Association of rs662799 in APOA5 with CAD in Chinese Han population

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Abstract

Background: CAD (Coronary Artery Disease) is a complex disease that influenced by various environmental and genetic factors. Previous studies have found many single nucleotide polymorphisms (SNPs) associated with the risk of CAD occurrence. However, the results are inconsistent. In this study, we aim to investigate genetic etiology in Chinese Han population by analysis of 7 SNPs in lipid metabolism pathway that previously has been reported to be associated with CAD.

Methods: A total of 631 samples were used in this study, including 435 CAD cases and 196 normal healthy controls. SNP genotyping were conducted via multiplex PCR amplifying followed by NGS (next-generation sequencing).

Results: Rs662799 in APOA5 (Apolipoprotein A5) gene was associated with CAD in Chinese Han population (Odds-ratio = 1.374, P-value = 0.03). No significant association was observed between the rest of SNPs and CAD. Stratified association analysis revealed rs5882 was associated with CAD in non-hypertension group (Odds-ratio = 1.593, P-value = 0.023). Rs1800588 was associated with CAD in smoking group (Odds-ratio = 1.603, P-value = 0.035).

Conclusion: The minor allele of rs662799 was the risk factor of CAD occurrences in Chinese Han population.

Keywords: Coronary artery disease, Chinese Han population, Case-control study, Single nucleotide polymorphisms

Background

Coronary Artery Disease (CAD) is a common type of cardiovascular disease which is the leading cause of death worldwide [1]. In China, it was estimated that 700,000 people died from CAD each year [2]. Many environmental factors have been identified to be associated with CAD, including diabetes, hypertension, smoking, TG (Triglyceride), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), TC (Serum total cholesterol) [3]. Genetic variants also contribute to CAD risk [4, 5]. To date, Genome Wide Association Study (GWAS) studies have identified many SNPs to be associated with CAD among different populations [6–10], including SNPs located within or nearby lipid metabolism genes. Some of them were successfully replicated in different populations, but there are still inconsistent results in some researches.

In this study, we aim to investigate the association of 7 SNPs of lipid metabolism genes (LIPC rs1800588, LPL rs320, APOC3 rs5128, CETP rs5882, PON1 rs662, APOA5 rs662799, APOB rs693) with the risk of developing CAD in Chinese Han population.

Methods

Patients and controls

The participants in this study were recruited from Wuhan General Hospital of Guangzhou Military Region between 2010 and 2016. Individuals with incomplete information were excluded. A total of 435 CAD patients and 196 non-CAD controls were involved in the study. All the participants were unrelated Chinese Han individuals. This study was approved by the Medical Ethics Committee of Southern Medical University and compliant with the principles set forth by the Declaration of Helsinki Principles.

Data collection

Subjects were defined as smokers if they smoked more than 100 cigarettes in lifetime. Subjects were diagnosed...
hypertension as systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure (DBP) >90 mmHg [11]. Diabetes mellitus was defined as either fasting plasma glucose levels of ≥7.0 mmol/L or plasma glucose levels of ≥11.1 mmol/L [12]. Total cholesterol, HDL cholesterol, LDL cholesterol, C-reactive protein (CRP) and triglyceride (TG) levels were measured according to standard laboratory methods in Southern Medical University. The diagnostic criteria for CAD cases were defined as followings: at least one of the major segments of coronary arteries (right coronary artery, left circumflex, or left anterior descending arteries) with more than or equal to 50% organic stenosis based on coronary angiography.

**SNP genotyping**

Blood samples (5 ml) were collected from participants. DNA extraction was conducted by TianGen DNA extraction kit (TianGen Ltd., Beijing, China) according to the manufacture instruction. DNA quality was analyzed by Electrophoresis and NanoDrop (NanoDrop Technologies, Houston, TX, USA) quantification. 20 ng DNA was used for PCR amplification. Chimeric specific primers were designed using Oligo 6.0, which contain target sequences and universal sequences. The product sizes of PCR reaction were between 107 and 160 bp, and the primer length was 37–38 bp, with melting temperature (Tm) 55–65 °C and the GC content between 20 and 80%. Multiplex PCR was conducted and followed by adaptor adding. Final products were purified by polyacrylamide gel electrophoresis (PAGE) and then sequenced on MiSeq platform (Illumina, USA).

