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Genetically Determined Plasma Lipid Levels and Risk of Diabetic Retinopathy: A Mendelian Randomization Study

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Results from observational studies examining dyslipidemia as a risk factor for diabetic retinopathy (DR) have been inconsistent. We evaluated the causal relationship between plasma lipids and DR using a Mendelian randomization approach. We pooled genome-wide association studies summary statistics from 18 studies for two DR phenotypes: any DR ($N = 2,969$ case and 4,096 control subjects) and severe DR ($N = 1,277$ case and 3,980 control subjects). Previously identified lipid-associated single nucleotide polymorphisms served as instrumental variables. Meta-analysis to combine the Mendelian randomization estimates from different cohorts was conducted. There was no statistically significant change in odds ratios of having any DR or severe DR for any of

the lipid fractions in the primary analysis that used single nucleotide polymorphisms that did not have a pleiotropic effect on another lipid fraction. Similarly, there was no significant association in the Caucasian and Chinese subgroup analyses. This study did not show evidence of a causal role of the four lipid fractions on DR. However, the study had limited power to detect odds ratios less than 1.23 per SD in genetically induced increase in plasma lipid levels, thus we cannot exclude that causal relationships with more modest effect sizes exist.

Diabetic retinopathy (DR) is a major microvascular complication of diabetes and is the leading cause of blindness in

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working-aged adults (1). It has been estimated that the global prevalence for any DR and proliferative DR (PDR) to be 34.6% and 7.0%, respectively (2).

Dyslipidemia is a major cardiovascular risk factor and has been suggested also as a potential risk factor for DR, in particular the more severe end points such as PDR and diabetic macular edema (DME) (2,3). However, in contrast to tight glycemic and blood pressure control, which have been shown in clinical trials to reduce DR progression (4,5), therapies targeted at dyslipidemia have not shown similar results (6,7). In this regard, fenofibrate, a peroxisome proliferator-activated receptor α (PPAR- α) agonist, has shown benefits in reducing requirements for laser treatment of DR and DME (8), but the therapeutic effects of fenofibrate may not be lipid dependent. The association of dyslipidemia with DR has been inconsistent among observational studies (9–12). Possible reasons for this include confounding (e.g., with obesity), reverse causation, and measurement biases. As such, there is difficulty in establishing a causal relationship between plasma lipids and DR.

Mendelian randomization (MR) is a study design using genetic variants as instrumental variables (IVs) to evaluate the causal relationship between a biomarker and an outcome of interest (13). Because it takes advantage of the natural randomization of genetic variants inherited independent of confounding factors such as lifestyle and environmental factors (14,15), MR avoids the issues of confounders and reverse causality and serves as a practical approach to evaluate the relationship between plasma lipids and DR.

In this study, we used an MR approach pooling multiple studies to evaluate the causal relationship between plasma lipids and two DR phenotypes, 1) any DR and 2) severe DR, by using genetic variants associated with plasma lipids as IVs.

RESEARCH DESIGN AND METHODS

Study Participants

We included a total of 18 genome-wide association studies (GWAS) on DR: African American Proliferative Diabetic Retinopathy Study (AAPDR); Age Gene/Environment Susceptibility–Reykjavik Study (AGES Reykjavik); Australian Genetics of Diabetic Retinopathy Study (AUST); Blue Mountains Eye Study (BMES); Cardiovascular Health Study–African American (CHS-AA); Cardiovascular Health Study–Whites

(CHS-Whites); Genetic Center, China Medical University Hospital, Taiwan; Genetics of Latinos Diabetic Retinopathy (GOLDR); Jackson Heart Study (JHS); Multi-Ethnic Study of Atherosclerosis–African American (MESA-AA); Multi-Ethnic Study of Atherosclerosis–Chinese (MESA-CHN); Multi-Ethnic Study of Atherosclerosis–European (MESA-EU); Multi-Ethnic Study of Atherosclerosis–Hispanic (MESA-HIS); Singapore Chinese Eye Study (SCES); Singapore Malay Eye Study (SiMES); Singapore Indian Eye Study (SINDI); Starr County Health Studies; and Taiwan–US Diabetic Retinopathy Study (TUDR). Details of the individual studies have been previously described (16–31). Of them, 17 had phenotype information on any DR and 11 on severe DR. Genotyping was performed on either the Illumina (San Diego, CA) or Affymetrix (Santa Clara, CA) platforms. Imputation was done using the Markov Chain Haplotyping software IMPUTE2 or MaCH with 1000 Genomes or HapMap Phase II as reference panels (Table 1). Details about imputation quality control and adjustment are provided in Table 1. Informed consent was obtained from all participants, ethics approval was obtained from the local ethics committee, and recommendations of the Declaration of Helsinki were adhered to.

