Genetic Susceptibility to Lipid Levels and Lipid Change Over Time and Risk of Incident Hyperlipidemia in Chinese Populations

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Background—Multiple genetic loci associated with lipid levels have been identified predominantly in Europeans, and the issue of to what extent these genetic loci can predict blood lipid levels increases over time and the incidence of future hyperlipidemia remains largely unknown.

Methods and Results—We conducted a meta-analysis of genome-wide association studies of lipid levels in 8344 subjects followed by replication studies including 14739 additional individuals. We replicated 17 previously reported loci. We also newly identified 3 Chinese-specific variants in previous regions (HLA-C, LIPC, and LDLR) with genome-wide significance. Almost all the variants contributed to lipid levels change and incident hyperlipidemia >8.1-year follow-up among 6428 individuals of a prospective cohort study. The strongest associations for lipid levels change were detected at LPL, TRIB1, APOA1-C3-A4-A5, LIPC, CETP, and LDLR (P range from 4.84×10^{-4} to 4.62×10^{-18}), whereas LPL, TRIB1, ABCA1, APOA1-C3-A4-A5, CETP, and APOE displayed significant strongest associations for incident hyperlipidemia (P range from 1.20×10^{-3} to 4.67×10^{-16}). The 4 lipids genetic risk scores were independently associated with linear increases in their corresponding lipid levels and risk of incident hyperlipidemia. A C-statistics analysis showed significant improvement in the prediction of incident hyperlipidemia on top of traditional risk factors including the baseline lipid levels.

Conclusions—These findings identified some evidence for allelic heterogeneity in Chinese when compared with Europeans in relation to lipid associations. The individual variants and those cumulative effects were independent risk factors for lipids increase and incident hyperlipidemia.

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Key Words: genetic loci ◼ genome-wide association study ◼ hyperlipidemia ◼ incidence ◼ lipids

Plasma concentrations of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides are the most important risk factors for cardiovascular diseases and are targets for therapeutic intervention. Genotype-association studies have successfully identified multiple genetic loci associated with blood lipids. However, almost all these loci were identified initially in European ancestry populations, and few such genetic studies have been conducted in Asian populations. Moreover, most of these studies are based on cross-sectional data, and little data exist on their genetic loci predicting lipid levels variation over time and hyperlipidemia incidence. Lifestyle factors, including poor diet, obesity, and lack of exercise, are known to increase the risk of hyperlipidemia. The differences in environmental exposures and genetic background between Chinese and Europeans might suggest potential different pathways of blood lipids. Some variants may be more common in specific ethnic groups, thereby providing greater statistical power, or the effects of genetic variants on lipid levels may be enlarged in specific ethnic groups. Therefore, large-scale studies in Chinese are needed not only to evaluate whether the previous reported loci could be generalized to Chinese population but also to identify new loci or Chinese-specific variants for blood lipids.
Herein, we conducted a genome-wide association study (GWAS) of blood lipid levels that included a meta-analysis of GWAS from 8344 samples at the discovery stage and additional 14739 samples in an independent replication study, involving a total of 23,083 subjects from Chinese Han ancestry. We subsequently investigated whether the identified variants would contribute to lipids increase and incident hyperlipidemia in 6428 individuals who participated in a prospective cohort study.

**Materials and Methods**

**Study Population**

The study comprised 2-staged analyses performed separately for TC, LDL-C, HDL-C, and triglycerides. A detailed description of the sample characteristics and phenotype measurements for each study is provided in Table 1 and in the Methods in the Data Supplement. The discovery stage included meta-analysis of 4 independent GWAS in 8344 individuals from Chinese populations: the China Atherosclerosis Study (CAS),13 the Beijing Atherosclerosis Study (BAS),14 Genetic Epidemiology Network of Salt-Sensitivity (GenSalt) study,15 and the Guangxi Fangchenggang Area Male Health and Examination Survey (FAMHES).16 In stage 2, replication analyses were conducted in an independent sample with a total of 14739 individuals from China Cardiovascular Health Study (CCHS). CCHS was conducted to investigate the risk factors for cardiovascular diseases in China since 2006. Overnight fasting blood samples were drawn by venipuncture to measure lipid levels. Blood specimens were processed in the central clinical laboratory at the Department of Population Genetics at Fuwai Hospital of the Chinese Academy of Medical Sciences in Beijing. This laboratory participates in the Lipid Standardization Program of the US Centers for Disease Control and Prevention and National Heart, Lung, and Blood Institute.

