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Genetic Modulation of Lipid Profiles following Lifestyle Modification or Metformin Treatment: The Diabetes Prevention Program

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Abstract

Weight-loss interventions generally improve lipid profiles and reduce cardiovascular disease risk, but effects are variable and may depend on genetic factors. We performed a genetic association analysis of data from 2,993 participants in the Diabetes Prevention Program to test the hypotheses that a genetic risk score (GRS) based on deleterious alleles at 32 lipid-associated single-nucleotide polymorphisms modifies the effects of lifestyle and/or metformin interventions on lipid levels and nuclear magnetic resonance (NMR) lipoprotein subfraction size and number. Twenty-three loci previously associated with fasting LDL-C, HDL-C, or triglycerides replicated ($P = 0.04–1\times10^{-17}$). Except for total HDL particles ($r = -0.03$, $P = 0.26$), all components of the lipid profile correlated with the GRS (partial $|r| = 0.07–0.17$, $P = 5\times10^{-5}–1\times10^{-19}$). The GRS was associated with higher baseline-adjusted 1-year LDL cholesterol levels ($\beta = +0.87$, SEE ± 0.22 mg/dl/allele, $P = 8\times10^{-5}$, $P_{interaction} = 0.02$) in the lifestyle intervention group, but not in the placebo ($\beta = +0.20$, SEE ± 0.22 mg/dl/allele, $P = 0.35$) or metformin ($\beta = -0.03$, SEE ± 0.22 mg/dl/allele, $P = 0.90$; $P_{interaction} = 0.64$) groups. Similarly, a higher GRS predicted a greater number of baseline-adjusted small LDL particles at 1 year in the lifestyle intervention arm ($\beta = +0.30$, SEE ± 0.012 ln nmol/L/allele, $P = 0.01$, $P_{interaction} = 0.01$) but not in the placebo ($\beta = -0.002$, SEE ± 0.008 ln nmol/L/allele, $P = 0.74$) or metformin ($\beta = +0.013$, SEE ± 0.008 nmol/L/allele, $P = 0.12$; $P_{interaction} = 0.24$) groups. Our findings suggest that a high genetic burden confers an adverse lipid profile and predicts attenuated response in LDL-C levels and small LDL particle number to dietary and physical activity interventions aimed at weight loss.

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Introduction

Dyslipidemia is a strong risk factor for atherosclerotic heart disease [1–3], has a well-defined genetic basis [4], and is modifiable through therapeutic lifestyle changes and weight-loss interventions [5,6]. Individuals at risk for diabetes are also at high risk of cardiovascular disease [7], and individualized lifestyle intervention programs, like the one incorporated into the Diabetes Prevention Program (DPP), have a salutary effect on dyslipidemia and cardiovascular disease risk in this population. However, the cost of widespread implementation of such interventions has been highlighted as a major limitation [8] and not all benefit equally from such interventions. Identifying persons most likely to benefit from intensive lifestyle modification could provide justification for targeting this subpopulation first, making the clinical translation of findings from studies such as the DPP more feasible.

Selection of persons whose dyslipidemia is likely to respond well to lifestyle interventions or pharmacotherapy could help target resources and optimize prevention strategies. To do so requires knowledge of the underlying risk factors for the trait and knowledge of how personal characteristics interact with exercise, diet, and weight loss. Although the heritability of polygenic dyslipidemia [9–11] and its sequelae [12] have been elucidated, little is known of how lifestyle interventions modify the effects of these loci, singly or in combination, on lipid profiles. Thus, learning how a person's genetic background modulates his or her response to therapeutic lifestyle changes and weight-loss interventions might help optimize the targeting of interventions designed to mitigate cardiovascular and metabolic disease risk.

The purpose of this study was to examine whether loci reliably associated with polygenic dyslipidemia modified the response to cardio-protective interventions in the DPP, a randomized clinical trial of intensive lifestyle modification, metformin treatment, or placebo with standard care. We hypothesized i) that the baseline lipid profiles of DPP participants would be associated with gene variants known to associate with polygenic dyslipidemia and ii) that improvement in lipidemia following treatment would depend on these same genetic variants. We also used NMR spectroscopy to characterize the associations of these previously reported loci with lipoprotein subfractions.

Results

Table 1 shows participant characteristics stratified by DPP treatment arm. The effects of the DPP interventions on 1 yr changes in weight [14], insulin secretion [13], beta-cell function [13], and lipid traits [29] are reported in detail elsewhere.

Individual SNP Replication

Thirty-two SNPs previously associated with triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and/or high-density lipoprotein-cholesterol (HDL-C) levels were considered [10]. Thirty-one of these were successfully genotyped in the DPP, and two SNPs in *CETP*, serving as HapMap proxies ($r^2 \geq 0.90$) for rs173539, including rs247616, were subsequently successfully genotyped, with rs247616 retained as the replacement for rs173539. Twenty-three of these 32 non-redundant SNPs replicated with their respective traits in a directionally consistent manner ($P \leq 0.05$), including 8/11 for TG, 9/14 for HDL-C and 8/11 for LDL-C. Two of the SNPs, rs12678919 and rs964184, replicated for both HDL-C and TG (Table S1).

Association of Individual SNPs with All Four Lipid Traits and Ten Lipoprotein Traits

Additionally, we evaluated the associations of the 32 lipid loci with baseline lipids and nuclear magnetic resonance (NMR)-derived lipoprotein traits (Large HDL particles, Small HDL particles, Total HDL particles, HDL size, LDL size, Total LDL particles, Small LDL particles, Total VLDL particles, Large VLDL particles, VLDL size). Of all analyses of baseline traits, roughly one third of the tests were nominally significant associations, and 35 associations were significant after correcting for all 448 hypothesis tests; these involved 12 SNPs and 13 traits (Table S2). Interestingly, SNP rs10401969 did not replicate for LDL-C (C vs. T: $\beta \pm SEM = -0.1 \pm 1.6$ mg/dl, additive $P = 0.94$), but was associated with decreased large VLDL (mean 5.43, 4.26, 4.07 nmol/L for TT, TC, CC genotypes respectively, additive $P = 4 \times 10^{-5}$) and smaller VLDL size (53.18, 50.94, 49.55 nm, $P = 2 \times 10^{-6}$). SNP rs7679 did not quite reach nominal significance for decreased HDL-C (C vs. T: $\beta \pm SEM = -0.016 \pm 0.009$ ln mg/dl, additive $P = 0.07$), but was very strongly associated with increased small HDL particle number (17.93, 20.14 and 21.89 μmol/L for TT, CT and CC genotypes respectively; additive $P = 4 \times 10^{-18}$) and consequently total HDL particle number (34.07, 35.10 and 36.78 μmol/L, $P = 2 \times 10^{-5}$).