DR Assessment and Definition

DR was either assessed through retinal photography or clinical diagnosis in the studies involved. DR was graded using the Early Treatment of Diabetic Retinopathy Study (ETDRS) adaptation of the modified Airlie House classification system or the American Academy of Ophthalmology (AAO) International Clinical Diabetic Retinopathy Disease Severity Scale. On the ETDRS scale, grade 10 represents no DR, grades ≥ 20 indicates any DR, and grades ≥ 53 indicates severe nonproliferative DR (NPDR) and PDR. On the AAO scale, the category of no DR indicates absence of DR, the remaining four categories together indicate any DR, and the two highest categories together capture severe NPDR and PDR. As all the studies were graded by one of these two scales and it is straightforward to harmonize DR phenotypes across these two scales, it was possible to easily harmonize the DR phenotype across all the studies.

Two DR phenotypes were assessed in MR analyses: 1) any DR referred to participants with evidence of presence of DR and 2) severe DR referred to participants with severe

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Table 1—Details of each study population

| Study | Country | Year | Ethnicity | Genotyping platform | Imputation (reference pool/quality cut-off threshold/adjustment in association tests) | DR ascertainment method | DR grading method | DR phenotypes (number of case and control subjects) |
|-----------------------------------------------------------|-----------|--------------------------------------------|------------------|----------------------------------------|---------------------------------------------------------------------------------------|-------------------------|-------------------|------------------------------------------------------|
| AAPDR | U.S. | 2012–2013 | African American | Affymetrix 5.0 | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (274, 56) 2. PDR (255, 56) |
| AGES Reykjavik | Iceland | 2002–2006 | Caucasian | Illumina HumanCNV370-Duo BeadChip | HapMap Phase II/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (85, 222) |
| AUST | Australia | 2006–2011 | Caucasian | Illumina Human OmniExpress BeadChip | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Clinical diagnosis | ETDRS | 1. Any DR (522, 435) 2. PDR (187, 435) |
| BMES | Australia | 1992–1994, 1997–2000, 2002–2004, 2007–2010 | Caucasian | Illumina Human670-Quad Custom BeadChip | 1000 Genomes, HapMap Phase II/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (124, 208) |
| CHS-AA | U.S. | 1997–1998 | African American | Illumina HumanOmni1-Quad v1.0 BeadChip | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (22, 39) |
| CHS-Whites | U.S. | 1997–1998 | Caucasian | Illumina HumanCNV370-Duo BeadChip | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (28, 143) |
| Genetic Center, China Medical University Hospital, Taiwan | Taiwan | 2006–2007 | Chinese | Illumina HumanHap550-Duo BeadChip | 1000 Genomes/INFO >0.4, MAF >5%/no | Clinical diagnosis | AAO | 1. Any DR (177, 579) 2. Severe NPDR/PDR (78, 579) |
| GOLDR | U.S. | 2007–2011 | Hispanic | Illumina OmniExpress Chip | 1000 Genomes/INFO >0.3/yes | Retinal photography | ETDRS | 1. Any DR (292, 221) 2. Severe NPDR/PDR (78, 221) |
| JHS | U.S. | 2010–2012 | African American | Affymetrix 5.0 | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (91, 160) 2. PDR (12, 160) |
| MESA-AA | U.S. | 2002–2004 | African American | Affymetrix 6.0 | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (101, 258) 2. PDR (11, 258) |
| MESA-CHN | U.S. | 2002–2004 | Chinese | Affymetrix 6.0 | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (25, 79) |
| MESA-EU | U.S. | 2002–2004 | Caucasian | Affymetrix 6.0 | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (38, 200) |
| MESA-HIS | U.S. | 2002–2004 | Hispanic | Affymetrix 6.0 | 1000 Genomes/INFO >0.6, MAF >1%, or >5 copies in imputed data/no | Retinal photography | ETDRS | 1. Any DR (88, 179) |
| SCES | Singapore | 2009–2011 | Chinese | Illumina Human670-Quad BeadChip | 1000 Genomes/R ² >0.8/yes | Retinal photography | ETDRS | 1. Any DR (71, 168) 2. Severe NPDR/PDR (12, 168) |

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Table 1—Continued

| Study | Country | Year | Ethnicity | Genotyping platform | Imputation (reference pool/quality cut-off threshold/adjustment in association tests) | DR ascertainment method | DR grading method | DR phenotypes (number of case and control subjects) |
|-----------------------------|-----------|-----------|-----------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|-------------------------|-------------------|-------------------------------------------------------|
| SIMES | Singapore | 2004–2006 | Malay | Illumina Human610-Quad BeadChip | 1000 Genomes/ $R^2 > 0.8$ /yes | Retinal photography | ETDRS | 1. Any DR (198, 363) 2. Severe NPDR/PDR (37, 363) |
| SINDI | Singapore | 2007–2009 | Indian | Illumina Human610-Quad BeadChip | 1000 Genomes/ $R^2 > 0.8$ /yes | Retinal photography | ETDRS | 1. Any DR (304, 537) 2. Severe NPDR/PDR (49, 537) |
| Starr County Health Studies | U.S. | 1981–2009 | Hispanic | Affymetrix Genome-wide SNP Array 6.0 | 1000 Genomes/ $R^2 > 0.5$ /yes | Retinal photography | ETDRS | 1. Any DR (529, 249) 2. Severe NPDR/PDR (124, 654) |
| TUDR | Taiwan | 1996–2011 | Chinese | 1. Illumina OmniExpress 730 K Array 2. Illumina iSelect 200 K Cardio-MetaboChip | 1000 Genomes/INFO > 0.4 /no | Clinical diagnosis | AAO | 1. PDR (434, 549) |

