Letter to the Editor

A Risk Prediction Model for Ischemic Stroke in Southern Chinese Population: Impact of Multiple Genetic Variants and Clinical/Lifestyle Factors[*]

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With a global prevalence of 7.7 million, ischemic stroke (IS) is one of the leading causes of death and disability worldwide. In China, IS alone contributed to 69.6% of stroke events and accounted for 37.1% of the mortality/disability rate[1]. IS is a complex disease that is known to be associated with various genetic variants and clinical and lifestyle risk factors[2]. Genome -wide association studies (GWAS) provided evidence for the occurrence of more than 160 IS-associated single nucleotide polymorphisms (SNPs). In terms of clinical risk factors, patients with a history of chronic diseases like hypertension, dyslipidemia, and diabetes mellitus, display a higher risk of developing IS. Among the various lifestyle components, leading a sedentary lifestyle, smoking, and having an unhealthy diet are known to be associated with the risk of IS. Thus, integration of various genetic, clinical, and lifestyle variables might prove to be highly beneficial in the prediction and prevention of IS at the individual level[3].

The present study aimed to construct a risk prediction model for IS by simultaneous incorporation of both genetic variants and clinical/lifestyle indicators. The study involved a prospective cohort of the Southern Chinese population. It is expected that the proposed model could be validated externally, and the obtained significant features would assist in the interpretation of IS risk in the Chinese population, with a particular focus on IS risk prediction at the individual level.

The study was conducted on subjects belonging to four community health service centers in the Ningbo City of Zhejiang Province. Initially, a total of 2,349 participants aged ≥ 40 years without any history of IS were recruited from April–July 2013. Detailed inclusion and exclusion criteria followed in the present study are described in Supplementary Figure S1 (available in www.besjournal.com). The clinical and physical parameters included in the present study were strictly defined. The information regarding the demographic and lifestyle characteristics of the subjects was collected using a standard questionnaire. The salient features of the questionnaire are demonstrated in Supplementary Table S1 (available in www.besjournal.com). After a follow up period of three years, the updated records for each participant containing information regarding the occurrence of any IS incidence were obtained from the electronic health record database. During this 3-year follow up, the individuals that were first diagnosed with heart failure, atrial fibrillation, or myocardial infarction were excluded from the study as such diagnoses might lead to significant changes in their lifestyles, which might further act as confounding factors in the present study. Consequently, 236 participants were excluded, and a total of 2,113 individuals were included in the study.

Initially, the target SNPs were identified and selected from IS-related genetic studies using common databases. A standard SNP selection process was implemented, which was previously established by Li et al.[4]. For genotyping, a total of 102 SNPs were selected (Supplementary Table S2 available in www.besjournal.com). For blood sample collection, the participants were subjected to overnight fasting, and samples were drawn by venipuncture, collected in vials containing anticoagulant EDTA, and preserved at −80 °C. DNA

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was extracted using Tiangen Blood Genomic DNA extraction kits. For genotyping, the polymerase chain reaction (PCR)/ligase detection reaction (LDR) were adopted. The PCR reactions contained 1 μL genomic DNA, 1.5 μL 10× PCR buffer, 1.5 μL $MgCl_2$, 0.3 μL dNTPs, 0.15 μL each primer, and 0.2 μL Taq DNA polymerase in a total volume of 15 μL, and were performed in an ABI Prism 7000 Sequence Detection System with an initial melting at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 15 s, extension at 72 °C for 30 s, and final extension at 72 °C for 3 min. Each ligation reaction included 3 μL PCR product, 1 μL 10× Taq DNA ligase buffer, 5 U Taq DNA ligase, and 0.01 μL each discriminating probe in a total volume of 10 μL, and was carried out in 30 cycles at 94 °C for 30 s and 56 °C for 3 min. Re-sequencing results for 10% of the samples showed that the concordance rates were > 95% for all target SNPs.

In the present study, elastic net regression was adopted for the modeling process[5]. In particular, this method introduces ℓ2 -norm and ℓ1 -norm penalties into the regularization term to deal with high correlation variables and estimates a series of coefficients $\widehat{\beta}_{Elastic}$ as per the following Equation:

$$\widehat{\beta}_{Elastic} = argmin_{\beta}\left[-\sum_{i=1}^{n}\{y_i log(\pi_i) + (1-y_i)\,log(1-\pi_i)\} + \lambda_1\sum_{j=1}^{p}|\beta_j| + \lambda_2\sum_{j=1}^{p}\beta_j^2\right]$$

Here, $y_i \in \{0,1\}$ denotes the response variable and $\pi_i = p(y_i = 1\,|x_i) = \frac{exp\left(x_i^T\beta\right)}{1+exp\left(x_i^T\beta\right)}, i = 1, 2, \cdots, n$. The tuning parameters λ_1 and λ_2 determine the regularized logistic regression solution and coefficient estimates. In this study, 102 SNPs and 27 clinical/lifestyle covariates were used in the construction of IS risk prediction model. Individuals' final risk scores were obtained, and they were further classified into three IS risk categories (high/intermediate/low). In particular, positive predictive values (PPVs), sensitivity, and specificity were calculated. The discriminative ability of the constructed models was measured in terms of the area under curves (AUC). In terms of the given captured predictors, the impact of various risk profiles on IS was evaluated, and R software, version 3.6.1, was used for analysis. Further, for the identification of KEGG/Reactome pathways and gene ontology (GO) function interpretations, the enrichment analysis tool g:Profiler was adopted[6].

