**EHBP1 SNPs, Their Haplotypes, and Gene–Environment Interactive Effects on Serum Lipid Levels**

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**Introduction**

Hypertension, coronary artery disease (CAD), and carcinoma are not only the main contributors to the global economic burden of diseases but are also the main causes of death in the world.15 Hyperlipidemia is the initial risk factor as well as an independent risk factor for hypertension and almost all arteriosclerotic diseases, such as CAD.3,4 and it has even been shown to be an important risk factor for some carcinomas.5,6 In the past 10 years, increasing studies have suggested that serum lipid levels are influenced by various environmental and genetic factors, leading to hyperlipidemia and then triggering obesity, hypertension, and cardiovascular disease (CVD).7–9 The rapid increase in the hyperlipidemia morbidity highlights the need for continued focus on the surveillance of serum lipid levels. Therefore, we hope to monitor serum lipid levels through community check-ups and even predict early risks of hyperlipidemia in different ethnic groups through gene and/or single nucleotide polymorphism (SNP) screening.10–12

It is well established that gene mutations are a critical cause of epigenetic expression. The EH domain-binding protein 1 (EHBP1 [MIM 609922]) gene encodes an Eps15 homology domain-binding protein that may have a great effect on endocytic transport. This gene is highly expressed in fat tissues, and its alternate splicing results in multiple transcript variants.13 A previous study has shown that EHBP1 influences low-density lipoprotein cholesterol (LDL-C) and body mass index (BMI) and revealed that it may be a novel gene for predicting obesity and hypertension.14 Additionally, some genome-wide association studies (GWASes) have found that single nucleotide polymorphisms (SNPs) rs2710642 (G > A)
and rs10496099 \((T > C)\) of \(EHBP1\) were related to HDL-C.\textsuperscript{15,16}

The diverse phenotypes of the gene might be partly attributed to ethnic/racial specificity. The Maonan ethnicity is one of the minorities in China, with a total population of 107,166 people according to the sixth national census statistics of China in 2010. Maonan people live in the Huanjiang area of Guangxi Province and have special culture and customs, including fat-rich dietary habits, alcohol consumption, and intraethnic marriages. The higher prevalence of some diseases can be explained by a high inbreeding coefficient in this population\textsuperscript{17} due to the intragroup marriage commonly practiced in the Maonan population. Based on the ethnic features of the Maonan population, various studies have been conducted to reveal the differences in the epigenetic expression of lipid-related genes in Maonan people.\textsuperscript{18,19} However, the relationship between \(EHBP1\) SNPs and serum lipid levels in different ethnic groups is still unclear. Here, two \(EHBP1\) SNPs and several environmental risk factors were measured in the Han and Maonan populations to explore their effects on serum lipid levels.

\section*{RESULTS}

\subsection*{General Information about the Participants.}
As shown in Table 1, no statistical difference in the age and gender was observed between the Han and Maonan populations, as well as the height, weight, waist circumference (WC), BMI, pulse pressure (PP), fasting blood glucose (FBS), and the hyperlipidemia morbidity \((P > 0.05)\). However, systolic blood pressure (SBP), diastolic blood pressure (DBP), cigarette smoking and alcohol consumption, and hypertension morbidity showed statistical differences between the two ethnic groups \((P < 0.05)\). In addition, the total cholesterol (TC), LDL-C level, and apolipoprotein (Apo) A1/ApoB ratio were significantly higher in Maonan than those in the Han population \((P < 0.01)\). On the contrary, the high-density lipoprotein cholesterol (HDL-C) and ApoB levels were significantly lower in Maonan than those in the Han ethnic group \((P < 0.01)\).

\subsection*{Frequency of Genotypes and Alleles.}
As shown in Table 2, two SNPs rs2710642 and rs10496099 showed point mutations from G to A and T to C, respectively. The allelic and genotypic distribution of the \(EHBP1\) rs2710642 and rs10496099 SNPs maintained concordance with the Hardy–Weinberg equilibrium (HWE) in the Han and Maonan populations \((P > 0.05)\) and were different between the two ethnic groups \((P < 0.001)\). The minor allele was the reference allele, and the major allele was the alternate allele. The frequencies of the alternate alleles rs2710642A \((77.0 \% vs 65.0 \%)\) and rs10496099C \((78.0 \% vs 70.0 \%)\) and the wild homozygotes rs2710642AA \((58.0 \% vs 41.0 \%)\) and rs10496099CC \((60.0 \% vs 50.0 \%)\) were higher, but the reference alleles and the homozygotes were lower in Maonan compared to those in the Han ethnic group.

