SUPPLEMENTARY DATA

Case ascertainment

Ascertainment of incident type 2 diabetes involved a review of the existing EPIC datasets at each center using multiple sources of evidence including self-report, linkage to primary-care registers, secondary-care registers, medication use (drug registers), hospital admissions and mortality data. Information from any follow-up visit or external evidence with a date later than the baseline visit was used. Rather than self-report, cases in Denmark and Sweden were identified via local and national diabetes and pharmaceutical registers and hence all ascertained cases were considered to be verified. Some cases in centers other than Denmark and Sweden were based on only one source of information. To increase the specificity of the definition for these cases, we sought further evidence including review of individual medical records in some centers. Follow-up was censored at the date of diagnosis, 31 December 2007 or the date of death, whichever occurred first.

Measurement of biomarkers (except fetuin-A)

The measurements were performed on serum (except for participants in the Umeå center in Sweden, where only plasma samples were available) or erythrocyte samples that had been previously frozen in liquid nitrogen or at ultra-low-temperature freezers at -80°C (samples from Umea, Sweden). γ-glutamyltransferase (GGT), alanine transaminase (ALT), albumin, creatinine, C-reactive protein (CRP), high-density lipoprotein (HDL) cholesterol, total cholesterol, and triglycerides were measured using a Cobas® (Roche Diagnostics, Mannheim, Germany) assay on a Roche Hitachi Modular P analyzer. Erythrocyte HbA_{1c} was measured using Tosoh (HLC-723G8) ion exchange high-performance liquid chromatography on a Tosoh G8 analyzer.

Genotyping

DNA was extracted from buffy coat from a citrated blood sample using standard procedures on an automated Autopure LS DNA extraction system (Qiagen, Hilden, Germany) with PUREGENE chemistry (Qiagen). The DNA was hydrated overnight prior to further processing, quantified by PicoGreen assay (Quant-iT) and normalised to 50 ng/ µl. Samples were genotyped if they had sufficient DNA, could be successfully genotyped on Taqman or Sequenom platforms and had sex chromosome genotypes concordant with self-reported sex. Samples that failed one genotyping round were repeated in another if they failed for reasons that may not relate to sample quality (e.g. signal intensity outliers or plates/arrays with an unusually high failure rate). Samples to be genotyped on the Illumina 660 W-Quad BeadChip were randomly selected from the available samples with the number of individuals selected per center being proportional to the percentage of total cases in that center. Danish samples were not available at this stage. Genotyping was carried out at the Wellcome Trust Sanger Institute. The remaining samples were genotyped on either the Illumina HumanCoreExome-12 or HumanCoreExome-24 at Cambridge Genomic Services in the University of Cambridge, Department of Pathology.

Before imputation, SNPs were filtered to remove those with minor allele count < 2, call rate < 95% or Hardy Weinberg p-value < 1e-6. SNPs were also removed if they were not found in the Haplotype Reference Consortium (HRC) panel, were A/T or G/C with MAF > 0.4, had an allele frequency difference > 0.2 with reference panel, or were indels. Imputation was performed at the Wellcome Trust Centre for Human Genetics using the HRC panel version 1.0 and IMPUTE v2.3.2 software.

Instrumental variable construction

Unless variants are highly correlated, all variants which can be reasonably assumed to be valid instruments can be included in a Mendelian randomization analysis to improve the precision of the causal estimate (1). To account for the high correlation within the AHSG gene, we first identified tagging SNPs based on HapMap 22/phaseII CEPH population data applying stringent criteria (minor allele frequency >5%, pairwise $r^2 \geq 0.80$) as similarly done earlier (2). As this selection process does not

rule out high intercorrelation, the five SNPs identified (rs4917, rs2070635, rs2070633, rs2248690, rs4831) were subsequently included in the same regression model and gene scores as instrumental variables were based on those SNPs showing a significant independent association with fetuin-A in this model.
The recent GWAS by Jensen et al. (3) has identified several more SNPs to be univariately associated with fetuin-A levels. E.g. 5 SNPs showed very low p-values and were not in high LD with rs4917 in the GWAS (3). However, all 5 are well covered by rs2070633 (r^2 ranging from 0.811 to 0.875 based on HapMap CEPH) selected for our gene score. Overall, the considerable degree of LD among SNPs in the AHSG gene, the magnitude of association of other SNPs identified in the GWAS (3) besides those mentioned above (substantially weaker compared to rs4917), and the statistical power of our instrumental variables (see below) makes it unlikely that inclusion of additional SNPs would substantially improve our instrumental variable approach.

