Association of the NCAN-TM6SF2-CILP2-PBX4-SUGP1-MAU2 SNPs and gene-gene and gene-environment interactions with serum lipid levels

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Keywords: single nucleotide polymorphism, gene-gene interactions, gene-environment interactions, generalized multifactor dimensionality reduction, lipid level

Received: January 5, 2020   Accepted: May 1, 2020   Published: June 22, 2020

ABSTRACT

This study investigated the association of the NCAN-TM6SF2-CILP2-PBX4-SUGP1-MAU2 SNPs and gene-gene and gene-environment interactions with serum lipid levels in the population of Southwest China. Genotyping of 12 SNPs (i.e., rs2238675, rs2228603, rs58542926, rs735273, rs16996148, rs968525, rs17216525, rs12610185, rs10401969, rs8102280, rs73001065 and rs150268548) was performed in 1248 hyperlipidemia patients and 1248 normal subjects. The allelic and genotypic frequencies of the detected SNPs differed substantially between the normal and hyperlipidemia groups (P < 0.05-0.001), and the association of the 12 SNPs and hyperlipidemia was also observed (P < 0.004-0.0001). Four haplotypes (i.e., NCAN C-C, CILP2 G-T, PBX4-SUGP1 G-C, and MAU2 C-A-G-T) and 5 gene-gene interaction haplotypes (i.e., rs2238675C-rs2228603C, rs16996148G-rs17216525T, rs12610185G-rs10401969C, rs73001065G-rs8102280A-rs150268548G-rs968525C and rs73001065C-rs8102280A-rs150268548G-rs968525C) showed a protective effect, whereas four other haplotypes (i.e., TM6SF2 T-A, TM6SF2 C-A, MAU2 G-G-G-C and MAU2 C-G-A-T), as well as 4 gene-gene interaction haplotypes (i.e., rs58542926C-rs735273A, rs58542926T-rs735273A, rs73001065G-rs8102280G-rs150268548G-rs968525C, and rs73001065C-rs8102280G-rs150268548A-rs968525T), exhibited an inverse effect on hyperlipidemia (P < 0.05-0.0001). There were notable three-locus models comprising SNP-SNP, SNP-environment, and haplotype-haplotype interactions (P < 0.05-0.0001). The individuals with some genotypes and haplotypes reduced the prevalence of hyperlipidemia, whereas the individuals with some other genotypes and haplotypes augmented the prevalence of hyperlipidemia. The NCAN-TM6SF2-CILP2-PBX4-SUGP1-MAU2 SNPs and gene-gene and gene-environment interactions on hyperlipidemia were observed in the population of Southwest China.

INTRODUCTION

Cardiovascular diseases (CVDs) have been a notable cause of disability and death worldwide. Numerous epidemiological, clinical and experimental studies have demonstrated that hyperlipidemia is involved in the progression of atherosclerosis which, in turn, causes CVD. Hyperlipidemia has a vital function in the onset...
of atherosclerosis; it can lead to oxidative stress and chronic inflammation and induce damage to macromolecules, endothelial cell apoptosis, proliferation and migration of vascular smooth muscle cells, all of which involve the formation of atheroma, leading to the development of atherosclerosis [1]. Although people strive to change their lifestyle and take such medications as statins and other lipid-lowering drugs, the incidence of CVD is still increasing [2]. It is difficult for many individuals to reach standard serum lipid levels even after taking medications, or some of them may suffer from certain side effects [3]. Thus, it is essential to discover variants for new markers that regulate serum lipid profiles, which may facilitate efforts to further improve hyperlipidemia and thus may reduce the probability of CVD.

Recently, genome-wide association studies (GWAS) have identified numerous new loci at chromosome 19p13 that can modify lipid metabolism, such as the neurocan gene (NCAN, Gene ID:1463, OMIM: 600826), transmembrane 6 superfamily member 2 gene (TM6SF2, Gene ID: 53345, OMIM: 606563), cartilage intermediate layer protein 2 gene (CILP2, Gene ID:148113, OMIM: 612419), PBX homeobox 4 gene (PBX4, Gene ID:80714, OMIM: 608127), SURP and G-patch domain containing 1 gene (SUGP1, Gene ID:57794, OMIM: 607992, formerly known as F23858, RBP, SF4), and MAU2 sister chromatid cohesion factor gene (MAU2, Gene ID:23383, OMIM: 614560) [4, 5]. These loci are located in regions associated with morbidity due to coronary artery diseases (CAD) [6–8]. Kozlitina et al. [9] demonstrated that hepatic triglyceride content (HTGC) was associated with serum lipid levels. Configurations of the equilibrium (LD) and haplotype [24]. The causes of these variations have not been fully elucidated, but hyperlipidemia is considered to be a complex disease characterized by subtle interpatient variability, comprising host genetic factors and environmental interactions that generate disease phenotypes and establish disease advancement. Although a series of studies have revealed that environmental factors have determined the presence of dyslipidemia [20–22], it is also known that genetic factors have a vital role and can establish how an individual responds to challenges [6, 12]. Our previous study established that the BCL3-PVRL2-TOMM40 SNPs were located on chromosome 19 p11, the prevailing model of rs157580 and rs8100239 SNPs, and some haplotypes and gene-gene interaction haplotypes were involved in protection, although other haplotypes and gene-gene interaction haplotypes, including the prevailing model of rs6859, rs3810143, rs519113 and rs10402271 SNPs, indicated an augmented morbidity function [23]. Even though we have conducted substantial research and made extensive progress in identifying genetic modifiers, the relationship between hyperlipidemia and other gene polymorphisms has not been fully elucidated. In this study, we focus on the association of the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 single nucleotide variants, gene-environment interactions and gene-gene interactions with serum lipid levels. Configurations of the relationships among SNPs throughout the genome might be categorized with regard to linkage disequilibrium (LD) and haplotype [24].

