Dyslipidemia is a major risk factor for CVD, the leading cause of death in the United States (1). Dyslipidemia is characterized by high circulating concentrations of total cholesterol (TC), LDL cholesterol (LDL-C), and TGs, and/or low concentrations of HDL cholesterol (HDL-C), and is a target of primary prevention of CVD (2). The first line of treatment for dyslipidemia includes lifestyle alterations such as healthy diet, regular physical activity, and a healthy body weight (3). Importantly, underlying societal shifts in diet, physical activity, and body weight have occurred since the 1970s, resulting in an obesity epidemic, which represents a major environmental shift impacting lipid levels in the United States.

Clustering of both CVD and dyslipidemia in families has long been observed and shown to be under significant genetic control (4, 5). For example, genetic heritability has been estimated from 30% to 70% for TC, LDL-C, and HDL-C in family-based studies (6–15), with estimates for TG heritability in families somewhat lower (15% to 50%) (6–11, 13–15). However, it is important to note that previous heritability studies were typically conducted cross-sectionally among families living apart and primarily in white cohorts.

African Americans have long been recognized to have more favorable lipid profiles than whites, despite higher average BMI (16–18). Reasons for this discrepancy are not fully known but may be associated with race-specific genetic predisposition. The few studies in lipid heritability including both African American and white populations rarely test for differences in heritability by race, although some differences have been noted (6, 19). Previous research in the Lipid Research Clinics (LRC) study, including

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the Cincinnati site, showed that black and white nuclear families (during a period in which the parents and offspring were living in the same household) may differ in interfamilial correlations, with black families generally lower than whites (7, 20), except for HDL-C, with black families demonstrating a greater genetic effect (7). These previous studies suggest that lipid traits may be influenced by race-specific genetic architecture, and if the underlying etiology of lipid variation differs between African Americans and whites, this may impact the success of gene discovery efforts. Indeed, large comprehensive measured genotype studies have noted that only 40 of 95 loci identified in individuals of European ancestry have been replicated in African American populations (21), highlighting the potential for race-specific contributions to lipid genetics.

Unfortunately, longitudinal heritability studies have largely been restricted to white twins (22, 23). Further, they do not specifically address longitudinal changes through the obesity epidemic. Some of these studies have, however, noted evidence for changes in the genetic underpinnings of lipid traits over time, as twin pairs aged through adolescence and adulthood (22, 23). A study in the Bogalusa Heart Study noted that longitudinal area under the curve for lipid traits exhibits stronger heritability than single measurements in either childhood or adulthood in both black and white sibling pairs (24). These results suggest a need to specifically examine time as a factor in the genetic architecture of lipids, to avoid heterogeneity in lipid trait genetics introduced by effects of age or of secular changes in obesity rates.

Therefore, within this context, our purpose was to describe the race-specific development of heritability of lipids across ~30 years of follow-up from childhood to adulthood. The Princeton Lipid Follow-up Study (PFS), a 22–30 year follow-up of the Cincinnati LRC population, leverages longitudinal family data collected before and after the obesity epidemic of the 1980s and 1990s. This study design provides a unique opportunity to assess race-specific changes in heritability estimates prior to and after the US obesity epidemic. In addition, this study examines the relative influence of shared genes and/or shared environment on lipids over time and whether these relationships differ by race.

MATERIALS AND METHODS

The Cincinnati Clinic of the National Heart, Lung, and Blood Institute LRC Prevalence Study (1973–78) studied students in grades 1–12 (ages 6 through 19) of the public and parochial schools in the Princeton School District (Cincinnati, OH) and a random sample of their parents and has been described previously (25). The student population from which participants came was 73% white and 27% African American, and 52.3% male and 47.7% female (26).

Figure 1 depicts the flow of subjects for all phases of the study. At the first LRC visit (1973–75), basic demographic information was collected, and TC and TGs were measured after an overnight fast (25). Approximately 6 weeks after visit 1, a 15% random subset of subjects, independent of visit 1 lipid results, was recalled to visit 2 (“random” subjects). In addition, hyperlipidemic subjects were recruited to visit 2 if they had TC or TGs in the approximate top 5% (age specific) (27, 28). At visit 2, height, weight, fasting TGs, HDL-C, and LDL-C were measured, as well as blood pressure and questions about lifestyle (e.g., smoking and alcohol use) (26). The LRC Family Study (or visit 3) was conducted from 1976 to 1978, involving all first-degree relatives of selected probands seen at visit 2 (either parents or offspring as probands), which again were chosen either randomly (15% of the visit 2 random subset) or as hyperlipidemic cases (top 5% of TC, LDL-C, or TGs) (27, 29). At visit 3, the same variables were measured as at visit 2, with the exception of blood pressure, smoking, and alcohol use. All first-degree relatives of probands at visit 3 were invited to participate regardless of whether they had previously attended any LRC visits.

