

Replication of the association between variants in *WFS1* and risk of type 2 diabetes in European populations

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Abstract

Aims/hypothesis Mutations at the gene encoding wolframin (*WFS1*) cause Wolfram syndrome, a rare neurological condition. Associations between single nucleotide polymorphisms

(SNPs) at *WFS1* and type 2 diabetes have recently been reported. Thus, our aim was to replicate those associations in a northern Swedish case–control study of type 2 diabetes. We also performed a meta-analysis of published and previously unpublished data from Sweden, Finland and France, to obtain updated summary effect estimates.

Methods Four *WFS1* SNPs (rs10010131, rs6446482, rs752854 and rs734312 [H611R]) were genotyped in a type 2 diabetes case–control study ($n=1,296/1,412$) of Swedish adults. Logistic regression was used to assess the association between each *WFS1* SNP and type 2 diabetes, following adjustment for age, sex and BMI. We then performed a meta-analysis of 11 studies of type 2 diabetes, comprising up to 14,139 patients and 16,109 controls, to obtain a summary effect estimate for the *WFS1* variants.

Results In the northern Swedish study, the minor allele at rs752854 was associated with reduced type 2 diabetes risk [odds ratio (OR) 0.85, 95% CI 0.75–0.96, $p=0.010$]. Borderline statistical associations were observed for the remaining SNPs. The meta-analysis of the four independent replication studies for SNP rs10010131 and correlated variants showed evidence for statistical association (OR 0.87, 95% CI 0.82–0.93, $p=4.5 \times 10^{-5}$). In an updated meta-analysis of all 11 studies, strong evidence of statistical association was also observed (OR 0.89, 95% CI 0.86–0.92; $p=4.9 \times 10^{-11}$).

Conclusions/interpretation In this study of *WFS1* variants and type 2 diabetes risk, we have replicated the previously reported associations between SNPs at this locus and the risk of type 2 diabetes.

Keywords Association study · Genetic · Meta-analysis · Replication · Swedish · Type 2 diabetes · *WFS1* · Wolfram syndrome

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Abbreviations

DGI	Diabetes Genetics Initiative
DPP	Diabetes Prevention Program
FUSION	Finland–United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics genome-wide association
GWA	genome-wide association
HWE	Hardy–Weinberg equilibrium
LD	linkage disequilibrium
OR	odds ratio
SNP	single nucleotide polymorphism
VIP	Västerbotten Intervention Programme

Introduction

Wolfram syndrome (OMIM: #222300) is a rare, progressive, neurological disorder with an autosomal-recessive mode of inheritance, which frequently manifests in childhood [1]. Diabetes insipidus and (non-autoimmune) diabetes mellitus with optic atrophy and deafness are features of the syndrome, giving rise to its alternative name: DIDMOAD.

Positional cloning studies in families with Wolfram syndrome identified linkage peaks on the short arm of chromosome 4 (4p16.1) [2] and mutations in the gene encoding wolframin (*WFS1*), which maps to that region, have since been shown to cause the syndrome [3].

In a recent report, four common single nucleotide polymorphisms (SNPs) (rs10010131, rs6446482, rs752854 and rs734312 [H611R]) at the *WFS1* locus were shown to be convincingly associated with type 2 diabetes in six UK studies and one study of an Ashkenazi Jewish population, which together comprised 9,533 patients and 11,389 controls [4]. In that study, the summary effect estimates [odds ratios (ORs)] for the four *WFS1* polymorphisms ranged from 0.90 to 0.92 and all were statistically associated.

In an accompanying report in this issue of *Diabetologia* [5] from the Diabetes Prevention Program (DPP), three of the *WFS1* SNPs (rs10010131, rs752854, rs734312) were tested for association with incident type 2 diabetes. No statistical association was found overall, although when stratified by treatment arm, a modest effect on diabetes incidence was observed in the lifestyle intervention group.

In this study, we attempted to replicate the previously reported associations between *WFS1* SNPs and risk of type 2 diabetes in a case–control study from the county of Västerbotten in northern Sweden. We then sought support for the nominal associations with diabetes identified in the north Swedish cohort by conducting an updated meta-analysis of type 2 diabetes case–control studies using published and unpublished data.