INFO, imputation quality metric; MAF, minor allele frequency.

NPDR and/or PDR (Table 1). Control subjects in the GWAS analyses were defined as subjects with type 2 diabetes without DR; case subjects were subjects with type 2 diabetes with either of the defined DR phenotypes.

Genetic IVs

We selected lipid-associated single nucleotide polymorphisms (SNPs) at 157 loci, including 60 for HDL cholesterol, 30 for LDL cholesterol, 28 for triglycerides, and 39 for total cholesterol, previously identified by the Global Lipids Genetic Consortium (GLGC) (32) in individuals of European ancestry. Summary statistics data for the association between these 157 SNPs and plasma lipids were used as genetic IVs for MR analyses in all ethnicities and for Caucasian cohorts. The SNPs used as IVs were not in linkage disequilibrium ($R^2 < 0.2$) with each other as reported by the original report (32). We then tested the effects of these 157 SNPs on plasma lipid levels in East Asian populations from the Asian Genetic Epidemiology Network (AGEN) Consortium and identified 51 SNPs (28 for HDL cholesterol, 10 for LDL cholesterol, and 13 for triglycerides) associated with plasma lipids ($P < 0.05$) in East Asians and used them for MR analysis in Chinese groups.

As the goal was to estimate the unconfounded association of specific lipid fractions with the DR outcomes, any of the 157 SNPs that were also associated with another fraction by definition violates the MR assumption that each SNP IV has no pleiotropic effect and only acts on the outcome via the specific lipid fraction exposure. Therefore, for the primary analysis, we selected the subset of SNPs that were unique (independent) to each lipid fraction (i.e., did not also have pleiotropic effect on another lipid fraction) as reported by the GLGC (32). Using the Type 2 Diabetes Knowledge Portal (www.type2diabetesgenetics.org), we also examined whether any of these SNPs were significantly associated ($P < 5 \times 10^{-8}$) with other risk factors for DR (type 2 diabetes itself, related glycemic traits, and hypertension). We also eliminated those SNPs from the primary analysis (Supplementary Table 1). However, we were also concerned that the primary analysis would suffer from a significant loss of power and might overcorrect for pleiotropy among the different lipid fractions. Therefore, we also performed a secondary analysis with the entire set of 157 SNPs. Of note, the 157 SNPs were chosen such that each SNP was only assigned to be the IV for the lipid fraction for which it was most strongly associated. That is, if a SNP was significantly associated with both HDL and total cholesterol levels but the association with HDL levels was stronger, then it was only chosen as an IV for HDL levels. This eliminated some pleiotropic SNPs from the analysis, although it was not as conservative as the primary analysis that eliminated SNPs with any pleiotropic effects completely, e.g., they were not assigned as IVs for any lipid fraction.

Statistical Analysis

We obtained GWAS summary statistics data from individual studies for either or both DR phenotypes for the SNPs where genotype and imputed data were available. We then