RESULTS

Demographic and biochemical characteristics

Table 1 describes the typical characteristics of 2,496 participants from both groups. Systolic blood pressure, diastolic blood pressure, pulse pressure, TC, TG, high-density lipoprotein cholesterol (HDL-C) and LDL-C levels were substantially higher in hyperlipidemia than
Table 1. Comparison of demographic, lifestyle characteristics and serum lipid levels between the normal and hyperlipidemia groups.

| Parameter                          | Normal   | Hyperlipidemia | t (x²) | P    |
|------------------------------------|----------|----------------|--------|------|
| Number                             | 1248     | 1248           |        |      |
| Male/female                        | 478/770  | 487/761        | 0.137  | 0.742|
| Age (years)¹                       | 55.98±12.78 | 56.87±12.12     | 1.672  | 0.205|
| Height (cm)                        | 154.02±7.74  | 153.53±8.07    | 2.495  | 0.114|
| Weight (kg)                        | 53.01±8.92  | 52.95±10.60    | 23.359 | 2E-006|
| Body mass index (kg/m²)            | 22.31±3.22  | 22.36±3.70     | 3.630  | 0.057|
| Waist circumference                | 77.13±7.81  | 76.34±9.21     | 24.311 | 2E-007|
| Smoking status [n (%)]             |          |                |        |      |
| Non-smoker                         | 936(75.00) | 984(78.84)     |        |      |
| ≤ 20 cigarettes/day               | 276(22.11) | 233(18.66)     |        |      |
| > 20 cigarettes/day               | 36(2.89)   | 30(2.40)       | 5.378  | 0.068|
| Alcohol consumption [n (%)]        |          |                |        |      |
| Non-drinker                        | 1007(80.69) | 994(79.65)    |        |      |
| ≤ 25 g/day                        | 121(9.66)  | 136(10.90)      |        |      |
| > 25 g/day                        | 120 (9.65) | 118(9.45)      | 0.997  | 0.614|
| Systolic blood pressure (mmHg)     | 129.26±19.28 | 135.89±24.76 | 69.976 | 2E-016|
| Diastolic blood pressure (mmHg)    | 81.55±11.46 | 83.47±12.55   | 12.250 | E-005|
| Pulse pressure (mmHg)              | 47.71±15.29 | 52.42±18.56    | 50.587 | 4E-015|
| Glucose (mmol/L)                   | 6.18±1.91  | 6.15±1.43      | 21.278 | E-006|
| Total cholesterol (mmol/L)         | 4.97±1.05  | 5.21±1.09      | 6.203  | 0.012|
| Triglyceride (mmol/L)²             | 1.49(0.68) | 1.63(0.71)     | 7.036  | 0.005|
| HDL-C (mmol/L)                     | 1.75±0.50  | 1.81±0.60      | 12.497 | 2E-005|
| LDL-C (mmol/L)                     | 2.88±0.85  | 2.99±0.79      | 6.198  | 0.017|
| ApoA1 (g/L)                        | 1.35±0.26  | 1.39±0.32      | 0.361  | 0.548|
| ApoB (g/L)                         | 0.84±0.19  | 0.88±0.20      | 1.484  | 0.223|
| ApoA1/ApoB                         | 1.67±0.50  | 1.66±0.57      | 0.095  | 0.758|

HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol. Apo: Apolipoprotein. ¹Mean ± SD determined by t-test. ²Because the data were not normally distributed, the value of triglyceride was presented as median (interquartile range), and the difference between the two groups was determined by the Wilcoxon-Mann-Whitney test.

in normal groups ($P < 0.05$-$P < 0.001$ for all), whereas body weight, waist circumference, and blood glucose levels were significantly lower in hyperlipidemia than in normal groups ($P < 0.001$ for all). However, there was no substantial difference in age, sex ratio, height, body mass index (BMI), smoking status, alcohol consumption, ApoA1, ApoB levels, or the ApoA1/ApoB ratio between the two groups ($P > 0.05$ for all).

Genotypic and allelic frequencies in both groups

Figure 1 shows the locations, as well as the partial nucleotide sequences, of the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 SNPs, which are located on chromosome 19. The genotypes of 12 SNPs were confirmed by direct sequencing. As mentioned in Table 2, the genotypic distribution of 12 SNPs substantially conformed to Hardy-Weinberg equilibrium (HWE) in the hyperlipidemia and normal. The genotypic and allelic frequencies of 12 SNPs in the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 were substantially different between the hyperlipidemia and normal groups (Tables 2 and 3). The allelic frequencies of rs2238675C, rs2228603T, rs58542926T, rs735273G, rs16996148T, rs17216525T, rs12610185A, rs1040 1969T, rs73001065G, rs8102280G, rs150268548A, and rs968525T were substantially greater in hyperlipidemic individuals than in normal subjects ($P < 0.05$-$P < 0.001$, for all).

Genotypes and serum lipid profiles

The associations among the genotypes of the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 SNPs and
serum lipid concentrations are presented in Figure 2. The minor allele carriers had higher serum levels of TC (NCAN rs2238675, NCAN rs2228603, TM6SF2 rs5854292, TM6SF2 rs735273, CILP2 rs16996148, and MAU2 rs968525), TG (TM6SF2 rs5854292, TM6SF2 rs735273, CILP2 rs16996148, CILP2 rs17216525, PBX4 rs12610185, SUGP1 rs10401969, and MAU2 rs8102280), and LDL-C (CILP2 rs16996148, MAU2 rs73001065, and MAU2 rs150268548) than the minor allele noncarriers in both hyperlipidemia and normal groups (P < 0.004 for all).

**Haplotype-based association with hyperlipidemia**

As presented in Table 4, the most common haplotypes were the NCAN C-T, TM6SF2 T-A, PBX4-SUGP1 G-T and MAU2 C-G-A-T (≥ 30%, in all samples). The incidences of the NCAN C-C (G2), TM6SF2 T-A (G3), TM6SF2 C-A (G5), CILP2 G-T (G6), PBX4-SUGP1 G-G-A-G (G8), MAU2 G-G-G-C (G9), MAU2 G-A-G-C (G10), MAU2 C-G-A-T (G12), and MAU2 C-A-G-T (G13) haplotypes were significantly different between the hyperlipidemia and normal groups (P < 0.05 for all). In addition, the haplotypes of G2, G6, G8, and G13 showed a protective effect, whereas all of the G3, G5, G9 and G12 haplotypes showed an inverse effect (P < 0.05-0.001, respectively). The detected sites that were elucidated by multiple locus LD were not fully statistically independent in the participants. As presented in Figure 3, both the LD and the haplotypes block the combination of two groups. Figure 4 shows that carriers with the detected gene-gene interaction haplotypes had higher serum TC (rs58542926C-rs735273A and rs73001065C-rs8102280G-rs150268548A-rs968525T), LDL (rs73001065G-rs8102280G-rs150268548G-rs968525C, and rs73001065C-rs8102280G-rs150268548A-rs968525T), and TG (rs58542926T-rs735273A) levels than the haplotype non-carriers.