Association of Genetic Risk Score (GRS) with Baseline Lipid and Lipoprotein Traits

A lipid GRS was calculated for each individual by first replacing missing genotypes with ethnicity-specific imputed means and then adding up the number of risk alleles possessed for each of the 32 independent SNPs. Of the 32 SNPs evaluated, 11 were originally associated in the meta-analysis with LDL cholesterol, 10 with HDL cholesterol only, seven with triglycerides only, and four with both HDL cholesterol and triglycerides. A risk allele was defined as one associated with increased TG or LDL-C or decreased HDL in the original meta-analysis [10]. After adjustment for age, sex, ethnicity, and BMI, the GRS was significantly associated with all baseline traits evaluated except total HDL particles ($P = 0.26$, Table 2). The following are *P*-values for the effects of the GRS, as a quantitative covariate, and geometric means for the upper and

Author Summary

The study included 2,993 participants from the Diabetes Prevention Program, a randomized clinical trial of intensive lifestyle intervention, metformin treatment, and placebo control. We examined associations between 32 gene variants that have been reproducibly associated with dyslipidemia and concentrations of lipids and NMR lipoprotein particle sizes and numbers. We also examined whether genetic background influences a person's response to cardioprotective interventions on lipid levels. Our analysis, which focused on determining whether common genetic variants impact the effects of cardioprotective interventions on lipid and lipoprotein particle size, shows that in persons with a high genetic risk score the benefit of intensive lifestyle intervention on LDL and small LDL particle levels is substantially diminished; this information may be informative for the targeted prevention of dyslipidemia, as it suggests that genetics might help identify persons in whom lifestyle intervention is likely to be an effective treatment for elevated lipids and lipoproteins. The NMR subfraction analyses provide novel insight into the biology of dyslipidemia by illustrating how numerous genetic variants that have previously been associated with lipid levels also modulate NMR lipoprotein particle sizes and number. This information may be informative for the targeted prevention of cardiovascular disease.

lower ethnicity-specific GRS quartiles for each trait. A higher GRS was associated with elevated baseline levels of: total cholesterol ($P=4\times10^{-11}$, 206 vs. 195 mg/dl), LDL-C ($P=9\times10^{-8}$, 129 vs. 121 mg/dl; arithmetic means), TG ($P=4\times10^{-19}$, 160 vs. 127 mg/dl), total VLDL particles ($P=6\times10^{-14}$, 67 vs. 53 nmol/L), large VLDL particles ($P=1\times10^{-14}$, 6.57 vs. 4.21 nmol/L), total LDL particles ($P=2\times10^{-10}$, 1412 vs. 1262 nmol/L), small LDL particles ($P=2\times10^{-11}$, 743 vs. 543 nmol/L), small HDL particles ($P=0.0005$, 19.17 vs. 18.10 μmol/L), and VLDL particle size ($P=1\times10^{-5}$, 53.86 vs. 51.84 nm). A higher GRS was also associated with lower baseline levels of: HDL-C ($P=1\times10^{-15}$, 43 vs. 47 mg/dl), LDL particle size ($P=1\times10^{-19}$, 0.256 vs. 0.269 nm), large HDL particles ($P=2\times10^{-8}$, 2.98 vs. 3.68 μmol/L), and HDL particle size ($P=0.0003$, 8.82 vs. 8.90 nm). All of these results are consistent with a greater number of risk alleles increasing the atherogenicity of the lipoprotein profile.

GRS×Intervention Interactions of Baseline-Adjusted One-Year Traits

Two traits showed evidence of GRS×lifestyle interaction: LDL-C ($P=0.02$) and small LDL particles ($P=0.01$, Table 3; Figure 1; Figure S1a–S1f). For these two traits, there was a residual detrimental impact of GRS in the lifestyle (i.e., the GRS was associated with higher levels at one year even after adjusting for baseline levels) but not the metformin or placebo group, suggesting that the lifestyle intervention was less effective at lipid-lowering in those with a higher genetic burden. A unit (allele) GRS increase was associated with higher residual LDL-C levels in the lifestyle group (β+0.087, SEE±0.022 mg/dl, $P=8\times10^{-5}$) but not in the metformin (β−0.03, SEE±0.22 mg/dl, $P=0.90$) or placebo (β+0.20, SEE±0.22 mg/dl, $P=0.35$) groups (Figure 1). Similarly, the GRS was associated with higher residual ln-small LDL particles in the lifestyle group (β+0.030, SEE±0.0.012 ln nmol/L, $P=0.01$), but not in the metformin (β−0.013, SEE±0.008 ln nm/

Table 1. Baseline Characteristics of the Study Population by Treatment Group [Quantitative Traits Are Shown as Median (Interquartile Range)].

Trait	Placebo	Metformin	Lifestyle
n	947	939	962
Age	49 (43–57)	50 (44–57)	49 (42–58)
Sex [M:F (% male)]	290:657 (31% male)	321:618 (34% male)	308:654 (32% male)
White/AA/Hisp/Asian/AI: *n* (%)	515(54)/207(22)/157(17)/38(4)/30(3)	534(57)/194(21)/155(17)/33(4)/23(3)	517(54)/191(20)/173(18)/53(6)/28(3)
BMI (kg/m^2)	33.4 (29.2–38.3)	33.0 (29.1–37.7)	32.8 (29.0–37.3)
Waist Circumference (cm)	104.4 (95–114.7)	104.3 (94.7–114.0)	103.8 (95.0–113.6)
Total Cholesterol (mg/dl)	201 (178–227)	202 (177–225)	202 (179–227)
LDL-C (mg/dl)	123 (102–147)	123 (103–145)	124 (102–145)
HDL-C (mg/dl)	43 (37–50)	44 (38–52)	44 (37–53)
Triglycerides (mg/dl)	146 (102.5–205.5)	135 (97–195)	136 (94–200)
Large HDL particles (umol/L)	3.3 (2.1–5.5)	3.4 (2.2–5.3)	3.3 (2.2–5.4)
Small HDL particles (umol/L)	19 (16.1–22.2)	19.2 (16.3–22.6)	18.9 (15.8–21.8)
Total HDL particles (umol/L)	34.1 (30.4–38.5)	34.7 (31.2–38.9)	33.95 (30.2–38.1)
HDL size (nm)	8.8 (8.6–9.1)	8.8 (8.6–9.1)	8.8 (8.6–9.1)
LDL size (nm)	0.263 (0.237–0.289)	0.263 (0.237–0.289)	0.263 (0.237–0.289)
Total LDL particles (nmol/L)	1369 (1140–1629)	1367 (1108–1607)	1332 (1123–1591)
Small LDL particles (nmol/L)	788 (517–1059)	779 (525–1041)	764 (520–1040)
Total VLDL particles (nmol/L)	63.3 (43.9–88.1)	62.4 (42.6–86.2)	63.2 (42.1–88.5)
Large VLDL particles (nmol/L)	5.4 (2.8–10.8)	6.1 (2.8–11)	5.9 (2.7–10.8)
VLDL size (nm)	52.2 (47.0–58.9)	53.0 (46.9–59.4)	52.8 (47.0–59.0)

