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ewlinePOC3 may not be a predictor of risk of ischemic vascular
disease in the Chinese population [v1; ref status: indexed,
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Abstract
The genetic background of ischemic vascular disease is actively being
explored. Several studies have shown that inhibition of APOC3 significantly
reduces plasma levels of apolipoprotein C3 and triglycerides. Recently, the TG
and HDL Working Group and Jørgensen et al. reported that loss-of-function
mutations in APOC3 are associated with decreased triglyceride levels and a
reduced risk of ischemic vascular disease in European and African individuals.

We performed a replication study in 4470 Chinese participants. The coding
regions of APOC3 were amplified and re-sequenced. However, only
synonymous and intronic variants with no functional consequences were
identified. None of the loss-of-function mutations reported in European and
African individuals were observed. Therefore, APOC3 may not be an ideal
predictor for risk of ischemic vascular disease in the Chinese population.

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The genetic basis of ischemic vascular disease such as coronary artery disease is actively being explored. Most studies have focused on susceptibility factors contributing to an increased risk, while only few studies have identified protective variants conferring reduced risk. Recently, the TG and HDL Working Group and Jørgensen et al. reported that loss-of-function mutations in APOC3 are associated with decreased triglyceride levels and a reduced risk of ischemic vascular disease in individuals of European and African ancestry. Approximately 1 in 150 individuals were heterozygous carriers of at least one of the four mutations: R19X, A43T, IVS2+1G→A, and IVS3+1G→T. Heterozygous carriers for these mutations had a significantly lower incidence of ischemic vascular disease as compared to non-carriers (hazard ratio = 0.59). Triglyceride and circulating APOC3 levels in the carriers were only 61% and 54% of those in non-carriers, respectively. These critical findings prompted us to undertake a replication study in China, where over one million people are affected by cardiovascular diseases each year (http://www.nhfpc.gov.cn/).

A total of 4470 unrelated Chinese participants were enrolled, including 1488 healthy controls, 1050 patients with ischemic stroke, 628 patients with coronary artery disease, and 1304 patients with venous thrombosis, which could also be exacerbated by effects on the coagulation system resulting from elevated triglyceride levels. The 1488 healthy controls and 1050 patients with ischemic stroke were described in a previous study. Briefly, healthy individuals did not present any relevant medical history or family history of ischemic vascular disease. Ischemic stroke was confirmed by brain computed tomography (CT) and/or brain magnetic resonance imaging (MRI). The 1304 patients with venous thrombosis were described previously. Thrombosis was confirmed by objective investigations such as color Doppler ultrasonography and/or CT angiography. Patients with coronary artery disease were enrolled in our hospital from September 2013 to March 2014. Coronary artery disease was validated by angiographic evidence of at least one segment of a major coronary with over 50% organic stenosis. The characteristics of the 628 patients with coronary artery disease are summarized in Table 1. Written informed consent was obtained from all participants, and the study was approved by the Ethics Committee of Union Hospital affiliated with Huazhong University of Science and Technology (Approval number 2013-03-0052).

Blood samples were collected into a vacutainer tube containing 0.105 mol/L trisodium citrate and were then centrifuged at 2000 g for 15 minutes. Genomic DNA was isolated using a salt precipitation method and was then used for sequencing. The four exons and the flanking intronic regions of APOC3 were amplified by PCR and then sequenced on an Applied Biosystems ABI 3730 Genetic Analyzer, as previously described. The oligonucleotide pairs and annealing temperatures employed in PCR and sequencing are shown in Table 2. In this study, we identified only synonymous and intronic variants, with no functional consequences, and similar genotype distributions across the groups (Table 3). None of the loss-of-function mutations reported in European and African individuals were observed in the current cohort. Considering the relatively large sample size, we suggest that functional variants in APOC3 could be very rare in China. Therefore, the genetic background of ischemic vascular disease is highly variable among different ethnic groups, and APOC3 may not be an ideal predictor of risk of ischemic vascular disease in the Chinese population.

Table 1. Characteristics of the 628 patients with coronary artery disease.

| Characteristic                  | Number | Percentage |
|--------------------------------|--------|------------|
| Onset age (yr, mean)           | 61.6 ± 10.8 |           |
| <40                            | 11     | 1.7%       |
| ≥40 and <60                    | 256    | 40.8%      |
| ≥60                            | 361    | 57.5%      |
| Sex                            |        |            |
| male                           | 408    | 65.0%      |
| female                         | 220    | 35.0%      |
| Coronary artery disease        |        |            |
| angina pectoris                | 422    | 67.2%      |
| myocardial infarction          | 206    | 32.8%      |
| Family history                 |        |            |
| yes                            | 32     | 5.1%       |
| no                             | 596    | 94.9%      |
| Current smoker                 |        |            |
| yes                            | 157    | 25.0%      |
| no                             | 471    | 75.0%      |
| Drinking                       |        |            |
| yes                            | 79     | 12.6%      |
| no                             | 549    | 87.4%      |
| Hypertension                   |        |            |
| yes                            | 425    | 67.7%      |
| no                             | 203    | 32.3%      |
| Type 2 diabetes                |        |            |
| yes                            | 175    | 27.9%      |
| no                             | 453    | 72.1%      |
| Fasting serum lipid levels     |        |            |
| TC                             | 3.97 ± 0.99 mmol/L |
| TG                             | 1.55 ± 1.05 mmol/L |
| HDL-C                          | 1.19 ± 0.29 mmol/L |
| LDL-C                          | 2.07 ± 0.76 mmol/L |

TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

The diagnosis of myocardial infarction was based on typical chest pain with a duration over 30 min, on electrocardiographic patterns, and on increased creatine kinase MB isoenzyme and troponin I levels. Hypertension is defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg. Type 2 diabetes were clarified using the 1999 WHO criteria, including fasting plasma glucose ≥ 7.0 mmol/L, 2-hour oral glucose tolerance test plasma glucose ≥ 11.1 mmol/L or ongoing therapy for diabetes.
Table 3. APOC3 variants identified in the 4470 Chinese participants.

| Variables | dbSNP ID | Control group | Ischemic stroke | Coronary heart disease | Venous thrombosis |
|-----------|----------|---------------|----------------|------------------------|-------------------|
|           |          | n = 1488      | n = 1050        | n = 628                | n = 1304          |
| Age (yr, mean) | 61.2 ± 12.8 | 62.2 ± 12.3 | 61.6 ± 10.8 | 51.7 ± 13.8 |
| Male, n (%) | 978 (65.7%) | 691 (65.8%) | 408 (65.0%) | 638 (48.9%) |
| Variants, n (%) | | | | |
| c.10C>A (p.Arg4=) | novel | | | |
| CC | 1485 (99.8%) | 1048 (99.8%) | 627 (99.8%) | 1301 (99.8%) |
| CA | 3 (0.2%) | 2 (0.2%) | 1 (0.2%) | 3 (0.2%) |
| P value | 0.95 | 0.84 | 0.87 |
| c.99G>A (p.Gln33=) | rs200557528 | | | |
| GG | 1481 (99.5%) | 1046 (99.6%) | 625 (99.5%) | 1296 (99.4%) |
| GA | 7 (0.5%) | 4 (0.4%) | 3 (0.5%) | 8 (0.6%) |
| P value | 0.73 | 0.98 | 0.61 |
| c.102T>C (p.Gly34=) | rs4520 | | | |
| TT | 655 (44.0%) | 456 (43.4%) | 278 (44.3%) | 582 (44.6%) |
| TC | 641 (43.1%) | 449 (42.8%) | 272 (43.3%) | 566 (43.4%) |
| CC | 192 (12.9%) | 145 (13.8%) | 78 (12.4%) | 156 (12.0%) |
| P value | 0.80 | 0.95 | 0.75 |
| c.179+57G>A | rs2070667 | | | |
| GG | 1098 (73.8%) | 776 (73.9%) | 454 (72.3%) | 967 (74.2%) |
| GA | 329 (22.1%) | 235 (22.4%) | 148 (23.6%) | 286 (21.9%) |
| AA | 61 (4.1%) | 39 (3.7%) | 26 (4.1%) | 51 (3.9%) |
| P value | 0.88 | 0.76 | 0.96 |
| c.240G>A (p.Lys80=) | novel | | | |
| GG | 1483 (99.7%) | 1047 (99.7%) | 626 (99.7%) | 1301 (99.8%) |
| GA | 5 (0.3%) | 3 (0.3%) | 2 (0.3%) | 3 (0.2%) |
| P value | 0.82 | 0.95 | 0.60 |
| c.*40G>C | rs5128 | | | |
| GG | 763 (51.3%) | 535 (51.0%) | 328 (52.2%) | 665 (51.0%) |
| GC | 562 (37.8%) | 394 (37.5%) | 240 (38.2%) | 494 (37.9%) |
| CC | 163 (10.9%) | 121 (11.5%) | 60 (9.6%) | 145 (11.1%) |
| P value | 0.90 | 0.64 | 0.98 |
| c.*71G>T | rs4225 | | | |
| GG | 1006 (67.6%) | 703 (66.9%) | 416 (66.2%) | 880 (67.5%) |
| GT | 414 (27.8%) | 304 (29.0%) | 180 (28.7%) | 368 (28.2%) |
| TT | 68 (4.6%) | 43 (4.1%) | 32 (5.1%) | 56 (4.3%) |
| P value | 0.73 | 0.79 | 0.92 |

Table 2. Primers and annealing temperatures for PCR and sequencing.

| Exon | Forward primer (5'-3') | Reverse primer (5'-3') | AT (ºC) | Product size (bp) |
|------|------------------------|------------------------|---------|-------------------|
| 1    | GCC TT ACT CCA AAC AC CC | AG TG CCT TCC AGG CTT GCT | 58      | 602               |
| 2 and 3 | C C T C T G A G AG CC C C TTT ACC G | C CG G AC G C C T G AC A AA | 58      | 646               |
| 4    | G G G G CATA A AC AT C TT G G | CT ACC C AA G G T G G T GA G G | 58      | 693               |

AT, annealing temperature. The accession number of APOC3 reference sequence in GenBank is NG_008949.1.

dbSNP, single nucleotide polymorphism database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/projects/SNP). Comparisons between the controls and each case group were assessed with the use of the chi-square test. A two tailed P<0.05 was considered significant.
Further studies are warranted to understand the genetic basis governing triglyceride levels and conferring protective effects on ischemic vascular disease in the Chinese population.

