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**ORIGINAL ARTICLE**

# Lack of association between *STK39* and hypertension in the Chinese population

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Genome-wide association study (GWAS) has identified *serine/threonine kinase 39 (STK39)* as a candidate gene for hypertension. A replication study provided supporting evidence that *STK39* functional polymorphism rs35929607 was associated with hypertension. Recently, another study also showed rs6749447 within the *STK39* was associated with blood pressure responses. However, these studies were all conducted in Caucasians. Thus, we carried out a case–control study to test whether *STK39* is a common candidate gene for hypertension, and to examine the interaction of genetic factors and non-genetic risk factors in the Chinese population. Thousand twenty four hypertensive cases and 1024 controls were genotyped for five polymorphisms. Four single-nucleotide polymorphisms (SNPs) are located within *STK39*, and rs4977950, the SNP that showed the strongest signal is located in a gene desert. Results indicated that none of these SNPs was associated with hypertension in the Chinese population. Logistic regression analysis found body mass index (BMI) and triglyceride level were higher in the hypertension group when compared with the control group. Multifactor dimensionality reduction analysis indicated that the interaction between BMI and rs4977950 may have an impact on hypertension. Taken together, the present study found no evidence that *STK39* was associated with hypertension in the Chinese population. Instead, non-genetic risk factors such as BMI have an important role in Chinese hypertensive subjects, and the 'missing inheritability' requires further investigation.

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## INTRODUCTION

Genome-wide association study (GWAS) has identified *serine/threonine kinase 39* (*STK39*) as a candidate gene for hypertension in the American Old Order Amish.[1] Recently, a replication study in two Sweden cohorts showed that the *STK39* functional polymorphism rs35929607A>G was associated with blood pressure (BP) and hypertension.[2] Consequently, *STK39* becomes a very important 'susceptibility gene' for hypertension.

*STK39* encodes a serine/threonine kinase known as a Ste20-related proline–alanine-rich kinase (SPAK). When phosphorylated by the WNK1 (WNK lysine-deficient protein kinase 1) and WNK4 (WNK lysine-deficient protein kinase 4), SPAK can bind and phosphorylate two $Na^+$-dependent cation chloride cotransporters, including furosemide-sensitive $Na^+/K^+/2Cl^-$ channel (NKCC2) and thiazide-sensitive $Na^+/Cl^-$ channel (NCC).[3–5] This pathway has a critical role in salt homeostasis in renal physiology and is strongly implicated in BP regulation and hypertension development.[3] Recently, Donner and colleagues selected 19 single-nucleotide polymorphisms (SNPs) from five GWASs and studied their association with BP responses to four different antihypertensive drug monotherapies in Finnish population. The result indicated only rs6749447 (within the *STK39* gene) was significantly associated with BP responses.[6]

However, these studies were all conducted in Caucasians, and whether it is a common candidate gene for hypertension in other populations is not clear. Therefore, we carried out a case–control study and an interaction analysis to verify whether *STK39* is associated with hypertension in the Chinese population.

## METHODS

### Study population

The participants were chosen from our established community-based epidemiology study of common diseases. With informed consent, we collected >10 000 health records. Subsequently, participants who fulfilled the following criteria were put into our database: 30–75 years old, Han Chinese, living in Ningbo City (East side of China) for at least three generations and do not have a migration history. Thousand twenty four essential hypertension cases and 1024 controls were chosen from this database. The hypertensive subjects and control subjects were matched for age and gender. In addition, only subjects who do not have cardiovascular disease or other major chronic illnesses according to their health records were included in the control group.

### BP and clinical parameters

BP measurements were conducted in the morning. After the participant had been in the sitting position for 10 min, three BP measurements were obtained at 5-min intervals using standard mercury sphygmomanometer, and the average of last two measurements was taken as the BP for that participant. Hypertension in this study is defined as a sitting systolic BP (SBP) $\geqslant$140 mm Hg and/or a diastolic BP (DBP) $\geqslant$90 mm Hg, or self-reported use of antihypertension medication. Patients with secondary hypertension were excluded. Normal BP is defined with SBP $\leqslant$120 mm Hg and DBP $\leqslant$80 mm Hg.

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Blood samples were collected with informed consent. Subsequently, total cholesterol (TC), high-density lipoprotein (HDL), and triglyceride (TG) were measured from these blood samples. Simultaneously, clinical information including body mass index (BMI), smoking habit and alcohol abuse were also obtained. The protocol of this study was reviewed and approved by the ethics committees of Kunming Institute of Zoology, Chinese Academy of Sciences and Ningbo University.