A subset of CCHS individuals was from 2 prospective cohorts (the China Multicenter Collaborative Study of Cardiovascular Epidemiology (ChinaMUCA)17 and the International Collaborative Study of Cardiovascular Disease in ASIA (InterASIA)18) and used to investigate individual single nucleotide polymorphisms (SNPs) and those cumulative effects on blood lipid increase and incident hyperlipidemia. The individuals completed the baseline survey and examination in 1998, 2000, and 2001. For the present study, the analyses were limited to participants for whom complete data were available for both follow-up data and genetic risk score (GRS). These restrictions resulted in 6428 individuals. After exclusion of prevalent hyperlipidemia cases at baseline, 6022, 5814, 4977, and 5450 individuals for TC, LDL-C, HDL-C, and triglycerides, respectively, were eligible for the present analysis of lipid increase and incident hyperlipidemia.

The diagnosis criteria of hyperlipidemia were based on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) report20: hypercholesterolemia (TC ≥ 240 mg/dL), high levels of LDL-C (≥ 160 mg/dL), low levels of HDL-C (< 40 mg/dL), and hypertriglyceridaemia (triglycerides ≥ 200 mg/dL).

Each study obtained approval from the institutional review boards of local research institutions. All participants in each study gave written informed consent.

**Genotype Imputation and Genotyping of Selected SNPs**

Detailed descriptions of genotyping arrays and quality control filters applied to the 4 discovery studies are provided in Table I in the Data Supplement. To facilitate combining results of genome-wide association scans based on the different genotyping platforms, we imputed missing genotypes based on reference haplotypes from the phased CHB+JPT HapMap data release 22 reference data set using MACH25 or IMPUTE.26 Only imputed SNPs with high genotype information content (Rsquared ≥ 0.3, info ≥ 0.9) were used for the association analysis. After quality control, we obtained ≤ 2.5 million genotyped or imputed autosomal SNPs for subsequent association analysis.

After genome-wide association analyses for each of the 4 discovery studies and meta-analysis in the combined samples, SNPs representing the independent association signal were taken forward to replication if they showed potential association (P < 5.0 × 10⁻⁴) for lipid levels in the discovery meta-analysis. If an SNP could not be genotyped, alternative tagging SNPs were considered. In total, 27 SNPs were selected and genotyped using iPLEX Sequenom MassARRAY platform (Sequenom).

**Statistical Analysis**

Triglyceride values were log-transformed before analysis. Population stratification was estimated by a principal component approach, as implemented by EIGENSTRAT software.22 Within each of the 4 discovery studies, continuous lipid levels were adjusted for age, age², sex, body mass index, the first 2 principal components, and lipid-lowering drug prescriptions (if applicable) in linear regression models under an additive model. We used allele dosages from imputation to account for uncertainty in imputed genotypes. A fixed-effects inverse variance-weighted meta-analysis implemented in METAL23 was used to combine the 4 studies in the discovery stage and to obtain results for each SNP. A quantile–quantile plot generated using R was used to evaluate the overall significance of the GWAS results and the potential impact of population stratification. The genomic inflation factor (λ) was estimated from the median of the χ² statistic divided by 0.456. We detected the associations of SNPs in the replication populations, and additionally, we performed meta-analysis of the discovery and replications.

**Genetic Risk Score**

We assessed the cumulative effect of the SNPs by using lipids GRS, which was a weighted sum across the SNPs combining doses of the lipid increasing alleles (lipid decreasing alleles for HDL-C) and the effect size (β-coefficient) for lipid levels change over time in a prospective cohort. Four scores were calculated individually for each lipid level (TC, LDL-C, HDL-C, and triglycerides). We included in the GRS only those SNPs significantly associated with the corresponding lipid levels in our combined discovery and replication studies with genome-wide significance or P < 5.0 × 10⁻⁴ for the SNPs located in known lipid genes. There was no linkage disequilibrium (LD) between SNPs included in the GRS. A 14-SNP GRS was constructed for TC, an 8-SNP GRS for LDL-C, a 6-SNP GRS for HDL-C, and a 9-SNP GRS for triglycerides. Missing genotype data for each SNP were imputed using the average risk allele frequency. However, if > 2 SNP genotypes were missing for a given individual, the GRS was set as missing for that individual. The GRS was modeled as a continuous variable (per 1-SD increase) and as quartiles of TC (7.85–19.79, 19.79–22.28, 22.28–24.77, and 24.77–35.40), LDL-C (2.95–10.02, 10.02–11.60, 11.60–13.20, and 13.20–17.90), HDL-C (13.38 to −9.18, −9.18 to −8.25, −8.25 to −7.11, and −7.11 to −1.34), and triglycerides (0.05–0.14, 0.14–0.16, 0.16–0.19, and 0.19–0.26). Associations of the GRS with their corresponding lipid levels increase and incident hyperlipidemia were analyzed using linear and logistic regression, respectively, in models adjusting for sex, age, body mass index, and the baseline lipid levels corresponding to GRS. We tested the null hypothesis of no linear trends effect over the quintiles. We determined the effect estimates for quintiles of GRS with the bottom quintile serving as the reference group. P values are reported for the linear trends across the quintiles. To evaluate the improvement in risk discrimination by using the genetic information, we compared C-indices24 for the models with and without the GRS.

