

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Meigs JB, Shrader P, Sullivan LM, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. *N Engl J Med* 2008;359:2208-19.

Supplementary Appendix

Meigs et al: Genetic Prediction of Type 2 Diabetes Beyond Common Risk Factors: The Framingham Study

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Supplementary Figure Legends

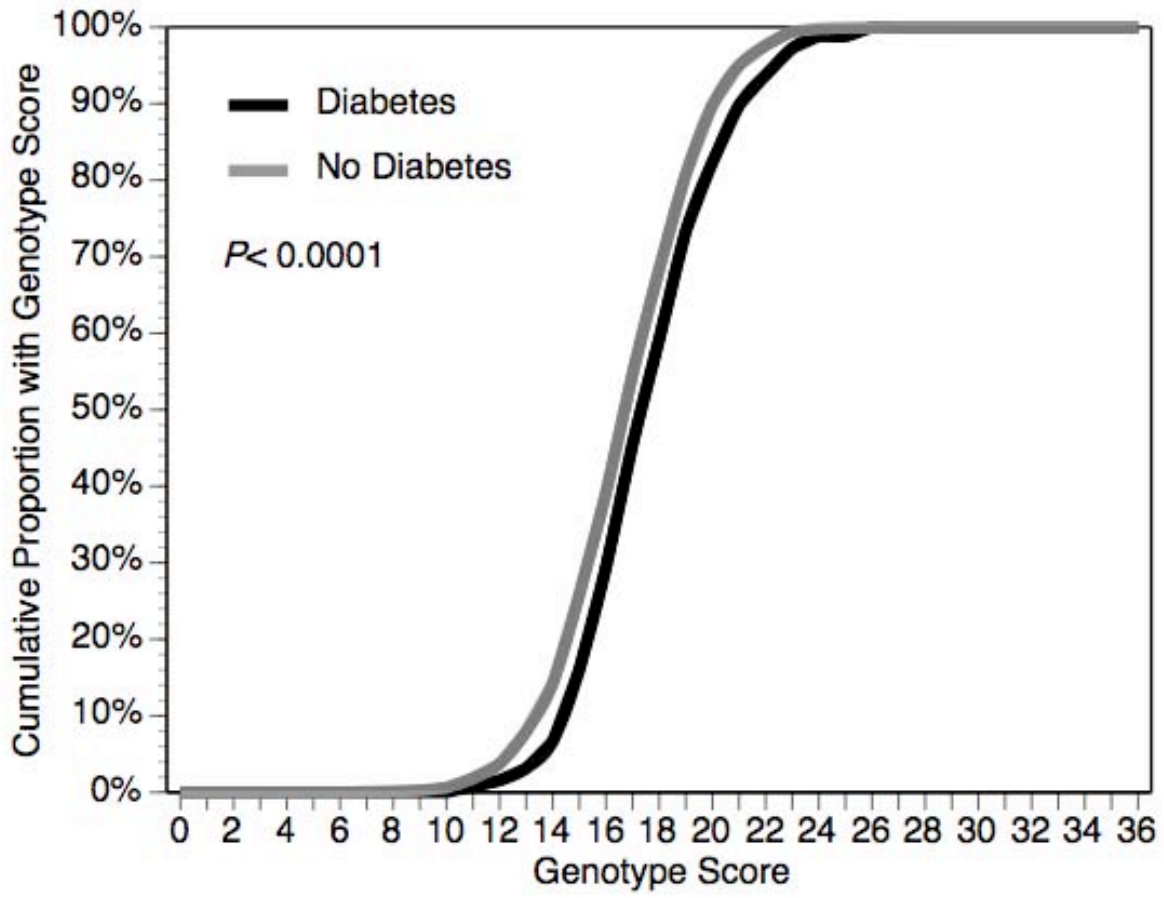
Supplementary Figure 1. The cumulative proportion of 2,377 Framingham Offspring Study subjects by genotype score, stratified by individuals who developed diabetes over 28 years of follow-up and by those who did not develop diabetes. The score distribution is shifted significantly ($P < 0.0001$) towards a higher genotype score among individuals with diabetes.

Supplementary Figure 2. The 28-year cumulative incidence of type 2 diabetes by genotype score. Individuals with a score ≤ 12 or ≥ 24 were grouped due to small numbers at the extremes of the genotype score distribution. The cumulative incidence of diabetes increases significantly ($P < 0.0001$) with increasing genotype score.