Among 2,113 participants recruited in this study, 3.17% were newly diagnosed with IS by August 2016. Supplementary Table S3 (available in www. besjournal.com) summarizes the baseline features, which were statistically compared between cases and controls. Interestingly, age, height, SBP, clinical history of hypertension, and dietary habits involving the consumption of egg, red meat, chicken, and fish showed significant differences between cases and controls, with $P < 0.05$.

At the initial genetic -based modeling stage, elastic net regression was applied on 102 SNPs selected from common databases. Further, the performance of all candidate models was evaluated using 10-fold cross -validation, and the model with the highest fitted AUC value was recognized as the best model. As shown in Figure 1, the derived/resulting genetic model captured 15 SNPs and was characterized by a fitted AUC of 0.691 (95% *CI*: 0.627–0.755). Following this, the model -driven genetic risk scores were calculated for each individual and utilized for the construction of the full model. In the next stage, 27 clinical/lifestyle covariates, including three demographic features, four anthropometric parameters, six clinical measurements, clinical history of two diseases, and 12 lifestyle variables, were used along with the model -driven genetic risk scores for further modeling. Finally, a complete model for IS risk prediction was generated, with a fitted AUC of 0.846 (95% *CI*: 0.803–0.89). This model identified four parameters, including age, model-driven genetic risk score, SBP, and fish intake, as important predictors of IS risk (Figure 1 and Supplementary Table S4 available in www.besjournal.com).

The resulting full model was further used to calculate the IS-risk scores for each participant. Subsequently, the participants were classified into three risk categories (Supplementary Table S5

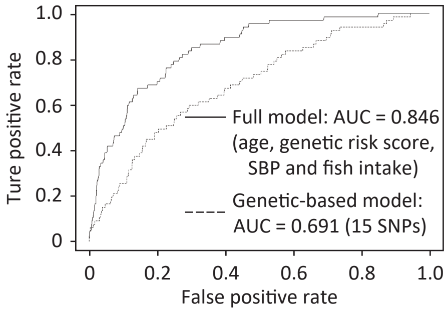

Figure 1. The ROC Curves of the genetic-based model and the full IS risk prediction model.

available in www.besjournal.com). Eventually, 68.34% of 2,113 participants corresponded to the low-risk group, with only 0.76% of these participants developing IS during a 3-year follow up period. In comparison to this, the intermediate and high -risk groups included 27.64% and 4.02% of the participants. Importantly, 5.82% and 25.88% of the subjects belonging to intermediate and high -risk groups, respectively, developed IS within three years. Interestingly, the risk of developing IS was found to be 34-times higher in participants belonging to the high-risk category as compared with those categorized into the low -risk group. These results further highlighted that the generated model displayed a good discriminatory ability to identify patients with a high risk of IS.

Further, the results of the univariate analysis revealed that the four recognized features were independently associated with IS (Supplementary Table S6 available in www.besjournal.com). In particular, individuals aged ≥ 60 years (*OR*: 2.39, 95% *CI*: 1.39–4.95), having elevated SBP (regression coefficient: 11.30, 95% *CI*: 6.66–15.93), or those with increased genetic risk (regression coefficient: 0.71, 95% *CI*: 0.51–0.91) displayed inflated IS risk. On the contrary, the dietary intake of fish reduced the risk of IS (*OR*: 0.70, 95% *CI*: 0.57–0.86). In order to investigate the relative risk of IS, individuals were further classified into various risk profiles. For three captured features of age, SBP, and fish intake, the individuals were defined as "healthy" for clinical/lifestyle exposures if the individual were aged < 60 years, had < 140 mmHg SBP, and consumed fish at least once a week, whereas the subjects were categorized as "intermediate" healthy ones if they fulfilled only two of the three criteria defined for "healthy" exposures. In cases where the subjects fulfilled either one or none of the aforementioned criteria, these were categorized as "unhealthy". Additionally, the derived genetic risk scores were used to generate three genetic risk strata, wherein the top third were treated as high genetic risk and the bottom third as low risk. As shown in Table 1, the relative risk of IS gradually increased as individuals' clinical/lifestyle exposures changed from healthy to unhealthy status and genetic susceptibility changed from low to high. Thus, the subjects with "unhealthy" clinical/lifestyle status and "high" genetic risk displayed the highest relative risk of IS (*RR*: 15.60, 95% *CI*: 3.75–64.96) as compared to the reference group. Interestingly, the participants with "low" genetic risk but "unhealthy" clinical/lifestyle status displayed higher IS risk than the individuals with "high" genetic risk but "healthy" non -genetic status (*RR*: 5.50, 95% *CI*: 1.20–25.20). These results highlighted a stronger cumulative impact of the non-genetic exposures on IS risk than the genetic profiles.