\subsection*{Genotypes of the Two \(EHBP1\) SNPs and Serum Lipid Levels.}
As shown in Figure 1, serum triglyceride (TG) levels were significantly higher in Maonan than those in the Han and control groups \((P < 0.01)\). On the contrary, the high-density lipoprotein cholesterol (HDL-C) and ApoB levels were significantly lower in Maonan than those in the Han ethnic group \((P < 0.01)\).

Table 1. General and Biochemical Characteristics of the Participants\textsuperscript{4}

| parameter                  | Han ethnic | Maonan ethnic | t (\(\chi^2\)) | P       |
|---------------------------|------------|---------------|---------------|---------|
| number                    | 564        | 796           |               |         |
| man/female                | 268/296    | 340/456       | 3.082         | 0.079   |
| age, years\textsuperscript{3} | 55.87 ± 11.54 | 54.48 ± 14.61 | 1.873         | 0.061   |
| height, cm                | 157.34 ± 8.63 | 157.16 ± 8.04 | 0.394         | 0.694   |
| weight, kg                | 56.89 ± 12.70 | 56.64 ± 10.75 | 0.394         | 0.694   |
| waistline, cm             | 78.25 ± 10.25 | 78.90 ± 9.16  | −1.218        | 0.223   |
| body mass index, kg/m\(^2\) | 22.90 ± 4.35 | 22.80 ± 3.12  | 0.481         | 0.630   |
| cigarette consumption, n (%)\textsuperscript{3} | | | | |
| 0 cigarette/day           | 435 (77.1) | 622 (78.1)    |               |         |
| ≤20 cigarettes/day        | 91 (16.2)  | 154 (19.4)    |               |         |
| >20 cigarettes/day        | 38 (6.7)   | 20 (2.5)      | 15.751        | 0.000   |
| alcohol consumption, n (%) | | | | |
| 0 g/day                   | 466 (82.6) | 620 (77.9)    |               |         |
| ≤25 g/day                 | 12 (2.2)   | 92 (11.5)     |               |         |
| >25 g/day                 | 86 (15.2)  | 84 (10.6)     | 45.137        | 0.000   |
| systolic blood pressure, mmHg | 131.50 ± 20.52 | 136.60 ± 23.42 | −4.162       | 0.000   |
| diastolic blood pressure, mmHg | 80.92 ± 11.52 | 84.77 ± 13.02 | −5.626       | 0.000   |
| pulse pressure, mmHg      | 50.64 ± 16.30 | 51.92 ± 16.69 | −1.412       | 0.158   |
| fasting blood glucose, mmol/L | 6.26 ± 1.38  | 6.17 ± 1.34   | 1.107         | 0.268   |
| total cholesterol, mmol/L | 4.90 ± 0.91 | 5.06 ± 0.99   | −3.037        | 0.002   |
| triglyceride, mmol/L\textsuperscript{4} | 1.38 (1.03,1.98) | 1.38 (0.98,1.93) | 0.022       | 0.883   |
| HDL-C, mmol/L             | 1.30 ± 0.26 | 1.26 ± 0.25   | 2.409         | 0.016   |
| LDL-C, mmol/L             | 3.06 ± 0.39 | 3.21 ± 0.54   | −5.691        | 0.000   |
| apolipoprotein (Apo)A1, g/L | 1.29 ± 0.24  | 1.29 ± 0.23   | 0.089         | 0.929   |
| ApoB, g/L                 | 0.97 ± 0.19 | 0.92 ± 0.18   | 4.983         | 0.000   |
| ApoA1/ApoB                | 1.40 ± 0.43 | 1.48 ± 0.44   | −3.299        | 0.001   |
| hypertension, n (%)       | 236 (41.7) | 410 (51.6)    | 13.095        | 0.000   |
| hyperlipidemia, n (%)     | 319 (56.4) | 425 (53.5)    | 1.071         | 0.301   |

