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COMMENTARY

Candidate gene studies reveal that the *WFS1* gene joins the expanding list of novel type 2 diabetes genes

J. Wasson · M. A. Permutt

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Abbreviations

ER	endoplasmic reticulum
GWA	genome-wide association
LD	linkage disequilibrium
OR	odds ratio
SNP	single nucleotide polymorphism

Wolfram syndrome

Wolfram syndrome, originally described in 1938, is a rare, autosomal recessive disease that is characterised by young onset insulin-dependent diabetes, progressive sensorineural deafness, diabetes insipidus, autonomic nervous system dysfunction and, ultimately, brainstem atrophy and premature death [1]. The Wolfram gene (*WFS1*), which encodes wolframin, was mapped to chromosome 4p in families with multiple affected individuals [2], and cloned in 1998 [3]. Wolframin is a protein of 890 amino acids that is produced in a wide variety of tissues, most prominently in pancreatic beta cells and brain. Over 100 missense and non-sense mutations have been described patients. As these mutations are associated with a non-immune loss of beta cells and diabetes, the gene was subsequently evaluated in more common forms of diabetes.

J. Wasson · M. A. Permutt (✉)
Division of Endocrinology, Metabolism,
and Lipid Research, Department of Medicine,
Washington University School of Medicine,
660 S. Euclid Ave., Campus Box 8127,
St Louis, MO 63110, USA
e-mail: apermutt@im.wustl.edu

Do variants of *WFS1* contribute to risk of type 2 diabetes?

Variants of *WFS1* have not convincingly been shown to contribute to the risk of type 1 diabetes [4]. The first study to address this question in type 2 diabetes sequenced DNA from 29 patients and uncovered 12 coding variants or single nucleotide polymorphisms (SNPs). These included five non-synonymous SNPs encoding amino acid changes [5]. The most abundant genetic variant alters the amino acid at position 611 from a histidine to an arginine (H611R). The arginine variant was present in 40% of diabetic cases and 45% of controls ($p<0.02$), suggesting that the variant was protective from diabetes. There were no functional studies, and the sample size in this initial study was relatively small. These observations suggested that variation in *WFS1* influences susceptibility to type 2 diabetes.

As beta cell dysfunction is a critical component of type 2 diabetes, a recent study genotyped 1,500 SNPs as markers for 84 beta cell candidate genes in a large number of UK Europid and Ashkenazi Jewish cases and controls [6]. Among all the genes examined, only SNPs in *WFS1* were consistently associated with type 2 diabetes. In this study by Sandhu et al. there were four SNPs associated with the disease, but all were in significant linkage disequilibrium (LD) in a 39 kb LD block that included the H611R variant. In this study there were six UK populations and one Ashkenazi population studied—a total of approximately

9,500 cases and 11,000 controls—yielding an odds ratio (OR) of 0.92 (95% CI 0.88–0.95, $p<10^{-4}$–10^{-7}), indicating once more a protective effect of the minor allele.

In this issue of the *Diabetologia*, two new studies attempt to replicate the previous observations. Franks et al. [7] genotyped the same four SNPs as Sandhu et al. [6] in northern Swedish Europid cases and controls. The minor allele at one SNP (rs752854) was statistically associated with reduced risk of type 2 diabetes (OR 0.85. 95% CI 0.75–0.96, $p=0.010$), while borderline statistical significance was observed for the other three SNPs. The direction and magnitude of the associations were consistent with the previous report. Franks et. al. [7] also conducted a meta-analysis of ~14,000 cases and an equal number of controls, which included their data, together with the results of the study by Sandhu et al. [6] and unpublished data for SNPs at the *WFS1* locus genotyped in three genome-wide association (GWA) studies (one Finnish, one French and one Swedish) [8–10]. This indicated that SNP rs10010131 at the *WFS1* locus is associated with a reduced risk of type 2 diabetes (OR 0.89, 95% CI 0.86–0.92; $p=4.9\times10^{-11}$).

In the second study in this issue, three of the previously studied SNPs at the *WFS1* locus were genotyped in a large number ($n=3,548$) of racially diverse members of the Diabetes Prevention Program (DPP), which was designed to compare lifestyle intervention or metformin with placebo [11]. This study differed from previous studies in that it determined the predictive value of genotypes at this locus for development of diabetes, and correlated this with different interventions. Additionally, phenotypes were obtained for all participants, including the insulin sensitivity index (reciprocal of the homeostasis model assessment of insulin resistance) and the insulinogenic index ([insulin at 30 min−insulin at 0 min]/[glucose at 30 min−glucose at 0 min], with insulin measured in pmol/l and glucose in mmol/l).