Methods

Västerbotten type 2 diabetes case–control study

We identified 1,296 adult type 2 diabetic patients through registries covering the county of Västerbotten in northern Sweden (Fig. 1); as controls we selected 1,412 non-diabetic individuals, who were group-matched for age, sex, examination date and geographic region of residence, from the Västerbotten Intervention Programme (VIP) (Table 1) [6]. Virtually all were European white individuals. Type 2 diabetes was determined using the 1999 diagnostic criteria of the World Health Organization [7]. Participants with fasting capillary glucose concentrations <7.0 mmol/l and no documented history of diabetes underwent a 75 g anhydrous oral glucose tolerance test. Accordingly, control participants were those without a documented history of diabetes and with glucose concentrations below the thresholds for type 2 diabetes [7]. Type 2 diabetes in the patient group was defined by clinical diagnoses. In a subsample of 1,013 cases, additional validation procedures were undertaken such as an independent OGTT or documented treatment with glucose-lowering drugs (Fig. 1). Analyses were repeated in this subsample to verify the genetic associations (see Results). All living participants provided written informed consent. Permission was obtained from the Local Research Ethics Committee of Umeå University, and approval for genetics investigations of this material was granted by the Swedish Data Inspection Board.

Clinical measurements The VIP survey is a population-based observational cohort study, in which, since 1985, all residents of the county of Västerbotten who are aged 40, 50 and 60 years have been invited to attend their primary healthcare centre for a clinical examination. Demographic and clinical data used in this report were collected as part of the VIP survey by trained research nurses using a protocol standardised across study centres [8]. Height and weight were measured using a calibrated wall-mounted stadiometer and scales, respectively. BMI was calculated as weight in kg/(height in m)². Plasma glucose was assayed using fresh capillary plasma on a benchtop analyser (Reflotron; Boehringer Mannheim, Mannheim, Germany). A threshold glucose value of ≤ 2 mmol/l was used to exclude potentially spurious values, resulting in the exclusion of three control individuals from the dataset.

Genetic analysis within the Västerbotten study DNA was extracted from peripheral white blood cells. Prior to genotyping, all genomic DNA samples were diluted to 4 ng/ μ l. SNPs were assayed at the Wellcome Trust Sanger Institute using the Taqman MGB chemistry (Applied Biosystems, Foster City, CA, USA) in accordance with the recommended

