Genetic association with lipids in Filipinos: waist circumference modifies an APOA5 effect on triglyceride levels

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Abstract Blood levels of lipoprotein cholesterol and triglycerides (TGs) are highly heritable and are major risk factors for cardiovascular disease (CVD). Approximately 100 lipid-associated loci have been identified in populations of European ancestry. We performed a genome-wide association study of lipid traits in 1,782 Filipino women from the Cebu Longitudinal Health and Nutrition Survey, and tested for evidence of interactions with waist circumference. We conducted additional association and interaction analyses in 1,719 of their young adult offspring. Genome-wide significant associations (P < 5 × 10⁻⁸) were detected at APOE for low density lipoprotein cholesterol and total cholesterol, and at APOA5 for TGs. Suggestive associations (P < 10⁻⁶) were detected at GCKR for TGs, and at CETP and TOM1 for high density lipoprotein cholesterol. Our data also supported the existence of allelic heterogeneity at APOA5, CETP, LIPC, and APOE. The secondary signal (Gly185Cys) at APOA5 exhibited a single nucleotide polymorphism (SNP)-by-waist circumference interaction affecting TGs (Pinteraction = 1.6 × 10⁻⁶), manifested by stronger SNP effects as waist circumference increased. These findings provide the first evidence that central obesity may accentuate the effect of the TG-increasing allele of the APOA5 signal, emphasizing that CVD risk could be reduced by central obesity control.—Wu, Y., A. F. Marvelle, J. Li, D. C. Croteau-Chonka, A. B. Feranil, C. W. Kuzawa, Y. Li, L. S. Adair, and K. L. Mohlke. Genetic association with lipids in Filipinos: waist circumference modifies an APOA5 effect on triglyceride levels. J. Lipid Res. 2013. 54: 3198–3205.

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Blood concentrations of lipoprotein cholesterol and triglycerides (TGs) are major risk factors for cardiovascular disease (CVD), the current leading cause of mortality worldwide (1). The most rapid increase in the prevalence of CVD is taking place in Asia, including the Philippines (1), although the obesity-related anthropometric measures remain lower in these Asian populations compared with those in populations of Europeans (2). Family-based studies have demonstrated that 40–50% of the total variation in lipid traits is genetically determined (3). Genome-wide association (GWA) studies have identified ~100 loci associated with fasting levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and TGs in Europeans (4–9). Many of these lipid-associated loci, including APOA5, LPL, GCKR, TRIB1, MLIPL, CETP, LIPC, and ABCA1, were further confirmed in individuals of non-European ancestries (10–12). More recently, a large-scale GWA study conducted in East Asians identified additional HDL-C signals near MYL2 and HECTD4 (C12orf51) (11). The full gene names corresponding to the locus symbols are listed in supplementary Table I.

The GWA signals identified to date have only explained ~10% phenotypic variation in each lipid trait, corresponding to ~25% of the trait heritability (8). Additional heritability may be explained by the incomplete coverage of functional variants and by allelic heterogeneity (13, 14).

Abbreviations: BMI, body mass index; CLHNS, Cebu Longitudinal Health and Nutrition Survey; CVD, cardiovascular disease; GWA, genome-wide association; HDL-C, high density lipoprotein cholesterol; LD, linkage disequilibrium; LDL-C, low density lipoprotein cholesterol; ln, log-transformed; PC, principal component; Q1(2, 3, 4), quartile 1(2, 3, 4); SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglyceride.
Gene-by-environment interactions may also account for some heritability (15) because the interactive effects of environmental modifiers may hinder the detection of genetic associations (16). Earlier studies have demonstrated that diet, alcohol, smoking, and physical activity can modulate genetic effects on lipid levels (12, 17–19). In addition, evidence exists that anthropometric traits, including body mass index (BMI), waist-hip ratio, and body composition, modify the association between genetic variants and lipid traits (20).

In recent decades, the rising prevalence of CVD shows a positive link with the increasing prevalence of central obesity (21, 22). Waist circumference is believed to be a stronger predictor of CVD than BMI, particularly in Asians, who tend to have a higher risk of CVD at any given level of BMI compared with Europeans (23). Intra-abdominal visceral fat accumulation has been shown to affect the lipid levels either directly or via insulin resistance (24, 25). Despite considerable evidence that genetic variants and obesity traits independently and jointly influence lipid levels, few studies have explicitly examined the modifying effect of waist circumference on lipid associations identified by GWA studies.