**Sequencing data analysis**

Sequencing data were separated by index sequences using FASTX-toolkit as each sample group has a unique index sequence. Then, the index and adapter sequences were trimmed out with cutadapt. And target sequences were mapped to human genome reference sequence (NCBI, dbSNP build 142) using BWA software. SNPs were called by samtools.

**Statistical analysis**

Continuous variables between cases and controls are calculated using Student’s t test and presented as mean ± SD. Categorical variables were calculated using chi-square test. Gene frequencies, allele frequencies, and differences in genotype and allele frequencies between different groups were also examined. Hardy-Weinberg equilibrium (HWE) test was performed by SHEsis [13]. P value >0.05 was considered in HWE. Allele distribution between cases and controls were analyzed using chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression analysis after adjusting for age, hypertension, type 2 diabetes, TG, TC, HDL-C and LDL-C. P value <0.05 was considered as statistically significant. Association analysis were conducted by SPSS version 21.0 (SPSS Inc., Chicago, Illinois, USA).

**Results**

**Clinical characteristics of cases and controls**

In this study, 435 CAD patients and 196 controls were included. Clinical characteristics are summarized in Table 1.

### Table 1 Baseline characteristics

|                | Cases             | Controls           | P    |
|----------------|-------------------|--------------------|------|
| Participant (n)| 435               | 196                |      |
| Gender (male, N (%)) | 144 (33.1%)  | 92 (46.9%)         | 0.001|
| Age (years)    | 60.8 ± 10.5       | 59.9 ± 10.4        | 0.322|
| EF             | 63.374 ± 8.058    | 64.757 ± 8.600     | 0.005|
| CRP            | 1.822 ± 2.891     | 1.137 ± 2.032      | 0.139|
| HDLC           | 1.116 ± 0.506     | 1.164 ± 0.303      | 0.006|
| LDL-C          | 2.335 ± 0.778     | 2.242 ± 0.659      | 0.296|
| TC             | 4.379 ± 1.055     | 4.211 ± 0.896      | 0.102|
| TG             | 1.949 ± 1.835     | 1.5 ± 0.933        | 0.001|
| ApoA           | 222.737 ± 236.699 | 162.74 ± 218.465   | 0.001|
| ApoA1          | 1.055 ± 0.213     | 1.155 ± 0.294      | 0.015|
| ApoB           | 0.784 ± 0.248     | 0.75 ± 0.203       | 0.609|
| GLU            | 6.046 ± 2.221     | 5.322 ± 1.375      | 0.001|
| GHb            | 5.924 ± 1.205     | 5.57 ± 0.903       | 0.007|
| Hypertension, yes, N (%) | 282 (65.6%) | 106 (54.1%)        | 0.006|
| Diabetes, yes, N (%) | 123 (28.5%) | 25 (12.8%)         | 0.000019|
| Smoker, yes, N (%) | 206 (47.7%) | 73 (37.6%)         | 0.019|
Samples in case group are more likely to have hypertension, diabetes and smoking. EF, HDLC and APOA1 levels are significantly lower in case group than in control group, while TG, APOA1, GLU and GHb levels are significantly higher in case group than in control group. These data indicate that male, EF, HDLC, TG, ApoA, APOA1, GLU, GHb, hypertension, diabetes and smoking are risk factors of developing CAD in this study.

