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Longitudinal Changes in Triglycerides According to *ANGPTL4* [E40K] Genotype and Longitudinal Body Weight Change in the Atherosclerosis Risk in Communities Study

JENNIFER A. NETTLETON, PhD, KELLY A. VOLCIK, PhD, ELLEN W. DEMERATH, PhD, ERIC BOERWINKLE, PhD, and AARON R. FOLSOM, MD

From the Division of Epidemiology and Disease Control (J.A.N., K.A.V., E.B.) and the Human Genetics Center, University of Texas Health Science Center, Houston, TX (K.A.V., E.B.), and the Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN (E.W.D., A.R.F.)

Abstract

PURPOSE—Allelic variation in the adipokine angiopoietin-like 4 gene (*ANGPTL4*[E40 K]) has been cross-sectionally associated with triglycerides, but the effects of genotype, or the interaction between genotype and body weight, on longitudinal triglyceride change have not been studied.

METHODS—Body weight, triglycerides, and *ANGPTL4*[E40 K] genotype were determined at baseline (1987–1989) and at 3 follow-up exams (1990–1992, 1993–1995, 1996–1998) in 7,939 white *ANGPTL4* [E40 K] G allele homozygotes and 344 A allele carriers. Changes in body weight and triglycerides were characterized as the difference between exam 4 and baseline measurements.

RESULTS—*ANGPTL4*[E40 K] A allele carriers had triglyceride concentrations that were 15 to 18 mg/dL lower than G allele homozygotes at all exams ($P < 0.0001$). Triglycerides increased in both genotype groups over the 9-year period ($+19.1 \pm 0.9$ and $+16.2 \pm 4.1$ mg/dL in GG and GA/AA, respectively; P difference, 0.48). Weight gain was associated with increased triglycerides to a comparable degree in both genotype groups ($+5.5 \pm 0.3$ and $+4.3 \pm 0.9$ mg/dL per 2-kg increase in body weight in GG and GA/AA, respectively, p interaction = 0.30).

CONCLUSIONS—Differences in triglyceride concentrations between *ANGPTL4*[E40 K] A allele carriers and G allele homozygotes are maintained over time, but the degree of increase in triglycerides was similar between groups and was not modified by body weight changes.

Keywords

ANGPTL4[E40 K]; Triglycerides; Body Weight; Atherosclerosis Risk in Communities (ARIC) study; Longitudinal Study

INTRODUCTION

The adipokine angiopoietin-like 4 (*ANGPTL4*) is thought to regulate fatty acid transport among tissues by inhibiting lipoprotein lipase, a key enzyme in triglyceride (TG) metabolism (1–3). Consistent with its suspected role, a recent cross-sectional analysis in white participants from three different cohorts showed a strong association between baseline TG levels and the

non-synonymous sequence variant [E40 K] in the *ANGPTL4* gene (4). Homozygous (AA) and heterozygous A allele carriers (GA) had significantly lower TG concentrations compared with their homozygous wild-type counterparts (GG). The influence of this polymorphism on longitudinal changes in TG has not yet been investigated. Given the relation between TG concentrations and the risk of cardiovascular disease (CVD), the effect of *ANGPTL4*[E40 K] polymorphisms may have important implications for risk of incident disease, especially if one considers the potential for lifetime low TG concentrations to have a greater impact than might be expected from a single measure of TG (analogous to that reported for low-density lipoprotein cholesterol and PCSK9 polymorphisms) (5).

Changes in body weight predict changes in TGs (6–9), but it is possible that the influence of body weight change on changes in TG concentration may be dampened in individuals with a genetic predisposition to lower TG concentrations (i.e., *ANGPTL4*[E40 K] A allele carriers). Therefore we characterized the 9-year longitudinal changes in TG in *ANGPTL4*[E40 K] GG and A allele carriers. We also examined the potential interaction between longitudinal body weight change and *ANGPTL4*[E40 K] genotype with respect to longitudinal TG changes. These analyses were conducted with data from white men and women who participated in the Atherosclerosis Risk in Communities (ARIC) study, one of the three cohorts involved in the original cross-sectional study demonstrating the strong effect of *ANGPTL4*[E40 K] variation and TG concentration (4).

