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Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes

John R.B. Perry¹, Michael N. Weedon¹, Claudia Langenberg², Anne U. Jackson³, Valeriya Lyssenko⁴, Thomas Sparsø⁵, Gudmar Thorleifsson⁶, Harald Grallert⁷, Luigi Ferrucci⁸, Marcello Maggio⁹, Giuseppe Paolisso¹⁰, Mark Walker¹¹, Colin N.A. Palmer¹², Felicity Payne¹³, Elizabeth Young², Christian Herder¹⁴, Narisu Narisu¹⁵, Mario A. Morken¹⁵, Lori L. Bonnycastle¹⁵, Katharine R. Owen¹⁶, Beverley Shields¹, Beatrice Knight¹, Amanda Bennett¹⁶, Christopher J. Groves¹⁶, Aimo Ruukonen¹⁷, Marjo Riitta Jarvelin¹⁸, Ewan Pearson¹², Laura Pascoe¹¹, Ele Ferrannini¹⁹, Stefan R. Bornstein²⁰, Heather M. Stringham³, Laura J. Scott³, Johanna Kuusisto²¹, Peter Nilsson⁴, Malin Neptin⁴, Anette P. Gjesing⁵, Charlotta Pisinger²², Torsten Lauritzen²³, Anelli Sandbaek²³, Mike Sampson²⁵, MAGIC, Ele Zeggini²⁶, Cecilia M. Lindgren^{16,27}, Valgerdur Steinthorsdottir⁶, Unnur Thorsteinsdottir⁶, Torben Hansen^{5,28}, Peter Schwarz²⁰, Thomas Illig⁷, Markku Laakso²¹, Kari Stefansson⁶, Andrew D. Morris¹², Leif Groop⁴, Oluf Pedersen^{5,24,29}, Michael Boehnke³, Inês Barroso¹³, Nicholas J. Wareham², Andrew T. Hattersley¹, Mark I. McCarthy^{16,27} and Timothy M. Frayling^{1,*}

¹Genetics of Complex Traits, Peninsula College of Medicine and Dentistry, University of Exeter, Magdalen Road, Exeter EX1 2LU, UK, ²MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK, ³Department of Biostatistics, Center for Statistical Genetics, University of Michigan School of Public Health, Ann Arbor, MI 48109, USA, ⁴Department of Clinical Sciences, Diabetes and Endocrinology, Lund University, University Hospital Malmö, Malmö, Sweden, ⁵Hagedorn Research Institute, 2820 Gentofte, Denmark, ⁶deCODE Genetics, 101 Reykjavik, Iceland, ⁷Institute of Epidemiology, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, 85764 Neuherberg, Germany, ⁸Longitudinal Studies Section, Clinical Research Branch, National Institute on Aging, NIH, Baltimore, MD, USA, ⁹Department of Internal Medicine and Medical Sciences, Section of Geriatrics, University of Parma, Parma, Italy, ¹⁰Department of Geriatrics and Metabolic Diseases Second, University of Naples, Naples, Italy, ¹¹Diabetes Research Group, School of Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne, UK, ¹²Biomedical Research Institute, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK, ¹³Metabolic Disease Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK, ¹⁴Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany, ¹⁵Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD 20892, USA, ¹⁶Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital Old Road, Headington Oxford OX3 7LJ, UK, ¹⁷Institute of Diagnostics, Clinical Chemistry and ¹⁸Institute of Health Sciences and Biocenter Oulu, University of Oulu, Box 5000, Fin90014, Finland, ¹⁹Department of Internal Medicine, University of Pisa, School of Medicine, Pisa, Italy, ²⁰Department of Medicine III, Division Prevention and Care of Diabetes, University of Dresden, 01307 Dresden, Germany ²¹Department of Medicine, University of Kuopio and Kuopio University Hospital, Kuopio 70210, Finland, ²²Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark, ²³Faculty of Health

*To whom correspondence should be addressed. Tel: +44 1392722935; Fax: +44 1392722926; Email: tim.frayling@pms.ac.uk

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Sciences and ²⁴Institute of Biomedical Science, University of Copenhagen, Copenhagen, Denmark ²⁵Department of Endocrinology and Diabetes, Norfolk and Norwich University Hospital NHS Trust, Norwich NR1 7UY, UK, ²⁶Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK, ²⁷Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK, ²⁸Faculty of Health Science, University of Southern Denmark, Odense, Denmark and ²⁹Faculty of Health, University of Aarhus, Aarhus, Denmark