Consent
Written informed consent to publish these data has been obtained by each participant.

Author contributions
YH and LT chose the article for correspondence and evaluated the data in the manuscript. LT, ZPC, QYW, WZ, HL, YYW, and BH performed experiments. LT and ZPC wrote the manuscript. YH supervised the process and critically edited the manuscript.

Competing interests
No competing interests were disclosed.

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References

1. Peden JF, Farrall M: Thirty-five common variants for coronary artery disease: the fruits of much collaborative labour. Hum Mol Genet. 2011; 20(R2): R198–205. PubMed Abstract | Publisher Full Text | Free Full Text
2. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, et al.: Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med. 2014; 371(1): 32–41. PubMed Abstract | Publisher Full Text
3. The TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute: Loss-of-function mutations in APOC3, triglycerides, and coronary disease. N Engl J Med. 2014; 371(1): 22–31. PubMed Abstract | Publisher Full Text | Free Full Text
4. Lu X, Tang L, Xu K, et al.: Novel association of a PROC variant with ischemic stroke in a Chinese Han population. Hum Genet. 2013; 132(1): 69–77. PubMed Abstract | Publisher Full Text
5. Tang L, Wang HF, Lu X, et al.: Common genetic risk factors for venous thrombosis in the Chinese population. Am J Hum Genet. 2013; 92(2): 177–187. PubMed Abstract | Publisher Full Text | Free Full Text
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The authors performed a confirmatory study for the recently reported protective effects of loss-of-function mutations in APOC3 in the Chinese population. Not a single loss-of-function mutation was identified in exons and exon-intron junctions of APOC3 in a total of 4470 study subjects. The authors concluded that APOC3 is not a good predictor of risk of ischemic vascular disease in the Chinese population.

This is a worthwhile study and the information, although negative, is certainly useful. However, based on the data reported in the paper, it is premature to conclude that functional variants in APOC3 are very rare and/or not related to ischemic vascular disease in the Chinese population. The data merely conclude that detrimental mutations are very rare in the sequenced regions of APOC3 gene in the Chinese population. Potential effects from large deletions of the gene and mutations in regulatory regions of the gene cannot be excluded. In fact, there have been reports of promoter polymorphisms that affect the expression of APOC3 (ref. 28-31 in Jørgensen et. al). Unless additional, more comprehensive mutational studies are performed in the cohort, conclusions should be modified to reflect the limitations of the current study.

There have been examples of synonymous mutations that affect the translation of mRNA and therefore affect the protein level. Also, intronic mutations distant from splice sites could affect splicing. Authors should clearly state reasons why they believe that the variants identified in the study have no functional consequences.

References should be added to the end of the second sentence of the paper (while only “a” few studies have identified protective variants conferring reduced risk).

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.
Chang-Geng Ruan
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Two very recent landmark large-scale studies show that loss-of-function mutations in APOC3 are associated with lower levels of plasma triglycerides, and carriers of these mutations have a reduced risk of coronary heart disease in European and African individuals. Tang et al. performed a timely replication study in 4,470 Chinese individuals. Unexpectedly, no loss-of-function mutations identified in the European and African populations were found in the Chinese population. This important study not only highlights the difference in genetic susceptibility to cardiovascular disease in different ethnic populations, but also suggests that APOC3 variants are not applicable to the Chinese population to predict risk for ischemic vascular disease.

APOC3 may still be an important regulator of lipid metabolism in Chinese, and novel variants of this gene remain to be identified in this ethnic population. Consequently, the authors need to change their conclusion from “Therefore, APOC3 may not be an ideal predictor for risk of ischemic vascular disease in the Chinese population” to “Therefore, APOC3 variants identified in the European and African population may not be an ideal predictor for risk of ischemic vascular disease in the Chinese population”.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Javier Corral
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Despite being a negative study, the information in this article is valuable. It describes a study that found no relevant mutation in APOC3 among a large number of Chinese subjects (4470), including 1488 healthy controls, 1050 patients with ischemic stroke, 628 patients with coronary artery disease, and 1304 patients with venous thrombosis. Actually, no one with the loss-of-function mutations reported in European and African individuals was identified in this study. Only synonymous and intronic variants were discovered. The authors indicated that these variants have no functional consequences, but no data are shown. At the least the TG levels according to the genotype might be shown in table 2.

The authors must indicate that other genetic defects, such a gross deletions or regulatory mutations not identified by sequencing methods or by analysis of exons, may cause a loss-of-function of APOC3 and might be associated with decreased triglyceride levels and a reduced risk of ischemic vascular disease. Actually, selection of subjects with decreased triglyceride levels and a deeper analysis of this gene might be a better strategy to identify these genetic variants potentially involved in TG levels and risk of ischemic vascular disease. Accordingly, I think the authors might change the title of the article.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.
