



Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2019 January ; 39(1): 97–106. doi:10.1161/ATVBAHA.118.311562.

Gene-based elevated triglycerides and T2D risk in the Women's Genome Health Study

Shafqat Ahmad^{1,2,3}, Samia Mora^{2,4,5}, Paul M Ridker^{4,5}, Frank B Hu^{1,6}, and Daniel I Chasman²

¹Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

²Preventive Medicine Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

³Department of Medical Sciences, Molecular Epidemiology, Uppsala University, Sweden

⁴Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

⁵Center for Lipid Metabolomics, Brigham and Women's Hospital, Harvard Medical School, Boston

⁶Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Abstract

OBJECTIVE: Higher triglyceride (TG) is a risk factor for incident type 2 diabetes mellitus (T2DM), but paradoxically, genetic susceptibility for higher TG has been associated with lower T2DM risk. There is also evidence that the genetic association may be modified by baseline TG. Whether such associations can be replicated and the interaction is selective for certain TG-rich lipoprotein particles (TRLP) remain to be explored.

APPROACH AND RESULTS: Cox regression involving TG, TRLPs, and genetic determinants of TG was performed among 15,813 participants with baseline fasting status in the Women's Genome Health Study (WGHS), including 1,453 T2DM incident cases over a mean 18.6 (SD=5.3) years of follow-up. A weighted, 40-SNP TG genetic risk score (TG-wGRS₄₀) was inversely associated with incident T2DM (HR [95%CI] =0.66 [0.58, 0.75]/10 TG risk alleles, *P*-value=<0.0001) with adjustment for baseline BMI, HDL-C and TG. TG-associated risk was higher among individuals in the low compared with the highest TG-wGRS₄₀ tertile (HR [95%CI] =1.98 [1.83, 2.14] vs. 1.68 [1.58, 1.80] per mmol/L, *P*_{interaction}=0.0007). In TG-adjusted analysis, large

Correspondence to: Dr. Daniel I Chasman, Preventive Medicine Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, DCHASMAN@research.bwh.harvard.edu.

Contribution statement

SA and DIC designed the study. SA and DIC conducted the statistical analyses. SA and DIC had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. SA wrote the first version of the manuscript and DIC reviewed the first manuscript draft. SA, SM, PMR, FBH and DIC interpreted the data and edited the manuscript. SA and DIC are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors critically revised the paper for important intellectual content and approved the final version.

Disclosures:

Samia Mora received research grant support from Atherotech Diagnostics for research outside the current work, served as a consultant to Quest Diagnostics, and has a patent application regarding the use of an NMR biomarker (not related to lipoproteins) in relation to colorectal cancer risk. All other coauthors have no conflict of interests.

and medium but not small TRLPs were associated with higher T2DM incidence for successively lower TG-wGRS₄₀ tertiles, $P_{\text{interaction}}=0.013, 0.012$ and 0.620 across tertiles, respectively.

CONCLUSIONS: Our results confirm the previous observations of the paradoxical associations of TG with T2D while focusing attention on the larger TRLP subfractions, suggesting their importance in clinical profiling of T2DM risk.

Keywords

Epidemiology; Biomarkers; Type 2 Diabetes; Risk Factors; Lipids and Cholesterol; Genetic Association Studies; Clinical Studies; Pathophysiology; Genetics

INTRODUCTION

Elevated serum triglyceride (TG) level is an independent risk factor for T2DM incidence, even after controlling for body mass index (BMI) and all other conventional risk factors^{1, 2}. From a clinical perspective, elevated TG levels may be observed as much as 10 years before diagnosis of T2DM^{3, 4}. In conventional analysis, TG is substantially confined to the chylomicron and very low density lipoprotein (VLDL) fractions, collectively termed as triglyceride rich lipoproteins particles (TRLP). However, TRLPs are highly heterogeneous, and technological developments using nuclear magnetic resonance (NMR) allow detection of five discrete TRLP subfractions including very large, large, medium, small, and very small as defined by lipoprotein particle diameter⁵, each of which may have distinct clinical properties. Therefore, further delineating associations between TG with T2DM according to TRLP subfractions may provide clinical insight.

Since 2007, through genome-wide association studies (GWAS), about 40 independent genetic loci have been discovered for association with TG levels^{6, 7}. When combined as a genetic risk score (GRS), the TG-raising alleles at these loci provide a quantitative and continuous measure of genetic susceptibility to elevated TG levels. Recently, Klimentidis *et al.*,⁸ reported a significant interaction between a TG-specific weighted GRS (TG-wGRS) and baseline TG for association with T2DM risk. The authors observed that TG was more strongly associated with T2DM incidence in low compared to high TG-wGRS individuals. Klimentidis *et al.*,⁸ and Li *et al.*,⁹ also reported that many of the TG-associated genetic variants at or near *CILP2*, *GCKR*, *NAT2*, *PLA2G6*, *CETP* and *APOA1* genes were positively associated with TG but negatively associated with glycemic traits and T2DM. Meanwhile, formal Mendelian randomization analysis using TG-specific genetic instruments found no evidence of causal association between plasma TG and T2DM, and instead suggested a protective effect of genetically raised TG levels on T2DM^{10, 11}.

In the current study, we seek to confirm and further explore these paradoxical findings by using the large, prospective population based sample of middle-aged women from the Women's Genome Health Study (WGHS) to evaluate whether established TG-associated loci modify the association of baseline TG with the incidence of T2DM over a mean 18.6 years of follow-up. We also aimed to investigate whether the relationship between TG with T2DM risk can be refined to specific TRLP subfractions, potentially informing which biological aspects of lipid metabolism influence development of T2DM.