Among the 15 captured SNPs, 6 SNPs or their genes, including rs1800961, rs2954029, rs17321515, rs2575876, rs7493, and rs693, were shown to be directly associated with IS, whereas the remaining 9 recognized SNPs or genes affected IS -related conditions (Supplementary Table S4). In particular, rs4939883 was reported to be associated with HDL-c level, rs10889353 contributed to variations in TG levels, and rs4299376, *PCSK9* (rs11583680), and *SLC12A3* (rs11643718) were LDL-associated variants. Besides these, rs1229984 was a risk factor associated with alcohol dependence that may increase the risk of IS *via* alcohol -induced sympathetic activation, whereas *ADCY3* (rs10187348) was a risk factor linked to obesity.

For the identification of KEGG/Reactome pathways and GO functional interpretations, enrichment analysis was performed for 15 captured SNPs *via* g:GOSt module of g:Profiler tool set (Figure 2)[6]. For multiple testing adjustments, g:SCS threshold was set to 0.001. Consequently, the captured SNPs were enriched to signaling pathways linked to cholesterol metabolism (KEGG: 04979, adjusted P = 2.471 × 10^{-6}), fat digestion and absorption (KEGG:04975, P_{adjusted} = 2.225 × 10^{-4}), plasma lipoprotein assembly (REAC: R-HSA-8963898, P_{adjusted} = 1.821 × 10^{-4}), and transport of small molecules (REAC:R -HSA -382551, P_{adjusted} = 4.604 × 10^{-4}). In terms of the biological domain, the captured

Table 1. Relative risk of IS for combined genetic and non-genetic profiles

Clinical/lifestyle exposure	Genetic risk		
	Low	Intermediate	High
Healthy	–	–	1 (reference)
Intermediate	–	2.83 (0.55–14.42) P = 0.21	6.74 (1.54–29.56) P = 0.011
Unhealthy	5.50 (1.20–25.20) P = 0.028	6.90 (1.56–30.51) P = 0.011	15.60 (3.75–64.96) P < 0.001

SNPs aggregated to cholesterol (GO: 0120020, P_{adjusted} = 4.505 × 10^{-5}), sterol (GO: 0120015, P_{adjusted} = 5.298 × 10^{-5}), and lipid (GO: 0120013, P_{adjusted} = 5.686 × 10^{-4}) transfer activities for molecular function (MF) domain. For the biological process (BP) domain, these SNPs functionally enriched to the lipid/cholesterol/sterol homeostasis (GO: 0055088, 0042632, 0055092, with $P_{\text{adjusted}} < 6 \times 10^{-6}$). According to human phenotype ontology, the identified SNPs were found to be significantly related to certain diseases, including premature coronary artery atherosclerosis, myocardial steatosis, and cerebral artery atherosclerosis.

In research settings, when collinear predictors greatly outnumber the available number of samples ($P > n$), ordinary regression is subjected to overfitting and coefficient instability. Comparatively, the use of the elastic net regression model allows us to control the total number of involved variables using the penalty parameter λ and capture groups of potentially highly correlated variables to build a sparse model that is immune to overfitting[5]. The robustness of elastic net regression in addressing multicollinearity and overfitting has been previously established[7]. To verify the same in this study, the data were further divided into construction and validation sets at a ratio of 9:1, and the validation procedure was introduced. The newly generated IS-risk model achieved a fitted AUC of 0.835 on the construction set and a validated AUC of 0.81 on the validation set. These values were slightly lower than the fitted AUC of the original model (0.846). These variations might be attributed to a certain degree of overfitting. Besides this, the occurrence of an insufficient number of cases (n = 60) and samples in the construction set after splitting the data could also have acted as contributing factor, resulting in a less-comprehensive prediction model that identified only a subset of important risk factors and had reduced power of prediction.

Several previous studies attempted the construction of risk prediction models for both "all-stroke" and/or "IS-only". Similar to the present case, these tools were also developed using common lifestyles, medical conditions, or genetic variants involved in lipid/cholesterol metabolism, statin pathways, or cerebral artery atherosclerosis[3,8,9]. These findings highlighted that different subtypes of IS could be induced by similar lifestyle and genetic risk factors[2]. Recently, an atrial substrate model was proposed by Kamel et al.[10] illustrating that aging and other common vascular risk factors like unhealthy lifestyles may simultaneously be involved in distinct etiologies underlying different IS subtypes. In particular, these factors could induce an abnormal atrial tissue substrate to cause AF and thromboembolic stroke, and also trigger large-artery atherosclerosis, ventricular systolic dysfunction, or in situ cerebral small -vessel occlusion, leading to thrombotic stroke. Nevertheless, different subtypes of IS are still characterized by their unique triggers and pathological mechanisms. Therefore, to reveal the unique etiologies of IS subtypes, it is important and necessary to build specific predictive tools for each subtype of IS.

