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Common variants in *WFS1* confer risk of type 2 diabetes

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Abstract

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AUTHOR CONTRIBUTIONS

I. Barroso, A.T.H., M.A.P., N.J.W. and M.S.S. designed this study; M.S.S. did the statistical analysis, supported by I. Barroso, A.D., S.L.D., K.A.F., H.L., R.J.N., P.D.P., R.S. and M.N.W. M.S.S. wrote the manuscript with I. Barroso, S.L.D. and K.A.F.; A.D. conducted the bioinformatic analysis; I. Blech., S.L.D., K.A.F. and C.K. arrayed and genotyped the replication studies; B.G., A.T.H., G.H., C.K., A.D.M., M.I.M., C.N.A.P., M.W., R.T., J.W. and N.J.W. provided study samples and all authors contributed to data interpretation and commented on the final manuscript.

COMPETING INTERESTS STATEMENT

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We studied genes involved in pancreatic β cell function and survival, identifying associations between SNPs in *WFS1* and diabetes risk in UK populations that we replicated in an Ashkenazi population and in additional UK studies. In a pooled analysis comprising 9,533 cases and 11,389 controls, SNPs in *WFS1* were strongly associated with diabetes risk. Rare mutations in *WFS1* cause Wolfram syndrome; using a gene-centric approach, we show that variation in *WFS1* also predisposes to common type 2 diabetes.

Progressive deterioration in β cell function is central to the pathophysiology of type 2 diabetes. Indeed, genes with important roles in pancreatic β cells have been shown to influence susceptibility to type 2 diabetes, including *KCNJ11* (refs. 1,2), *HNF4A* (refs. 3,4) and, most recently, *TCF7L2* (ref. 5). Therefore, we conducted a gene-centric association study for type 2 diabetes, genotyping 1,536 SNPs in 84 genes regulating pancreatic β cell development, growth, function and survival (Supplementary Methods online). We genotyped all 1,536 SNPs in four case-control studies of type 2 diabetes (three UK and one Ashkenazi population) and used a two-stage analysis (Supplementary Methods). Informed written consent was obtained from all study participants, and all studies were approved by the relevant ethics committees (Supplementary Methods).

We first assessed the association between candidate SNPs and diabetes risk in the three UK study sets, comprising up to 1,484 cases and 1,856 controls, using a log-additive (codominant) model. Of 1,367 SNPs that passed genotyping quality control (Supplementary Methods), 18 (1.3%) were associated with diabetes risk at $P < 0.01$, our threshold for defining association in this initial screening phase (Table 1 and Supplementary Table 1 online). In the second phase, to reduce the number of statistical tests, we restricted the analysis to assessing the association between these 18 SNPs and diabetes risk in the Ashkenazim study set, an ethnically distinct founder population comprising 930 cases and 461 controls. Two of the originally associated SNPs (rs10010131 and rs6446482) were associated in this independent study at $P < 0.05$ (Table 1).

As expected, the two replicated SNPs (rs10010131 and rs6446482) were associated with diabetes risk in a combined analysis of all four studies ($P = 1.3 \times 10^{-4}$ and $P = 2.7 \times 10^{-4}$, respectively; Supplementary Table 2 online). These SNPs were in strong LD with each other in our study populations ($r^2 = 0.98$) and located in the same gene, *WFS1*. For rs10010131 and rs6446482, the risk allele was the major allele, with a frequency of 60% for both SNPs.

Although only two of the original six variants typed across *WFS1* were associated with diabetes in our two-staged approach, in a pooled analysis of all four study sets, five SNPs showed statistical association (Supplementary Table 2). This was not unexpected, given the high linkage disequilibrium (LD) among these SNPs (Supplementary Table 3 online). Compared with the other *WFS1* SNPs, rs10010131 showed a marginally stronger association. Therefore, we used likelihood ratio tests (Supplementary Methods and Supplementary Table 4 online) to assess whether rs10010131 explained all the observed associations. Based on these analyses, we found that all the association signals were due to rs10010131 and rs6446482. We also found evidence of possible interdependency between these SNPs and rs752854 on disease risk (Supplementary Table 4). Because of their high correlation, these analyses suggest that rs10010131 or rs6446482 might be independent causal alleles, or that they are in LD with a causal allele, or both, and that the other SNPs do not independently contribute to disease risk.

