Body mass index interaction with *SCN10A* effects on cardiovascular autonomic neuropathy risk in a Chinese Han population

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Background: The aim of this study was to estimate the extent to which the interaction between *SCN10A* (rs7375096) and body mass index (BMI) affects susceptibility to cardiovascular autonomic neuropathy (AN) in a Chinese Han sample.

Method: We conducted a large-scale, population-based study including 791 overweight patients and 1186 controls to analyze the interaction between *SCN10A* and BMI with regard to CAN. All participants were genotyped for the presence of *SCN10A* (rs7375036) polymorphisms. Univariate and multiple linear regression analyses were employed to detect these relationships. The interaction between rs7375036 and BMI with regard to CAN susceptibility on an additive scale was estimated by using the relative excess risk due to interaction, the proportion attributable to interaction, and the synergy index.

Results: Univariate analyses found no significant association between rs7375036 single nucleotide polymorphism and CAN. After controlling for potential confounding factors, the multiple linear regression analysis showed a significant association between BMI and CAN ($P = 0.021$, OR = 1.121). In addition, a significant positive interaction effect of BMI and rs7375036 on CAN was detected ($P = 0.017$).

Conclusion: Our findings suggest that BMI and *SCN10A* (rs7375036) have interaction effects that influence the CAN.

Keywords: *SCN10A*; cardiovascular autonomic neuropathy; interaction; body mass index; Chinese Han population.

Introduction

Cardiovascular autonomic neuropathy (CAN) is a major complication of diabetes mellitus (DM), leading to increased morbidity and mortality in diabetic patients. CAN contributes to a high risk of cardiac arrhythmia, silent myocardial ischemia, and sudden death.^{1,2} It may occur when sympathetic and parasympathetic cardiovascular fibers are affected, which leads to neurohormonal compromise. The prevalence of CAN is estimated to be approximately 20–70%, depending on the test used and the population investigated.^{1,3} CAN also affects many other major segments of the general population, such

as individuals with hypertension (HTN) and metabolic syndrome (MetS).^{4,5} In diabetic patients, MetS has been reported to be associated with cardiovascular autonomic (CA) function indices or impaired CA function.^{5,6}

People classified as overweight or obese exhibit increased risk of cardiovascular disease compared to those of normal weight.⁷ A meta-analysis of five longitudinal cohort studies demonstrated that overweight or obese diabetic patients had a twofold greater relative risk of mortality than normal-weight patients.⁸ Obesity is often considered as an important component of MetS and an independent risk factor for DM in Chinese populations.⁹ Our previous studies indicated that

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MetS was associated with CAN in the general Chinese population.^{10,11} However, the documented association of obesity or overweight with CAN in several ethnic populations^{12,13} has not been replicated in the general Chinese population.

Several recent genome-wide association studies (GWAS)^{14,15} have linked *SCN10A* to the PR interval and QRS duration. The *SCN10A* gene encodes the neuronal sodium channel isoform, NaV1.8. The single nucleotide polymorphism (SNP) of rs6795970 within the *SCN10A* gene carries a higher risk for heart blockages associated with cardiac conduction abnormalities in patients with hypertrophic cardiomyopathy.¹⁶ Previous studies provided evidence for gene–obesity interactions in complex human diseases.^{17,18} It is evident that obesity/overweight is associated with CAN, and *SCN10A* is associated with cardiac electrophysiology. Our previous studies demonstrated that MetS interacted with estrogen receptor 1 (*ESR1*) and DM interacted with *SCN10A* on CAN,^{19,20} respectively. Based on our previous studies, we hypothesize that obesity/overweight may modify the association between *SCN10A* and CAN via the interactions of *SCN10A* and BMI with CAN. The aim of this study was to explore the risk factors for CAN and the interaction effects of *SCN10A* and body mass index (BMI) on CAN susceptibility in a large-scale clinical setting.

Methods

Study population

As described earlier,^{19–21} this study is based on a community-based dataset in Chinese population. We recruited participants from rural and urban communities in Shanghai, and those aged 30–80 years with undiagnosed CAN were included. Written consent was obtained from all participants before the study. This study was approved by the Ethics Committee of Shanghai Tongji Hospital of Tongji University, Shanghai, China. The methods were carried out in accordance with the approved guidelines. Our study invited a total of 3012 subjects to a screening visit between 2011 and 2013. The exclusion criteria were detailed in our earlier study^{19–21}; some subjects were excluded to eliminate potential confounding factors that may have influenced their CA function.