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**Figure 1.** Locations and partial nucleotide sequences of the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 SNPs. NCAN, the neurocan gene; TM6SF2, the transmembrane 6 superfamily member 2 gene; CILP2, the cartilage intermediate layer protein 2 gene; PBX4, the PBX homeobox 4 gene; SUGP1, the SURP and G-patch domain containing 1 gene; MAU2, the MAU2 sister chromatid cohesion factor gene.
Table 2. Comparison of the genotype frequencies between the normal and hyperlipidemia groups [n (%)].

| SNP     | Genotype | Normal (n=1248) | Hyperlipidemia(n=1248) | $\chi^2$ | $P$    |
|---------|----------|-----------------|-------------------------|----------|--------|
| NCAN rs2238675 C>T | CC       | 135(10.8)       | 164(13.1)               |          |        |
|         | CT       | 551(44.2)       | 578(46.3)               | 6.395    | 0.041  |
|         | TT       | 562(45.0)       | 506(40.5)               |          |        |
|         | $P_{HWE}$| 0.891           | 0.245                   |          |        |
|         | CC       | 454(36.4)       | 375(30.1)               |          |        |
| NCAN rs2228603 C>T | CT       | 597(47.8)       | 618(49.5)               | 15.334   | 3E-006 |
|         | TT       | 197(15.8)       | 255(20.4)               |          |        |
|         | $P_{HWE}$| 0.748           | 0.071                   |          |        |
|         | CC       | 713(57.1)       | 663(53.1)               |          |        |
| TM6SF2 rs58542926 C>T | CT       | 461(36.9)       | 484(38.8)               | 6.542    | 0.038  |
|         | TT       | 74(5.9)         | 101(8.1)                |          |        |
|         | $P_{HWE}$| 0.941           | 0.065                   |          |        |
|         | AA       | 989(79.3)       | 923(74.0)               |          |        |
| TM6SF2 rs735273 A>G | AG       | 244(19.6)       | 301(24.1)               | 10.317   | 0.006  |
|         | GG       | 15(1.2)         | 24(1.9)                 |          |        |
|         | $P_{HWE}$| 0.887           | 0.238                   |          |        |
|         | GG       | 984(78.9)       | 667(53.5)               |          |        |
| CILP2 rs16996148 G>T | GT       | 243 (19.5)      | 491(39.3)               | 187.550  | E-015  |
|         | TT       | 21 (1.7)        | 90(7.2)                 |          |        |
|         | $P_{HWE}$| 0.891           | 0.245                   |          |        |
|         | CC       | 64(5.1)         | 36(2.9)                 |          |        |
| CILP2 rs17216525 C>T | CT       | 437(35.0)       | 348(27.9)               | 26.428   | 3E-8   |
|         | TT       | 747(59.9)       | 864(69.2)               |          |        |
|         | $P_{HWE}$| 0.778           | 0.651                   |          |        |
|         | GG       | 241(19.3)       | 176(14.1)               |          |        |
| PBX4 rs12610185 G>A | GA       | 614(49.2)       | 584(46.8)               | 21.127   | E-7    |
|         | AA       | 393(31.5)       | 488(39.1)               |          |        |
|         | $P_{HWE}$| 0.886           | 0.628                   |          |        |
|         | TT       | 437(35.0)       | 476(38.1)               |          |        |
| SUGP1 rs10401969 T>C | TC       | 608(48.7)       | 613(49.1)               | 7.034    | 0.030  |
|         | CC       | 203(16.3)       | 159(12.7)               |          |        |
|         | $P_{HWE}$| 0.781           | 0.104                   |          |        |
|         | GG       | 63(5.0)         | 81(6.5)                 |          |        |
| MAU2 rs73001065 G>C | GC       | 435(34.9)       | 511(41.0)               | 14.640   | 0.001  |
|         | CC       | 750(60.1)       | 656(52.6)               |          |        |
|         | $P_{HWE}$| 0.884           | 0.111                   |          |        |
|         | GG       | 582(46.4)       | 640(51.3)               |          |        |
| MAU2 rs8102280 G>A | GA       | 540(43.3)       | 507(40.6)               | 6.546    | 0.038  |
|         | AA       | 126(10.3)       | 101(8.1)                |          |        |
|         | $P_{HWE}$| 0.872           | 0.566                   |          |        |
|         | GG       | 227(18.20)      | 171(13.7)               |          |        |
| MAU2 rs150268548 G>A | GA       | 611(49.0)       | 582(46.6)               | 16.568   | 7E-006 |
|         | AA       | 410 (32.9)      | 495(39.7)               |          |        |
Gene-gene (G × G) interaction-based association with hyperlipidemia

As shown in Table 5, the most common G × G interaction was C-C-A-G-C-A-T-C-G-A-T (H1, > 15%, in all samples). The frequencies of the C-C-C-A-G-C-A-T-C-G-A-T (H1), T-C-C-A-G-C-A-T-C-G-A-T (H2), T-T-C-A-G-C-A-T-C-G-A-T (H3), C-C-C-A-G-C-A-T-C-A-G-C (H5), C-C-C-A-G-C-G-C-G-A-G-C (H6), T-T-A-G-C-G-C-G-C-G-A-T (H7), T-T-A-G-C-G-C-G-C-G-A-G-C (H8), and T-T-A-G-C-A-T-C-A-G-C (H9) G × G interactions were significantly different between the normal and hyperlipidemia groups (P < 0.05 for all). Meanwhile, the G × G interactions of H1, H2, H6, H8 and H9 contributed to a protective effect, while the G × G interaction of H3, H5 and H7 showed an inverse effect. The H2, H6, H8 and H9 carriers had low TC levels, but the H5 and H7 carriers had high TC levels; the H1 carriers had low TG levels, but the H3 carriers had high TG levels; the H7 carriers had high LDL-C levels, and the H9 carriers had low LDL-C levels; and the H5 carriers had high ApoA1 levels in both the normal and hyperlipidemia groups (Figure 5; P < 0.006 for all).