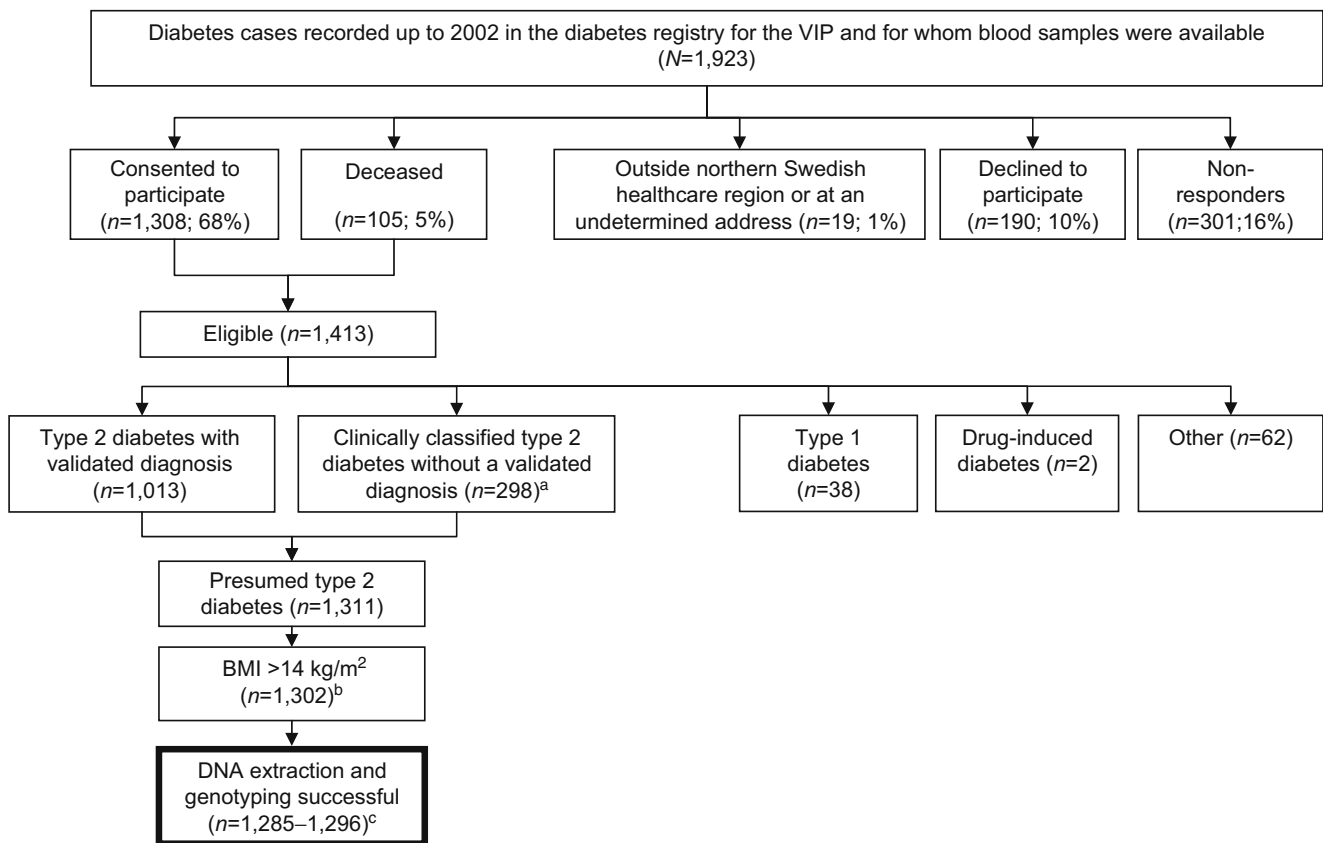


Fig. 1 Case selection criteria for participants in the Västerbotten diabetes case-control study; ^aCases in whom the clinician had documented a diagnosis of type 2 diabetes on the medical chart, but where corroboration of this diagnosis through other means such as an

independent OGTT or record of glucose-lowering drug treatment was absent; ^bBMI data were missing in eight individuals; ^c*n* varies according to genotype

protocol [9]. Genotyping success and concordance rates were >98 and 100% for all SNPs, respectively.

described by Sandhu et al. [4]; the correlated SNPs are therefore direct proxies for rs10010131.

Additional case-control studies for meta-analysis

To increase statistical accuracy, we added relevant data from recent genome-wide association (GWA) scans of type 2 diabetes. We contacted the relevant investigators of these GWA scans [10–13] and requested summary statistics (OR and 95% CI) for SNPs at *WFS1* that were correlated in HapMap at an r^2 of 1.0 with SNP rs10010131, the SNP showing the strongest statistical association in the study

Statistical analysis

Statistical analyses were conducted using SAS software 9.1 (SAS Institute, Cary, NC, USA). Hardy–Weinberg equilibrium (HWE) was assessed using the likelihood ratio test with 1 *df*. Linkage disequilibrium (LD), expressed as r^2 , was calculated using Haploview 4.0 (available from <http://www.broad.mit.edu/mpg/haploview>, last accessed in November 2007). Power calculations were performed using Quanto 1.1.1

Table 1 Characteristics of participants in the Västerbotten type 2 diabetes case-control study

	Patients (n=1,296)	Controls (n=1,412)	<i>p</i> value
Age (years)	53.6 (7.57)	53.1 (8.55)	0.230
Sex (no. of men/women)	754/542	689/698	<0.001
BMI (kg/m ²)	29.5 (4.76)	25.8 (3.75)	0.132
Fasting blood glucose (mmol/l)	8.04 (3.19)	5.27 (0.63)	0.017
2 h blood glucose (mmol/l) ^a	9.83 (4.90)	6.55 (1.48)	0.040

Data are means (SD) unless otherwise indicated

The *p* values were calculated using *t* tests for continuous traits or the Pearson correlation statistic for frequencies

^aIn the patient group, 2 h glucose measurements were available for only 846 patients

(available from <http://hydra.usc.edu/gxe>, last accessed in November 2007). Conditional logistic regression models were fitted to assess the associations between each of the *WFS1* genotypes and type 2 diabetes. Models were adjusted for age, sex and BMI. Glucose variables were log-transformed to correct skewness; anti-logged means and 95% CIs are presented for the respective results. For the descriptive statistics, central tendency and variance are reported as means and SD, respectively. A value of $p < 0.05$ was considered statistically significant. Meta-analysis of studies was performed using STATA version 8.2 (StataCorp, College Station, TX, USA) and a fixed effects model and inverse-variance-weighted averages of $\log(\text{ORs})$ to obtain a combined estimate of the overall OR. Between-study heterogeneity was assessed using the χ^2 statistic.

Results

WFS1 genotype associations in the Västerbotten case–control study

Table 1 shows participant characteristics for the Västerbotten case–control study; patients and controls were generally overweight or obese, middle-aged adults. The ratio of men to women was higher in the patient group.