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L, $P=0.12$) or placebo ($\beta-0.002$, SEE$\pm$0.008, $P=0.74$) groups (Figure 1). There were no metformin×GRS interactions significant at the $P=0.05$ level. In addition to the three traits discussed, several traits showed residual detrimental effects of the GRS in one or more strata (total cholesterol, TG, LDL size, total VLDL particles, large HDL particles, and HDL size) without any statistical evidence of treatment×GRS interaction (Table 3).

Discussion

In the present study, the majority of previously associated SNPs replicated for baseline lipid traits, and there was a statistically significant relationship between the GRS and the vast majority of standard lipid traits and NMR lipoprotein subfractions. Importantly, in several cases the evidence for association with NMR subfractions was much stronger than for the original standard lipid trait, which may be owing to the relative proximity of the subfractions to the genetic loci. For example, SNP rs7679, originally associated with total HDL–C levels in previous GWASs, in the DPP was not significantly associated with HDL-C ($P=0.07$) but was strongly associated with small HDL particle levels ($P=4\times10^{-18}$). This SNP is near *PLTP*, encoding phospholipid transfer protein, a molecule directly influencing HDL particle size [14]. Such findings extend our understanding of lipid biology and suggest that, compared to standard lipid levels, measurements of lipoprotein subfractions may provide a more effective way of capturing genetically influenced risk. This is particularly important, as recent studies have shown that HDL-C is a heterogeneous trait, which in the context of clinical use may benefit from substratification by genotype [15].

We also observed that within the lifestyle group but not the placebo or metformin groups, the GRS was associated with higher LDL-C and small LDL particle levels after one year of intervention. These findings suggest that the genetic burden on these traits cannot be completely overcome by lifestyle modification. However, even those with the greatest genetic burden benefit to a limited extent from lifestyle intervention in terms of LDL-C reduction (Figure 1A), although the effect on LDL particle size reduction is almost completely ablated (Figure 1B). Even a true residual effect of lifestyle on LDL-C in people with the highest GRS does not negate the clinical relevance of our findings in terms of potential to facilitate tailored treatment decisions. Seeing less of an effect of lifestyle in a particular patient subgroup indicates that these persons may benefit from more frequent surveillance, more intense lifestyle interventions, or aggressive pharmaceutical interventions to supplement lifestyle interventions. Conversely, knowing that lifestyle intervention is likely to be adequate in persons with the lowest genetic burden may maximize the patient's diet adherence and potentially reduce the costs and side effects associated with prescribing lipid-lowering medications unnecessarily. The availability of information on genetic background may also facilitate patient-provider dialogue, owing to improved diagnostic accuracy. This is similar to the strategy used to control cholesterol levels in patients with a monogenic disorder such as familial hypercholesterolemia due to a severe loss of function mutation in LDLR, where lifestyle intervention combined with pharmacotherapy is needed to bring LDL-C levels within an acceptable range (Third Report of the NCEP-ATP III on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults).

It is also important to bear in mind that the interaction effects may be underestimated in our paper. This is because the majority of SNPs included in the GRS are likely to be imperfect proxies for unobserved functional variants, resulting in some degree of genotype misclassification. Moreover, all 32 SNPs were included within the GRS, even though not all SNPs convey statistically significant effects in the DPP and do not individually modify the effects of the interventions. A parsimonious GRS including only those SNPs that are statistically significant in the DPP would likely be overfitted to our data, resulting in biased conclusions about the strength and magnitude of gene×treatment interactions.

Dyslipidemia is a long-established risk factor for CVD [1–3]. Thus, the primary and secondary prevention of atherosclerotic CVD often involves intervening on lipid levels [16]. Lifestyle interventions [17] and metformin treatment [18] that result in weight loss have the potential to improve lipid profiles; nevertheless, as long recognized [19], changes in lipid profiles following interventions vary greatly from one person to the next. Some of the variability in response to interventions may be because genotypes modulate the effects of preventive interventions on lipid homeostasis and CVD risk [20].

Of the many known dyslipidemia-predisposing loci discovered so far [10], only a handful have been the focus of studies testing hypotheses of gene×treatment interactions [21–28], and most of these studies are small ($N<150$), non-randomized trials of dietary intervention. Although some of these studies have focused on genomic regions that are confirmed to harbor dyslipidemia-predisposing loci, such as *APOB*, *CETP*, *LIPC* and *LPL* [21,22,24–28], no exhaustive studies testing whether GWAS-discovered loci [10,29,30] modify response to treatments have been previously reported.

The GRS used in this study attenuated the impact of the DPP lifestyle intervention on LDL levels and small LDL particle number. This suggests that a genetic predisposition to high LDL levels and more small LDL particles is difficult to overcome through lifestyle intervention alone. These data also unmask the effects of an underlying genetic defect of LDL levels and small LDL particles found in individuals with a high genetic burden, which becomes visible when adiposity and blood TG content are reduced through lifestyle intervention. This information may justify the combination of lifestyle and lipid lowering drug treatment from the outset in these individuals, rather than the usual approach of stepping from lifestyle to drug therapy when the former fails.