G × G and gene-environment (G × E) interactions on hyperlipidemia

Entropy-based interaction dendrogram (Figure 6) and proportional hazard model results (Figure 7) show the strongest synergy of SNP-SNP interaction between rs735273 and rs16996148 and haplotype-haplotype interaction between G10 and G6. However, these results showed a redundancy effect in SNP-environment interaction (rs16996148 GT/TT and diabetes, rs16996148 TT and diabetes increased the risk of hyperlipidemia. The haplotype-haplotype interaction showed that G10 (MAU2 G-A-G-C) and G6 (CILP2 G-T) carriers could reduce the risk of hyperlipidemia compared with G10 or G6 carriers. With regard to the gene-gene interaction between H3 (T-T-C-A-G-C-A-T-C-G-A-T) and H6 (C-C-C-A-G-C-G-C-G-A-G-C) carriers, we found that the latter showed an inferior risk of hyperlipidemia, while the former indicated an augmented probability of hyperlipidemia. As a genotype-environment interaction was considered, G6 (CILP2 G-T) carriers and diabetes increased the risk of hyperlipidemia. A similar result was shown in the gene-environment interaction between H6 (C-C-C-A-G-C-G-C-G-A-G-C) carriers and diabetes.

**DISCUSSION**

The major new findings in this study were as follows: (1) The study showed the single nucleotide mutation frequencies, haplotype frequencies and interaction of G × G interlocus frequencies among 12 NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 SNPs in the people from Southwest China for the first time; (2) It also presented new evidence that single nucleotide mutation, haplotype, G × G and G × E interactions among the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 SNPs are probably closely associated with serum lipid levels; (3) We established some new diversity effects from the interactions of SNP-SNP, SNP-environment, haplotype-haplotype, haplotype-environment, G × G and G × E; and (4) We also found different interactions that augmented the risk of hyperlipidemia.

Hyperlipidemia is the main risk factor that can result in CVD, which accounts for approximately 4 million deaths each year worldwide [25, 26]. High levels of TC can contribute to the risk for CAD [27], ischemic cerebrovascular accident [28], aortic dissection and peripheral arterial disease [29]. It has been demonstrated that TG levels have an intense association with non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome [30]. NAFLD and metabolic
Table 3. Comparison of the allele frequencies between the normal and hyperlipidemia groups [n (%)].

| SNP     | Allele | Normal (n=1248) | Hyperlipidemia (n=1248) | $\chi^2$ | P    |
|---------|--------|----------------|-------------------------|---------|------|
| NCAN    | C/T    | 821(33.0)/1675(67.1) | 906(36.3)/1590(63.7) | 6.396   | 0.011|
| NCAN    | G/A    | 1505(60.3)/991(39.7) | 1368(54.8)/1128(45.2) | 15.390  | E-005|
| TM6SF2  | C/T    | 1887(75.6)/609(24.4) | 1810(72.5)/686(27.5) | 6.182   | 0.013|
| TM6SF2  | A/G    | 2222(89.0)/274(11.0) | 2147(86.0)/349(14.0) | 10.316  | 0.001|
| CILP2   | G/T    | 2211(88.6)/285(11.4) | 1825(73.1)/671(26.9) | 192.770 | 4E-012|
| CILP2   | C/T    | 565(22.6)/1931(77.4) | 420(16.8)/2078(83.2) | 26.592  | 3E-007|
| PBX4    | G/A    | 1096(43.9)/1400(56.1) | 936(37.5)/1560(62.5) | 21.247  | 2E-007|
| SUGP1   | T/C    | 1482(59.4)/1014(40.6) | 1565(62.7)/931(37.3) | 5.803   | 0.016|
| MAU2    | G/C    | 561(22.5)/1935(77.5) | 673(27.0)/1823(73.0) | 13.503  | 9E-004|
| MAU2    | G/A    | 1704(68.3)/792(31.7) | 1787(71.6)/709(28.4) | 6.563   | 0.010|
| MAU2    | G/A    | 1065(42.7)/1431(57.3) | 924(37.0)/1572(63.0) | 16.616  | 6E-005|
| MAU2    | C/T    | 1031(41.3)/1465(58.7) | 951(38.1)/1545(61.9) | 5.355   | 0.021|

NCAN: the neurocan gene. TM6SF2: the transmembrane 6 superfamily member 2 gene. CILP2: the cartilage intermediate layer protein 2 gene. PBX4: the PBX homeobox 4 gene. SUGP1: the SURP and G-patch domain containing 1 gene. MAU2: the MAU2 sister chromatid cohesion factor gene.

Dyslipidemia is the result of a combination of genetic and environmental factors that have been universally recognized worldwide [51, 52]. China is a multiethnic country with 56 ethnic groups [53]. Han nationality is the largest ethnic group, and the rest of 55 ethnic groups are closely connected with abnormal serum lipid levels [45]. Compared with the normal groups, there was a higher percentage of smoking and alcohol intake in the hyperlipidemia group. A large number of Southwest Chinese adults enjoy drinking. Most people who live in rural areas usually make wine themselves by using corns, cereals and cassava. It has been documented that alcohol could elevate serum levels of HDL-C and benefit CAD [46, 47]. However, it has also been reported that the elevation of HDL-C levels was set off by increased smoking levels. Smoking could increase the serum concentrations of TC, TG and LDL-C, but it could decrease serum levels of HDL-C [48, 49]. This phenomenon may be a suitable explanation for the current results of serum lipid levels between the two groups. There might be an effect of modifiable or non-modifiable risk factors on genetic variants identified in GWAS of disease. Recently, a number of variants have been identified to be connected with lifestyle behaviors and health outcomes in GWAS. From the example of tobacco and alcohol research that we discussed above, behavioral phenotypes can be predicted by a genetic variant, which has been shown in GWAS of disorders that informally interact with these activities. It is important to explain GWAS findings [50].
distributed in different areas of the country. The genotypic and allelic frequencies of many SNPs in some genes were inconsistent in diverse racial/ethnic groups [54–56]. There may also be an ethnic difference in lifestyle and environmental factors, as well as in genetic background. To the best of our knowledge, the \textit{TM6SF2} rs58542926 SNP increased the risk of NAFLD in the eastern Chinese Han population [57]. The SNP of rs16996148 in \textit{NCAN-CILP2} or \textit{NCAN/CILP2/PBX4} was significantly associated with dyslipidemia in the midlands and east of the Chinese Han population [8, 58]. The studies mentioned above suggested that genetic variants of those genes in chromosome 19p13 confer susceptibility to dyslipidemia in the Chinese populations. However, the relationship between dyslipidemia and \textit{SUGP1} and \textit{MAU2} is not clear in the Chinese populations, and the association between SNPs, gene-gene, and gene-environment interactions and dyslipidemia is still limited. With the rapid development of biomedicine technology, we are entering a precision medicine era, and precision medicine seeks to identify and classify individual patients such that optimal treatment decisions can be made. It is essential to explore the \textit{NCAN-TM6SF2-CILP2-PBX4-SUGP1-MAU2} SNPs, gene-gene and gene-environment interactions on serum lipid levels in Southeast China and other areas of Chinese populations. These results may help us to take precise treatment for dyslipidemia and decrease the risk of CVD.