The PFS was conducted between 1998 and 2003, 22–30 years after the last LRC visit. The PFS focused on changes in the familial correlations of LDL-C, so LRC subjects at visit 2 and visit 3 with at least one first-degree relative at one of these visits were targeted. Methods for PFS have been described previously (30, 31), but briefly, participants underwent a physical examination (height, weight, and blood pressure), fasting blood draw for clinical chemistries and DNA extraction, and questionnaires regarding lifestyle (e.g., smoking, alcohol use, and diet) and personal and family medical history. The study protocol was approved by the Children’s Hospital Institutional Review Board, and all participants gave signed, informed consent.

All individuals and families that attended the PFS visit were eligible for inclusion in the present analysis (n = 1,454). Potential LRC participants who otherwise would have been eligible for PFS but who died prior to follow-up (n = 184) or otherwise were not seen at PFS (n = 983) were not included in analysis. For this analysis, PFS participants from the LRC study were classified as being in random or hyperlipidemic families according to the classification of the family’s proband at LRC (visit 2 or visit 3). Visit 2 families were classified as random if all family members at visit 2 were random subjects; otherwise, they were classified as hyperlipidemic families because the family group included at least one hyperlipidemic subject. Refer to Fig. 1 for depiction of random and hyperlipidemic family types.

Clinical measures

In both the LRC and PFS, data were collected using standard protocols (32). Height and weight were measured with subjects in light, indoor clothing and shoes removed. In the LRC, one measurement of height and weight was made. In the PFS, two measurements of height and weight were made, with a third measurement made if the first two differed by more than 0.5 cm (height) and/or 0.3 kg (weight). The mean of replicate measurements was used for analyses. The BMI (kg/m²) was used to characterize body habitus. Prior to each visit, participants were instructed to fast for 12 h prior to screening. In each study, fasting blood was drawn into vacutainers containing EDTA, kept on wet ice (LRC) or cold packs (PFS), and delivered to the laboratory within 3 h for processing. Lipid profiles were measured in LRC-Centers for Disease Control standardized laboratories (33). LDL-C was calculated using the Friedewald formula but was measured by direct determination for all participants with TGs >400 mg/dl (LRC) or >250 mg/dl (PFS).

For individuals on lipid-lowering therapy at PFS, both the TC and LDL-C values were multiplied by 1.506 to model a 33.6% reduction in TC and LDL-C due to treatment as shown previously (34, 35). This represents the average treatment effect for LDL-C across a broad range of drug classes and doses targeting lipid reduction (36). Use of lipid-lowering therapy was rare at LRC, so unaltered lipid values were used.
Genotyping

Genomic DNA was extracted from buffy coats collected at PFS by a simple salting out procedure using the Puregene isolation kit (Gentra Systems, Minneapolis, MN) (37). The ApoE gene includes two polymorphisms (rs7412 and rs429358), which, in combination, code for the three major isoforms: E2, E3, and E4. ApoE polymorphisms were assessed by restriction fragment length polymorphism-PCR by the method of Hixson with minor modifications using the following primer sequences: 5'-ACA GAA TTC GCC CCG GCC TGG CAC CTG CTA AGG A-3' (forward) and 5'-TAA GCT TGG CAC GGC TGT CCA AGG A-3' (reverse) (38). Amplified PCR products (244 bp) were digested with HhaI restriction enzyme (New England Biolabs, Ipswich, MA) overnight at 37°C, separated by electrophoresis on 12% acrylamide gel, and visualized after ethidium bromide staining. Accuracy of genotypes was assured by incorporating a known sample on each acrylamide gel plate for each of the three possible genotypes. A random sample of 10% was repeated, and results were compared with original values. Mendelian inconsistencies for ApoE genotypes in family pedigrees were identified using pedsys and resolved by removing inconsistent individuals' genotypes (n = 14). Individuals were classified using indicator variables for ApoE2 and ApoE4 carrier status, with the E3/E3 genotype as reference.

Statistical methods

Women who were pregnant at either LRC or PFS were excluded from analyses of that visit (n = 2 at PFS, n = 0 at LRC). For individuals seen at both visit 2 and visit 3, visit 2 data were used. Continuous variables were examined for normality using the Shapiro-Wilk test, and BMI, TC, HDL-C, and TGs were natural-log transformed for analysis to improve normality at both time points; however, nontransformed values are presented.

