

A Polygenic Lipodystrophy Genetic Risk Score Characterizes Risk Independent of BMI in the Diabetes Prevention Program

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Context: There is substantial heterogeneity in insulin sensitivity, and genetics may suggest possible mechanisms by which common variants influence this trait.

Objectives: We aimed to evaluate an 11-variant polygenic lipodystrophy genetic risk score (GRS) for association with anthropometric, glycemic and metabolic traits in the Diabetes Prevention Program (DPP). In secondary analyses, we tested the association of the GRS with cardiovascular risk factors in the DPP.

Design: In 2713 DPP participants, we evaluated a validated GRS of 11 common variants associated with fasting insulin-based measures of insulin sensitivity discovered through genome-wide association studies that cluster with a metabolic profile of lipodystrophy, conferring high metabolic risk despite low body mass index (BMI).

Results: At baseline, a higher polygenic lipodystrophy GRS was associated with lower weight, BMI, and waist circumference measurements, but with worse insulin sensitivity index (ISI) values. Despite starting at a lower weight and BMI, a higher GRS was associated with less weight and BMI reduction at one year and less improvement in ISI after adjusting for baseline values but was not associated with diabetes incidence. A higher GRS was also associated with more atherogenic low-density lipoprotein peak-particle-density at baseline but was not associated with coronary artery calcium scores in the Diabetes Prevention Program Outcomes Study.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAC, coronary artery calcification; DPP, Diabetes Prevention Program; DPPPOS, Diabetes Prevention Program Outcomes Study; GRS, genetic risk score; GWAS, Genome-wide association studies; ISI, insulin sensitivity index; SNP, single nucleotide polymorphism.

Conclusions: In the DPP, a higher polygenic lipodystrophy GRS for insulin resistance with lower BMI was associated with diminished improvement in insulin sensitivity and potential higher cardiovascular disease risk. This GRS helps characterize insulin resistance in a cohort of individuals at high risk for diabetes, independent of adiposity.

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Freeform/Key Words: insulin resistance, obesity, metformin, lifestyle intervention, type 2 diabetes

The underlying pathogenesis of type 2 diabetes involves relative insufficiency in insulin secretion through β cell dysfunction in the context of increased secretory demand because of insulin resistance [1]. Genome-wide association studies (GWAS) have established that common genetic variation influences insulin resistance by identifying 19 single nucleotide polymorphisms (SNPs) that have reached genome-wide associations with fasting insulin-based measures of insulin resistance [2–4]. However, despite the importance of insulin resistance as a risk factor for type 2 diabetes, there is variation in the pathological pathways leading to diabetes, and there is considerable heterogeneity in the genetic architecture of insulin resistance. Studies have also shown that insulin resistance is an independent predictor of cardiovascular disease risk [5]. Although increased body mass index (BMI) remains an overwhelming risk factor for insulin resistance and the development of type 2 diabetes, it is now established that for a given BMI, the pattern of fat storage is associated with modification of this risk [6]. However, the role of insulin resistance independent of BMI in the pathogenesis of type 2 diabetes and cardiovascular disease risk has been difficult to comprehend, largely because of the strong correlation between obesity and insulin resistance.

Rare monogenic disorders may shed some light on the relationship between insulin resistance and diabetes in the absence of the confounding effects of BMI. For example, in primary lipodystrophy, severe insulin resistance develops in lean individuals in association with generalized or regional lack of adipose tissue. Patients with lipodystrophy exhibit severe insulin resistance, metabolic dyslipidemia, and diabetes resulting from impaired adipose tissue function [7]. To define the polygenic correlate of lipodystrophy, Yaghootkar *et al.* [8] selected 19 common genetic variants associated with fasting insulin-based measures of insulin resistance and used hierarchical clustering and results from GWAS of eight non-disease outcomes of monogenic insulin resistance to group these variants. A cluster of 11 common genetic variants associated with fasting insulin-based measures of insulin resistance in GWAS was associated with chronic disease outcomes, including type 2 diabetes and coronary artery disease, in directions consistent with a lipodystrophy phenotype: high metabolic disease risk despite low BMI [8].