The present study had certain limitations. The original model derived in this study could not be validated owing to the limited number of cases and sample size. Thus, future studies should apply this model to an independent dataset to verify its accuracy. Additionally, the measurements or classifications of some lifestyle factors used in this study were not standardized, which might further limit the application of this model in external settings.

For fatal diseases that affect populations all across the globe, the development of robust, individualized disease risk assessment tools is the first step toward precision medicine and health care. In the present study, a new tool was generated for IS risk prediction, which involved 15 captured SNPs and three clinical/lifestyle predictors. The results of the study highlighted the suitability of this new tool in IS

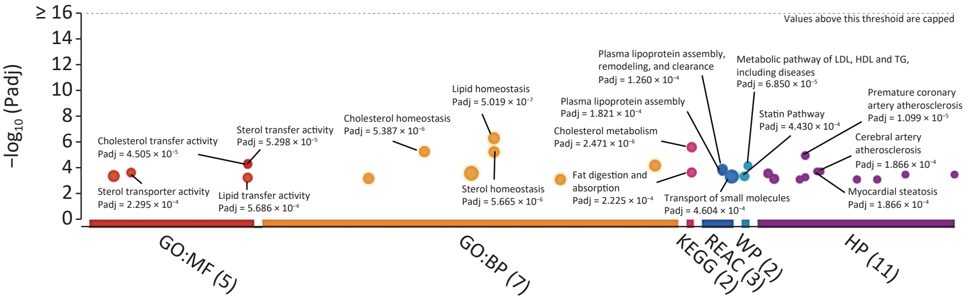

Figure 2. The P-value plot of enriched pathways and functional domains for the 15 captured SNPs.

risk recognition in the Chinese population at the individual level. Additionally, this tool might assist in providing valuable information regarding the implications of various factors in IS etiology.

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The authors declare no conflict of interest.

The study was approved by the Medical Ethics Committee of Hangzhou Normal University (No. 2013020).

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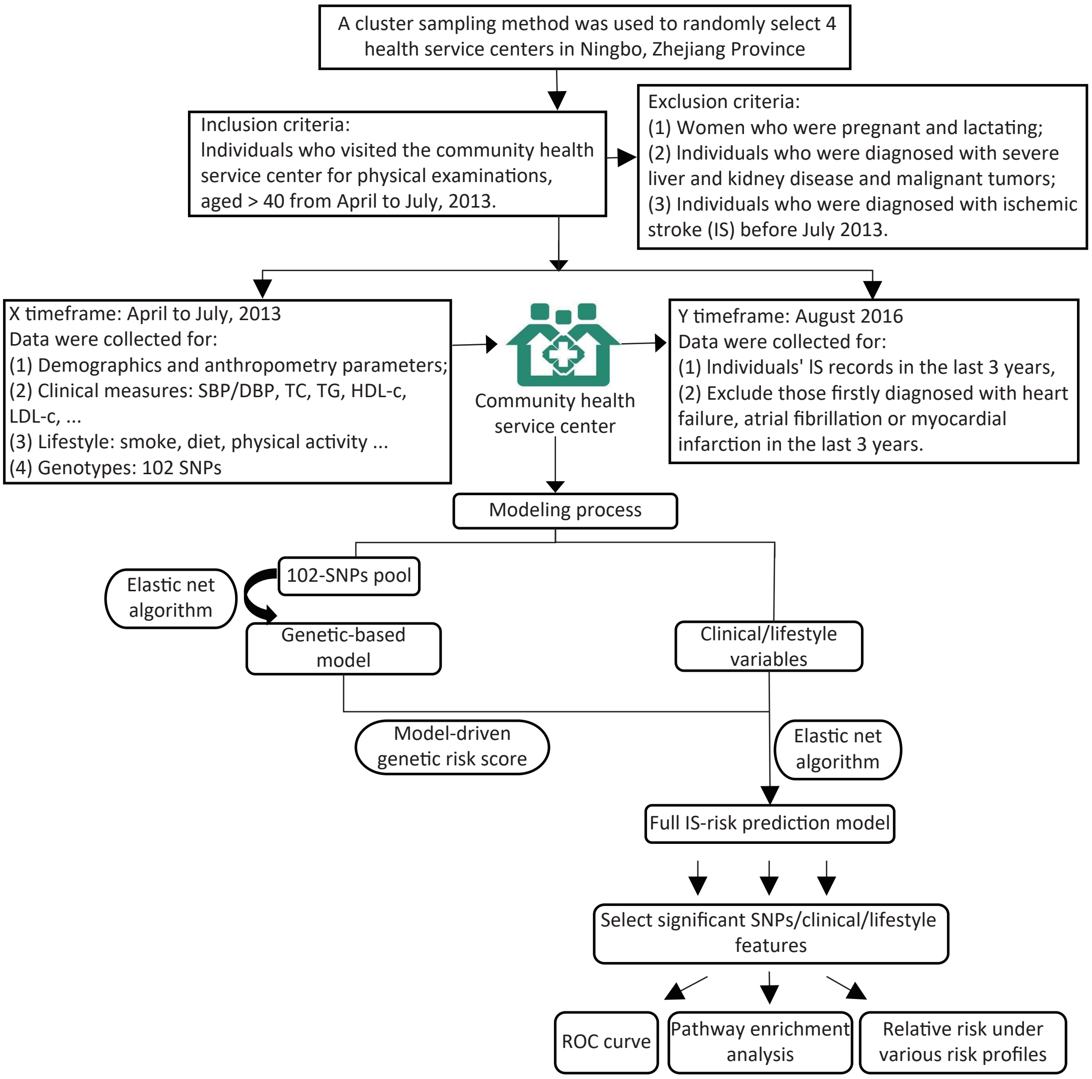
A cluster sampling method was used to randomly select 4 health service centers in Ningbo, Zhejiang Province
Inclusion criteria:
Individuals who visited the community health service center for physical examinations, aged > 40 from April to July, 2013.
Exclusion criteria:
(1) Women who were pregnant and lactating;
(2) Individuals who were diagnosed with severe liver and kidney disease and malignant tumors;
(3) Individuals who were diagnosed with ischemic stroke (IS) before July 2013.
X timeframe: April to July, 2013
Data were collected for:
(1) Demographics and anthropometry parameters;
(2) Clinical measures: SBP/DBP, TC, TG, HDL-c, LDL-c, ...
(3) Lifestyle: smoke, diet, physical activity ...
(4) Genotypes: 102 SNPs
Community health service center
Y timeframe: August 2016
Data were collected for:
(1) Individuals' IS records in the last 3 years,
(2) Exclude those firstly diagnosed with heart failure, atrial fibrillation or myocardial infarction in the last 3 years.
Modeling process
Elastic net algorithm
102-SNPs pool
Genetic-based model
Clinical/lifestyle variables
Model-driven genetic risk score
Elastic net algorithm
Full IS-risk prediction model
Select significant SNPs/clinical/lifestyle features
ROC curve
Pathway enrichment analysis
Relative risk under various risk profiles