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Epidemiological studies consistently show that circulating sex hormone binding globulin (SHBG) levels are lower in type 2 diabetes patients than non-diabetic individuals, but the causal nature of this association is controversial. Genetic studies can help dissect causal directions of epidemiological associations because genotypes are much less likely to be confounded, biased or influenced by disease processes. Using this Mendelian randomization principle, we selected a common single nucleotide polymorphism (SNP) near the *SHBG* gene, rs1799941, that is strongly associated with SHBG levels. We used data from this SNP, or closely correlated SNPs, in 27 657 type 2 diabetes patients and 58 481 controls from 15 studies. We then used data from additional studies to estimate the difference in SHBG levels between type 2 diabetes patients and controls. The *SHBG* SNP rs1799941 was associated with type 2 diabetes [odds ratio (OR) 0.94, 95% CI: 0.91, 0.97; $P = 2 \times 10^{-5}$], with the SHBG raising allele associated with reduced risk of type 2 diabetes. This effect was very similar to that expected (OR 0.92, 95% CI: 0.88, 0.96), given the *SHBG*-SNP versus SHBG levels association (SHBG levels are 0.2 standard deviations higher per copy of the A allele) and the SHBG levels versus type 2 diabetes association (SHBG levels are 0.23 standard deviations lower in type 2 diabetic patients compared to controls). Results were very similar in men and women. There was no evidence that this variant is associated with diabetes-related intermediate traits, including several measures of insulin secretion and resistance. Our results, together with those from another recent genetic study, strengthen evidence that SHBG and sex hormones are involved in the aetiology of type 2 diabetes.

INTRODUCTION

Circulating sex hormone levels are associated with type 2 diabetes, but the causal nature of these associations is controversial. Many observational epidemiological studies have described associations between type 2 diabetes and androgens (primarily testosterone), estrogens (estradiol) and their circulating binding protein, sex hormone binding protein (SHBG) (1). Meta-analyses of these studies show consistent sex-specific associations. In men, lower testosterone, higher estradiol and lower SHBG levels are associated with type 2 diabetes. In women, higher testosterone, higher estradiol and lower SHBG are associated with type 2 diabetes (1).

The differences in sex hormone levels between type 2 diabetes patients and controls are complicated by increased adiposity, which is also associated with reduced SHBG. However, differences in adiposity cannot fully explain the associations between sex hormones and type 2 diabetes. In both men and women, the differences in testosterone, estradiol and SHBG levels between type 2 diabetes patients and non-diabetic individuals remain very similar before and after controlling for BMI, age, ethnic background and, in females, menopausal status (1).

There is evidence that the associations between sex hormones and type 2 diabetes may be causal. First, a recent study described an association between two SNPs in the *SHBG* gene and type 2 diabetes (2). Second, prospective studies show that the levels of sex hormones are altered in individuals

diagnosed many years later (1). Third, hyper-androgenic disorders such as polycystic ovarian syndrome (PCOS) in women result in an increased risk of type 2 diabetes and are very strongly associated with insulin resistance (3). In men, androgen supplementation in the presence of central obesity and low testosterone levels increases insulin sensitivity (4–6). Fourth, male rats lacking the androgen receptor are insulin resistant (7) and female rats treated with testosterone after oophorectomy become insulin resistant (8), findings that are consistent with the sex-specific associations seen in humans.

Despite some evidence pointing towards a causal role of sex hormones in type 2 diabetes, further evidence is required. The recent genetic study involved a relatively small number of patients (359 women and 170 men), the effect sizes, with odds ratios (ORs) of 1.52 and 1.39, are similar to those for SNPs in the *TCF7L2* gene (9), which raises doubt as to why these associations were not found by genome-wide association approaches, and the evidence of association between *SHBG* SNPs and type 2 diabetes was not strong ($P = 0.02$ for each SNP under the best model) (2). It is well known that even well-conducted, well-controlled epidemiological associations can often be confounded by imperfect measurement of known, or unknown, related factors. Prospective studies establish whether or not a risk factor is present prior to disease diagnosis, but do not necessarily solve the concerns about epidemiological studies. Disease processes can start many years before disease diagnosis, which means that altered hormone levels observed prospectively could be a

consequence rather than cause of early disease processes. The causal direction of the associations between hyper-androgenism and insulin resistance in PCOS is not known. Studies in animals may not be relevant to humans and human trials of androgen therapy have focused on people with extreme levels of obesity or circulating hormones and conflicting results have been reported (10,11). Finally, insulin lowering interventions in non-diabetic men and women (without PCOS) lead to increased SHBG levels, suggesting that insulin can causally influence sex hormone dynamics (12,13).

Genetic studies can be a valuable additional tool which help to dissect causal directions of epidemiological associations (14). In most situations, alleles at genetic variants are randomly distributed between individuals who differ by conventional confounding factors. This 'Mendelian randomization' principle has been used recently in the context of metabolic traits to provide evidence that lifelong exposure to slightly raised C-reactive protein (CRP) (15), Interleukin-18 (IL18) (16), regulated on activation, normal T-cell expressed and secreted protein (RANTES) (17) or beta-carotene (18) levels are unlikely to alter the risk of type 2 diabetes, whereas some evidence for an aetiological association has been suggested for macrophage migration inhibitory factor (MIF) (19). Other examples of this approach provide mechanistic insight to disease. For example the association between variation in the *FTO* gene, that is associated with adiposity, and some cancers, provides some evidence that adiposity has an aetiological role in cancer (20).

In this study, we used the principle of Mendelian Randomization to provide evidence for or against an aetiological role of raised SHBG levels and reduced risk of type 2 diabetes. Our results indicate that higher SHBG levels may reduce the risk of type 2 diabetes.