Table 2—Baseline characteristics of participants in each study

| Study | Any DR | | | | | | Severe DR | | | | | |
|-------------------------------------|---------------|-------------|-------------|------------------|-------------|-------------|---------------|-------------|-------------|------------------|-------------|-------------|
| | Case subjects | | | Control subjects | | | Case subjects | | | Control subjects | | |
| | Age, years | Sex, % male | Sample size | Age, years | Sex, % male | Sample size | Age, years | Sex, % male | Sample size | Age, years | Sex, % male | Sample size |
| AAPDR | 59.4 | 40.9 | 274 | 61.5 | 32.1 | 56 | 59.5 | 41.2 | 255 | 61.5 | 32.1 | 56 |
| AGES Reykjavik | 76.2 | 52.9 | 85 | 76.0 | 56.3 | 222 | - | - | - | - | - | - |
| AUST | 66.4 | 58.6 | 522 | 67.3 | 52.4 | 435 | 64.6 | 60.8 | 187 | 67.3 | 52.4 | 435 |
| BMES | 64.3 | 50.0 | 124 | 63.8 | 48.1 | 208 | - | - | - | - | - | - |
| CHS-AA | 77.0 | 9.1 | 22 | 78.3 | 46.2 | 39 | - | - | - | - | - | - |
| CHS-Whites | 78.1 | 46.4 | 28 | 77.4 | 43.4 | 143 | - | - | - | - | - | - |
| Genetic Center, China | | | | | | | | | | | | |
| Medical University Hospital, Taiwan | 62.0 | 50.3 | 177 | 58.0 | 53.9 | 579 | 62.2 | 50.0 | 78 | 58.0 | 53.9 | 579 |
| GOLDR | 53.4 | 40.4 | 292 | 54.0 | 32.6 | 221 | 53.6 | 47.4 | 78 | 54.0 | 32.6 | 221 |
| JHS | 61.2 | 34.1 | 91 | 63.7 | 36.2 | 160 | 64.4 | 30.8 | 12 | 63.7 | 36.2 | 160 |
| MESA-AA | 63.2 | 50.6 | 101 | 63.6 | 45.6 | 258 | 68.3 | 50.0 | 11 | 63.6 | 45.6 | 258 |
| MESA-CHN | 67.0 | 48.0 | 25 | 66.9 | 51.9 | 79 | - | - | - | - | - | - |
| MESA-EU | 61.9 | 55.6 | 38 | 65.3 | 61.2 | 200 | - | - | - | - | - | - |
| MESA-HIS | 66.4 | 54.6 | 88 | 64.0 | 47.5 | 179 | - | - | - | - | - | - |
| SCES | 62.0 | 57.7 | 71 | 62.5 | 57.7 | 168 | 61.6 | 75.0 | 12 | 62.5 | 57.7 | 168 |
| SIMES | 63.2 | 41.9 | 198 | 62.9 | 47.7 | 363 | 63.1 | 35.1 | 37 | 62.9 | 47.7 | 363 |
| SINDI | 61.8 | 55.9 | 304 | 60.9 | 51.8 | 537 | 62.4 | 46.9 | 49 | 60.9 | 51.8 | 537 |
| Starr County Health Studies | 60.3 | 40.6 | 529 | 57.8 | 40.2 | 249 | 62.0 | 47.6 | 124 | 59.0 | 39.1 | 654 |
| TUDR | - | - | - | - | - | - | 61.2 | 44.2 | 434 | 66.8 | 58.3 | 549 |

performed inverse variance-weighted, fixed-effect meta-analyses with METAL software to pool available GWAS summary data for each SNP for both DR phenotypes from individual studies. Individual SNP data were pooled from all studies, as well as studies from Caucasian and Chinese cohorts separately.

Next, the association between plasma lipids and DR at each SNP was calculated as $\beta_{(lipid-DR)} = \beta_{(SNP-lipid)}/\beta_{(SNP-DR)}$ (33), where $\beta_{(lipid-DR)}$ represents the estimated effect size (logarithm of the odds ratio [OR]) of 1 SD of genetically determined plasma lipid levels on DR. To assess the association between each lipid trait and DR, we combined the $\beta_{(lipid-DR)}$ estimates across multiple SNPs using fixed-effect meta-analysis. Cochran Q test was applied to assess heterogeneity across SNPs. Heterogeneity across SNPs was found to be low ($I^2 < 40\%$) among studies (Supplementary Table 2), hence random-effect meta-analysis was not carried out.

We performed the same analysis for two subgroups of studies for each DR phenotype where the IVs were presumed to be stronger on account of similar ancestry backgrounds: 1) among studies of Caucasian ancestry using the SNPs identified by the GLGC as IVs and 2) among studies of Chinese ancestry using SNPs from the AGEN Consortium as IVs. Of note, $\beta_{(SNP-lipid)}$ estimates differed between GLGC and AGEN Consortium, thus supporting the separate analyses in these two populations. All statistical analyses were performed using Stata 14 (StataCorp LP, College Station, TX).

RESULTS

The baseline characteristics of the participants in each study are shown in Table 2. A total of 2,969 case and 4,096 control subjects were included in the analysis of the any DR phenotype and 1,277 case and 3,980 control subjects were included in the analysis of the severe DR phenotype. A summary of the 157 lipid-associated SNPs used as IVs for MR analysis and the SNP pooled association with DR are shown in Supplementary Tables 3 and 4.

Tables 3 and 4 show the results of the MR analysis for the any DR phenotype in all cohorts and the subgroup Caucasian and Chinese cohort analyses. We did not find any significant association between plasma lipids and DR. In the primary analysis (Table 3), for each 1-SD increase in genetically induced increase in plasma lipid profiles, the OR of having any DR was 0.91 (95% CI 0.67–1.23) for HDL, 2.50 (0.91–6.87) for LDL, 1.00 (0.86–1.15) for triglycerides, and 0.83 (0.53–1.31) for total cholesterol in the all ethnicities analysis.