Measurements

As described in our earlier study,^{19–21} the participants were interviewed to document their medical histories, medications, and smoking habits. Laboratory assessments of cardiovascular disease risk factors were completed along with standardized examinations for heart rate variability (HRV). The evaluation of demographic information, glucose profiles, lipid profiles, renal function, HRV indices, and medical

histories was described previously.^{19,20} The definitions of BMI, HTN, DM, and MetS were provided in our previous study.^{19,20} The genotyping for SNP of rs7375036 was detailed earlier.²⁰ Briefly, the genomic DNA was isolated from whole blood by proteinase K digestion followed by phenol–chloroform extraction. The rs7375036 in 2092 Chinese Han participants was genotyped. Of these subjects, 1977 participants with complete clinical and genotype data were available for data analysis in this study.

As mentioned in our earlier study,^{19,20} short-term HRV was applied to evaluate CA function. HRV was measured non-invasively by power spectral analysis. CAN was diagnosed based on at least two abnormal CA reflex test results based on short-term HRV tests.^{20,22,23} A total of 1977 participants with complete clinical and genotype data were available for data analysis in this study.

Statistical analysis

Differences in variables between obesity and normal BMI participants were determined with an unpaired *t*-test. Between groups, differences in properties were assessed with the χ^2 analysis. Multivariable logistic regression (MLR) was carried out to control potential confounders to determine the independent associations of variables with CAN. Interaction analyses were detailed in our earlier study,^{19,20} MLR was conducted to include two main variables and its interaction item to evaluate the interaction effect. The odds ratio (OR) with a 95% confidence interval (CI) was calculated for the relative risk of BMI and SNP with regard to CAN. Three parameters, relative excess risk due to interaction (RERI), proportion attributable to interaction (AP), and synergy index (*S*), were used to estimate measures of interaction on an additive scale. The results were analyzed using the Statistical Package for Social Sciences for Windows version 16.0 (SPSS, Chicago, IL, USA). Tests were two-sided, and a *P*-value of <0.05 was considered significant.

Results

Baseline characteristics of participants

The baseline clinical characteristics of the 1977 participants were described earlier,^{19–21} and are listed in Table 1. Participants with excess weight had significantly higher weights than those with normal BMI (71.84 kg versus 57.70 kg, *P* < 0.01). The prevalence rates of HTN, DM, MetS, and CAN were also significantly higher in participants with excess weight (*P* < 0.001 for all). Systolic blood pressure (SBP), diastolic blood pressure (DBP), glucose levels, lipid levels, and heart rate were higher in participants with excess weight compared to those with normal BMI, while there were

Table 1. The baseline characteristic of individuals.

Variable	Normal BMI	Excess weight	Total sample	P-value*
Demographic information				
N	1186	791	1977	—
Age years	59.99 ± 8.63	61.27 ± 8.77	60.5 ± 8.71	<0.001
Gender male, %	368(31.03%)	299(37.8%)	667(33.74%)	<0.001
Height cm	161.45 ± 7.83	161.54 ± 7.87	161.49 ± 7.85	0.729
Weight kg	57.7 ± 7.7	71.84 ± 8.77	63.3 ± 10.68	<0.001
SBP mmHg	124.57 ± 18.19	132.46 ± 18.77	127.71 ± 18.82	<0.001
DBP mmHg	78.14 ± 9.33	82.28 ± 9.7	79.79 ± 9.69	<0.001
HRV indices				
HR bpm	72.2 ± 10.32	72.99 ± 9.89	72.52 ± 10.16	0.018
TP ms ²	894.05 ± 724.14	857.28 ± 685.73	879.34 ± 709.16	0.11
LF ms ²	194.82 ± 205.7	188.49 ± 214.59	192.29 ± 209.3	0.351
HF ms ²	185.64 ± 234.32	183.27 ± 201.17	184.69 ± 221.63	0.743
LFHF	1.75 ± 2.04	1.62 ± 1.88	1.7 ± 1.98	0.051
Laboratory assays				
FPG mmol/L	5.34 ± 1.71	5.69 ± 1.86	5.48 ± 1.78	<0.001
PBG mmol/L	7.17 ± 3.45	8.26 ± 3.66	7.6 ± 3.58	<0.001
FINS uml/L	6.18 ± 9.19	8.87 ± 15.44	7.26 ± 12.16	<0.001
TC mmol/L	5.34 ± 0.99	5.28 ± 1.02	5.32 ± 1	0.049
TG mmol/L	1.59 ± 0.86	1.89 ± 1.12	1.71 ± 0.99	<0.001
HDL mmol/L	1.41 ± 0.34	1.27 ± 0.28	1.36 ± 0.33	<0.001
LDL mmol/L	3.17 ± 0.78	3.22 ± 0.77	3.19 ± 0.78	0.027
SCr μmol/L	77.18 ± 29.37	78.18 ± 21.77	77.58 ± 26.59	0.249
UA μmol/L	269.08 ± 81	297.66 ± 85.93	280.51 ± 84.17	<0.001
Medical history				
Smoking yes,%	163(13.74%)	141(17.83%)	304(15.38%)	<0.001
HTN yes,%	455(38.36%)	473(59.8%)	928(46.94%)	<0.001
DM yes,%	192(16.2%)	227(28.7%)	419(21.2%)	<0.001
MetS yes,%	308(25.97%)	475(60.05%)	783(39.61%)	<0.001
CAN yes,%	196(16.53%)	167(21.11%)	363(18.36%)	<0.001
SNP				
rs7375036 (C)	20(1.70%)	17(2.09%)	37(1.96%)	0.367