**Results**

**Discovery Meta-Analysis and Replication Study**

The discovery stage included 4 independent GWAS in 8344 individuals from Chinese populations (Table 1). The discovery meta-analysis evaluated separately associations of TC, LDL-C, HDL-C, and triglycerides with 2 573 667 genotyped or imputed autosomal SNPs. All genotyped and imputed autosomal SNPs passed quality control filters in each of the 4 data sets before conducting the meta-analysis (Table I in the Data Supplement).
Table 1. Baseline Characteristics of the Participants

| Stage of Study | Discovery | Replication |
|---------------|-----------|-------------|
| Sample size   | CAS       | BAS         | GenSalt | FAMHES | CCHS |
|               | 3998      | 466         | 1881    | 1999   | 14739|
| Male/female   | 1870/2128 | 363/103     | 993/888 | 1999/0 | 4222/10517|
| Age, y        | 52.53±7.69 | 51.07±9.53  | 38.72±9.53 | 37.5±11.1 | 52.49±8.70 |
| BMI, kg/m²    | 25.18±3.79 | 24.64±3.36  | 23.35±3.18 | 23.3±3.4 | 24.77±3.69 |
| TC, mg/dL     | 185.84±35.79 | 195.34±37.05 | 170.70±33.65 | 220.42±40.22 | 183.17±34.93 |
| HDL-C, mg/dL  | 49.92±12.42 | 49.78±12.38 | 50.97±11.31 | 54.52±12.76 | 51.20±12.42 |
| TG, mg/dL     | 159.24±115.52 | 128.04±98.84 | 123.47±78.20 | 137.24±158.49 | 149.20±110.61 |
| LDL-C, mg/dL  | 105.58±30.35 | 119.79±32.94 | 95.25±27.30 | 114.46±30.94 | 103.82±29.73 |
| Cigarette smoking, % | 32.74 | 62.23 | 34.13 | 50.8 | 16.29 |
| Alcohol consumers, % | 24.71 | 46.78 | 29.24 | 82.6 | 20.24 |

BAS indicates Beijing Atherosclerosis Study; BMI, body mass index; CAS, China Atherosclerosis Study; CCHS, China Cardiovascular Health Study; GenSalt, Guangxi Fangchenggang Area Male Health and Examination Survey; GenSalt, the Genetic Epidemiology Network of Salt-Sensitivity study; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; and TG, triglycerides.

Quantile–quantile plots for lipid levels are presented in Figure I in the Data Supplement. The genomic control inflation factor (λc) for the meta-analysis was modest (λc=1.030–1.038). As shown in the Manhattan plots of the −log_{10} P values for lipid levels in Figure II in the Data Supplement, the meta-analysis identified 9 well-established loci (GCKR, HMGR, LPL, ABO, APOAI-C3-A4-A5, LIPC, CETP, LIPG, and LDLR) at genome-wide significance (defined as P<5.0×10^{−8}). We then selected 27 SNPs that were associated with TC, LDL-C, HDL-C, and triglycerides at P<5.0×10^{−5} in the discovery analysis and genotyped them in an independent sample comprising 14739 Chinese individuals. In replication analysis, of these 27 SNPs, 18 SNPs showed significant association with lipid after adjustment for multiple testing (P<0.0018=0.05/27), including 11 SNPs with genome-wide significance. In addition, 2 SNPs showed nominal significance (P<0.05; Table II in the Data Supplement).