The DPP lifestyle intervention prioritized weight loss, daily fat gram intake and physical activity goals over intake of saturated fat, cholesterol, viscous fiber and plant stanols/sterols; this may have influenced the nature of the changes in the lipid profile. When compared to the metformin and placebo groups, the lifestyle intervention group reported improved physical activity levels and reductions in calorie intake, resulting in significantly greater weight losses [31], each of which has major influences on TG levels. The lifestyle intervention group reported significantly greater reductions in percent calories from total fat and saturated fat than the metformin and placebo groups [31]. However, they did not, on average, achieve the National Cholesterol Education Program target for saturated fat intake and did not focus on the other therapeutic lifestyle changes, such as the additional dietary changes mentioned above, that often have the largest effects on LDL concentrations. The ethnic diversity of the DPP cohort facilitates the generalizability of results, but may also lead to confounding by population stratification in genetic analyses. However, Sensitivity analyses in the European White sub-cohort of the DPP yielded comparable effect estimates to the results obtained in the entire DPP genetics cohort (results for baseline traits shown in Table S3), supporting the conclusion that confounding by population stratification is unlikely to explain our findings.

Table 2. Association of 32-SNP GRS with Baseline Lipid and Lipoprotein Traits (n≤2,843).

Trait	Q1	Q2	Q3	Q4	%diff**	Partial r*	Beta±SE/unit	p-value***
	(22–33*)	(33–35*)	(35–37*)	(37–44*)				
n	875	751	636	586	–	–	–	–
Chol (mg/dl)	195 (192–197)	199 (196–202)	201 (199–204)	206 (204–209)	6%	0.12	+0.007±0.001	$\mathbf{4\times10^{-11}}$
LDL-C (mg/dl)	121 (119–123)	124 (122–127)	126 (124–129)	129 (127–132)	7%	0.11	+1.01±0.19	$\mathbf{9\times10^{-8}}$
HDL-C (mg/dl)	47 (47–48)	46 (45–46)	45 (44–46)	43 (42–44)	9%	−0.15	−0.011±0.001	$\mathbf{1\times10^{-15}}$
TG (mg/dl)	127 (123–131)	140 (135–145)	146 (141–152)	160 (153–166)	26%	0.14	+0.026±0.003	$\mathbf{4\times10^{-19}}$
LDL size (nm)	0.269 (0.267–0.271)	0.264 (0.262–0.266)	0.260 (0.258–0.262)	0.256 (0.254–0.258)	5%	−0.17	−0.0059±0.0007	$\mathbf{1\times10^{-19}}$
Total VLDL particles (nmol/L)	53 (51–55)	59 (56–61)	62 (59–65)	67 (64–71)	26%	0.17	+0.027±0.004	$\mathbf{6\times10^{-14}}$
Large VLDL particles (nmol/L)	4.21 (3.91–4.54)	4.93 (4.54–5.35)	5.87 (5.37–6.42)	6.57 (5.99–7.2)	56%	0.15	+0.052±0.057	$\mathbf{1\times10^{-14}}$
Total LDL particles (nmol/L)	1262 (1234–1292)	1304 (1272–1337)	1345 (1308–1382)	1412 (1373–1453)	12%	0.13	+0.013±0.002	$\mathbf{2\times10^{-10}}$
Small LDL particles (nmol/L)	543 (511–577)	621 (581–664)	703 (654–756)	743 (689–801)	37%	0.17	+0.037±0.005	$\mathbf{2\times10^{-11}}$
Large HDL particles (μmol/L)	3.68 (3.52–3.86)	3.31 (3.15–3.48)	3.21 (3.04–3.39)	2.98 (2.82–3.15)	19%	−0.15	−0.023±0.004	$\mathbf{2\times10^{-8}}$
Small HDL particles (μmol/L)	18.10 (17.71–18.49)	18.42 (17.99–18.85)	18.57 (18.1–19.05)	19.17 (18.67–19.69)	6%	0.08	+0.007±0.002	**0.0005**
HDL size (nm)	8.90 (8.87–8.93)	8.87 (8.83–8.9)	8.83 (8.8–8.87)	8.82 (8.78–8.85)	1%	−0.07	−0.0011±0.0003	**0.0003**
VLDL size (nm)	51.84 (51.21–52.48)	52.34 (51.66–53.04)	53.65 (52.88–54.43)	53.86 (53.07–54.66)	4%	0.10	+0047±0.0011	$\mathbf{1\times10^{-5}}$
Total HDL particles (μmol/L)	34.62 (34.17–35.07)	34.19 (33.71–34.68)	34.52 (33.99–35.06)	34.17 (33.63–34.73)	1%	−0.03	−0.0013±0.0012	0.26

*Quartiles were assigned separately in each ethnic group, leading to slight overlap in quartile ranges of number of risk alleles. Traits are age-, sex-, ethnicity- and BMI-adjusted geometric means and 95% confidence intervals, except LDL-C, for which arithmetic mean and 95% confidence interval are shown. Ethnic specific quartile upper limits are 32, 34, 36, 44 alleles for both Whites and African Americans, 32, 35, 37, 43 alleles for Hispanics, 32, 35, 37, 42 alleles for Asians/Pacific Islanders and 33, 34, 36, 40 alleles for American Indians.

**Percent difference between Q4 and Q1 in reference to Q1.

***Partial r and p-value based on analysis of GRS as a quantitative covariate with adjustment for age, sex, ethnicity and BMI.

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Table 3. Association of 32-SNP GRS with Baseline-Adjusted One-Year Lipid and Lipoprotein Traits (n = 2,686).

Trait	Placebo	Metformin	p	ILS	p
	$\beta_{GRS} \pm SE$	$\beta_{GRS} \pm SE$	GRSxMet	$\beta_{GRS} \pm SE$	GRS×ILS
n	895	884	–	907	–
Chol (mg/dl)	+0.001±0.001	−0.0002±0.0012	0.42	***+0.004±0.001***	0.09
LDL-C (mg/dl)	+0.20±0.22	−0.03±0.22	0.64	***+0.87±0.22***	**0.02**
HDL-C (mg/dl)	−0.002±0.001	−0.001±0.001	0.78	−0.001±0.002	0.61
TG (mg/dl)	**+0.008±0.004**	+0.004±0.004	0.50	+0.005±0.004	0.59
LDL size (nm)	−0.0004±0.0009	**−0.0022±0.0009**	0.17	***−0.0024±0.0009***	0.22
Total VLDL particles (nmol/L)	+0.0003±0.0058	+0.0077±0.0054	0.49	**+0.0142±0.0059**	0.12
Large VLDL particles (nmol/L)	−0.0036±−0.0096	+0.0106±0.0106	0.46	+0.0085±0.0123	0.61
Total LDL particles (nmol/L)	−0.0028±0.0027	+0.0013±0.0027	0.34	+0.0035±0.0030	0.10
Large HDL particles (μmol/L)	**−0.014±0.006**	**−0.011±0.006**	0.90	−0.009±0.006	0.89
Small HDL particles (μmol/L)	−0.001±0.003	+0.001±0.003	0.56	+0.001±0.006	0.45
Small LDL particles (nmol/L)	−0.002±0.008	+0.013±0.008	0.24	***+0.030±0.012***	**0.01**
HDL size (nm)	−0.0004±0.0004	**−0.0009±0.0004**	0.38	**−0.0011±0.0005**	0.16
VLDL size (nm)	−0.00005±0.00165	+0.00015±0.00169	0.97	−0.00096±0.00189	0.54
Total HDL particles (μmol/L)	−0.0003±0.0015	−0.0021±0.0016	0.36	−0.0010±0.0017	0.76

All traits except LDL-C ln-transformed prior to analysis and presentation of beta coefficients and standard errors. Treatment-specific results in **bold** indicate p≤0.05; ***bold italics*** indicates p<0.01; ***underlined bold italics*** indicate p<0.001.