To test the hypothesis that central obesity would influence the association between genetic variation and lipid levels, we performed a GWA study to investigate single nucleotide polymorphism (SNP) associations with lipoprotein cholesterol and TG levels in 1,782 Filipino mothers from the CLHNS and tested for interactions between lipid-associated variants and waist circumference affecting lipid levels. We also conducted analyses in 1,719 young adult offspring of the CLHNS mothers using Metabochip genotypes to confirm SNP associations and interactions with waist circumference.

METHODS AND PROCEDURES

Study subjects and phenotypes

The CLHNS is an ongoing population-based birth cohort study that began in 1983. The original study population, study design, and recruitment protocols have been described in detail previously (26). Briefly, the baseline survey randomly recruited 3,327 pregnant women from the Metropolitan Cebu area of the Philippines in 1983–1984 (3,080 singleton live births), and since followed them and their offspring to the present. Trained fielders, we applied MACH2QTL to perform the GWA analyses, adjusting for age, age², household assets (a score that counts the possession of land and house and household amenities, such as a TV or refrigerator), natural log household income, and the first seven PCs were included as covariates. To examine the association in the combined cohort of mothers and offspring, we applied a general linear mixed model that accounted for the correlation of the outcome trait between mother-child pairs due to shared genetic and environmental

Statistical analysis

Values of TC and TGs were natural log-transformed (ln) to approximate the normal distributions of the traits. We applied multiple linear regression models and assumed an additive mode of inheritance to test for the association between genotypes and HDL-C, LDL-C, ln TC, and ln TGs. We constructed 10 principal components (PCs) of population substructure using EIGENSOFT (32). In the CLHNS mothers, we applied MACH2QTL to perform the GWA analyses, adjusting for age, age², household assets, ln household income, and the first seven PCs. To control for the effects of other potential factors that could confound the SNP-lipid associations, we also conducted additional analyses with additional covariates, including menopausal status, current smoking, and type 2 diabetes, in the CLHNS mothers. In the CLHNS offspring, we used SAS version 9.2 (SAS Institute, Cary, NC) to test the association between lipid traits and selected SNPs that were reported in previous GWA studies. As the ages of all offspring were within two years of each other, only sex, household assets, ln household income, and the first seven PCs were included as covariates. To examine the association in the combined cohort of mothers and offspring, we applied a general linear mixed model that accounted for the correlation of the outcome trait between mother-child pairs due to shared genetic and environmental

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exposures. Age, age$^2$, sex, household assets, ln household income, the first seven PCs, and generation (mother/offspring) were used as covariates.

For each SNP that showed evidence of a main effect association with a lipid trait in the CLHNS ($P < 0.1$ in both mothers and offspring analyzed separately), we further tested for a genotype interaction with waist circumference by including a SNP-by-waist circumference interaction term in the linear regression model or in the linear mixed model. The same sets of covariates used in the main effect analyses were included in the interaction analyses. For each SNP that showed significant evidence of interaction ($P_{\text{interaction}} < 0.05$ in both mother and offspring cohorts), we conducted a stratified analysis by categorizing the study samples based on quartiles of waist circumference and testing for the main effects of the SNP on lipids within quartiles. All interaction analyses were conducted in SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

The general characteristics of the 1,782 mothers and 1,719 young adult offspring from the CLHNS studied in these genetic analyses are shown in Table 1. Association analyses revealed genome-wide significant associations ($P < 5 \times 10^{-8}$) at APOE for both LDL-C (rs7412, $P = 1.6 \times 10^{-25}$) and TC (rs7412, $P = 1.8 \times 10^{-11}$), and at APOA5 for TGs (rs662799, $P = 3.1 \times 10^{-15}$) in the CLHNS mothers (Table 2). Suggestive evidence of association at a less stringent threshold of $P < 10^{-6}$ was detected at the TG locus GCKR (rs780099, $P = 1.6 \times 10^{-7}$) and the HDL-C locus CETP (rs1800775, $P = 6.3 \times 10^{-7}$). In addition, we observed that variants at TOM1, a new HDL-C locus recently identified in a large-scale meta-analysis in individuals of European ancestry (9), also exhibited suggestive evidence of association in the mothers (rs138777, $P = 5.0 \times 10^{-7}$).