MATERIALS AND METHODS

Study Population

Individual level data for the WGHS is not currently publicly available but scripts for performing the analysis described in this report will be provided upon request. The study participants of the WGHS are derived from the Women's Health Study (WHS) that was designed as a placebo-controlled randomized trial evaluating the impact of low-dose of aspirin and vitamin E for the primary prevention of CVD^{12, 13} among initially healthy middle age (45 years or older) health-care professionals. Subsequent to the conclusion of the trial, the study population has been followed in observational mode. The WGHS consists of the approximately 72% of WHS participants with a baseline blood sample. For the current analysis, we have 23,294 participants with available genotype data and self-reported European ancestry that was also confirmed through genetics¹². Klimentidis *et al.*,⁸ performed analyses in the fasting sample, and considering this, for the current primary analyses we have 15,813 of 21,848 participants without diabetes at baseline who also had more than 8 hours of fasting status at baseline. Secondary analyses were performed among all participants (fasting and non-fasting). Analysis in the WGHS was approved by institutional review board of Brigham and Women's Hospital, Boston.

At baseline, clinical characteristics were collected through self-reported questionnaire regarding age and body mass index (BMI), as described previously¹⁴. All lipid levels including TG and HDL cholesterol (HDL-C) were enzymatically measured through Hitachi 917 analyzer (Roche Diagnostics) using baseline plasma. TGs were measured enzymatically with correction for endogenous glycerol.

Incident T2DM ascertainment

Incident T2DM cases were initially identified by self-report using annual questionnaires that asked if the participant had been diagnosed with diabetes since baseline¹⁵. Self-reported incident T2DM cases were confirmed through physician-administered telephone interviews or the questionnaires that inquired about clinical manifestations of diabetes including diabetes medications, diagnostic diabetes testing and disease symptoms¹⁶.

Measurement of Triglyceride-rich Lipoprotein (TRLP) Subfractions

NMR-based measures were performed for quantifying TRLPs, as has been reported previously^{17, 18}. Briefly, H-NMR spectra (400 MHz) of plasma were deconvoluted with the LipoProfile IV assay (LipoScience) to resolve concentrations (nmol/L) of distinct TRLP subfractions, differentiated according to diameter, i.e. particle size. For the current analysis, we considered very large (90–240 nm), large (50–89 nm), medium (37–49 nm), small (30–36 nm) and very small (24–29 nm) TLRPs. Mean particles size (TRLZ) ranged from 30–100 nm. The TG and cholesterol content of the combined TRLPs, designated as TRLTG and TRLTC respectively, were also determined by NMR.

Genotyping and Genetic Risk Score

In the WGHS, genotyping was performed with the HumanHap300 Duo array using the Infinium II assay^{19, 20}. Imputation for the SNPs that were not present in the genotyping

array was performed using MACH (v. 1.0.16) using the 1000 genomes phase I v. 3 (March 2012) cosmopolitan reference panel. For the current analyses, we used 40-TG associated genetic variants that have been previously reported in association with TG^{6,7}, as our primary genetic predictor regarding analyses because 40-TG associated variants are similar to the 31-TG associated genetic variants in the publication by Klimentidis et al⁸. In addition, we performed secondary analyses using recently published 127-TG associated genetic variants²¹. Most of the SNPs were directly genotyped. The SNPs that were imputed had high imputation quality score (MACH $r^2 \geq 0.85$). For the 40 TG-associated SNPs, effect allele frequencies, chromosomal position and gene names have been described previously²² and for the 127-TG associated SNPs²¹, this information is shown in Supplemental Table I. Weighted genetic risk scores (TG-wGRSs) were created using published effect estimates from the 40-TG (TG-wGRS₄₀) and 127-TG (TG-wGRS₁₂₇) associated genetic variants separately and scaled to such that each unit reflects 10 alleles, as described previously²².

Statistical Analysis

Cox proportional regression models were used to assess the association of TG, TRLPs, SNPs, and the TG-wGRSs with the incident T2DM. Interaction analyses were performed by additionally introducing a term of TG-associated SNPs or the TG-wGRSs \times TG in the Cox models. For interaction analyses, the TG-wGRSs were used as continuous variables or as a tertiles (coded as 0, 1 and 2). TG was used as a continuous variable. The basic models were adjusted with principle components (PCs) (to control for European population substructure), age, and randomization to treatment in the WHS parent cohort (vitamin E and aspirin). The fully adjusted models included covariates from the basic model plus measures of HDL-C and BMI, similar to Klimentidis *et al.*,⁸. All clinical covariates were determined at baseline. For the single SNP analysis, we considered a significant threshold $p = 0.00125$ ($=0.05/40$) after Bonferroni correction for the 40 single SNPs tests performed. Very large and large TRLP variables were not normally distributed as the distribution of these variables were left skewed. To assess the significance of associations with these variables, we used permutation procedures to derive empirical association *P*-values. We repeated the Cox regression over 10,000 iterations, each time resampling the TG-wGRS₄₀ at random without replacement to generate a null distribution of beta coefficients. The empirical *p*-values were derived as the proportion of beta coefficients from the empirical null distribution with absolute value greater than the absolute value of the beta coefficient from the unpermuted model. Also, because many of the study participants have lower even undetectable concentrations of very large TRLP particles, we created variable combining the very large and large TRLP particles and tested that combined variable in interaction with TG-wGRS₄₀ in predicting T2DM incidence. In sensitivity analyses, we generated trait residuals by regressing TG (and other TRLPs) against the TG-wGRS₄₀ to eliminate collinearity, and used the residuals as an interactor variable with the TG-wGRS₄₀ to assess association with T2DM. To investigate whether interactions with the TG-wGRS₄₀ were consistent with the implicit assumption that the ratio of SNP effects between strata of TG was similar across all SNPs, we regressed SNP effects for incident T2DM (as beta coefficients) in the group with TG below or equal to median TG against the group with TG above median. SNPs with disproportionate influence on the regression relationship were identified using the R function “influence.measures” that

flags extreme points for any of a series of regression diagnostics, including Cook's D, leave-one-out analysis, covariance ratio, and leverage.

RESULTS

Clinical and general characteristics of the study participants are shown in Table 1. For the current analyses, there were N=15,813 with fasting status (and N=21,848 in total) female participants from the WGHS among whom 1,453 experienced incident cases for T2DM during follow-up (N=1,944 incident T2DM cases in total sample). The mean follow-up duration for T2DM incident was 18.6 (SD=5.3) years.