Associations at Previously Reported Loci and Chinese-Specific Variants

We evaluated the evidence of association for these SNPs by combined results of the discovery and replication study. We confirmed 14 previously reported loci of TC, LDL-C, HDL-C, and triglycerides (PCSK9, ANGPTL3, GCKR, HMGR, MLXIPL, LPL, TRIB1, ABCA1, ABO, APOAI-C3-A4-A5, ALDH2, LIPC, CETP, and APOE) at the level of genome-wide significance (P<5.0×10^{−8}). Moreover, suggestive associations were replicated for APOB, HPR, and ABCA8 loci (Table 2). We also identified 3 novel ethnic-specific variants in previously reported regions (HLA-C, LIPG, and LDLR) in European populations at the level of genome-wide significance. These SNPs were not in LD (r²<0.2 in JPT+CHB) with the previously reported SNPs. These include SNPs in HLA-C (TC, P=1.50×10^{−10}; LDL-C, P=3.28×10^{−10}), LIPC (HDL-C, P=2.35×10^{−9}), and LDLR (TC, P=2.39×10^{−16}; LDL-C, P=1.44×10^{−13}).

Single Variants and Lipid Levels Change and Incident Hyperlipidemia

Of these 20 SNPs in Chinese shown in Table 2, 17 were associated in at least 2 lipid phenotypes, which resulted in a total of 37 associations of those SNPs with the corresponding lipid levels change and incident hyperlipidemia (Table III in the Data Supplement). For lipid levels change, all the SNPs displayed a positive association in a direction consistent with their effect on lipid. We compared the effect sizes of lipids change over time with those observed in GWAS shown in Table 2 and found a high degree of correlation (r values from 0.83 to 0.92; Figure III in the Data Supplement). Of these 37 associations, 24 (64.9%) showed nominal significance (P<0.05), whereas 10 (27.0%) displayed significant even at a Bonferroni-corrected threshold (P range from 4.84×10^{−4} to 4.62×10^{−18}; Table 3). For incident hyperlipidemia, directions of effect were consistent for all SNPs, but the association of LIPC with hypercholesterolemia was not. Twenty-one (56.8%) displayed were significantly associated with the corresponding incident hyperlipidemia at nominal significance (P<0.05). ABCA1, APOAI-C3-A4-A5, and CETP with low levels of LDL-C, and LPL, TRIB1, APOAI-C3-A4-A5, and APOE with hypertriglyceridemia displayed significant associations after accounting for multiple testing (P range from 1.20×10^{−3} to 4.67×10^{−18}; Table 4).

GRS and the Corresponding Lipid Levels Change and Incident Hyperlipidemia

Over the mean follow-up period of 8.1 years, 404, 221, 476, and 807 individuals developed incident hypercholesterolemia, high levels of LDL-C, low levels of HDL-C, and hypertriglyceridemia, respectively. For each lipid outcome (TC, LDL-C, HDL-C, and triglycerides), the SNPs associated with the corresponding lipid levels (Table III in the Data Supplement) were used to calculate GRS. The GRS for 4 lipids were independently associated with linear increases in their corresponding lipid levels over time (P range from 6.55×10^{−11} to 1.42×10^{−28}) and incident hyperlipidemia (P range from 5.33×10^{−4} to 5.33×10^{−18}; Table 4). For example, individuals in the highest quartile of risk score had an increase of 9.930 mg/dL in TC levels, and 93.5% increased risk for incident hypercholesterolemia, compared with those in the lowest quartile. An increase of 1 SD of the GRS was associated
with increases of 3.724 mg/dL ($P=6.59\times10^{-22}$) in TC, 2.252 mg/dL ($P=4.72\times10^{-12}$) in LDL-C, and 0.033 mg/dL ($P=5.71\times10^{-36}$) in log-transformed triglycerides and a decrease of 1.699 mg/dL ($P=2.06\times10^{-28}$) in HDL-C. The Figure shows the distribution of GRS by their corresponding incident hyperlipidemia. There was significant difference of GRS between individuals with hyperlipidemia and without hyperlipidemia ($P$ from $4.87\times10^{-5}$ to $1.57\times10^{-27}$). Each SD increase of the GRS resulted in 28.6% to 48.2% increased risk for the corresponding incident hyperlipidemia ($P$ values range from $8.46\times10^{-4}$ to $2.00\times10^{-21}$).