The SNPs showing independent associations or interdependency (rs10010131, rs6446482 and rs752854) are intronic, with no obvious evidence for biological function. Therefore, we conducted a more detailed examination of variation in this gene. Using data from HapMap, and based on sequence spanning 63.4 kb (chromosome 4, 6374656-6438055), including 15

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kb extending both 5′ and 3′ from *WFS1*, we detected strong LD across the region. The entire gene was defined by a single haplotype block of 39 kb (Supplementary Fig. 1 online). Within this block, 53 SNPs had a minor allele frequency (MAF) of ≥1%. The six SNPs we typed in our studies were all located in this region and together tagged 88% of the common variation in this block (47 of the 53 SNPs, MAF ≥1%, $r^2 \geq 0.8$) with a mean r^2 of 0.97. One nonsynonymous SNP (R611H, rs734312) is highly correlated with SNPs rs10010131 (r^2 = 0.92) and rs6446482 (r^2 = 0.88) and thus may be a causal variant. Consistent with this LD structure, a previous study based on 479 cases and 509 controls (a subset of our samples) has shown suggestive association between rs734312 and type 2 diabetes6.

To extend support for an association between variation at *WFS1* and diabetes risk, we attempted further replication, typing rs10010131, rs6446482, rs752854 and the highly correlated nonsynonymous SNP (rs734312) in three further case-control studies, ADDITION (926 cases and 1,497 controls), Warren 2 (2,465 cases and 3,843 controls) and Tayside (3,728 cases and 3,732 controls). For rs10010131, rs6446482 and rs752854, we found independent evidence for association in each study (Table 2).

Because of the possible interdependency between rs10010131 and rs752854 in the secondary analysis of the four initial studies, we assessed this association in a combined analysis of the ADDITION, Warren 2 and Tayside studies. We did not find any evidence for interdependency in these studies (data not shown). We also conducted haplotype analysis using log-linear modeling. Based on these two loci, we did not find any consistent evidence for a haplotype association that was independent of the underlying SNP associations among these studies (data not shown).

We also found some evidence for association of the nonsynonymous SNP rs734312 in the ADDITION, Warren 2 and Tayside studies (Table 2). Therefore, we genotyped this variant in our original studies and conducted a pooled analysis of all seven studies, comprising up to 9,533 cases and 11,389 controls (Table 2). In this analysis, rs734312 was associated with diabetes risk ($P = 2.0 \times 10^{-5}$). However, likelihood ratio tests showed that rs734312 did not contribute to a model including rs10010131 ($P = 0.88$), whereas rs10010131 substantially improved the fit of a model including rs734312 ($P = 4.9 \times 10^{-3}$), suggesting that rs734312 is unlikely to be the functional variant explaining these associations. We also did not find any consistent evidence for interdependency between rs10010131 or rs6446482 and rs752854 and diabetes risk in the combined study sets (data not shown).

By contrast, we found that rs10010131 (MAF = 40%) and rs6446482 (MAF 41%) were strongly associated with diabetes risk at $P = 1.4 \times 10^{-7}$ and $P = 3.4 \times 10^{-7}$, respectively, in the pooled study set (Table 2). Furthermore, the magnitude of this association was highly consistent across studies (Supplementary Fig. 2 and Supplementary Table 5 online), with no heterogeneity among studies (P (six degrees of freedom) = 0.59 and 0.68 for rs10010131 and rs6446482, respectively).

We provide strong evidence for an association between variation at *WFS1* and risk of type 2 diabetes. *WFS1* encodes wolframin, a membrane glycoprotein that maintains calcium homeostasis of the endoplasmic reticulum. Mutations in this gene cause Wolfram syndrome (DIDMOAD, OMIM 222300), which is characterized by diabetes insipidus, juvenile-onset non-autoimmune diabetes mellitus, optic atrophy and deafness7,8. Disruption of *Wfs1* in mice causes overt diabetes or impaired glucose tolerance, depending on genetic background9,10. Both humans and mice deficient in Wolframin show pancreatic β cell loss, possibly as a result of an enhanced endoplasmic reticulum stress response leading to increased β cell apoptosis9,11,12. Thus, *WFS1* is critical for survival and function of insulin-producing pancreatic β cells. In line with this evidence, chemical enhancement of

endoplasmic reticulum function has been suggested as a treatment for the metabolic abnormalities associated with diabetes13.

Our results indicate that variation in *WFS1* not only results in a rare syndrome partly characterized by early-onset non-autoimmune diabetes but also is associated with susceptibility to common type 2 diabetes. For rs10010131, based on a risk allele frequency of 60%, we estimate that the population attributable fraction is 9%, explaining 0.3% of the excess familial risk. Other examples of such polygenic loci that are also involved in monogenic and syndromic forms of type 2 diabetes include *KCNJ11* (ref. 1), *PPARG*14 and *HNF4A*[3,4].

