CORRESPONDENCE

REVISED

APOC3 may not be a predictor of risk of ischemic vascular disease in the Chinese population [v2; ref status: indexed, http://f1000r.es/4xr]

Liang Tang[1,2*], Zhi-Peng Cheng[1,2*], Qing-Yun Wang[1], Wei Zeng[1,2], Hui Liu[1], Ying-Ying Wu[1], Bei Hu[1,2], Yu Hu[1,2]

[1]Institute of Hematology, Tongji Medical College, Union Hospital, Huazhong University of Science and Technology, Wuhan, 430022, China
[2]Collaborative Innovation Center of Hematology, Union Hospital, Huazhong University of Science and Technology, Wuhan, 430022, China

[*] Equal contributors

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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.

Open Peer Review

Referee Status: ✓ ✓ ✓

	Invited Referees 1	2	3
REVISED version 2 published 22 Dec 2014			✓ report
version 1 published 07 Nov 2014	✓ report	✓ report	? report

1 **Javier Corral**, Universidad de Murcia Spain

2 **Chang-Geng Ruan**, Ministry of Health China

3 **Bin Zhang**, Lerner Research Institute of Cleveland Clinic USA

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Comments (0)

Corresponding author: Yu Hu (dr_huyu@126.com)

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REVISED **Amendments from Version 1**

In response to the reviewers' comments, we have added some important limitations to the study in the revised text.

See referee reports

Correspondence

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[1], while only a few studies have identified protective variants conferring reduced risk[2]. 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[3,4]. 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[5]. 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[6]. 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[3]. 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. Therefore, the genetic background of ischemic vascular disease is highly variable among different ethnic groups, and *APOC3* may not be an

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 2. Primers and annealing temperatures for PCR and sequencing.

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	AT (°C)	Product size (bp)
1	GCCTTTACTCCAAACACCCC	AGTGCTTCTCCAGGCTTGCT	58	602
2 and 3	CCTTCTGAGAGCCCGTATTAGC	CCGCAGCAGCCTGACAAA	58	646
4	GGGGCATAAACATCTGAGGT	CTACCAGAAGGTGGATAGAGC	58	693

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

Table 3. *APOC3* variants identified in the 4470 Chinese participants.

Variables	dbSNP ID	Control group n = 1488	Ischemic stroke n = 1050	Coronary heart disease n = 628	Venous thrombosis 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 (%)[b]					
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

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.

ideal predictor of risk of ischemic vascular disease in the Chinese population.

However, there are some limitations in this study. First, we only analyzed the coding sequence, as in the studies carried out in European and African individuals. Potential effects from large deletions and mutations in regulatory regions of the gene cannot be excluded. Previous studies have revealed two promoter polymorphisms (T-455C and C-482T) that affect the expression of *APOC3*. However, their relationship with coronary artery disease was not observed in a Han Chinese population[7]. Second, the consequences of the synonymous mutations and the intronic mutations identified here were only predicted by an on-line bioinformatics tool (http://www.fruitfly.org/seq_tools/splice.html). Third, the triglyceride levels were not available for the patients with venous thrombosis. Thus, the triglyceride levels according to the genotypes were not further analyzed. Nevertheless, considering the relatively large sample size, we suggest that functional variants in *APOC3* could be very rare in China. 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.

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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PubMed Abstract | Publisher Full Text | Free Full Text

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Current Referee Status: ✓ ✓ ✓

Version 2

Referee Report 23 December 2014

doi:10.5256/f1000research.6399.r7130

Bin Zhang
Genomic Medicine Institute, Lerner Research Institute of Cleveland Clinic, Cleveland, OH, USA

Authors addressed the main concerns. No further comments.

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.

Version 1

Referee Report 10 December 2014

doi:10.5256/f1000research.6066.r6866

Bin Zhang
Genomic Medicine Institute, Lerner Research Institute of Cleveland Clinic, Cleveland, OH, USA

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.

Referee Report 05 December 2014

doi:10.5256/f1000research.6066.r6920

Chang-Geng Ruan
Key Laboratory of Thrombosis and Haemostasis, Ministry of Health, Suzhou, China

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.

Referee Report 13 November 2014

doi:10.5256/f1000research.6066.r6688

Javier Corral
Centro Regional de Hemodonación, Universidad de Murcia, Murcia, Spain

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.