RESULTS

Observed association between *SHBG* SNP and type 2 diabetes risk

We observed evidence of association between the *SHBG* SNP rs1799941 and type 2 diabetes (OR 0.94, 95% CI: 0.91, 0.97; $P = 2 \times 10^{-5}$), with the SHBG raising allele associated with reduced risk of type 2 diabetes (Figs 1 and 2). This result included correction for age, sex and BMI across 14 of the 15 studies (one study is uncorrected), but the result was similar when correcting for age and sex only (OR 0.94, 95% CI: 0.91, 0.97; $P = 2 \times 10^{-5}$). The result was also similar in men (OR 0.95, 95% CI: 0.91, 0.99; $P = 0.009$); and women (OR 0.93, 95% CI: 0.89, 0.98; $P = 0.003$). There was no evidence of heterogeneity in any analysis, either between or within sexes or with and without correction for BMI (all heterogeneity $P > 0.15$, maximum I²—a measure of the variation in effect size attributable to heterogeneity—29%). There was a trend towards an increased effect in carriers of two copies of the SHBG raising allele, compared with carriers of two copies of the SHBG lowering allele (OR 0.91, 95% CI: 0.85, 0.97; $P = 0.006$) (uncorrected). The SHBG lowering (G) allele at rs1799941 ranged in allele frequency from 0.72 to 0.75 in individual study controls and from 0.73 to 0.77 in individual study type 2 diabetes patients.

Observed differences in SHBG levels between type 2 diabetes patients and controls

Details of the differences in SHBG levels between type 2 diabetes patients and controls are given in Table 2 and in Figures 1 and 3. SHBG levels were 0.233 (95% CI: -0.35, -0.115) standard deviations lower in type 2 diabetes patients compared with controls, when controlling for BMI. This estimate differed little between men (OR -0.218, 95% CI: -0.360, -0.076) and women (OR -0.264, 95% CI: -0.475, -0.054).

Approximate expected association between *SHBG* SNP and type 2 diabetes risk

We next tested whether or not the observed association between the *SHBG* SNP and type 2 diabetes was consistent with that expected if SHBG levels alter the risk of type 2 diabetes. We used the estimates of the effect of rs1799941 on circulating SHBG levels and the difference in SHBG levels between type 2 diabetes patients and controls to calculate an approximate expected effect of rs1799941 with type 2 diabetes (Fig. 1). The expected associations were: for men and women combined (OR 0.92, 95% CI: 0.88, 0.96); for men (OR 0.92, 95% CI: 0.88, 0.97); and for women (OR 0.91, 95% CI: 0.84, 0.98). These estimates were similar to those observed.

Associations between *SHBG* SNPs and type 2 diabetes related intermediate traits

We did not detect any association between the *SHBG* SNP rs1799941 (or its close proxy rs12150660) and any measures of intermediate type 2 diabetes related traits in population-based studies. These included fasting insulin ($P = 0.11$, $N = 37\,864$), the HOMAIR measure of insulin resistance ($P = 0.24$, $N = 36\,601$), fasting glucose ($P = 0.52$, $N = 45\,691$), the HOMAB measure of beta-cell function ($P = 0.04$, $N = 36,135$) [all rs12150660], insulin secretion as assessed by oral glucose tolerance test-based measures of glucose, insulin and C-peptide at 30 and 120 min (all $P > 0.14$, $N = 5771$) or insulin resistance as measured by hyperinsulinaemic-euglycaemic clamps ($P = 0.94$, $N = 1229$) [all rs1799941].

DISCUSSION

Our study provides evidence that raised circulating SHBG levels reduce the risk of type 2 diabetes. Our results, together with those from the recent study by Ding *et al.* (2), provide insight into the role of sex hormones in the aetiology of type 2 diabetes. It has been known for some time that circulating SHBG levels are lower in people with type 2 diabetes compared with non-diabetic individuals, both in cross-sectional comparisons and prospectively. Despite this association, there has been considerable doubt as to the causal relevance of sex hormones in type 2 diabetes. The two genetic studies therefore add considerably to the argument that sex hormones are involved in the aetiology of the disease. This may have important implications for the treatment of diabetes or hypo- and hyper-androgenism in the two sexes.