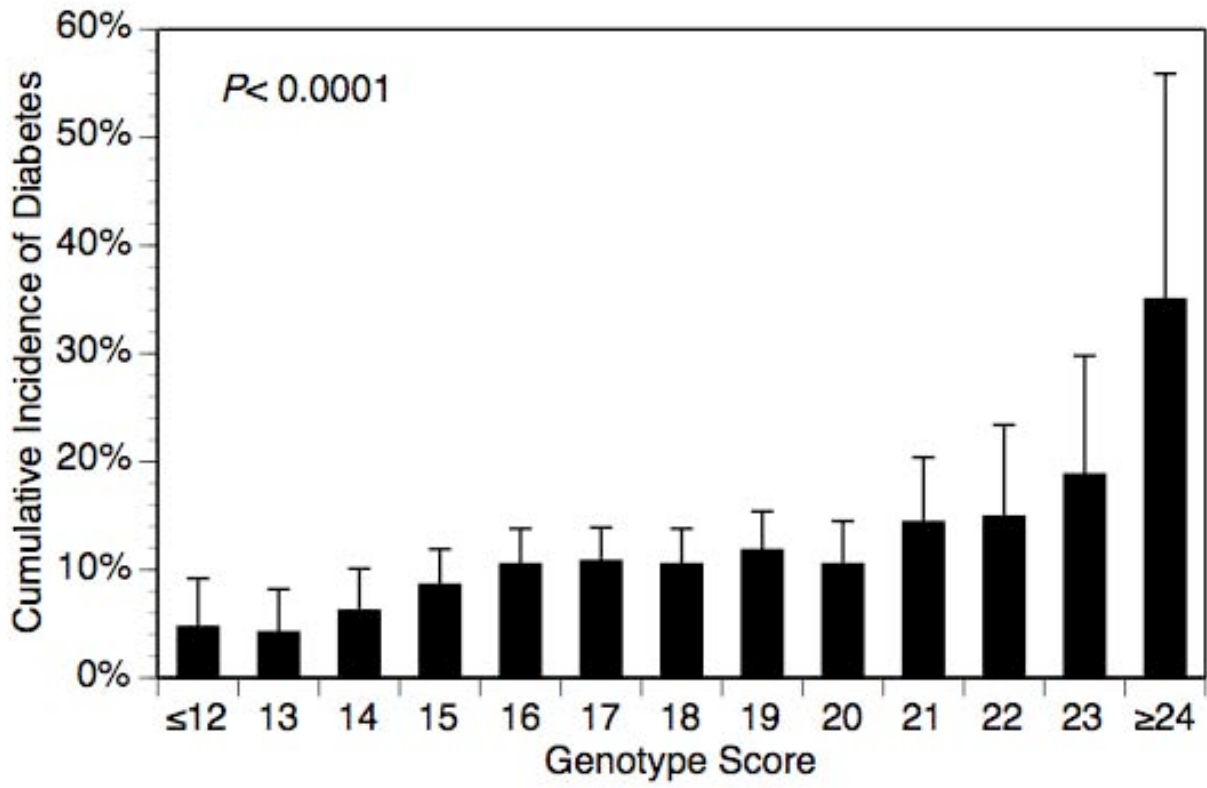
Supplementary Figure 3. Calibration plots for the sex-adjusted, sex- and self-reported family history-adjusted, and simple clinical model-adjusted regression models shown in Table 3.

Supplementary Figure 4. The predicted risk for type 2 diabetes based on the simple clinical model-adjusted model (manuscript Table 3 and reference #5). The dashed vertical lines indicate the cut-points defining low predicted risk ($< 2\%$, 66.9% of the sample), medium predicted risk (2 to $< 8\%$, 20.8% of the sample) and high predicted risk ($\geq 8\%$, 12.3% of the sample) used in the net reclassification improvement matrices.