We further evaluated these SNP associations in the CLHNS young adult offspring genotyped using the MetaboChip (Table 2). We observed significant associations ($P < 5 \times 10^{-8}$) for the variants at APOE ($P = 1.0 \times 10^{-23}$ for LDL-C and $1.7 \times 10^{-20}$ for TC) and APOA5 ($P = 2.6 \times 10^{-15}$), and nominal association for SNPs at GCKR ($P = 3.5 \times 10^{-8}$) and CETP ($P = 1.7 \times 10^{-8}$). For the SNP rs138777 at TOM1, we found marginal association with HDL-C in the offspring ($P = 0.058$). In the combined analysis including both mothers and offspring, all of these loci, except TOM1 ($P = 4.0 \times 10^{-5}$), reached significance at $P < 5 \times 10^{-8}$ (Table 2).

We next assessed whether additional SNP-lipid associations identified in previous GWA studies could be extended to Filipino middle-aged and young adults, groups that have not been widely studied. As all the loci were previously reported, we defined the evidence of association as $P < 0.1$ for the reported GWA index SNPs or their proxies in both mothers and offspring with a consistent direction of effect. Among the 95 previously reported lipid loci (8), 14 reached this threshold in the CLHNS. In addition to the four loci (APOE, APOA5, GCKR, and CETP) described in Table 2, the additional loci included three for TGs (LPL, MLXIPL, and ANGPTL3), three for HDL-C (LIPC, MMAB-MVK, and LIPG), two for LDL-C (ABO and APOB), and two for TC (TIMD4 and DNAH11) (Table 3). Analyses including both mothers and offspring showed that the variant rs588136 at LIPC also exhibited genome-wide significant association with HDL-C levels in the combined samples ($P = 1.5 \times 10^{-15}$).

Given prior evidence that up to 10 lipid-associated loci exhibited two or more signals (7, 8, 14), and in particular, that at least four lipid loci have been reported to contain multiple signals in East Asians (APOA5 for TG, CETP for HDL-C, and APOE and ABO for LDL-C) (14), we next tested for the association with these additional variants in the CLHNS samples. We conditioned on the SNP genotype of the initial signal and defined a secondary signal as the most strongly associated SNP with a $P_{\text{conditional}} < 5 \times 10^{-4}$ in combined mothers and offspring. After accounting for the effects of the most strongly associated SNPs at each of the five loci that reached GWA significance in the combined mothers and offspring, we confirmed the presence of secondary signals at four loci including the TG locus APOA5, the LDL-C loci CETP and LIPC, and the LDL-C locus APOE (Table 4). Except at the LDL-C locus APOE, the evidence of association of the second signals was attenuated when we conditioned on the first signals at the corresponding loci [$\Delta(-\log_{10}P) > 2$], suggesting that these additional signals at APOA5, CETP, and LIPC were not completely independent of the first signals (Table 4). In further analyses adjusting for the additional covariates of menopausal status, current smoking, and type 2 diabetes status (supplementary Table II), we observed similar results for the SNPs described above [all $\Delta(-\log_{10}P) < 1$, supplementary Table III].

| Characteristic | Mothers (n = 1,782) | Offspring (n = 1,719) |
|----------------|---------------------|-----------------------|
| Female (%)     | 100                 | 47.6                  |
| Age in 2005 (years) | 48.4 ± 6.1       | 21.5 ± 0.3            |
| Household assets in 2005 (0 to 11) | 5.2 ± 2.0         | 5.2 ± 2.0             |
| Household income in 2005 (pesos/week) | 396.4 (244.6, 623.9) | 357.7 (213.6, 586.7) |
| TC (mmol/l) | 4.79 (4.09, 5.44) | 3.94 (3.44, 4.58) |
| HDL-C (mmol/l) | 1.06 ± 0.27       | 1.09 ± 0.29           |
| LDL-C (mmol/l) | 3.10 ± 0.87       | 2.44 ± 0.75           |
| TGs (mmol/l)  | 1.25 (0.91, 1.78) | 0.99 (0.74, 1.41) |
| Waist circumference (cm) | 80.0 (73.5, 88.5) | 69.0 (65.0, 74.1) |