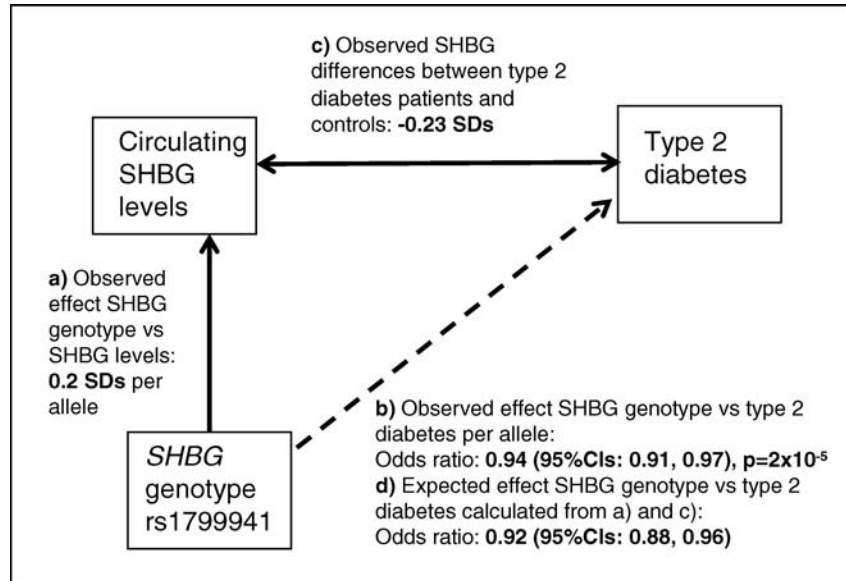


Figure 1. Triangulation approach to assess how the observed association of the *SHBG* SNP rs1799941 with type 2 diabetes compares with that expected given the association between rs1799941 and circulating SHBG levels and the difference in SHBG levels between type 2 diabetes patients and controls.

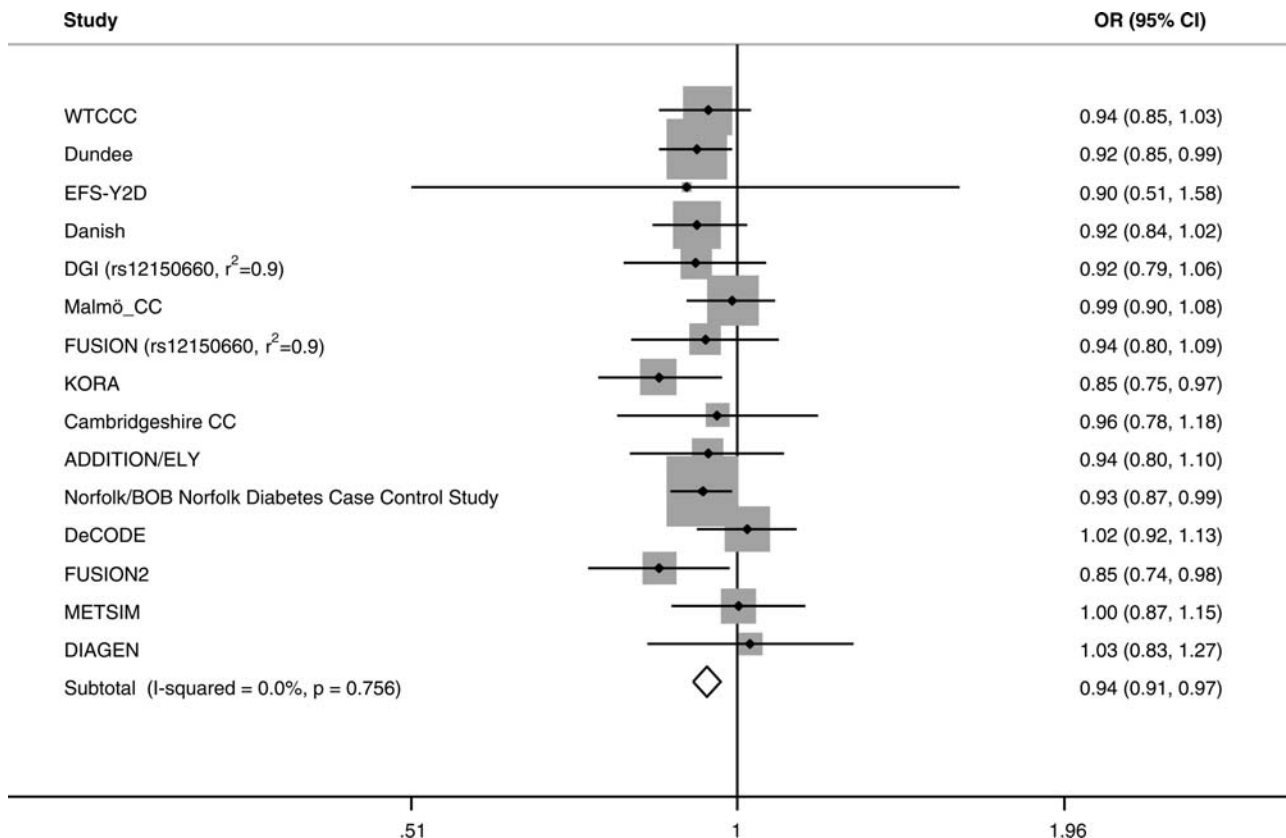


Figure 2. Meta-analysis of rs1799941 in type 2 diabetes case-control studies correcting for or matching for BMI, age and sex. OR, odds ratio.