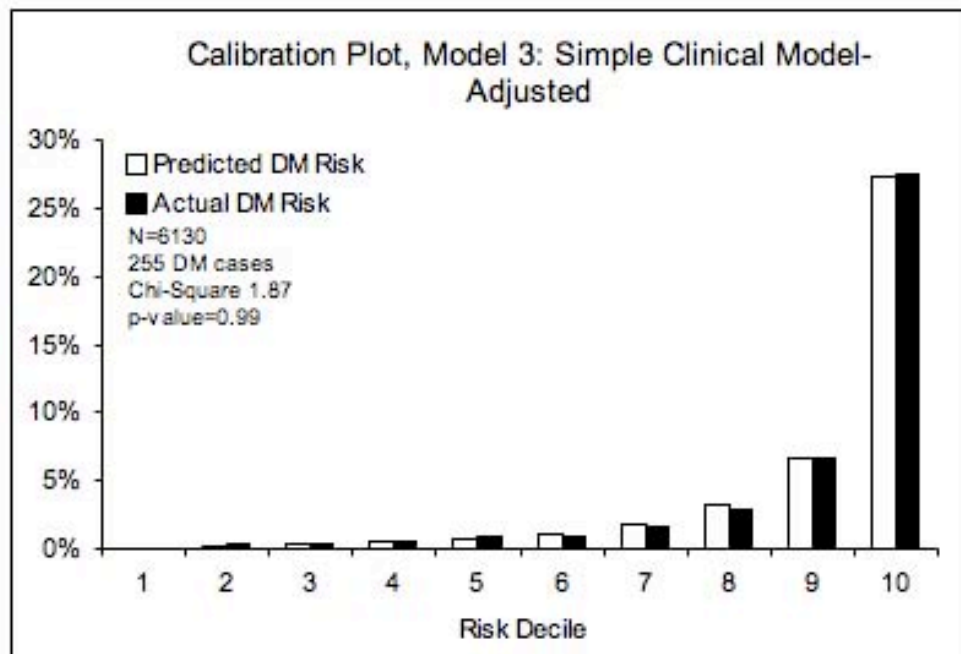
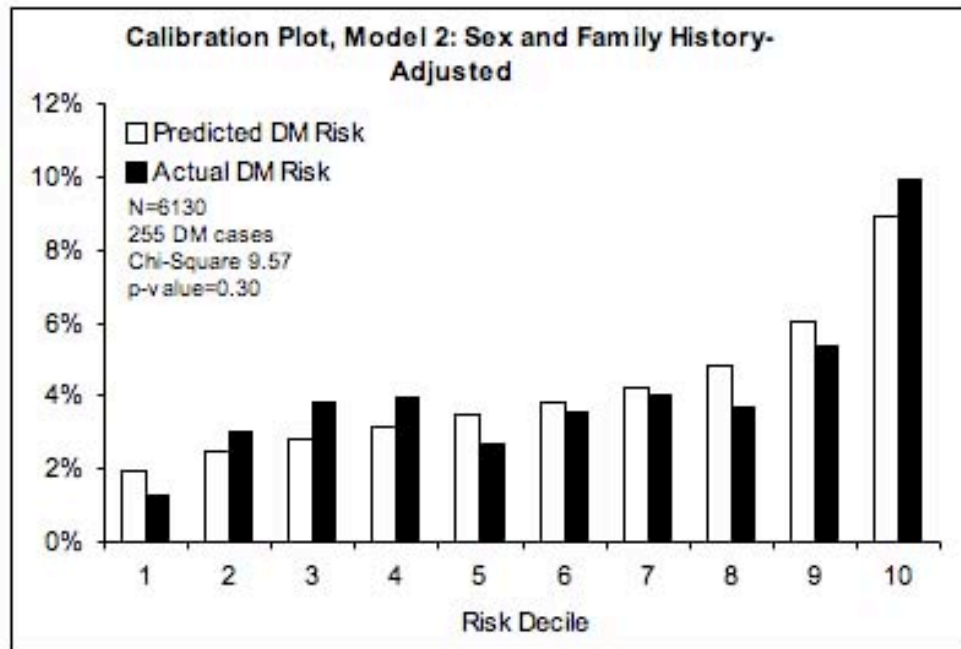
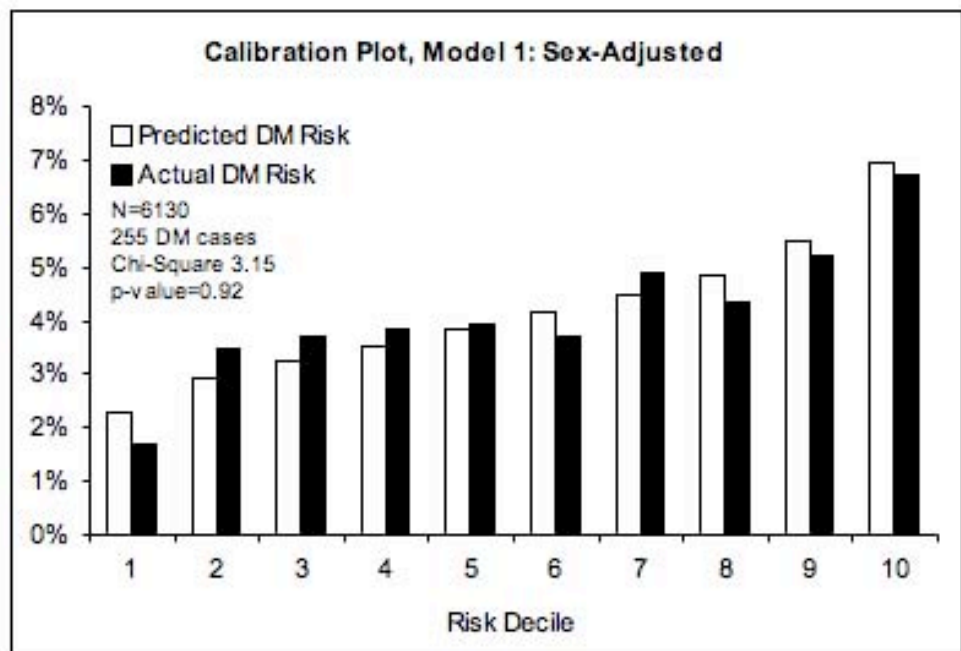
Supplementary Figure 1.