### SNP genotyping

Genomic DNA was obtained from the whole blood by standard phenol/chloroform extraction. Genotyping was performed by the GenomeLab SNPstream Genotyping System (Beckman Coulter, Fullerton, CA, USA) according to the protocols provided by the manufacturer.[7] Of the five candidate SNPs, rs12692877 was replaced by a nearby SNP (rs4667569, $D'=1$, $r^2=1$ in CHB) due to unavailability of appropriate primers (www.autoprimer.com). The primers for SNPstream were listed in Supplementary Table 1. rs4977950 was genotyped by PCR-restriction fragment length polymorphism using mismatched primers (forward primer 5′-CTGGCATTTGTATTACTTTC-3′, reverse primer 5′-TGAACAGGTCCCA TACTC-3′), and the PCR products were digested by the restriction enzyme SpeI (New England Biolabs, Beverly, MA, USA).

### Statistics

Continuous variables are presented as the mean ± s.d. and analyzed by *t*-test between the two groups. Statistical analysis of allele and genotype frequencies between case and control groups and among different sex groups were performed by $\chi^2$-test. Effect of confounding variables were identified by logistic regression (SPSS 16.0, SPSS Inc., Chicago, IL, USA). Hardy–Weinberg equilibrium was determined by the software PEDSTATS V0.6.8 (http://www.sph.umich.edu/csg/abecasis/).[8] Multifactor dimensionality reduction (MDR) was used to identify and characterize interactions among the SNPs and the non-genetic factors, including BMI, serum HDL, TC and TG level, as well as frequency of smoking and alcohol abuse.[9] The software used for MDR is distributed in a JAVA platform with a graphical user interface and is freely available (http://www.epistasis.org/mdr.html).

All tests were two-sided and *P*-values $<0.05$ were considered statistically significant. For $\chi^2$-test, the *P*-values were adjusted for the total number of tested SNPs using the Bonferroni correction method ($\alpha=0.05/5=0.01$).

## RESULTS

The baseline characteristics of the participants are summarized in Table 1. In this study, 1024 hypertensive participants and 1024 controls, who have all been long-term residents of Ningbo City, were analyzed. The male to female ratio was equal in both groups, and mean age of hypertensive participants and controls were similar, demonstrating that the experimental and control groups were well-matched and are appropriate for the following analyses.

BMI, serum TG and TC level in the hypertensive group were significantly greater than the control group ($P<0.01$). Furthermore, significantly more subjects drank alcohol regularly in hypertensive group than those in control group ($P<0.01$). However, HDL level and smoking habit were similar in both groups.

As shown in Supplementary Figures S1–S5, the results of SNPstream and PCR-restriction fragment length polymorphism were clear and reliable. Table 2 shows the genotypes and minor allele frequency of each SNP. The genotyping success rate was 99.7%, and all five SNPs did not deviate from Hardy–Weinberg equilibrium ($P>0.05$). In addition, with the prevalence, odds ratio, and minor allele frequency in this study, the Genetic Power Calculator (available online http://pngu.mgh.harvard.edu/~purcell/gpc/) indicated that the sample size is big enough to do case–control analysis with 80% power.[10] According to the $\chi^2$-test *P*-values and odds ratios, none of the SNPs was shown to associate with hypertension, even after stratification by gender (Table 2).

Considering the effect of confounding variables, we further carried out logistic regression analysis with genetic and non-genetic factors. The result indicated that only BMI and TG were associated with hypertension ($P<0.001$), and none of the SNPs was positive (Table 3).

Finally, MDR was used to analyze the interaction among different SNPs and non-genetic risk factors for hypertension. After input the five SNPs together with information of TG, TC, HDL, BMI, smoking and alcohol drinking, the software output the best model for BMI with 10/10 cross-validation consistency, and 'BMI, rs4977950' with 9/10 cross-validation consistency (Table 4).

## DISCUSSION

*STK39* encodes SPAK, and the WNK-SPAK-NKCC2/NCC pathway is involved in salt renal reabsorption and BP homeostasis.[3] Therefore, it is not surprising to see that SNPs within *STK39* were identified to associate with hypertension through GWAS. To avoid the possibility of false-positive results, Fava *et al.* conducted a replication study in two independent Sweden cohorts.[2] Their results indicated that the functional rs35929607A>G polymorphism was associated with higher SBP and DBP values in the Malmö Diet and Cancer ($n=5634$), but not in the Malmö Preventive Project ($n=17\,894$). After stratification for sex, the results remained statistically significant only in women.[2]

**Table 1.** Baseline characters of the investigated participants

| *Variables* | *Case* | *Control* | P-*value* |
|---|---|---|---|
| Number | 1024 | 1024 | NA |
| Sex (male) (%) | 43.6 | 43.6 | NA |
| Age (years) | 57.23 ± 7.24 | 57.01 ± 7.39 | 0.41 |
| TG (mM) | 2.02 ± 1.69 | 1.64 ± 1.12 | <0.01 |
| HDL (mM) | 1.41 ± 0.35 | 1.40 ± 0.32 | 0.81 |
| TC (mM) | 5.34 ± 1.01 | 5.18 ± 0.93 | <0.01 |
| BMI (kg m$^{-2}$) | 24.65 ± 3.25 | 23.22 ± 2.88 | <0.01 |
| Smoking (%) | 18.90 | 16.37 | 0.12 |
| Alcohol drinking (%) | 22.86 | 19.23 | <0.01 |

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; TC, total cholesterol; TG, triglyceride.