Meta-Analysis of DIAGRAM and EPIC-InterAct data
We have used summary statistics of a meta-analysis consisting of 12,171 type 2 diabetes cases and 56,862 controls across 12 GWAS as published elsewhere (4). All studies participating in DIAGRAM included men and women of European descent. DIAGRAM data are publicly available at http://diagram-consortium.org/downloads.html. We extracted the odds ratio and corresponding 95% CIs for each of the three SNPs that were used to create the genetic scores (rs4917, rs2070633, rs2248690).

Power calculation
The power for the Mendelian Randomization analysis at a two-sided α of 0.05 was calculated based on the available sample size, the association of the genetic variables with fetuin-A concentration, and the expected causal effect estimate using the online tool mRnd (http://glimmer.rstudio.com/kn3in/mRnd/).
Given the HR of 1.18 per 50 μg/ml higher fetuin-A in our observational analysis, we would have expected a HR of 1.19 per allele of the weighted genetic score based on the association of the genetic variables with fetuin-A concentration in the subcohort of the Potsdam center. In EPIC-InterAct, we have 99.9% power to detect this expected HR with the weighted genetic score or with the single SNPs (rs4917, rs2070633, and rs2248690). Combining our data with those from the DIAGRAM consortium further increased the power enabling us to detect a HR of 1.05 with sufficiently high power (89% power for rs4917, 89% for rs2070633, and 81% for rs2248690).

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SUPPLEMENTARY DATA

Supplementary Table 1. Association of individual AHSG SNPs with fetuin-A concentration in the Potsdam part of the EPIC-InterAct study subcohort

SNP	Allele 1	Allele 2	Mean level (μg/ml) of fetuin-A by *AHSG* genotype			β (SE) for fetuin concentration	*p*	r^2
			11	12	22			
rs4917	T	C	214	257	299	44.1 (2.6)	3.40×10^{-56}	0.24
rs2070635	A	G	238	272	308	37.0 (2.6)	1.49×10^{-41}	0.18
rs2070633	T	C	223	267	307	42.3 (2.5)	1.56×10^{-57}	0.24
rs2248690	T	A	212	252	291	41.8 (2.9)	2.59×10^{-43}	0.19
rs4831	G	C	270	267	272	4.0 (4.4)	0.3719	0.0009

n=965

β obtained from single univariate linear regression models for each SNP with SNPs modelled per fetuin-A-increasing allele and fetuin-A expressed in μg/ml

Supplementary Table 2. Association of weighted genetic score with potential confounders

Continuous traits	β (SE)	*p*
Age, years	-0.26 (0.13)	0.05
Alcohol intake, g/d	0.38 (0.30)	0.21
GGT, U/l	0.49 (0.63)	0.44
ALT, U/l	0.08 (0.21)	0.71
Albumin, g/l	-0.03 (0.05)	0.50
Creatinine, umol/l	-0.17 (0.24)	0.49

Binary traits	Odds ratio (95% CI)	*p*
Sex, male	0.97 (0.91, 1.04)	0.41
Current smoking	0.93 (0.87, 1.00)	0.06
Physically active	1.01 (0.93, 1.09)	0.83
Longer education (incl. university)	0.94 (0.87, 1.02)	0.13

β obtained from linear regression (age, alcohol intake, GGT, ALT, albumin, creatinine) and ORs obtained from logistic regression (sex, current smoking, physically active, longer education); estimates are per fetuin-A-increasing allele of the weighted genetic score, adjusted for country among 10,850 subcohort participants

SUPPLEMENTARY DATA

Supplementary Table 3. Association of weighted genetic score with potential mediators

Mediators (continuous)	β (SE)	*p*
BMI, kg/m^2	0.04 (0.06)	0.52
Waist circumference, cm	0.19 (0.19)	0.33
CRP, mg/l	-0.06 (0.06)	0.38
HDL cholesterol, mmol/l	0.0009 (0.007)	0.90
Total cholesterol, mmol/l	-0.03 (0.02)	0.12
Triglycerides, mmol/l	-0.002 (0.01)	0.91
HbA_{1c}, mmol/mol	0.08 (0.08)	0.31