The current study has several limitations. First, the sample size is comparatively small. Thus, additional studies with large sample sizes are necessary. Second, lower numbers of individuals are obtainable for minor allele frequency (MAF) of certain variants, and it is relatively weak in calculating a strong power. Third, numerous unmeasured environmental and genetic factors must be determined, such as dietary patterns, physical exercises, and energy intake. Finally, we should define the relevance of this finding with a high criterion in further studies, including incorporating the genetic information of the \textit{NCAN}, \textit{TM6SF2}, \textit{CILP2}, \textit{PBX4}, \textit{SUGP1} and \textit{MAU2} single nucleotide mutations, haplotypes, interactions of G × G and G × E from \textit{in vivo} to \textit{in vitro}, and testing the effect of genetic variants with different molecular biological levels, such as genetic transcription and expression.

Figure 2. Association of the \textit{NCAN}, \textit{TM6SF2}, \textit{CILP2}, \textit{PBX4}, \textit{SUGP1} and \textit{MAU2} genotypes and serum lipid parameters in the normal and hyperlipidemia groups. TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; TG, triglyceride. $^a$P < 0.004, $^b$P < 0.001, and $^c$P < 0.0001.
Table 4. Comparison of the haplotype frequencies between the normal and hyperlipidemia groups [n (frequency)].

| NoO. | Haplotype          | Hyperlipidemia | Normal   | χ²   | P      | OR (95% CI) |
|------|--------------------|----------------|----------|------|--------|-------------|
| G1   | NCAN C-T           | 815.80(0.3)    | 818.97(0.3) | 0.091 | 0.762  | 1.0 (0.9-1.2) |
| G2   | NCAN C-C           | 0.08(0.0)      | 0.20(0.0) | 20.637 | 6E-006 | 0.7 (0.7-0.8) |
| G3   | TM6SF2 T-A         | 626.97(0.3)    | 611.80(0.3) | 16.728 | 4E-005 | 1.4 (1.2-1.6) |
| G4   | TM6SF2 C-G         | 20.95(0.0)     | 0.00(0.0) | 0.446  | 0.504  | 1.1 (0.9-1.2) |
| G5   | TM6SF2 C-A         | 84.00(0.0)     | 211.15(0.1) | 10.752 | 0.001  | 1.3 (1.1-1.5) |
| G6   | CILP2 G-T          | 0.00(0.0)      | 12.00(0.0) | 14.534 | 0.001  | 0.7 (0.6-0.8) |
| G7   | PBX4-SUGP1 G-T     | 1524.00(0.6)   | 1523.83(0.6) | 0.049  | 0.826  | 1.0 (0.9-1.1) |
| G8   | PBX4-SUGP1 G-C     | 35.96(0.3)     | 36.16(0.0) | 12.128 | 5E-004 | 0.8 (0.7-0.9) |
| G9   | MAU2 G-G-G-C       | 480.04(0.2)    | 371.85(0.2) | 15.976 | 6E-005 | 1.4 (1.2-1.6) |
| G10  | MAU2 G-A-G-C       | 61.44(0.3)     | 86.22(0.0) | 3.662  | 0.036  | 0.7 (0.5-1.0) |
| G11  | MAU2 C-A-A-T       | 203.71(0.0)    | 252.01(0.1) | 4.376  | 0.557  | 0.8 (0.7-0.9) |
| G12  | MAU2 C-G-A-T       | 1461.50(0.6)   | 1341.77(0.5) | 20.71  | 5E-006 | 1.3 (1.2-1.5) |
| G13  | MAU2 C-A-G-T       | 645.79(0.3)    | 753.77(0.3) | 8.189  | 0.004  | 0.8 (0.7-0.9) |

NCAN: the neurocan gene. TM6SF2: the transmembrane 6 superfamily member 2 gene. CILP2: the cartilage intermediate layer protein 2 gene. PBX4: the PBX homeobox 4 gene. SUGP1: the SURP and G-patch domain containing 1 gene. MAU2: the MAU2 sister chromatid cohesion factor gene. The haplotype is combined with NCAN rs2238675-rs2228603, TM6SF2 rs58542926-rs735273, CILP2 rs16996148-rs17216525, PBX4-SUGP1 rs12610185-rs10401969, and MAU2 rs73001065-rs8102280-rs150268548-rs968525.

In conclusion, this study shows potential interactions among the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2, environment and serum lipid levels in hyperlipidemia subjects. Our findings also showed that the interactions increased the risk of hyperlipidemia over single-locus tests. In addition, these factors exhibit distinctive collaboration or redundancy effects on morbidity.