There are several strengths to our study. First, genotypes are randomly distributed in ethnically homogenous populations, meaning our results are very unlikely to be confounded or biased or secondary to diabetes disease processes. Second,

the statistical confidence of the association between the *SHBG* variant and type 2 diabetes is very strong—of approximately 1 million independent common variants in European genomes, we would only expect 20 to reach this level of

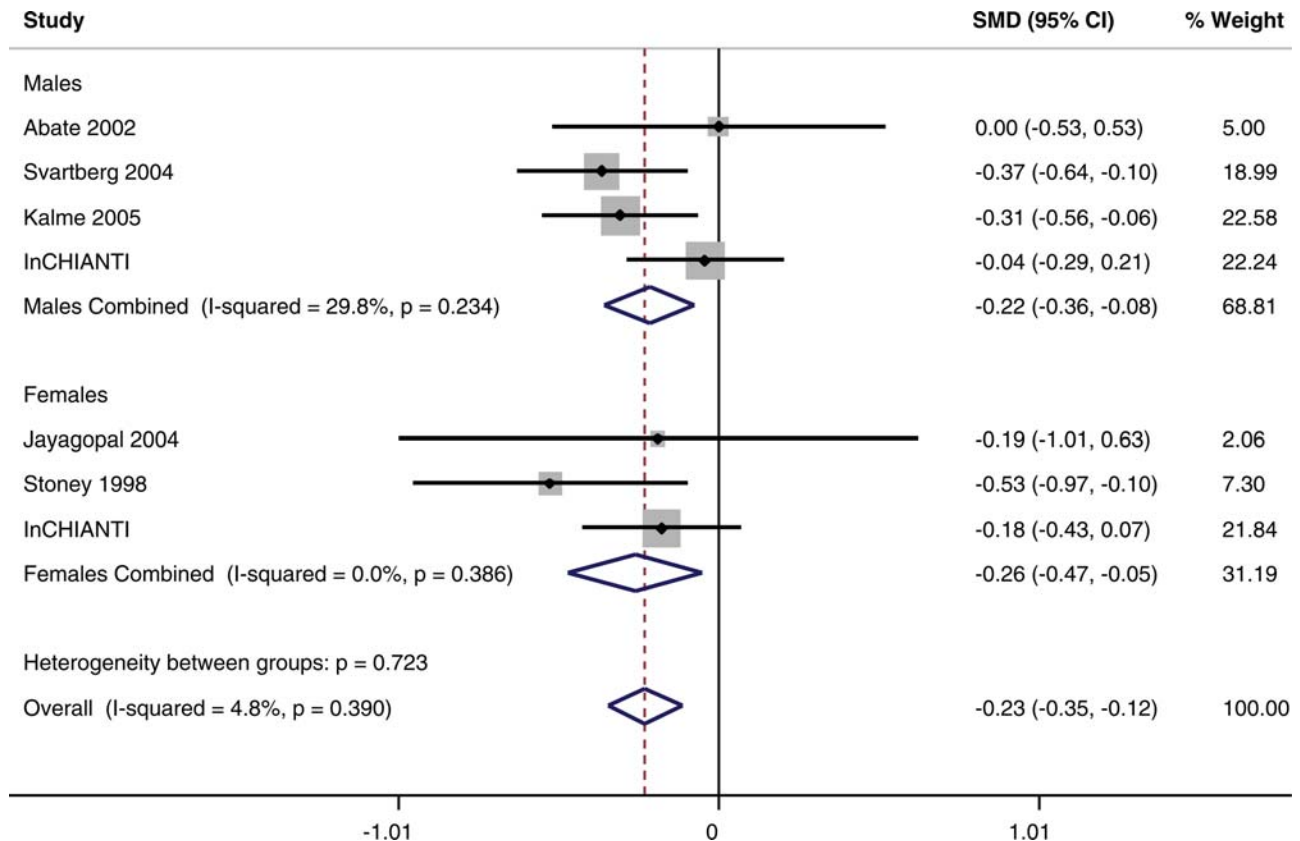


Figure 3. Details of the correlations between SHBG levels and type 2 diabetes from seven cross-sectional studies of European individuals correcting or matching for BMI or waist circumference.

statistical confidence by chance. Our results are consistent across 15 studies comprising 27 657 type 2 diabetic patients and 58 481 controls, with all but two studies showing an OR in the expected direction. Third, the ORs we observe between the *SHBG* rs1799941 SNP and type 2 diabetes are consistent with those expected from a triangulation approach. Fourth, the use of a SNP at the *SHBG* gene locus strongly associated with circulating SHBG levels means the association is very unlikely to be due to a pleiotropic effect of the variant on another pathway.

There are a few limitations to our study. Most notably we have not found any evidence for the mechanism behind the association between SHBG levels and type 2 diabetes. Despite the large numbers available, we did not observe any associations with various measures of insulin resistance or insulin secretion. This lack of association with intermediate type 2 diabetes traits is not inconsistent with the association with type 2 diabetes. The OR of 0.94 (equivalent to 1.06 for the SHBG lowering allele) is small and previous studies of variants of this magnitude have often not identified any associations with measures of insulin resistance or insulin secretion (21). This may in part be due to the reproducibility of measures based on insulin, which has a very short half life and fluctuates appreciably within individuals. We note, however, that the small effect size does not mean the finding has any less relevance to the question of aetiology, which relies only on the statistical confidence of the findings.

