Diabetologia. Author manuscript; available in PMC 2009 April 17.

Published in final edited form as:
Diabetologia. 2008 March ; 51(3): 458–463. doi:10.1007/s00125-007-0887-6.

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

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Abstract

Aims/hypothesis: Mutations at the Wolframin encoding gene, *WFS1,* cause Wolfram syndrome, a rare neurological condition. Associations between single nucleotide polymorphisms (SNPs) at *WFS1* and type 2 diabetes have recently been reported. In the present study, we sought to replicate those associations in a northern Swedish case-control study for type 2 diabetes. We also meta-analyzed published and previously unpublished data from Sweden, Finland and France to obtain updated summary effect estimates.

Methods: Four *WFS1* SNPs (rs10010131, rs6446482, rs752854, rs734312 [R611H]) 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 body mass index. We then performed a meta-analysis of 11 studies of type 2 diabetes, comprising up to 14,139 cases 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 (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, or its proxy variants, showed evidence for statistical association (OR=0.87; 95% CI=0.82-0.93; $p=4.5\times10^{-5}$). In an updated meta-analysis of all 11 studies, comprising 14,139 cases and 16,109 controls, strong evidence for statistical association was also observed (OR=0.89; 95% CI=0.86-0.92; $p=4.9\times10^{-11}$).

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CONFLICT OF INTEREST STATEMENT:
None declared by any coauthor

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

Keywords

Wolfram Syndrome; WFS1; Genetic; Replication; Type 2 diabetes; Association study; Swedish; Meta-analysis

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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 Wolframin encoding gene (*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, rs734312 [R611H]) at the *WFS1* gene locus were shown to be convincingly associated with type 2 diabetes in six UK studies and one study of Ashkenazi Jews, which together comprised 9,533 cases and 11,389 controls [4]. In that study, the summary effect estimates (odd ratios) for the four *WFS1* polymorphisms ranged from 0.90-0.92, and all were statistically associated.

In 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 a direction consistent with previous reports (JC Florez, accompanying manuscript).

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 northern Swedish cohort by conducting an updated meta-analysis of type 2 diabetes case-control studies using published and unpublished data.

MATERIALS AND METHODS

Västerbottens type 2 diabetes case-control study

Twelve-hundred-ninety-six adults with type 2 diabetes were identified through registries covering the county of Västerbotten in northern Sweden (Figure 1), and 1,412 non-diabetic individuals, group matched on age, sex, examination date and geographic region of residence, were selected from the Västerbotten Intervention Programme (VIP) as controls (Table 1). Virtually all of these individuals were European whites. Type 2 diabetes was determined using the 1999 diagnostic criteria of the World Health Organization [5]. Participants with fasting capillary glucose concentrations <7.0mmol/l and no document history of diabetes underwent a 75g anhydrous oral glucose tolerance test. Accordingly, control subjects were those without a document history of diabetes and with glucose concentrations below the thresholds for type 2 diabetes [5]. Type 2 diabetes in the case 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 (see Figure 1). Analyses were repeated in this sub-sample to verify the genetic associations (see *Results* section). All living participants

provided written informed consent. Ethics permission was obtained from the Local Research Ethics Committee of Umeå University and approval for genetics investigations in this material was granted by the Swedish Data Inspection Board.

Clinical measurements—The VIP survey is a population-based observational cohort study where all residents of the county of Västerbotten aged 40, 50 and 60 years have, since 1985, been invited to attend their primary health care center 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 standardized across study centers [6]. Height and weight were measured using a calibrated wall-mounted stadiometer and scales, respectively. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Plasma glucose was assayed using fresh capillary plasma on a Reflotron benchtop analyzer (Boehringer Mannheim, Mannheim, Germany). A threshold glucose value of ≤2mmol/l was used to exclude potentially spurious values, resulting in the exclusion of three control individuals from the dataset.

Genetic analysis within the Västerbottens study—DNA was extracted from peripheral white blood cells. Prior to genotyping, all genomic DNA samples were diluted to 4ng/μl. SNPs were assayed at the Wellcome Trust Sanger Institute using the Taqman® MGB chemistry (Applied Biosystems, Foster City, CA), following the recommended protocol [7]. Genotyping success and concordance rates were >98% and 100% for all SNPs, respectively.