\textsuperscript{4}HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. \textsuperscript{4}Quantitative data of Gaussian distribution used t-test describing mean ± standard deviation (SD). \textsuperscript{4}Categorical data used the \(\chi^2\) test. \textsuperscript{4}Quantitative data of skewed distribution used the Wilcoxon–Mann–Whitney test describing the median (interquartile range). \(P < 0.05\) indicated statistically significant difference.
in the Maonan population were different among the three genotypes of the rs2710642 and rs10496099 SNPs ($P = 0.003$ and 0.010, respectively). The rs2710642G and rs10496099T allele carriers had higher TG levels than those of the rs2710642G and rs10496099T allele noncarriers. In both of the Han and Maonan groups, no differences in the remaining serum lipid indexes were found between rs2710642 and rs10496099 SNPs.

Interestingly, after we adjusted the risk factors including age, gender, alcohol consumption, smoking, blood glucose, BMI, and hypertension, the association between the two EHBP1 SNPs and serum lipid levels was changed (shown in Table 3). Overall, in the Han, serum LDL-C levels were lower in the rs2710642GA carriers than the rs2710642AA carriers ($P = 0.015$), and ApoA1 levels were lower in the rs10496099TT carriers than the rs149099CC carriers ($P = 0.042$). In the Maonan, serum LDL-C levels were higher but HDL-C levels were lower in the rs2710642GG carriers than the rs2710642AA carriers ($P = 0.003$ and 0.037; respectively).

### Haplotypes Frequencies between the Two EHBP1 SNPs.

There was moderate linkage disequilibrium (LD) between the EHBP1 rs2710642 and rs10496099 SNPs in the Han and Maonan populations ($D^′ = 0.820$ or $r^2 = 0.537$ and $D^′ = 0.807$ or $r^2 = 0.620$, respectively; Table 4). Thus, the frequencies of the major haplotypes (frequency threshold for rare haplotypes >0.03) in the two ethnic groups are presented in Table 5. The frequencies of the rs2710642A−rs10496099C, rs2710642G−rs10496099T, and rs2710642G−rs10496099C haplotypes were different between the two ethnic groups ($P < 0.001$ for all). However, the frequency of the rs2710642A−rs10496099T haplotype was not different between the two ethnic groups.

### Haplotypes and Serum Lipid Levels.

The associations of haplotypes and serum lipid levels in the two ethnic groups are summarized in Figure 2. The rs2710642A−rs10496099C haplotype carriers in the Han group had higher ApoA1 levels than those of the rs2710642A−rs10496099C haplotype noncarriers ($P = 0.009$), and the rs2710642G−rs10496099C haplotype carriers had higher TG levels than those of the rs2710642G−rs10496099C haplotype noncarriers ($P < 0.001$).

In contrast, the rs2710642A−rs10496099C haplotype carriers in the Maonan group had lower TG levels than those of the rs2710642A−rs10496099C haplotype noncarriers ($P = 0.006$), and the rs2710642G−rs10496099C haplotype carriers had lower HDL-C levels than those of the rs2710642G−rs10496099C haplotype noncarriers ($P = 0.014$).

After gender, age, smoking, alcohol consumption, BMI, blood glucose, and hypertension were adjusted, serum TG levels in the Han population were higher in the rs2710642G−rs10496099C haplotype carriers than those in the rs2710642G−rs10496099C haplotype noncarriers ($P = 0.015$).

### Table 2. Genotypic and Allelic Frequencies of the Two EHBP1 SNPs in the Han and Maonan Ethnic Groups [n (%)]a

| SNP     | genotype (allele) | Han (n = 564) | Maonan (n = 796) | $\chi^2$ | $P$  |
|---------|-------------------|---------------|-----------------|----------|-----|
| rs2710642 | GG                | 57 (10)       | 34 (4)          |          |     |
|         | GA                | 276 (49)      | 300 (38)        |          |     |
|         | AA                | 231 (41)      | 462 (58)        | 45.563   | 0.000|
|         | A                 | 738 (65)      | 1224 (77)       |          |     |
|         | G                 | 390 (35)      | 368 (23)        | 43.126   | 0.000|
|         | $P_{HWE}$         | 0.062         | 0.11            |          |     |
| rs10496099| TT                | 52 (9)        | 32 (4)          |          |     |
|         | TC                | 231 (41)      | 290 (36)        |          |     |
|         | CC                | 281 (50)      | 474 (60)        | 21.839   | 0.000|
|         | C                 | 793 (70)      | 1238 (78)       |          |     |
|         | T                 | 335 (30)      | 354 (22)        | 19.438   | 0.000|
|         | $P_{HWE}$         | 0.69          | 0.15            |          |     |

