



# DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD

## Genetic Predisposition to Dyslipidemia and Type 2 Diabetes Risk in Two Prospective Cohorts

The Harvard community has made this article openly available.  
[Please share](#) how this access benefits you. Your story matters.

<b>Citation</b>	Qi, Qibin, Liming Liang, Alessandro Doria, Frank B. Hu, and Lu Qi. 2012. Genetic predisposition to dyslipidemia and type 2 diabetes risk in two prospective cohorts. <i>Diabetes</i> 61(3): 745-752.
<b>Published Version</b>	<a href="https://doi.org/10.2337/db11-1254">doi:10.2337/db11-1254</a>
<b>Accessed</b>	February 19, 2015 12:00:03 PM EST
<b>Citable Link</b>	<a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:10642217">http://nrs.harvard.edu/urn-3:HUL.InstRepos:10642217</a>
<b>Terms of Use</b>	This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a>

*(Article begins on next page)*

# Genetic Predisposition to Dyslipidemia and Type 2 Diabetes Risk in Two Prospective Cohorts

Qibin Qi,<sup>1</sup> Liming Liang,<sup>2</sup> Alessandro Doria,<sup>3,4</sup> Frank B. Hu,<sup>1,2,5</sup> and Lu Qi<sup>1,5</sup>

Dyslipidemia has been associated with type 2 diabetes, but it remains unclear whether dyslipidemia plays a causal role in type 2 diabetes. We aimed to examine the association between the genetic predisposition to dyslipidemia and type 2 diabetes risk. The current study included 2,447 patients with type 2 diabetes and 3,052 control participants of European ancestry from the Nurses' Health Study and the Health Professionals Follow-up Study. Genetic predisposition to dyslipidemia was estimated by three genotype scores of lipids (LDL cholesterol, HDL cholesterol, and triglycerides) on the basis of the established loci for blood lipids. Linear relation analysis indicated that the HDL cholesterol and triglyceride genotype scores, but not the LDL cholesterol genotype score, were linearly related to elevated type 2 diabetes risk. Each point of the HDL cholesterol and triglyceride genotype scores was associated with a 3% (odds ratio [OR] 1.03 [95% CI 1.01–1.04]) and a 2% (1.02 [1.00–1.04]) increased risk of developing type 2 diabetes, respectively. The ORs were 1.39 (1.17–1.65) and 1.19 (1.01–1.41) for type 2 diabetes by comparing extreme quartiles of the HDL cholesterol genotype score and triglyceride genotype score, respectively. In conclusion, genetic predisposition to low HDL cholesterol or high triglycerides is related to elevated type 2 diabetes risk. *Diabetes* 61:745–752, 2012

**D**yslipidemia has been associated with type 2 diabetes (1), and the most common patterns of dyslipidemia in diabetic patients are reduced HDL cholesterol and elevated triglyceride levels. Prospective studies also have shown that low HDL cholesterol and high triglyceride levels, but not LDL cholesterol levels, are independent risk factors for type 2 diabetes (2–7), and the values of HDL cholesterol and/or triglycerides have been used in the risk-scoring systems for predicting incident diabetes (4,6,7). However, it remains unclear whether low HDL cholesterol/high triglyceride levels play a causal role in the development of type 2 diabetes.

Information on the associations of genetic predisposition to dyslipidemia with risk of type 2 diabetes might help clarify the causality. A recent study reported that a genotype score for triglyceride levels was not associated with type 2 diabetes risk (8). However, the less extensive inclusion of the susceptibility loci (nine loci) might limit the

causal inference. Moreover, the study did not address other patterns of dyslipidemia (high LDL cholesterol and low HDL cholesterol levels).

Recently, a meta-analysis of 46 lipid genome-wide association studies comprising >100,000 individuals of European ancestry has established more comprehensive genetic profiles for various blood lipids, including LDL cholesterol, HDL cholesterol, and triglycerides (9). In the current study, we calculated three genotype scores on the basis of 31, 41, and 25 well-established single nucleotide polymorphisms (SNPs) for LDL cholesterol, HDL cholesterol, and triglycerides, respectively, as proxies of genetic predisposition to dyslipidemia. We examined the effects of these dyslipidemia genotype scores on type 2 diabetes risk in women and men of European ancestry from two prospective cohorts: the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS).

## RESEARCH DESIGN AND METHODS

The NHS is a prospective cohort study of 121,700 female registered nurses who were aged 30–55 years at study inception in 1976 when all of them completed a mailed questionnaire on their medical history and lifestyle (10). A total of 32,826 women provided blood samples between 1989 and 1990. The HPFS is a prospective cohort study of 51,529 U.S. male health professionals who were aged 40–75 years at study inception in 1986 (11). Between 1993 and 1999, 18,159 men provided blood samples. In both cohorts, information about health and disease has been collected biennially by self-administered questionnaires every 2 years since inception. The study was approved by the human research committee at the Brigham and Women's Hospital (Boston, MA), and all participants provided written informed consent.