Additional case-control studies for meta-analysis

To increase statistical precision, we added relevant data from recent genome-wide association (GWA) scans of type 2 diabetes. We contacted relevant investigators of these GWA scans [8-11] and requested summary statistics (odds ratios and 95% confidence intervals) 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 [4], and therefore are a direct proxy for this SNP.

Statistical analysis

Statistical analyses were conducted using the SAS software v9.1 (SAS Inst., Carey, NC). 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 v4.0 (http://www.broad.mit.edu/mpg/haploview). Power calculations were performed using Quanto v1.1.1 (http://hydra.usc.edu/gxe). 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 (ln) to correct skewness; anti-logged means and 95% confidence limits are presented for the respective results. For the descriptive statistics, central tendency and variance are reported as means and standard deviations (SD), respectively. A p-value <0.05 was considered statistically significant. Meta-analysis of studies was performed using STATA v8.2 using a fixed effects model and inverse-variance-weighted averages of log odds ratios to obtain a combined estimate of the overall odds ratio. Between-study heterogeneity was assessed using the χ^2 statistic.

RESULTS

WFS1 genotype associations in the Västerbottens case-control study

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

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

In logistic regression analyses modeling 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 confidence limits were slightly wider for rs10010131, rs6446482 and rs752854, reflecting the reduced sample size (results not shown).

Results of the meta-analysis of WFS1 genotypes on 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 additional studies included in the meta-analysis have been reported previously [8-10]. 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. [8], this was SNP rs4416547, typed in 686 type 2 diabetes cases and 669 controls. For the DGI study [9], it was SNP rs10012946, typed in 1,464 cases and 1,467 controls. SNP rs10010131 was available for analysis from the candidate gene panels from the FUSION study, which comprised 1,160 cases and 1,172 controls [10].

In a meta-analysis of the Västerbotten study and the three additional studies, which comprised a total of 4,606 cases and 4,720 controls, we found strong evidence for a statistical association between the rs10010131 variant at *WFS1* and type 2 diabetes, (OR=0.87 (95% CI=0.82-0.93) at $P = 4.5 \times 10^{-5}$) (Figure 3a), providing clear evidence for independent replication. The magnitude of association was highly consistent among studies with no material heterogeneity among studies (p (3 df) = 0.17). Finally, we conducted an updated meta-analysis of all 11 published and unpublished studies. As anticipated, this analysis showed robust evidence for statistical association $p = 5.4 \times 10^{-11}$ (p for heterogeneity [10 df] = 0.42) (Figure 3b).

DISCUSSION

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

WFS1 SNP rs752854 was statistically associated with type 2 diabetes in our northern Swedish study, and the direction and magnitude of the association is 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 northern Swedish study, 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 attributable 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, and the LD between the two SNPs showing the strongest statistical associations with type 2 diabetes were correlated at r^2=0.98. In this report, the LD between these two SNPs was similar (r^2=0.97), but the remaining pair-wise comparisons ranged between r^2=0.46-0.97 (Figure 2). The difference in LD structure across the *WFS1* locus within the Västerbotten population and those studied elsewhere may reflect different admixture patterns and also highlights that it is unlikely that we are directly assessing the causal SNP that underlies these association signals. It may also be that these differences are attributable to statistical fluctuations.

Given the relatively small magnitude of these associations and the nominally significant p-values in the Västerbotten study, it is likely that our failure to reproduce the associations for three 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. reported odds ratios of approximately 0.90-0.92 per copy of the minor alleles at each locus [4]. 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 in order to be able to detect associations where the effects on disease are modest.

The protein encoded by the *WFS1* gene, Wolframin, is a 100-kD 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 recticulum of neurons and pancreatic beta-cells [12, 13]; disruption of these processes is believed to cause the progressive pancreatic beta-cell loss and neuronal degeneration observed in Wolfram syndrome [14].

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 (JC Florez, accompanying manuscript). 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 variation 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 in that study, 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.

Acknowledgments

We are grateful to the participants who took part in this study. We thank Kerstin Enquist for expert technical assistance with sample preparation and Ruth Loos for helpful comments during the preparation of the manuscript. We also thank Åsa Ågren, Sara Nilsson and Hubert Sjodin for help with data organization and Amanda Thompson for genotyping technical assistance. Some of the data from the Västerbottens study originates from the Västerbotten Intervention Programme; we express our gratitude to Lars Weinehall and others involved in that project. The study was funded via grants from the Swedish Diabetes Association (DIA2006-013 to PWF), Novo Nordisk (370579201 to PWF), Västerbotten County Council, and the NIH (DK62370 to FUSION and DK72193 to KLM). IB, FP, KAF are funded by The Wellcome Trust. OR was funded by Västerbotten County Council. PWF was funded in part by a strategic appointment grant from the Västerbottens ALF Committee (2006-09).