*Categorical data used the $\chi^2$ test. $P < 0.05$ indicated statistically significant difference. HWE means Hardy–Weinberg equilibrium. $P_{HWE} > 0.05$ indicated statistically significant difference."
0.001) and serum ApoA1 levels were also higher in the rs2710642A-ctrs10496099C haplotype carriers than those in the rs2710642A-ctrs10496099C haplotype noncarriers (P = 0.031). Serum TC levels in the Maonan population were lower in the rs2710642A-ctrs10496099C haplotype carriers and higher in the rs2710642G-ctrs10496099C haplotype carriers than those in the corresponding haplotype noncarriers (P = 0.020 and 0.007, respectively). Serum HDL-C levels were higher in the rs2710642A-ctrs10496099C haplotype carriers and lower in the rs2710642G-ctrs10496099C haplotype carriers than those in the corresponding haplotype noncarriers (P = 0.021 and 0.016, respectively; Table 6).

**Genotypes and Haplotypes with Hyperlipidemia.** The associations of the genotypes and haplotypes of the two SNPs with the risks of hyperlipidemia are shown in Tables 7-9. The rs2710642G-ctrs10496099C haplotype carriers were more likely to develop hyperlipidemia than the haplotype noncarriers (adjusted odds ratio [OR] = 2.64, 95% confidence interval [CI] = 1.52-4.59, P = 0.001) and were 2.25 times more than the rs2710642A-ctrs10496099C haplotype carriers to lead to hyperlipidemia in the Han group (95% CI = 1.47-4.33, P = 0.0008), but the difference was hardly observed in genotype models and the remaining haplotype carriers in the Han and Maonan populations.

**Interaction of Haplotypes and the Stratified Risk Factors for Hyperlipidemia.** As presented in Figure 3, the prevalence of hyperlipidemia in the Han group was significantly changed when under the common effects on the haplotype rs2710642G-ctrs10496099C interaction with other risk factors, like the rs2710642G-ctrs10496099C-female (adjusted OR = 2.39, 95% CI = 1.09-5.24, P = 0.029), rs2710642G-ctrs10496099C-hypertension (adjusted OR = 2.90, 95% CI = 1.16-7.22, P = 0.022), rs2710642G-ctrs10496099C-smoking (adjusted OR = 3.44, 95% CI = 1.10-10.76, P = 0.034), and the rs2710642G-ctrs10496099C-FBS ≥ 7.0 mmol/L (adjusted OR = 2.97, 95% CI = 1.08-8.14, P = 0.035).

**Risk Factors Related to Serum Lipid Levels.** Pearson correlation analysis of the heat map (Figure 4) demonstrated that some of the genotypes and haplotypes of the EHBP1 rs2710642 and rs10496099 SNPs were correlated with serum lipid levels and validated that some nongene risk factors, including age, gender, smoking, alcohol consumption, blood pressure, BMI, and FBS levels, were also associated with serum lipid levels in the two ethnic groups.

## DISCUSSION

Hyperlipidemia, a sensitive risk factor predisposing individuals to CAD or hypertension, is a common disease with a complicated pathogenesis. Hyperlipidemia can emerge from genetic factors such as lipid-associated gene mutations and interactions among various factors, such as age, gender, alcohol consumption, diet, exercise, and gene–environmental interactions. The EHBP1 SNPs rs2710642 and rs10496099 on chromosome 2 started at position 6292422 and 62578767, and the lengths of them were 214 and 220, respectively. There was 343 kb pairwise distance between them. Thus, there were more chance for them to compose haplotypes. In this study, the probe detected these two targeted sequences in the participants of the Maonan and Han.