In the secondary analysis (Table 4), for each 1-SD increase in genetically induced increase in plasma lipid profiles, the OR of having DR was 0.94 (95% CI 0.79–1.14) for HDL, 0.95 (0.75–1.20) for LDL, 1.08 (0.96–1.22) for triglycerides, and 0.92 (0.74–1.14) for total cholesterol in the all ethnicities analysis.

Tables 5 and 6 show the results of the MR analysis for the severe DR phenotype. For the primary analysis (Table 5), the OR (95% CI) for the association between plasma lipids and severe DR was 0.98 (0.74–1.31) for HDL, 0.95

Table 3—MR estimate of the association between lipids and any DR using SNPs unique to each lipid fraction and independent of glycemic traits# (primary analysis)

| | All ethnicities (N = 2,969 case and 4,096 control subjects) | | | | Caucasian (N = 797 case and 1,208 control subjects) | | | | Chinese (N = 273 case and 826 control subjects) | | | |
|-------------------|----------------------------------------------------------------|------------------|---------|---------------------------|--------------------------------------------------------|-------------------|---------|---------------------------|----------------------------------------------------|-------------------|---------|---------------------------|
| | No. of SNPs* | OR† (95% CI) | P value | <i>F</i> ² , % | No. of SNPs* | OR† (95% CI) | P value | <i>F</i> ² , % | No. of SNPs* | OR† (95% CI) | P value | <i>F</i> ² , % |
| HDL | 44 | 0.91 (0.67–1.23) | 0.539 | 0.0 | 44 | 0.99 (0.61–1.60) | 0.960 | 8.3 | 21 | 1.36 (0.29–6.44) | 0.699 | 0.0 |
| LDL | 9 | 2.50 (0.91–6.87) | 0.075 | 0.0 | 9 | 3.93 (0.53–29.33) | 0.182 | 0.0 | 3 | 0.70 (0.01–88.38) | 0.885 | 0.0 |
| Triglycerides | 15 | 1.00 (0.86–1.15) | 0.983 | 0.0 | 15 | 1.05 (0.67–1.65) | 0.828 | 0.0 | 4 | 1.09 (0.05–25.66) | 0.959 | 5.1 |
| Total cholesterol | 18 | 0.83 (0.53–1.31) | 0.424 | 0.0 | 18 | 0.74 (0.42–1.30) | 0.293 | 0.0 | – | – | – | – |

$\beta_{(SNP-lipid)}$ estimates taken from the GLGC for Caucasians and the AGEN Consortium for Chinese differed. Therefore, we performed analyses in these two populations separately. *Number of SNPs included in meta-analysis. †ORs are for a SD of genetically induced increases in plasma lipid profiles. #Two SNPs that showed genome-wide significant association with glycemic traits were also excluded: rs9686661 and rs12328675.

Table 4—MR estimate of the association between lipids and any DR for all SNPs (secondary analysis)

| | All ethnicities (N = 2,969 case and 4,096 control subjects) | | | Caucasian (N = 797 case and 1,208 control subjects) | | | Chinese (N = 273 case and 826 control subjects) | | | | | |
|-------------------|----------------------------------------------------------------|------------------|---------|--------------------------------------------------------|---------------|------------------|----------------------------------------------------|-----------|---------------|------------------|---------|-----------|
| | No. of SNPs** | OR* (95% CI) | P value | f^2 , % | No. of SNPs** | OR* (95% CI) | P value | f^2 , % | No. of SNPs** | OR* (95% CI) | P value | f^2 , % |
| HDL | 60 | 0.94 (0.79–1.14) | 0.543 | 0.0 | 60 | 1.02 (0.76–1.35) | 0.917 | 12.8 | 28 | 1.16 (0.51–2.63) | 0.728 | 0.0 |
| LDL | 30 | 0.95 (0.75–1.20) | 0.651 | 0.0 | 30 | 0.87 (0.60–1.28) | 0.487 | 0.0 | 10 | 1.44 (0.31–6.70) | 0.641 | 0.0 |
| Triglycerides | 28 | 1.08 (0.96–1.22) | 0.227 | 0.0 | 28 | 1.11 (0.85–1.44) | 0.453 | 0.0 | 13 | 1.55 (0.70–3.41) | 0.280 | 0.0 |
| Total cholesterol | 39 | 0.92 (0.74–1.14) | 0.438 | 0.0 | 39 | 0.98 (0.70–1.37) | 0.889 | 5.2 | – | – | – | – |

$\beta_{\text{SNP-lipid}}$ estimates taken from the GLGC for Caucasians and the AGEN Consortium for Chinese differed. Therefore, we performed analyses in these two populations separately. *ORs are for a SD of genetically induced increases in plasma lipid profiles. **Number of SNPs included in meta-analysis.