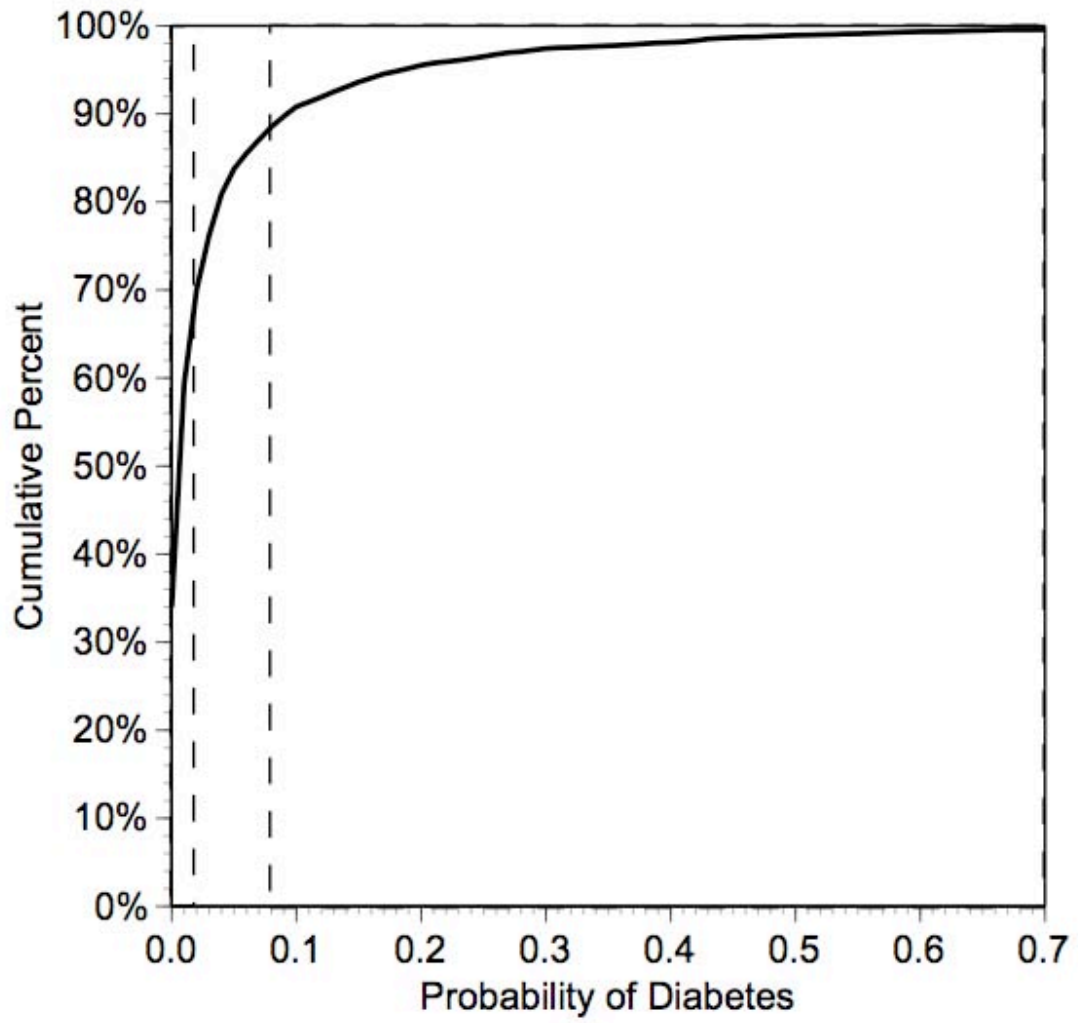
Supplementary Figure 2.



Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Table 1. Effects of Age on Risk for Incident Type 2 Diabetes Associated with Genotype Score