**Table 2.** Association statistics for five SNPs

| *SNP* | *Group* | *MAF* | P-*value* | *OR* | *95% CI* |
|---|---|---|---|---|---|
| rs6749447 | Case | 0.30 | 0.65 | 1.03 | 0.90–1.18 |
| | Control | 0.31 | | | |
| | Male | 0.31 | 0.49 | 1.07 | 0.88–1.31 |
| | Female | 0.30 | 0.99 | 1.00 | 0.84–1.19 |
| rs3754777 | Case | 0.26 | 0.80 | 1.02 | 0.89–1.17 |
| | Control | 0.25 | | | |
| | Male | 0.26 | 0.63 | 0.95 | 0.77–1.17 |
| | Female | 0.25 | 0.44 | 1.08 | 0.89–1.30 |
| rs35929607 | Case | 0.44 | 0.82 | 1.01 | 0.90–1.15 |
| | Control | 0.44 | | | |
| | Male | 0.45 | 0.83 | 0.98 | 0.81–1.18 |
| | Female | 0.43 | 0.62 | 1.04 | 0.88–1.23 |
| rs4667569 | Case | 0.47 | 0.60 | 0.97 | 0.86–1.09 |
| | Control | 0.46 | | | |
| | Male | 0.47 | 0.80 | 1.02 | 0.85–1.23 |
| | Female | 0.46 | 0.36 | 0.93 | 0.79–1.09 |
| rs4977950 | Case | 0.22 | 0.07 | 0.87 | 0.75–1.01 |
| | Control | 0.20 | | | |
| | Male | 0.20 | 0.30 | 0.88 | 0.70–1.12 |
| | Female | 0.22 | 0.14 | 0.86 | 0.71–1.05 |

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

The population studied in the initial GWAS from Wang *et al.*[1] is American Old Order Amish, which is a closed founder population emigrated from Switzerland in the early 1700s. The study populations in articles of Fava *et al.* and Donner *et al.* are from Swede and Finland. These populations are all Caucasians and close in geographical location. When we checked the details of the positive SNPs (rs6749447, rs3754777 and rs4977950) in Wang's GWAS, we found that the minor allele frequency differs greatly between Europeans and Asians. To determine whether *STK39* is a common candidate gene for hypertension, we set to find out whether it is associated with hypertension in the Chinese population, as China has now become a nation with rapidly increasing hypertension prevalence.

We first studied two SNPs (rs35929607 and rs12692877) within *STK39*, which were putative functional SNPs based on a multispecies sequence alignment.[1] However, neither SNP was significantly associated with hypertension in the Chinese population. As the result was quite different from Fava's replication study,[2] we additionally genotyped two tag SNPs (rs6749447 and rs3754777) within *STK39*. These two SNPs belong to LD bin 1 and bin 2. Both SNPs were significant with SBP in Amish ($P<0.0001$), marginally significant in population from diabetes genetics initiative ($P=0.02$ for rs6749447 and 0.03 for rs3754777), but not significant in populations from Framingham Heart Study from, GenNet and Hutterites.[1] However, in the present study, these two tag SNPs were still not associated with hypertension. We further genotyped rs4977950 as it has the minimum *P*-value in Wang's GWAS. This SNP rs4977950 is not a *STK39* polymorphism, but a SNP that is located in a gene desert 900 kb away from known genes. In our study, the *P*-value for rs4977950 is 0.074, and the odds ratio is 1.15 (95% confidence interval: 0.99–1.33). Even after stratification with gender, none of the five SNPs showed association with hypertension.

Hereto, five genome-wide significant SNPs genotyped in this study did not show a positive association with hypertension in the Chinese population. Similarly, Cunnington *et al.*[11] also did not find any association between the three SNPs (rs6749447, rs3754777, rs35929607) within *STK39* and SBP or DBP from a cohort of 1372 British Caucasians. Furthermore, in Fava's study, after exclusion of 2398 individuals from the Malmö Preventive Project cohort, who also participated in the Malmö Diet and Cancer study, G allele of rs35929607 was no longer associated with hypertension.[2] Thus, it is still questionable whether *STK39* can be accepted as a common susceptibility gene for hypertension.