Adding GRS to the models including traditional risk factors significantly improved risk discrimination of incident

| Gene       | SNP       | CHR | Position | Code/Other Allele | Code Allele Frequency | Trait         | β (SE)     | n          | $P$ Value     |
|------------|-----------|-----|----------|-------------------|-----------------------|---------------|------------|------------|---------------|
| Previously reported loci in GWAS |           |     |          |                   |                       |               |            |            |               |
| PCSK9      | rs7525649 | 1   | 55271744 | T/C               | 0.72                  | TC            | 2.212 (0.34) | 22612      | 1.62$\times10^{-10}$          |
|            |           |     |          |                   |                       | LDL-C         | 1.954 (0.29) | 22376      | 2.69$\times10^{-11}$          |
| ANGPTL3    | rs12042319| 1   | 62822407 | G/A               | 0.81                  | TC            | 2.229 (0.404) | 23028      | 4.85$\times10^{-8}$           |
|            |           |     |          |                   |                       | TG            | 0.02 (0.003)  | 23027      | 8.33$\times10^{-12}$          |
| APOB       | rs312949  | 2   | 21187788 | C/G               | 0.28                  | TC            | 1.594 (0.355) | 23031      | 6.92$\times10^{-10}$          |
|            |           |     |          |                   |                       | LDL-C         | 1.415 (0.303) | 22788      | 2.87$\times10^{-9}$           |
| GCXCR      | rs1260333 | 2   | 27602128 | A/G               | 0.52                  | TC            | 1.773 (0.32)  | 22873      | 2.94$\times10^{-9}$           |
|            |           |     |          |                   |                       | TG            | 0.022 (0.002) | 22873      | 2.16$\times10^{-21}$          |
| HMGCRC     | rs6871667 | 5   | 74640498 | A/G               | 0.55                  | TC            | 2.214 (0.324) | 22668      | 7.99$\times10^{-12}$          |
|            |           |     |          |                   |                       | LDL-C         | 2.014 (0.278) | 22427      | 7.41$\times10^{-13}$          |
| MLXIPL     | rs13231516| 7   | 72501185 | T/G               | 0.87                  | TC            | 2.128 (0.324) | 23008      | 1.16$\times10^{-11}$          |
| LPL        | rs12678919| 8   | 19888502 | A/G               | 0.91                  | HC-LDL        | -1.954 (0.204) | 22648      | 1.86$\times10^{-21}$          |
| TRIB1      | rs2954029 | 8   | 126560154| A/T               | 0.41                  | TC            | 2.218 (0.324) | 23008      | 1.16$\times10^{-11}$          |
| ABOC       | rs2575876 | 9   | 10670560 | G/A               | 0.79                  | TC            | 3.224 (0.392) | 23027      | 4.03$\times10^{-13}$          |
| ABO        | rs579459  | 9   | 135143989| C/T               | 0.20                  | TC            | 3.038 (0.480) | 18474      | 2.72$\times10^{-15}$          |
| APOA1-C3-A4-A5 | rs662799     | 11  | 116168917| G/A               | 0.28                  | HDL-C         | -2.516 (0.126) | 23021      | 1.84$\times10^{-21}$          |
| ALDH2      | rs11066280 | 12  | 111302166| T/A               | 0.83                  | TG            | 0.017 (0.003)  | 22589      | 9.79$\times10^{-10}$          |
| LPC        | rs1800588  | 15  | 56510967 | T/C               | 0.38                  | TC            | 2.719 (0.33)  | 22845      | 8.45$\times10^{-16}$          |
| CETP       | rs3764261 | 16  | 55550825 | A/C               | 0.17                  | TC            | 4.439 (0.447) | 20576      | 9.69$\times10^{-23}$          |
| HPR        | rs12927205 | 16  | 70582578 | A/G               | 0.74                  | TC            | 1.784 (0.362) | 23005      | 6.94$\times10^{-12}$          |
| ABCA8      | rs12453914 | 17  | 64650473 | A/C               | 0.42                  | TC            | 1.237 (0.279) | 23032      | 1.67$\times10^{-16}$          |
| APOE       | rs157582  | 19  | 50088059 | T/C               | 0.18                  | TG            | 0.078 (0.008)  | 12320      | 3.92$\times10^{-22}$          |

Novel Chinese-specific variants at the previous reported loci

| HLA-C      | rs9357121 | 6   | 31348458 | T/G               | 0.85                  | TC            | 2.062 (0.330) | 23001      | 1.50$\times10^{-10}$          |
|            |           |     |          | (tagging rs3177928; $r^2=0.02$) |                       | LDL-C         | 1.842 (0.301) | 22759      | 3.28$\times10^{-10}$          |
| LIPG       | rs12970066| 18  | 45361150 | G/C               | 0.28                  | HDL-C         | 0.784 (0.128) | 22650      | 3.25$\times10^{-08}$          |
|            |           |     |          | (tagging rs7241918; $r^2=0.017$) |                       | LDL-C         | 0.993 (0.250) | 22788      | 3.66$\times10^{-05}$          |
| LDLR       | rs7258950 | 19  | 11111139 | G/A               | 0.78                  | TC            | 3.168 (0.383) | 22495      | 2.39$\times10^{-16}$          |
|            |           |     |          | (tagging rs6511720; $r^2=0$) |                       | LDL-C         | 3.387 (0.326) | 22252      | 1.44$\times10^{-24}$          |