Recently, Willer et al. reported that EHBP1 rs2710642 significantly correlated with LDL-C (n = 173, P = 6.00 × 10⁻⁶) in the European population. Likewise, a previous GWAS identified an association between the EHBP1 rs10496099 and arterial stiffness in the European population (P = 7.28 × 10⁻⁵), suggesting that the EHBP1 rs10496099 was associated with blood pressure and lipid metabolism. However, rarely studied exhibited the relationship of the rs2710642 and rs10496099 SNPs with serum lipid levels, in the Han and Maonan populations.

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Figure 2. Associations of serum lipid levels and haplotypes in the Han and Maonan populations. *P < 0.017 indicated statistically significant difference after Bonferroni correction.

Table 6. Association of the Haplotypes between the Two SNPs and Serum Lipid Levels

| Lipid     | Haplotype       | Han            | Maonan          |
|-----------|-----------------|----------------|-----------------|
|          |                 | carriers/noncarriers | std. error | β     | t   | P    | carriers/noncarriers | std. error | β     | t   | P    |
| TG       | rs2710642G−rs10496099C | 0.168          | 0.095          | 3.362          | 0.001 |
|          | rs10496099C−rs2710642A | 0.015          | 0.064          | 2.165          | 0.031 |
| ApoA1    | rs2710642G−rs10496099C | 0.014          | 0.057          | 2.303          | 0.021 |
|          | rs10496099C−rs2710642A | 0.031          | −0.060         | −2.419         | 0.016 |
| TC       | rs2710642A−rs10496099C | 0.055          | −0.057         | −2.321         | 0.020 |
|          | rs10496099C−rs2710642A | 0.124          | 0.067          | 2.709          | 0.007 |
| HDL-C    | rs2710642A−rs10496099C | 0.007          | 0.053          | 2.156          | 0.031 |
|          | rs10496099C−rs2710642A | 0.168          | 0.095          | 3.362          | 0.001 |
| LDL-C    | rs2710642G−rs10496099C | 0.055          | −0.057         | −2.321         | 0.020 |
|          | rs10496099C−rs2710642A | 0.124          | 0.067          | 2.709          | 0.007 |

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; and std. error, standard error. The P value was calculated by multivariable linear regression, and genotype, age, gender, drinking, smoking, fasting blood glucose, body mass index, and hypertension were adjusted. P < 0.05 indicated statistically significant difference.

Table 7. Frequencies and Risk for Gene Models in Each SNP between the Normal and Hyperlipidemia Populations

| SNP          | model | genotype | Han                  | Maonan               |
|--------------|-------|----------|----------------------|----------------------|
| rs2710642G > A | codominant | AA       | 1.876 (1.80−2.69)       | 1.47 (0.80−2.69)       |
|              | dominant | AA + GA  | 0.150 (0.67−1.50)       | 0.055 (0.67−1.50)       |
|              | recessive | AA + GG  | 0.150 (0.67−1.50)       | 0.055 (0.67−1.50)       |
|              | overdominant | AA + GA  | 1.054 (1.80−2.69)       | 1.47 (0.80−2.69)       |
|              | log-additive | GA       | 1.732 (1.13−2.73)       | 1.099 (1.02−2.73)       |
| rs10496099T > C | codominant | CC       | 0.055 (0.67−1.50)       | 0.055 (0.67−1.50)       |
|              | dominant | CC + TC  | 0.893 (0.80−1.80)       | 0.893 (0.80−1.80)       |
|              | recessive | CC + TT  | 0.463 (0.46−1.45)       | 0.463 (0.46−1.45)       |
|              | overdominant | CC + TT  | 0.314 (0.31−1.73)       | 0.314 (0.31−1.73)       |
|              | log-additive | TC       | 0.160 (0.87−1.12)       | 0.160 (0.87−1.12)       |