METHODS

Subjects

The ARIC study was initiated in 1987 to investigate the etiology and occurrence of atherosclerosis and atherosclerotic diseases (10). Men and women, 45 to 64 years of age, were recruited from four U.S. communities: Forsyth County, North Carolina; Jackson, Mississippi (African Americans only); northwest Minneapolis suburbs, Minnesota; Washington County, Maryland. After providing informed consent, 15,792 participants were enrolled in the study (8,710 women and 7,082 men). Study protocols were approved by the institutional review boards at each center. Because of limited variation among non-whites in *ANGPTL4* at the locus studied, non-white participants ($n = 4,314$) were excluded from the current investigation. The current investigation also excluded participants who had a history of diabetes ($n = 1,046$), who were taking cholesterol-lowering medications ($n = 386$), who were not typed for *ANGPTL4* [E40 K] ($n = 973$), who fasted less than 8 hours ($n = 234$), or who regularly consumed large quantities of alcohol (>20 g/d for women and >30 g/d for men) ($n = 840$). Participants missing baseline measures of TG concentration or with no follow-up measures of TG concentration were also excluded ($n = 401$). After these exclusions, 3,708 white men and 4,575 white women remained for analysis.

TG Measurement and Anthropometry Measurements

Plasma TGs were measured by enzymatic procedures (11), with reagents from Boehringer Mannheim Biochemical (analysis adapted for use in Cobas-Bio Analyzer, Roche). External control consisted of successful participation in the Lipid Standardization Program of the Centers for Disease Control and Prevention. The coefficient of variation for TGs was 7% (12). Changes in TGs were calculated as the difference between baseline and exam 4 measurements.

Body mass index (BMI) was calculated from measured weight (in kilograms)/height (in meters).² Waist circumference was measured to the nearest centimeter. Change in body weight and waist circumference were calculated as the difference between baseline and exam 4 measurements.

Genotyping

The *ANGPTL4*[E40 K] polymorphism was measured as previously described (4) using isolated genomic DNA and the TaqMan assay (Applied Biosystems, Foster City, CA). The TaqMan assays were read on a 7900HT real-time PCR instrument (Applied Biosystems, Foster City, CA) and genotypes were called using a semi-automated clustering routine. The common G allele encodes the glutamic acid (E) residue in the *ANGPTL4* protein, while the lower frequency A allele encodes a lysine (K). Because of small numbers of A allele homozygotes, G/A heterozygotes ($n = 338$) and A/A homozygotes ($n = 6$) were combined and their values compared with those of G/G homozygotes ($n = 7,939$).

Statistical Analysis

Participant characteristics at the baseline exam were compared between *ANGPTL4*[E40 K] genotype group (SAS proc GLM). TG concentration at each of the four ARIC exams, as well as the change in TG across exams, was calculated separately by *ANGPTL4*[E40 K] genotype group. Changes in TGs according to 2-cm changes in waist circumference or 2-kg changes in body weight were also calculated separately by *ANGPTL4*[E40 K] genotype group. Interactions between genotype group and body weight change or between genotype group and waist circumference change were estimated with cross-product terms. Unadjusted and multivariable adjusted values of these outcomes were calculated. The multivariable model included study center (Minnesota, Maryland, North Carolina), age (years), sex (male/female), smoking (current/former/never smoker and cigarette years), education level (up to and including grade school, high school without diploma, high school graduate, vocational school, college graduate, graduate school/professional school), physical activity (Baecke sport score) (13), and alcohol intake (g/d).

All analyses were performed with SAS version 9.1. Because of skewed distribution, TG values were transformed to the natural log scale for analysis and were back-transformed to the original scale for presentation. When calculating the change between exam 1 and exam 4 TGs, untransformed TG values were used.

RESULTS

Participant demographic and lifestyle characteristics, as well as baseline body weight, BMI, and waist circumference, did not differ between *ANGPTL4*[E40 K] G allele homozygotes and A allele carriers with the exception of greater alcohol intake in carriers ($P = 0.02$) (Table 1). Plasma concentrations of TGs were 14 to 23 mg/dL lower in A allele carriers than in G allele homozygotes at each exam, but the degree of change (increase) between the baseline exam and exam 4 was similar: +17 to 19 mg/dL. Adjusting for waist circumference or BMI (change over follow-up or measured at the exam of interest) did not alter these results (data not shown). Adjustment for basic demographic and lifestyle factors (study center, age, sex, smoking status, cigarette years, physical activity, alcohol intake) did not change these associations (data not shown).

In all participants, a 2-kg increase in body weight between baseline and exam 4 was associated with a 5.4 mg/dL increase in TGs ($\beta \pm \text{SE}$: 5.4 ± 0.3), and a 2-cm increase in waist circumference was associated with a 2.3 mg/dL increase in TG (2.3 ± 0.2), adjusted for demographic and lifestyle factors. Table 2 shows the TG changes associated with 2-kg body weight and 2-cm waist circumference changes in each *ANGPTL4*[E40 K] genotype group. These data showed no evidence of interaction between *ANGPTL4*[E40 K] genotype and either measure of anthropometric change in terms of TG concentration change (p for interaction ≥ 0.30). If only baseline data were considered, there was also no evidence of interaction between *ANGPTL4* [E40 K] genotype and BMI, body weight, or waist circumference (p for interaction > 0.71 , data

not shown). Adjustment for hormone use or diagnosis of diabetes over follow-up also did not change these results.