Associations of TG, TG-wGRS and incident T2DM

Although 40 SNPs have been identified through GWAS for TG, Klimentidis *et al.*⁸ performed analysis using a TG-wGRS based on 31 TG-associated SNPs as they observed that this TG-wGRS was more strongly associated with TG. However, by reviewing the literature, we found that three SNPs, *COBL1* rs10195252, *APOB* rs1042034, and *APOE* rs439401 were not primarily or secondarily associated with TG in the most recent GWAS⁶. We therefore constructed a TG-wGRS and performed all of our primary analyses using 40-TG associated SNPs after excluding these three inconsistent SNPs but including 12 additional SNPs (*PIGV-NROB2* rs12748152, *LRPAP1* rs6831256, *VEGFA* rs998584, *RSPO3* rs719726, *MIR148A* rs4719841, *MET* rs38855, *AKR1C4* rs1832007, *PDXDC1* rs3198697, *FTO* rs1121980, *MPP3* rs8077889, *INSR* rs7248104 and *PEPD* rs731839). All of the included genetic variants were primarily or secondarily associated with TG at genome-wide significance⁶.

The TG-wGRS₄₀ was positively associated with TG (β [SE]=0.38[0.01] mmol/L per 10-TG associated risk alleles, *P*-value 0.0001) when the regression model was adjusted with age, BMI, HDL-C and PCs. TG was positively associated with T2DM incidence (HR [95% CI] =1.70[1.63, 1.77] per mmol/L, *P*-value 0.0001) when the regression analysis was adjusted with age and PCs. The association of TG with T2DM was attenuated (HR [95% CI] = 1.37[1.30, 1.43] per 1mmol/L, *P*-value 0.0001) when the regression analysis was additionally adjusted with BMI and HDL-C (Table 2). In a Cox model adjusted with age and PCs, we observed that the TG-wGRS₄₀ was associated with a non-significant reduction in T2DM incidence (HR [95% CI] =0.94 [0.83, 1.06] per 10-TG associated risk alleles, *P*-value=0.30). However, when the model was additionally adjusted for baseline age, TG, BMI and HDL-C, the TG-wGRS₄₀ was significantly associated with reduced T2DM incidence (HR [95% CI] =0.66 [0.58, 0.75] per 10-TG associated risk alleles, *P*-value 0.0001). Similarly, we observed that the TG-wGRS₁₂₇ was negatively but non-significantly (HR [95% CI] =0.98 [0.93, 1.03] per mmol/L, *P*-value= 0.39) associated with the incidence of T2DM when the analyses were adjusted with basic model covariates. In the fully adjusted regression models, TG-wGRS₁₂₇ was significantly negatively associated with T2DM incidence (HR [95% CI] =0.87 [0.83, 0.92] per mmol/L, *P*-value 0.0001).

We observed a significant interaction of baseline TG levels and the TG-wGRS₄₀ ($P_{\text{interaction}}= 0.002$) for incident T2DM when the Cox model was adjusted for age and PCs. This interaction was also observed when the regression model was additionally adjusted for

baseline BMI and HDL-C ($P_{\text{interaction}}=0.030$) (Table 2). Through residual trait analysis, we observed that the TG-wGRS₄₀ explained 4.99% trait variance in TG, similar to previously reported estimates²². When we replaced the TG with GRS-residualized TG in the analysis, the TG-wGRS₄₀ × trait-residual interactions for incident T2DM remained significant ($P_{\text{interaction}}=0.005$ for the basic model and $P_{\text{interaction}}=0.05$ for the fully adjusted model). In the Cox model adjusted for age and PCs, we observed that TG was associated with higher incidence of T2DM in the low TG-wGRS₄₀ tertile (HR [95% CI] =1.98 [1.83, 2.14] per mmol/L) compared to the high TG-wGRS₄₀ tertile (HR [95% CI] =1.68 [1.58, 1.80] per mmol/L), with a statistically significant interaction ($P_{\text{interaction}}=0.0007$) (Figure S1). Similar interactions were observed when the regression models were additionally adjusted with BMI and HDL-C (Table 3). Similar to the finding with the TG-wGRS₄₀, we observed significant interactions of TG-wGRS₁₂₇ and TG levels in the risk of T2DM in the basic model ($P_{\text{interaction}}=0.0157$) and in the fully adjusted model ($P_{\text{interaction}}=0.0432$) (Table 2)

TG-rich lipoproteins, TG-wGRS and T2DM risk

In Cox models adjusted for basic covariates, total TRLP, very large, large, medium, very small TRLPs, TRLZ, TRLTG and TRLTG (except small TRLP) were significantly associated with T2DM (all P -values < 0.0001), as has been published previously in a similar WHS sample¹⁶. Regarding the TG-wGRS₄₀, when the regression models were fully adjusted for BMI and HDL-C along-with total TG at baseline, we observed significant interactions between TG-wGRS₄₀ and large TRLP ($P_{\text{interaction}}=0.016$) and medium TRLP ($P_{\text{interaction}}=0.047$) with T2DM (Table 2). No significant interactions were observed for very large ($P_{\text{interaction}}=0.107$), very small ($P_{\text{interaction}}=0.891$) or small ($P_{\text{interaction}}=0.828$) TRLPs. Similarly, a variable combining large and very large TRLP particles showed significant interaction with TG-wGRS₄₀ in the incident T2DM ($P_{\text{interaction}}=0.0035$). For both the large TRLPs and the combination of large and very large TRPLs, in models adjusted with basic covariates, we also observed significant interaction using a resampling approach to derive an empirical procedure to address the skew in the TRLP distribution ($P_{\text{interaction, empirical}}=0.0024$ and 0.0057, respectively). Similarly, we observed concordant interactions for the different TG-rich lipoprotein subfractions (as shown in Table 2).