OR, odds ratio; CI, confidence interval. The value for both P and *P < 0.05 indicated statistically significant difference.
report that the rs2710642G lipidemia through interaction with environmental factors. altered the lipid levels and even the prevalence of hyper-risk factor for hyperlipidemia in the Han population and it rs10496099C exhibited markedly discrepancies among different ethnic groups. A previous GWAS with Chinese Han in Beijing (CHB) according to the HapMap Project database demonstrated that the Beijin (CHB) according to the HapMap Project database of the HapMap Project. These observed results might be attributed to developmental or environmental stimuli and 68.9%, respectively. Although admixture is a known confounding factor for GWASes, it is unlikely to affect the conclusions of this study. Because the high-throughput sequencing results of this study indicated that the base ratio of each sequencing target of rs2710642A or rs10496099C in this study were closer to the frequencies in the CHB, but they were different from the rates in Europeans, Americans, Africans, and Japanese people according to the HapMap Project database. These observed results might be attributed to developmental or environmental stimuli and to differences in sample sizes and regions. Many studies have

Maonan populations in Guangxi Province of China. Therefore, there were three major merits of this study: (1) it provided more information about the EHBPI SNPs for the sample library, creating the foundation to explore further genomics research on different populations; (2) it demonstrated a novel report that the rs10496099 genotypes were related to serum TG levels in the Maonan population and the haplotypes of EHBPI rs2710642A and rs10496099C alleles in the Han population were closer to or more than 50% and some of them were as high as 100%. Moreover, to data of this study, the frequencies of the rs2710642A and rs10496099C alleles in the Han population were close to or more than 50% and some of them were as high as 100%. Moreover, to data of this study, the frequencies of the rs2710642A and rs10496099C allel...
It is well known that gene–environment interactions modify multiple blood lipid fractions.18,30–33 The Han and Maonan ethnicities are two of the S6 ethnic groups in China, the former is the largest with 1226 million people in 2010. The Maonan group is one of the S5 official ethnic minorities. Although both the Han and Maonan participants came from the same region in our study, they still had differences in dietary habits and lifestyle, especially long-term dietary habits such as a high-fat diet (HFD) mainly coming from pork, beef, or animal offal; pickled products; and daily alcohol consumption in the Maonan ethnic group. A recent study reported that HFD consumption significantly increased the adiposity index.34 Another study suggested that the pickling process using brine negatively impacted the abundance of secondary metabolites in both onion and lemon to decrease their health benefits.35 Additionally, Klop et al. elaborated that high alcohol intake would increase the plasma level of TGs by inducing increased secretion of very low density lipoprotein in the liver and transferring it to the peripheral blood.36 Some previous studies demonstrated that excessive consumption of alcohol (>280 g/week)37,38 was a risk factor for dyslipidemia, while low and moderate drinking did not increase hyperlipidemia9,39 or CVD risks,40 but our study did not find that alcohol consumption has an effect on the incidence of hyperlipidemia in the two SNP carriers. On the other hand, opinions on the correlation between smoking and lipid profiles were not consistent,41–43 while this study did not find that smoking is directly associated with hyperlipidemia. Therefore, we speculated that these different environmental exposures sometimes would integrate with lipid-related gene effect, leading to a variation in serum lipid profiles between the Han and Maonan populations.

Here, the regression analysis models in our study exhibited that hypertension and gene mutations were independent risk factors increasing but age decreasing the incidence of hyperlipidemia in the Han population. Meanwhile, the four risk factors observed, including gender, smoking status, fasting glucose, and hypertension, particularly interacted with the haplotype rs2710642G–rs10496099C of the two EHBPI SNPs elevating the incidence of hyperlipidemia. Morgan et al. reported that the declining clearance rate of LDL-C with aging was a major cause of hyperlipidemia.44 In contrast, we found that the rs2710642G–rs10496099C-aged ≥65 year interaction subgroup had normal serum lipid levels, and the rs2710642G–rs10496099C-smoking interaction subgroup increasing the risk of hyperlipidemia. Thus, we inferred that smoking but not age was an aggravating factor to increase the haplotype rs2710642G–rs10496099C effect on elevating the serum lipid concentration. In addition, a previous animal trial demonstrated that estrogen and XX chromosome have positive effects on elevating the serum lipid concentrations.
effects on elevating HDL-C levels.\textsuperscript{45} This study showed that the interaction subgroup of rs2710642G\textsuperscript{-}rs10496099C-female had a lower odds ratio of hyperlipidemia than that of the corresponding subgroup, which also demonstrated that the female factor could attenuate the effect of haploid rs2710642G\textsuperscript{-}rs10496099C on increasing lipid levels. Additionally, for the past decades, many statistics have shown a positive relationship between diabetes mellitus (DM) and hyperlipidemia. High FBS was especially recognized as a warning of prediabetes (pre-DM) and DM; however, there were limited data to support the association between FBS and serum lipid traits, except a new study reported that the pre-DM was correlated with hyperlipidemia.\textsuperscript{46} In this study, we showed that the integrative effect of rs2710642G\textsuperscript{-}rs10496099C-FBS $\geq$ 7.0 mmol/L significantly led to hyperlipidemia. Finally, several published studies have suggested that lipid-related gene interactions with environmental factors could change blood lipid profiles and even modulate blood pressure levels.\textsuperscript{7,47} The outcomes of this study revealed that the interaction of rs2710642G\textsuperscript{-}rs10496099C-hypertension resulted in a higher prevalence of hyperlipidemia than that of the corresponding subgroups. Therefore, we identified that rs2710642\textsuperscript{-}rs10496099\textsuperscript{-}environment interactions changed the serum lipid concentrations and the hyperlipidemia morbidity in the Han ethnic population.