(0.39–2.36) for LDL, 0.84 (0.33–2.12) for triglycerides, and 0.68 (0.25–1.87) for total cholesterol. In the secondary analysis (Table 6), the OR (95% CI) for the association between plasma lipids and severe DR was 1.02 (0.81–1.29) for HDL, 0.94 (0.80–1.10) for LDL, and 0.69 (0.41–1.16) for total cholesterol, respectively. In the secondary analysis, there was stronger evidence that genetically determined plasma triglycerides levels conferred an increased risk of having severe DR (OR 1.37 [95% CI 0.99–1.88]), although the results did not achieve statistical significance ($P = 0.056$). We did not find any association between plasma lipids and severe DR in the subgroup Caucasian and Chinese cohort analyses.

Of note, in the primary analysis using only strictly defined independent IVs, the risk of genetically determined plasma triglycerides levels on having severe DR was greatly reduced (OR 0.84 [95% CI 0.33–2.12]), suggesting that the association in the secondary analysis was due to pleiotropic triglyceride-related SNPs. Given this finding, we also repeated the analysis for triglycerides and severe DR using the 12 SNPs that have effects on triglycerides and at least one other lipid fraction (Table 6). The risk of genetically determined plasma triglycerides levels on having severe DR was strengthened (OR 1.42 [95% CI 1.01–2.00], $P = 0.044$) when only these 12 pleiotropic SNPs were used. Because the PPAR- α agonist fenofibrate has shown benefits in reducing requirements for laser treatment of DR and DME (8) that are not explained by its therapeutic effects on triglyceride levels, we examined whether any of these 12 SNPs were in or near PPAR- α target genes (34). We found that 3 of these 12 SNPs are near PPAR- α target genes involved in lipoprotein uptake/metabolism and lipogenesis (Supplementary Table 5).

We calculated the power for this study using all 157 SNPs. We determined power for varying ORs for DR per SD of the exposure variable (plasma lipid), with the assumption that the proportion of lipid variance explained by SNP IVs is $R^2 \sim 10\%$ and with a type 1 error of 0.05 (Supplementary Table 6) (35). The minimum OR for which the study has 80% power is 1.23 for the any DR outcome and approximately 1.3 for the severe DR outcome.

DISCUSSION

To the best of our knowledge, our study is the most comprehensive MR study to evaluate the causal role of plasma lipids in DR development by combining multiethnic cohorts from different countries. We did not see clear evidence of a causal relationship between lipid measures and DR in the group as a whole or in the subgroup analyses in Caucasian and Chinese cohorts using stronger IVs. Our findings may help shed light on the considerable variability in previous observational studies exploring the association between plasma lipids and DR (36). In previous studies, HDL (37,38), LDL (39,40), triglycerides (41), and total cholesterol (38) have been inconsistently shown to be associated with DR. Our findings suggest that these associations previously observed may overall be noncausal, partially due to residual confounders. Our findings were generally consistent throughout

the subgroup analyses and across populations as we found no heterogeneity across different populations. However, this study was not powered to detect modest (OR <1.23) effect sizes, and thus we cannot exclude the possibility that more modest causal associations between lipid levels and DR may exist.

Our findings did suggest a possible causal relationship between a pleiotropic pathway that includes the triglyceride pathway and severe DR. In a subanalysis examining the SNPs that have effects on triglycerides and at least one other lipid fraction, there was a marginally significant ($P = 0.044$) association between the genetically determined plasma lipid levels and severe DR risk. This finding must be interpreted cautiously given the multiple hypotheses tested in this study, but it is an interesting finding that should be followed up in future studies.

Previous studies have shown an association between dyslipidemia and severe DR (2), as well as beneficial effects of fenofibrate treatment on DR (42). Fenofibrate acts mainly to lower plasma triglycerides levels, but the mechanism of its effect on DR is unclear (43). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, treatment with fenofibrate reduced the need for laser treatment for DR and showed a reduction in two-step progression in DR among those with preexisting DR (8). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study similarly showed that fenofibrate reduced DR progression in combination with statins, although this effect could not be entirely explained based on plasma lipid-altering effects (5). Our data suggest that the SNPs that influence triglyceride levels but also influence other plasma lipid fractions may have the strongest influence on DR risk, suggesting pleiotropic effects of SNPs may be important. In particular, further examination of the effects of the three triglyceride SNPs near PPAR- α target genes (Supplementary Table 5) may help to further explain how fenofibrate reduces DR progression with a mechanism other than change in plasma lipid profile.

It is possible that the traditional lipid measures of total, HDL, and LDL cholesterol and triglycerides may not accurately measure the effects of dyslipidemia on DR. Previous studies have suggested a more direct relationship between apolipoprotein AI (ApoAI) and apolipoprotein B (ApoB) with DR compared with traditional lipid measures. ApoAI can be found in HDL and is overexpressed in the retina of patients with diabetes (44). ApoB is a structural protein for VLDL, IDL, and LDL (45) and may reflect the atherogenic potential of lipid metabolism (46). Observational studies have found ApoAI, ApoB, and ApoB-to-ApoAI ratio to be significantly associated with DR with higher discriminating abilities for DR compared with traditional lipid measures (47). Our study did not evaluate genetically determined apolipoprotein levels as IVs for MR analysis, which may yet reveal possible causal relationships between dyslipidemia and DR.