![Figure 3. Results of linkage disequilibrium (LD) analyses of the NCAN, TM6SF2, CILP2, PBX4, SUGP1 and MAU2 SNPs.](image-url)
MATERIALS AND METHODS

SNP selection

Twelve SNPs in the *NCAN, TM6SF2, CILP2, PBX4, SUGP1* and *MAU2* were selected as follows: (1) *NCAN*, which was associated with serum lipid levels, was selected from a previous GWAS. The gene clusters of *TM6SF2-CILP2-PBX4-SUGP1-MAU2* were closely associated with lipid metabolism and *NCAN*. (2) Information regarding Tagging SNPs, functional SNPs, and predicted SNPs can be found in our previous article [23]. (3) SNP information was obtained from NCBI dbSNP Build 132 (http://www.ncbi.nlm.nih.gov/SNP/), which can be found in Supplementary Table 1. The MAF was restricted to greater than 1% in SNPs. (4) There might be some association between those SNPs and serum lipid levels or cardio cerebral vascular diseases in previous studies. (5) *NCAN* rs2238675-rs2228603, *TM6SF2* rs58542926-rs735273, *CILP2* rs16996148-rs17216525, *PBX4-SUGP1* rs12610185-rs10401969, and *MAU2* rs73001065-rs8102280-rs150268548-rs968525 were chosen by the block-based method. This strategy is facilitated by the associations among tagging SNPs and is demonstrated as LD ($D' > 0.7$).

Subjects

The sample sizes were calculated by Quanto software (Version 1.2, https://quanto.software.informer.com/1.2/) at the beginning of this study, and they were sufficient to satisfy the statistical power. A total of 1248 unrelated patients with hyperlipidemia were enrolled from the First Affiliated Hospital, Guangxi Medical University from Sep. 1, 2016 to Dec. 31, 2018. Participants were 18 to 80 years old (mean 55.98 ± 12.78 years), and patients with a family history of hyperlipidemia were excluded. Meanwhile, a total of 1248 randomly selected adults served as the control group. They underwent periodical medical check-ups, and their age, gender and ethnic group were matched to the patients. They were 18 to 80 years old (mean 56.87 ± 12.12 years). There was no history of major diseases in any participants.

Figure 4. Association of the *NCAN, TM6SF2, CILP2, PBX4, SUGP1* and *MAU2* haplotypes and serum lipid parameters in the normal and hyperlipidemia groups. TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; TG, triglyceride. \(^{a}P < 0.006, ^{b}P < 0.001. \text{and } ^{c}P < 0.0001.\)
Table 5. Comparison of G × G interaction frequencies between the normal and hyperlipidemia groups [n (frequency)].

| No | G × G interaction | Hyperlipidemia | Normal | $x^2$ | P | OR (95%CI) |
|----|-------------------|----------------|--------|-------|---|-----------|
|    | A                 | B              | C      | D     | E  | F         | G      | H     | I     | J         | K       | L         |
| H1 | C                 | C              | A      | G     | C  | A       | T      | C      | G     | A       | T        | 440.78 (0.177)  |
|    | H2                | T              | C      | A     | G  | C       | A     | T      | C      | G     | A       | T        | 219.96 (0.088)  |
|    | H3                | T              | T      | C     | A  | G       | C     | A     | T      | C      | G       | A       | 162.55 (0.065)  |
|    | H4                | T              | T      | T     | C  | A       | G     | C     | A     | T      | C       | 119.10 (0.048)  |
|    | H5                | C              | C      | C     | A  | G       | C     | A     | T      | C     | A       | G       | 109.61 (0.044)  |
|    | H6                | C              | C      | C     | A  | G       | C     | G     | C     | G     | A       | G       | 55.83 (0.022)  |
|    | H7                | T              | T      | T     | A  | G       | C     | G     | C     | C     | G       | A       | 95.07 (0.038)  |
|    | H8                | T              | T      | T     | A  | G       | C     | C     | A     | G     | C       | C       | 42.20 (0.017)  |
|    | H9                | T              | T      | T     | A  | G       | C     | A     | T      | C     | A       | G       | 16.45 (0.007)  |

A: NCAN rs2238675 C>T.  
B: NCAN rs2228603 C>T.  
C: TM6SF2 rs58542926 C>T.  
D: TM6SF2 rs735273 A>G.  
E: CILP2 rs16996148 G>T.  
F: CILP2 rs17216525 C>T.  
G: PBX4 rs12610185 G>A.  
H: SUGP1 rs10401969 T>C.  
I: MAU2 rs73001065 G>C.  
J: MAU2 rs8102280 G>A.  
K: MAU2 rs15026854 G>A.  
L: MAU2 rs968525 C>T.

None of the participants took any medications that might have any impact on lipid metabolism. This study design was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (No. Lunshen 2014-KY-Guoji-001; Mar. 7, 2014). Informed consent was obtained from all participants.

**Clinical data**

The clinical data were obtained by means of a universally standardized technique [38]. Standardized questionnaires were administered to acquire details of demographics, socioeconomic standing and lifestyle dynamics. The status of cigarette smoking was categorized into ≤ 20 cigarettes per day and > 20 cigarettes per day [59]. Alcohol intake was classified based on the grams of alcohol per day: ≤ 25 and > 25 [23]. Details regarding other factors, such as height, weight, waist circumference, blood pressure, and BMI (kg/m²), were also acquired.

**Biochemical measurements**

Venous blood samples were acquired following 12 h of fasting. TC, HDL-C, LDL-C and TG concentrations in serum were detected by means of Tcho-1, TG-LH (RANDOX Laboratories, UK), Cholestest N HDL, and Cholestest LDL (Daiichi Pure Chemicals Co., Ltd., Japan) kits, respectively. ApoA1 and ApoB concentrations in serum were determined by immunoassay (RANDOX Laboratories). Detection of all samples was completed with an autoanalyzer (Hitachi Ltd., Japan) [60].

**Diagnostic criteria**

The standard values of serum lipid levels in our clinical biochemistry laboratory were as follows: TC (3.10–5.17 mmol/L), TG (0.56–1.70 mmol/L), HDL-C (1.16–1.42 mmol/L), LDL-C (2.70–3.10 mmol/L), ApoA1 (0.80–1.05 g/L), and the ApoA1/ApoB ratio (1.00–2.50). Hyperlipidemia was diagnosed with serum levels of TC > 5.17 mmol/L and/or TG > 1.70 mmol/L [61, 62]. The diagnosis of hypertension was made as per the Seventh Report of Joint National Committee (JNC-7) [63]. BMI was classified as normal (< 24 kg/m²), overweight (24–28 kg/m²) or obese (> 28 kg/m²).