However, still, some limitations should be mentioned in our study. (1) A lack of the intake information of pickled products and fat-rich food, which may bring bias to explore the serum lipid-related dietary factors;\textsuperscript{48} (2) larger sample size is needed compared with that in the previous GWASes; (3) no stratified statistics were performed to explore the association of each risk factor with serum lipid levels; and (4) no investigation was conducted to analyze the effects of many other EHBP1 SNPs and multiple gene–gene interactions on serum lipid levels.

\section*{Conclusions}

The frequencies of EHBP1 rs2710642 and rs10496099 alterate alleles and genotypes showed differences in the Han and Maonan populations. The serum lipid levels were closely related to core haplotypes (rs2710642A\textsuperscript{-}rs10496099C and rs2710642G\textsuperscript{-}rs10496099C) and other risk factors including age, levels of FBS, and blood pressure. In particular, our results revealed a strong positive correlation between the rs2710642G\textsuperscript{-}rs10496099C haplotype and hyperlipidemia morbidity in the Han ethnic group. Therefore, we recognized that the differences in serum lipid levels in the Han and Maonan populations were attributed to the influence of interethnic EHBP1 SNPs, their haplotypes, and gene–environment interactions.

\section*{Experimental Section}

\textbf{Participants.} A total of 1360 unrelated subjects, who were descendants from three generations of the Maonan or Han ethnic, were randomly selected from our previous study in 2015 using stratified random sampling;\textsuperscript{49} all of them lived in Guangxi Zhuang Autonomous Region of southern China. They were divided into Han and Maonan groups; no statistical difference in the age and gender was observed between the two groups. In Han group, 564 subjects aged 55.87 $\pm$ 11.54 years were studied, of which 268 subjects (47.52%) were males and 296 subjects (52.48%) were females; in the Maonan group, 796 subjects aged 54.48 $\pm$ 14.61 years were studied, of which 340 subjects (42.71%) were males and 456 subjects were females (57.29%). All of them were generally healthy, and those with a history of CVD, stroke, diabetes, renal disease, thyroid disease, autoimmune disease, carcinoma, or mental illness were excluded. The participants did not use medications, including medicine and various supplements, such as vitamins and fish oil. After four professional investigators explained the experimental project, those who met additional entry criteria and signed informed consent were selected.

\textbf{Detection of Serum Lipid Levels.} All of the subjects received the detection of serum lipid levels, and blood samples (3 mL) were collected after fasting for 8 h. The detections were performed by an autoanalyzer (type 7170A; Hitachi Ltd., Tokyo, Japan);\textsuperscript{39} serum TC, TGs, HDL-C, and LDL-C were detected using commercially enzymatic assay kits, and serum ApoA1 and ApoB were detected using turbidimetric immunoassay.\textsuperscript{47,50,51}