To estimate the proportion of phenotypic variation explained by additive genetic effects (heritability), we used mixed models as implemented in SOLAR (39) on lipid traits during the period of shared households (1970s) and after the period of shared households (2000s). Briefly, fixed effects account for covariate effects on the mean while random effects disentangle covariance between relative pairs into genetic and environmental components. This is operationalized in equation 1:

\[ y = \mu + \beta \times (\text{covariate}) + g + \epsilon \]  
\[ \Omega = \Phi \sigma^2_g + \Gamma \sigma^2_e \]  

(Eq. 1)

where \( \mu \) is the grand mean, \( \beta \) is the vector of measured covariate effects, and \( g \) and \( \epsilon \) are genetic and environmental deviations.
Assuming that $g$ and $e$ are uncorrelated random normal variables with expectation 0, the phenotypic covariance of relative pairs ($\Omega$) can be partitioned into additive genetic and environmental components, where $\Phi$ is the kinship matrix, $I$ is the identity matrix, and $\sigma_g^2$ and $\sigma_e^2$ are the variance due to additive genetic ($g$) and residual ($e$) effects. The significance of covariates is determined by comparing the log likelihood from a model where the covariate effect is estimated with one where it is constrained to 0. To test the significance of the additive genetic component, the log likelihood of a model where $\sigma_g^2$ is estimated is compared with the log likelihood of a model where $\sigma_g^2$ is constrained to 0. Twice the difference in log likelihoods of these models yields a test statistic distributed as a $1/2:1/2$ mixture of a $\chi^2$ variable with 1 degree of freedom and a point mass at 0 (40).

Covariates considered for inclusion in models included BMI, age, age$^2$, sex, race by sex interaction, age$^2$ by sex interaction, ApoE2 carrier, ApoE4 carrier, family ascertainment status, smoking, alcohol use, prescription medication use (for diabetes, hypertension, thyroid, heart, and cholesterol, as well as birth control pills and steroids), insulin use for diabetes, nonprescription weight-reducing medication, special diets (to control cholesterol and diabetes), and dietary intake of the ratio of polyunsaturated to saturated fat ($P/S$ ratio, PFS). Each covariate was evaluated for each lipid trait in univariate analyses, and covariates with $P \leq 0.10$ were included in the final models. Of note, due to analytic procedures, individuals must have complete covariate data in addition to the outcome variables to enter into the final models. Thus, due to missing data, each outcome had different numbers of individuals entering the analyses.

To estimate the expected shared contribution of genes and environments to circulating lipid concentrations both in the 1970s and 30 years later, we performed bivariate analysis in this model, the phenotype covariance is further decomposed to include the genetic correlation between traits due to additive genetic effects, such that the covariation between two individuals for the two time points is given by

$$
\Omega = \begin{bmatrix}
\Omega_{11} & \Omega_{12} \\
\Omega_{21} & \Omega_{22}
\end{bmatrix}
$$


(Eq. 2)

where $\Omega$ is a covariance matrix of $2 \times 2$ covariance matrices. The matrix elements are defined by

$$
\Omega_{ab} = 2\Phi_{ab} \sigma_g \sigma_g + I_{ab} \sigma_e \sigma_e
$$


(Eq. 3)

where $a$ and $b$ represent the two time points, and $\rho_a$ and $\rho_b$ are the additive genetic and environmental correlations between the two time points, respectively. The genetic correlation estimates the proportion of variance that lipid traits at the two time points share due to genetic causes and is independent of trait heritability. If $a = b$, then $\rho_a = 1$, and the covariance of a pair of relatives simplifies to equation 1. Likewise, the environmental correlation estimates the proportion of unmeasured environmental effects shared in common between the two time points. For bivariate models, covariates that were significant in the univariate models were included.

To understand whether differences in heritability estimates were due to differences in the amount of variation explained by genes, formal models tested whether the genetic variance differed by race. This was operationalized by estimating models where the genetic variation was allowed to differ by race and comparing the model fit with a model where the genetic variation by race was constrained to be equal. The difference in the $-2 \log$ likelihood between these models was tested for significance. For all analyses, $P \leq 0.05$ was considered significant.

### RESULTS

A total of 1,454 individuals (mean age 14.1 and 40.6 years for children and parents in 1970s; 39.6 and 66.5 years in the 2000s) in 373 families (286 white, 87 African American) were included in the analysis, with data available at both LRC and PFS. The large majority of participants ($n = 1,441$) had data from both time points, with 3 participants having data only from the LRC visit and 10 participants having data only from the PFS visit. The individuals with a single time point were retained as they were informative for both the univariate and bivariate analyses. Table 1 presents the distribution of family structure of the analysis sample by race. Approximately one-third of these families (31.5% of white families and 28.7% of African American families) were selected randomly, with the remaining two-thirds selected based on a proband with high lipid values.

Table 2 presents the demographic and clinical values for the cohort at LRC and PFS visits by race and generation. Differences in these characteristics were tested by race group and study visit (not shown in the table). Racial differences in sex distribution in both generations ($P = 0.011$ for parents, $P = 0.0007$ for offspring) and ApoE4 carrier status ($P = 0.0001$) were evident. White parents were older than African American parents at both time points ($P = 0.052$ at LRC, $P = 0.036$ at PFS), and white offspring were older than African American offspring at PFS ($P = 0.0075$). At LRC, African American participants had significantly higher HDL-C and lower TGs than white participants (both $P < 0.0001$). At PFS, African American participants had higher BMI ($P < 0.0001$), HDL-C ($P = 0.0009$), dietary $P/S$ ratio ($P < 0.0001$), and current smoking ($P = 0.001$), but lower TGs ($P < 0.0001$), frequent alcohol use ($P = 0.0013$), and treatment with cholesterol medications ($P = 0.015$) or birth control or hormone replacement ($P = 0.006$) than whites. TC, LDL-C, ApoE2 carrier status, and dietary control of diabetes at PFS did not significantly differ by race.