SNP IDs and chromosomal positions are based on NCBI Build 36 of the genome. CHR, chromosome; GWAS, genome-wide association study; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; $r^2$ are obtained from 1000 Genomes data in JPT+CHB; SNP, single nucleotide polymorphisms; and TC, total cholesterol.
hypercholesterolemia (C-index change, 1%; \( P = 0.035 \)), hypertriglyceridemia (C-index change, 2%; \( P = 2.0 \times 10^{-4} \)), and low levels of HDL-C (C-index change, 2%; \( P = 5.0 \times 10^{-3} \); Table IV in the Data Supplement), whereas C-index analysis showed no improvement in the prediction of incident high levels of LDL-C (\( P = 0.122 \)).

### Discussion

The present study systematically investigated genetic susceptibility to lipid levels and its relevance to lipid change over time and risk of incident hyperlipidemia in Chinese populations. Our GWAS for lipids in Chinese replicated 17 loci previously identified in populations of European ancestry. We also identified Chinese-specific variants at 3 loci (HLA-C, LIPC, and LDLR). The study is the first investigation to address effect of variants on both lipid changes over time and risk of incident hyperlipidemia in East Asians. In a sample of 6418 Chinese participants from a prospective cohort study, during >8.1 years of follow-up, we found that this genetic information in Chinese could improve the prediction of incident hyperlipidemia beyond baseline lipid levels.

Recent GWAS have identified 157 significantly lipid-associated loci in individuals of European ancestry, and ancestry-specific analyses identified some associated SNPs clearly distinct from the original GWAS variants. As these studies were conducted almost exclusively in populations of European descent, Table 3. Significant Variants Associated With Lipid Levels Change and Incident Hyperlipidemia

| SNP | Gene | CHR | Position | Code | Allele | Other Allele | \( \beta \) (SE) | \( P \) Value | OR (SE) | \( P \) Value |
|-----|------|-----|----------|------|--------|-------------|-------------|-------------|---------|-----------|
| rs1800588 | LIPC | 15 | 56510967 | T | C | 1.985 (0.569) | 4.84 \times 10^{-4} | 1.222 (0.075) | 7.76 \times 10^{-3} |
| rs3764261 | CETP | 16 | 55550825 | A | C | 2.596 (0.730) | 3.83 \times 10^{-4} | 1.281 (0.092) | 7.15 \times 10^{-3} |
| rs7258950 | LDLR | 19 | 11111139 | G | A | 2.849 (0.655) | 1.39 \times 10^{-5} | 1.240 (0.093) | 2.08 \times 10^{-2} |

LDL-C change and incident high levels of LDL-C

| SNP | Gene | CHR | Position | Code | Allele | Other Allele | \( \beta \) (SE) | \( P \) Value | OR (SE) | \( P \) Value |
|-----|------|-----|----------|------|--------|-------------|-------------|-------------|---------|-----------|
| rs2575876 | ABCA1 | 9 | 106705560 | A | G | -0.847 (0.271) | 1.77 \times 10^{-3} | 1.310 (0.082) | 9.14 \times 10^{-4} |
| rs662799 | APOA1-C3-A4-A5 | 11 | 11168917 | G | A | -1.256 (0.252) | 6.24 \times 10^{-7} | 1.282 (0.107) | 1.0 \times 10^{-3} |
| rs1800588 | LIPC | 15 | 56510967 | C | T | -1.246 (0.225) | 1.38 \times 10^{-3} | 1.286 (0.074) | 3.8 \times 10^{-3} |
| rs3764261 | CETP | 16 | 55550825 | C | T | -1.959 (0.284) | 1.80 \times 10^{-4} | 1.452 (0.100) | 1.0 \times 10^{-3} |

Hypertriglyceridemia (C-index change, 1%; \( P = 0.035 \)), hypertriglyceridemia (C-index change, 2%; \( P = 2.0 \times 10^{-4} \)), and low levels of HDL-C (C-index change, 2%; \( P = 5.0 \times 10^{-3} \); Table IV in the Data Supplement), whereas C-index analysis showed no improvement in the prediction of incident high levels of LDL-C (\( P = 0.122 \)).