Data are mean ± SD, median (25th percentile, 75th percentile), or percent. Only one of the CLHNS mothers and one of the young adult offspring were taking lipid-altering medication.
As shown in supplementary Table IV, individuals with larger waist circumference have more atherogenic lipoprotein profiles. To investigate whether the associations in Tables 2–4 were affected by measures of waist circumference, a known predictor of CVD risk, we tested the SNP-lipid associations again while also adjusting for waist circumference in addition to the covariates included in the initial model. Results from analyses in the CLHNS mothers showed that the association evidence did not change substantially for most of the loci (|Δlog_{10}P| < 2) (supplementary Table III). However, adjusting for waist circumference appeared to influence the association of the APOA5 variant rs662799 with TGs (unadjusted P = 3.1 × 10^{-18}; adjusted P = 2.8 × 10^{-22}) (supplementary Table III).

We next assessed whether the lipid-associated SNPs exhibited evidence of interaction with waist circumference on lipid levels (supplementary Table V). Among the 20 signals at 15 loci with main effect associations shown in Tables 2–4, we observed SNP-by-waist circumference interactions (Pinteraction < 0.05) affecting TGs for APOA5 variants rs3741297 (Pinteraction = 9.6 × 10^{-3}) and rs662799 (Pinteraction = 0.032), and affecting TC level for the APOE variant rs7412 (Pinteraction = 0.034) in CLHNS mothers. Additional analyses in the offspring confirmed the interaction for APOA5 rs3741297 (Pinteraction = 7.4 × 10^{-5}). In a mixed model that combined the mothers and offspring together, the interaction remained significant (Pinteraction = 1.6 × 10^{-5}), even after a conservative Bonferroni correction for multiple testing (P < 8.3 × 10^{-4}, 0.05/60 tests).

Further analyses stratified by quartiles of waist circumference supported the interaction between APOA5 variant rs3741297 and waist circumference affecting TG levels. In the mothers, the SNP had no effect on TGs in the lowest quartile of waist circumference (<73.5 cm (quartile (Q)1: geometric mean of TG level in individuals carrying CC = 0.85 mmol/l and CT+TT = 0.98 mmol/l, P = 0.93, n = 440), but the minor allele was significantly associated with higher TG level in higher quartiles of waist circumference (Q2: CC = 1.29 mmol/l and CT+TT = 1.59 mmol/l, P = 2.6 × 10^{-3}, n = 444; Q4: CC = 1.56 mmol/l and CT+TT = 2.52 mmol/l, P = 4.5 × 10^{-3}, n = 443; Fig. 1A). The magnitudes of the association became stronger as waist circumference increased (Q1: β = −0.01, Q2: β = 0.24, Q3: β = 0.51, and Q4: β = 0.52). Notably, we observed a consistent pattern of interaction in the offspring, with stronger SNP effects in individuals with higher waist circumference (Q1: β = 0.21, n = 408; Q2: β = 0.04, n = 411; Q3: β = 0.14, n = 415 and Q4: β = 0.37, n = 415; Fig. 1B). The mothers had a higher median waist circumference than the offspring; we next stratified the samples using a threshold of 74.0 cm, the median of waist circumference in the combined cohort. In the combined mothers and offspring, individuals carrying the T allele of rs3741297 had significantly higher