**Genotyping**

Extraction of genomic DNA was accomplished by utilizing the conventional phenol-chloroform method in venous blood leukocytes. Genotyping of the 12 variants was performed on the Snapshot of next generation sequencing technology platform HiSeq XTen (Illumina, USA) in Sangon Biotech Co., Ltd. (Shanghai, China). The details regarding sense and antisense primers are provided in Supplementary Table 2.
Figure 5. $G \times G$ haplotype-based association with serum lipid levels in normal and hyperlipidemic individuals. TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; TG, triglyceride. $^{a} P < 0.006$, $^{b} P < 0.001$, and $^{c} P < 0.0001$. 
Figure 6. Various sorts of gene-gene and gene-environment interaction dendrograms. Elements with strong interactions appear close together, and elements with weak interactions appear distant from each other.

| Subgroup                          | Variable 1 | Variable 2 | Hazard Ratio (95% CI) | P-value |
|-----------------------------------|------------|------------|-----------------------|---------|
| SNP-SNP interactions              | rs73573    | rs16996148 |                       |         |
|                                   | AA         | GG         | 1                     |         |
|                                   | AA         | GT+TT      | 1.23(0.92-1.66)       | 0.1323  |
|                                   | AG+GG      | TT         | 0.62(0.44-1.16)       | 0.2182  |
|                                   | AG+GG      | GT+TT      | 0.81(0.57-1.23)       | 1.70E-03|
| SNP-Environment interactions      | rs16996148 | Diabetes   |                       |         |
|                                   | GG         | No         | 1                     |         |
|                                   | TT         | Yes        | 1.24(0.88-1.43)       | 0.611   |
|                                   | GT+TT      | No         | 0.81(0.64-1.28)       | 0.068   |
|                                   | GT+TT      | Yes        | 1.14(0.77-1.93)       | 0.0009  |
| Haplotype-Haplotype interactions  | G10        | G6         |                       |         |
| Non-carriers                      | Non-carriers| 1         |                       |         |
| Carriers                          | Non-carriers| 0.87(0.78-1.25)| 3.09E-04|         |
| Non-carriers                      | Carriers   | 0.64(0.53-1.18)| 0.0278  |         |
|                                   | Carriers   | 0.47(0.37-1.09)| 4.30E-05|         |
| Haplotype-Environment interactions| G6         | Diabetes   |                       |         |
| Non-carriers                      | No         | 1          |                       |         |
| Carriers                          | No         | 0.77(0.54-1.24)| 0.422   |         |
| Carriers                          | Yes        | 1.18(0.76-2.38)| 3.40E-05|         |
| Gene-Gene interactions            | H3         | H6         |                       |         |
| Non-carriers                      | Non-carriers| 1         |                       |         |
| Carriers                          | Non-carriers| 1.31(0.84-2.17)| 1.50E-04|         |
| Non-carriers                      | Carriers   | 0.77(0.54-1.03)| 0.0019  |         |
|                                   | Carriers   | 0.89(0.64-1.22)| 2.30E-05|         |
| Gene-Environment interactions     | H6         | Diabetes   |                       |         |
| Non-carriers                      | No         | 1          |                       |         |
| Carriers                          | No         | 1.24(0.87-1.76)| 0.212   |         |
| Carriers                          | 0.73(0.56-1.15)| 0.431   |         |
| Carriers                          | Yes        | 1.12(0.75-1.97)| 2.20E-05|         |

Figure 7. SNP-SNP, SNP-environment, haplotype-haplotype, haplotype-environment, gene-gene and gene-environment interactions on the risk of hyperlipidemia.
Statistical analyses

SPSS 22.0 (IBM SPSS Inc., USA) was employed to analyze the data. Quantitative variables of normally distributed data are represented as the mean ± SD, while serum TG levels of non-normally distributed data are represented as medians and interquartile ranges. Typical features between the normal and hyperlipidemia groups were compared by means of analysis of covariance. Distribution of the genotypes and interactions of alleles, haplotypes, G × G between normal and hyperlipidemia groups were examined by chi-square test; the HWE, pairwise LD, haplotype frequencies and G × G interaction containing the variants were computed by means of Haploview (version 4.2; Broad Institute of MIT and Harvard). The pattern of pairwise LD among 12 SNPs was tested by $D'$ using Haploview software. We employed Univariate to test associations between genotypes, haplotypes, G × G interactions and lipid phenotypic variations. $P < 0.004$ represented statistical significance in the association between any variants and lipid phenotypic variations (equivalent to $P < 0.05$ after adjusting for 12 independent tests by the Bonferroni correction). The association between genotypes, alleles, haplotypes, G × G interactions and lipid phenotypic variants was performed using unconditional logistic regression evaluation. Other parameters were adjusted for the data analysis. The greatest interaction pattern among genes, SNPs and environmental exposures was screened by means of generalized multifactor dimensionality reduction [64]. The cross-validation consistency score was performed to identify the best model of selected interaction among all probabilities. The testing balanced accuracy was a measure of the degree to which the interaction precisely calculates case-control status with scores between 0.50 (representing that the model projects no better than chance) and 1.00 (representing impeccable prediction). Finally, to evaluate whether an identified model is significant, we used a sign test or a permutation test for accuracy of prediction.

Availability of data and materials

The datasets generated during the present study are not publicly available, because detailed genetic information of each participant was included in these materials.

Abbreviations

Apo: apolipoprotein; BMI: body mass index; CAD: coronary artery disease; CILP2: the cartilage intermediate layer protein 2 gene; CVDs: cardiovascular diseases; DNA: deoxyribonucleic acid; E: environment; G: gene; GWAS: genome-wide association studies; HDL-C: high-density lipoprotein cholesterol; HMGCR: 3-hydroxy-3-
methylglutaryl coenzyme A (HMG CoA) reductase; HTGC: hepatic triglyceride content; HWE: Hardy-Weinberg equilibrium; JNC-7: the Seventh Report of Joint National Committee; LD: linkage disequilibrium; LDL-C: low-density lipoprotein cholesterol; MAF: minor allele frequency; MAU2: the MAU2 sister chromatid cohesion factor gene; NAFLD: non-alcoholic fatty liver disease; NCAN: the neurocan gene; PBX4: the PBX homeobox 4 gene; siRNA: small interfering Ribonucleic Acid; SNP: single nucleotide polymorphism; SUGP1: the SURP and G-patch domain containing 1 gene; TC: total cholesterol; TG: triglyceride; TM6SF2: the transmembrane 6 superfamily member 2 gene.