The major conclusion of this well-performed study is that, overall, the *WFS1* variants do not predict the onset of diabetes for the group as a whole. However, the differences in phenotypes observed in the two intervention groups may give clues as to the aetiology of the risk of diabetes conveyed by variants at this locus, i.e. impairment of beta cell function. Individuals with the variant allele H611R exhibited lower glucose-stimulated insulin secretion, yet were more insulin sensitive. Another variant was associated with reduced insulinogenic index with no difference in insulin sensitivity, suggesting that variants of *WFS1* primarily affect beta cell function, consistent with patterns of expression of the gene. It is fair to say at this point as the authors do state that more detailed physiological studies need to be performed on larger samples.

Why do candidate gene studies identify genes missed by GWA studies?

There are several possible explanations. Candidate gene studies use more densely spaced SNPs to target the gene in question. More recently, because of the expense of GWA studies, candidate gene studies have included more participants and are therefore more likely to uncover lower risk alleles. Finally, in a GWA study the results are corrected for the large number of tests performed, and the threshold p value for positive associations is higher, leaving room for more type 2 error. In fact, in the study by Franks et al. [7], the meta-analysis used data from GWA studies, which increased the probability that *WFS1* is a risk factor for type 2 diabetes.

How does *WFS1* deficiency contribute to beta cell dysfunction?

Immunocytochemistry and subcellular fractionation studies have indicated that wolframin is localised in the endoplasmic reticulum (ER) membrane and has nine transmembrane segments. Antibody studies indicate that the amino terminus extends into the cytoplasm, while the carboxy terminus is in the ER lumen [12]. Expression studies in vitro suggest that *WFS1* encodes a protein with an important role in the regulation of intracellular Ca^{2+} homeostasis [13]. N-linked glycosylation is necessary for biogenesis and protein stability. Fibroblasts from a patient with a non-sense mutation in the gene revealed that protein absence was caused by rapid decay of the transcript, and a patient with compound missense mutations exhibited markedly reduced protein stability [12]. Thus, in patients with Wolfram syndrome mutations the protein is present at reduced levels rather than dysfunctional, although the mechanisms are completely unknown as to how the more common variants predispose/protect against type 2 diabetes.

Several studies have demonstrated a relationship between ER stress and *WFS1* [14–16]. Both the mRNA and protein are induced by ER stress. Glucose-induced insulin secretion also activates *WFS1* expression and wolframin production, and a reduction in *WFS1* mRNA results in the activation of ER stress. ER stress as a cause of diabetes has been demonstrated in the mouse by knockout of eIF2-α kinase (PERK), resulting in beta cell loss and diabetes [17], and in a rare genetic disease characterised by mutation of this gene, resulting in early-onset diabetes and epiphyseal dysplasia, the Wolcott–Rallison syndrome [18]. A mouse model of Wolfram syndrome with a conditional deletion of *WFS1* in beta cells revealed, by electron microscopy, a reduction in beta cell mass, enhanced apoptosis, elevation

of a marker of ER stress and dilated ER with decreased secretory granules [14]. These results indicate that lack of expression of *WFS1* in beta cells appears to be a significant contributor to the reduction in beta cell survival [14, 19].

How can variants in *WFS1* contribute to type 2 diabetes?

The most common *WFS1* variant, H611R, may be a causative variant, yet we know nothing of its potential function. Like the protective effect of the minor allele at *PPARG* (Pro12Ala), the minor allele at *WFS1* (H611R) appears protective. In its initial phase, ER stress serves to slow down protein synthesis. During periods of starvation it might be advantageous to decrease the rate protein synthesis and reduce insulin secretion, thus accounting for the higher frequency of this variant. During periods of excess nutrition, however, this *WFS1* variant may predispose to ER stress-mediated apoptosis. With regard to the five non-synonymous coding variants that were described after sequencing DNA from diabetic patients [5], any or all of which could contribute to *WFS1* dysfunction, we know nothing about their potential biological effects Perhaps the best estimate of the effects of these variants can be determined by cell-based assays, yet to be performed. Additionally, these variants are in a large LD block and may be in LD with yet to be discovered regulatory variants that might alter levels of expression of this gene.

What have we learned? Monogenic disease genes and multifactorial diabetes

We now know that genes responsible for monogenic forms of diabetes are good candidates to contribute low risk for the more common forms of diabetes. These low-risk alleles require large sample sizes and rather densely spaced SNPs to be defined. When we finally identify a susceptibility locus, the biological correlates are often missing. The future lies in translating genomic observations into the contexts of gene–gene interactions, effects of environment, tissue expression, and functional biological studies.

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

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