Although this provides evidence for a genetic signature for metabolic disease, it is unknown whether these variants predict metabolic disease features in a multiethnic cohort of subjects at high risk for the development of diabetes, and whether they predict change in glycemic and metabolic parameters over time in the context of diabetes interventions designed to ameliorate insulin resistance and reduce the risk of type 2 diabetes. The Diabetes Prevention Program (DPP), a randomized controlled trial of metformin and lifestyle modification *vs* placebo, can be analyzed to address these questions. In the current study, we aimed to evaluate the same 11 variant polygenic lipodystrophy genetic risk score (GRS) [8] for association with relevant anthropometric, glycemic and metabolic traits, and incident diabetes in the DPP's multiethnic cohort of individuals who are at high metabolic risk at baseline. Additionally, we aimed to evaluate the association of the GRS with progression to diabetes and response to metformin and intensive lifestyle interventions. In secondary analyses, we also tested the association of the GRS with cardiovascular disease risk factors that are intermediate measurable phenotypes that are associated with cardiovascular disease [9].

1. Materials and Methods

A. Description of DPP Study Design and Participants

The study design of the DPP (ClinicalTrials.gov Identifier: NCT00004992) and characteristics of the participants at baseline have been described previously [10, 11]. Briefly, the DPP was a multicenter trial in the United States that assessed whether intensive lifestyle intervention or metformin therapy prevented or delayed the onset of diabetes in individuals who were at high risk of developing diabetes. The DPP enrolled 3234 overweight or obese individuals without diabetes but with impaired glucose tolerance and elevated fasting glucose, and randomly assigned them to placebo, metformin (850 mg twice daily), or an intensive lifestyle intervention. The DPP showed that after a mean follow-up of 2.8 years, metformin and lifestyle interventions reduced the incidence of diabetes by 31% (95% CI 17 to 43) and 58% (95% CI 48 to 66) respectively, *vs* placebo [12].

All 3150 surviving DPP participants who had not withdrawn consent were eligible for a follow-up study, the Diabetes Prevention Program Outcomes Study (DPPOS), a study initiated to establish the longer term effects of the DPP interventions on the development of diabetes [13]. At the end of the DPP, after a brief metformin and placebo washout study [13], the participants in the placebo and metformin groups were subsequently unmasked to their treatment assignment and placebo was stopped. In view of the clear evidence of benefit of the lifestyle intervention, all participants were offered the lifestyle intervention in a group format during a one-year bridge period between DPP and DPPOS [14]. During DPPOS, as in DPP, metformin was provided to the group originally assigned to it, however, metformin was now unmasked. In this study, except for coronary artery calcification (CAC) scores, which were obtained at year 14 of follow-up in DPPOS, all measurements were obtained during the original DPP trial. Institutional review board approval was obtained by each participating center and all subjects included in this study provided written informed consent for the main studies and for subsequent genetic investigations.

B. Measurements

The methods for measuring glucose, insulin, total cholesterol, and triglyceride levels have been described previously [11]. Participants were excluded from the DPP if they had markedly elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) concentrations at baseline as defined by age and sex: for age <47 years, ALT >46 U/L for women and >118 U/L for men; for age \geq 47 years, ALT >58 U/L for both men and women; for AST \geq 66 U/L per criteria established by the DPP central laboratory. After baseline measurements, follow-up ALT and AST levels were not measured in the intensive lifestyle group. Mean AST concentrations increased in 1999 by \sim 4 U/L regardless of study visit (baseline, 3, 6 months, etc.) consistent with assay drift. A similar change in ALT concentrations was not found [15]. For these reasons, this analysis was limited to ALT.

The insulin sensitivity index (ISI) was calculated as the reciprocal of homeostasis model assessment of insulin resistance using the equation $[(\text{FI (mU/L)} \times \text{fasting glucose (mmol/L)})/22.5]$ [16] based on glucose and insulin levels during the oral glucose tolerance test at baseline and one-year follow-up. Participants were asked not to take metformin or placebo on the morning of the oral glucose tolerance test. For longitudinal analyses, the one-year end point was chosen because the sample size was largest at that time point (95% of participants completed the one-year follow-up visit) and weight loss was the most pronounced at one year in the intervention arms.

DPP participants from 18 of the 27 sites ($n = 1106$) volunteered for measurement of adipose tissue by CT at baseline and after one year of the study. The instruments used included the GE High Speed Advantage (General Electric, Milwaukee, WI), at five centers, the Picker PQ 5000 (Picker, Groton, CT) at five centers, the Siemens and Siemens Somatom Plus (Siemens and Siemens, New York, NY) at two centers, the GE 9800 (General Electric) at three centers, and the GE Highlite (General Electric) at two centers. Two 10-mm thick axial images were obtained at the L4-5 spaces. The data obtained were submitted to a central

reading facility at the University of Colorado in Denver. The reading center calculated the total visceral adipose area on each scan, delineated visceral fat from subcutaneous fat by circumscribing the transversalis fascia, and calculated subcutaneous adipose tissue by subtracting the visceral adipose tissue from the total cross-sectional area for fat [17]. We report results using measurement of both visceral and subcutaneous fat in 618 participants.

