4 (119.3–149.9)	
	GG	146.1 (135.1–157.3)		141.9 (132.5–151.5)		138.5 (128.7–148.4)		146.9 (132.1–162.0)	
rs752854[b]									
Insulin sensitivity index	AA	0.168 (0.161–0.176)	0.61	0.196 (0.188–0.205)	0.59	0.231 (0.220–0.242)	0.53	0.233 (0.215–0.252)	0.56
	AG	0.174 (0.165–0.184)		0.203 (0.193–0.214)		0.235 (0.221–0.249)		0.223 (0.202–0.245)	
	GG	0.169 (0.150–0.190)		0.200 (0.181–0.221)		0.217 (0.191–0.246)		0.208 (0.166–0.261)	
Insulinogenic index	AA	143.8 (134.1–153.7)	0.86	140.3 (131.9–148.9)	0.66	140.0 (131.0–149.2)	0.70	144.7 (131.5–158.2)	0.35
	AG	146.8 (135.0–158.8)		146.1 (135.5–156.8)		137.6 (126.6–148.9)		131.6 (115.8–147.9)	
	GG	150.7 (123.9–178.8)		146.6 (127.3–166.5)		148.9 (125.2–173.7)		155.2 (117.3–195.9)	
rs734312[c]									
Insulin sensitivity index	AA	0.166 (0.156–0.177)	0.63	0.199 (0.187–0.211)	0.99	0.239 (0.224–0.255)	0.02	0.226 (0.203–0.252)	0.99
	AG	0.172 (0.163–0.181)		0.199 (0.189–0.209)		0.238 (0.225–0.252)		0.228 (0.209–0.249)	
	GG	0.173 (0.163–0.183)		0.200 (0.189–0.211)		0.213 (0.200–0.228)		0.226 (0.202–0.253)	
Insulinogenic index	AA	139.2 (125.3–153.5)	0.57	135.8 (124.2–147.6)	0.007	130.5 (118.6–142.7)	0.06	147.5 (129.0–166.6)	0.54
	AG	146.7 (135.7–158.0)		135.9 (126.1–145.9)		138.8 (128.5–149.3)		134.1 (119.2–149.4)	
	GG	149.4 (136.3–162.9)		157.5 (146.5–168.6)		151.5 (138.8–164.6)		141.9 (122.7–161.8)	

The insulin sensitivity index (reciprocal of insulin resistance by HOMA-IR) and the insulinogenic index ([pmol/l]/[mmol/l]) were estimated at 1 year after treatment with placebo, metformin, lifestyle or troglitazone in DPP participants

Least-squares geometric means (adjusted for baseline values) were compared across genotypes at each *WFS1* variant by ANOVA (*F* test)

The previously reported protective alleles are A for rs10010131 and G for both rs752854 and rs734312

[a] Placebo, *n*=835; metformin, *n*=878; lifestyle, *n*=919; troglitazone, *n*=339

[b] Placebo, *n*=836; metformin, *n*=875; lifestyle, *n*=918; troglitazone, *n*=338

[c] Placebo, *n*=833; metformin, *n*=875; lifestyle, *n*=918; troglitazone, *n*=336