**Association with the risk of developing CAD**

Seven SNPs were genotyped in 435 cases and 196 controls. Allele frequency distribution was shown in Table 2. All SNPs were accorded with HWE. In these 7 SNPs, rs5882 and rs662799 were associated with CAD ($p \leq 0.05$), in which rs662799 was explored by Ye et al. of the association between coronary heart disease (CHD) and the APOA5 rs662799 polymorphism [14]. After adjustment of age, gender, smoking, diabetes and hypertension, only rs662799 was significantly associated with increasing risk of developing CAD (risk allele T, OR = 1.374, 95% CI = 1.032–1.83, $P = 0.03$) (Table 3). Minor (T) allele frequency of SNP rs662799 in control and case group were 33.9 and 38.2% respectively. The results remained significant when HDLC, LDLC, TC and TG were introduced into the model (data not shown). No significant differences were observed in other polymorphisms after adjustment.

### Table 2 Hardy-Weinberg equilibrium

| SNP   | Genotype | Cases      | Controls   | χ² | P   | Cases      | Controls   | χ² | P   |
|-------|----------|------------|------------|----|-----|------------|------------|----|-----|
| rs1800588 | CC       | 149        | 147.1273   | 0.163509 | 0.685946 | 78         | 78.67222   | 0.050014 | 0.823039 |
|       | CT       | 178        | 181.7455   | 82  | 80.65556 | 20         | 20.67222   | 0.000125 | 0.991075 |
|       | TT       | 58         | 56.12727   | 20  | 20.67222 | 119        | 116.6667   | 0.048    | 0.992822 |
| rs320  | GG       | 12         | 11.31992   | 0.059714 | 0.806948 | 7          | 4.666667   | 1.68     | 0.194924 |
|       | GT       | 107        | 108.3602   | 42  | 46.66667 | 42         | 46.66667   | 0.000125 | 0.991075 |
|       | TT       | 260        | 259.3199   | 119 | 116.6667 | 119        | 116.6667   | 0.000125 | 0.991075 |
| rs5128 | CC       | 44         | 46         | 0.195652 | 0.658253 | 21         | 16.8342    | 2.076069 | 0.149625 |
|       | CT       | 188        | 184        | 72  | 80.33161 | 100        | 95.8342    | 0.000125 | 0.991075 |
|       | GG       | 182        | 184        | 100 | 95.8342  | 100        | 95.8342    | 0.000125 | 0.991075 |
| rs5882 | AA       | 116        | 120.1608   | 0.681031 | 0.409232 | 74         | 68.04188   | 3.21016  | 0.073182 |
|       | AG       | 213        | 204.6784   | 80  | 91.91623 | 80         | 91.91623   | 0.803    | 0.963  |
|       | GG       | 83         | 87.1608    | 37  | 31.04188 | 37         | 31.04188   | 0.803    | 0.963  |
| rs662  | AA       | 51         | 50.9484    | 0.000125 | 0.991075 | 26         | 30.48262   | 1.854151 | 0.173301 |
|       | AG       | 186        | 186.1032   | 99  | 90.03476 | 99         | 90.03476   | 0.803    | 0.963  |
|       | GG       | 170        | 169.9484   | 62  | 66.48262 | 62         | 66.48262   | 0.803    | 0.963  |
| rs662799| AA      | 211        | 206.1553   | 1.235773 | 0.266287 | 111        | 108.7513   | 0.759702 | 0.383422 |
|       | AG      | 170        | 179.6894   | 67  | 74.9747  | 67         | 74.9747    | 0.803    | 0.963  |
|       | GG      | 44         | 39.15529   | 14  | 11.7513  | 14         | 11.7513    | 0.803    | 0.963  |
| rs693  | CC      | 349        | 348.889    | 0.015305 | 0.901541 | 147        | 147.8421   | 0.974012 | 0.323682 |
|       | CT      | 35         | 35.22208   | 24  | 22.31579 | 24         | 22.31579   | 0.000125 | 0.991075 |
|       | TT      | 1          | 0.888961   | 0    | 0.842105 | 0          | 0.842105   | 0.000125 | 0.991075 |