Total circulating adiponectin was measured using a latex particle-enhanced turbidimetric assay (Otsuka Pharmaceutical, Tokyo, Japan) [18]. In participants with triglyceride >4.5 mmol/L, lipoprotein fractions were separated using preparative ultracentrifugation of plasma by β quantification. C-reactive protein and fibrinogen levels in plasma were immunochemically measured using the Behring Nephelometer auto-analyzer (Dade Behring; Marburg, Germany). Tissue plasminogen activator levels were measured in citrated plasma using an ELISA (Asserachrom tPA; Diagnostica Stago, Parsippany, NJ [19]), which measures total tissue plasminogen activator antigen [20].

Given the multiethnic demographics of the study population, cardiovascular disease risk was computed using the American Heart Association/American College of Cardiology 10-year risk score [21]. Subclinical atherosclerosis by coronary artery calcification (CAC) was measured in year 14 of DPPOS in 2029 subjects using a multidetector CT according to methods that have been published previously [22].

C. Genotyping

DNA was extracted from peripheral blood leukocytes and genotyping was performed on the customized MetaboChip (Illumina, San Diego, CA). The MetaboChip contains ~200,000 SNPs chosen based on previous GWAS meta-analyses of 23 metabolic traits related to T2D, obesity, and cardiovascular diseases. Study participants with sex discrepancy (4 subjects) or familial relatedness (6 subjects) were excluded. SNPs were excluded if the call rate was less than 95% or if they failed Hardy-Weinberg equilibrium testing ($P < 1.0 \times 10^{-7}$) within each ethnic group. The overall genotyping success rate was excellent at >99.85%.

D. SNP Selection and Construction of Genetic Risk Score

Eleven common variants associated with fasting insulin-based measures of insulin resistance were selected for the GRS based on their association with features of metabolic syndrome including increased risk of type 2 diabetes, coronary artery disease, and higher systolic and diastolic blood pressures despite low BMI, similar to a lipodystrophic profile [8]. All these variants have been associated with fasting insulin at the accepted level of genome-wide significance ($P < 5 \times 10^{-8}$) in GWAS previously published by the MAGIC investigators [2–4]. The GRS was computed based on the assumption of an additive genetic effect. The GRS was calculated by accounting for the number of risk alleles present per SNP and summing the results over the 11 SNPs. Participants with more than three missing SNPs were excluded ($n = 281$). For participants with 1, 2, or 3 missing SNPs (total 120 individuals), the GRS was calculated by multiplying the GRS from the available SNPs by 22 and dividing by twice the number of successfully genotyped SNPs. We used an unweighted score in which equal weight was given to all the risk alleles because of the minimal differences in the published effect sizes for fasting insulin published by MAGIC (Table 1). We divided the subjects into four groups of genetic risk only for descriptive purposes in the baseline table (Table 2) and in the plots for illustration purposes (Fig. 1). Based on the distribution of the GRS, a GRS of <12 defined the first group, a GRS of ≥ 12 but <14 defined the second group, a GRS of ≥ 14 but <15 defined the third group, and a GRS of ≥ 15 defined the fourth group. There were numerous tied values at the boundaries of the groups, leading to uneven sample sizes in the four groups. However, this did not affect statistical analyses because the analyses were done considering the GRS as a continuous variable.

E. Statistical Analyses

The GRS was analyzed in general linear models predicting baseline and one-year change from baseline for measured variables. Variables with a non-normal distribution were log

Table 1. Effect Sizes for Fasting Insulin Along With Frequencies of Genetic Variants in Published Reports from MAGIC and in the DPP Participants

Gene	SNP	β Estimates for ln FI ($\mu\text{U/mL}$) in MAGIC	Risk Allele Frequency in MAGIC (%)	Frequency of High-Risk Alleles in DPP (%)					
				Overall N = 2713	Caucasian N = 1503	Black N = 554	Hispanic N = 458	Asian/Pacific Islanders N = 120	American Indian N = 78
<i>IRSI</i>	rs2943634	0.027	66	67	68	45	77	92	92
<i>COBLL1/GRB14</i>	rs7607980	0.027	88	88	87	85	90	98	97
<i>ARL15</i>	rs4865796	0.011	67	74	70	75	82	81	94
<i>FAM13A</i>	rs3822072	0.013	48	49	49	53	42	54	40
<i>LYPLALI</i>	rs2785980	0.015	67	70	67	84	53	71	44
<i>PEPD</i>	rs731839	0.014	34	34	34	39	40	58	47
<i>PDGFC</i>	rs4691380	0.017	67	59	66	29	61	64	71
<i>RSPO3</i>	rs2745353	0.011	39	35	50	63	56	60	66
<i>PPARG</i>	rs18001282	0.022	87	91	90	98	91	90	81
<i>TET2</i>	rs9884482	0.012	39	35	38	11	45	58	40
<i>ANKRD55</i>	rs459193	0.013	73	70	75	58	73	53	74

Abbreviation: FI, fasting insulin.