Supplementary Figure S1. Study design.

Supplementary Table S1. Definition and classification of features collected in our study

Features	Definition/Classification
Ischemic Stroke	Diagnostic criteria from the American Heart Association/American Stroke Association in 2013; Diagnostic tools: brain computerized tomography (CT) and/or magnetic resonance imaging (MRI) and clinical characterization.
Anthropometry parameters	Height, weight and waist circumference were measured by regular methods during physical examination; Body Mass Index (BMI) = weight (kg)/height square (m^2).
Systolic/diastolic blood pressure (SBP/DBP)	Measured by regular methods during physical examination.
Plasma concentrations of TC, TG, HDL-c and LDL-c	Measured by a Hitachi 7180 biochemistry automatic analyzer.
Dyslipidemia	Determined by one of the following four criteria: 1.Low density lipoprotein cholesterol (LDL-c) ≥ 3.37mmol/L; 2.High density lipoprotein cholesterol (HDL-c) ≤ 1.04mmol/L' 3.Total cholesterol (TC) ≥ 5.18mmol/L; 4.Triglyceride (TG) ≥ 1.7mmol/L.
Hypertension	Systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg or having a history of antihypertensive-drug consumption.
Smoking	"Yes": Smoke ≥ 1cigarette or more per day in the last 12 months. "No": Smoke < 1 cigarette per day in the last 12 months.
Egg intake	3 levels: Eat < 1/week; 1-4/week; > 5/week in the last 12 months.
Salt intake	3 levels: Intake < 6g/day; 6-9g/day; > 9g/day in the last 12 months.
Fruit/vegetable intake	"Seldom": consuming less than 50g/day in the last 12 months. "Regular": consuming 50g/day in the last 12 months.
Mile/soymilk intake	"Seldom": consuming less than 200ml/day in the last 12 months; "Regular": consuming 200ml/day in the last 12 months.
Red meat/fish/chicken/ dessert intake	"Seldom": consuming this food less than once a week in the last 12 months. "≥ 1 times/week": consuming this food at least once a week in the last 12 months.
Physical activity	"Sedentary": occupations that require little exercise at work, such as office workers; "Light": occupations that need to stand for a relatively long time at work, such as salesmen, waiters and teachers; "Moderate": occupations that required long hours of walking, pushing or pulling at work, such as cleaning services; "Heavy": occupations that required strenuous effort and extensive total body movements such as dancers, construction workers.