### Secular (time) trends in heritability and shared genetic effects

Overall, the heritability of lipids decreased between the LRC and the PFS study visits, with the exception of TGs. Heritability of lipids at LRC, representing the period of shared households and prior to the US obesity epidemic, was generally high (Table 3). TC had a heritability of 0.65 ± 0.07 ($P < 0.0001$), and LDL-C and HDL-C were also highly heritable during this period (LDL: 0.59 ± 0.07, HDL: 0.58 ± 0.07).

| TABLE 1. Family size and structure by race |
|---------------------------------------------|
| White | African American |
|---|---|
| N families (2+ individuals) | 286 | 87 |
| Random ascertainment (%) | 31.5 | 28.7 |
| Family size (median [IQR]) | 3 [2, 4] | 2 [2, 4] |
| Range: 2–11 | Range: 2–11 |
| Relative pairs in analysis | | |
| Parent-offspring | 598 | 150 |
| Sibling pairs | 469 | 154 |
| Half-siblings | 5 | 41 |
| IQR, Interquartile Range. | | |

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TABLE 2. Demographic and clinical characteristics of participants

|                              | White LRC (1970s) |          | African American LRC (1970s) |          | White PFS (2000s) |          | African American PFS (2000s) |          |
|------------------------------|-------------------|----------|-----------------------------|----------|-------------------|----------|-----------------------------|----------|
|                              | Parents           | Offspring| Parents                     | Offspring| Parents           | Offspring| Parents                     | Offspring|
|                              | n                 |          | n                           |          | n                 |          | n                           |          |
| Age (years)                  | 41.0 ± 6.3        | 14.3 ± 9.4 | 39.1 ± 8.1                  | 13.8 ± 5.3 | 66.9 ± 6.2        | 39.9 ± 4.8 | 64.9 ± 8.0                  | 38.9 ± 5.1 |
| Sex (% male)                 | 42.3              |          | 27.6                        |          | 42.3              |          | 27.6                        |          |
| BMI (kg/m²)                  | 25.9 ± 4.7        | 20.0 ± 4.1 | 27.7 ± 5.7                  | 20.6 ± 5.3 | 29.4 ± 5.8        | 28.0 ± 6.3 | 32.3 ± 7.6                  | 30.8 ± 7.8 |
| Cholesterol (mg/dl)          | 209.6 ± 40.1      | 171.1 ± 31.5 | 206.3 ± 37.4                | 182.5 ± 33.4 | 195.3 ± 39.0      | 193.4 ± 41.8 | 197.2 ± 43.5                | 189.0 ± 42.1 |
| ApoE4 (%)                    | –                 |          | –                           |          | 32.7              |          | 35.0                        |          |
| ApoE2 (%)                    | –                 |          | –                           |          | 13.5              |          | 15.6                        |          |
| ApoE2 (%)                    | –                 |          | –                           |          | 15.1              |          | 13.9                        |          |
| ApoE4 (%)                    | –                 |          | –                           |          | 37.2              |          | 41.3                        |          |
| Sex (% male)                 | 42.3              |          | 27.6                        |          | 42.3              |          | 27.6                        |          |
| Cholesterol (mg/dl)          | 209.6 ± 40.1      | 171.1 ± 31.5 | 206.3 ± 37.4                | 182.5 ± 33.4 | 195.3 ± 39.0      | 193.4 ± 41.8 | 197.2 ± 43.5                | 189.0 ± 42.1 |
| ApoE4 (%)                    | –                 |          | –                           |          | 32.7              |          | 35.0                        |          |
| ApoE2 (%)                    | –                 |          | –                           |          | 13.5              |          | 15.6                        |          |
| ApoE2 (%)                    | –                 |          | –                           |          | 15.1              |          | 13.9                        |          |
| ApoE4 (%)                    | –                 |          | –                           |          | 37.2              |          | 41.3                        |          |
| ApoE4 (%)                    | –                 |          | –                           |          | 15.1              |          | 13.9                        |          |
| ApoE4 (%)                    | –                 |          | –                           |          | 37.2              |          | 41.3                        |          |
| Sex (% male)                 | 42.3              |          | 27.6                        |          | 42.3              |          | 27.6                        |          |
| Treatments (%)               | –                 |          | –                           |          | –                 |          | –                           |          |
| Cholesterol medications (%)  | 1.3               |          | 0                           |          | 36.0              |          | 6.0                         |          |
| Diet for diabetes (%)        | –                 |          | –                           |          | 13.7              |          | 1.7                         |          |
| Birth control/hormone (%)    | 20.5              |          | 9.5                         |          | 43.2              |          | 26.1                        |          |
| Mean ± SD or percent presented. |                  |          |                              |          |                  |          |                              |          |

Mean ± SD or percent presented.

a Data for APOE genotype carrier status reported only at PFS visit. Dietary P/S ratio and diet for diabetes only assessed at PFS visit.

b At PFS, medication-adjusted cholesterol and LDL represents the average of all cholesterol or LDL values after the following adjustments: for participants reporting use of cholesterol medications, observed values were multiplied by 1.506, while lipid values were not modified for participants not taking cholesterol medications.

c Frequent alcohol use (≥3 times per week) and treatments at LRC reported for parents only.