As an alternative representation, we also calculated the difference in TG change between genotype groups stratified by four weight change categories (percent change in body weight = [(exam 4 weight – exam 1 weight)/exam 1 weight]*100): 1) weight loss or no weight gain, 2) >0–5% weight gain 3) >5%–10% weight gain, and 4) >10% weight gain. Again, the change in TGs within each percentage weight change category did not significantly differ between *ANGPTL4*[E40 K] G allele homozygotes and A allele carriers (Table 3).

DISCUSSION

The current longitudinal investigation, including 8,283 white men and women from the ARIC study, showed that both body weight and *ANGPTL4*[E40 K] genotype independently influence plasma TG concentrations, but together genotype and body weight do not interactively determine TG concentration. Persons carrying the *ANGPTL4*[E40 K] A allele had lower concentrations of TGs than non-carriers at all exams, but TG concentrations increased as body weight increased uniformly in both genotype groups. These data indicate that while *ANGPTL4* [E40 K] variation may confer CVD protection via maintenance of lower TG concentrations (14,15), other environmental and lifestyle factors still operate as they would regardless of genotypic tendency. Having the “protective” genotype does not preclude the potent effects of lifestyle factors, such as increasing body weight, on a CVD risk factor like TG.

Our results are in contrast to those of previous studies investigating gene-environment (body weight) interactions with respect to lipid concentrations. Two cross-sectional studies reported interactions between measures of adiposity and the hepatic lipase 514C >T polymorphism, with both showing the T allele associated with greater high-density lipoprotein only in individuals who had a healthy body weight (BMI <25 kg/m²) (16) or less visceral adiposity (visceral adipose tissue <130 cm²) (17). Since the association between *ANGPTL4*[E40 K] and TGs was reported only recently, there are no published studies of the interactive effects of environmental factors and *ANGPTL4*[E40 K] genotype. *ANGPTL4*[E40 K] variation is hypothesized to decrease TG concentrations due to a loss of function of the *ANGPTL4* protein, a reversal of lipoprotein lipase inhibition, and an increase in TG clearance (1–3). Fat mass, or changes in fat mass, reflected by various measures of adiposity also influence TG concentration via lipoprotein lipase and also through modulation of hepatic lipase activity. For example, weight loss has been shown to increase LPL mRNA (18) and BMI has been positively associated with hepatic lipase activity (10,19), resulting in lower HDL and greater TG.

Because the mechanisms by which *ANGPTL4*[E40 K] variation and body weight influence TG concentrations are similar, we hypothesized that carriers of the *ANGPTL4*[E40 K] variant allele might be resistant to the influences of changes in adiposity (reflected here by body weight or waist circumference change). However, data showed that the positive slope of TG change associated with increasing body weight or waist circumference was similar in both genotype groups (i.e., the TG differential between genotype groups did not materially change as body weight and waist circumference changed over follow-up).

Strengths of this analysis include a large sample size and longitudinal measures of TG, body weight, and waist circumference. There are also potential limitations to this analysis. Although careful internal and external quality control procedures were followed in the current study (12), TG concentrations are measured with a certain degree of error due to intraindividual variability, which could complicate the interpretation of changes in TG concentration over time. However, this should be nondifferential. Our measure of total adiposity (body weight)

also includes lean body mass and thus may not detect possible body fat differences between the genotype groups; however, use of waist circumference, which is more highly correlated with total and central body adiposity than is weight, also showed no differential effect on TG change by genotype. The methods of modeling “change” in epidemiological analyses are debated, but we believe the approach used here was most appropriate, given the strong association between genotype and TG at baseline and the potential for measurement error in the outcome of interest (TG) (20). Finally, these data were exclusively from white men and women from the United States and, therefore, cannot be generalized with confidence to other race groups or other geographical areas.

In conclusion, changes in plasma TG over time are similar between *ANGPTL4*[E40 K] A allele carriers and non-carriers, with A allele carriers maintaining relatively lower TG concentrations despite increases in TG concentration over 9 years of follow-up in both groups. Furthermore, *ANGPTL4*[E40 K] body weight and waist circumference changes impart a similar influence on TG regardless of genotype. As with all studies of gene x environment interaction, these findings require investigation in other large cohorts before definitive conclusions can be drawn, but they do suggest that to maintain low TG concentration and, thus, lower CVD risk, maintenance of healthy body weight and waist circumference are important, regardless of *ANGPTL4*[E40 K] genotype.