Table 2. Characteristics of DPP Participants at Baseline by Quartile of Polygenic Lipodystrophy GRS

Baseline Variable	Group of GRS ^{a,b}				P ^c
	1 st	2 nd	3 rd	4 th	
N ^d	394	773	476	1070	
Sex, n, %					
Women	286 (16.4)	504 (28.8)	325 (18.6)	712 (40.7)	0.072
Men	108 (12.2)	269 (30.4)	151 (17.0)	358 (40.4)	
Self-reported race/ethnicity, n, %					
Caucasian	195 (13.0)	420 (27.9)	276 (18.4)	612 (40.7)	0.115
Black	141 (25.5)	207 (37.7)	93 (16.8)	113 (20.4)	
Hispanic	51 (11.1)	116 (25.3)	75 (16.4)	216 (47.2)	
American Indian				46 (59.0)	
Asian American		16 (13.3)	19 (15.8)	83 (69.2)	
Age at randomization, y	50.9 (49.9-52.0)	51.1 (50.4-51.9)	50.6 (49.7-51.6)	50.3 (49.7-51.0)	0.423

^aData are reported as mean (95% CI) for continuous variables and as frequency (%) for categorical variables.

^bEmpty cells indicate sample sizes ≤ 15 that are not reported per DPP policy for concern of compromise of confidentiality and limitations with interpretation of findings.

^cP values from logistic regression for categorical variables and general linear models for continuous variables.

^dThe sample sizes in each group are uneven because of the many tied values at the boundaries of the groups.

transformed. The one-year analysis included a test for treatment \times GRS interaction. The effect of the GRS on diabetes incidence over the course of the main trial (mean follow-up time of 3.2 years) was tested using a Cox proportional hazards model with genotype, treatment arm, and a genotype-treatment arm interaction test as the independent variables predicting time to diabetes. The association between quantitative traits and genotype under an additive genetic model was tested in each treatment arm using analysis of covariance (ANCOVA). CAC severity was analyzed by Tobit regression [23] of the CAC score using the QLIM procedure in SAS to account for the skewness resulting from the relatively large number of individuals with a CAC score of 0. The Tobit regression coefficient represents the log ratio of the geometric mean CAC score per unit increase in the covariate, assuming some measurable calcification for all subjects, including subjects with undetectable levels. All tests performed were two sided, and an α -level of 0.05 was used to determine statistical significance. The Statistical Analysis Software (SAS) version 9.3 was used for all analyses (SAS Institute, Inc., Cary, NC)

2. Results

The 2713 participants from the DPP analyzed in this study had a mean (\pm SD) baseline age of 50.7 ± 10.7 years; 67% of the participants were women and 45% were nonwhite. At baseline, the mean BMI of the participants was 34.1 ± 6.7 kg/m², and waist circumference was 105.4 ± 14.6 cm. The characteristics of the DPP participants at baseline divided into four groups for descriptive purposes based on the polygenic lipodystrophy GRS is listed in Table 2. The list of common genetic variants used in the GRS along with their published effect size for fasting insulin is shown in Table 1. This table also shows the frequencies of the risk alleles in the DPP participants by ethnicity as well as published frequencies reported in MAGIC. The distribution of the GRS across the DPP participants was normal with a mean score of 13.9 (SD \pm 2.2) and a median of 14.0 without substantial differences across all three treatment groups. The baseline characteristics of the participants predicted by the GRS are presented in Table 3. At baseline, a higher GRS was associated with higher ALT levels, higher fasting insulin, and with lower ln ISI values after adjustment for age at randomization, sex, self-reported race/ethnicity, and waist circumference. We chose adjustment for waist circumference because central adiposity as measured by waist circumference instead of BMI is a better predictor of type 2 diabetes, metabolic syndrome, and cardiovascular disease and

