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Original Article

Genetic polymorphisms of INSIG2 were associated with coronary artery disease in Uygur Chinese population in Xinjiang, China

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Abstract: Background: Dyslipidemia is a major and independent risk factor for the development of Coronary artery disease (CAD). The protein which is encoded by insulin induced gene2 (INSIG2) plays an important role in the mediation of the feedback control of cholesterol synthesis, lipogenesis and glucose homeostasis. The aim of the present study was to assess the association between the human INSIG2 gene and CAD in Han Chinese and Uygur Chinese population of Xinjiang, China. Methods: A total of 832 CAD patients (334 Han, 498 Uygur) and 919 controls (346 Han, 573Uygur) were selected for the present Case-control study. Three tagging SNPs (rs1261829, rs21613329 and rs17047757) of INSIG2 gene were genotyped using TaqMan® assays from Applied Biosystems following the manufacturer's instructions and analyzed in an ABI 7900HT Fast Real-Time PCR System. Results: In the Uygur Chinese population, for total, men and women the rs17047757 was associated with CAD by analyses of a dominant model (all, $P < 0.001$) and the difference remained significant after multiple adjustment in a dominant model (all, $P < 0.001$). This relationship was also observed in rs2161829 for total and women by analyses of a recessive model (for total: $P = 0.002$; for women: $P = 0.001$, respectively) the difference remained significant after multiple adjustment in a recessive model (for both, $P = 0.001$). However, this relationship was not observed in this three tagging SNPs before and after multiple adjustment in Han Chinese population. Conclusion: Our results indicated that both rs17047757 and rs21613329 in the INSIG2 gene were associated with CAD in Uygur Chinese population in Xinjiang, China.

Keywords: Genetics, INSIG2 gene, single nucleotide polymorphism, coronary artery disease, case-control study

Introduction

Coronary artery disease (CAD) is one of the leading causes of disability and mortality worldwide [1], the etiology and pathogenesis of CAD are that of a multi-factorial disorder that results from both genetic and environmental risk factors. Dyslipidemia is a major and independent risk factor for the development of CAD and accounts for approximatively 50% of CAD cases in the population [2, 3]. Accumulated evidences suggest that heritable factors range from 40%~60% for the variation in concentration and components of the plasma lipids [4].

Cholesterol is essential component of mammalian cell membranes and it plays important roles in the biosynthesis of steroid hormones and the maintenance of membrane integrity [5]. Whole-body cholesterol homeostasis reflects a balance between dietary uptake, endogenous synthesis, reverse cholesterol transport and removal from the body via biliary and intestinal excretion. There are several genes such as insulin induced gene (INSIG1 and INSIG2) that are involved in the feedback control of lipid synthesis at the transcriptional levels. INSIG is not only one of the endoplasmic reticulum proteins (ER), but also a kind of oxysterol-binding pro-

teins [6, 7] and plays an important role in the mediation of the feedback control of cholesterol synthesis, lipogenesis, glucose homeostasis [5, 8]. Studies conducted by Yabe D et al in vitro showed that when sterols are present in the cell ,INSIG2 blocks further cholesterol synthesis [6]; and studies in vivo also have demonstrated that over expression or down regulation of INSIG2 could significantly affect Cholesterol homeostasis and body weight of the animals [9, 10]. Krapivner et al showed that INSIG2 is also expressed in adipocytes and this expression involved in adipocyte metabolism and body weight regulation [11].

Human INSIG2 is a ~21.5 Kb gene was identified by Yabe et al and mapped on the long arm of chromosome 2, localized to band p14.1, and contains 225 amino acids [12]. Since Herbert et al [13] discovered in a genome-wide association study that genetic variation of rs7566605 in the upstream of the INSIG2 gene associated with BMI, a several studies have explored genetic polymorphisms of INSIG2 gene with related metabolic traits and CAD, but studies of the association between genetic polymorphisms of the INSIG2 gene and cardiovascular disease in diverse ethnicities remain controversial. Several studies have found that genetic polymorphisms of INSIG2 is not only associated with CAD but also related to the major risk factors of CAD, namely, overweight, obesity, hypercholesterolemia, diabetes while others have suggested that genetic polymorphisms of INSIG2 was not associated with CAD or the risk factors of the CAD. However, the relationship between genetic polymorphisms of the INSIG2 gene and CAD in Han and Uygur Chinese population of Xinjiang Uygur Autonomous Region northeast of China is remains unknown.

The aim of the present study is to determine the relationship between genetic polymorphism of INSIG2 gene and coronary artery disease in Han Chinese and Uygur Chinese population of China.

Material and methods

Ethical approval

This study was approved by the ethics committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) and was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from each participant for collection and analysis of relevant clinical data.

Subjects

A total of 1451 unrelated Han Chinese and Uygur Chinese subjects (680 Han, 771 Uygur) who lived in Xinjiang Uygur Autonomous Region of China were included in this study. We recruited 832 cases (334 Han, 498 Uygur) with CAD from The First Affiliated Hospital of Xinjiang Medical University between January 2009 and October 2013. Patients underwent coronary angiography and diagnosed with CAD based on the evidence of at least > 50% stenos is in one major coronary artery. Patients with congenital heart disease, cardiomyopathy, valvular disease and multiple organ failure syndrome were excluded from this group. A total of 919 control subjects (346 Han, 573 Uygur) were randomly selected from the Cardiovascular Risk Survey (CRS) in Xinjiang, northwest of China. The detailed description of the study population and the methods were described previously [14, 15]. Briefly, the CRS consisted of 14,618 subjects (5,757 Hans, 4,767 Uygurs and 4,094 Kazakhs) and was a multiple-ethnic, community-based, cross-sectional study was designed to investigate the prevalence and risk factors for cardiovascular diseases and to determine the genetic and environmental contributions to atherosclerosis, CAD, and cerebral infarction of the Chinese Han, Uygur, and Kazakh populations in the Xinjiang northwest of China from October 2007 to March 2010. Individuals with myocardial infarction, CAD, coronary stenting, multiple organ failure syndrome, and those whose data were incomplete were excluded from control group. Data and information about traditional risk factors of CAD (including hypertension, diabetes mellitus, and dislipidemia) were collected from all participants. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, and/or taking antihypertensive medication. Diabetes mellitus was defined on the basis of the World Health Organization (WHO) criteria (fasting plasma glucose level ≥ 7.0 mM and/or self-reported current treatment with anti-diabetes medication). Hyperlipidemia was defined as a total plasma cholesterol > 6.22 mmol or plasma triglycerides > 2.26 mmol and/or the current use of lipid-lowering drugs [16].