P < 0.0001; HDL: 0.74 ± 0.07, P < 0.0001), adjusting for covariates. Heritability of TGs was much lower, but still significant (0.36 ± 0.08, P < 0.0001). After the period of shared households (PFS), heritability for TC, LDL-C, and HDL-C all declined somewhat but remained significant, while heritability for TGs increased slightly, adjusting for covariates.

For those seen at visit 3 only, data on smoking and alcohol use were not collected. To determine the potential impact of smoking and alcohol use as covariates, the analysis was limited to the subset seen at visit 2 and repeated to evaluate significance of these variables and changes to results. Results within this subset analysis were very similar and did not affect interpretation among those with smoking and alcohol use data (data not shown), so these covariates were not included in final models to maximize sample size.

Overall, bivariate analyses support shared genetic effects through time for each of the lipid traits (Table 3). For TC, we find a strong genetic correlation (ρg) between visits of 0.83 ± 0.06 (P < 0.0001), with nonsignificant shared environmental correlations (ρe = 0.12 ± 0.10) and a derived estimate of the phenotypic correlation (ρp) of 0.51, accounting for family structure. LDL-C, HDL-C, and TGs all show similar results, with strong shared genetic correlations (ρg ranging from 0.82 for TGs to 0.87 for HDL-C), weak and nonsignificant environmental correlations (ρe ranging from 0.12 for HDL-C to 0.18 for TGs), and moderate phenotypic correlations ranging from 0.42 (TGs) to 0.55 (HDL-C) across time.

African Americans show lower heritability and higher environmental contributions through time

Racial differences in heritability are clear (Table 4), with both TC and LDL-C notably less heritable for African American families than whites, both during shared households (before the obesity epidemic) and separate households (after the obesity epidemic). In African American families, LDL-C heritability was nonsignificant (0.17 ± 0.17) at LRC and 0.27 ± 0.13 (P < 0.05) at PFS, adjusting for significant covariates, while similar figures for whites were 0.65 ± 0.07 and 0.46 ± 0.08 (both P < 0.0001). TC heritability demonstrates the same pattern (Table 4). For HDL and TGs, a different pattern emerges, with similarly high heritability of HDL in both groups at both time points and similar but lower heritability in both groups for TGs. Further, when exploring the reason for the differences in heritability by race, LDL-C genetic variance was significantly lower in African Americans than whites (P = 0.015) at the LRC time point; for the other phenotypes, no significant differences in genetic variance were identified.

Bivariate analysis also reveals differences by race (Table 4). In whites, the vast majority of the genetic variance in all lipid traits is shared between time points (ρg > 0.80 for all traits). However, none of the lipid traits in white families demonstrated shared environmental contributions to lipid traits across time (ρe < 0.14 for all traits; Table 4). Among African American families, a large proportion of genetic variance of TC and LDL-C is also shared between the 1970s and 2000s (ρg > 0.90 for both), while shared genetic effects
are somewhat lower for HDL ($\rho = 0.66$) and nonsignificant for TGs. Unlike whites, however, African American families also demonstrate a significant shared environmental contribution to all lipid traits between the 1970s and 2000s, with $\rho_e$ accounting for 0.35–0.48 of the variability within families (all $P \leq 0.05$).

**DISCUSSION**

Using a unique cohort of white and African American nuclear families whose lipids were measured both in the 1970s (LRC) and the 2000s (PFS), we were able to examine heritability through time and longitudinally shared genetic and environmental factors by race. In this study, we demonstrate that African Americans have similar or lower heritability for lipids than whites (Table 4) but with evidence of durable environmental effects on lipid variation that were not present in whites. This novel finding has implications for the design of measured genotype studies in lipids in African American and white populations.

Overall, the heritability estimates for white families in the present study (e.g., 0.31–0.75) are consistent with estimates of heritability for plasma lipids from previous studies in white populations [e.g., 0.30–0.50 for LDL-C and HDL-C (11, 41–43), somewhat lower for TGs, and somewhat higher for TC (42)]. Previous analyses reported in the 1980s within the LRC study noted genetic heritability in whites ranging from 0.194 for TGs to 0.624 for LDL-C (10, 12).