A further caveat to our study is that we used a different and much smaller set of patients and controls to estimate the magnitude of the association between circulating SHBG levels and type 2 diabetes compared with the association between the *SHBG* SNP and type 2 diabetes. This difference meant that the estimated effect is very approximate and may be biased by the availability of data from previous publications. The confidence intervals of our estimated effect do not take into account the error in the estimate of the SNP-SHBG levels and so this adds further to the uncertainty of this calculation. Despite these concerns, the estimated and observed ORs for the *SHBG* SNP effect on type 2 diabetes were very similar.

There are some notable differences between our study and that of Ding *et al.* (2). While both studies support an association between *SHBG* SNPs and type 2 diabetes, the effect sizes are very different. Ding *et al.* report ORs of 1.52 (0.66 inverted) and 1.39 for rs6259 and rs6257, respectively. These ORs are similar to those for SNPs in the *TCF7L2* gene, which represent the most strongly associated SNPs in genome wide association studies of type 2 diabetes. There is no evidence of association between rs6259 (a non-synonymous SNP, D356N) and type 2 diabetes in data from 4549 cases and 5579 controls [$P=0.72$, OR 1.02 [0.92–1.12], all of European ancestry] (22), and this SNP appears to be less strongly associated with SHBG levels than rs1799941, based on data from previous studies (23,24). The other SNP reported by Ding *et al.*, rs6257, is not in HapMap

but previously published data (25) indicates that rs1799941 and rs6257 are partially correlated and that rs1799941 drives the association with SHBG levels. These observations, together with our much larger sample size, suggest that the small OR of 0.94 is a closer reflection of the true effect size than that reported by Ding *et al.*

How might genetically lowered SHBG levels increase the risk of type 2 diabetes? Previous studies suggest that rs1799941 has different effects on total testosterone levels in men and women. Dunning *et al.* showed that the SHBG lowering allele at rs1799941 is not associated with total testosterone levels in postmenopausal women ($N = 1975$) (23). This means that women with genetically lower SHBG levels are exposed to proportionally more of the adverse effects of the biologically active unbound testosterone. In contrast, higher testosterone levels are thought to protect against adverse metabolic effects in men and Ahn *et al.* (25) showed that the SHBG raising allele at rs1799941 is strongly associated with higher total testosterone levels in men ($N = 4678$). This explanation is complicated by the fact that free testosterone levels are maintained in men by a strong feedback loop whereby luteinizing hormone (LH) production from the pituitary gland in the presence of low androgen levels stimulates testosterone production in the testes. This should mean that genetically lower SHBG levels will not greatly affect free testosterone levels in men. However, there is recent evidence that bound testosterone may be biologically active (26). If this is the case, then men with lower total testosterone due to lower SHBG will be exposed to less of the protective metabolic effects of androgens, despite similar levels of unbound testosterone.

There are additional implications of our results that require further study. First, our results and those of Ding *et al.* are not altered by adjustment or matching for BMI despite the fact that increased BMI and adiposity are correlated with reduced SHBG levels. This could mean that altered SHBG levels are part of the reason why increased BMI causally increases the risk of type 2 diabetes. Second, a SNP (rs6259) in the *SHBG* gene has been associated with prostate cancer and there is increasing evidence that there may be a causal link to the inverse association between prostate cancer and type 2 diabetes (27), although our results suggest that this requires additional studies of rs1799941 in prostate cancer (28).

In conclusion, a large type 2 diabetes case–control study provides strong statistical support for a role of SHBG and sex hormones in the aetiology of type 2 diabetes.

MATERIALS AND METHODS

Our methods were based on the triangulation approach outlined in Figure 1. In summary we used (i) a common single nucleotide polymorphism (SNP) near the *SHBG* gene, rs1799941, previously shown to be very strongly associated with SHBG levels; (ii) 27 657 type 2 diabetes patients and 58 481 controls from 15 studies to test the association between this SNP and type 2 diabetes risk; (iii) data from six studies to estimate the magnitude of the association between SHBG levels and type 2 diabetes and (iv) existing

data to calculate an approximate expected effect of the *SHBG* SNP on type 2 diabetes given the magnitude of the SNP versus SHBG levels association and the magnitude of the SHBG levels versus type 2 diabetes association.

Selection of a SNP strongly associated with SHBG levels

Several genetic studies have established unequivocal evidence that common variation close to the *SHBG* gene influences circulating SHBG levels. We used previously published information to quantify the association between a SNP, rs1799941, 67 base pairs upstream of the *SHBG* gene and circulating SHBG levels. Several studies have shown consistent associations between rs1799941 and SHBG levels ($P < 1 \times 10^{-16}$) (23–25). Each copy of the A allele is associated with a 0.20 SDs increase in SHBG levels, based on Finnish (NFBC66) and Italian (InCHIANTI) population-based studies, with similar effect sizes in men and women (24).