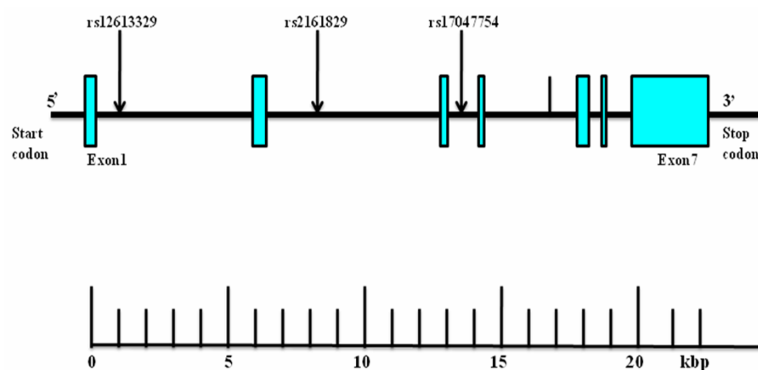

Figure 1. Structure of the human INSIG2 gene. The gene consists of seven exons (boxes) separated by six introns (lines; intergenic regions). Arrows indicate the locations of single-nucleotide polymorphisms (SNPs). kbp, kilobase pairs.

Anthropometric and biochemical variables measurement

Weight and height were measured in a standard method, and body mass index (BMI) was calculated. After 5 min of rest, blood pressure was measured three times within 10 min and the median value was used in the statistical analysis. Smoking and drinking was self-reported using a questionnaire described previously [17]. After 12-hour overnight fasting, 5 mL of venous blood was collected into tubes containing EDTA, sent to Xinjiang coronary artery disease VIP laboratory and analyzed within 4 hours. Genomic DNA was extracted from peripheral leukocytes using a standard phenol-chloroform method and stored at -80°C for future analysis. Biochemical markers in serum such as total cholesterol (TC), triglycerides (TG), glucose, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) were measured by Clinical Laboratory Department of the First Affiliated Hospital of Xinjiang Medical University with a standard method (AR/AVL Clinical Chemistry System; Dimension, Newark, NJ, USA).

SNP selection and Genotyping of INSIG2 gene

Using Haploview 4.2 software and International HapMap Project website phase I&II data base (http://hapmap.ncbi.nlm.nih.gov/i), we obtained three tagging SNPs (rs12613329, rs2161829, rs17047757) for the Han Chinese population by using minor allele frequency (MAF) ≥ 0.05 , linkage disequilibrium (D') across this three SNPs was D' = 1. The numbering of the three SNPs (rs12613329, rs2161829, rs17047757) was by order of increasing distance from the INSIG2 gene 5'end (**Figure 1**). Genotyping in this case-control study was performed by using an Applied Biosystems (ABI, Foster City, CA) TaqMan 7900 system. SNPs primers and probes were provided by ABI Assay-on-demand (http://myscience.appliedbiosystems.com). Thermal cycling was performed using the Applied Biosystems 7900HT Fast Real-Time PCR system (Applied Biosystems). PCR amplification was performed using 2.5 μL of TaqMan Universal Master Mix, No AmpErase UNG (2×) (Applied Biosystems) in a 5 μL final reaction volume, along with 2 ng DNA, 2.375 μL ultrapure water, 0.079 μL Tris-EDTA (TE) buffer (1×), 0.046 μL TaqMan SNP Genotyping Assay Mix (40×) containing a 331.2 nmol/L final concentration of primers and a 73.6 nmol/L final concentration of the probes. The thermal cycling conditions were as follows: 50°C for 2 min; 95°C for 10 min; 50 cycles of 95°C for 15 s; and 60°C for 1 min. Biosystems). Finally the plates were read on the sequence detection system 9700 instrument with the end-point analysis mode of the sequence detection system version 1.6.3 software package (Applied Biosystems).

Statistical analyses

All statistical analyses were performed using SPSS 17.0 software for Windows (SPSS Institute, Chicago, IL, USA). Deviation from the Hardy-Weinberg equilibrium (HWE) of the SNPs was tested by the χ^2 analysis. Continuous variables were compared using the general linear model and represented as means±standard deviation (SD). Logistic regression analyses with effect ratios (odds ratio [OR] and 95% CI) were used to assess contribution of major risk factors. Analyses of traits were adjusted for sex and age; fasting triglycerides were log-transformed using natural logarithms for analysis. Two-tailed *P*-values of 0.05 were considered significant.