AUTHOR CONTRIBUTIONS

G.-X.D. conceived the study, participated in the design, collected the clinical data and samples, performed the statistical analyses, and drafted the manuscript. R.-X.Y. conceived the study, participated in the design, collected the clinical data and samples, and helped to draft the manuscript. Y.-Z.G., C.-X.L., P.-F.Z., B.-L.W., J.-Z.W. and L.M. collected the clinical data and samples. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We acknowledge and appreciate our colleagues for their valuable efforts and comments on this paper.

CONFLICTS OF INTEREST

The authors have no potential financial or non-financial conflicts of interest to report.

FUNDING

The authors acknowledge the essential role of the Funding of the National Natural Science Foundation of China (No: 81460169), Guangxi Self-financing Research Projects (Z20190025), Guangxi Medical and Health Key Discipline Construction Project (2020-2024) and the Project of Liuzhou People’s Hospital (LRY202007) in this motif.

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PMID:27096864
### Supplementary Table 1. Characteristics of the 19p13.11 gene mutations.

| SNV ID (rs#) | HGVS Name | Chr: Position | Contig | Contig Pos | SNP to Chr | Allele | MAF/Minor | Map Methods |
|--------------|-----------|---------------|--------|------------|------------|--------|-----------|-------------|
| **NCAN**     |           |               |        |            |            |        |           |             |
| rs2238675    | NM_004386.2: c.1072+529 C>T | 19:19225799 | NT_011295.12 | 19165799    | Fwd | T | T=0.0960/481 (1000 Genomes) | mapup        |
| rs2228603    | NM_004386.2: c.274 C>T | 19:19159115 | NT_011295.12 | 19159115    | Fwd | C | T=0.0439/220 (1000 Genomes) | mapup        |
| **TM6SF2**   |           |               |        |            |            |        |           |             |
| rs58542926   | NM_001001524.2: c.499 C>T | 19:19208740 | NT_011295.12 | 19208740    | Fwd | T | T=0.0667/334 (1000 Genomes) | mapup        |
| rs735273     | NM_001001524.2: c.-1387 A>G | 19:19214602 | NT_011295.12 | 19214602    | Fwd | G | C=0.4655/2331 (1000 Genomes) | mapup        |
| **CILP2**    |           |               |        |            |            |        |           |             |
| rs16996148   | NC_000019.9: g.19658472 G>T | 19:19487663 | NT_011295.12 | 19487663    | Fwd | G | T=0.1156/579 (1000 Genomes) | mapup        |
| rs17216525   | NC_000019.9: g.19662220 C>T | 19:19491411 | NT_011295.12 | 19491411    | Fwd | C | T=0.0815/408 (1000 Genomes) | mapup        |
| **PBX4**     |           |               |        |            |            |        |           |             |
| rs12610185   | NM_025245.2: c.119+7598 C>T | 19:19550913 | NT_011295.12 | 19550913    | Fwd | C | T=0.1132/567 (1000 Genomes) | mapup        |
| **SUGP1**    |           |               |        |            |            |        |           |             |
| rs10401969   | NM_172231.3: c.1243+80 A>G | 19:19236909 | NT_011295.12 | 19236909    | Fwd | A | G=0.1176/589 (1000 Genomes) | mapup        |
| **MAU2**     |           |               |        |            |            |        |           |             |
| rs73001065   | NM_015329.3: c.1548+296 G>C | 19:19289732 | NT_011295.12 | 19289732    | Fwd | G | C=0.0319/160 (1000 Genomes) | mapup        |
| rs8102280    | NM_015329.3: c.1155+15 G>A | 19:19284941 | NT_011295.12 | 19284941    | Fwd | G | A=0.0333/167 (1000 Genomes) | mapup        |
| rs150268548  | NC_000019.9: g.19494483 G>A | 19:19323674 | NT_011295.12 | 19323674    | Fwd | G | A=0.0260/130 (1000 Genomes) | mapup        |
| rs968525     | NM_015329.3: c.1309-483 C>T | 19:19288406 | NT_011295.12 | 19288406    | Rev | C | T=0.3033/1519 (1000 Genomes) | mapup        |
Supplementary Table 2. The sequences of forward and backward primers of the 19p13.11 gene mutations.

| Gene   | Primer sequence                      |
|--------|--------------------------------------|
| NCAN   | TGGAAGAGAGATAATGCCTCAATTGGC          |
|        | GGTAGTGTCCAACCTCTCATGAACCTTG         |
| rs2238675 | TCCAACCCAGGCACACAGGATAT              |
| rs2228603 | GCCACCCCTCAGCAGCATTTGTC              |
| TM6SF2 | CCTCCCCCTTCTTTCTTTGACACAA          |
| rs58542926 | CCTGCACCATGGAAGGCAATAA              |
| rs735273 | CTGCACTGGACAAATCTCTAAC              |
|        | CCCGCTTACAGAAGGCTCATTTTA            |
| CILP2  | CCGATCTCATCATTACCCACTC              |
| rs16996148 | GTCCACCCTAGGGAAGGAAG                |
| rs17216525 | CAGCCAGGAGGATAGAAGATACT              |
| PBX4   | TTCTTTGAGCTGCACCATTCTG              |
| rs12610185 | TGTCAAACACAAAAAACCAACACAATT          |
| SUGP1  | GGGAATTTTATGAGGAAATTTCCCAGA         |
| rs10401969 | ATTGCAATAGGCCCAGCAATTCC              |
|        | TTGGAAGGCTCTGACTTCTCTTCAC           |
| MAU2   | GCATGGCAGTTTTCATCTATGC              |
| rs73001065 | CCCTCAGGTGCCACACACAG               |
| rs8102280 | CAGTTTGGTACAGACAGGACATG             |
| rs150268548 | GAAAGTTCTGGATAGGTAACTTTAC           |
|        | GGCAAAGTGCGCTTTTTTCT                |
| rs968525 | GGCGTGATCTGACTGATAATTGTGACT        |