Observed association between *SHBG* SNP and type 2 diabetes risk

Through collaboration between 15 studies, we assembled a set of 27 657 cases and 58 481 controls. These studies varied in characteristics and ascertainment criteria and details are given in Table 1. All patients and controls were of European ancestry. The *SHBG* SNP rs1799941 was directly genotyped in all but three studies, two of which used a strongly correlated SNP (rs12150660 $r^2 = 0.9$ with rs1799941), available as part of genome wide association data (FUSION1 and DGI), and a third used a mixture of directly genotyped and imputed data (Decode). Genotyping methods and quality control steps are given in Supplementary Material, Table S1. Individual studies calculated a per allele OR for the A allele at rs1799941, correcting for age, sex and BMI (except publicly available DGI data where cases and controls were matched for gender and BMI, and WTCCC samples, where data for BMI in controls was not available). The FUSION2 study additionally corrected for birth province and used 5 year age category instead of age. We next used the ‘metan’ command in STATAv10 to meta-analyse these results across all 15 studies. This function weighs all studies by the inverse of their variance (a function of SNP frequency and study size) to obtain a per allele estimate of the type 2 diabetes OR for all studies. We quote the OR effect of the SHBG raising allele, which, because raised SHBG levels are correlated with reduced risk of type 2 diabetes, we expected to be < 1.0 , if SHBG levels are involved in the aetiology of type 2 diabetes. Given the different levels of SHBG in men and women we repeated analyses stratified by sex.

Observed differences in SHBG levels between type 2 diabetes patients and controls

SHBG levels are not routinely measured in type 2 diabetes patients and controls. Instead, we used data from type 2 diabetes studies (four of men only and three of women only) that had measured SHBG levels. These studies were men and women from the InCHIANTI study (24) and the cross-sectional studies published in the meta-analysis conducted

Table 1. Characteristics of type 2 diabetes patients and controls from 15 studies

Study	Type 2 diabetes					Control				
	N	Age Mean	SD	BMI Mean	SD	N	Age Mean	SD	BMI Mean	SD
Men										
WTCCC	1334	50.15	9.08	30.43	5.32	1042	N/A	N/A	N/A	N/A
Dundee	2205	55.38	8.81	30.78	5.36	2283	59.98	11.41	27.16	3.95
EFS-Y2D	102	40	5.45	30.63	4.9	861	32.7	5.97	26.59	3.87
Danish	2049	56.55	8.87	30.24	4.88	2252	47.06	9.16	26.11	3.52
DGI ^a	742	N/A	N/A	N/A	N/A	707	N/A	N/A	N/A	N/A
Malmö CC	1641	56.76	11	29.09	4.9	1287	57.7	6.08	25.52	3.08
FUSION1 ^{a,b}	623	53.59	9.09	29.44	4.02	573	63.42	7.62	27.02	3.52
KORA ^b	669	60.4	9.1	29.7	4.7	840	60.5	9	28	3.9
Cambridgeshire CC ^b	346	63.4	7.9	28.7	3.8	343	63.7	7.9	27.1	3.5
ADDITION/ELY ^b	553	60.2	7.5	32.3	5.4	725	61.2	9.2	27.1	3.9
NDCCS ^b	3,575	66.4	10.6	29.3	4.8	3,038	58.9	9.1	26.3	3.1
DeCODE	832	55	12.1	29.7	4.9	7603	64.3	16.4	27.4	4.5
FUSION2	646	58.3	8.82	30.23	4.96	724	57.45	7.58	26.74	3.41
METSIM ^c	818	60.84	5.99	30.07	5.2	3372	57.98	6.4	26.25	3.46
DIAGEN	255	62.55	11.06	29.25	4.46	267	55.87	14.38	26.52	3.48
Total	16 390					25 917				
Women										
WTCCC	951	49.82	9.56	32.86	7.08	1039	N/A	N/A	N/A	N/A
Dundee	1666	56.36	8.98	32.82	6.83	2414	56.97	12.04	26.89	5.17
EFS-YT2D	89	37.88	7.18	34.14	7.78	901	30.44	5.25	23.97	4.39
Danish	1398	57.35	9.89	31.28	6.28	2607	46.81	8.97	25.08	4.39
DGI ^a	722	N/A	N/A	N/A	N/A	760	N/A	N/A	N/A	N/A
Malmö CC	1150	59.46	11.89	30.46	6.13	2210	57.34	5.96	24.79	3.83
FUSION1 ^{a,b}	470	54.03	8.98	31.2	5.25	599	63.71	7.27	27.24	4.14
KORA ^b	538	61.6	9.4	31.8	5.8	678	61.4	9.2	27.9	4.7
Cambridgeshire CC ^b	202	63.3	7.9	31.6	6.8	191	63.5	7.7	27.8	5.1
ADDITION/ELY ^b	339	62.9	6.4	34.2	6	885	60.5	9.1	27.1	5.4
NDCCS ^b	2,481	67.1	11.4	30.8	6.5	3,390	57.8	9.3	25.9	4
DeCODE	582	55.2	12.7	30.6	6	16007	57.2	18.2	26.5	5.2
FUSION2	437	61.09	7.45	31.75	5.76	463	60.92	7.34	27.14	4.6
DIAGEN	242	67.49	12.3	31.09	7	420	55.93	14.09	26.62	4.78
Total	11 267					32 564				

N/A, clinical characteristics not available separated by sex. Age is the age at diagnosis for diabetic patients unless otherwise stated.