Result

Clinical and metabolic characteristics of the subjects

In Uygur Chinese population, for total, including men and women, body mass index, plasma concentration of glucose, triglyceride, LDL-c,

Table 1. Demographic and clinical characteristics of study participants (Uygur Chinese population)

	Uygur			Men			Women		
	CAD	Control	*P* value	CAD	Control	*P* value	CAD	Control	*P* value
Number (n)	498	573		243	279		255	294	
Age (year)	54.452±0.450	53.534±0.420	0.142	56.469±0.662	55.538±0.618	0.304	52.435±0.611	51.531±0.569	0.279
EH (%)	279 (56.0)	192 (34.0)	< 0.001	141 (58.0)	108 (39.6)	0.011	138 (54.1)	84 (28.9)	< 0.001
Diabetes (%)	126 (25.3)	36 (6.3)	< 0.001	75 (30.9)	24 (8.6)	< 0.001	51 (20.0)	12 (4.1)	0.004
Smoking (%)	72 (14.5)	51 (8.9)	0.073	69 (28.4)	51 (18.3)	0.150	3 (1.2)	0 (0.0)	0.999
Drinking (%)	36 (7.2)	18 (3.1)	0.994	36 (14.8)	15 (5.4)	0.548	0 (0)	3 (1.0)	0.999
BMI (Kg/m^2)	27.200±0.178	25.871±0.166	0.043	28.082±0.257	26.646±0.234	0.031	26.464±0.243	25.266±0.226	0.036
GLU (mmol/L)	5.676±0.098	5.109±0.90	0.001	6.230±0.171	5.330±0.155	< 0.001	5.232±0.097	4.865±0.090	0.001
Hyperlipidemia (%)	174 (34.9)	78 (13.6)	< 0.001	105 (43.2)	57 (20.4)	< 0.001	69 (27.1)	22 (7.5)	< 0.001
TG (mmol/L)	0.196±0.011	0.073±0.010	0.020	0.227±0.017	0.057±0.016	0.041	0.153±0.014	0.034±0.013	0.037
TC (mmol/L)	4.823±0. 059	4.194±0. 055	0.767	5.029±0.092	4.421±0.086	0.021	4.629±0.072	4.133±0.067	0.093
HDL-c (mmol/L)	0.055±0.077	0.047±0.007	0.015	0.072±0.009	0.040±0.008	0.276	1.185±0.034	1.252±0.031	0.058
LDL-c (mmol/L)	3.160±0. 046	2.644±0. 043	0.002	3.259±0.092	2.478±0.055	0.032	3.118±0.067	2.781±0.063	0.012

Continuous variables are expressed as mean ±Std. Error. Categorical variables are expressed as percentages. BMI, body mass index; Glu, glucose; TG, triglyceride; TC, total cholesterol; HDL-c, high density lipoprotein; LDL-c, low density lipoprotein; EH, essential hypertension.

Table 2. Demographic and clinical characteristics of study participants (Han Chinese population)

	Han			Men			Women		
	CAD	Control	*P* value	CAD	Control	*P* value	CAD	Control	*P* value
Number (n)	334	346		193	175		141	171	
Age (year)	62.456±0.388	62.399±0.381	0.916	62.104±0.517	62.046±0.543	0.938	62.939±0.588	62.760±0.543	0.882
EH (%)	215 (64.4)	159 (46.4)	< 0.001	116 (60.1)	75 (47.2)	0.018	99 (70.2)	84 (55.6)	0.011
Diabetes (%)	97 (29.0)	37 (10.7)	< 0.001	55 (28.5)	23 (13.1)	0.001	42 (29.8)	14 (8.2)	< 0.001
Smoking (%)	6 (1.8)	0 (0.0)	0.999	5 (2.6)	0 (0.0)	0.999	1 (0.7)	0 (0.0)	0.999
Drinking (%)	1(0.3)	0 (0.0)	1.000	1 (0.5)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	1.000
BMI (Kg/m^2)	26.35±0.183	25.44±0.179	0.002	26.410±0.224	25.722±0.109	0.018	25.690±0.278	24.73±0.106	0.023
GLU (mmol/L)	6.309±0.112	5.482±0.109	< 0.001	6.01±2.18	5.29±1.46	0.013	6.48±2.77	5.20±1.07	0.023
Hyperlipidemia (%)	100 (29.9)	88 (25.5)	0.176	62 (50.5)	50 (49.5)	0.065	50 (56.2)	39 (43.8)	0.017
TG (mmol/L)	0.245±0.012	0.202±0.012	0.001	0.234±0.017	0.104±0.018	0.024	0.648±0.008	0.408±0.008	0.002
TC (mmol/L)	4.264±0.055	4.438±0.054	0.541	3.936±0.068	4.237±0.071	0.293	4.60±1.09	4.64±1.02	0.386
HDL-c (mmol/L)	6.309±0.112	5.482±0.109	0.850	0.017±0.008	0.025±0.008	0.177	0.050±0.009	0.096±0.008	0.195
LDL-c (mmol/L)	2.570±0.050	2.519±0.048	0.994	2.258±0.059	2.439±0.062	0.511	2.878±0.084	2.602±0.077	0.939

Continuous variables are expressed as mean ±Std. Error. Categorical variables are expressed as percentages. BMI, body mass index; Glu, glucose; TG, triglyceride; TC, total cholesterol; HDL-c, high density lipoprotein; LDL-c, low density lipoprotein; EH, essential hypertension.

and prevalence of conventional risk factors for CAD including hypertension, diabetes mellitus and hyperlipidemia were significantly higher in subjects with CAD than in controls. No significant differences were found in age, sex, smoking, drinking, and plasma concentration of triglyceride between CAD subjects and controls; compared with women, plasma concentration of total cholesterol was significantly higher in men subjects with CAD than in controls (**Table 1**).

In Han Chinese population, for total, including men and women BMI, plasma concentration of glucose, triglyceride, the prevalence of hypertension and diabetes mellitus were significantly higher in subjects with CAD than in controls. No significant differences were found in age, sex, drinking, smoking, plasma concentration of cholesterol, HDL-c and LDL-c between CAD subjects and controls; for women, prevalence of hyperlipidemia was higher in subjects with CAD than in controls (**Table 2**).