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Interestingly, no significant interaction was observed between the GRS and other biochemical components of the lipid profile in the present study. It is important to bear in mind, however, that despite being the largest clinical trial of its kind, the DPP is only moderately powered to detect gene×treatment interactions [32]; it is likely, therefore, that gene×treatment interactions that are small in magnitude will have been overlooked here. Moreover, during the course of writing this paper, many smaller impact lipid loci have been discovered [9,11]. Thus, it is possible that with a larger sample size and the inclusion of some or all of these additional loci, we may have discovered interaction effects on other lipid traits.

In summary, we have shown that common genetic loci that influence polygenic dyslipidemia also modify the effects of clinical interventions designed to mitigate cardiovascular and metabolic risk. This report is the first comprehensive effort to examine validated lipid loci within the context of a large randomized clinical trial. The findings of this study may facilitate the implementation of complex trait genetics into the clinical setting.

Methods

Participants

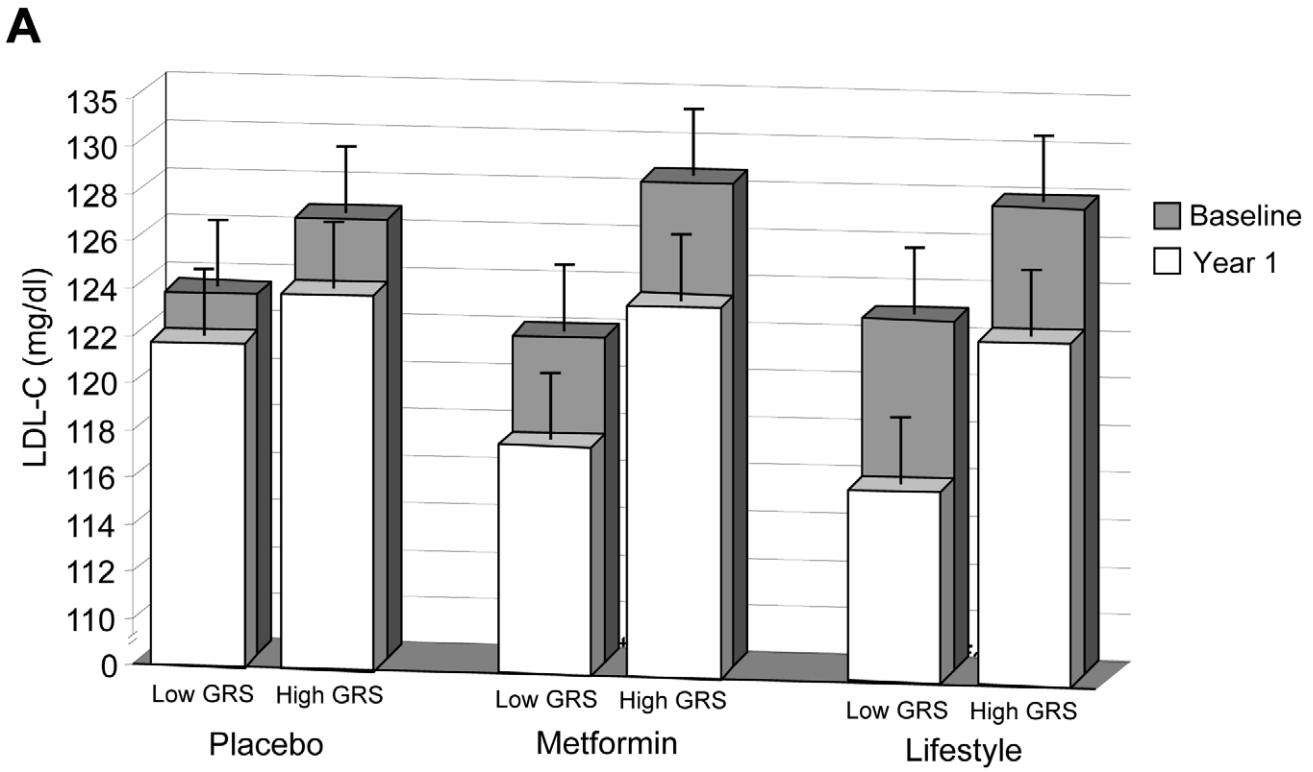
The DPP was a multi-center randomized controlled trial that examined the effects of metformin or intensive lifestyle modification on the incidence of type 2 diabetes [33,34]. Briefly, overweight persons with elevated but non-diabetic fasting and post-challenge glucose levels were randomized to receive placebo, metformin (850 mg twice daily) or a program of intensive lifestyle modification. The lifestyle intervention was designed to achieve ~150 min/wk of physical activity and ~7% weight loss via focus on daily fat gram goals. Fat gram goals were based on initial weight and 25% of calories from fat using a calorie level estimate to produce a weight loss of ~0.5–1 kg/wk. The principal endpoint was the development of diabetes by confirmed semi-annual fasting plasma glucose or annual oral glucose tolerance testing (OGTT). Other phenotypes, such as changes in weight, waist circumference, lipids, insulin and glucose, were also ascertained. Written, informed consent was obtained from each participant, and each of the 27 DPP centers obtained institutional review board approval prior to initiation of the study protocol. A total of 2,993 participants in the placebo, lifestyle and metformin groups had DNA available and provided consent for genetic analysis. Individuals taking lipid lowering medications at baseline (n = 145) were excluded from all analyses.