### Table 4. Association of Genetic Risk Scores for Lipid Levels With Their Corresponding Lipid Levels Change and Incident Hyperlipidemia

| Lipid levels change | Continuous GRS (Per SD)* | Q2 vs Q1 | Q3 vs Q1 | Q4 vs Q1 | \( P \) for Trend |
|---------------------|---------------------------|----------|----------|----------|------------------|
| TC GRS vs TC change | 3.724 (0.386) | 4.488 (1.082) | 7.04 (1.085) | 9.930 (1.092) | 9.72 \times 10^{-2} |
| LDL-C GRS vs LDL-C change | 2.252 (0.325) | 2.012 (0.918) | 4.53 (0.922) | 5.529 (0.924) | 6.55 \times 10^{-1} |
| HDL-C GRS vs HDL-C change | -1.699 (0.153) | -1.174 (0.445) | -2.129 (0.440) | -4.178 (0.433) | 4.77 \times 10^{-2} |
| TG GRS vs TG change | 0.033 (0.003) | 0.021 (0.007) | 0.038 (0.007) | 0.081 (0.007) | 1.42 \times 10^{-2} |

Incident hyperlipidemia

| Lipid levels change | Continuous GRS (Per SD)* | Q2 vs Q1 | Q3 vs Q1 | Q4 vs Q1 | \( P \) for Trend |
|---------------------|---------------------------|----------|----------|----------|------------------|
| TC GRS vs HyperTC | 1.312 (0.055) | 1.292 (0.175) | 1.645 (0.168) | 1.935 (0.162) | 1.09 \times 10^{-3} |
| LDL-C GRS vs HyperLDL-C | 1.286 (0.075) | 0.794 (0.238) | 1.392 (0.210) | 1.701 (0.203) | 5.33 \times 10^{-4} |
| HDL-C GRS vs LowHDL-C | 1.351 (0.052) | 1.200 (0.129) | 1.582 (0.135) | 2.146 (0.145) | 1.72 \times 10^{-3} |
| TG GRS vs HyperTG | 1.482 (0.041) | 1.319 (0.126) | 1.563 (0.123) | 2.661 (0.118) | 5.33 \times 10^{-3} |

The OR and \( \beta \) for continuous GRS are for 1-SD unit increase in GRS. GRS indicates genetic risk score; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; OR, odds ratio; SNP, single nucleotide polymorphism; TC, total cholesterol; and TG, triglycerides.

*The covariates were sex, age, body mass index, and the baseline lipid levels corresponding to the GRSs.
studies in non-European populations would allow us to assess the relevance of the findings to other ethnic groups. In the present study, we found both shared and population-specific lipid susceptibility were commonly present. We not only replicated 17 previously reported loci but also identified 3 Chinese-specific variants (HLA-C, LIPG, and LDLR). Of those, the association of LIPG with HDL-C has been found not to be generalized to diverse populations including African, Indians, Mexican, and Hispanics.12 We observed that the reported lead SNPs at these 3 loci in Europeans were monomorphic or had low minor allele frequency (<0.1) in the Chinese Han population, whereas these SNPs were polymorphic in European populations (Table V in the Data Supplement). For example, in Europeans, a prominent association was reported for rs6511720, which is not polymorphic in the Chinese. These data suggest that genetic heterogeneity in these loci may be because of different LD structure and minor allele frequency. As expected, the ethnic-specific variants we detected in Chinese were not in LD with the initially reported lead SNPs in Europeans. Conversely, we also investigated whether the 3 Chinese-specific variants identified in our samples were associated with lipid in Europeans, using the results from the Global Lipids Genetics Consortium, a meta-analysis of 188,577 individuals.3 The rs12970066 at LIPG showed suggestive significant association with HDL-C in the population of European ancestry (P=4.77×10–7), whereas rs9357121 at HLA-C and rs7258950 at LDLR showed no associations with lipid levels. These differences in European and Chinese populations may facilitate the fine mapping of common causal variants. We used HaploReg to search for evidence of the functional role for 3 Chinese-specific variants. Rs9357121 (HLA-C) and rs12970066 (LIPG) were in strong LD with rs1131151 (r²=0.91 in 1000 genomes East Asian) and rs2000813 (r²=0.81), respectively, which were nonsynonymous variants. The SNP rs7258950 in LDLR lies within the promoter and enhancer histone marks as well as DNase hypersensitive sites. To gain further understanding of the lipid susceptibility loci, we tested their associations with the traditional risk factors of cardiovascular diseases in the replication samples. After Bonferroni correction for 20 independent tests, LPL showed significant associations (P<2.5×10–7=0.05/20) with diastolic blood pressure and hypertension, whereas GCKR was significantly associated with plasma glucose (Table VI in the Data Supplement).