The strengths of this study include pooled data from multiple population-based studies, allowing us to increase

Table 5—MR estimate of the association between lipids and severe DR using SNPs unique to each lipid fraction and independent of glycemic traits* (primary analysis)

| | All ethnicities (N = 1,277 case and 3,980 control subjects) | | | | | Caucasian (N = 187 case and 435 control subjects) | | | | | Chinese (N = 524 case and 1,296 control subjects) | | | | |
|-------------------|----------------------------------------------------------------|------------------|---------|-----------|--------------|------------------------------------------------------|---------|-----------|--------------|-------------------|------------------------------------------------------|-----------|--|--|--|
| | No. of SNPs* | OR† (95% CI) | P value | I^2 , % | No. of SNPs* | OR† (95% CI) | P value | I^2 , % | No. of SNPs* | OR† (95% CI) | P value | I^2 , % | | | |
| HDL | 44 | 0.98 (0.74-1.31) | 0.909 | 0.0 | 44 | 1.71 (0.59-5.02) | 0.325 | 0.0 | 21 | 1.06 (0.33-3.42) | 0.925 | 0.0 | | | |
| LDL | 9 | 0.95 (0.39-2.36) | 0.917 | 0.0 | 9 | 0.85 (0.28-2.60) | 0.782 | 0.0 | 3 | 1.42 (0.03-62.62) | 0.855 | 0.0 | | | |
| Triglycerides | 15 | 0.84 (0.33-2.12) | 0.712 | 0.0 | 15 | 0.61 (0.06-6.36) | 0.678 | 11.6 | 4 | 0.66 (0.05-8.85) | 0.754 | 0.0 | | | |
| Total cholesterol | 18 | 0.68 (0.25-1.87) | 0.454 | 0.0 | 18 | 0.53 (0.06-4.79) | 0.568 | 0.0 | — | — | — | — | | | |

† I^2 estimates taken from the GLGC for Caucasians and the AGEN Consortium for Chinese differed. Therefore, we performed analyses in these two populations separately. *Number of SNPs included in meta-analysis. †ORs are for a SD of genetically induced increases in plasma lipid profiles. #Two SNPs that showed genome-wide significant association with glycemic traits were also excluded: rs9686661 and rs12328675.

Table 6—MR estimate of the association between lipids and severe DR for all SNPs (secondary analysis)

| | All ethnicities (N = 1,277 case and 3,980 control subjects) | | | Caucasian (N = 187 case and 435 control subjects) | | | Chinese (N = 524 case and 1,296 control subjects) | | | | | |
|-------------------|----------------------------------------------------------------|------------------|---------|------------------------------------------------------|---------------|------------------|------------------------------------------------------|-----------|---------------|------------------|---------|-----------|
| | No. of SNPs** | OR* (95% CI) | P value | I^2 , % | No. of SNPs** | OR* (95% CI) | P value | I^2 , % | No. of SNPs** | OR* (95% CI) | P value | I^2 , % |
| HDL | 60 | 1.02 (0.81–1.29) | 0.861 | 0.3 | 60 | 1.49 (0.77–2.87) | 0.235 | 0.0 | 28 | 1.00 (0.54–1.85) | 0.991 | 0.0 |
| LDL | 30 | 0.94 (0.80–1.10) | 0.440 | 0.0 | 30 | 0.93 (0.73–1.18) | 0.538 | 0.0 | 10 | 0.62 (0.21–1.89) | 0.401 | 0.0 |
| Triglycerides | 28 | 1.37 (0.99–1.88) | 0.056 | 0.0 | 28 | 1.27 (0.58–2.79) | 0.552 | 21.9 | 13 | 0.90 (0.48–1.67) | 0.730 | 0.0 |
| Triglycerides† | 12 | 1.42 (1.01–2.00) | 0.044 | 2.5 | 12 | 1.43 (0.62–3.31) | 0.403 | 38.0 | 9 | 0.91 (0.48–1.73) | 0.780 | 0.0 |
| Total cholesterol | 39 | 0.69 (0.41–1.16) | 0.159 | 0.0 | 39 | 1.18 (0.36–3.90) | 0.788 | 0.0 | – | – | – | – |

$\beta_{\text{SNP-lipid}}$ estimates taken from the GLGC for Caucasians and the AGEN Consortium for Chinese differed. Therefore, we performed analyses in these two populations separately. *ORs are for a SD of genetically induced increases in plasma lipid profiles. **Number of SNPs included in meta-analysis. †The SNPs are rs6831256 (pleiotropic with total cholesterol, LDL), rs998584 (HDL), rs731839 (HDL), rs2131925 (LDL, total cholesterol), rs1260326 (total cholesterol), rs17145738 (HDL), rs1495741 (total cholesterol), rs12678919 (HDL), rs2954029 (total cholesterol, LDL, HDL), rs174546 (LDL, total cholesterol, HDL), rs964184 (total cholesterol, HDL, LDL), and rs11613352 (HDL).