Measurements

All participants fasted for ≥12 hrs the night before blood was drawn from an antecubital vein. Standard blood lipid measurements (triglyceride [TG], total cholesterol, HDL-C, calculated LDL-C) were performed at the DPP central biochemistry laboratory. TG and total cholesterol levels were measured using enzymatic methods standardized to the Centers for Disease Control and Prevention reference methods [35]. HDL fractions for cholesterol analysis were obtained by the treatment of whole plasma with dextran sulfate Mg^{2+} [36]. LDL cholesterol was calculated by the Friedewald equation [37]. In participants with TGs>4.5 mmol/l, the lipoprotein fractions were separated using preparative ultracentrifugation of plasma by β quantification [38]. Lipoprotein subclass particle concentrations and average VLDL, LDL, and HDL particle diameters were measured by NMR spectroscopy at LipoScience, Inc (Raleigh, NC) with modification of existing methods [39].

Genotyping

Thirty-two SNPs previously associated with lipid concentrations in GWAS meta-analyses [10] were selected. DNA was extracted from peripheral blood leukocytes using standard methods. Genotyping was performed by allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight mass spectrometry on a Sequenom iPLEX platform [40]. The mean genotyping success


A
LDL-C (mg/dl)
135
130
128
126
124
122
120
118
116
114
112
110
0
Low GRS
High GRS
Placebo
Low GRS
High GRS
Metformin
Low GRS
High GRS
Lifestyle
Baseline
Year 1

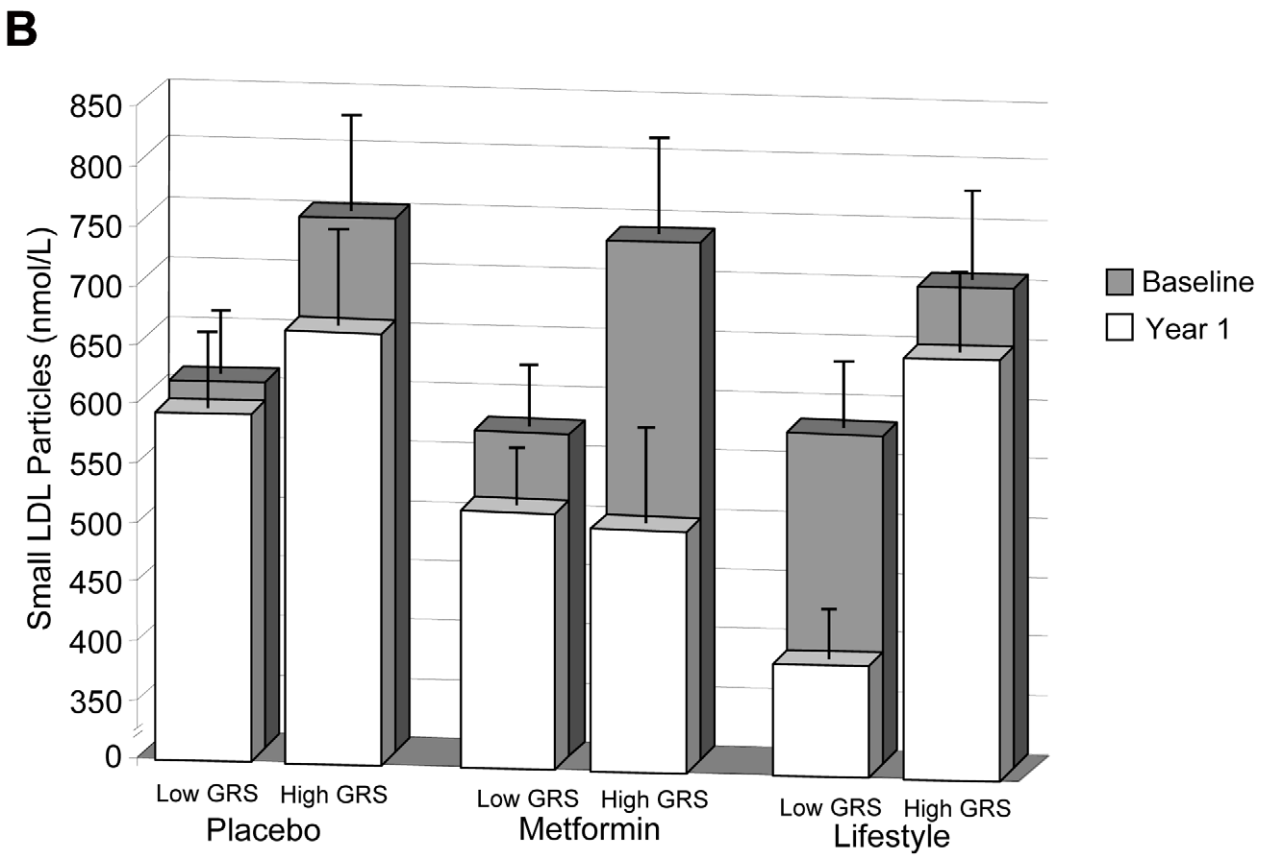

B
Small LDL Particles (nmol/L)
850
800
750
700
650
600
550
500
450
400
350
0
Low GRS
High GRS
Placebo
Low GRS
High GRS
Metformin
Low GRS
High GRS
Lifestyle
Baseline
Year 1

Figure 1. LDL-C levels. LDL-C levels at baseline and 1 year (A) and small LDL particle levels at baseline and 1 year (B) stratified by treatment group and lipid GRS. Each column shows ethnicity-adjusted arithmetic (for LDL-C) or geometric (for small LDL particles) means (with upper 95% confidence), stratified above and below (less than or equal to) the ethnic-specific median GRS value. Ethnic-specific median GRS values are 34 alleles for Caucasian, African American and American Indian ethnicities and 35 for Hispanic and Asian/Pacific Islander ethnicities.
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rate was 96.7%. The minimum call rate was 94.0%. All SNPs were in Hardy-Weinberg equilibrium within each self-reported ethnic group.

Statistical Analysis

The SAS software v9.2 (SAS, Carey, NC) was used for analyses. Baseline total cholesterol, HDL-C, TG and all lipoprotein sub-fraction levels were natural log transformed for non-normality, and LDL-C was evaluated directly. For replicating the previously reported associations of SNPs with baseline traits and evaluating the association of the individual SNPs with NMR lipoprotein particle sizes and numbers, measurements were compared across genotypic groups by ANCOVA (general model, 2 df *F* test for three possible genotypes), and evidence for an additive effect of genotype was also evaluated using the measured genotype approach, in which each genotype was assigned a value of 0, 1 or 2 according to the number of minor alleles. Analyses of baseline traits were adjusted for age, sex, self-reported ethnicity (to minimize confounding due to potential differences in both allele frequency and lipid traits across ethnicities) and BMI. For the individual SNP analyses, the Bonferroni-corrected *P*-value for significance was set at $P<0.0001$ to account for multiple comparisons (32 SNPs×14 traits = 448 tests; 0.05/448 = 0.0001).