^aDGI and FUSION1 studies used proxy SNP ($r^2 = 0.9$) from GWAS data rs12150660.

^bAge at recruitment given for these studies.

^cMETSIM is a study of men only.

by Ding *et al.* (1), for which we were able to retrieve mean and standard deviation measures of SHBG in cases and controls, were restricted to individuals of European ancestry, and had matched or corrected SHBG levels for body mass index or waist circumference. Details of these studies are given in Table 2. For each study, we tabulated six variables: number of cases, mean case SHBG levels, standard deviation of case SHBG levels, number of controls, mean control SHBG levels and standard deviation of control SHBG levels, using ngml^{-1} as the units of SHBG. We performed an inverse variance weighted meta-analysis of these studies, both overall and within sexes, using the 'metan' command in STATAv10, to derive a standardized mean difference (SMD) of SHBG levels between type 2 diabetes cases and controls.

Approximation of the expected association between SHBG SNP and type 2 diabetes risk

We used the estimates of the effect of rs1799941 on SHBG levels in (i) and the correlation between SHBG levels and type 2 diabetes estimated in (iii) to calculate an approximate

expected effect of rs1799941 on type 2 diabetes, assuming that there is an aetiological association between SHBG levels and type 2 diabetes risk. We used the formula:

$$\text{OR}_{\text{expected}} = \exp(1.81 \times (\text{SD}_A \times \text{SMD}_B))$$

where SD_A is the per allele effect of rs1799941 on circulating SHBG levels expressed as a standard deviation and SMD_B is the standardized mean difference (SMD) of SHBG levels between type 2 diabetes cases and controls. This approach has previously been described in the context of diabetes-related traits (29) and the rationale for the formula for approximating SMDs to ORs is described in more detail in Chinn *et al.* (30). The figure 1.81 is the scaling factor used to approximate $\ln(\text{ORs})$ to and from standardized mean differences (30).

Associations between SHBG SNP and type 2 diabetes related traits

To test for associations between the SHBG SNP rs1799941 (or its close proxy rs12150660) and type 2 diabetes related traits,

Table 2. Details of SHBG levels in type 2 diabetes patients and normal glycaemic individuals in seven cross-sectional studies

	Type 2 diabetic patients					Controls				
	N	Age (SD)	BMI (SD)	SHBG Mean	SD	N	Age (SD)	BMI (SD)	SHBG Mean	SD
Men										
Abate <i>et al.</i> (34) ^a	33	55 (9)	27.6 (5.0)	19.10	13.00	24	45 (9)	28.2 (7.6)	19.10	13.00
Svartberg <i>et al.</i> (35) ^b	55	N/A	N/A	43.70	21.40	1364	N/A	N/A	51.80	22.00
Kalme <i>et al.</i> (36) ^c	109	N/A	N/A	63.20	29.23	152	N/A	N/A	71.60	25.20
InCHIANTI ^d	71	72.0 (9.4)	27.80 (3.45)	85.12	46.83	476	66.6 (16.2)	26.91 (3.36)	87.22	47.56
TOTAL	268					2016				
SMD (95%CI)										-0.218 (-0.360, -0.076)
Women										
Jayagopal <i>et al.</i> (37) ^e	12	62 (50–73)	31.6 (25.1–35.7)	38.80	18.20	11	56 (48–70)	32.0 (26.6–44.4)	42.20	17.10
Stoney <i>et al.</i> (38) ^f	42	64 (6.5)	28.8 (5.2)	27.80	20.50	42	63 (6.5)	28.9 (4.5)	40.30	26.12
InCHIANTI (4)	67	75.7 (9.8)	29.34 (5.05)	102.92	75.47	650	68.4 (15.8)	27.04 (4.58)	118.22	85.17
TOTAL	121					703				
SMD (95% CI)										-0.264 (-0.475, -0.054)

Data are from individuals of European ancestry matched or corrected for BMI or waist circumference. Women are postmenopausal.

SMD, standardized mean difference; N/A, not available from original publications.

^aControls selected to have the same range of BMI as cases. Controls include two individuals and cases include eight individuals of Hispanic ancestry.

^bSvartberg *et al.*, SHBG means and SDs are corrected for age and waist circumference.

^cKalme *et al.*, SHBG means and SDs are corrected for BMI.

^dInCHIANTI SHBG values are geometric values derived from lnSHBG levels corrected for age and BMI.

^eControls weight matched to cases. For BMI and age, median and range given.

^fStoney *et al.* controls selected to match cases for BMI and age.

we used data from a variety of sources. For measures of fasting glucose and homeostatic model assessment (HOMA) based measures of insulin resistance, we used data from MAGIC of between 36 135 and 45 691 individuals, details of which are described in detail elsewhere (31). For measures of insulin secretion, we used 5771 individuals from the Danish study, details of which are described in detail elsewhere (32). For measures of whole body/peripheral insulin resistance, we used 1229 individuals from the RISC study (33). All statistical tests were performed using appropriate transformations (described in the relevant publications) and a per allele 1.d.f. test (in keeping with the additive effect of rs1799941 on SHBG levels).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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