### Table 3 association analysis

| rs    | Control OR  | Case OR  | OR  | 95%CI  | FDR P  | P adj | OR  | 95%CI  |
|-------|-------------|----------|-----|--------|--------|-------|-----|--------|
| rs1800588 | 0.661/0.339 | 0.618/0.382 | 0.163 | 1.205 | 0.927–1.566 | 0.228 | 0.104 | 1.257 | 0.954–1.656 |
| rs320  | 0.833/0.167 | 0.827/0.173 | 0.803 | 1.045 | 0.741–1.472 | 0.803 | 0.963 | 0.991 | 0.693–1.418 |
| rs5128 | 0.705/0.295 | 0.667/0.333 | 0.187 | 1.193 | 0.918–1.55 | 0.218 | 0.31 | 1.152 | 0.876–1.514 |
| rs5882 | 0.597/0.403 | 0.536/0.464 | 0.048 | 1.281 | 1.002–1.638 | 0.168 | 0.128 | 1.222 | 0.944–1.581 |
| rs662  | 0.596/0.404 | 0.646/0.354 | 0.098 | 0.809 | 0.629–1.04 | 0.229 | 0.123 | 0.813 | 0.624–1.058 |
| rs662799 | 0.753/0.247 | 0.696/0.304 | 0.043 | 1.326 | 1.008–1.744 | 0.301 | 0.03 | 1.374 | 1.032–1.83 |
| rs693  | 0.93/0.07  | 0.952/0.048 | 0.135 | 0.669 | 0.394–1.137 | 0.236 | 0.168 | 0.679 | 0.392–1.177 |
Table 4 Stratified analysis

|                     | rs1800588 | n 320 | 95% CI | rs5128 | n 5128 | 95% CI | rs5882 | n 5882 | 95% CI | rs662 | n 662 | 95% CI | rs662799 | n 662799 | 95% CI | rs693 | n 693 | 95% CI |
|---------------------|-----------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-----------|-----------|--------|--------|--------|--------|
| Male                | 0.059     | 1.417 | 0.987–2.036 | 0.399 | 1.233 | 0.758–2.006 | 0.285 | 1.215 | 0.85–1.736 | 0.25 | 1.218 | 0.87–1.705 | 0.392 | 0.858 | 0.604–1.218 | 0.003 | 1.8 | 1.215–2.668 | 0.356 | 0.711 | 0.345–1.467 |
| Female              | 0.835     | 1.047 | 0.68–1.61 | 0.294 | 0.747 | 0.433–1.288 | 0.742 | 1.075 | 0.7–1.651 | 0.32 | 1.227 | 0.819–1.839 | 0.146 | 0.739 | 0.492–1.111 | 0.936 | 0.982 | 0.637–1.514 | 0.316 | 0.646 | 0.275–1.519 |
| Diabetes            | 0.961     | 0.982 | 0.472–2.042 | 0.447 | 1.484 | 0.536–4.106 | 0.686 | 1.154 | 0.576–2.309 | 0.105 | 1.738 | 0.891–3.394 | 0.591 | 0.831 | 0.422–1.635 | 0.226 | 1.576 | 0.754–3.291 | 0.091 | 0.323 | 0.087–1.197 |
| No diabetes         | 0.073     | 1.313 | 0.975–1.767 | 0.695 | 0.926 | 0.629–1.363 | 0.374 | 1.145 | 0.849–1.543 | 0.362 | 1.14 | 0.86–1.51 | 0.155 | 0.811 | 0.608–1.082 | 0.067 | 1.34 | 0.98–1.832 | 0.392 | 0.77 | 0.423–1.401 |
| Hypertension        | 0.206     | 1.269 | 0.877–1.835 | 0.313 | 1.28 | 0.792–2.069 | 0.158 | 1.297 | 0.903–1.863 | 0.929 | 0.985 | 0.7–1.385 | 0.429 | 0.869 | 0.613–1.232 | 0.004 | 1.792 | 1.199–2.679 | 0.233 | 0.615 | 0.277–1.366 |
| No hypertension     | 0.24      | 1.285 | 0.846–1.954 | 0.214 | 0.7 | 0.399–1.228 | 0.969 | 0.992 | 0.649–1.516 | 0.023 | 1.593 | 1.067–2.379 | 0.14 | 0.736 | 0.49–1.106 | 0.992 | 1.002 | 0.657–1.529 | 0.515 | 0.775 | 0.36–1.668 |
| Smoking             | 0.035     | 1.603 | 1.035–2.482 | 0.702 | 0.895 | 0.506–1.581 | 0.402 | 1.197 | 0.786–2.824 | 0.393 | 1.191 | 0.798–1.779 | 0.115 | 0.715 | 0.472–1.084 | 0.108 | 1.456 | 0.921–2.301 | 0.235 | 0.631 | 0.295–1.35 |
| No smoking          | 0.625     | 1.094 | 0.763–1.57 | 0.811 | 1.058 | 0.667–1.677 | 0.511 | 1.13 | 0.785–1.626 | 0.273 | 1.209 | 0.861–1.699 | 0.451 | 0.876 | 0.621–1.236 | 0.123 | 1.339 | 0.924–1.941 | 0.552 | 0.783 | 0.35–1.753 |
Stratified association analysis
Gender, smoking, diabetes and hypertension status were stratified for further investigation (Table 4). Rs662799 was associated with CAD in male and hypertension group. Rs5882 was associated with CAD in non-hypertension group. Rs1800588 was associated with CAD in smoking group.