Accordingly, we observed a stronger association of large TRLPs with incident of T2DM among individuals in the lower TG-wGRS₄₀ tertile (HR [95% CI] =1.02 [0.97–1.06]) compared to the individuals in the higher TG-wGRS₄₀ tertile (HR [95% CI] = 0.97 [0.93–1.01]) with a significant interaction ($P_{\text{interaction}}=0.013$). Similar interactions were observed regarding medium TRLPs ($P_{\text{interaction}}=0.012$) (Table 3). However, the interactions with tertiles of TG-wGRS₄₀ were non-significant for small and very small TRLPs ($P_{\text{interaction}}=0.620$) and ($P_{\text{interaction}}=0.648$), respectively. We observed materially similar results when the analyses were performed in the total WGHS sample, including both fasting and non-fasting participants (Supplemental Table I).

Justification of using a genetic risk score for testing interactions with TG

One assumption in using a genetic risk score (GRS) for gene-environment interaction studies, is that the ratio of individual SNP effects across environmental strata should be approximately similar for all SNPs. We tested this assumption by comparing SNP-T2DM

association between two strata of TG (dichotomous variable was created based upon the median value: low < 1.29 mmol/L vs high > 1.29 mmol/L). In a linear model of SNP effects in the two strata, we observed that four of the SNPs (*KLHL8* rs442177, *APOA1* rs964184, *CAPN3* rs2412710 and *CILP2* rs10401969) had disproportionate influence on the regression fit (Supplemental Table II and Figure S2). We performed sensitivity analysis of TG-wGRS₄₀ × TG interactions in T2DM after removing the most influential SNPs (*KLHL8* rs442177, *APOA1* rs964184, *CAPN3* rs2412710 and *CILP2* rs10401969) from the TG-wGRS₄₀ and observed significant interactions with TG ($P_{\text{interaction}}=0.016$) for the basic model but not for the fully adjusted model ($P_{\text{interaction}}=0.27$).

Pleiotropic effects

In order to reduce the potential influence of known pleiotropic effects on our conclusions, we removed genetic variants from the TG-wGRS₄₀ that associate at genome-wide significance with T2DM related traits (*GCKR* rs1260326 [T2DM, fasting glucose, beta-cell function, fasting insulin], *IRS1* rs2972146 [fasting glucose, T2DM, homeostatic model assessment of β -cell function, homeostatic model assessment of insulin resistance], *FADS1-2-3* rs174546 [fasting glucose, T2DM, homeostatic model assessment of β -cell function], *FTO* rs1121980 [T2DM], *CILP2* rs10401969 [T2DM] and *PEPD* rs731839 [T2DM])²³. As was found for the primary 40 SNP TG-wGRS, this 34 SNP TG-wGRS was associated with a non-significant reduction in T2DM incidence (HR [95% CI] =0.96 [0.84, 1.10] per 10-TG associated risk alleles, P -value=0.57). However, in models additionally adjusted for baseline TG, HDL-C and BMI, the 34 SNP TG-wGRS was negatively associated with T2DM (HR [95% CI] =0.67 [0.58, 0.77] per 10-TG associated risk alleles, P -value = 0.0001). Similar to the 40 SNP TG-wGRS, we observed significant interactions of the 34 SNP TG-wGRS with very large TRLPs ($P_{\text{interaction}}=0.016$) and large TRLPs ($P_{\text{interaction}}= 0.001$) for association with T2DM outcomes (Supplemental Table III). In order to remove any of the potential pleiotropic effects from the TG-wGRS₁₂₇, we excluded 15 genetic variants that associate with T2DM related traits. As with the TG-wGRS₁₂₇, the 112 SNPs TG-wGRS was associated with non-significant decrease in T2DM (HR [95% CI] =0.97 [0.91, 1.02] per 10-TG associated risk alleles, P -value=0.26) but a significant decrease in the fully adjusted model (HR [95% CI] =0.86 [0.82, 0.91] per 10-TG associated risk alleles, P -value = 0.0001). As was found for the TG-wGRS₁₂₇, we observed significant interactions between 112 SNPs TG-wGRS and TG for the T2DM outcome in the basic model ($P_{\text{interaction}}=0.021$) or and the fully adjusted model ($P_{\text{interaction}}=0.047$).

Individual SNPs, TG and T2DM incidence

In single SNP analyses, we observed that 5 (out of 40) SNPs including *FADS1-2-3* rs174546, *APOA1* rs964184, *CETP* rs3764261, *CILP2* rs10401969 and *PLA2G6* rs5756931 showed significantly negative association (P -value < 0.05) with T2DM incidence but after correction for multiple testing, only *APOA1* rs964184 remained statistically significant (Table 4). Moreover, 18 of the 40 SNPs were directionally negatively associated with T2DM in models adjusted for age, PCs, BMI and HDL. Regarding single SNPs × TG interactions on T2DM incidence, we observed that a significant excess ($P < 0.001$) of 9 (out of 40) variants showed nominally significant interactions ($P < 0.05$) but after correction for multiple testing none of the variants remained statistically significant (Table 4).

DISCUSSION

By using data from a well-powered prospective sample of initially healthy US females with homogeneous European ancestry, our study provides a compelling evidence that genetic risk for higher TG derived from a 40-SNP TG-wGRS is associated with lower T2DM risk, contrary to the positive observational associations with TG levels. Further, there was a significant interaction between the TG-wGRS₄₀ and TG levels for predicting incident T2DM such that individuals with lower genetic predisposition to elevated TG had relatively stronger TG-based risk of T2DM than individuals with high genetic predisposition to elevated TG. We observed similar findings when the analyses were performed using a TG-wGRS based upon 127 TG-associated genetic variants. These results confirm findings from Klimentidis *et al.*,⁸ who studied a TG-wGRS based on 31 SNPs and found both reduced risk of T2DM with increased genetic risk of TG and an interaction between the TG-wGRS and TG levels for T2DM. The 40 SNPs in our TG-wGRS excluded three SNPs from the TG-wGRS of Klimentidis that only had weak evidence of TG association while including 12 additional SNPs with primary association for TG.