Supplementary Table S2. The reported association between 102 SNPs or its genes and IS or IS-related diseases/conditions

SNP	Associated with IS or IS-related diseases/conditions (SNP)	Gene	Associated with IS or IS-related diseases/ conditions (Gene)
rs11646692	—	*BCO1*	Coronary atherosclerosis, dyslipidemia
rs6564851	Carotenoid and tocopherol levels		
rs12934922	—	*BCO1*	Coronary atherosclerosis, dyslipidemia
rs7501331	—	*BCO1*	Coronary atherosclerosis, dyslipidemia
rs671	Coronary artery disease, Body mass index (BMI), triglycerides (TG)	—	—
rs1229984	Cardiovascular disease, systolic blood pressure (SBP),	—	—
rs2479409	Low density lipoprotein cholesterol levels (LDL-c), et al.	—	—
rs17111503	LDL-c, total cholesterol levels (TC), at al.	—	—
rs2483205	—	*PCSK9*	Coronary atherosclerotic lesion extension and calcification
rs662145	—	*PCSK9*	Coronary atherosclerotic lesion extension and calcification
rs11583680	—	*PCSK9*	Coronary atherosclerotic lesion extension and calcification
rs111563724	—	*PCSK9*	Coronary atherosclerotic lesion extension and calcification
rs2738466	—	*LDLR*	Coronary heart disease
rs1003723	—	*LDLR, MIR6886*	Coronary artery disease, plasma lipid levels

Continued

SNP	Associated with IS or IS-related diseases/conditions (SNP)	Gene	Associated with IS or IS-related diseases/ conditions (Gene)
rs6413504	—	*LDLR*	Coronary artery disease, plasma lipid levels
rs17845226	—	*ANXA2*	LDL-c, Coronary heart disease
rs8025278	—	*SLC12A1, LOC107984755*	Hypertension
rs12438818	—	*SLC12A1, LOC107984755*	Hypertension
rs11643718	—	*SLC12A3*	Coronary atherosclerotic lesion extension and calcification
rs5805	—	*SLC12A3*	Coronary atherosclerotic lesion extension and calcification
rs3812963	—	*SLC12A3*	Coronary atherosclerotic lesion extension and calcification
rs4784733	—	*SLC12A3*	Coronary atherosclerotic lesion extension and calcification
rs3782724	Obesity (early onset extreme)		
rs2228576	—	*SCNN1A*	Insulin resistance
rs7205273	—	*SCNN1B*	stroke
rs7200183	—	*SCNN1G*	Hypertension
rs675759	—	*KCNJ1*	Serum Lipid Profile
rs675388	—	*KCNJ1*	Serum Lipid Profile
rs2846679	—	*KCNJ1, LOC107984409*	Serum Lipid Profile
rs1148058	—	*KCNJ1*	Serum Lipid Profile
rs4299376	Coronary artery disease	—	—
rs964184	Coronary artery disease	—	—
rs693	TC, et al.	—	—
rs515135	Coronary artery disease, LDL-c, et al.	—	—
rs5167	TG, high density lipoprotein cholesterol levels (HDL-c)	—	—
rs4420638	Coronary artery disease, LDL-c, et al.	—	—
rs3764261	HDL-c, TG, et al.	—	—
rs10401969	LDL-c, et al.	—	—
rs10889353	TG, et al.	—	—
rs174547	HDL-c, TG, et al.	—	—
rs4846914	HDL-c, et al.	—	—
rs1260326	Cardiovascular disease risk factors, TG, et al.	—	—
rs12654264	LDL-c, et al.	—	—
rs1800961	HDL-c, et al.	—	—
rs16942887	HDL-c, TG, et al.	—	—
rs5929	—	*LDLR*	Coronary artery disease, plasma lipid levels
rs2650000	LDL-c	—	—
rs1800588	HDL-c, TG, et al.	—	—
rs4939883	HDL-c, TC, et al.	—	—
rs7241918	HDL-c, TC, et al.	—	—
rs328	TG, HDL-c, et al.	—	—
rs17145738	TG, et al.	—	—
rs16996148	LDL-c, TG	—	—
rs3812316	TG, et al.	—	—
rs12130333	TG, TC	—	—
rs17321515	TG, TC, et al.	—	—
rs7493	Yu-Zhi constitution type in type 2 diabetes	—	—
rs629301	LDL-c, et al.	—	—