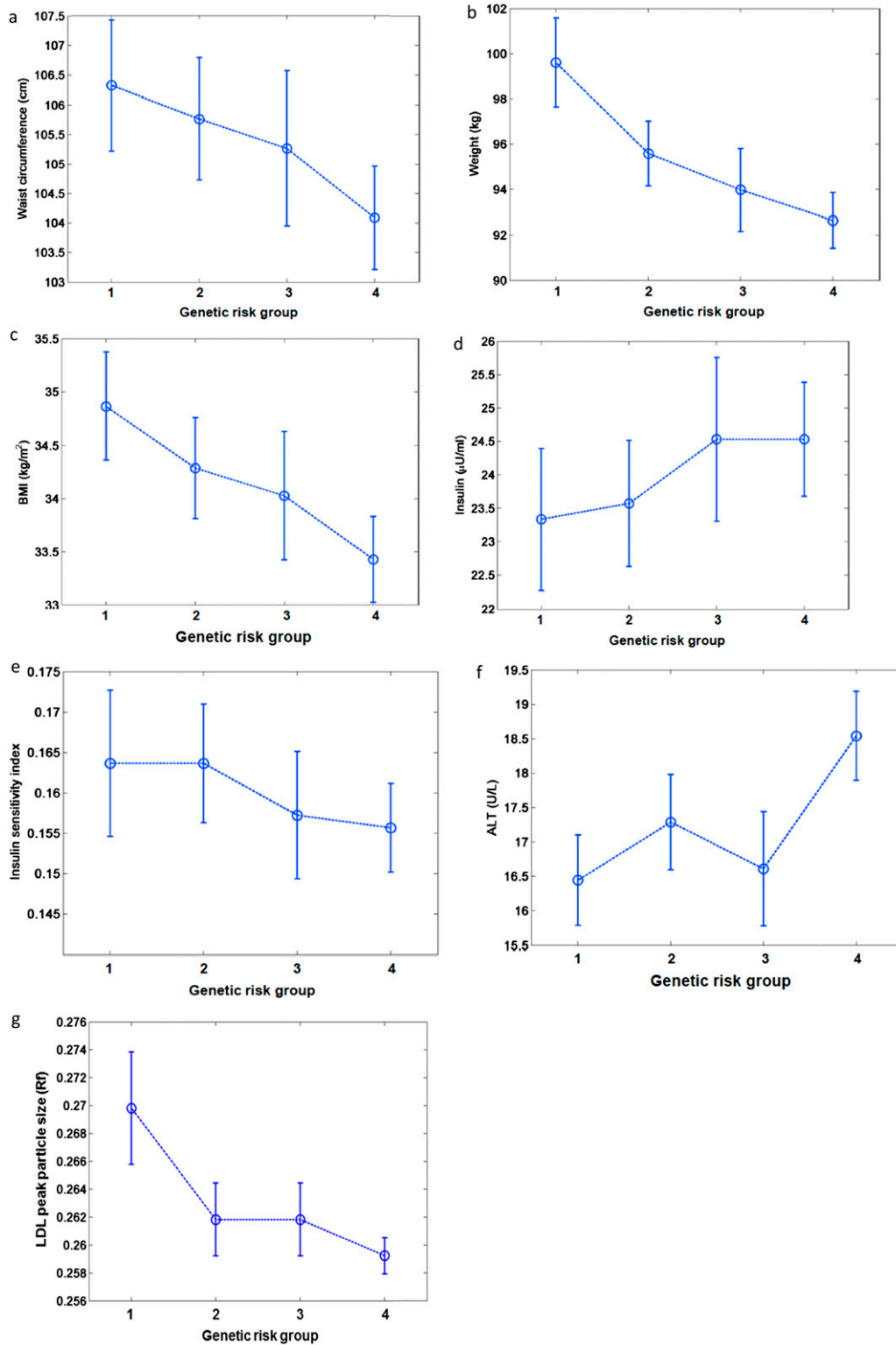


Figure 1. Relation of baseline variables (a) weight, (b) BMI, (c) waist circumference, (d) fasting insulin, (e) ISI, (f) ALT, and (g) LDL peak particle size with the GRS across four groups (G1, G2, G3, G4) of genetic risk is shown. The circles represent the means and the heights of the bars represent the 95% CI. Log transformed variables have been reverse transformed for descriptive purposes. All measures were adjusted for age, sex, and race/ethnicity. Fasting insulin, ISI, ALT, and LDL peak particle density measurements were additionally adjusted for waist circumference.