Participants for the current study were selected among those with a blood sample using a nested case-control study design (12,13). Diabetes cases were defined as self-reported diabetes confirmed by a validated supplementary questionnaire (14,15). For cases before 1998, we used the National Diabetes Data Group criteria to define diabetes (16), which included one of the following: one or more classic symptoms (excessive thirst, polyuria, weight loss, hunger, pruritus, or coma) plus a fasting plasma glucose level of  $\geq 7.8$  mmol/L (140 mg/dL), a random plasma glucose level of  $\geq 11.1$  mmol/L (200 mg/dL), or a plasma glucose level 2 h after an oral glucose tolerance test of  $\geq 11.1$  mmol/L (200 mg/dL); at least two elevated plasma glucose levels (as described previously) on different occasions in the absence of symptoms; or treatment with hypoglycemia medication (insulin or oral hypoglycemic agent). We used the American Diabetes Association diagnostic criteria for diabetes diagnosis from 1998 onward (17). These criteria were the same as those proposed by the National Diabetes Data Group, except for the elevated fasting plasma glucose criterion, for which the cut point was changed from

From the <sup>1</sup>Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts; the <sup>2</sup>Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; the <sup>3</sup>Research Division, Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts; the <sup>4</sup>Department of Medicine, Harvard Medical School, Boston, Massachusetts; and the <sup>5</sup>Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.

Corresponding author: Lu Qi, nhlqi@channing.harvard.edu.

Received 8 September 2011 and accepted 3 December 2011.

DOI: 10.2337/db11-1254

This article contains Supplementary Data online at <http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-1254/-/DC1>.

© 2012 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

7.8 mmol/L (140 mg/dL) to 7.0 mmol/L (126 mg/dL). Only case subjects with diagnosed diabetes after the cohort baseline were included. Control subjects were defined as those free of diabetes at the time of the diagnosis of case and remained unaffected through follow-up (2006). After applying a quality-control filter in the NHS and HPFS T2D GWA scans (13), duplicate samples, samples with misidentified sex, related samples (siblings or possible first cousins), samples with evidence of contamination, samples with highly variable intensity data, and samples with missing call rates  $\geq 2\%$  were excluded. Population structure was investigated by principal component analysis (18). In this analysis, participants clustered with the HapMap CEU samples were genetically inferred to have European ancestry. Finally, a total of 3,088 (1,334 case and 1,754 control subjects) women and 2,411 (1,113 case and 1,298 control subjects) men of genetically inferred European ancestry were included in the current analysis.

**Assessment of plasma lipids and covariates.** Plasma lipid levels were measured in 718 women and 753 men with type 2 diabetes (after the onset of diabetes). LDL cholesterol concentration was measured by a homogenous direct method from Genzyme (Cambridge, MA), with coefficients of variation of  $< 3.1\%$ . Concentrations of triglycerides and HDL cholesterol were measured simultaneously on the Hitachi 911 analyzer using reagents and calibrators from Roche Diagnostics (Indianapolis, IN), with coefficients of variation of  $< 1.8\%$ .

Information about anthropometric data, lifestyle factors, menopausal status and postmenopausal hormone therapy (women only), and medication use was derived from the baseline questionnaires (10,11). BMI was calculated as weight in kilograms divided by the square of height in meters. For men, physical activity was expressed as metabolic equivalents per week by using the reported time spent on various activities, weighting each activity by its intensity level. For women, physical activity was expressed as hours per week because metabolic equivalent task hours were not measured at baseline in the NHS. The validity of the self-reported body weight and physical activity data have been described previously (19–21).

**Genotyping.** SNPs genotyping and imputation have been described in detail elsewhere (the NHS and HPFS T2D GWA scans) (13). In brief, samples were genotyped and

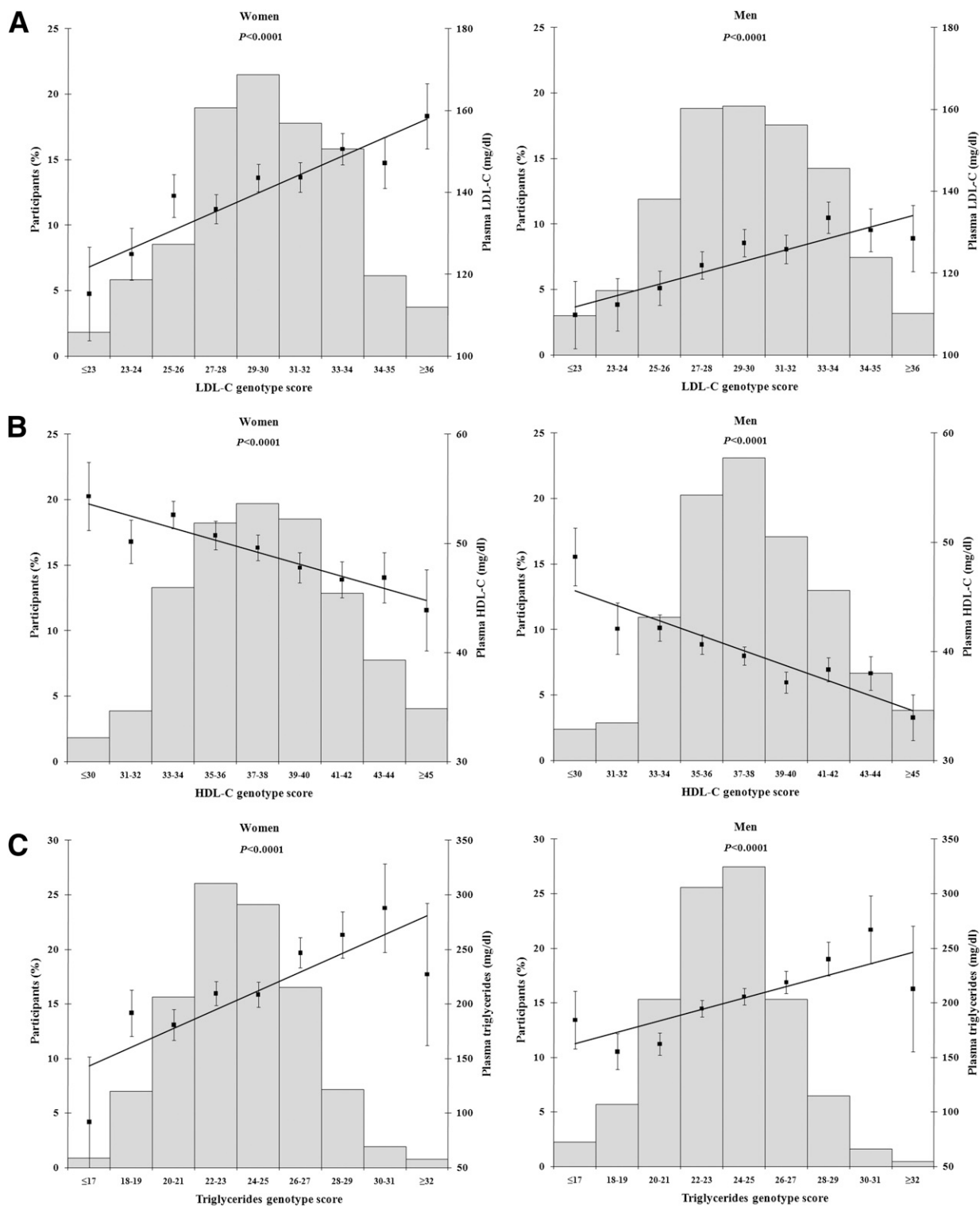
analyzed using the Affymetrix Genome-Wide Human 6.0 array (Santa Clara, CA) and the Birdseed calling algorithm. All samples used in the current study achieved a call rate of  $> 98\%$ . Individual SNPs were excluded if they were monomorphic, had a missing call rate of  $\geq 2\%$ , had more than one discordance in the multiple genotyped samples (one HapMap control sample was genotyped 12 times), or had a Hardy-Weinberg equilibrium  $P$  value of  $< 1 \times 10^{-4}$  or a minor allele frequency of  $< 0.02$ . We used MACH (<http://www.sph.umich.edu/csg/abecasis/mach>) to impute SNPs on chromosomes 1–22, with National Center for Biotechnology Information build 36 of phase II HapMap CEU data (release 22) as the reference panel.