The distribution of genotypes and alleles of the three tagging SNPs

The genotype distributions for each of the three tagging SNPs were in good agreement with the predicted Hardy-Weinberg equilibrium values

Table 3. Uygur Chinese population: Genotype and allele distributions in patients with CAD and control participants

Variants	Uygur			Men			Women		
	CAD n (%)	Control n (%)	*P* value	CAD n (%)	Control n (%)	*P* value	CAD n (%)	Control n (%)	*P* value
rs12613329(SNP1)									
Genotype									
C/C	18 (3.6)	30 (5.2)		12 (4.9)	21 (7.5)		6 (2.4)	9 (3.1)	
C/G	225 (45.2)	239 (41.7)		111 (45.7)	125 (45.2)		114 (44.7)	113 (38.4)	
G/G	255 (51.2)	304 (53.1)	0.297	120 (49.4)	132 (47.3)	0.475	135 (52.9)	172 (58.5)	0.332
Dominant model									
GG	255 (51.2)	304 (53.1)		120 (49.4)	132 (47.3)		135 (52.9)	172 (58.5)	
CG+CC	243 (48.8)	269 (46.9)	0.581	123 (50.6)	147 (52.7)	0.661	120 (47.1)	122 (41.5)	0.197
Recessive model									
CC	18 (3.6)	30 (5.2)		12 (4.9)	21 (7.5)		6 (2.4)	9 (3.1)	
CG+GG	480 (96.4)	543 (94.8)	0.237	231 (95.1)	258 (92.5)	0.280	249 (97.6)	285 (96.9)	0.794
Allele									
C	261 (26.2)	299 (26.1)		135 (27.8)	167 (30.0)		126 (24.7)	131 (22.3)	
G	735 (73.8)	847 (73.9)	0.961	351 (72.2)	389 (70.0)	0.452	384 (75.3)	457 (77.7)	0.354
rs2161829 (SNP2)									
Genotype									
A/A	105 (21.1)	131 (22.9)		45 (18.5)	53 (19.0)		60 (23.5)	78 (26.5)	
A/G	237 (47.6)	310 (54.4)		135 (55.6)	163 (58.4)		102 (40.0)	147 (50.0)	
G/G	156 (31.3)	132 (23.0)	0.009	63 (25.9)	63 (22.6)	0.674	93 (36.5)	69 (23.5)	0.003
Dominant model									
AA	105 (21.1)	131 (22.9)		45 (18.5)	53 (19.0)		60 (23.5)	78 (26.5)	
GG+AG	393 (78.9)	442 (77.1)	0.506	198 (81.5)	226 (81.0)	0.911	195 (76.5)	216 (73.5)	0.432
Recessive model									
GG	156 (31.3)	132 (23.0)		63 (25.9)	63 (22.6)		93 (36.5)	69 (23.5)	
AA+AG	342 (68.7)	441 (77.0)	0.002	180 (74.1)	216 (77.4)	0.412	162 (63.5)	225 (76.5)	0.001
Allele									
A	447 (44.9)	572 (49.9)		225 (46.3)	269 (48.2)		222 (43.5)	303 (51.5)	
G	549 (55.1)	574 (50.1)	0.022	261 (53.7)	289 (51.8)	0.576	288 (56.6)	285 (48.5)	0.009
rs17047757 (SNP3)									
Genotype									
A/A	255 (51.2)	429 (74.9)		129 (53.1)	222 (79.6)		126 (49.4)	207 (70.4)	
A/G	201 (40.4)	135 (23.6)		84 (34.6)	57 (20.4)		117 (45.9)	78 (26.5)	
G/G	42 (8.4)	9 (1.6)	< 0.001	30 (12.3)	0 (0.00)	< 0.001	12 (4.7)	9 (3.1)	< 0.001
Dominant model									
AA	255 (51.2)	429 (74.9)		129 (53.1)	222 (79.6)		126 (49.4)	207 (70.4)	
AG+GG	243 (48.8)	144 (25.1)	< 0.001	114 (46.9)	57 (20.4)	< 0.001	129 (50.6)	87 (29.6)	< 0.001
Recessive model									
GG	42 (8.4)	9 (1.6)		30 (12.3)	0 (0.00)		12 (4.7)	9 (3.1)	
AG+AA	456 (91.6)	564 (98.4)	< 0.001	213 (87.7)	279 (100)	0.003	243 (95.3)	285 (96.6)	0.357
Allele									
A	711 (71.4)	993 (86.6)		342 (70.4)	501 (89.8)		369 (72.4)	492 (83.7)	
G	285 (28.6)	153 (13.4)	< 0.001	144 (29.6)	57 (10.2)	< 0.001	141 (27.6)	96 (16.3)	< 0.001

CAD, Coronary artery disease; n, number of participants; SNP, single-nucleotide polymorphism.

(Data not shown). **Tables 3** and **4** shows the distribution of genotypes and alleles of the three tagging SNPs of Chinese Han and Uygur population for INSIG2 gene.

In Uygur Chinese population, for total, men and women, the distribution of SNP3 (rs17047757) genotypes, allele frequency and dominant model (AA vs. GG+AG) showed significant differ-

Table 4. Han Chinese population: Genotype and allele distributions in patients with CAD and control participants