| Locus  | Trait | SNP Reported Previously | SNP Analyzed in CLHNS | LD 2 | EAF | β (SE) | P  | EAF | β (SE) | P  | EAF | β (SE) | P  |
|-------|-------|------------------------|-----------------------|------|-----|-------|----|-----|-------|----|-----|-------|----|
| LPL   | TGs   | rs12678919             | rs328                 | 0.99 | 0.95 | 0.11 (0.04) | 7.9E-03 | 0.95 | 0.07 (0.04) | 0.10 | 0.09 (0.03) | 1.6E-03 |
| MLXPL | TGs   | rs17145738             | rs17145738            | 1.00 | 0.10 | −0.05 (0.03) | 0.077 | 0.10 | −0.06 (0.03) | 0.040 | −0.06 (0.02) | 4.2E-03 |
| ANGPTL3 | TGs   | rs17131925             | rs17131925            | 1.00 | 0.71 | 0.04 (0.02) | 0.036 | 0.69 | 0.04 (0.02) | 0.034 | 0.04 (0.01) | 5.1E-03 |
| LIPC  | HDL-C | rs2070895              | rs588136              | 1.00 | 0.69 | −0.05 (0.01) | 5.1E-06 | 0.65 | −0.06 (0.01) | 1.1E-09 | −0.05 (0.01) | 1.5E-12 |
| LIPG  | HDL-C | rs22491818             | rs2156552             | 0.94 | 0.14 | −0.02 (0.01) | 0.075 | 0.13 | −0.03 (0.01) | 0.031 | −0.03 (0.01) | 4.6E-03 |
| MMAB-MVK | HDL-C | rs2383104             | rs10774708            | 1.00 | 0.62 | −0.02 (0.01) | 0.076 | 0.61 | −0.03 (0.01) | 3.6E-03 | −0.02 (0.01) | 0.011 |
| ABO   | LDL-C | rs9411489              | rs2519093             | 0.95 | 0.85 | −0.10 (0.05) | 0.035 | 0.83 | −0.13 (0.03) | 1.6E-04 | −0.12 (0.03) | 3.0E-05 |
| APOB  | LDL-C | rs1367117              | rs1367117             | 0.99 | 0.88 | −0.09 (0.05) | 0.047 | 0.87 | −0.09 (0.04) | 0.023 | −0.08 (0.03) | 7.4E-03 |
| TIMD4 | TC    | rs6882076              | rs6882076             | 1.00 | 0.65 | 0.01 (0.01) | 0.060 | 0.65 | 0.02 (0.01) | 0.035 | 0.02 (0.01) | 4.0E-03 |
| DNAH11 | TC    | rs12670798             | rs5008148             | 0.94 | 0.55 | 0.02 (0.01) | 0.072 | 0.58 | 0.01 (0.01) | 0.052 | 0.02 (0.01) | 0.012 |

Results are shown if P < 0.1 in both CLHNS mothers and offspring with a consistent direction of effect. EAF, effect allele frequency.

aSNPs were previously reported in (8), except MMAB-MVK rs2383104 was reported in (7).

bLD 2 between the SNP pairs was estimated based on the 1000 Genomes Project phase I EUR data (November 2010 release).
TG levels compared with the CC carriers only in the larger waist circumference group (≥74.0 cm; β = 0.41, 0.38, and 0.38 in mothers, offspring, and combined cohorts, respectively), but not in the smaller waist group (<74.0 cm; β = −0.01, 0.14, and 0.12 in mothers, offspring, and combined cohorts, respectively) (Fig. 2).

As sex may influence the SNP association with lipid traits, we further tested the evidence of sex differences among the SNPs with main effect association. As shown in supplementary Table VI, all P values for SNP-by-sex interaction were >0.05, except for a variant rs7499892 at CETP, which exhibited suggestive evidence of sexual dimorphic association. The association at CETP was stronger in women than in men (β = 0.098, P = 1.4 × 10⁻⁵) than in men (β = 0.051, P = 5.5 × 10⁻³).

**DISCUSSION**

In this study of genetic associations with lipoprotein and TG levels in Filipinos from the CLHNS, we observed the associations at 15 loci, which accounted for 3.1, 4.7, 6.8, and 6.2% of the phenotypic variation of TC, HDL-C, LDL-C, and TGs, respectively, in the mothers from the CLHNS. Consistent with previous findings (7, 8), in particular those from populations of East Asians (14), our data also supported the existence of allelic heterogeneity at APOA5, CETP, LIPC, and APOE; the four secondary signals observed at these loci led to an average 23% increase in the amount of phenotypic variance compared with that explained by the most strongly associated variants alone. In addition, the findings of these consistent SNP-lipid associations in a young adult population aged ~20 years, who had healthier lipid levels compared with middle-aged or older individuals, suggests a shared genetic influence on lipid levels across adulthood despite differing durations of environmental exposures. While we did not confirm the other previously reported loci, this might reflect our modest power to detect the association, differences in linkage disequilibrium (LD) between analyzed variants and underlying functional variants, or population-specific loci. Furthermore, the modifying effects of environmental factors could have impeded the detection of some associations (16).