**Genotype score calculation.** To estimate the genetic predisposition to dyslipidemia, three lipid (LDL cholesterol, HDL cholesterol, and triglycerides) genotype scores were calculated on the basis of the well-established SNPs in 95 loci for blood lipids reported by a recent meta-analysis of genome-wide association studies (9). Only SNPs with genotyped data or high imputation quality scores (MACH  $r^2 \geq 0.8$ ) were included. To minimize the influence of pleiotropic effects, the SNPs or their correlated SNPs ( $r^2 \geq 0.80$ ), which have been reported to be associated with type 2 diabetes risk or fasting glucose at a genome-wide significance level, including the SNPs in *GCKR* (22), *FADS1* (22), *IRS1* (23), and *KLF14* (24) loci, were excluded, leaving 31, 41, and 25 SNPs for LDL cholesterol, HDL cholesterol, and triglycerides, respectively, in the current analysis (Supplementary Table 1). We assumed that each SNP in the panel acts independently in an additive manner, and the genotype scores were calculated by using a weighted method. Each SNP was weighted by its relative effect size ( $\beta$ -coefficient) obtained from the reported meta-analysis data (9). The genotype scores were calculated by multiplying each  $\beta$ -coefficient by the number of corresponding risk alleles and then summing the products. Because this produced an LDL cholesterol genotype score out of 115.88 (twice the sum of the  $\beta$ -coefficients), an HDL cholesterol genotype score out of 59.46 (twice the sum of the  $\beta$ -coefficients), and a triglyceride genotype score out of 247.36 (twice the sum of the  $\beta$ -coefficients), the values were divided by 115.88, 59.46, and 247.36 and multiplied by 62, 82, and 50 (the total number of the risk alleles), respectively, to make the genotype scores easier to interpret.

TABLE 1  
Characteristics of the participants at baseline

	Women		<i>P</i>	Men		<i>P</i>
	Case subjects	Control subjects		Case subjects	Control subjects	
<i>n</i>	1,334	1,754		1,113	1,298	
Age (years)	43.4 (6.7)	43.2 (6.7)	0.44	55.5 (8.5)	55.5 (8.4)	0.83
BMI (kg/m <sup>2</sup> )	27.3 (4.9)	23.9 (3.0)	$< 0.001$	27.8 (4.0)	25.0 (2.7)	$< 0.001$
Current smokers (%)	30.1	20.9	$< 0.001$	12.0	7.6	$< 0.001$
Alcohol intake (g/day)	4.5 (9.3)	6.6 (10.0)	$< 0.001$	11.2 (16.2)	12.1 (15.3)	0.19
Physical activity (h/week)	3.7 (2.8)	4.1 (2.9)	$< 0.001$	—	—	—
Physical activity (MET h/week)	—	—	—	14.6 (19.0)	21.1 (25.2)	$< 0.001$
Postmenopausal (%)	34.6	30.6	0.02	—	—	—
Postmenopausal hormone use (%)	30.7	28.9	0.52	—	—	—
LDL cholesterol genotype score	30.5 (3.7)	30.4 (3.7)	0.21	30.6 (3.7)	30.5 (3.6)	0.85
HDL cholesterol genotype score	37.5 (3.8)	37.1 (3.8)	0.002	37.5 (3.6)	37.3 (3.7)	0.21
Triglyceride genotype score	24.2 (2.9)	24.0 (2.9)	0.05	24.2 (2.9)	24.1 (3.0)	0.37

Data are means (SD) or percent, unless otherwise indicated.