Heritability for plasma lipids in our African American families (e.g., 0.17 for LDL-C to 0.62 for TGs and HDL-C) is also similar to the limited previous reports in African American or Caribbean populations (e.g., 0.28 in TGs to 0.55 for HDL-C) (6, 8, 44), none of which were recruited prior to the obesity epidemic. Some differences occur by lipid trait, for example TGs, where African American heritability was strong (0.62) in our study, but weak to moderate (0.28–0.37) and sometimes not significant in others (6, 8, 44). The current study’s estimates for African American families at LRC are lower for TC (0.22) and LDL-C (0.17) than those reported in an early analysis of a subset of this same cohort (0.39 and 0.54, respectively) (7), possibly due to differences in analysis techniques. Strikingly, we found that the heritability estimates for LDL-C and TC were lower for the African American families compared with the white families. Lower heritability in one population versus another can result from a situation where the genetic variances are equal, but the environmental variance is larger in the first. However, the present analysis specifically demonstrated differences in genetic variance in LDL-C by race, suggesting a different genetic architecture. A previous study of African American and Caucasian twin sets also reported significant racial difference in heritability for LDL-C but noted that African American twins had significantly higher heritability (0.92) than whites (0.69) (19), in direct contrast to the present study. It is possible that differences in family structure (e.g., family vs. twin) and study design (e.g., cross-sectional vs. longitudinal) account for differences between these findings, but additional work is clearly needed to understand

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**TABLE 3.** Lipid trait heritability at LRC and PFS, and bivariate genetic ($\rho_g$) and nongenetic ($\rho_n$) correlations between LRC and PFS for the entire cohort.

| Trait | $h^2$ LRC | $b$ Bivariate ($\rho_g$) | $b$ Bivariate ($\rho_n$) |
|-------|-----------|-------------------------|-------------------------|
| TC    | 0.65 ± 0.07 | 0.83 ± 0.06 | 0.12 ± 0.10* |
| LDL-C | 0.47 ± 0.07 | 0.82 ± 0.07 | 0.14 ± 0.09* |
| HDL-C | 0.59 ± 0.07 | 0.82 ± 0.07 | 0.12 ± 0.11' |
| TGs   | 0.41 ± 0.07 | 0.87 ± 0.06 | 0.10 ± 0.08* |

All heritability, bivariate genetic correlation ($\rho_g$), and bivariate nongenetic correlation ($\rho_n$) estimates presented in this table were tested for difference from 0, adjusting for covariates listed below. All $P$ values are <0.0001 unless otherwise noted.

*Overall heritability estimates were derived from models adjusting for the following covariates: TC (LRC): age, age$^2$, race, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment; TC (PFS): age, age$^2$, sex, race, ApoE2 carrier, ApoE4 carrier, family ascertainment; LDL-C (LRC): age, sex, age$\times$sex, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment, race; LDL-C (PFS): age, sex, age$\times$sex, age$^2$, BMI, ApoE2 carrier, ApoE4 carrier, family ascertainment; HDL-C (LRC): age, sex, age$\times$sex, age$^2$, ApoE2 carrier, ApoE4 carrier, race, ApoE2 carrier, BMI; HDL-C (PFS): sex, race, ApoE2 carrier, BMI; TGs (LRC): age, sex, age$\times$sex, age$^2$, ApoE2 carrier, BMI, family ascertainment; TGs (PFS): sex, age$\times$sex, age$^2$, race, ApoE2 carrier, BMI, family ascertainment.

$\rho_g$ and $\rho_n$ estimates provided for bivariate relationships between LRC and PFS for each lipid trait. Models were adjusted for the following covariates: TC: race, sex, age, ApoE2 carrier, ApoE4 carrier, family ascertainment, age$^2$, age$\times$sex, age$^2\times$sex, BMI; LDL-C: race, age, ApoE2 carrier, ApoE4 carrier, family ascertainment, age$^2$, age$\times$sex, age$^2\times$sex, BMI; HDL-C: race, sex, ApoE2 carrier, age$^2$, age$\times$sex, age$^2\times$sex, BMI; TGs: race, sex, age, ApoE2 carrier, family ascertainment, age$^2$, age$\times$sex, age$^2\times$sex, BMI.

*Nonsignificant ($P > 0.05$).

*Overall heritability was strong (0.62) in our study, but weak to moderate (0.28–0.37) and sometimes not significant in others (6, 8, 44).

**DISCUSSION**

Using a unique cohort of white and African American nuclear families whose lipids were measured both in the 1970s (LRC) and the 2000s (PFS), we were able to examine heritability through time and longitudinally shared genetic and environmental factors by race. In this study, we demonstrate that African Americans have similar or lower heritability for lipids than whites (Table 4) but with evidence of durable environmental effects on lipid variation that were not present in whites. This novel finding has implications for the design of measured genotype studies in lipids in African American and white populations.

Overall, the heritability estimates for white families in the present study (e.g., 0.31–0.75) are consistent with estimates of heritability for plasma lipids from previous studies in white populations [e.g., 0.30–0.50 for LDL-C and HDL-C (11, 41–43), somewhat lower for TGs, and somewhat higher for TC (42)]. Previous analyses reported in the 1980s within the LRC study noted genetic heritability in whites ranging from 0.194 for TGs to 0.624 for LDL-C (10, 12).
the likely different genetic architecture of LDL-C in African American and white populations.