Notes: *Difference analysis between non-MetS and MetS groups; SBP-systolic blood pressure, DBP-diastolic blood pressure, FPG-fasting plasma glucose, PBG-plasma blood glucose, FINS-fasting blood insulin, TC-serum total cholesterol, TG-triglyceride, UA-uric acid, HDL-high-density lipoprotein cholesterol, LDL-low-density lipoprotein cholesterol, SCr-serum creatinine, UA-uric acid, HR-heart rate, TP-total power of variance, LF-low frequency, HF-high frequency, HTN-hypertension, MetS-metabolic syndrome, DM-diabetes mellitus, CAN-cardiovascular autonomic neuropathy, SNP-single nucleotide polymorphism.

lower high-density lipoprotein (HDL) cholesterol levels in participants with excess weight. As mentioned in our previous study,²⁰ the minor allele (C) frequencies of rs7375036 within the *SCN10A* gene were 1.70% and 2.09% in participants with excess weight and those with normal BMI, respectively.

Univariate and multiple variable analysis for diabetes

Univariate logistic regression models were developed to estimate the associations between various clinical factors and CAN. As described earlier,^{20,21} the univariate analyses revealed that age, BMI, SBP, DBP, glucose level, triglyceride (TG), and medical history were significantly associated with CAN ($P < 0.05$ for all); however, there were no significant associations between rs7375036 SNP and CAN ($P = 0.627$).

The MLR that included BMI and rs7375036 SNP, after controlling for the potential confounding factors of age, gender, blood pressure, glucose level, TG, and medical history, indicated a significant association between BMI and CAN ($P = 0.021$, OR = 1.121, 95%CI: 1.018–1.234, Table 2). However, there was no significant association between rs7375036 SNP and CAN ($P = 0.699$).

BMI and SCN10A interaction analysis for CAN

MLR models were developed to include the two main effect variables of BMI and rs7375036 SNP and their interaction (BMI*rs7375036). The interaction item among them was detected in the MLR model after adjustment for the relevant potential confounders of age, gender, blood pressure, glucose level, TG, and medical history ($P = 0.017$) (Table 3 and Fig. 1). In participants with normal BMI, the CAN

Table 2. Multiple variable analysis for BMI and rs7375036 for CAN.

Variables	β	SE	P-value	OR	95.0% CI
BMI	0.114	0.049	0.021	1.121	1.018–1.234
rs7375036	0.116	0.301	0.699	1.124	0.623–2.028

Notes: DM-diabetes mellitus, BMI-body mass index, multiple variables logistic model adjusted for age, gender, blood pressure, laboratory assays, and medical history.

Table 3. The interaction analysis of BMI and SNPs for CAN.

Variables	β	SE	P-value	OR	95.0% CI
BMI	0.266	0.084	0.002	1.304	1.106–1.537
rs7375036	−0.006	0.013	0.133	0.994	0.878–1.127
BMI by rs7375036	1.687	0.706	0.017	5.404	1.355–21.558
RERI		5.706			3.781–8.613
AP		0.815			−0.185–1.814
S		20.149			9.567–32.433

Notes: DM-diabetes mellitus, RERI-relative excess risk due to interaction, AP-proportion attributable to interaction, S-synergy index.

prevalence was lower in those with minor allele C and those with allele T (10.21% and 15.54%, respectively). In contrast, in participants with excess weight, the CAN prevalence was significantly higher in those with minor allele C compared to those with allele T (37.29% versus 19.79%). The interaction effect was estimated ($OR_{Int} = 5.404$, 95%CI: 1.355–21.558) as was the interaction on an additive scale (RERI = 5.706, 95%CI: 3.781–8.613; AP = 0.815, 95%CI: −0.185–1.814; S = 20.149, 95%CI: 9.567–32.433).

Discussion

We conducted a large-scale, community-based, cross-sectional study to evaluate the interaction effect of BMI and *SCN10A* on

CAN in a large case-control sample of Chinese individuals. To the best of our knowledge, this is the first investigation with an interaction analysis of CAN with regard to BMI and *SCN10A* in a Chinese population. The short-term HRV was measured non-invasively by a power spectral analysis to evaluate CA function. This test is suitable for population-based studies. The prevalence of HTN, MetS, and CAN in the general population was similar to the previous studies. Our sample was a good representation across the country, so our findings might work well outside of the studied areas in China.