**FIG. 1.** Lipid genotype scores and plasma lipids in diabetic women and men. **A:** LDL cholesterol (LDL-C). **B:** HDL cholesterol (HDL-C). **C:** Triglycerides. The histograms represent the percentage of participants, and the means  $\pm$  SE of LDL cholesterol, HDL cholesterol, and triglycerides are plotted, with the trend lines across the respective genotype score, adjusted for age and BMI.

In sensitivity analysis, four SNPs in *ANGPTL3*, *PPP1R3B*, *TRIB1*, and *APOA1-C3-A4-A5* loci for LDL cholesterol; five SNPs in *APOB*, *MLXIPL*, *TRIB*, *APOA1-C3-A4-A5*, and *LRP1* loci for HDL cholesterol; and four SNPs in *GALNT2*, *ZNF664*, *LIPC*, and *PLTP* loci for triglycerides were further excluded from the calculation of the respective

genotype scores, because these SNPs had the largest effects on other two blood lipid traits (9).

**Statistical analysis.**  $\chi^2$  Tests and *t* tests were used for comparison of proportions and means between case patients and control participants. General linear regression models were applied to test the association between the

plasma lipid levels and the respective genotypes scores in 673 women and 633 men with type 2 diabetes who were not taking lipid-lowering drugs. We used logistic regression to estimate odd ratios (ORs) for type 2 diabetes risk, adjusting for age and BMI. To examine the accumulative effects of the genotype scores, we compared the type 2 diabetes risk across the quartiles of the genotype scores. In multivariate analysis, we further adjusted for smoking (never, past, or current), alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, or ≥15.0 g/day), menopausal status (pre- or postmenopausal [never, past, or current hormone use], women only), and physical activity (quintiles). Results in women and men were pooled by using inverse variance weights under a fixed model because there was no heterogeneity. Linear relation analysis between the genotype scores (as continuous variables) and risk of type 2 diabetes was performed by using a restricted cubic spline regression model (25). All reported *P* values are nominal and two sided. Statistical analyses were performed in SAS version 9.1 (SAS Institute, Cary, NC).

**RESULTS**

**Characteristics of study participants.** Table 1 shows the baseline characteristics of participants of two nested case-control studies from the NHS (women) and HPFS (men). The patients were incident cases diagnosed during the follow-up through 2006 in these cohorts. Patients with type 2 diabetes had a significantly higher BMI, engaged in less physical activity, and were more likely to smoke and have a family history of diabetes than control subjects, among both women and men. In addition, among women, diabetic patients consumed less alcohol and were more likely to be postmenopausal than control subjects. In addition, the genotype scores were not associated with age, BMI, or lifestyle factors including smoking, alcohol intake, and physical activity (all *P* > 0.05).

**Lipid genotype scores and plasma lipid levels.** Figure 1 presents the relationship between the three lipid genotype scores and the respective plasma lipid levels among diabetic women and men. The three genotype scores were all

normally distributed among women and men. As expected, higher genotype scores were associated with higher plasma LDL cholesterol and triglyceride levels but lower HDL cholesterol levels, respectively, among both women and men (all *P* < 0.0001).