	Model without Genotype Score		Model with Genotype Score	
	OR	(95% CI)	OR	(95% CI)
Model 1: age and sex-adjusted				
Men (vs. women)	1.28	(0.99 - 1.65)	1.30	(1.00 - 1.68)
Age (per year)	1.07	(1.06 - 1.09)	1.07	(1.06 - 1.09)
Genotype Score	-	- -	1.12	(1.07 - 1.18)
C-statistic (95% CI)	0.729	(0.701 - 0.757)	0.741	(0.714 - 0.769)
P value for difference in c-statistic			0.05	
Calibration Chi Square and P value				15.22 0.05
Model 1a and 1b: age stratified, sex-adjusted				
1a: Age <50 years				
Men (vs. women)	1.29	(0.89 - 1.87)	1.29	(0.89 - 1.86)
Genotype Score	-	- -	1.17	(1.09 - 1.25)
C-statistic (95% CI)	0.532	(0.486 - 0.578)	0.609	(0.557 - 0.660)
P value for difference in c-statistic			0.009	
Calibration Chi Square and P value				7.26 0.51
Net Reclassification Improvement				11.9%
P value				0.009
1b: Age ≥50 years				
Men (vs. women)	1.27	(0.90 - 1.81)	1.29	(0.91 - 1.83)
Genotype Score	-	- -	1.07	(1.00 - 1.15)
C-statistic (95% CI)	0.530	(0.487 - 0.574)	0.558	(0.508 - 0.608)
P value for difference in c-statistic			0.20	
Calibration Chi Square and P value				3.03 0.93
Net Reclassification Improvement				0.47%
P value				0.92
P value for Age x Genotype Score				0.37
Model 2: age-, sex-, and self-reported family history adjusted				
Men (vs. women)	1.34	(1.04 - 1.73)	1.36	(1.05 - 1.76)
Family History of diabetes (vs. no family history)	2.64	(1.99 - 3.51)	2.55	(1.92 - 3.39)
Age (per year)	1.08	(1.07 - 1.09)	1.08	(1.07 - 1.09)
Genotype Score	-	- -	1.11	(1.06 - 1.17)
C-statistic (95% CI)	0.752	(0.725 - 0.778)	0.759	(0.732 - 0.786)
P value for difference in c-statistic			0.16	
Calibration Chi Square and P value				8.94 0.35
Model 2a and 2b: age stratified, sex and self-reported family history-adjusted				
2a: Age <50 years				
C-statistic (95% CI)	0.622	(0.571 - 0.674)	0.650	(0.597 - 0.703)
P value for difference in c-statistic			0.15	
Net Reclassification Improvement				8.08%
P value				0.09
2b: Age ≥50 years				
C-statistic (95% CI)	0.579	(0.530 - 0.628)	0.596	(0.544 - 0.647)
P value for difference in c-statistic			0.24	
Net Reclassification Improvement				2.84%
P value				0.43
P value for Age x Genotype Score				0.43
Model 3a and 3b: simple clinical model-adjusted				
3a: Age <50 years				
C-statistic (95% CI)	0.901	(0.874 - 0.929)	0.904	(0.875 - 0.933)
P value for difference in c-statistic			0.48	
Net Reclassification Improvement				1.14%
P value				0.74
3b: Age ≥50 years				

C-statistic (95% CI)	0.865	(0.831 - 0.899)	0.865	(0.830 - 0.899)
P value for difference in c-statistic			0.87	
Net Reclassification Improvement				0.86%
P value				0.47
P value for Age x Genotype Score				0.34

Odds ratios (OR), 95% confidence intervals (CI) and c-statistics for 255 cases of diabetes in 6,130 person-exams were calculated using pooled logistic regression with generalized estimating equations.

Among subjects <50, there were 117 cases of diabetes over 4,4489 person exams. Among subjects ≥50, there were 138 cases of diabetes over 1,641 person exams.

The body-mass index is the weight in kilograms divided by the square of the height in meters HDL denotes high-density lipoprotein.

Supplementary Table 2 . Risk for Incident Type 2 Diabetes Associated with the 3-level Genotype Score, Family History of Diabetes, and Simple Clinical Risk Factors