	Han			Men			Women		
Variants	CAD n (%)	Control n (%)	*P* value	CAD n (%)	Control n (%)	*P* value	CAD n (%)	Control n (%)	*P* value
rs12613329 (SNP1)									
Genotype									
C/C	36 (10.8)	38 (11.0)		25 (13.0)	22 (12.6)		11 (7.8)	16 (9.4)	
C/G	137 (41.0)	150 (43.4)		75 (38.9)	76 (43.4)		62 (44.0)	74 (43.3)	
G/G	161 (48.2)	158 (45.7)	0.786	93 (48.2)	77 (44.0)	0.642	68 (48.2)	81 (47.4)	0.899
Dominant model									
GG	161 (48.2)	158 (45.7)		93 (48.2)	77 (44.0)		68 (48.2)	81 (47.4)	
CG+CC	173 (51.8)	188 (54.3)	0.539	100 (51.8)	98 (56.0)	0.464	73 (51.8)	90 (52.6)	0.910
Recessive model									
CC	36 (10.8)	38 (11.0)		25 (13.0)	22 (12.6)		11 (7.8)	16 (9.4)	
CG+GG	298 (89.2)	308 (89.0)	1.000	168 (87.0)	153 (87.4)	1.000	130 (92.2)	155 (90.6)	0.689
Allele									
C	209 (31.3)	226 (32.7)		125 (32.4)	120 (34.3)		84 (29.8)	106 (31.0)	
G	459 (68.7)	466 (67.3)	0.601	261 (67.6)	230 (65.7)	0.638	198 (70.2)	236 (69.0)	0.793
rs2161829 (SNP2)									
Genotype									
A/A	97 (29.0)	91 (26.3)		62 (32.1)	52 (29.7)		35 (24.8)	39 (22.8)	
A/G	154 (46.1)	176 (50.9)		91 (47.2)	88 (50.3)		63 (44.7)	88 (51.5)	
G/G	83 (24.9)	79 (22.8)	0.464	40 (20.7)	35 (20.0)	0.846	43 (30.5)	44 (25.7)	0.470
Dominant model									
GG	83 (24.9)	79 (22.8)		40 (20.7)	35 (20.0)		43 (30.5)	44 (25.7)	
AA+AG	251 (75.1)	276 (77.2)	0.589	153 (79.3)	140 (80.0)	0.897	98 (69.5)	127 (74.3)	0.376
Recessive model									
AA	97 (29.0)	91 (26.3)		62 (32.1)	52 (29.7)		35 (24.8)	39 (22.8)	
GG+AG	237 (71.0)	255 (73.7)	0.441	131 (67.9)	123 (70.3)	0.652	106 (75.2)	132 (77.2)	0.690
Allele									
A	348 (52.1)	358 (51.7)		215 (55.7)	192 (54.9)		133 (47.2)	166 (48.5)	
G	329 (47.9)	334 (48.3)	0.914	171 (44.3)	158 (45.1)	0.824	149 (52.8)	176 (51.5)	0.748
rs17047757 (SNP3)									
Genotype									
A/A	198 (59.3)	197 (56.9)		107 (55.4)	97 (55.4)		91 (64.5)	100 (58.5)	
A/G	119 (35.6)	128 (37.0)		80 (41.5)	64 (36.6)		39 (27.7)	64 (37.4)	
G/G	17 (5.1)	21 (6.1)	0.780	6 (3.1)	14 (8.0)	0.098	11 (7.8)	7 (4.1)	0.102
Dominant model									
AA	198 (59.3)	197 (56.9)		107 (55.4)	97 (55.4)		91 (64.5)	100 (58.5)	
AG+GG	136 (40.7)	149 (43.1)	0.586	86 (44.6)	78 (44.6)	1.000	50 (35.5)	71 (41.5)	0.295
Recessive model									
GG	17 (5.1)	21 (6.1)		6 (3.1)	14 (8.0)		11 (7.8)	7 (4.1)	
AG+AA	317 (94.9)	325 (93.9)	0.629	187 (96.9)	161 (92.0)	0.063	130 (92.2)	164 (95.9)	0.222
Allele									
A	515 (77.1)	522 (75.4)		294 (76.2)	258 (73.7)		221 (78.4)	264 (77.2)	
G	153 (22.9)	170 (24.6)	0.484	92 (23.8)	92 (26.3)	0.495	61 (21.6)	78 (22.8)	0.772

CAD, Coronary artery disease; N, number of participants; SNP, single-nucleotide polymorphism.

ence between CAD and control subjects (all $P < 0.05$, respectively), the G allele of SNP3 (rs17047757) was significantly higher in CAD patients than in control participants (total: 28.6% vs. 13.4%; men: 29.6% vs. 10.2%; women 27.6% vs. 16.3%). For total and women,

Table 5. Multiple logistic regression analysis for CAD patients and control subjects of Uygur Chinese population

	Total			Men			Woman		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
SNP2 (rs2161829)									
Recessive model (GG vs. AG+AA)	2.550	1.845-3.525	< 0.001	1.445	0.901-2.316	0.126	3.130	2.000-4.900	< 0.001
Sex	1.208	0.904-1.614	0.201						
Age	1.006	0.992-1.021	0.393	1.007	0.986-1.027	0.529	0.997	0.978-1.017	0.801
Hypertension	1.702	1.279-2.265	< 0.001	1.383	0.915-2.092	0.124	2.010	1.331-3.034	0.001
Diabetes	14.934	7.378-30.225	< 0.001	8.650	3.573-20.942	< 0.001	5.246	1.725-15.957	0.003
BMI	1.076	1.035-1.119	< 0.001	1.025	0.969-1.085	0.381	1.087	1.029-1.148	0.003
Glu	0.003	0.001-0.021	< 0.001	0.808	0.714-0.915	0.001	.830	.684-1.007	0.059
Hyperlipidemia	1.992	1.296-3.061	0.002	2.405	1.278-4.529	0.007	2.496	1.290-4.830	0.007
TG	3.240	1.534-6.843	0.002	1.670	0.564-4.944	0.354	3.527	1.185-10.503	0.024
LDL-c	1.705	1.477-1.967	< 0.001	2.260	1.791-2.853	< 0.001	1.475	1.211-1.707	< 0.001
SNP3 (rs17047757)									
Dominant model (AA vs. GG+AG)	0.104	0.066-0.163	< 0.001	0.121	0.070-0.211	< 0.001	0.052	0.025-0.110	< 0.001
Sex	1.443	0.555-1.013	0.060						
Age	1.006	0.991-1.021	0.469	1.013	0.991-1.036	0.235	0.997	0.976-1.019	0.815
Hypertension	1.830	1.357-2.466	< 0.001	1.968	1.250-3.098	0.003	1.834	1.191-2.827	0.006
Diabetes	1.677	0.786-3.580	0.182	5.086	1.921-13.463	0.001	0.210	0.058-0.765	0.018
BMI	1.091	1.048-1.136	< 0.001	1.070	1.006-1.139	0.033	1.094	1.031-1.160	0.003
Glu	123.226	7.452-2037.64	0.001	1.013	0.851-1.205	0.887	3.247	2.075-5.081	< 0.001
Hyperlipidemia	0.814	0.783-1.926	0.871	1.104	0.559-2.180	0.776	2.045	1.012-4.132	0.046
TG	4.629	2.059-10.407	0.001	4.385	1.265-15.205	0.020	4.303	1.273-14.548	0.019
LDL-c	1.636	1.410-1.897	< 0.001	2.091	1.617-2.706	< 0.001	1.459	1.187-1.793	< 0.001