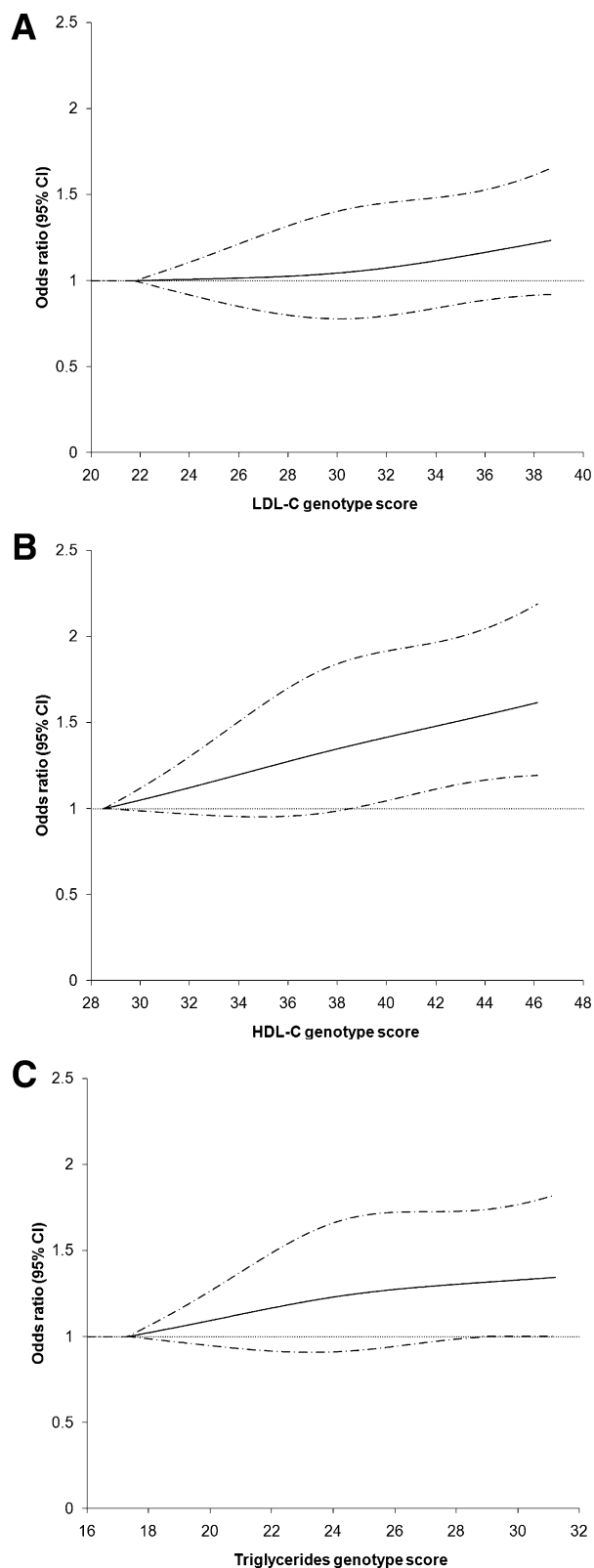
**LDL cholesterol genotype score and type 2 diabetes.** In women, the OR for type 2 diabetes was 1.01 (95% CI 0.98–1.03) with each point (corresponding to one unfavorable allele) of the LDL cholesterol genotype score, after adjusting for age and BMI (Table 2). In men, the corresponding OR for type 2 diabetes was 1.00 (0.98–1.03). Pooled results between women and men showed that the LDL cholesterol genotype score was not associated with type 2 diabetes (*P* for trend = 0.24). Linear relation analysis also indicated that there was no significant linear relationship between the LDL cholesterol genotype score and risk of type 2 diabetes (Fig. 2A). In sensitivity analysis, we excluded four SNPs (*ANGPTL* rs3850634, *PPP1R3B* rs2126259, *TRIB1* rs2954022, and *APOA1-C3-A4-A5* rs964184) that had the largest effects on HDL cholesterol or triglyceride levels from the calculation of the LDL cholesterol genotype score, and we observed similar results (pooled OR 1.00 [0.99–1.02] with each point of the genotype score; *P* = 0.51).

**HDL cholesterol genotype score and type 2 diabetes.** As shown in Table 3, the HDL cholesterol genotype score was significantly associated with an increased type 2 diabetes risk in women (OR 1.03 [95% CI 1.01–1.05] with each point of the genotype score). We observed a similar result in men (1.02 [0.99–1.04]), and the pooled OR for type 2 diabetes between women and men was 1.02 (1.01–1.04) with each point of the HDL cholesterol genotype score, adjusted for age and BMI. The ORs for type 2 diabetes increased across the quartiles of the HDL cholesterol genotype score (*P* for trend = 0.002). Compared with those in the lowest quartile of the HDL cholesterol genotype score, participants in the highest quartile had an OR of 1.37 (1.16–1.61). Multivariate adjustment did not change the associations. In addition, there was a linear relationship between the HDL cholesterol genotype score and increased risk of type 2 diabetes (Fig. 2B). In sensitivity analysis, we excluded five SNPs (*APOB* rs1042034, *MLXIPL* rs17145738, *TRIB1*

TABLE 2  
Association between the LDL cholesterol genotype score and risk for type 2 diabetes

	Continuous	Quartile				<i>P</i> for trend
		1	2	3	4	
<b>Women</b>						
<i>n</i> (case/control subjects)		302/436	348/437	314/436	354/437	
Median (range)		26.1 (16.6–27.9)	29.2 (28.0–30.5)	31.6 (30.6–32.9)	34.6 (33.0–43.2)	
OR (95% CI)						
Age and BMI adjusted	1.01 (0.98–1.03)	1.00	1.20 (0.95–1.49)	1.05 (0.84–1.32)	1.13 (0.91–1.42)	0.46
Multivariate adjusted*	1.01 (0.98–1.03)	1.00	1.17 (0.93–1.47)	1.08 (0.86–1.36)	1.15 (0.92–1.45)	0.33
<b>Men</b>						
<i>n</i> (case/control subjects)		275/323	247/323	292/323	292/323	
Median (range)		26.2 (19.1–27.9)	29.2 (28.0–30.4)	31.7 (30.5–33.0)	34.6 (33.1–43.6)	
OR (95% CI)						
Age and BMI adjusted	1.00 (0.98–1.03)	1.00	0.92 (0.72–1.19)	1.03 (0.81–1.32)	1.10 (0.86–1.40)	0.35
Multivariate adjusted*	1.01 (0.98–1.03)	1.00	0.92 (0.71–1.18)	1.02 (0.80–1.31)	1.09 (0.85–1.40)	0.36
<b>Pooled OR (95% CI)†</b>						
Age and BMI adjusted	1.01 (0.99–1.02)	1.00	1.07 (0.90–1.26)	1.04 (0.88–1.23)	1.12 (0.95–1.32)	0.24
Multivariate adjusted*	1.01 (0.99–1.02)	1.00	1.05 (0.88–1.24)	1.05 (0.89–1.25)	1.13 (0.95–1.33)	0.18

\*Adjusted for age, BMI, smoking, alcohol intake, physical activity, and menopausal status (women only). †Results were pooled between women and men using inverse variance weights under a fixed model, because there was no heterogeneity between women and men (all *P* for heterogeneity >0.13).