A novel aspect of the present study is also the ability to examine longitudinal patterns of heritability and correlations in genetic and environmental contributions to heritability of lipid traits over time. Our results showed that the heritability of lipid traits generally decreased between the LRC and the PFS study visits, with the exception of TGs. These declines in heritability are not unexpected because at LRC the families were sharing a household, while at PFS the offspring were adults largely living in separate households. Shared household effects have been demonstrated to inflate the estimates of heritability in nuclear family studies in which families were living in a single household.

Bivariate analysis between time periods in white families indicates that similar sets of genes contribute to variability of lipid traits during and after the period of shared households (and before and after the obesity epidemic). In the whole cohort, we note significant shared genetic contributions to heritability between the 1970s and the 2000s, and weak shared environmental contributions, with only TGs demonstrating a significant environmental component. However, these findings were strikingly different in race-specific analyses.

In white families, between 81% and 92% of variability in lipids is likely due to the same genes both before and after the obesity epidemic, with little contribution of durable shared environment to the genetic architecture of lipid values across time. This suggests that different (nonshared) components of the environment contribute to variance in lipid traits in the 1970s and 2000s among whites in this study. A study of two sets of twins of different ages controlling for parent-child correlations (45) also noted significant shared genetic effects across different ages, ranging from 46% for TGs to 80% for HDL-C. Longitudinal twin studies in whites also confirm some shared genetic contributions but a limited role of shared environmental factors over time as twins aged (22, 23). Confirmation in the present study of such longitudinal patterns in families before and after the obesity epidemic is both novel and practical in relation to identifying genetic variants associated with lipid traits in whites over time.

By contrast, the genetic architecture in African American families suggests a smaller heritability of TC and LDL-C that is nonetheless strongly shared over time, with >90% overlap in the genetic etiology between the 1970s and 2000s. HDL-C and TGs demonstrate higher heritability at each time point, but the specific genes contributing to these phenotypes are not as strongly consistent, suggesting changes in the genetic underpinning of TGs and HDL-C over time. Furthermore, African American families demonstrate a significant contribution of shared environmental variability to lipid traits over time, which is not observed in white families. This study represents the first to our knowledge to describe these longitudinal patterns in African American cohorts.

The environmental contribution to African American lipid traits may include many potential (unmeasured) constructs, including such possibilities as geography, diet, physical activity, or environmental exposures. Geographically,

### TABLE 4. Heritability and bivariate analysis of lipids at LRC and PFS by race

|       | White                | African American   |
|-------|----------------------|--------------------|
|       | $h^2$  | Bivariate ($\rho_b$) | Bivariate ($\rho_b$) | $h^2$  | Bivariate ($\rho_b$) | Bivariate ($\rho_b$) |
| TC    |        |                     |                    |        |                     |                    |
| LRC   | 0.70 ± 0.07 | 0.81 ± 0.07 | 0.04 ± 0.13$^d$ | 0.22 ± 0.15$^f$ | 0.90 ± 0.22$^f$ | 0.36 ± 0.14$^f$ |
| PFS   | 0.54 ± 0.08 |                      |                    | 0.25 ± 0.14$^f$ |
| LDL-C | 0.65 ± 0.07 | 0.82 ± 0.07 | 0.05 ± 0.12$^d$ | 0.17 ± 0.17$^f$ | 0.91 ± 0.29$^f$ | 0.35 ± 0.12$^f$ |
| HDL-C | 0.46 ± 0.08 |                      |                    | 0.27 ± 0.13$^f$ |
| LRC   | 0.75 ± 0.07 | 0.92 ± 0.06 | 0.04 ± 0.13$^d$ | 0.61 ± 0.16$^f$ | 0.66 ± 0.15$^f$ | 0.48 ± 0.17$^f$ |
| PFS   | 0.44 ± 0.07 |                      |                    | 0.48 ± 0.13 |
| TGs   | 0.31 ± 0.09 | 0.92 ± 0.11 | 0.14 ± 0.09$^d$ | 0.38 ± 0.19$^f$ | 0.49 ± 0.24$^d$ | 0.39 ± 0.16$^f$ |
| PFS   | 0.40 ± 0.08 |                      |                    | 0.62 ± 0.12 |

All heritability, bivariate genetic correlation ($\rho_b$), and bivariate nongenetic correlation ($\rho_e$) estimates presented in this table were tested for difference from 0, adjusting for covariates listed below. All $P$ values are <0.0001 unless otherwise noted.

$^a$White heritability estimates were derived from models adjusting for the following covariates: TC (LRC and PFS): age, age$^2$, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment; LDL-C (LRC): age, sex, age $\times$ sex, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment; HDL-C (LRC): age, sex, age $\times$ sex, age$^2$, age$^2 \times$ sex, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment; TGs (LRC): age, sex, age $\times$ sex, age$^2$, age$^2 \times$ sex, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment.