the distribution of SNP2 (rs2161829) genotypes, allele frequency and recessive model (GG vs. AA+AG) showed significant difference between CAD and control subjects (all $P < 0.05$, respectively), the G allele of SNP2 (rs2161829) was significantly higher in CAD patients than in control participants (total: 55.1% vs. 50.1%; women: 56.6% vs. 48.5%). For men, the distribution of SNP2 (rs2161829) genotypes, allele frequency, dominant model (AA vs. GG+AG) and recessive model (GG vs. AA+AG) showed no significant difference between CAD and control subjects (all $P > 0.05$, respectively) and also the distribution of SNP1 (rs12613329) genotypes, allele frequency, dominant model (GG vs. CC+CG) and recessive model (CC vs. GG+CG) showed no significant difference between CAD and control subjects(all $P > 0.05$, respectively) (**Table 3**).

For the Han population, the distribution of the three tagging SNPs genotypes and alleles showed no significant difference between the CAD patients and control subjects (**Table 4**).

Multiple logistic regression analysis for CAD patients and control subjects from Uygur Chinese population

We used BMI, Glucose, plasma concentration of TG and LDL-c, incidence of hypertension, diabetes, hyperlipidemia, rs17047757 (AA vs. GG+AG), rs2161829 (GG vs. AA+AG), which exhibited differences in the univariate analysis, and Putative confounders (age and sex) as the independent variables, CAD as the dependent variable to perform a multiple logistic regression analysis (**Table 5**).

For total and women, after multiple adjustment SNP2 (rs2161829) remained significantly associated with CAD in recessive model (for total: OR = 2.550, 95% confidence interval [CI]: 1.845-3.525, $P < 0.001$; for women: OR = 3.130, 95% confidence interval [CI]: 2.000-4.900, $P < 0.001$). For total, men and women, after multiple adjustment SNP3 (rs17047757) remained significantly associated with CAD in dominant model (for total: OR = 0.014, 95% confidence interval [CI]: 0.066-0.163, $P < 0.001$; for men: OR = 0.121, 95% confidence

interval [CI]: 0.070-0.211, $P < 0.001$; for women: OR = 0.052, 95% confidence interval [CI]: 0.025-0.110, $P < 0.001$) (**Table 5**).

Discussion

We found that variation in the INSIG2 gene is associated with CAD in Uygur Chinese population, but were not associated with CAD in Han Chinese population. We could hypothesized that there may exist ethnic difference between genetic polymorphism of the INSIG2 gene with CAD. To best of our knowledge, this was the first study to investigate the common allelic variants in INSIG2 gene and its association with CAD in Uygur Chinese population.

INSIG proteins are required for feedback regulation of cholesterol synthesis. INSIG proteins have two isoforms, designated as INSIG1 and INSIG2. INSIG1 was originally cloned by Peng et al. in regenerating liver [18] and was subsequently shown to be dramatically elevated in the fat tissue of rats at the onset of diet-induced obesity [19]. In 2003, Yabe et al. reported the discovery of a liver-specific transcript of INSIG2 in rodents, named INSIG-2a, which differs from the ubiquitous transcript, called INSIG-2b, in the non-coding first exons that splice into a common second exon through the use of different promoters [6]. These two proteins were essential for feedback inhibition of cholesterol synthesis by virtue of their sterol dependent interaction with two other ER membrane proteins: sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) and hydroxymethylglutaryl coenzyme A (HMG-CoA). (1) SCAP is an escort protein required for the proteolytic processing and activation of sterol regulatory element binding proteins (SREBPs) which are the transcription factors that activate genes encoding enzymes required for synthesis of cholesterol, unsaturated fatty acids, triglycerides, and phospholipids in liver and other organs [5, 8]; (2) HMG-CoA reductase (HMGR), the enzyme that catalyzes the rate-determining step of the cholesterol biosynthetic pathway The HMG- CoA reductase is a rate-controlling enzyme in cholesterol biosynthesis [20, 21]. These two proteins share a polytopic intramembrane sequence called the sterol-sensing domain (SSD), through which the sterols cause SCAP and HMG-CoA reductase to bind INSIG proteins [22-26]. When cells are depleted of sterols, SREBPs are transported by Scap from ER to Golgi, where they are processed proteolytically to yield active nuclear fragments (nSREBPs), Sterol-induced binding of SCAP to INSIG proteins leads to ER retention of SCAP, as a result, delivery of SCAP/SREBP complex to the Golgi will be prevented, causing transcriptional rates of SREBP target genes to decline and leading to a reduction in cholesterol synthesis and uptake [25, 26]. Binding of HMG-CoA reductase to INSIG proteins leads to the ubiquitination and degradation of the reductase [12, 27]; finally by virtue of these dual activities, INSIG proteins cause coordinated links in both transcription of relevant genes and sterol pathway activity.

Human INSIG2 gene was identified in 2003, up to date, accumulated evidence generated from different study groups has suggested that genetic polymorphisms of the INSIG2 gene was associated with CAD and other major risk factors for CAD, such as hypercholesterolemia, obesity and insulin resistance. Since the genetic variant of INSIG2 that was implicated in obesity through a genome-wide association study performed in the Framingham Heart Study [13], a series of studies have explored the association of genetic polymorphisms of INSIG2 gene with related metabolic traits such as components of plasma lipids and glucose homeostasis [28-42]. Results from these studies are not consistent either. Some researchers have found that polymorphism (rs7566605) of INSIG2 was associated with serum level of triglycerides or the body mass index [28-33], but this association was not replicated in other studies [34-39], lack of replication may be due to gene-gene or gene-environment interactions. Relatively few studies investigated the association between INSIG2 gene polymorphisms and coronary artery disease and previous studies have failed to find a significant association between the INSIG2 genetic polymorphisms with CAD. For example Liu et al. have selected four SNPs (rs10197745, rs4848492, rs17047757 and rs9308762) of the INSIG2 gene as a htSNPs and found that any of these SNPs were not associated with CAD [41] and other studies also demonstrated that a common single nucleotide polymorphism (SNP rs7566605) of the INSIG2 gene was not associated with the CAD [34] or a weak association combined with other risk factors of the CAD [41].