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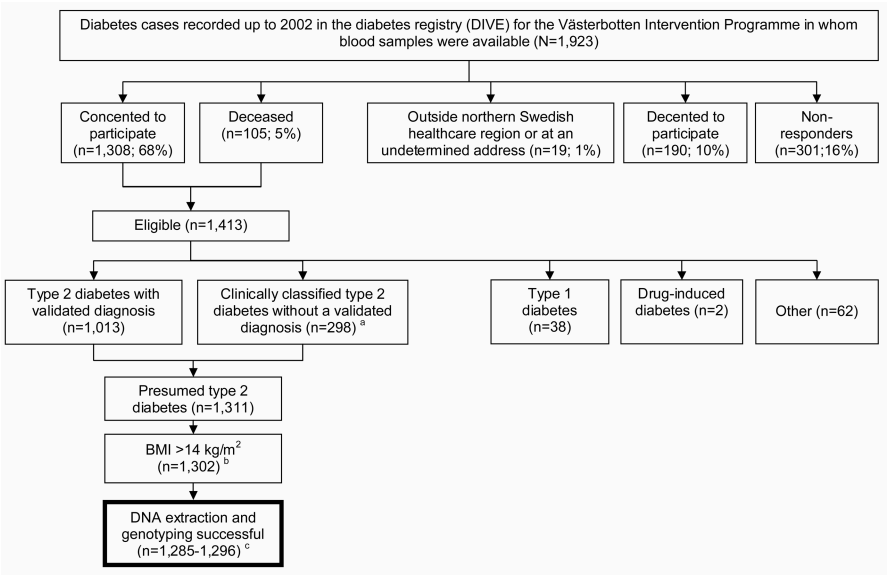

Figure 1.

Case selection criteria for participants in the Västerbottens type 2 diabetes case-control study. [a] Cases 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. [b] BMI data were missing in eight individuals. [c] *n* varies by genotype.

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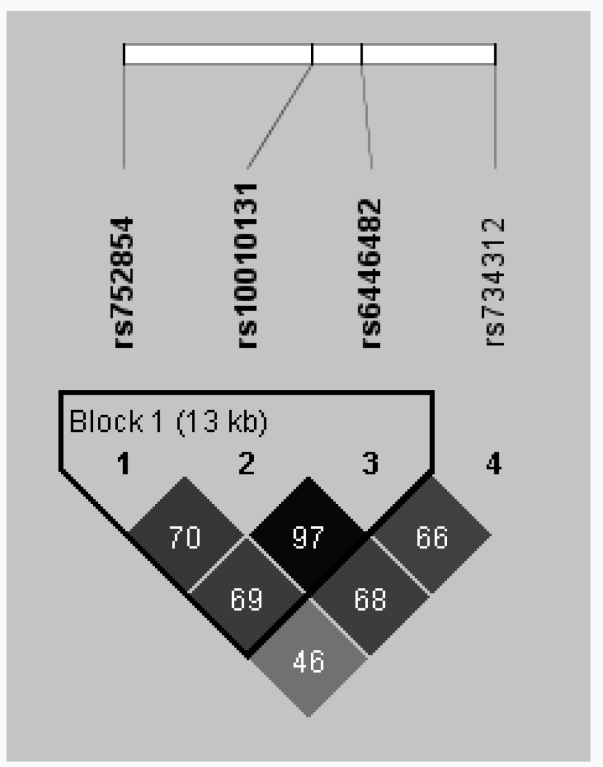

Figure 2.
Linkage disequilibrium (r^2) between *WFS1* genotypes in the control sample of the Västerbottens type 2 diabetes case-control study (N=1,412)