The current study also extends the previously reported discordant effects of a TG-wGRS on TG and T2DM incidence and the interaction of TG with the TG-wGRS in Klimentidis *et al.*,⁸. Our investigation focuses on the interaction effects with TG to the large, and medium but not small or very small TRLPs, even after adjustment with TG. Given the significant interaction of medium and large TRLPs, it may have been surprising that there was no significant interaction with the very large TRLPs (findings are summarized in Figure 1). However, approximately 20 % of the sample had no detectable very large TRLPs leading to lower variability in very large TRLPs compared to the others. The combined variable of large and very large TRLP particles showed significant interaction with TG-wGRS₄₀ for incident T2DM and the significance was affirmed by the empirically *p*-values.

Previously, Mora *et al.*,¹⁶ using the same sample, reported that large TRLPs were associated with higher risk of T2DM compared to the smaller TRLPs, which was further confirmed in two independent cohorts of multiethnic men and women^{24, 25}. Similarly, Garvey *et al.*,²⁶ reported that higher insulin resistance was associated with large but not small TRLPs. Festa *et al.*,²⁷ reported that larger but not smaller TRLPs were associated with increased risk of T2DM among 800 individuals of the Insulin Resistance Atherosclerosis Study who were followed-up for 5-years. Although the interactions persisted in TG adjusted analysis, the biochemical mechanism of our observed interactions may nevertheless be related to the larger amount of TG that is carried by larger compared with smaller TRLPs. Comparing individuals having insulin sensitivity to those with insulin resistant phenotypes, hepatic overproduction of larger TRLPs is a key feature of dyslipoproteinemia of insulin resistance and T2DM²⁸. It has been reported through *in vivo* lipoprotein kinetic experiments that increased larger TRLPs can change the composition of HDL particles through the action of cholesteryl ester transfer protein (CETP) and hepatic lipase, which targets TG, and this process leads to formation of small dense HDL particles and also increased catabolism of these particles²⁹. In turn, catabolism of HDL particles is associated with the formation of small density LDL (sdLDL) particles, which have been associated with insulin resistance and T2DM related phenotypes³⁰.

It is common in GRS interaction effects analysis to draw SNP weights in constructing GRS from single SNP marginal association effects. This approach assumes that the magnitude of main and interaction effects across SNPs are highly correlated, and indeed, genetic variants that show main effect association with a trait are generally good candidates for interaction effects³¹. In a previous interaction analysis with adiposity, we have shown that the assumption was largely valid for the TG-wGRS₄₀ in relation to TG as the outcome across normal compared to overweight/obese strata²². By adopting that same approach, we demonstrate that the results here were largely valid also for interaction with TG in predicting T2DM, although the assumption of proportionality was challenged for four of the genetic variants (*KLHL8* rs442177, *APOA1* rs964184, *CAPN3* rs2412710 and *CILP2* rs10401969). Excluding these SNPs, the interaction of the GRS and TG-wGRS₄₀ for T2DM remained significant.

The biological and pathophysiological mechanisms through which higher TG-wGRSs were protective for the risk of T2DM, after adjusting with baseline TG and HDL, are not clear. The effects of the TG-associated genetic variants on T2D may reflect their role in hepatic de-novo lipogenesis³² through which they might regulate glucose uptake and glycolysis and may subsequently increase plasma TG levels³³. A potential explanation of the discordant effects of TG and T2DM is that higher glucose utilization and glycolytic flux leads to downregulation of glucose-6-phosphatase and upregulation of glucokinase (GCK), phosphofructokinase and fatty acid synthases. These changes lead to higher glycogen synthesis, increased concentration of malonyl CoA concentration and production of larger TRLP subfractions³⁴. Our observation that TG-wGRSs positively associate with TG but with lower risk to T2DM is consistent with this hypothesis.

Our study has many strengths, including a large number of incident T2DM cases, the homogenous study sample, a comprehensive collection of TG-associated genetic variants, longitudinal study design, and complete profiles of TRLP subfractions and standard lipids. The long follow-up duration for incident T2D also reduces the likelihood of reverse causation. Our study has several limitations including that our sample was based upon European ancestry women participants only; the generalizability of the current findings still needs to be established by testing among ancestrally diverse populations. TG, HDL and LDL are highly correlated lipids and it is difficult to distinguish the specific effects of one lipid fraction versus another. To limit such potential pleiotropic effects, TG-wGRS and T2DM incidence analyses were additionally adjusted with HDL and LDL and the results were materially similar. However, the findings from Klimentidis *et al.*,⁸ were based upon both male and female participants and were similar to our findings suggesting that they are independent of sex.

In summary, our findings provide novel information understanding the complex relationship between TG and T2DM by focusing this relationship on the large TRLP component of TG. At the clinical level, the gene-based relationship between TG and T2DM related traits was opposite to the observational relationship, suggesting a complex gene regulation and cardiometabolic interplay between TG and T2DM related traits. One expects that the mechanisms underlying our associations most likely occur in tissues where lipids and T2DM related biomolecules are synthesized and stored. Our findings thus suggests future studies

employing systematic biological approaches using liver, muscle and adipose tissues are warranted to help further understand the causal links between lipids, glycemic traits, and their consequences to T2DM risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

The WGHS is supported by the National Cancer Institute (CA047988 and UM1CA182913) and the National Heart, Lung, and Blood Institute (HL043851 and HL080467) with collaborative scientific support and funding for genotyping. Dr. Shafqat Ahmad was supported through a fellowship from Nutricia Research Foundation (2016-T1), Swedish Heart-Lung Foundation (20150711) and Henning och Johan Throne-Holst stiftelse Fellowship Sweden. Dr. Mora was supported by the research grants from American Heart Association (0670007N), the Sandra A. Daugherty Foundation and the NHLBI (K08 HL094375) regarding NMR measurements, as well as from the National Heart, Lung, and Blood Institute (R01HL134811 and K24 HL136852), and the National Institute of Diabetes and Digestive and Kidney Diseases (DK112940). Also, without additional costs, LabCorp provided the LipoProfile IV results. Daniel Chasman is supported by the NINDS (R21NS092963, R21NS104398) and the NEI (R01EY021900).