Continued

SNP	Associated with IS or IS-related diseases/conditions (SNP)	Gene	Associated with IS or IS-related diseases/ conditions (Gene)
rs2954029	Coronary artery disease, Coronary artery disease, et al.	—	—
rs4149269	—	*ABCA1*	Coronary artery disease
rs2472433	—	*ABCA1*	Coronary artery disease
rs2740486	—	*ABCA1*	Coronary artery disease
rs2515616	Waist-to-hip ratio adjusted for BMI	—	—
rs2472386	HDL-c	—	—
rs2740479	C-reactive protein levels	—	—
rs4149264	—	*ABCA1*	Coronary artery disease
rs4149339	—	*ABCA1*	Coronary artery disease
rs2515617	—	*ABCA1*	Coronary artery disease
rs2254884	—	*ABCA1*	Coronary artery disease
rs2065412	—	*ABCA1*	Coronary artery disease
rs2472377	—	*ABCA1*	Coronary artery disease
rs4149336	—	*ABCA1*	Coronary artery disease
rs2297406	—	*ABCA1*	Coronary artery disease
rs6479282	—	*ABCA1*	Coronary artery disease
rs4743764	—	*ABCA1*	Coronary artery disease
rs2740484	—	*ABCA1*	Coronary artery disease
rs11789818	—	*ABCA1*	Coronary artery disease
rs2575876	HDL-c in current drinkers, TG in current drinkers, et al.	—	—
rs2482433	—	*ABCA1*	Coronary artery disease
rs2515614	—	*ABCA1*	Coronary artery disease
rs4743763	—	*ABCA1*	Coronary artery disease
rs2000069	—	*ABCA1*	Coronary artery disease
rs10820743	—	*ABCA1*	Coronary artery disease
rs2472510	—	*ABCA1*	Coronary artery disease
rs4665273	—	*ADCY3*	Carotid plaque formation, obesity
rs1127568	—	*ADCY3*	Carotid plaque formation, obesity
rs6751537	—	*ADCY3*	Carotid plaque formation, obesity
rs7608976	—	*ADCY3*	Carotid plaque formation, obesity
rs11689546	—	*ADCY3*	Carotid plaque formation, obesity
rs7604576	—	*ADCY3*	Carotid plaque formation, obesity
rs7593130	—	*ADCY3*	Carotid plaque formation, obesity
rs2241759	—	*ADCY3*	Carotid plaque formation, obesity
rs2278485	—	*ADCY3, LOC105377626*	Carotid plaque formation, obesity
rs1344840	—	*ADCY3*	Carotid plaque formation, obesity
rs4077678	BMI, Childhood obesity	—	—
rs10187348	—	*ADCY3*	Carotid plaque formation, obesity
rs10431036	—	*BCO2*	Ischemic stroke
rs11214109	—	*BCO2*	Ischemic stroke
rs12420476	—	*BCO2*	Ischemic stroke
rs13328843	—	*BCO2*	Ischemic stroke
rs11641677	—	*BCO1*	Coronary atherosclerosis
rs6939861	—	*TFEB, MIR10398*	Cardiovascular diseases
rs11759908	Estimated glomerular filtration rate, estimated glomerular filtration rate in non-diabetics	—	—

Supplementary Table S3. Comparison of baseline features in individuals with or without IS

Characteristics	Case (*n* = 67)	Control (*n* = 2,046)	$t/z/\chi^2$	*P* value
Age, Mean (IQR), y	70.7 (12)	58.6 (17)	8.88	< 0.001
Sex, *n* (%)			0.16	0.88
Male	30 (44.78)	936 (54.25)		
Female	37 (55.22)	1,110 (45.75)		
Education level, *n* (%)			−1.92	0.05
≤ Primary school	57 (85.07)	1,456 (71.16)		
Middle school	7 (10.45)	517 (25.27)		
≥ High school	3 (4.48)	73 (3.57)		
Height, Mean (IQR), cm	157.6 (12.5)	160.4 (11)	−2.76	0.005
Weight, Mean (IQR), kg	57.94 (12.5)	59.97 (13)	−1.65	0.10
Waist, Mean (IQR), cm	82.4 (13)	81.3 (11)	1.13	0.26
BMI, Mean (IQR), kg/m^2	23.3 (4.01)	23.3 (3.95)	0.08	0.93
SBP, Mean (IQR), mmHg	145.1 (26.5)	133.9 (28)	4.77	< 0.001
DBP, Mean (IQR), mmHg	83.4 (13)	81.66 (17)	1.14	0.25
TC, Mean (IQR), mmol/L	5 (1.445)	4.9 (1.238)	1.12	0.26
TG, Mean (IQR), mmol/L	1.4 (0.84)	1.4 (0.78)	−0.18	0.85
HDL-c, Mean (IQR), mmol/L	1.2 (0.28)	1.3 (0.37)	−1.5	0.13
LDL-c, Mean (IQR), mmol/L	3.2 (1.19)	3.1 (1.1)	0.91	0.36
Dyslipidemia, *n* (%)	38 (56.72)	1,163 (56.84)	−0.02	0.98
Hypertension, *n* (%)	46 (68.66)	939 (45.9)	3.56	< 0.001
Smoking, *n* (%)			0.37	0.71
Yes	18 (26.87)	538 (26.3)		
No	49 (73.13)	1,508 (73.7)		
Egg intake, *n* (%)			−2.09	0.04
< 1 /week	28 (41.8)	558 (27.3)		
1–4 /week	31 (46.3)	1,206 (58.9)		
> 5 /week	8 (11.9)	282 (13.8)		
Salt intake, n (%)			1.2	0.23
< 6 g/day	39 (58.2)	1,389 (67.9)		
6–9 g/day	25 (37.3)	546 (26.7)		
> 9 g/day	3 (4.5)	111 (5.4)		
Fruit intake, *n* (%)			−1.78	0.07
Seldom (< 50 g/day)	29 (43.3)	671 (32.8)		
Regular (≥ 50 g/day)	38 (56.7)	1,375 (67.2)		
Vegetable intake, *n* (%)			0.02	0.99
Seldom (< 50 g/day)	28 (1.4)	0 (0)		
Regular (≥ 50 g/day)	2018 (98.6)	67 (100)		
Milk intake, *n* (%)			0.02	0.98
Seldom (< 200 mL/day)	42 (62.69)	1,312 (64.13)		
Regular (≥ 200 mL/day)	25 (37.31)	734 (35.87)		