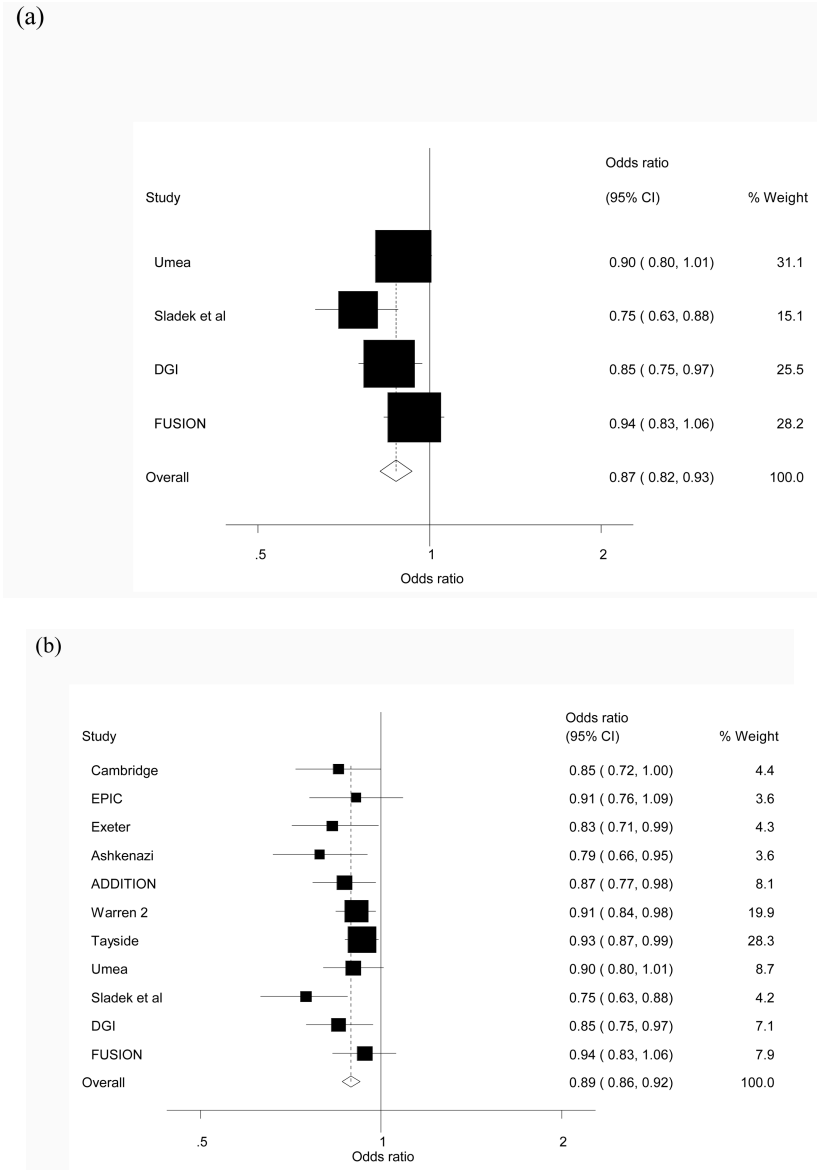

Figure 3.

(a) Association between *WFS1* SNPs and risk of type 2 diabetes in previously unpublished studies. (b) Association between *WFS1* SNPs and risk of type 2 diabetes in all currently available studies. Summary data for SNP rs10010131 was used in the meta-analysis with the exception of Sladek et al [8] and DGI [9] where rs4416547 and rs10012946 were substituted as proxy markers. These SNPs are perfectly correlated ($r^2 = 1.0$) with rs10010131 in HapMap samples.

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Table 1

Participant characteristics for the Västerbottens type 2 diabetes case-control study

	Cases (N=1,296)	Controls (N=1,412)	p-value
Age (years)	53.6 (7.57)	53.1 (8.55)	0.230
Sex (male/female) [a]	754/542	689/698	<0.001
Body mass index (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
2hr blood glucose (mmol/l) [b]	9.83 (4.90)	6.55 (1.48)	0.040

Data are means (SD) or

[a] *n*. p-values were calculated using t-tests for continuous traits or the Pearson correlation statistic for frequencies.

[b] In cases, 2hr glucose was available in only n=846.

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Table 2

Effect estimates for each of the *WFS1* SNPs in relation to type 2 diabetes in the Västerbottens type 2 diabetes case-control study

	Adjusted geometric means or Odds ratios (95% CI)			p-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

Results are odds ratios (OR: type 2 diabetes) and 95% confidence intervals from additive genetic models. . Data are adjusted for age, sex and BMI. p-values are from tests for linear trend as reported in the *Results*.