All SNPs were consistent with HWE ($p > 0.1$). Minor allele frequencies in control participants for each SNP were: rs10010131=0.43; rs6446482=0.44; rs752854=0.35; and rs734312=0.48. The LD between SNPs in control participants here was generally lower than the values reported in other populations [4], ranging from 0.46 to 0.97 (Fig. 2).

In logistic regression analyses modelling log additive genetic effects, borderline statistically significant associations were observed between SNPs rs10010131, rs6446482 and rs734312 and type 2 diabetes (Table 2); the magnitude and direction of these associations are consistent with the initial study [4]. SNP rs752854 was statistically associated with type 2 diabetes, with the minor allele conveying a protective effect (OR 0.85, 95% CI 0.75–0.96, $p=0.010$; Table 2).

All analyses were re-run in the subset of individuals with validated type 2 diabetes diagnoses ($n=1,013$). In these models, the point estimates for each of the SNPs were consistent with the full dataset, although the CIs were slightly wider for rs10010131, rs6446482 and rs752854, reflecting the reduced sample size (results not shown).

Results of the meta-analysis of *WFS1* genotypes with regard to type 2 diabetes risk

For the meta-analysis, we included data from three of the first five type 2 diabetes GWA scans; a fourth was included in the original study [4]. The characteristics of each of the

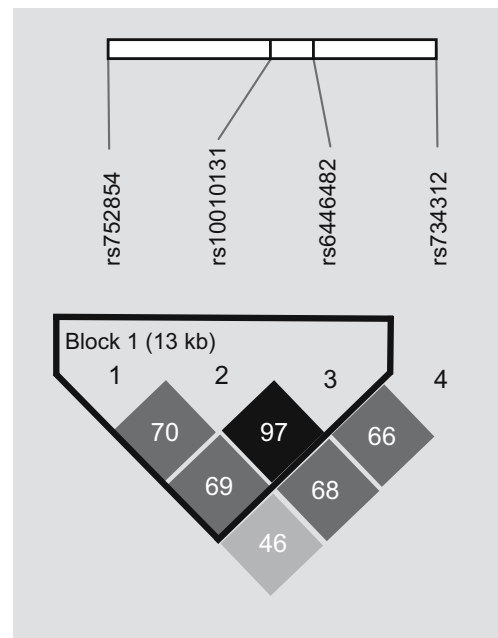


Fig. 2 Linkage disequilibrium (r^2) between *WFS1* genotypes in the control sample of the Västerbotten type 2 diabetes case–control study ($n=1,412$)

additional studies included in the meta-analysis have been reported previously [10–12]. We based these effect estimates on SNPs that were highly correlated with SNP rs10010131, which showed the strongest signal in the original report. For Sladek et al. [10], this was SNP rs4416547, typed in 686 type 2 diabetes patients and 669 controls. For the Diabetes Genetics Initiative (DGI) study [11], it was SNP rs10012946, typed in 1,464 patients and 1,467 controls. SNP rs10010131 was available for analysis from the candidate gene panels from the Finland–United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION), which comprised 1,160 patients and 1,172 controls [12].

In a meta-analysis of the Västerbotten study and the three additional studies, which comprised a total of 4,606 patients and 4,720 controls, we found strong evidence for a statistical association between variation at *WFS1* and type 2 diabetes (OR 0.87, 95% CI 0.82–0.93, $p=4.5 \times 10^{-5}$; Fig. 3a), providing clear evidence of independent replication. The magnitude of association was highly consistent among studies, with no material heterogeneity among studies ($p=0.17$ with 3 *df*). Finally, we conducted an updated meta-analysis of all 11 published and previously unpublished material. The unpublished data were obtained by contacting the authors of recently published genome-wide scans in which the relevant variants were genotyped (C. Dina, P. Froguel, K. L. Mohlke, C. Willer) [10–12]. As expected, this analysis showed robust evidence for statistical association ($p=5.4 \times 10^{-11}$; p for heterogeneity=0.42 with 10 *df*; Fig. 3b).