**FIG. 2.** Spline plot of lipid genotype scores and risk for type 2 diabetes. **A:** LDL cholesterol (LDL-C) genotype score. **B:** HDL cholesterol (HDL-C) genotype score. **C:** Triglyceride genotype score. Data are ORs (solid lines) and 95% CIs (dashed lines), adjusted for age, sex, and BMI, for pooled data from women and men.

rs10808546, *APOA1-C3-A4-A5* rs964184, and *LRP1* rs11613352) that had the largest effects on LDL cholesterol or triglyceride levels from the calculation of the HDL cholesterol genotype score, and we observed similar results (pooled OR 1.02 [1.01–1.04] with each point of the genotype score;  $P = 0.01$ ).

**Triglyceride genotype score and type 2 diabetes.** The ORs for type 2 diabetes were both 1.02 (95% CI 0.99–1.05) with each point of the triglyceride genotype score in women and men, and the pooled OR was 1.02 (1.00–1.04), after adjustment for age and BMI (Table 4). The ORs for type 2 diabetes increased across the quartiles of the triglyceride genotype score ( $P = 0.007$  for trend). Participants in the highest quartile of the triglyceride genotype score had an OR of 1.19 (1.00–1.40) compared with those in the lowest quartile. Multivariate adjustment did not change the associations. Linear relation analysis also indicated that the triglyceride genotype score showed a linear relationship with increasing type 2 diabetes risk (Fig. 2C). In sensitivity analysis, we excluded four SNPs (*GALNT2* rs1321257, *ZNF664* rs12310367, *LIPC* rs261342, and *PLTP* rs4810479) that had the largest effects on LDL or HDL cholesterol levels from the calculation of the triglyceride genotype score, and we observed similar results (pooled OR 1.03 [1.01–1.05] with each point of the genotype score;  $P = 0.015$ ).

## DISCUSSION

In two well-established prospective, nested case-control studies of U.S. women and men, we found that the genetic predisposition to dyslipidemia of low HDL cholesterol or high triglyceride levels significantly increases type 2 diabetes risk. In consideration of the fact that the individual SNPs may have quite moderate effects on type 2 diabetes risk, we examined the collective contribution of the multiple genetic variants by computing three lipid genotype scores, which emphasized the overall genetic susceptibility to dyslipidemia of high LDL cholesterol, low HDL cholesterol, or high triglycerides. As greater genetic variation was explained by multiple variants, the genetic predisposition might be estimated more accurately by using this approach. Each additional risk allele in the genotype scores of HDL cholesterol or triglycerides only was associated with an ~2–3% increased risk for type 2 diabetes. By accumulation, participants with an HDL cholesterol genotype score in the highest quartile had an ~40% greater risk of developing type 2 diabetes than those in the lowest quartile.

Consistent with the previously reported additive effects of multiple genetic variants on plasma lipid levels (9), we confirmed that the genotype scores summarizing the number of LDL cholesterol- or triglyceride-raising alleles or HDL cholesterol-lowering alleles were associated with higher LDL cholesterol and triglyceride levels and lower HDL cholesterol levels, respectively, in diabetic men and women. Our data indicated that the genotype scores for low HDL cholesterol or high triglycerides were associated with elevated type 2 diabetes risk, whereas the LDL cholesterol genotype score was not related to diabetes risk. These findings are largely in line with previous observations from the prospective studies showing that low HDL cholesterol and high triglyceride levels, but not high LDL cholesterol levels, significantly predicted the risk of type 2 diabetes (2–7). Because genetic variants are randomly assigned and generally uncorrelated with environmental

TABLE 3  
Association between the HDL cholesterol genotype score and risk for type 2 diabetes

	Continuous	Quartile				P for trend
		1	2	3	4	
<b>Women</b>						
<i>n</i> (case/control subjects)		287/434	321/439	304/436	405/435	
Median (range)		32.9 (25.1–34.5)	35.9 (34.6–37.1)	38.3 (37.2–39.5)	41.5 (39.6–56.3)	
OR (95% CI)						
Age and BMI adjusted	1.03 (1.01–1.05)	1.00	1.17 (0.93–1.47)	1.16 (0.92–1.46)	1.40 (1.12–1.75)	0.004
Multivariate adjusted*	1.03 (1.01–1.05)	1.00	1.18 (0.93–1.48)	1.16 (0.92–1.47)	1.39 (1.11–1.75)	0.006
<b>Men</b>						
<i>n</i> (case/control subjects)		232/324	319/323	264/320	291/324	
Median (range)		33.1 (24.4–34.7)	36.1 (34.7–37.4)	38.5 (37.5–39.8)	41.5 (39.9–51.9)	
OR (95% CI)						
Age and BMI adjusted	1.02 (0.99–1.04)	1.00	1.40 (1.09–1.79)	1.11 (0.86–1.43)	1.32 (1.03–1.70)	0.14
Multivariate adjusted*	1.02 (1.00–1.05)	1.00	1.42 (1.10–1.82)	1.12 (0.87–1.46)	1.39 (1.07–1.79)	0.07
<b>Pooled OR (95% CI)†</b>						
Age and BMI adjusted	1.02 (1.01–1.04)	1.00	1.27 (1.07–1.50)	1.14 (0.96–1.35)	1.37 (1.16–1.61)	0.002
Multivariate adjusted*	1.03 (1.01–1.04)	1.00	1.28 (1.08–1.52)	1.15 (0.96–1.35)	1.39 (1.17–1.65)	0.001

\*Adjusted for age, BMI, smoking, alcohol intake, physical activity, and menopausal status (women only). †Results were pooled between women and men using inverse variance weights under a fixed model, because there was no heterogeneity between women and men (all *P* for heterogeneity >0.30).

factors, the observed genetic associations should be unaffected by confounding factors and also free of the inverse effect of type 2 diabetes. Thus, our results support potentially causal roles of HDL cholesterol and triglycerides in type 2 diabetes. However, we could not exclude the possibility that the genotype scores might be related to other unknown variables in the causal pathway. Our data are different from a previous study that did not find the significant association between a triglyceride genotype score and type 2 diabetes risk (8). Of note, the previous study included fewer loci (which only accounted for 3–5% of the variation in circulation triglycerides [8]) than our study. The current triglyceride genotype score included more loci, which jointly explained ~10% of the variation of plasma triglycerides (9).