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Selected Abbreviations and Acronyms

TG	triglyceride
<i>ANGPTL4</i>[E40 K]	adipokine angiopoietin-like 4 (gene)
ARIC	Atherosclerosis Risk in Communities (study)
G	glycine
A	alanine

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TABLE 1

Selected baseline characteristics and plasma triglycerides across all exams in white men and women from the ARIC study stratified by *ANGPTL4*[E40 K] genotype*

	<i>ANGPTL4</i> [E40 K] genotype		<i>p</i> Value
	GG	GA/AA	
Number	7,939	344	
Age, yr	54.1 ± 0.06	54.2 ± 0.3	0.65
Sex (% female)	55.2	57.0	0.51
Education (% ≥ high school degree)	84.4	86.7	0.25
Physical activity(% in quintile 5 of Baecke sport score)	16.7	16.3	0.85
Current smoking (%)	22.2	22.7	0.83
Cigarette years	305 ± 5	323 ± 23	0.42
Alcohol intake (g/d)	3.7 ± 0.1	4.5 ± 0.3	0.02
Body mass index(kg/m ²)	26.7 ± 0.1	26.3 ± 0.3	0.22
Body weight (kg)	75.9 ± 0.2	75.1 ± 0.9	0.39
Waist circumference(cm)	95.0 ± 0.1	93.6 ± 0.7	0.06
Baseline/exam 1 TG(geometric mean[95% CI], mg/dL)	113.5 (112.2–114.7)	94.0 (91.1–101.1)	<0.001
Exam 2 TG (mg/dL)	119.8 (118.5–121.1)	105.6 (91.8–101.7)	<0.001
Exam 3 TG (mg/dL)	128.2 (126.8–129.6)	105.2 (99.9–10.8)	<0.001
Exam 4 TG (mg/dL)	130.5 (129.1–131.9)	110.3 (104.7–116.1)	<0.001
Nine-year change in TG (mg/dL)	19.1 ± 0.8	17.0 ± 4.1	0.61

ARIC = Atherosclerosis Risk in Communities (study); GG = ***; GA/AA = ***; TG = triglyceride; CI = confidence interval.

* Data represent unadjusted means ± standard error or percentages unless otherwise noted. *p* for difference between genotype categories was calculated by *t* test (continuous variables) or chi-square analysis (categorical outcomes). Distribution of TG concentrations was skewed and, therefore, values were transformed to the natural log scale for analysis and back transformed to the geometric scale for presentation.

TABLE 2

Nine-year change in plasma triglyceride concentrations associated with a 2-cm increase in waist circumference or a 2-kg increase in body weight by *ANGPTL4*[E40 K] genotype group

	GA	GA/AA	<i>p</i> Interaction
Change in TG (mg/dL) between exam 1 and exam 4 per 2-cm change in waist circumference	3.2 ± 0.2	2.8 ± 0.6	0.69 [*]
	2.3 ± 0.2	2.1 ± 0.8	0.69 [†]
Change in TG (mg/dL) between exam 1 and exam 4 per 2-kg change in body weight	5.8 ± 0.3	4.6 ± 0.9	0.33 [*]
	5.5 ± 0.3	4.3 ± 0.9	0.30 [†]

GA = ***; GA/AA = ***; TG = triglyceride.

* Data are unadjusted $\beta \pm$ standard error.

[†] Data are $\beta \pm$ standard error adjusted for study center, age (years), sex, smoking (never/current/former and cigarette years), physical activity (Baecke sport score), and alcohol intake (g/d).

TABLE 3

Nine-year change in plasma triglyceride concentrations by *ANGPTL4*[E40 K] genotype and percent weight gain category*

% Body weight gain category	Change in TG (mg/dL) between exam 1 and exam 4		<i>p</i> Value
	GG	GA/AA	
Weight loss or no change	3.0 ± 1.4 (n = 2,483)	−0.41 ± 6.9 (n = 109)	0.71
>0–5%	15.9 ± 1.5 (n = 2,189)	12.5 ± 6.9 (n = 101)	0.63
>5%–10%	28.1 ± 1.9 (n = 1,705)	25.9 ± 9.8 (n = 65)	0.83
>10%	49.0 ± 2.0 (n = 1,562)	37.8 ± 9.2 (n = 69)	0.23

TG = triglyceride; GG = ***; GA/AA = ***.

* Data are means (± standard errors) adjusted for study center, age, sex, smoking (never/current/former and cigarette years), physical activity (Baecke sport score), and alcohol intake.