A novel and important finding of this study is the evidence of a significant interaction between an APOA5 (apolipoprotein A-V) variant and waist circumference affecting TG levels in both mother and offspring cohorts in the CLHNS. Waist circumference, a measure of abdominal adiposity, may predict CVD risk better than BMI (34). Waist circumference is strongly associated with CVD and its risk factors including TGs (35). The increasing difference in TG levels between rs3741297 CC and CT+TT carriers with increasing waist circumference (Fig. 1) suggests that a larger waist circumference augments the genetic effect.
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Gly185Cys in other populations (minor allele frequency < 0.005 in Europeans and African Americans) compared with Asians (minor allele frequency < 0.03) may explain why its association with TGs has only been detected in Asians to date (14). As Gly185Cys was not present on the GWA genotyping array or the 1000 Genomes Project reference panel (June 2010 release), the variant was not directly genotyped or imputed in the CLHNS mothers; however, our results based on the Metabochip genotyped data in the offspring showed the same significant main effect association on TG level and interaction with waist circumference as for the tested proxy variant rs3741297. In the CLHNS mothers, 4% of individuals carrying the TG-increasing T allele of the proxy SNP rs3741297 (LD r² = 1.0 with 185Cys), had significantly higher TG levels (geometric mean ± SE: 1.63 ± 0.09 mmol/l, approximately to the upper limit of 1.70 mmol/l for normal TG levels) compared with nonrisk allele carriers (1.28 ± 0.01 mmol/l). The modifying effect of waist circumference on the APOA5 association with TGs is biologically plausible. The APOA5 185Cys variant's role in reducing LPL activation may be exacerbated by the effect of abdominal obesity on LPL mass and activity. Intra-abdominal visceral fat accumulation has been shown to affect LPL mass and activity either directly or via insulin resistance, thereby impairing the hydrolysis of TGs (24, 25). Similarly, earlier studies demonstrated an interaction of the LPL-HindIII genotype with abdominal obesity on TG levels (41, 42) and hypothesized that the presence of central obesity exacerbates the alterations in LPL activity caused by genetic variation, thus preventing the possible compensatory effects that may occur in nonobese individuals (42). Our identification of a SNP-by-waist circumference interaction suggests that future studies should analyze genetic associations in different strata of anthropometric, lifestyle, and other environmental factors.
In addition to the two APOA5 variants that we described in this study, many other variants at APOA5 have been shown to exhibit significant association with TG levels in diverse populations (4–8, 12, 14). Among these reported SNPs, several variants including rs964184 (7, 8), rs5741298 (14), and rs651821 (12, 14) also showed genome-wide significant association (all \( P < 1 \times 10^{-11} \)) in the CLHNS mothers; however, the significant level of these associations were substantially attenuated by conditioning on the most strongly associated variant rs662799 (all \( P_{\text{conditional}} > 0.45 \), suggesting that these variants and rs662799 would represent a same TG signal. Other variants, including rs12272004 (6), rs28927680 (5), rs12286037 (4), and rs315506 (14), which were common and significantly associated with TGs in European or African ancestry populations, were found not to be variable in the 1000 Genomes Project ASN samples.

A previous study in the CLHNS reported that compared with women from the USA, the Filipino women had significantly lower levels of HDL-C at all BMI levels and exhibited a higher prevalence of isolated HDL-C phenotype, which is defined as a combination of low HDL-C (<35 mg/dl) but normal TGs (<200 mg/dl) (2). Although these findings suggested potentially different effects of genetic variants across populations, we did not identify any additional loci/variants that were specific to Filipinos or had substantially larger genetic effects in this population compared with those in Europeans (8, 14). Our study may not have sufficient power to detect such association, but the unusually low HDL-C level in the Filipinos is possibly due to the different lifestyle and/or environmental exposures across populations.

In conclusion, our findings emphasize the importance of central obesity prevention in public health efforts to reduce CVD risk, particularly in individuals who are genetically susceptible to the disease.

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