In the present study, however, we found that polymorphisms of INSIG2 gene were associated with risk of CAD in a Uygur population in China. There was significant difference in genotype distribution of SNP2 (rs2161829) and SNP3 (rs17047757) between CAD patients and control subjects, the GG genotype of both rs17047757 and rs2161829 were significantly higher in CAD patients than in control participants, but these associations did not found in Chinese Han population in our study, this result was in line with the study of Liu et al [41]. The possible reason for these differences may be due to the interaction between ethnic differences and environmental factors; Uygur population were mainly Caucasian and East Asian [42], according to the Statistics, the total Uygur population was 8.4 million in 2000, among whom 99.4% live in the Xinjiang Uygur Autonomous Region which is located in the center of Asia. There are some diet difference between Han Chinese and Uygur Chinese populations, the dietary patterns of Uygur Chinese population primarily characterized by high intakes of pasta, salt, beef, mutton, dairy products and milk products, drink coffees or tea and low intakes of vegetable, fruit and rice than Han Chinese population. Despite diet difference, ethnic difference may contribute to the different results between Han Chinese and Uygur Chinese populations. If we take the genetic diversity across different populations into consideration, the extent of linkage disequilibrium among the genetic variants are likely to vary, and this could also be another explanation of our study results. Thus further studies are necessary to deepen our understanding of different gene polymorphism among different ethnic groups. Interestingly, in the univariate analysis, there have significant difference for hyperlipidemia between Uygur Chinese case-control subjects, but this difference was not retained after multiple logistic regression analysis, maybe it is because of interaction between the risk factors which we included our multiple testing.

Study limitations

Current two independent case-control studies, however, harbors some limitations. First, the source of CAD patients was limited to The First Affiliate Hospital of Xinjiang Medical University, and these subjects may possess some risk factors of cardiovascular disease. Second, our CAD patients comes from the First Affiliate Hospital of Xinjiang Medical University and our control subjects comes from the CRS; when patients admitted in our hospital or when we conducted the CRS study we have not collect the dietary information, and we know that dietary information could be quite insightful, this was our another study limitations . Finally, the Uygur are an admixed population, originate from intermarriage between Caucasians and East Asian, mainly living in the Xinjiang Uygur Autonomous Region of China, lack of individual genetic back ground information was also our study limitations.

Conclusions

In conclusion, polymorphisms of INSIG2 gene were associated with CAD in Uygur Chinese population in China. Additional studies will need to be undertaken in order to clarify the underlying molecular mechanism which associates polymorphism of INSIG2 gene with CAD among different ethnicity.

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Disclosure of conflict of interest

None.

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References

[1] Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: Systematic analysis of population health data. Lancet 2006; 367: 1474-1457.

[2] Arsenault BJ, Boekholdt SM and Kastelein JJ. Lipid parameters for measuring risk of cardiovascular disease. Nat Rev Cardiol 2011; 8: 197-206.

[3] Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington

S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009; 302: 1993-2000.

[4] Weiss LA, Pan L, Abney M, Ober C. The sex specific genetic architecture of quantitative traits in humans. Nat Genet 2006; 38: 218-222.

[5] Brown MS, Goldstein JL. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997; 89: 331-340.

[6] Yabe D, Brown MS, Goldstein JL. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element binding proteins. Proc Natl Acad Sci U S A 2002; 99: 12753-12758.

[7] Radhakrishnan A, Ikeda Y, Kwon HJ, Brown MS, Goldstein JL. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig. Proc Natl Acad Sci U S A 2007; 104: 6511-6518.

[8] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002; 109: 1125-1131.

[9] Takaishi K, Duplomb L, Wang MY, Li J, Unger RH. Hepatic insig-1 or-2 overexpression reduces lipogenesis in obese Zucker diabetic fatty rats and in fasted/refednormal rats. Proc Natl Acad Sci U S A 2004; 101: 7106-7111.

[10] Engelking LJ, Liang G, Hammer RE, Takaishi K, Kuriyama H, Evers BM, Li WP, Horton JD, Goldstein JL, Brown MS. Schoenheimer effect explained-feedback regulation of cholesterol synthesis in mice mediated by Insig proteins. J Clin Invest 2005; 115: 2489-2498.

[11] Krapivner S, Popov S, Chernogubova E, Hellénius ML, Fisher RM, Hamsten A, van't Hooft FM. Insulin-induced gene 2 involvement in human adipocyte metabolism and body weight regulation. J Clin Endocrinol Metab 2008; 93: 1995-2001.

[12] Jo Y, Debose-Boyd RA. Control of cholesterol synthesis through regulated ER-associated degradation of HMG CoA reductase. Crit Rev Biochem Mol Biol 2010; 45: 185-198.

[13] Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeufer A, Illig T, Wichmann HE, Meitinger T, Hunter D, Hu FB, Colditz G, Hinney A, Hebebrand J, Koberwitz K, Zhu X, Cooper R, Ardlie K, Lyon H, Hirschhorn JN, Laird NM, Lenburg ME, Lange C, Christman MF. A common genetic variant is associated with adult and childhood obesity. Science 2006; 312: 279-283.

[14] Xie X, Ma YT, Yang YN, Fu ZY, Li XM, Huang D, Ma X, Chen BD, Liu F. Polymorphisms in the SAA1/2 gene are associated with carotid intima media thickness in healthy Han Chinese subjects: the Cardiovascular Risk Survey. PLoS One 2010; 5: e13997.