Discussion
CAD was a complex disease that influenced by a combination of genetic and environmental factors, but so far the molecular mechanisms of CAD were only partially revealed [15]. Lipoprotein metabolism was associated with CAD susceptibility in general population. LDLc, TC, TG, HDLc were associated with CAD status in many studies.

Many genes involving in lipid and lipoprotein metabolism pathway had been revealed to be related with CAD. Notably, SNPs of some genes that involved in lipid metabolism were identified to be associated with CAD developing risk. Many studies investigate the relationship between polymorphisms in or near these genes and CAD development. However, the results were inconsistent in different races or populations. The present study was performed to investigate if the polymorphisms in lipid metabolism genes were associated with CAD occurrences.

A significant association of the SNP rs662799 in APOA5 genes with CAD was observed after adjustment of gender, age, smoking, diabetes, hypertension and lipid status. However, no association was found between other SNPs and CAD susceptibility.

Apoa5 played an important role in TG metabolism (synthesis and removal of TG) [16]. TG was also a risk factor for CAD. APOA5 gene expression level was associated with TG plasma concentration [16]. Rs662799 was a polymorphism located in the promoter region (−1131 T > C) that influences the expression level of APOA5 gene [17]. The allele frequency of rs662799 in HapMap database was 1.7, 13.3, 26.7 and 28.9% in European, African, Chinese and Japanese respectively.

In previous researches, rs662799 was associated with TG level [18, 19]. In some studies, no association was detected between rs662799 and CAD [20], possibly due to lack of power.

There are limitations to the study. Our analysis only includes the study of Chinese Han population, and the sample size is small. So, it might not apply to other populations.

Conclusion
In conclusion, we confirmed that rs662799 in APOA5 gene was significantly associated with CAD development. As the cohort size was limited, there may not be sufficient power to detect the effects of other SNPs. Besides of SNPs, rare mutations might also contribute to CAD development. Further studies with larger population and different technology were needed in order to provide more insights into the biological relevance of CAD.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
SFD designed the study, HC performed the experiments, and MZ, XYW, XL, YW, DCL analyzed the data and prepared the manuscript. All authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate
This study was approved by the Medical Ethics Committee of Southern Medical University and compliant with the principles set forth by the Declaration of Helsinki Principles. Written informed consent was provided by all patients.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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