We further assessed the individual effect of lipid-related SNPs on increases in lipids and risk of incident hyperlipidemia. All the SNPs indicated a directionality-consistent association with increases in lipids for lipid-raising alleles. These SNPs were associated with mild increases in TC (range, 0.456–2.849 mg/dL per allele), LDL-C (range, 0.664–2.464 mg/dL per allele), and log-transformed triglycerides (range, 0.005–0.036 mg/dL per allele) and decrease in HDL-C (range, 0.460–1.959 mg/dL per allele). As expected, almost all of these SNPs were also observed to increase the risk of incident hyperlipidemia. Although each SNP exerts a modest effect, a combination of SNPs, in aggregate, can have a substantial influence on incident hyperlipidemia. Consistent with single variants finding, the variants in aggregate were significantly associated with linear increases in lipids and risk of incident hyperlipidemia. These associations were largely independent on lipid levels measured at baseline. By accumulation, for example, individuals in the top compared with bottom quintiles of GRS differed by an increase of 9.930 mg/dL in TC and

![Graph](image_url)
a 93.5% increased risk for incident hypercholesterolemia in a follow-up period of 8.1 years. We also found GRS improved risk discrimination of incident hypercholesterolemia, hyper-triglyceridemia, and low levels of HDL-C over the traditional risk factors. Several European studies also showed the lipid-associated SNPs were associated with the longitudinal lipid changes.\textsuperscript{26–29} Varga et al\textsuperscript{28} further demonstrated that the ability of the 157 establish lipid-loci to predict incident dyslipidemia was modest in the Swedish populations with 10-year follow-up. The C-index values were increased by 2% to 4%, which were slightly higher than those (1%–2%) reported by our study, given that only 20 variants were involved in our GRS. Although the variants have modest effects on lipids, their presence may act over the entire life course and translate into comparatively large effects. It has been shown that joint effect of LDL-C–related SNPs was an independent risk factor for incident cardiovascular diseases in Europeans.\textsuperscript{30,31} However, the results might not be generalized to populations with genetic backgrounds different from that of European populations. Thus, it is necessary to further evaluate the predictive abilities of the genetic predisposition to higher lipids for risk of cardiovascular diseases in Chinese population.

The major strength of our study includes the use of a population-based cohort for the assessment of effect of variants on both lipid change over time and risk of incident hyperlipidemia. The effect size estimated in published GWAS is the most frequently used to create the GRS in published studies of genetic variants and risk prediction, but it may be overestimated because of winner’s curse. In the present study, we constructed the GRS using the $\beta$ coefficient as determined in our prospective cohort to increase the accuracy of the GRS. Our results should be interpreted in the context of potential limitations. First, our discovery sample size is modest relative to other previous consortia of European descent. This means that some signals especially with a low minor allele frequency and weaker genetic effects may have been missed. Second, although our genetic predisposition score captured the combined information from the established genetic variants for lipids in Chinese to date, it may account for only a small proportion of lipid variation. For the 20 significantly associated variants in Chinese, trait variance explained was 5.0% for TC, 4.7% for LDL-C, 6.9% for triglycerides, and 6.1% for HDL-C, respectively, which was small when compared with 12% to 15% explained by 157 variants discovered in European populations.\textsuperscript{3,4} The identification of the variants with small effect in large-scale studies would be expected to capture additional genetic variance. Finally, our study was undertaken in individuals of Chinese, and hence the results might not be generalized to populations with genetic backgrounds different from that of our population.

In conclusion, we replicated 17 loci previously identified in populations of European for lipid levels. At HLA-C, LIPG, and LDLR, we also identified some evidence for allelic heterogeneity in Chinese when compared with Europeans. The individual SNPs and those aggregate effects were independent risk factors for lipid increase and incident hyperlipidemia.

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Disclosures
None.

Appendix
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Genetic studies have identified multiple genetic loci associated with lipid levels. However, genetic predisposition to higher lipid levels on lipid changes over time and risk of future hyperlipidemia is uncertain, particularly among Chinese who may have different genetic and environmental exposures from Europeans. Here, we systematically evaluated genetic susceptibility to lipid levels in 23,083 Chinese populations. We not only replicated 17 lipid-related loci previously identified in populations of European but also identified some evidence for allelic heterogeneity in 3 genetic region in Chinese when compared with Europeans. We then observed these lipid-related genetic variations could contribute to lipids increase and incident hyperlipidemia, as our better understanding of the genetic architecture of blood lipid, the genetic information might be used clinically in cardiovascular diseases risk prediction in the future.