Table 3. Baseline Characteristics Predicted by the Polygenic Lipodystrophy GRS

Baseline Variable	Sample Size (n)	β Estimate per Allele	SE	<i>P</i>
Weight, kg ^a	2712	-0.6341	0.1705	<0.001
BMI, kg/m ^{2a}	2712	-0.2342	0.0574	<0.001
Waist circumference, cm ^a	2712	-0.4670	0.1288	<0.001
FI, μ U/mL, ln	2709	0.0116	0.0045	0.010
Fasting glucose, mg/dL	2712	0.1035	0.0734	0.159
ISI, ln	2709	-0.0125	0.0046	0.007
Systolic blood pressure (mm Hg)	2712	0.0531	0.1271	0.676
Diastolic blood pressure (mm Hg)	2712	0.0334	0.0836	0.690
HDL-cholesterol, mg/dL	2707	-0.0670	0.0983	0.496
LDL-cholesterol, mg/dL	2707	0.0554	0.3003	0.854
Triglycerides, mg/dL, ln	2707	0.0069	0.0046	0.128
ALT, U/L, ln	2709	0.0113	0.0047	0.015
Visceral adipose tissue, cm ² , L4-L5 ln	791	-0.0062	0.0048	0.196
Subcutaneous adipose tissue, cm ² , L4-5 ln	791	-0.0025	0.0035	0.480

All measures adjusted for age at randomization, sex, self-reported race/ethnicity and waist circumference except where indicated.

Abbreviation: FI, fasting insulin.

^aAdjusted for age at randomization, sex, and self-reported race/ethnicity.

because this is the anthropometric measure most strongly associated with outcomes in the DPP [24]. In contrast, a higher GRS was associated with lower weight, lower BMI and lower waist circumference measurements. Figure 1a-1f demonstrates the relation of the some of the baseline variables with the GRS across four groups of genetic risk.

Table 4 lists the association of the GRS with change in measured traits at one year. Over the first year, a higher GRS was associated with a smaller magnitude of weight loss, despite starting with lower weight and BMI measurements at baseline. Additionally, over one year, a higher GRS was associated with less improvement in the ln ISI after adjustment for baseline value, age at randomization, sex, self-reported race/ethnicity, waist circumference, and treatment group. These results remained noteworthy after adjusting for BMI in our analysis. There was no interaction of the GRS with the treatment arms on the change in ISI over the first year ($P = 0.617$).

We also evaluated the association of the GRS with diabetes incidence over the course of the main DPP trial using a Cox proportional hazards model with a mean follow-up of 3.2 years. We did not find interactions between either treatment arm and the GRS, therefore, we pursued our multivariable models in the full cohort adjusting for treatment arms. There was no association with diabetes incidence [hazard ratio = 1.03, $P = 0.229$, 95% CI (0.98 to 1.07)] with the model including adjustment for major risk factors for type 2 diabetes including age at randomization, sex, waist circumference, and self-reported race/ethnicity.

In secondary analyses, we tested the association of the GRS with cardiovascular disease risk factors in 2708 participants at baseline and at one year. Table 5 shows the association of the GRS with the cardiovascular disease risk factors tested at baseline. A higher GRS was associated with lower low-density lipoprotein (LDL) peak-particle size at baseline after adjusting for age, sex, self-reported race/ethnicity, and waist circumference (Fig. 1g). Over one year, the GRS was associated with lowering in the fibrinogen levels after adjustment for baseline value, age, sex, self-reported race/ethnicity, waist circumference, and treatment group. We found no association of the GRS with CAC scores at 14 years in the DPPOS follow-up.

3. Discussion

With the use of a large-scale genetic study and the phenotypic accuracy of clinical trial data, we have extended the genetic link between various components of the metabolic syndrome resembling a lipodystrophy phenotype in a multiethnic cohort of individuals who are at high

Table 4. Association of the GRS with Change in Traits at 1 Year

Year 1-Baseline	Sample Size (n)	β Estimate per Allele	SE	P
Weight, kg ^a	2568	0.1234	0.0529	0.020
BMI, kg/m ^{2a}	2568	0.0461	0.0190	0.015
Waist circumference, cm ^a	2568	0.0754	0.0593	0.204
FI, μ U/mL, ln	2568	0.0130	0.0043	0.002
Fasting glucose, mg/dL, ln ^a	2568	0.0003	0.0009	0.722
ISI, ln ^b	2506	-0.0136	0.0047	0.004
Systolic blood pressure, mm Hg	2568	-0.0874	0.1134	0.441
Diastolic blood pressure, mm Hg	2568	0.0277	0.0740	0.708
HDL-cholesterol, mg/dL	2559	0.0043	0.0591	0.942
LDL-cholesterol, mg/dL	2559	0.0067	0.2033	0.974
Triglycerides, mg/dL, ln +	2559	0.0047	0.0033	0.154
ALT, U/L, ln+	1857	0.0801	0.1211	0.509
Visceral adipose tissue, cm ² , L4-5	618	-0.0006	0.0017	0.721
Subcutaneous adipose tissue, cm ² , L4-5	618	0.4760	1.0300	0.644

All measures adjusted for baseline value, age at randomization, sex and self-reported race/ethnicity, waist circumference and treatment group except where indicated.