β obtained from linear regression; estimates are per fetuin-A-increasing allele of the weighted genetic score, adjusted for country among 10,850 subcohort participants

Supplementary Table 4. Instrumental variable estimates for the weighted genetic score in strata of age, sex, waist circumference, and HbA_{1c} in the EPIC-InterAct study

Strata	HR (95% CI) per 50μg/ml increase in fetuin-A concentration
Age≤55 years (*n*=11,953)	1.01 (0.92, 1.11)
Age>55years (*n*=10,426)	1.04 (0.97, 1.10)
Men (*n*=9,691)	1.03 (0.93, 1.13)
Women (*n*=12,688)	1.03 (0.94, 1.11)
Low waist circumference (*n*=10,450) (Men: ≤98 cm, Women: ≤84 cm)	1.01 (0.93, 1.10)
High waist circumference (*n*=10,163) (Men: >98 cm, Women: >84 cm)	1.02 (0.96, 1.09)
HbA_{1c}≤5.6% (38 mmol/mol) (*n*=12,064)	1.03 (0.93, 1.12)
HbA_{1c}>5.6% (38 mmol/mol) (*n*=9,865)	0.97 (0.89, 1.07)

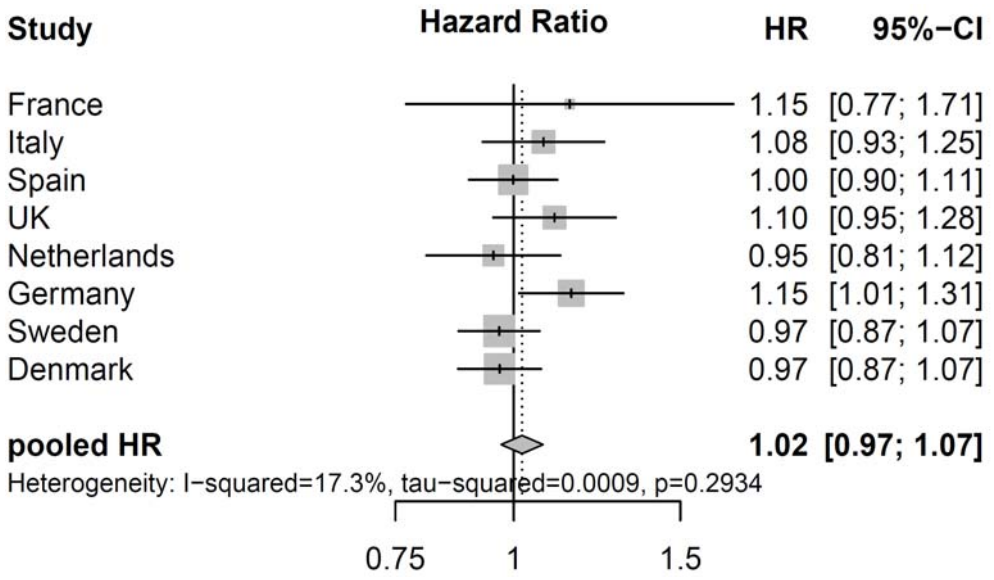
Instrumental variable estimates were obtained from two-stage least squares procedure. Data are adjusted for age, sex, study center, and genotyping source. Analyses have been performed stratified by country and combined with random-effects meta-analysis.

SUPPLEMENTARY DATA

Supplementary Figure 1. Forest plot of the association of the weighted genetic score with diabetes risk in the EPIC-InterAct study by country

Data are HRs and 95% CIs per fetuin-A-increasing allele, adjusted for age, sex, study center, and genotyping source. Analyses have been performed stratified by country and combined with random-effects meta-analysis.

n=22,379 including 12,975 incident cases of type 2 diabetes



Supplementary Figure 2. Forest plot of the association of the unweighted genetic score with diabetes risk in the EPIC-InterAct study by country

Data are HRs and 95% CIs per fetuin-A-increasing allele, adjusted for age, sex, study center, and genotyping source. Analyses have been performed stratified by country and combined with random-effects meta-analysis.

n=22,379 including 12,975 incident cases of type 2 diabetes