A genetic risk score (GRS) was calculated from the 32 SNPs using the direction of association from the initial association seen in the published meta-analysis [10]; for each SNP, an allele was designated as a risk allele if it was associated with higher TG or LDL-C and/or lower HDL-C. In order to be able to incorporate all individuals in the analysis, including those missing genotypes at one or more loci, a simple imputation procedure within each self-reported ethnic group was implemented (in order to account for allele frequency differences across ethnicities) prior to score calculation. First, after coding the genotype as the number of minor alleles (0, 1 or 2), an ethnicity-specific mean genotype was calculated and rounded to the nearest whole number. Missing genotypes were replaced by the appropriate rounded mean genotype [41]. We calculated a GRS for each individual by adding up the number of risk alleles for each of the 32 tested SNPs, where a risk allele was defined as one associated with increased TG or LDL-C or decreased HDL. The GRS was then included as a quantitative independent variable in a multiple regression model for each baseline lipid/lipoprotein trait to test for association, adjusted for age, sex, self-reported ethnicity, and BMI. GRS quartiles were constructed separately within each self-reported ethnicity prior to calculating quartile-specific arithmetic means or geometric means and 95% confidence intervals. To test for interaction of the risk score with treatment, a multiple regression model was constructed with the 1 year value as the outcome variable and including GRS, lifestyle and metformin treatment and GRS×lifestyle and GRS×metformin terms, along with adjustments for the corresponding baseline trait, baseline age, sex and self-reported ethnicity.

Sample Size and Power

A priori power calculations are an important study-planning tool, providing relevant information on likely effect sizes and variances is accessible. It is possible to obtain a broad understanding of the power constraints of our study by extrapolating results from other experimental settings (as described in detail in [42]), but specific *a priori* power calculations could not be performed for the current study because reliable effect estimates and variances for tests of gene×treatment interactions for the index genotypes and phenotypes were unavailable in the published literature at the time this study was planned. *Post-hoc* power calculations were not performed, as these are well known to cause bias when interpreting a study's results [43–46]. However, confidence intervals are included in the figures, which give insight into the precision of the GRS effect estimates and hence the power to detect those estimates in the DPP cohort.

Supporting Information

Figure S1 a: Box plots for Small LDL particles measured at baseline in the placebo arm of the Diabetes Prevention Program stratified by level of the genetic risk score. b: Box plots for Small LDL particles measured at baseline in the metformin arm of the Diabetes Prevention Program stratified by level of the genetic risk score. c: Box plots for Small LDL particles measured at baseline in the lifestyle arm of the Diabetes Prevention Program stratified by level of the genetic risk score. d: Box plots for Small LDL particles measured at 1 yr follow-up in the placebo arm of the Diabetes Prevention Program stratified by level of the genetic risk score. e: Box plots for Small LDL particles measured at 1 yr follow-up in the metformin arm of the Diabetes Prevention Program stratified by level of the genetic risk score. f: Box plots for Small LDL particles measured at 1 yr follow-up in the lifestyle arm of the Diabetes Prevention Program stratified by level of the genetic risk score.
(PPTX)

Table S1 Details of individual SNP replication results (n≤2,843). The table compares results for each of the SNP loci in published meta-analysis and elsewhere with those reported here in the DPP. Analyses and means adjusted for age, sex, ethnicity and BMI.
(DOCX)

Table S2 Associations of individual SNPs with lipid or lipoprotein traits significant at the Bonferroni-significant p-value of <0.0001 for additive model (see Table 1 for units). Shown are geometric means for all traits except LDL-C, for which arithmetic means are shown. All analyses and means adjusted for age, sex, ethnicity and BMI.
(DOCX)

Table S3 Associations of individual SNPs with lipid or lipoprotein traits measured at baseline in White participants from the DPP (n = 1,565). Analyses were performed to determine whether population stratification owing to the mutliethnic nature of the DPP is likely to confound the associations reported in the main analyses. The comparability of the results in White DPP participants with the main analyses indicates that confoudning by population stratification is unlikely to underly the main results reported here. Analyses and means adjusted for age, sex, and BMI.
(DOCX)

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Conceived and designed the experiments: TIP TI JCF PWF. Performed the experiments: ESH LMD ARS RBG. Analyzed the data: TIP KAJ. Contributed reagents/materials/analysis tools: ESH LMD ARS RBG KAJ AT JM QP DA PIWdB. Wrote the paper: TIP TI PWF. Critiqued and approved the manuscript: TIP TI KAJ PIWdB AT JM QP ESH LMD DA ARS RBG JCF PWF.