Abbreviation: FI, fasting insulin.

^aAdjusted for age at randomization, sex, and race/ethnicity

^bln (Year 1 measurement) - ln (Baseline measurement)

metabolic risk at baseline. In the DPP, a select cluster of common genetic variants associated with insulin sensitivity as captured by fasting insulin-based measures was associated with worse measures of insulin resistance and higher alanine transaminase levels but with lower weight, BMI, and waist circumference measurements at baseline. The association of the GRS with alanine transaminase levels is noteworthy despite the exclusion of participants with markedly elevated levels in the DPP [15]. These findings expand on previous results examining the genetics of insulin resistance from the DPP [25] and highlight the unique metabolic signature of this genetic risk score of common genetic variants linked to insulin resistance.

The DPP also allows us to examine how this GRS influences change in our variables of interest over one year of intervention with either metformin, intensive lifestyle intervention, or placebo. Despite starting at a lower weight and BMI, participants with a high genetic burden for lipodystrophic insulin resistance were less likely to lose weight or show improvement in their BMI. Additionally, despite having lower weight and BMI, participants with the highest genetic burden for the lipodystrophy phenotype were less likely to improve their insulin sensitivity after accounting for demographic characteristics including waist circumference. The change in one-year analyses for the all the end points tested took into account the baseline value. The lack of association of the GRS with CT-based adipose tissue

Table 5. Association of the Polygenic Lipodystrophy GRS With Cardiovascular Disease Risk Factors at Baseline

Baseline Variable	Sample Size (n)	β Estimate per Allele	SE	P
Adiponectin, μ g/mL, ln	2691	-0.0027	0.0033	0.407
Fibrinogen, mg/dL, ln	2704	-0.0008	0.0019	0.661
C-reactive protein, mg/dL, ln	2708	-0.0143	0.0085	0.094
Tissue plasminogen activator, ng/mL, ln	2698	-0.0009	0.0032	0.789
LDL particle size, Rf, ln	2708	-0.0022	0.0010	0.034
ACC/AHA 10 y risk score ln	2708	-0.0009	0.0085	0.916

All measures adjusted for age at randomization, sex, self-reported race/ethnicity and waist circumference.

Abbreviations: ACC, American College of Cardiology; AHA, American Heart Association.

measurements is likely because these measurements were only available in a small subset ($n = 618$) of the DPP participants, limiting the power of the analyses.

We have previously shown in the DPP, that a GRS of 17 established insulin resistance variants was associated with decreased insulin sensitivity at baseline and diminished improvement in the ISI over one year of the study [25]. This current study builds upon that body of work by examining a more refined GRS, based on 11 of the 17 insulin-resistance SNPs used in the previous study [25] but with the key difference being that this 11-SNP GRS (in contrast with the complete 17-SNP score) was associated with a lipodystrophy phenotype, capturing the critical feature of lower adiposity but higher metabolic disease risk. Our results, in comparison with the previous DPP report, highlight the relative importance of the subset of these 11 variants. Our results also indicate that in the DPP participants, the genetic burden of even a subset of insulin resistance variants was associated with diminished insulin sensitivity at baseline and over one year of interventions. We have also extended our previous exploration by characterizing the association of the new GRS against cardiovascular endophenotypes in the DPP. The genetic basis of the phenotype of high insulin resistance with low body adiposity was also recently examined by Lotte *et al* [26]. They combined GWAS results for fasting insulin, high-density lipoprotein (HDL) cholesterol, and triglyceride levels and identified 53 genomic regions associated with high fasting insulin, high triglyceride, and low HDL cholesterol levels, a subset of which have been previously implicated in insulin resistance. Because the DPP is a cohort of participants at risk for diabetes at baseline and is therefore enriched for insulin resistance, we chose to use a score that was purely derived from genetic variants associated with insulin resistance.

We have also previously shown that a GRS derived from known type 2 diabetes variants predicts diabetes incidence [27]. Because most of the risk alleles at loci associated with fasting insulin are not associated with type 2 diabetes in large population-based studies, it is not surprising that the polygenic lipodystrophy GRS was not associated with diabetes incidence in the current analyses. This is consistent with current evidence that common genetic variants associated with β -cell function have greater predictive power for type 2 diabetes [28], and it highlights the important role of β -cell secretory function in the pathogenesis of type 2 diabetes [1].