	Model without 3-level Genotype Score		Model with 3-level Genotype Score	
	OR	(95% CI)	OR	(95% CI)
Sex-Adjusted Model				
Men (vs. women)	1.31	(1.02 - 1.68)	1.31	(1.02 - 1.68)
Genotype Score ≤15	-	- -	1.00	- -
Genotype Score 16-20	-	- -	1.62	(1.14 - 2.29)
Genotype Score ≥21	-	- -	2.60	(1.68 - 4.02)
C-statistic (95% CI)	0.534	(0.502 - 0.565)	0.579	(0.545 - 0.613)
P value for difference in c-statistic			0.01	
Calibration Chi Square and P value				0.86 0.83
Sex- and Self-reported Family History-Adjusted Model				
Men (vs. women)	1.35	(1.05 - 1.74)	1.35	(1.05 - 1.74)
Family History of diabetes (vs. no family history)	2.26	(1.72 - 2.97)	2.15	(1.63 - 2.83)
Genotype Score ≤15	-	- -	1.00	- -
Genotype Score 16-20	-	- -	1.55	(1.09 - 2.19)
Genotype Score ≥21	-	- -	2.37	(1.53 - 3.67)
C-statistic (95% CI)	0.595	(0.560 - 0.630)	0.615	(0.579 - 0.651)
P value for difference in c-statistic			0.11	
Calibration Chi Square and P value				2.08 0.72
Simple Clinical Model-adjusted Model				
Age (per year)	1.05	(1.03 - 1.06)	1.05	(1.03 - 1.06)
Men (vs. women)	0.66	(0.48 - 0.91)	0.66	(0.47 - 0.90)
Family History of diabetes (vs. no family history)	1.72	(1.24 - 2.38)	1.62	(1.17 - 2.25)
Body mass index (per kg/m ²)	1.14	(1.10 - 1.17)	1.14	(1.11 - 1.18)
Fasting plasma glucose (per mg/dL)	1.14	(1.12 - 1.16)	1.13	(1.11 - 1.15)
Systolic blood pressure (per mm Hg)	1.01	(1.00 - 1.02)	1.01	(1.00 - 1.02)
HDL cholesterol (per mg/dL)	0.99	(0.97 - 1.00)	0.99	(0.97 - 1.00)
Fasting triglycerides (per mg/dL)	1.00	(1.00 - 1.00)	1.00	(1.00 - 1.00)
Genotype Score ≤15	-	- -	1.00	- -
Genotype Score 16-20	-	- -	1.56	(1.04 - 2.33)
Genotype Score ≥21	-	- -	2.46	(1.47 - 4.11)
C-statistic (95% CI)	0.900	(0.880 - 0.919)	0.901	(0.881 - 0.920)
P value for difference in c-statistic			0.52	
Calibration Chi Square and P value				5.25 0.73

Odds ratios (OR), 95% confidence intervals (CI) and area-under-the-receiver-operator-characteristic curves (the c-statistic) for 255 cases of diabetes in 6,130 person-exams were calculated using pooled logistic regression with generalized estimating equations. The referent group for the genotype score is individuals with a genotype score ≤15.

Supplementary Table 3. Posterior Probabilities of Type 2 Diabetes According to Prior Probabilities and Level of Genetic Score

Genetic Score	Likelihood Ratio (95% CI)	Prior Probability of Diabetes						Posterior Probability of Diabetes		
		3%	5%	7%	10%	12%	15%	20%		
≤15	0.62 (0.47 - 0.83)	1.9%	3.2%	4.5%	6.5%	7.8%	9.9%	13.4%		
16-20	1.04 (0.94 - 1.14)	3.1%	5.2%	7.2%	10.3%	12.4%	15.5%	20.6%		
≥21	1.73 (1.29 - 2.32)	5.1%	8.4%	11.5%	16.1%	19.1%	23.4%	30.2%		

Likelihood ratios were calculated by dividing the proportion of subjects with diabetes in each Genetic Score strata by the proportion of all subjects without diabetes in the same stratum; 95 percent confidence intervals for likelihood ratios were calculated from a Taylor series approximation.

Probabilities of diabetes were calculated from odds of diabetes based on Bayes Theorem: prior odds = prior probability / (1 - prior probability) and posterior probability = posterior odds / (1 + posterior odds) where posterior odds = prior odds multiplied by the likelihood ratio.

Supplementary Table 4. SNPs Ranked by SNP Weight for Weighted Genotype Score

SNP	Locus	Chromosome	Risk Allele	Published Odds Ratio	Published P Value	Standard Error	SNP Weight
rs7903146	<i>TCF7L2</i>	10	T	1.37	1.00E-48	0.022	14.08
rs1470579	<i>IGF2BP2</i>	3	C	1.14	8.90E-16	0.016	8.40
rs10811661	<i>CDKN2A/B</i>	9	T	1.20	7.80E-15	0.023	7.76
rs864745	<i>JAFZ1</i>	7	T	1.10	4.62E-05	0.014	6.85
rs5219	<i>KCNJ11</i>	11	T	1.14	6.70E-11	0.020	6.53
rs12779790	<i>CDC123,CAMK1D</i>	10	G	1.11	4.74E-05	0.016	6.46
rs7578597	<i>THADA</i>	2	T	1.15	1.09E-04	0.022	6.30
rs7754840	<i>CDKAL1</i>	6	C	1.12	4.10E-11	0.018	6.22
rs7961581	<i>TSPAN8,LGR5</i>	12	C	1.09	3.68E-05	0.014	6.14
rs4607103	<i>ADAMTS9</i>	3	C	1.09	3.13E-04	0.014	6.14
rs1111875	<i>HHEX</i>	10	C	1.13	5.70E-10	0.020	5.99
rs10923931	<i>NOTCH2</i>	1	T	1.13	6.86E-06	0.020	5.99
rs13266634	<i>SLC30A8</i>	8	C	1.12	5.30E-08	0.021	5.50
rs1153188	<i>DCD</i>	12	A	1.08	3.21E-05	0.014	5.43
rs1801282	<i>PPARG</i>	3	C	1.14	1.70E-06	0.027	4.87
rs9472138	<i>VEGFA</i>	6	T	1.06	4.10E-05	0.012	4.86
rs10490072	<i>BCL11A</i>	2	T	1.05	4.89E-05	0.012	4.03
rs689	<i>INS</i>	11	T	1.89	4.00E-02	0.315	2.02