There are several lines of evidence supporting the probably causal relationship between low HDL cholesterol/high triglyceride levels and type 2 diabetes risk. Recent data indicated that HDL may influence β-cell function by its abilities of cholesterol efflux, antioxidation, anti-inflammation, and antiapoptosis (26), and accumulation of triglycerides ectopically in other tissues than adipose may impair insulin signaling as well as insulin secretion (27). Findings from previous prospective studies in large populations from different ethnical groups have shown that plasma HDL cholesterol and triglyceride levels are independent predictors of future type 2 diabetes (2–7). For instance, a previous study comprising >14,000 participants found that the risk of new-onset diabetes decreased by 28% and increased by 12% per mmol/L increase in baseline HDL cholesterol and triglyceride levels, respectively (5). The

TABLE 4  
Association between the triglyceride genotype score and risk for type 2 diabetes

	Continuous	Quartile				P for trend
		1	2	3	4	
<b>Women</b>						
<i>n</i> (case/control subjects)		308/436	302/437	337/436	371/437	
Median (range)		20.7 (13.2–22.1)	23.1 (22.2–24.0)	24.8 (24.1–25.8)	27.3 (25.9–35.3)	
OR (95% CI)						
Age and BMI adjusted	1.02 (0.99–1.05)	1.00	1.01 (0.81–1.27)	1.09 (0.87–1.36)	1.22 (0.97–1.52)	0.06
Multivariate adjusted*	1.02 (0.99–1.05)	1.00	1.00 (0.79–1.26)	1.07 (0.85–1.34)	1.20 (0.96–1.50)	0.09
<b>Men</b>						
<i>n</i> (case/control subjects)		264/323	239/322	331/324	274/322	
Median (range)		20.9 (13.1–22.2)	23.1 (22.3–23.9)	24.9 (24.0–26.1)	27.6 (26.2–35.5)	
OR (95% CI)						
Age and BMI adjusted	1.02 (0.99–1.05)	1.00	0.92 (0.71–1.18)	1.30 (1.02–1.65)	1.15 (0.90–1.48)	0.05
Multivariate adjusted*	1.03 (1.00–1.06)	1.00	0.92 (0.71–1.19)	1.28 (1.01–1.64)	1.18 (0.92–1.52)	0.04
<b>Pooled OR (95% CI)†</b>						
Age and BMI adjusted	1.02 (1.00–1.04)	1.00	0.97 (0.82–1.15)	1.18 (1.00–1.39)	1.19 (1.00–1.40)	0.007
Multivariate adjusted*	1.02 (1.00–1.04)	1.00	0.96 (0.81–1.14)	1.17 (0.99–1.38)	1.19 (1.01–1.41)	0.008

\*Adjusted for age, BMI, smoking, alcohol intake, physical activity, and menopausal status (women only). †Results were pooled between women and men using inverse variance weights under a fixed model, because there was no heterogeneity between women and men (all *P* for heterogeneity >0.30).

observed associations between the genetic predisposition to low HDL cholesterol or high triglycerides and elevated type 2 diabetes risk in the current study provide further evidence for the true association, because they are unlikely to be the results of confounding factors. Moreover, importantly, data from some clinical trials have shown that bezafibrate, a drug used to treat dyslipidemia by raising HDL and lowering triglycerides, could improve insulin resistance (28) and lower the hazard for incident diabetes (29,30). Taken together, these findings support that contention that dyslipidemia of low HDL cholesterol/high triglycerides plays a causal role in the development of type 2 diabetes, which suggests the potential importance of therapeutic implications of dyslipidemia, either pharmacologically or through lifestyle intervention, in preventing type 2 diabetes.

The major strengths of our study include the prospective study design, high-quality genotype data, and minimal population stratification (13). We acknowledge that plasma lipids were not measured in control participants. Although our lipid genotype scores captured the combined information from most of the established genetic variants for blood lipids, these variants only explained ~10% variation of each lipid trait (9). This may explain the observed moderate effect of the lipid genotype scores on type 2 diabetes risk. There were several overlapped SNPs included in these three lipid genotype score, because these SNPs have been associated with more than one lipid trait (9). However, in our sensitivity analyses, when the SNPs that had the largest effects on other two lipid traits were excluded from the calculation of the genotype score, we observed similar results. In addition, our study is restricted to white subjects, and whether the genotype scores of HDL cholesterol and triglycerides are significantly associated with type 2 diabetes in other ethnic groups remains to be investigated.

In conclusion, we found that the genetic predisposition to low HDL cholesterol or high triglycerides, estimated by the lipid genotype scores, were associated with an increased risk of type 2 diabetes among women and men from two prospective cohorts. Our findings support a potentially causal relationship between low HDL cholesterol or high triglyceride levels and type 2 diabetes.