Metformin and intensive lifestyle modification both improved insulin sensitivity over one year in DPP participants irrespective of genetic risk burden. This is again consistent with previous results from the DPP and highlights the effectiveness of these preventive interventions across the gradient of genetic risk [25, 27]. The lack of statistical significance achieved with the use of this GRS compared with previous scores in the DPP suggests pathophysiological differences in this particular subset of genetic variants and should be explored in future studies.

A major goal of diabetes prevention and treatment is to prevent microvascular and macrovascular events. In the DPP, those who did not develop diabetes had a lower prevalence of microvascular complications than those who did develop diabetes, supporting the importance of diabetes prevention [29]. However, there have not been a sufficient number of cardiovascular events in the DPPOS to permit meaningful analysis of macrovascular events [30]. Because this polygenic lipodystrophy GRS has previously been associated with coronary artery disease [8], we tested the GRS for association with cardiovascular disease risk factors in the DPP. These risk factors are quantitative laboratory-based measures that are heritable intermediate phenotypes and are related to the disease outcome of interest, in this case, cardiovascular disease. Our results show that among individuals who are at risk for diabetes, a higher GRS for insulin resistance with lower BMI was associated with higher cardiovascular disease risk profile, specifically smaller or more dense LDL peak particle measurements after adjustment for relevant demographic and anthropometric measurements [8]. LDL peak particle density is an emerging risk factor that seems to be an important predictor of cardiovascular events and progression of coronary artery disease [26]. Of note, the GRS was not associated with the CAC scores that predict total coronary atherosclerotic burden at year 14 in DPPOS. However, because we do not have baseline CAC data, our

interpretation of results is based on the assumption that as a result of randomization to treatment groups, the distribution of CAC scores at baseline would have been similar among the treatment groups. Further evaluation of this GRS awaits results of cardiovascular events in the DPP, but our results suggest that this GRS may help characterize cardiovascular disease risk in a seemingly homogenous group of individuals at risk for diabetes.

One of the main strengths of our study is that the DPP randomized controlled trial design enabled extensive in-depth phenotyping and comprehensive longitudinal measurements as well as standardized therapeutic interventions to characterize the effects of genetic variants on various outcomes including diabetes incidence and response to in a multiethnic cohort with high metabolic risk at baseline. However, we recognize that our study also has limitations. The genetic variants in our score are associated with fasting insulin, which is not the most accurate measure of insulin sensitivity. However, more accurate clamp-based measures of insulin sensitivity are not feasible in a large-scale study such as the DPP. Also, our insulin measurements are posthepatic and do not reflect portal insulin secretion, which was not obtained in the DPP. DPP participants are adults ascertained by the presence of risk factors for the development of diabetes but do not have diabetes, therefore, their glycemic variables fall within a narrow range. Additionally, the GRS is based on variants that have been discovered in populations of European descent and therefore may not adequately capture genetic variation in individuals of non-European descent. For the analyses related to cardiovascular endophenotypes, we acknowledge the concern for multiple hypothesis testing, although the outcomes tested are closely related and likely not independent; we are therefore careful to interpret our findings related to these traits as emerging from exploratory analyses.

In conclusion, our results provide further evidence for a genetic link between various risk factors that contribute to cardiovascular disease risk and highlight the potential association of adipose tissue dysfunction independent of BMI with worsening insulin resistance and potential increased cardiovascular disease risk. We hope that these results advance physiological understanding and will inform future functional studies to explore the underlying mechanisms behind these associations. Individuals at high risk of type 2 diabetes who had a high genetic burden of lipodystrophic insulin resistance had less improvement in their insulin sensitivity over time. We confirm that metformin treatment and intensive lifestyle modification are effective in improving insulin sensitivity regardless of genetic risk. A full evaluation of the effects of this GRS on cardiovascular disease awaits the accrual of hard cardiovascular event outcomes in the DPPOS.

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Data Availability: DPP and DPPOS data are available in the NIDDK repository (www.niddkrepository.org/home/) and can be requested by any researcher. In accordance with the NIH Public Access Policy, we continue to provide all manuscripts to PubMed Central including this manuscript. DPP/DPPOS has provided the protocols and lifestyle and medication intervention manuals to the public through its public website (www.dppos.org). The DPPOS abides by the NIDDK data sharing policy and implementation guidance as required by the NIH/NIDDK.

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