sample size and thus statistical power. Despite this, our study is still limited by sample size. It is possible that a larger, better powered study in the future could reveal a positive finding. We also used multiple lipid-associated SNPs to increase the ability to detect an association between each lipid trait and DR, as effects of individual SNPs on DR may be modest. The IVs used for the European analysis (all genome-wide significant SNPs) were quite strong with an estimated F-statistic of greater than 10, given the $R^2 \sim 10\%$ in the original report (32). For Asian subanalysis, the IVs were weaker, but the sensitivity analysis using the strong IVs (genome-wide significant SNPs) did not change the results materially (Supplementary Tables 2 and 7).

Limitations to this study include differing DR grading methodologies among pooled studies, but harmonization was straightforward because all studies were graded on one of two widely accepted scales. Another limitation is that the traditional meta-analysis techniques used do not completely take into account the variability in allelic effects between ethnic groups. Fixed-effects meta-analysis assumes the allelic effect to be the same in all populations. Conversely, random-effects meta-analysis assumes that each population has a different underlying allelic effect, which is also suboptimal as populations from the same ethnic group tend to be more homogenous than those that are more distantly related. We found little evidence of heterogeneity, and therefore we feel that the fixed-effect meta-analysis approach is justified and that heterogeneity is not a likely explanation for the negative results. However, we cannot exclude the possibility that some trans-ethnic heterogeneity may decrease the power of this study slightly. The variation in imputation thresholds and adjustment among the cohorts is another limitation of the study, as whether a SNP was imputed and imputation accuracy can affect the precision, variance explained, and power of the study. In addition, our study did not explore the relationship between plasma lipids and DME, which has been suggested in previous studies (48).

The SNPs chosen as IVs for MR analysis in all ethnicities were identified from a previous study of individuals from European ancestry, which explained only 10–15% of total lipid trait variance (32), and this might also have weakened the IV strength in our non-European cohorts. However, when we compare findings from that in the largest European GWAS for lipid levels to the findings from genetic association studies performed in African Americans, Hispanics, and Asians, we find great consistency with regard to effect size and direction among ethnicities (Supplementary Tables 8–11). Although there may be some loss of power from potential interancestry differences in SNPs affecting lipid levels, it is likely outweighed by the gain in power by using the larger number of SNPs from the European lipid GWAS, which explains a greater amount of lipid level variation.

In addition, the SNPs chosen as IVs from MR analysis were derived from a study of mainly subjects without diabetes, which may also decrease the validity of the measures in our study. However, a recent GWAS of lipid levels performed

exclusively in patients with type 2 diabetes identified all of the top findings that had been previously found in populations without diabetes, indicating that there is significant alignment of the genetic architecture of lipid levels between populations with and without diabetes (Supplementary Table 12) (49). We did not establish the association of the SNPs with lipid levels directly in our own cohorts because we only had lipid level data on a subset of patients. This is a limitation, but we note that other MR studies of lipid SNPs have also used the approach we used here with positive results (50), and so we do not think this methodologic limitation is likely to explain our negative results.

One final limitation of this study is the inability to convert risk estimates into more clinically meaningful estimates. This is a limitation of all MR studies using the summary statistics from large GWAS studies, but it does not invalidate the main aim of these studies, which is to garner evidence for causality (50). In the GLGC GWAS, the statistical analysis was a linear regression with the inverse normal transformed lipid trait as the dependent variable (32). The effect estimates were provided in SD units. Unfortunately, the raw lipid value data from this study are not available. Therefore, we are not able to convert our findings to a more clinically meaningful outcome such as SD of raw plasma lipid levels. The GLGC GWAS does provide the average SD for LDL (36.8 mg/dL), HDL (14.7 mg/dL), triglycerides (92.3 mg/dL), and total cholesterol (42.7 mg/dL) in its Supplementary Table 1 (32). But the SD of the raw plasma lipid values cannot be derived directly from the SD of the inverse normalized values without access to raw data.

In conclusion, our findings did not find clear evidence of a causal role of dyslipidemia on the risk for DR, suggesting that the inconsistently observed associations from previous studies were noncausal and may also have been affected by confounders. We did find a nominal association between pleiotropic triglyceride IVs and severe retinopathy, which should be explored in further studies, particularly given that some of these IVs are in loci near genes that are targets for PPAR- α and that fenofibrate, a PPAR- α agonist, has been shown to decrease DR progression. Our study provides further understanding of the relative contribution of plasma lipids to the pathogenesis of diabetic complications.

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