#### ACKNOWLEDGMENTS

This study was supported by grants HL-71981, HL-073168, U01-HG-004399, DK-58845, and DK-46200 from the National Institutes of Health. L.Q. is a recipient of the American Heart Association Scientist Development Award (0730094N).

No potential conflicts of interest relevant to this article were reported.

Q.Q. designed the study, researched data, and wrote, edited, and reviewed the manuscript. L.L. and F.B.H. researched data, contributed to the discussion, and edited and reviewed the manuscript. A.D. contributed to the discussion and edited and reviewed the manuscript. L.Q. designed the study, researched data, contributed to the discussion, and edited and reviewed the manuscript. L.Q. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in abstract form at the 71st Scientific Sessions of the American Diabetes Association, San Diego, California, 24–28 June 2011.

The authors thank all the participants of the NHS and the HPFS for their continued cooperation.

#### REFERENCES

- Haffner SM; American Diabetes Association. Dyslipidemia management in adults with diabetes. *Diabetes Care* 2004;27(Suppl. 1):S68–S71
- D'Agostino RB Jr, Hamman RF, Karter AJ, Mykkanen L, Wagenknecht LE, Haffner SM; Insulin Resistance Atherosclerosis Study Investigators. Cardiovascular disease risk factors predict the development of type 2 diabetes: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 2004;27:2234–2240
- Schmidt MI, Duncan BB, Bang H, et al.; Atherosclerosis Risk in Communities Investigators. Identifying individuals at high risk for diabetes: the Atherosclerosis Risk in Communities study. *Diabetes Care* 2005;28:2013–2018
- Wilson PWF, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Arch Intern Med* 2007;167:1068–1074
- Gupta AK, Dahlof B, Dobson J, Sever PS, Wedel H, Poulter NR; Anglo-Scandinavian Cardiac Outcomes Trial Investigators. Determinants of new-onset diabetes among 19,257 hypertensive patients randomized in the Anglo-Scandinavian Cardiac Outcomes Trial: Blood Pressure Lowering Arm and the relative influence of antihypertensive medication. *Diabetes Care* 2008;31:982–988
- Chien K, Cai T, Hsu H, et al. A prediction model for type 2 diabetes risk among Chinese people. *Diabetologia* 2009;52:443–450
- Kahn HS, Cheng YJ, Thompson TJ, Imperatore G, Gregg EW. Two risk-scoring systems for predicting incident diabetes mellitus in U.S. adults age 45 to 64 years. *Ann Intern Med* 2009;150:741–751
- De Silva NMG, Freathy RM, Palmer TM, et al. Mendelian randomization studies do not support a role for raised circulating triglyceride levels influencing type 2 diabetes, glucose levels, or insulin resistance. *Diabetes* 2011;60:1008–1018
- Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010;466:707–713
- Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health* 1997;6:49–62
- Rimm EB, Giovannucci EL, Willett WC, et al. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* 1991;338:464–468
- Cornelis MC, Qi L, Zhang C, et al. Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry. *Ann Intern Med* 2009;150:541–550
- Qi L, Cornelis MC, Kraft P, et al.; Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC); Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium. Genetic variants at 2q24 are associated with susceptibility to type 2 diabetes. *Hum Mol Genet* 2010;19:2706–2715
- Manson JE, Rimm EB, Stampfer MJ, et al. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. *Lancet* 1991;338:774–778
- Hu FB, Leitzmann MF, Stampfer MJ, Colditz GA, Willett WC, Rimm EB. Physical activity and television watching in relation to risk for type 2 diabetes mellitus in men. *Arch Intern Med* 2001;161:1542–1548
- National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979;28:1039–1057
- American Diabetes Association. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997;20:1183–1197
- Patterson N, Price AL, Reich D. Population structure and eigenanalysis. *PLoS Genet* 2006;2:e190
- Willett W, Stampfer MJ, Bain C, et al. Cigarette smoking, relative weight, and menopause. *Am J Epidemiol* 1983;117:651–658
- Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology* 1990;1:466–473
- Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol* 1994;23:991–999
- Dupuis J, Langenberg C, Prokopenko I, et al.; DIAGRAM Consortium; GIANT Consortium; Global BPgen Consortium; Anders Hamsten on behalf of Procardis Consortium; MAGIC investigators. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 2010;42:105–116
- Rung J, Cauchi S, Albrechtsen A, et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. *Nat Genet* 2009;41:1110–1115



24. Voight BF, Scott LJ, Steinthorsdottir V, et al.; MAGIC investigators; GIANT Consortium. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet* 2010;42:579–589
25. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med* 1989;8:551–561
26. Kruit JK, Brunham LR, Verchere CB, Hayden MR. HDL and LDL cholesterol significantly influence beta-cell function in type 2 diabetes mellitus. *Curr Opin Lipidol* 2010;21:178–185
27. Szendroedi J, Roden M. Ectopic lipids and organ function. *Curr Opin Lipidol* 2009;20:50–56
28. Tenenbaum A, Fisman EZ, Boyko V, et al. Attenuation of progression of insulin resistance in patients with coronary artery disease by bezafibrate. *Arch Intern Med* 2006;166:737–741
29. Tenenbaum A, Motro M, Fisman EZ, et al. Peroxisome proliferator-activated receptor ligand bezafibrate for prevention of type 2 diabetes mellitus in patients with coronary artery disease. *Circulation* 2004;109:2197–2202
30. Tenenbaum A, Motro M, Fisman EZ, et al. Effect of bezafibrate on incidence of type 2 diabetes mellitus in obese patients. *Eur Heart J* 2005;26:2032–2038