Table 2 Effect estimates for each of the *WFS1* SNPs in relation to glucose levels or type 2 diabetes in the Västerbotten type 2 diabetes case–control study

SNP	Adjusted geometric means or odds ratio (95% CI)			<i>p</i> value
	Common allele homozygotes	Heterozygotes	Minor allele homozygotes	
rs10010131				
Type 2 diabetes (yes vs no)	1.00	0.87 (0.69–1.01)	0.81 (0.63–1.03)	0.083
rs6446482				
Type 2 diabetes (yes vs no)	1.00	0.93 (0.75–1.16)	0.83 (0.65–1.05)	0.098
rs752854				
Type 2 diabetes (yes vs no)	1.00	0.84 (0.64–1.11)	0.72 (0.55–0.96)	0.010
rs734312				
Type 2 diabetes (yes vs no)	1.00	0.81 (0.66–1.00)	0.80 (0.64–1.01)	0.066

Data are presented as ORs and 95% CIs for type 2 diabetes from additive genetic models, and are adjusted for age, sex and BMI. *p* values are from tests for linear trend as reported in the Results.

Discussion

In this study we assessed the effects of *WFS1* polymorphisms on type 2 diabetes in populations from the northern and southern regions of Sweden, northern and western Finland, and France. The purpose of this investigation was to replicate the *WFS1* genotype associations with type 2 diabetes that have been reported recently in several UK studies and one of an Ashkenazi Jewish population [4].

WFS1 SNP rs752854 was statistically associated with type 2 diabetes in our northern Swedish study from Västerbotten, the direction and magnitude of the association being consistent with the previous report [4]. Although the remaining three SNPs were not statistically associated with type 2 diabetes, the effects are similar in direction and magnitude to those reported previously. In the original report, rs10010131 was the variant most strongly associated with diabetes, whereas in the Västerbotten cohort, the associated variant was rs752854. Both variants are non-coding. Therefore, it is probable that they tag the true functional locus and the difference in statistical associations between studies is attrib-

utable to different genetic substructures of the populations. This possibility is supported by the lower pair-wise LD between SNPs in the Västerbotten case–control study compared with the studies included in the original report. For example, LD in the original study populations ranged between $r^2=0.75$ – 0.98 for pair-wise comparisons of the four associated SNPs, with the LD between the two SNPs showing the strongest statistical associations with type 2 diabetes being correlated at $r^2=0.98$. In this report, the LD between these two SNPs was similar ($r^2=0.97$), but for the remaining pair-wise comparisons the r^2 value ranged from 0.46 – 0.97 (Fig. 2). The differences in LD structure across the *WFS1* loci within the population of Västerbotten and in the populations from elsewhere that we studied may reflect different admixture patterns. Given the very high genotyping success and concordance rates for the Västerbotten cohort, it is unlikely that genotyping error explains these differences. It is, however, also possible that these differences in LD structure are attributable to statistical fluctuations.

Given the relatively small magnitude of these associations and the nominally significant *p* values in the Västerbotten

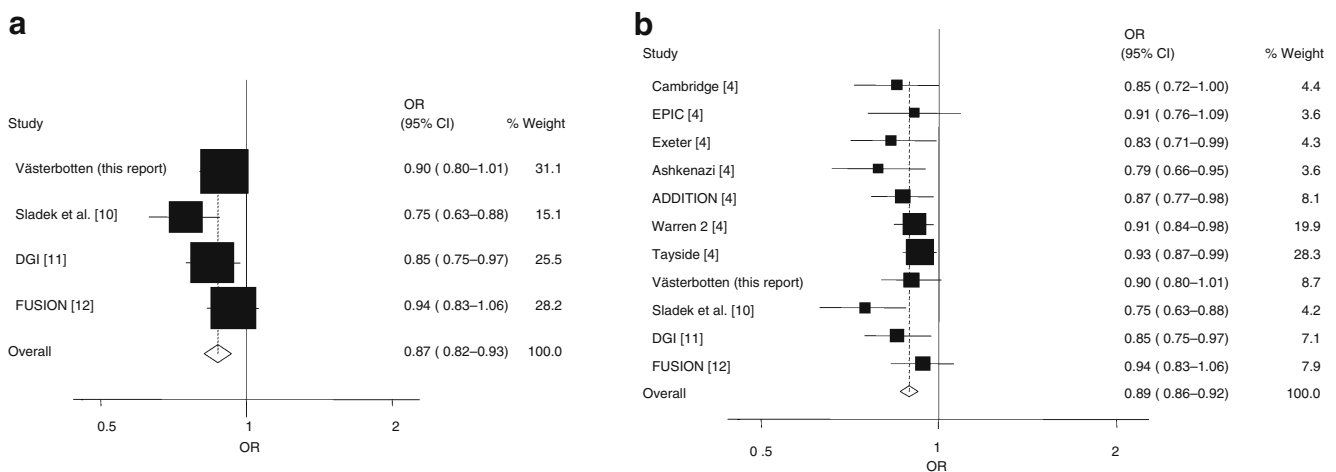


Fig. 3 **a** Association between *WFS1* SNPs and the risk of type 2 diabetes in previously unpublished studies. **b** Association between *WFS1* SNPs and the risk of type 2 diabetes in all currently available studies. Summary data for SNP rs10010131 were used in the meta-