Nonstandard Abbreviations and Acronyms

TRLP	Triglyceride-rich lipoprotein subfraction particles
TG-wGRS	Triglyceride-associated weighted genetic risk score
WGHS	Women's Genome Health Study
TRLZ	Triglyceride rich lipoprotein particles size
TRLTG	TG rich TRLP particles
TRLTC	Cholesterol rich TRLP particles

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Highlights

- Previous results had shown that while higher plasma triglycerides (TG) is a risk for type 2 diabetes (T2DM), genetic predisposition to higher TG was paradoxically protective for T2DM.
- Moreover, a significant interaction between a genetic risk score of TG-associated variants (TG-wGRS) and baseline TG for association with T2DM risk has been observed. In the interaction, plasma TG was more strongly associated with T2DM incidence among individuals with lower compared to higher genetic predisposition to elevated TG.
- By using TG-wGRSs based on 40-triglyceride and 127-triglyceride associated genetic variants, we tested whether such associations can be replicated and also whether the interaction is selective for certain triglyceride-rich lipoprotein particles (TRLP).
- In our cohort with 18.6 years of follow-up observation, our results confirm the paradoxical associations of TG with incident T2DM as well as the genetic interactions with TG. Moreover, the results add novelty by demonstrating the role of larger but not smaller TRLP subfractions in the interaction
- The results focus attention on the influence of large TRLPs on incident T2DM and may ultimately contribute to identifying population groups with greater susceptibility to T2DM through a combination of genetic risk and plasma lipid profile.

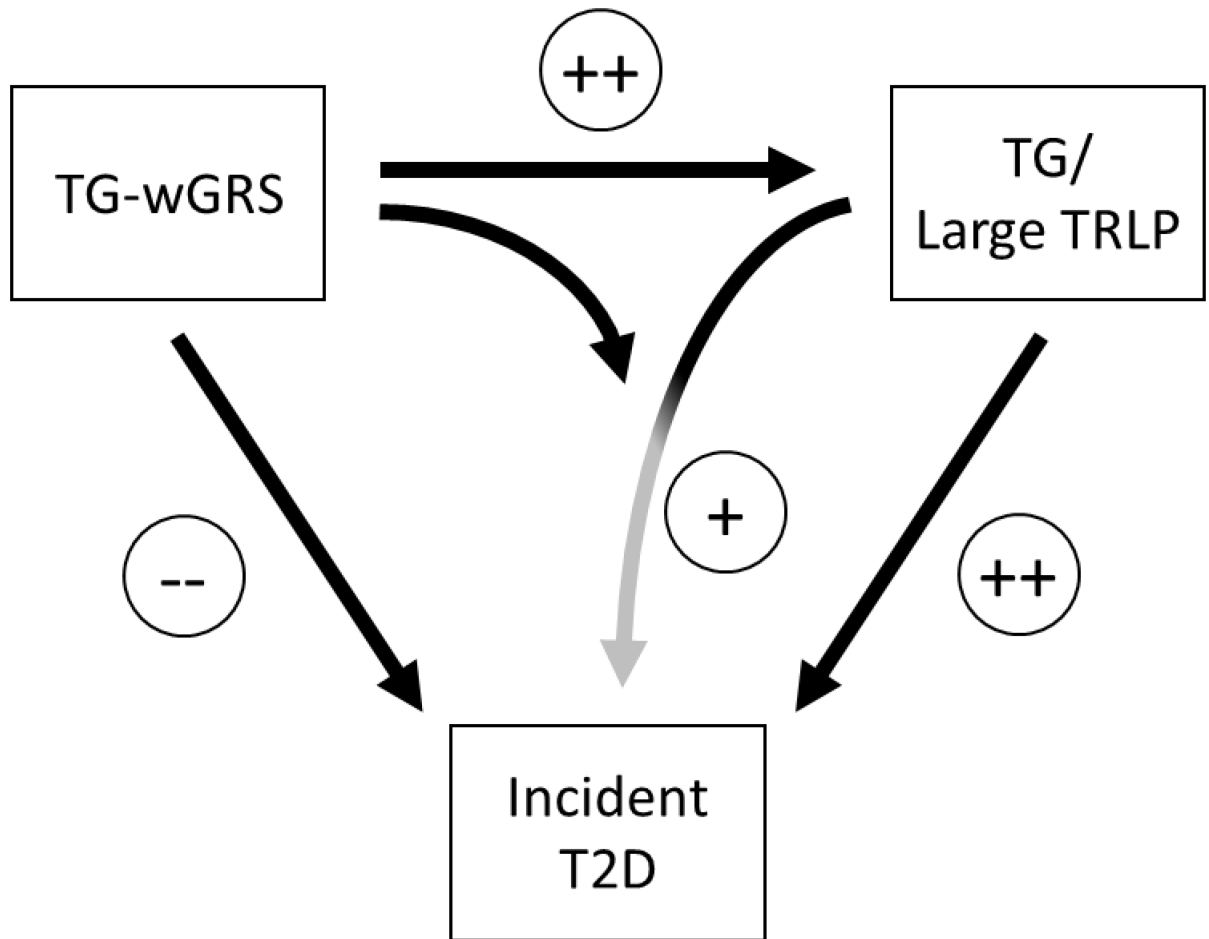


Figure 1. Central figure describes the TG-wGRS association with TG, TRLPs and the risk of T2DM.

Table 1

Baseline Characteristics of the total and with fasting Participants a in the WGHS Cohort

	Fasting sample		Total sample	
	Mean	SD	Mean	SD
Number of participants, N	15813		21848	
Age (years)	54.91	7.2	54.62	7.1
Triglycerides (mmol/L)	1.49	0.8	1.55	0.9
BMI, kg/m ²	25.75	4.9	25.76	4.9
HDL, mmol/L	1.41	0.4	1.40	0.4
TRLP (nmol/L)	173.42	62.2	172.25	60.8
Very large TRLP (nmol/L)	0.23	0.5	0.25	0.5
Large TRLP (nmol/L)	2.55	3.0	2.70	3.0
Medium TRLP (nmol/L)	18.03	13.3	18.45	13.3
Small TRLP (nmol/L)	62.14	38.1	61.76	37.7
Very small TRLP (nmol/L)	90.47	42.9	89.08	42.2
TRLZ (nm)	43.72	7.3	44.08	7.5
TRLTG (mmol/L)	2.38	1.3	2.33	1.3
TRLTC (mmol/L)	0.77	0.3	0.77	0.3
Genetic-predisposition score	40.09	4.2	40.11	4.2

BMI: body mass index; HDL: high density lipoproteins; TRLP: triglycerides rich lipoprotein particles;

TRLZ: mean triglyceride-rich particles size; TRLTG: TG rich TRLP particles;

TRLTC: cholesterol rich TRLP particles.