Rather than assume that each SNP conferred an identical degree of risk, we created a weighted genotype score. Weights for the weighted genotype score were generated by multiplying individual SNP scores by the published odds ratio for diabetes associated with the SNP divided by the standard error of the odds ratio. For instance, one risk allele in *TCF7L2* adds 14.08 to the weighted genetic score, and two risk alleles add 28.16 to the score, while one risk allele in *INS* contributes 2.02 to the score, and two risk alleles contribute 4.04.

The mean weighted genotype score was 109.3 (SD 19.5) among subjects who developed diabetes and 104.1 (18.2) among those who did not ($P < 0.0001$).

Supplementary Table 5. Risk for Incident Type 2 Diabetes Associated with the Weighted Genotype Score, Family History of Diabetes, and Simple Clinical Risk Factors

	Model without Weighted Genotype Score		Model with Weighted Genotype Score	
	OR	(95% CI)	OR	(95% CI)
Sex-Adjusted Model				
Men (vs. women)	1.31	(1.02 - 1.68)	1.31	(1.02 - 1.68)
Weighted Genotype Score	-	- - -	1.01	(1.01 - 1.02)
C-statistic (95% CI)	0.534	0.502 - 0.565)	0.576	(0.539 - 0.613)
P value for difference in c-statistic			0.02	
Calibration Chi Square and P value			12.77	0.12
Sex- and Self-reported Family History-Adjusted Model				
Men (vs. women)	1.35	(1.05 - 1.74)	1.35	(1.05 - 1.73)
Family History of diabetes (vs. no family history)	2.26	(1.72 - 2.97)	2.17	(1.65 - 2.86)
Weighted Genotype Score	-	- - -	1.01	(1.01 - 1.02)
C-statistic (95% CI)	0.595	(0.560 - 0.630)	0.614	(0.577 - 0.651)
P value for difference in c-statistic			0.09	
Calibration Chi Square and P value			8.75	0.36
Simple Clinical Model-adjusted Model				
Age (per year)	1.05	(1.03 - 1.06)	1.05	(1.03 - 1.06)
Men (vs. women)	0.66	(0.48 - 0.91)	0.65	(0.47 - 0.90)
Family History of diabetes (vs. no family history)	1.72	(1.24 - 2.38)	1.64	(1.18 - 2.27)
Body mass index (per kg/m ²)	1.14	(1.10 - 1.17)	1.14	(1.11 - 1.18)
Fasting plasma glucose (per mg/dL)	1.14	(1.12 - 1.16)	1.13	(1.11 - 1.15)
Systolic blood pressure (per mm Hg)	1.01	(1.00 - 1.02)	1.01	(1.00 - 1.02)
HDL cholesterol (per mg/dL)	0.99	(0.97 - 1.00)	0.99	(0.97 - 1.00)
Fasting triglycerides (per mg/dL)	1.00	(1.00 - 1.00)	1.00	(1.00 - 1.00)
Weighted Genotype Score	-	- - -	1.13	(1.11 - 1.15)
C-statistic (95% CI)	0.900	(0.880 - 0.919)	0.901	(0.882 - 0.920)
P value for difference in c-statistic			0.41	
Calibration Chi Square and P value			3.44	0.90

Odds ratios (OR), 95% confidence intervals (CI) and c-statistics for 255 cases of diabetes in 6,130 person-exams were calculated using pooled logistic regression with generalized estimating equations.

To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for HDL cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. The body-mass index is the weight in kilograms divided by the square of the height in meters HDL denotes high-density lipoprotein.

Supplementary Table 6. Reclassification of Risk by the Weighted Genotype Score for Individuals Who Did and Did Not Develop Diabetes