Our study demonstrates that with sufficiently powered studies, the gene-centric approach is an effective strategy to identify disease susceptibility loci that contribute modest risk, complementing genome-wide approaches. Because of strong LD across *WFS1* in European populations and the difficulty of resolving association signals in an epidemiological context, our study also highlights the need for appropriately powered studies in populations with greater genetic diversity. In conclusion, these data provide strong evidence that variation in *WFS1*, a gene with an essential role in the endoplasmic reticulum stress response in insulin-producing pancreatic β cells, contributes to risk of common type 2 diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Associations ($P < 0.01$) between SNPs in genes involved in β cell development, growth, function and survival and risk of type 2 diabetes in UK populations and a replication study in an Ashkenazi population

		Stage 1 (UK population)		Replication study (Ashkenazi population)	
Gene	**SNP**	**Odds ratio (95% c.i.)**	***P* value[a]**	**Odds ratio (95% c.i.)**	***P* value**
CHGA	rs941584	0.80 (0.72-0.90)	2.8×10^{-4}	1.03 (0.87-1.22)	0.75
NFATC1	rs643705	1.30 (1.12-1.52)	5.4×10^{-4}	0.85 (0.71-1.02)	0.08
PAX6	rs628224	0.82 (0.71-0.93)	2.8×10^{-3}	0.95 (0.74-1.22)	0.71
NFKB1	rs1609798	0.85 (0.77-0.95)	2.9×10^{-3}	1.04 (0.87-1.24)	0.68
NFKB1	rs11722146	0.85 (0.76-0.95)	3.0×10^{-3}	1.03 (0.86-1.22)	0.75
NFKB1	rs230498	0.86 (0.77-0.95)	3.0×10^{-3}	1.03 (0.87-1.21)	0.74
WFS1	**rs10010131**	**0.86 (0.78-0.95)**	**3.1×10^{-3}**	**0.79 (0.66-0.94)**	**0.01**
CACNA1D	rs4687736	1.18 (1.06-1.32)	3.3×10^{-3}	1.00 (0.82-1.22)	0.97
EGFR	rs2075112	1.15 (1.04-1.27)	5.1×10^{-3}	1.03 (0.87-1.22)	0.74
PBX1	rs7535186	0.87 (0.79-0.96)	5.3×10^{-3}	0.97 (0.83-1.14)	0.76
WFS1	**rs6446482**	**0.87 (0.79-0.96)**	**5.7×10^{-3}**	**0.79 (0.66-0.95)**	**0.01**
NFKB1	rs230539	0.87 (0.78-0.96)	5.9×10^{-3}	1.03 (0.87-1.21)	0.77
TCF2	rs7501939	1.15 (1.04-1.27)	6.6×10^{-3}	1.04 (0.89-1.22)	0.64
CACNA1D	rs3796347	1.15 (1.04-1.26)	7.0×10^{-3}	0.96 (0.81-1.13)	0.60
CAMK2A	rs3822607	0.87 (0.78-0.96)	7.1×10^{-3}	1.06 (0.89-1.26)	0.50
NFATC1	rs3826567	1.30 (1.07-1.58)	7.3×10^{-3}	0.88 (0.69-1.11)	0.27
FOXA3	rs11669442	1.15 (1.04-1.28)	7.6×10^{-3}	0.92 (0.77-1.08)	0.31
FGF2	rs1048201	1.19 (1.05-1.36)	8.2×10^{-3}	1.19 (0.98-1.45)	0.08

The UK population comprised up to 1,484 cases and 1,856 controls, and the Ashkenazi population comprised up to 930 cases and 461 controls. All SNPs were in Hardy-Weinberg equilibrium ($P > 0.01$ in controls). c.i., confidence interval.

[a]Based on a log-additive model adjusting for study.

Table 2

Association between SNPs located in *WFS1* and risk of type 2 diabetes: replication studies and pooled analysis

SNP	Odds ratio (95% c.i.)	*P* value
ADDITION study (926 cases, 1,497 controls)		
rs10010131	0.87 (0.77-0.98)	0.020
rs6446482	0.87 (0.77-0.98)	0.021
rs752854	0.86 (0.76-0.97)	0.013
rs734312	0.92 (0.82-1.03)	0.163
Warren 2 study (2,465 cases, 3,843 controls)		
rs10010131	0.91 (0.84-0.98)	0.011
rs6446482	0.92 (0.86-0.99)	0.027
rs752854	0.93 (0.86-1.00)	0.060
rs734312	0.93 (0.87-1.00)	0.061
Tayside study (3,728 cases, 3,732 controls)		
rs10010131	0.93 (0.87-0.99)	0.029
rs6446482	0.92 (0.87-0.99)	0.019
rs752854	0.93 (0.86-0.99)	0.032
rs734312	0.93 (0.87-0.99)	0.019
All seven pooled studies, comprising up to 9,533 cases and 11,389 controls[a]		
rs10010131	0.90 (0.86-0.93)	1.4×10^{-7}
rs6446482	0.90 (0.87-0.94)	3.4×10^{-7}
rs734312	0.92 (0.88-0.95)	2.0×10^{-5}
rs752854	0.92 (0.88-0.96)	1.3×10^{-4}

c.i., confidence interval. Boldface represents replicated SNPs.

[a]Based on a single-locus log-additive model adjusted for study.