Table 2

Association of TG and TG-rich lipoproteins with the incidence of T2DM and TG-wGRS x biomarkers interactions in the incidence of T2DM in the WGHS (fasting sample)

	Model	HR	HR (95% CIs)		P-value	$\#P_{\text{interaction}}$	$\#P_{\text{interaction}}$
Triglycerides (mmol/L)	Basic	1.70	1.63	1.77	<0.0001	0.002	0,008
	Full	1.37	1.30	1.43	<0.0001	0.030	0,043
	Full + TG	1.00	1.00	1.00	0.0163	0.429	0,089
TRLP (nmol/L)	Basic	1.01	1.01	1.01	<0.0001	0.182	0,129
	Full	1.00	1.00	1.00	<0.0001	0.824	0,294
	Full + TG	1.00	1.00	1.00	0.0163	0.429	0,089
Very large TRLP	Basic	1.56	1.49	1.64	<0.0001	0.598	0,111
	Full	1.31	1.24	1.39	<0.0001	0.171	0,195
	Full + TG	1.08	1.01	1.17	0.034	0.107	0,120
Large TRLP	Basic	1.15	1.14	1.17	<0.0001	0.002*	0,700
	Full	1.07	1.06	1.09	<0.0001	0.043	0,385
	Full + TG	0.99	0.96	1.01	0.298	0.016	0,076
Medium TRLP (nmol/L)	Basic	1.02	1.02	1.03	<0.0001	0.014	0,035
	Full	1.00	1.00	1.01	0.0446	0.141	0,159
	Full + TG	0.98	0.97	0.98	<0.0001	0.047	0,039
Small TRLP (nmol/L)	Basic	1.00	1.00	1.00	0.1178	0.041	0,009
	Full	1.00	1.00	1.00	0.0008	0.518	0,083
	Full + TG	1.00	1.00	1.00	0.0091	0.891	0,231
Very small TRLP (nmol/L)	Basic	1.01	1.01	1.01	<0.0001	0.514	0,864
	Full	1.00	1.00	1.01	<0.0001	0.325	0,688
	Full + TG	1.00	1.00	1.00	0.0923	0.828	0,696
TRLZ (nm)	Basic	1.06	1.06	1.07	<0.0001	0.746	0,682
	Full	1.04	1.03	1.04	<0.0001	0.396	0,630
	Full + TG	1.02	1.01	1.03	<0.0001	0.132	0,258
TRLTG (mmol/L)	Basic	2.02	1.89	2.15	<0.0001	0.012	0,022
	Full	1.39	1.28	1.51	<0.0001	0.181	0,127
	Full + TG	0.69	0.59	0.80	<0.0001	0.045	0,013
TRLTC (mmol/L)	Basic	3.26	2.79	3.82	<0.0001	0.11	0,074
	Full	1.47	1.22	1.77	<0.0001	0.621	0,192
	Full + TG	0.60	0.48	0.76	<0.0001	0.211	0,036

Basic model was adjusted with age, PCs, Vitamin E and Aspirin

Fully adjusted model was adjusted with basic model + BMI, HDL

Full + TG: Fully adjusted model + TG; *Empirical Pinteraction = 0.0024 after 10,000 permutations (Methods).

Table 3 Association of TG with the incidence of T2DM across tertiles of GRS in the WGHHS (fasting sample)

	Model	1st GRS tertile		2nd GRS tertile		3rd GRS tertile		$P_{interaction}$			
		HR	HR (95% CIs)	HR	HR (95% CIs)	HR	HR (95% CIs)				
Triglycerides (mmol/L)	Basic	1.98	1.83	2.14	1.72	1.60	1.85	1.68	1.58	1.80	0.0007
	Full	1.55	1.41	1.71	1.35	1.24	1.48	1.40	1.30	1.51	0.032
TRLP (nmol/L)	Basic	1.01	1.01	1.01	1.01	1.00	1.01	1.01	1.01	1.01	0.161
	Full	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.650
Full + TG	Basic	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.336
	Full	1.67	1.54	1.82	1.45	1.32	1.58	1.64	1.52	1.76	0.824
Very large TRLP	Basic	1.42	1.30	1.56	1.20	1.08	1.35	1.38	1.26	1.50	0.504
	Full	1.11	0.98	1.26	0.95	0.82	1.10	1.14	1.01	1.28	0.319
Large TRLP	Basic	1.21	1.18	1.24	1.15	1.13	1.18	1.14	1.12	1.17	0.0006
	Full	1.11	1.08	1.15	1.07	1.04	1.09	1.07	1.04	1.10	0.025
Full + TG	Basic	1.02	0.97	1.06	0.99	0.94	1.03	0.97	0.93	1.01	0.013
	Full	1.03	1.02	1.04	1.03	1.02	1.03	1.02	1.01	1.03	0.007
Medium TRLP (nmol/L)	Basic	1.01	1.00	1.02	1.01	1.00	1.01	1.00	1.00	1.01	0.039
	Full	0.98	0.97	0.99	0.98	0.98	0.99	0.97	0.97	0.98	0.012
Small TRLP (nmol/L)	Basic	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.018
	Full	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.317
Full + TG	Basic	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	0.620
	Full	1.01	1.00	1.01	1.00	1.00	1.00	1.01	1.01	1.01	0.298
Very small TRLP (nmol/L)	Basic	1.01	1.00	1.01	1.00	1.00	1.00	1.01	1.01	1.01	0.253
	Full	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.01	0.648
TRLZ (nm)	Basic	1.07	1.06	1.08	1.06	1.05	1.07	1.07	1.06	1.08	0.675
	Full	1.05	1.04	1.06	1.03	1.02	1.04	1.04	1.03	1.05	0.429
Full + TG	Basic	1.03	1.01	1.04	1.01	0.99	1.02	1.02	1.01	1.04	0.171
	Full	2.64	2.29	3.04	1.97	1.77	2.20	2.03	1.83	2.27	0.004
TRLTG (mmol/L)	Basic	1.65	1.39	1.95	1.37	1.19	1.58	1.47	1.28	1.68	0.111
	Full										