The present study reconfirmed the fact that BMI is significantly associated with CAN. Univariate and multiple variable analyses provided evidence to support this finding ($P < 0.001$), and the results were consistent with the previous reports.^{12,13} However, no associations between *SCN10A* and CAN were found in the present study ($P = 0.699$). The functional presence of *SCN10A*/NaV1.8 in intracardiac neurons indicates a novel role for this neuronal sodium channel in the regulation of cardiac electric activity; however, there is no evidence suggesting that *SCN10A* polymorphisms are associated with an increased relative risk of CAN. In neural tissues, the sodium channel isoform NaV1.8 (encoded by the *SCN10A* gene) is highly expressed in small- and medium-diameter nociceptive sensory neurons of the dorsal root ganglia and cranial sensory ganglia.²⁴ There is little evidence to demonstrate *SCN10A* (rs7375096) to be an independent risk factor for CAN. This may be attributed to the limited number of participants, which resulted in insufficient statistical power to detect a slight effect of the common polymorphism in *SCN10A* on CAN susceptibility. Therefore, it is necessary to determine the association between this *SCN10A* genetic variant and CAN in a larger sample size. Another possible explanation for this puzzling phenomenon is the diverse ethnic/regional backgrounds of the participants as the findings might vary by population because of underlying, unobserved genetic variation.

These interesting findings showed that there was a significant interaction effect of BMI and *SCN10A* on CAN in a Chinese population. Our study is the first analysis of the interaction of BMI and *SCN10A* variants on CAN susceptibility. Among several factors, autonomic dysregulation has been advocated as a causal link between unhealthy lifestyles, such as overeating and sedentariness, and metabolic abnormalities, as modeled by MetS.²⁵ Considering that lifestyle changes can modify the risk of CAN, the likely effect of metabolic quantitative traits on CAN susceptibility needs to be addressed as well as their interactions with various gene variants. NaV1.8 function has mostly been associated with pain perception,²⁶ but several GWAS have also linked *SCN10A* to PR interval and QRS duration on ECG.^{14,15,27} The association between the *SCN10A* locus and atrial fibrillation has consistently been reported.¹⁴ These findings

Figure 1. Interaction analysis of *SCN10A* (7375036) and BMI for CAN. Interaction of BMI and *SCN10A* (rs7375036), $BMI * rs7375036$ $OR_{GEI} = 5.404$, 95%CI: 1.355–21.558, $P = 0.017$.

indicate that *SCN10A* might be a potential genetic marker for CAN. In the present study, the interaction of rs7375096 (*C* allele) and BMI conferred a high risk for CAN. Additionally, our previous study demonstrated that DM interacts *SCN10A* to affect CAN in this sample.²⁰ These findings offered evidence what *SCN10A* associated with CAN was modified by environmental factors such as metabolic factors.

Autonomic dysfunction depends not only on autonomic nervous system function, but also on the integrity of the CA reflex loops. CAN has been attributed to increased tunica intima or media thickness at the site of the baroreceptors, reduced vascular distensibility, endothelial dysfunction, and impaired cardiac contractility, which also contributes to CA dysfunction.²⁸ It has already been demonstrated that lifestyle modifications and improvements in HTN and/or weight control can prevent the progression of CAN.²⁹ In this study, we focused on evaluating the relationships of *SCN10A* variants and BMI to CAN. It is noteworthy that the risk imparted by the minor *C* allele was higher than that by the *T* allele in overweight subjects, thus prompting speculation about a possible interaction between *SCN10A* (rs7375096) and BMI in determining overall CAN risk. However, the underlying mechanism is still unknown.

This study had several limitations. Firstly, the participants were all recruited from Shanghai, so they may not have been representative of China as a whole. Additionally, this study did not cover age groups outside of 30–80 years, so it is less practical for younger or elderly populations. Furthermore, the present study was based on a cross-sectional design, so it was not possible to detect individual changes in autonomic regulation related to lifestyle changes, or to perform a comparative simultaneous evaluation of interventions on BMI and CA function. Finally, it is important to confirm these findings with additional investigations using larger samples to systematically evaluate the likely interaction effect of *SCN10A* variants and BMI on CAN risk. The synergistic results of this study need to be verified by future follow-up studies.

Conclusion

Our findings suggested that BMI is independently and significantly associated with CAN, and offered evidence of an interactive effect of *SCN10A* (rs7375096) and BMI on CAN. The environmental factor of BMI may modify the *SCN10A* gene to associate it with the CAN. A functional study, such as gene-targeting in mice, is needed to clarify the role of *SCN10A* as a whole.

Conflict of Interest

The authors have no conflict of interest to declare.

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