References

1. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, et al. (1998) Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. JAMA 279: 1615–1622.
2. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, et al. (1996) The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 335: 1001–1009.
3. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, et al. (1995) Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. N Engl J Med 333: 1301–1307.
4. Namboodiri KK, Kaplan EB, Heuch I, Elston RC, Green PP, et al. (1985) The Collaborative Lipid Research Clinics Family Study: biological and cultural determinants of familial resemblance for plasma lipids and lipoproteins. Genet Epidemiol 2: 227–254.
5. Yu-Poth S, Zhao G, Etherton T, Naglak M, Jonnalagadda S, et al. (1999) Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. Am J Clin Nutr 69: 632–646.
6. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, et al. (2002) Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med 347: 1483–1492.
7. Cederberg H, Saukkonen T, Laakso M, Jokelainen J, Harkonen P, et al. (2010) Postchallenge Glucose, HbA1c, and Fasting Glucose as Predictors of Type 2 Diabetes and Cardiovascular Disease: A 10-year Prospective Cohort Study. Diabetes Care.
8. Benjamin SM, Valdez R, Vinicor F (2002) Diabetes prevention. N Engl J Med 346: 1829–1830; author reply 1829–1830.
9. Chasman DI, Pare G, Mora S, Hopewell JC, Peloso G, et al. (2009) Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet 5: e1000730. doi:10.1371/journal.pgen.1000730
10. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, et al. (2009) Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 41: 56–65.
11. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, et al. (2010) Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466: 707–713.
12. Kathiresan S, Melander O, Anevski D, Guiducci C, Burtt NP, et al. (2008) Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 358: 1240–1249.
13. Kitabchi AE, Temprosa M, Knowler WC, Kahn SE, Fowler SE, et al. (2005) Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: effects of lifestyle intervention and metformin. Diabetes 54: 2404–2414.
14. Yazdanyar A, Yeang C, Jiang XC (2011) Role of phospholipid transfer protein in high-density lipoprotein- mediated reverse cholesterol transport. Curr Atheroscler Rep 13: 242–248.
15. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, et al. (2012) Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet.
16. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, et al. (2004) European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of eight societies and by invited experts). Atherosclerosis 173: 381–391.
17. Ratner R, Goldberg R, Haffner S, Marcovina S, Orchard T, et al. (2005) Impact of intensive lifestyle and metformin therapy on cardiovascular disease risk factors in the diabetes prevention program. Diabetes Care 28: 888–894.
18. Despres JP (2003) Potential contribution of metformin to the management of cardiovascular disease risk in patients with abdominal obesity, the metabolic syndrome and type 2 diabetes. Diabetes Metab 29: 6S53–61.
19. Keys A (1965) Effects of Different Dietary Fats on Plasma-Lipid Levels. Lancet 1: 318–319.
20. Corella D, Ordovas JM (2009) Nutrigenomics in cardiovascular medicine. Circ Cardiovasc Genet 2: 637–651.
21. Bernstein MS, Costanza MC, James RW, Morris MA, Cambien F, et al. (2003) No physical activity×CETP 1b.-629 interaction effects on lipid profile. Med Sci Sports Exerc 35: 1124–1129.
22. Kilpelainen TO, Lakka TA, Laaksonen DE, Mager U, Salopuro T, et al. (2008) Interaction of single nucleotide polymorphisms in ADRB2, ADRB3, TNF, IL6, IGF1R, LIPC, LEPR, and GHRL with physical activity on the risk of type 2 diabetes mellitus and changes in characteristics of the metabolic syndrome: The Finnish Diabetes Prevention Study. Metabolism 57: 428–436.
23. Nettleton JA, Steffen LM, Schulze MB, Jenny NS, Barr RG, et al. (2007) Associations between markers of subclinical atherosclerosis and dietary patterns derived by principal components analysis and reduced rank regression in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr 85: 1615–1625.
24. Spielmann N, Leon AS, Rao DC, Rice T, Skinner JS, et al. (2007) CETP genotypes and HDL-cholesterol phenotypes in the HERITAGE Family Study. Physiol Genomics 31: 25–31.
25. Teran-Garcia M, Rankinen T, Koza RA, Rao DC, Bouchard C (2005) Endurance training-induced changes in insulin sensitivity and gene expression. Am J Physiol Endocrinol Metab 288: E1168–1178.
26. Todorova B, Kubaszek A, Pihlajamaki J, Lindstrom J, Eriksson J, et al. (2004) The G-250A promoter polymorphism of the hepatic lipase gene predicts the conversion from impaired glucose tolerance to type 2 diabetes mellitus: the Finnish Diabetes Prevention Study. J Clin Endocrinol Metab 89: 2019–2023.
27. Tucker AJ, Mackay KA, Robinson LE, Graham TE, Bakovic M, et al. (2010) The effect of whole grain wheat sourdough bread consumption on serum lipids in healthy normoglycemic/normoinsulinemic and hyperglycemic/hyperinsulinemic adults depends on presence of the APOE E3/E3 genotype: a randomized controlled trial. Nutr Metab (Lond) 7: 37.
28. Zhang C, Lopez-Ridaura R, Rimm EB, Rifai N, Hunter DJ, et al. (2005) Interactions between the −514C→T polymorphism of the hepatic lipase gene and lifestyle factors in relation to HDL concentrations among US diabetic men. Am J Clin Nutr 81: 1429–1435.

29. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, et al. (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 40: 161–169.
30. Sandhu MS, Waterworth DM, Debenham SL, Wheeler E, Papadakis K, et al. (2008) LDL-cholesterol concentrations: a genome-wide association study. Lancet 371: 483–491.
31. Mayer-Davis EJ, Sparks KC, Hirst K, Costacou T, Lovejoy JC, et al. (2004) Dietary intake in the diabetes prevention program cohort: baseline and 1-year post randomization. Ann Epidemiol 14: 763–772.
32. Jablonski KA, McAteer JB, de Bakker PI, Franks PW, Pollin TI, et al. (2010) Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program. Diabetes 59: 2672–2681.
33. The Diabetes Prevention Program Research Group (1999) Design and methods for a clinical trial in the prevention of type 2 diabetes. Diabetes Care 22: 623–634.
34. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, et al. (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 346: 393–403.
35. Warnick GR (1986) Enzymatic methods for quantification of lipoprotein lipids. Methods Enzymol 129: 101–123.
36. Warnick GR, Benderson J, Albers JJ (1982) Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 28: 1379–1388.
37. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499–502.
38. Hainline AJ, Karon J, Lippel K (1983) Manual of Laboratory Operations. 2nd ed. . Washington, DC, , US.: Lipid Research Clinics Program, Lipid and Lipoprotein Analysis, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health
39. Otvos JD (2002) Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. Clin Lab 48: 171–180.
40. Tang K, Fu DJ, Julien D, Braun A, Cantor CR, et al. (1999) Chip-based genotyping by mass spectrometry. Proc Natl Acad Sci U S A 96: 10016–10020.
41. Fontaine-Bisson B, Renstrom F, Rolandsson O, Payne F, Hallmans G, et al. (2010) Evaluating the discriminative power of multi-trait genetic risk scores for type 2 diabetes in a northern Swedish population. Diabetologia.
42. Moore AF, Jablonski KA, McAteer JB, Saxena R, Pollin TI, et al. (2008) Extension of type 2 diabetes genome-wide association scan results in the diabetes prevention program. Diabetes 57: 2503–2510.
43. Goodman SN, Berlin JA (1994) The use of predicted confidence intervals when planning experiments and the misuse of power when interpreting results. Ann Intern Med 121: 200–206.
44. Smith AH, Bates MN (1992) Confidence limit analyses should replace power calculations in the interpretation of epidemiologic studies. Epidemiology 3: 449–452.
45. Detsky AS, Sackett DL (1985) When was a "negative" clinical trial big enough? How many patients you needed depends on what you found. Arch Intern Med 145: 709–712.
46. Greenland S (1988) On sample-size and power calculations for studies using confidence intervals. Am J Epidemiol 128: 231–237.