Model Without Weighted Genotype Score	Model With Weighted Genotype Score					
	0 to <2%		2 to <8%		≥8%	
	N	row%	N	row%	N	row%
Model 1: sex-adjusted						
Individuals who developed diabetes						
0 to <2%	0	0.00%	0	0.00%	0	0.00%
2 to <8%	1	0.39%	248	97.25%	6	2.35%
≥8%	0	0.00%	0	0.00%	0	0.00%
Total N	1		248		6	
Individuals who did not develop diabetes						
0 to <2%	0	0.00%	0	0.00%	0	0.00%
2 to <8%	31	0.53%	5804	98.79%	40	0.68%
≥8%	0	0.00%	0	0.00%	0	0.00%
Total N	31		5804		40	
Net Reclassification Improvement						1.81%
P value						0.08
Model 2: sex- and self-reported family history-adjusted						
Individuals who developed diabetes						
0 to <2%	0	0.00%	0	0.00%	0	0.00%
2 to <8%	3	1.41%	200	93.90%	10	4.69%
≥8%	0	0.00%	10	23.81%	32	76.19%
Total N	3		210		42	
Individuals who did not develop diabetes						
0 to <2%	0	0.00%	0	0.00%	0	0.00%
2 to <8%	152	2.79%	5203	95.63%	86	1.58%
≥8%	0	0.00%	178	41.01%	256	58.99%
Total N	152		5381		342	
Net Reclassification Improvement						2.98%
P value						0.12
Model 3: Simple clinical model-adjusted						
Individuals who developed diabetes						
0 to <2%	21	87.5%	3	12.50%	0	0.00%
2 to <8%	3	5.56%	45	83.33%	6	11.11%
≥8%	0	0.00%	1	0.56%	176	99.44%
Total N	24		49		182	
Individuals who did not develop diabetes						
0 to <2%	3923	97.47%	102	2.53%	0	0.00%
2 to <8%	115	9.02%	1107	86.82%	53	4.16%
≥8%	0	0.00%	49	8.52%	526	91.48%
Total N	4038		1258		579	
Net Reclassification Improvement						2.11%
P value						0.14

Net reclassification improvement for 255 cases of diabetes in 6,130 person-exams were based on pooled logistic regression models with generalized estimating equations including or not including the weighted genotype score

Supplementary Table 7. Framingham Offspring Categorical Characteristics at Baseline for Each of Three Time Periods in the Pooled Analysis

	Period 1	Period 2	Period 3
Number of Subjects	2188	1916	2026
Directly Observed Parental History of Diabetes (%)	16.9	17.6	17.1
Age Category (%)			
<50	92.8	74.1	51.2
50-64	7.2	25.5	40.5
≥65	0.0	0.4	8.3
BMI, mean kg/m ² (%)			
<25	56.1	53.4	40.0
25-29	33.4	34.8	40.8
≥30	10.6	11.8	19.2
Blood pressure >130/85 mm Hg or hypertension therapy (%)	31.1	32.2	44.6
HDL-C <40 mg/dL in men or <50 mg/dL in women (%)	33.9	38.8	38.6
Fasting triglyceride >150 mg/dL (%)	11.7	14.8	23.2
IFG* (%)	16.2	16.9	14.3

* Impaired fasting glucose (IFG) defined as 110-125 mg/dl at Offspring exam 1, 105-125 mg/dl at Offspring exam 2, and 100-125 mg/dl at Offspring exams 3 to 7.

The body-mass index is the weight in kilograms divided by the square of the height in meters HDL denotes high-density lipoprotein.

Supplementary Table 8. Risk for Incident Type 2 Diabetes Associated with the Genotype Score and Categorical Simple Clinical Risk Factors

	Model without Genotype Score		Model with Genotype Score	
	OR	(95% CI)	OR	(95% CI)
Categorical Simple Clinical Model-Adjusted Model				
Age 50-64 years	2.04	(1.51 - 2.74)	2.05	(1.52 - 2.76)
Age ≥65 years	2.75	(1.56 - 4.85)	2.72	(1.54 - 4.80)
Men (vs. women)	0.61	(0.46 - 0.82)	0.61	(0.45 - 0.82)
Self-reported Family History of diabetes (vs. no family history)	1.79	(1.32 - 2.44)	1.71	(1.25 - 2.33)
Body mass index 25-29.9 kg/m ²	3.15	(2.01 - 4.93)	3.11	(1.99 - 4.88)
Body mass index >30 kg/m ²	6.43	(4.04 - 10.3)	6.64	(4.16 - 10.6)
Impaired Fasting Glucose	7.08	(5.31 - 9.44)	6.89	(5.16 - 9.21)
Blood pressure > 130/85 or Hypertension therapy	1.89	(1.36 - 2.61)	1.91	(1.38 - 2.65)
HDL-C <40 mg/dL in men or <50 mg/dL in women	1.43	(1.06 - 1.94)	1.45	(1.07 - 1.96)
Fasting triglyceride >150 mg/dL	1.70	(1.24 - 2.32)	1.70	(1.25 - 2.33)
Genotype Score	-	- -	1.11	(1.05 - 1.17)
C-statistic (95% CI)	0.877	(0.856 - 0.899)	0.879	(0.857 - 0.901)
P value for difference in c-statistic			0.60	
Calibration Chi Square and P value				4.28 0.83

Odds ratios (OR), 95% confidence intervals (CI) and c-statistics for 255 cases of diabetes in 6,130 person-observations were calculated using pooled logistic regression with generalized estimating equations.

The body-mass index is the weight in kilograms divided by the square of the height in meters HDL denotes high-density lipoprotein.