Continued

Characteristics	Case (n = 67)	Control (n = 2,046)	t/z/χ^2	*P* value
Soymilk intake, *n* (%)			−0.62	0.54
Seldom (< 200 mL/day)	50 (74.63)	1,514 (74)		
Regular (≥ 200 mL/day)	17 (25.37)	532 (26)		
Red meat intake, *n* (%)			−4.13	< 0.001
Seldom	16 (23.88)	172 (8.4)		
≥ 1 times/week	51 (76.12)	1,874 (91.6)		
Fish intake, *n* (%)			−4.13	< 0.001
Seldom	13 (19.4)	149 (7.28)		
≥ 1 times/week	54 (80.6)	1,897 (92.72)		
Chicken intake, *n* (%)			−2.1	0.04
Seldom	27 (40.3)	581 (28.4)		
≥ 1 times/week	40 (59.7)	1,465 (71.6)		
Dessert intake, *n* (%)			−0.8	0.43
Seldom	24 (35.8)	639 (31.2)		
≥ 1 times/week	43 (64.2)	1,407 (68.8)		
Physical activity, *n* (%)			0.12	0.90
Sedentary	13 (19.4)	259 (12.66)		
Light	34 (50.75)	1,182 (57.78)		
Moderate	9 (13.43)	426 (20.82)		
Heavy	11 (16.42)	179 (8.74)		

Supplementary Table S4. The coefficients of 15 SNPs adopted by the genetic-based IS-risk model and 4 significant features identified by the full IS-risk model

Genetic-based IS-risk model				
SNP	**Coefficient**	***Gene***	**Associated with IS or IS-related diseases/ conditions**	**PMID**
rs1800961	0.56226531	*HNF4A*	IS	22403240
rs4299376	0.39578499	*ABCG8*	LDL-c	26043746
rs2278485	0.23077208	*ADCY3*	Cardiovascular disease	28985495
rs10889353	0.13749869	*DOCK7*	TG, LDL-c	26744084
rs2954029	0.10619992	*TRIB1*	IS	31250580
rs17321515	0.08371697	*TRIB1*	IS	30787327
rs5167	0.08041245	*APOC2*	Cardiovascular disease	29367937
rs10187348	0.02155788	*ADCY3*	Obesity	30704512
rs11583680	0.02063195	*PCSK9*	LDL-c	28577571
rs1229984	0.01210608	*ADH1B*	Alcohol dependence	30994927
rs2575876	0.011446	*ABCA1*	IS	28865324
rs4939883	−0.14570917	*LOC105372112*	HDL-c	22174694
rs11643718	−0.10686348	*SLC12A3*	LDL-c	28166833
rs7493	−0.07784675	*PON2*	IS	28566152
rs693	−0.01463697	*APOB*	IS	29416768
Full IS-risk model				
Features		**Coefficients**		**PMID**
Age		0.427610541		30010821
Genetic risk score		0.332308739		——
SBP		0.00413839		28097354
Fish intake		−0.004976849		15155968

Note. "——" Genetic risk score is a combined feature derived from the genetic-based IS-risk model, thus its PMID is not available.

Supplementary Table S5. The performance of the full IS-risk model in the three risk categories (high/intermediate/low)

Risk category	Low risk	Intermediate risk	High risk	Total
Number of Observations	1444	584	85	2113
Cases	11	34	22	67
PPV	0.76%	5.82%	25.88%	3.17%
Observation%	68.34%	27.64%	4.02%	100.00%
Sensitivity	16.42%	50.74%	32.84%	100.00%
Specificity	70.04%	26.88%	3.08%	100.00%
Relative Risk	0.23	1.89	10.66	1

Supplementary Table S6. The univariate analysis results for the captured features.

Characteristics	Case (N = 67)	Control (N = 2,046)	OR/Regression coefficient (95% *CI*)	*P*
Genetic risk score, Mean (IQR)	3.854 (1.161)	3.148 (0.95)	0.71 (0.51–0.91)	< 0.001
Age, *n* (%)				
40–49	3 (4.48)	546 (26.69)	—	—
50–59	3 (4.48)	565 (27.61)	0.97 (0.18–5.24)	0.97
60–69	19 (28.36)	604 (29.52)	2.39 (1.39–4.95)	0.01
> 70	42 (62.68)	331 (16.18)	2.85 (2.03–4.58)	< 0.001
SBP, Mean (IQR)	145.1 (26.5)	133.9 (28)	11.30 (6.66–15.93)	< 0.001
Fish intake, *n* (%)			0.70 (0.57–0.86)	< 0.001
Never	13 (19.40)	149 (7.28)		
> 1 times/week	54 (80.60)	1,897 (92.72)		