$^b$White and African American bivariate estimates were each derived from models adjusting for the following covariates: TC: age, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment; LDL-C: age, sex, age $\times$ sex, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment; HDL-C: age, sex, age $\times$ sex, age$^2$, ApoE2 carrier, ApoE4 carrier, BMI, family ascertainment; TGs: age, sex, ApoE2 carrier, ApoE4 carrier, family ascertainment, age$^2$, age $\times$ sex, age$^2 \times$ sex, BMI.

$^c$African American heritability estimates were derived from models adjusting for the following covariates: TC (LRC): age$^2$, ApoE2 carrier, family ascertainment; TC (PFS): age, age$^2$, ApoE2 carrier, family ascertainment; LDL-C (LRC and PFS): age, ApoE2 carrier, ApoE4 carrier, family ascertainment; HDL-C (LRC): age $\times$ sex, ApoE4 carrier, BMI; HDL-C (PFS): sex, BMI; TGs (LRC): age, BMI, family ascertainment; TGs (PFS): age, sex, ApoE2 carrier, ApoE4 carrier, family ascertainment, age$^2$,

$^d$Nonsignificant.

$^e P < 0.05.$

$^f P < 0.001.$
we note that 89% of the African American participants in this study still resided in the 452 zip code prefix (encompassing the Princeton City School District and central Hamilton County, OH), while only 48% of white participants still resided in this area, suggesting greater geographic stability in our African American families. We may also speculate that African American families maintained a more stable household environment throughout life, or that children were more likely to recreate aspects of their childhood households, such as diet, when they become adults. At the Princeton LRC visit, parent-child correlations for carbohydrate intake were higher among black families than white families (46), with nutrient intake having a small but significant association with plasma lipids in this cohort (47–49). However, to our knowledge, longitudinal data to support this supposition are lacking. Interestingly, the white families demonstrate nearly no contribution of shared environmental factors occurring before and after the obesity epidemic, which may suggest that white families experienced environmental changes in factors affecting lipid traits differently between these two time points.

Implications of differing genetic architecture on measured genotype studies

In white families, strong overall heritability and shared genetic etiology over time may contribute to successful identification of genes contributing to lipid phenotypes. This finding also implies that genes identified in studies in white individuals prior to the obesity epidemic would remain reasonable targets for genetic studies in lipids in more recent cohorts. By contrast, the genetic architecture of lipid traits, especially TGs and HDL-C, in African Americans may need to be defined relative to time-varying factors, such as shared versus independent households, secular time, or participant age, and require larger sample sizes to compensate for lower heritability and relatively higher environmental impact. With respect to TC and LDL-C in African Americans, the lower overall heritability of these traits may significantly hamper efforts to detect genetic contributions to these traits, despite strong sharing of genetic contribution to lipid variance over time.

Large-scale studies in multietnic populations have recently noted that white and African American cohorts may share many underlying genetic loci for lipid traits (50, 51). However, several genetic variants affecting lipids are race specific (50–53), and not all results in whites can be replicated in African Americans (21). Furthermore, gene × environment (54) or gene × gene interactions (55) may differentially affect whites and African Americans in assessing the etiology of lipid traits. These findings highlight the need to incorporate understanding of race-specific genetic architecture into these studies.

The strengths of the current study are a large size, biregional composition, and longitudinal follow-up of families, which permits evaluation of genetics and environment both during and after the period of shared households, and in relation to the intervening obesity epidemic. Certain limitations should also be acknowledged. Only visit 2 collected data on smoking and alcohol use, which typically correlate with plasma lipid concentrations. However, analyses including and excluding these factors in the subset with visit 2 data did not change the results. In addition, the study design included only nuclear families, so it was not possible to estimate the effect of shared households versus nonshared environmental contribution to plasma lipids in the 1970s. Also, the lower proportion of African American families in this study may not have provided sufficient power to detect significance of the lower heritability estimates or permitted identification of subter differences in genetic architecture between whites and African Americans.

CONCLUSIONS

In conclusion, although this study confirms that plasma lipids are significantly influenced by genetics in both white and African American families, the consistency of genetic architecture of lipids appears to differ by race and across time, such that the specific genetic etiology of plasma lipids may be more difficult to identify in African American families. The present study highlights that a persistent environment in African Americans, coupled with generally lower heritability and less sharing of genetic etiology over time, may need to be directly considered in measured genotype studies aiming to identify genetic variants associated with lipid traits in African Americans. This may further complicate studies already constrained by smaller sample sizes and shorter segments of linkage disequilibrium in African American populations. However, the discovery of durable environmental effects across the obesity epidemic opens up new areas for research on the environmental factors contributing to lipid variability. Knowledge of these genetic architecture differences may also help guide the effective use of environmental data in race-specific genetic studies to delineate why African Americans are relatively protected from dyslipidemia in the face of increased obesity and CVD risk.

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