Pickering and colleagues have initially suggested that hypertension and BP are complex traits.[12] Before the era of GWAS, epidemiological studies have found dozens of risk factors, such as smoking, alcohol abuse, excessive salt intake, obesity, mental stress and others to associate with high BP.[13,14] Although no positive SNP was found within this study, we found interesting interactions between genetic factors and non-genetic risk factors. High BMI and serum TG level were already believed to be risk factors for hypertension,[15,16] and were also confirmed by logistic regression in current study. The MDR analysis further demonstrated that these important risk factors interacted with the genetic factor. Thus, the present interaction analysis gave a little more information than the single genetic study. The participants in recent GWASs are mainly from well-organized cohorts (Global BPgen and Cohorts for Heart and Aging Research in Genomic Epidemiology), which means the epidemiology data are readily available.[17–19] With the development of statistic methods for evaluation of gene–environment interaction, more missing inheritability will be found.[20,21]

All in all, studies from Wang *et al.*, Fava *et al.* and Donner *et al.* have demonstrated that *STK39* is associated with hypertension or BP regulation in three different Caucasian populations,[1,2,6] but this result could not be replicated in the Chinese population. In addition, in our interaction analysis, a significant interaction was found between genetic and non-genetic factors, demonstrating that GWAS alone is not a sufficient indicator for hypertension. To decipher causal factors leading to the development and the pathogenesis of hypertension, future work will require analysis of both genetic (epigenetic) and non-genetic (environmental) factors.

**Table 3.** Logistic regression for genetic and non-genetic factors

| *Variables* | *B* | P-*value* | *Exp (B)* | *95% CI* |
|---|---|---|---|---|
| Gender | −0.122 | 0.326 | 0.885 | 0.693–1.129 |
| Age | 0.010 | 0.132 | 1.010 | 0.997–1.024 |
| rs6749447 | 0.074 | 0.576 | 1.076 | 0.832–1.393 |
| rs3754777 | 0.087 | 0.556 | 1.091 | 0.815–1.461 |
| rs35929607 | 0.036 | 0.767 | 1.037 | 0.817–1.315 |
| rs4667569 | 0.140 | 0.347 | 1.150 | 0.859–1.540 |
| rs4977950 | 0.130 | 0.120 | 1.139 | 0.967–1.341 |
| BMI | 0.146 | 0.000 | 1.157 | 1.118–1.198 |
| TG | 0.158 | 0.000 | 1.171 | 1.073–1.278 |
| HDL | 0.285 | 0.087 | 1.330 | 0.959–1.845 |
| TC | 0.039 | 0.484 | 1.040 | 0.932–1.161 |
| Smoking | 0.227 | 0.152 | 1.255 | 0.919–1.713 |
| Alcohol | 0.257 | 0.088 | 1.293 | 0.963–1.735 |
| Constant | −5.405 | 0.000 | 0.004 | |

Abbreviations: B, β-regression coefficient; CI, confidence interval.

*What is known about the topic*

- Genome-wide association study has identified *STK39* as a candidate gene for hypertension.
- A replication study in two Sweden cohorts provided supporting evidence that *STK39* functional polymorphism rs35929607 was associated with hypertension. Another study conducted in Finnish population also showed rs6749447 within the *STK39* was associated with blood pressure responses.

*What this study adds*

- None of the SNPs within the *STK39* was associated with hypertension in the Chinese population.
- Logistic regression showed that only body mass index (BMI) and TG were associated with hypertension.
- MDR analysis indicated that the interaction among BMI and rs4977950 may have an impact on hypertension.
- The present study found no evidence that *STK39* was associated with hypertension in the Chinese population. Instead, interaction analysis indicated that non-genetic risk factors such as BMI have an important role for Chinese hypertensive subjects, and the 'missing inheritability' requires further investigation.

**Table 4.** MDR analysis of gene-environment interaction

| *Best model* | *Testing accuracy* | *Testing sensitivity* | *Testing odds ratio* | *Testing* $\chi^2$ | *Cross-validation consistency* |
|---|---|---|---|---|---|
| BMI | 0.58 | 0.42 | 2.14 (95% CI: 1.14–4.02) | 5.74 ($P=0.0166$) | 10/10 |
| BMI, rs4977950 | 0.59 | 0.61 | 2.08 (95% CI: 1.15–3.77) | 5.99 ($P=0.0144$) | 9/10 |
| BMI, TC, rs4977950 | 0.61 | 0.54 | 2.45 (95% CI: 2.00–2.99) | 77.63 ($P<0.0001$) | 5/10 |

Abbreviations: BMI, body mass index; CI, confidence interval; MDR, multifactor dimensionality reduction; TC, total cholesterol.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Journal of Human Hypertension website (http://www.nature.com/jhh)