\textbf{Diagnostic Criteria.} The normal serum TC, TGs, HDL-C, LDL-C, ApoA1, and ApoB levels and the ApoA1/ApoB ratio are defined as 3.10–5.17 mmol/L, 0.56–1.70 mmol/L, 0.90–1.81 mmol/L, 2.70–3.20 mmol/L, 1.00–1.78 g/L, and 0.63–1.14 g/L and 1.00–2.50, respectively. Hyperlipidemia is defined as TC greater than 5.17 mmol/L, or TG greater than 1.78 mmol/L.\textsuperscript{52} Hypertension is defined as systolic blood pressure $\geq$ 140 mmHg, and/or diastolic blood pressure $\geq$ 90 mmHg.\textsuperscript{39} Obesity in China is defined as BMI greater than 28 kg/m$^2$,\textsuperscript{54,55} and the BMI is calculated according to the following formula: BMI = weight/(height$^2$).

\textbf{SNP Selection.} The basic principles of SNP selection were as follows: (1) \textit{EHBP1} was selected from a previous GWAS associated with lipid metabolism. (2) Tagging SNPs were selected by Haplovire and correlated with lipids (Broad Institute of MIT and Harvard, Cambridge, MA, version 4.2). (3) SNP information was obtained from NCBI dbSNP Build 132 (http://www.ncbi.nlm.nih.gov/snp/). (4) The minor allele frequency (MAF) of the SNPs was higher than 5%. (5) The \textit{EHBP1} and its SNPs (rs2710642 and rs10496099) might have been associated with blood lipids in previous studies.\textsuperscript{5,16,56}

\textbf{Genotyping.} DNA was extracted by a phenol–chloroform method and then sent to the Department for Next-Generation Sequencing, Sangon Biotech Co., Ltd. (Shanghai, China). Genotyping of the SNPs was performed on HiSeq XT En sequencers (Illumina, San Diego, CA). The sense and antisense primers of the two SNPs were as follows: rs2710642F 5′-TCTTTGTCCTTTTCATTTATGTG-TAGATA-3′ \textsuperscript{rs2710642R} 5′-GTCTTTTACCTTCCAACG-TATTGTGCTTT-3′; and rs10496099F 5′-TGGAACTCA-CATCTGGACAGATTTCG-3′, \textsuperscript{rs10496099R} 5′-CATTTTCTCCTTTGGCTTCTGATC-3′.

\textbf{Statistical Analyses.} SPSS software, version 25 (SPSS Inc., Chicago, IL), was employed to perform the statistical analysis. Quantitative variables with a normal distribution were described as the mean $\pm$ standard deviation (SD) and were analyzed using Student’s unpaired t-test and one-way analysis of variance (ANOVA) to compare differences. Nonnormally distributed data were expressed as medians and interquartile ranges and were compared by Mann–Whitney nonparametric tests. Qualitative variables were analyzed using $\chi^2$ analysis to evaluate the different ratios between the groups. The associations between genotypes or haplotypes and continuous serum lipid variables were analyzed by multivariable linear
regression. The difference in serum lipid levels associated with genotypes was considered statistically significant at a value of $P < 0.025$, and $P < 0.017$ was considered a significant difference in the association of lipids with haplotypes (corresponding to $P < 0.05$ after adjusting for two or three independent tests by the Bonferroni correction). The OR values were calculated by the binary logistic regression model—enter method, and a 95% CI was also provided. Stratified risk factors, including gender, age, smoking, alcohol consumption, BMI, hypertension, and fasting glucose, were adjusted in the model. The HWE, frequencies of genotype and haplotype, and the LD for pairs of selected mutations measured by $D'$ and $r^2$ were calculated by SHEsis software (http://analysis.bio-x.cn). Using R software (version 3.3.6), we calculated the $P$ of Pearson correlation analysis and drew a heat map of the correlated risk factors for serum lipid levels. The other graphics were drawn by GraphPad Prism (version 8.0.0).

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**Author Contributions**

C.-X.L. conceived the study, participated in the design, collected data, performed statistical analyses, and drafted the manuscript. R.-X.Y. conceived the study, participated in the design, organized the investigation of the epidemiology, collected the samples, and helped to modify the manuscript. Z.-H.S. performed statistical analyses. G.-X.D., P.-F.Z., B.-L.W., and Y.-Z.G. collected the samples. All authors read and approved the final manuscript.

**Notes**

The authors declare no competing financial interest.

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