analysis, with the exception of Sladek et al. [10] and DGI [11], where rs4416547 and rs10012946 were substituted as proxy markers. These SNPs are perfectly correlated ($r^2=1.0$) with rs10010131 in HapMap samples

study, it is likely that our failure to reproduce the associations for two of the *WFS1* SNPs is attributable to insufficient statistical power, rather than the absence of a true effect. In the original report on *WFS1* genotypes and type 2 diabetes risk, Sandhu et al. [4] reported ORs of approximately 0.90 to -0.92 per copy of the minor alleles at each locus. We calculated the sample size that would be required to detect these associations. At a level of statistical association of $p=0.05$ and a power of 80%, more than 3,000 case–control pairs would be required to detect an association of this magnitude, thus highlighting the need for large studies when seeking to detect associations where the effects on disease are modest.

Wolframin is a 100 kDa transmembrane protein that is expressed in neurons and pancreatic beta cells. The proposed functions of wolframin include the regulation of membrane trafficking, protein processing and calcium homeostasis in the endoplasmic reticulum of neurons and pancreatic beta cells [14, 15]. Disruption of these processes is believed to cause the progressive pancreatic beta cell loss and neuronal degeneration observed in Wolfram syndrome [16].

In the present study, we were unable to assess the effects of the *WFS1* genotypes on measures of insulin sensitivity or secretion. However, given the known mechanisms through which wolframin functions on pancreatic beta cells, it is possible that defects in insulin production underlie the genetic associations reported here and elsewhere with type 2 diabetes. In the DPP, nominal evidence of association between *WFS1* genotypes and measures of insulin secretion was observed [5]. Future studies focusing on this phenotype may help determine the mechanisms through which common variants at the *WFS1* gene and type 2 diabetes are related.

In conclusion, we have replicated the association between SNPs at the *WFS1* locus and risk of type 2 diabetes in a Swedish case–control study. Although we only found evidence for a statistical association with SNP rs752854, the direction and magnitude of the associations for the other three SNPs are consistent with previous reports. Furthermore, by undertaking a meta-analysis of additional data from European individuals, collectively comprising up to 12,979 cases and 14,937 controls, we have been able to robustly confirm the association of a second *WFS1* locus, rs10010131, with risk of type 2 diabetes.

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

References

- Barrett TG, Bunday SE, Macleod AF (1995) Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. *Lancet* 346:1458–1463
- Polymeropoulos MH, Swift RG, Swift M (1994) Linkage of the gene for Wolfram syndrome to markers on the short arm of chromosome 4. *Nat Genet* 8:95–97
- 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
- Sandhu MS, Weedon MN, Fawcett KA et al (2007) Common variants in *WFS1* confer risk of type 2 diabetes. *Nat Genet* 39:951–953
- Florez JC, Jablonski KA, McAteer J et al (2007) Testing of diabetes-associated *WFS1* polymorphisms in the Diabetes Prevention Program. *Diabetologia* DOI 10.1007/s00125-007-0891-x
- Weinehall L, Hallgren CG, Westman G, Janlert U, Wall S (1998) Reduction of selection bias in primary prevention of cardiovascular disease through involvement of primary health care. *Scand J Prim Health Care* 16:171–176
- World Health Organization (1999) Definitions, diagnosis and classification of diabetes mellitus and its complications: Part 1: Diagnosis & classification of diabetes mellitus. World Health Organization, Geneva
- Hallmans G, Agren A, Johansson G et al (2003) Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort—evaluation of risk factors and their interactions. *Scand J Public Health (Suppl 61)*:18–24
- Ranade K, Chang MS, Ting CT et al (2001) High-throughput genotyping with single nucleotide polymorphisms. *Genome Res* 11:1262–1268
- 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
- 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
- Steinthorsdottir V, Thorleifsson G, Reynisdottir I et al (2007) A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. *Nat Genet* 39:770–775
- 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
- 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
- Osman AA, Saito M, Makepeace C, Permutt MA, Schlesinger P, Mueckler M (2003) Wolframin expression induces novel ion channel activity in endoplasmic reticulum membranes and increases intracellular calcium. *J Biol Chem* 278:52755–52762