	Model	1st GRS tertile		2nd GRS tertile		3rd GRS tertile		<i>P</i> _{interaction}
		HR	HR (95% CIs)	HR	HR (95% CIs)	HR	HR (95% CIs)	
TRLTC (mmol/L)	Full + TG	0.73	0.56	0.69	0.51	0.72	0.56	0.038
	Basic	4.60	3.38	3.10	2.37	3.46	2.63	0.078
	Full	1.81	1.28	1.44	1.05	1.74	1.26	0.414
	Full + TG	0.60	0.39	0.61	0.40	0.72	0.48	0.132

Basic model was adjusted with age, PCs, VE and ASA

Fully adjusted model was adjusted with basic model + BMI, HDL

Full + TG: fully adjusted model + TG

Table 4

List of 40-triglyceride associated regarding their genomic position, effect allele, TG effect size (weights used for GRS), association with T2DM incidence and interaction with TG in WGHS (fasting sample)

SNP	Locus	Effect Allele	Main Effect on T2DM incidence			Interaction	
			HR	HR (95% CIs)		P-value	P _{interaction}
rs12748152	<i>PIGV-NR0B2</i>	T	1.12	0.98	1.28	0.091	0.553
rs2131925	<i>ANGPTL3</i>	T	0.95	0.88	1.03	0.211	0.406
rs4846914	<i>GALNT2</i>	G	0.95	0.88	1.03	0.184	0.781
rs1260326	<i>GCKR</i>	T	1.02	0.95	1.10	0.602	0.140
rs2972146	<i>IRS1</i>	T	1.00	0.92	1.08	0.934	0.460
rs645040	<i>MSL2L1</i>	T	1.04	0.95	1.14	0.380	0.243
rs6831256	<i>LRPAP1</i>	G	1.02	0.94	1.09	0.703	0.370
rs442177	<i>KLHL8</i>	T	1.01	0.93	1.08	0.901	0.001
rs9686661	<i>MAP3K1</i>	T	0.96	0.87	1.05	0.369	0.467
rs6882076	<i>TIMD4</i>	C	1.05	0.98	1.14	0.186	0.027
rs998584	<i>VEGFA</i>	A	1.05	0.95	1.16	0.321	0.200
rs719726	<i>RSPO3</i>	T	1.01	0.93	1.09	0.908	0.012
rs4719841	<i>MIR148A</i>	G	1.00	0.93	1.08	0.941	0.346
rs13238203	<i>TYW1B</i>	C	0.06	0.00	2.16	0.125	0.337
rs17145738	<i>MLXIPL</i>	C	0.96	0.86	1.08	0.522	0.045
rs38855	<i>MET</i>	A	0.98	0.91	1.06	0.589	0.002
rs11776767	<i>PINX1</i>	C	0.96	0.88	1.04	0.282	0.622
rs1495741	<i>NAT2</i>	G	1.05	0.96	1.15	0.252	0.883
rs12678919	<i>LPL</i>	A	0.96	0.85	1.09	0.554	0.029
rs2954029	<i>TRIB1</i>	A	1.03	0.95	1.11	0.496	0.827
rs1832007	<i>AKR1C4</i>	A	1.06	0.96	1.18	0.245	0.089
rs10761731	<i>JMJD1C</i>	A	0.95	0.88	1.03	0.195	0.492
rs2068888	<i>CYP26A1</i>	G	0.98	0.91	1.06	0.619	0.954
rs174546	<i>FADS1-2-3</i>	T	0.90	0.83	0.97	0.007	0.716
rs964184	<i>APOA1</i>	G	0.77	0.69	0.87	<.0001	0.010
rs11613352	<i>LRP1</i>	C	0.96	0.88	1.04	0.318	0.284
rs4765127	<i>ZNF664</i>	G	1.04	0.96	1.12	0.332	0.602
rs2412710	<i>CAPN3</i>	A	1.23	0.96	1.59	0.105	0.845
rs2929282	<i>FRMD5</i>	T	1.12	0.94	1.33	0.224	0.963
rs1532085	<i>LIPC</i>	A	1.00	0.93	1.08	0.910	0.124
rs3198697	<i>PDXDC1</i>	C	1.01	0.91	1.11	0.932	0.485
rs11649653	<i>CTF1</i>	C	1.03	0.95	1.12	0.494	0.630
rs1121980	<i>FTO</i>	A	1.01	0.94	1.09	0.790	0.047
rs3764261	<i>CETP</i>	C	0.91	0.83	1.00	0.040	0.578
rs8077889	<i>MPP3</i>	C	0.96	0.87	1.05	0.315	0.800

SNP	Locus	Effect Allele	Main Effect on T2DM incidence			Interaction	
			HR	HR (95% CIs)		P-value	P _{interaction}
rs7248104	<i>INSR</i>	G	1.03	0.95	1.11	0.500	0.690
rs10401969	<i>CILP2</i>	T	0.83	0.73	0.95	0.007	0.487
rs731839	<i>PEPD</i>	G	1.06	0.98	1.15	0.135	0.566
rs6065906	<i>PLTP</i>	C	0.98	0.89	1.07	0.618	0.047
rs5756931	<i>PLA2G6</i>	T	0.90	0.83	0.97	0.007	0.469

Models were adjusted with age, PCs, BMI and HDL

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