[15] Xie X, Ma YT, Yang YN, Fu ZY, Li XM, Zheng YY, Huang D, Ma X, Chen BD, Liu F. Polymorphisms in the SAA1 gene are associated with ankle-to-brachial index in Han Chinese healthy subjects. Blood Press 2011; 20: 232-238.

[16] Joint Committee for Developing Chinese guidelines on Prevention and Treatment of Dyslipidemia in Adults. Chinese Guidelines on prevention and treatment of dyslipidemia in adults. Zhonghua Bing Xin Xue Guan Za Zhi 2007; 35: 390-419.

[17] Xie X, Ma YT, Fu ZY, Yang YN, Xiang Ma, Chen BD, Wang YH, Fen Liu. Haplotype Analysis of the CYP8A1 gene associated with myocardial infarction. Clin Appl Thromb Hemost 2009; 15: 574-580.

[18] Peng Y, Schwarz EJ, Lazar MA, Genin A, Spinner NB, Taub R. Cloning, human chromosomal assignment, and adipose and hepatic expression of the CL-6/INSIG1 gene. Genomics 1997; 43: 278-284.

[19] Li J, Yu X, Pan W, Unger RH. Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. Am J Physiol Metab 2002; 282: E1334-1341.

[20] Gil G, Faust JR, Chin DJ, Goldstein JL, Brown MS. Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. Cell 1985; 41: 249-258.

[21] Skalnik DG, Narita H, Kent C, Simoni RD. The membrane domain of 3-hydroxy-3-methylglutaryl-coenzyme A reductase confers endoplasmic reticulum localization and sterol-regulated degradation onto β-galactosidase. J Biol Chem 1988; 263: 6836-6841.

[22] Hua X, Nohturfft A, Goldstein JL, Brown MS. Sterol resistance in CHO cells traced to point mutation in SREBP cleavage activating protein (SCAP). Cell 1996; 87: 415-426.

[23] Yang T, Goldstein JL, Brown MS. Over expression of membrane domain of SCAP prevents sterols from inhibiting SCAP/ SREBP exit from endoplasmic reticulum. J Biol Chem 2000; 275: 29881-29886.

[24] Sever N, Yang T, Brown MS, Goldstein JL, DeBose-Boyd RA. Accelerated degradation of HMG CoA reductase mediated by binding of Insig-1 to its sterol-sensing domain. Mol Cell 2003; 11: 25-33.

[25] Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, Goldstein JL, Brown MS. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. Cell 2002; 110: 489-500.

[26] Ye J, DeBose-Boyd RA. Regulation of cholesterol and fatty acid synthesis. Cold Spring Harb Perspect. Biol 2011; 3.

[27] Sever N, Song BL, Yabe D, Goldstein JL, Brown MS, DeBose-Boyd RA. Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. J Biol Chem 2003; 278: 52479-52490.

[28] Talbert ME, Langefeld CD, Ziegler JT, Haffner SM, Norris JM, Bowden DW. INSIG2 SNPs associated with obesity and glucose homeostasis traits in Hispanics: the IRAS Family Study. Obesity (Silver Spring) 2009; 17: 1554-1562.

[29] Oki K, Yamane K, Kamei N, Asao T, Awaya T, Kohno N. The single nucleotide polymorphism upstream of insulin-induced gene 2 (INSIG2) is associated with the prevalence of hypercholesterolaemia, but not with obesity, in Japanese American women. Br J Nutr 2009; 101: 322-327.

[30] Fornage M, Papanicolaou G, Lewis CE, Boerwinkle E, Siscovick DS. Common INSIG2 polymorphisms are associated with age-related changes in body size and high-density lipoprotein cholesterol from young adulthood to middle age. Metabolism 2010; 59: 1084-1091.

[31] Do R, Bailey SD, Pare G, Montpetit A, Desbiens K, Hudson TJ, Yusuf S, Bouchard C, Gaudet D, Pérusse L, Anand S, Vohl MC, Pastinen T, Engert JC. Fine mapping of the insulin induced gene 2 identifies a variant associated with LDL cholesterol and total apolipoprotein B levels. Circ Cardiovasc Genet 2010; 3: 454-461.

[32] Wang HJ, Zhang H, Zhang SW, Pan YP, Ma J. Association of the common genetic variant upstream of INSIG2 gene with obesity related phenotypes in Chinese children and adolescents. Biomed Environ Sci 2008; 21: 528-536.

[33] Baylin A, Deka R, Tuitele J, Viali S, Weeks DE, McGarvey ST. INSIG2 variants, dietary patterns, and metabolic risk in Samoa. Eur J Clin Nutr 2013; 67: 101-107.

[34] Wiedmann S, Neureuther K, Stark K, Reinhard W, Kallmünzer B, Baessler A, Fischer M, Linsel-Nitschke P, Erdmann J, Schunkert H, Hengstenberg C. Lack of association between a common polymorphism near the INSIG2 gene and BMI, myocardial infarction, and cardiovascular risk factors. Obesity (Silver Spring) 2009; 17: 1390-1395.

[35] Cha S, Koo I, Choi SM, Park BL, Kim KS, Kim JR, Shin HD, Kim JY. Association analyses of the INSIG2 polymorphism in the obesity and cholesterol levels of Korean populations. BMC Med Genet 2009; 10:96.

[36] Hubacek JA, Kuthanova L, Bohuslavova R, Adámková V, Lánská V, Meitinger T, Pfeufer A. INSIG2 promoter variant, obesity markers and lipid parameters No association in a large Slavonic Caucasian population sample. Folia Biol (Praha) 2010; 56: 131-134.

[37] Andreasen CH, Mogensen MS, Borch-Johnsen K, Sandbaek A, Lauritzen T, Sørensen TI, Hansen L, Almind K, Jørgensen T, Pedersen O, Hansen T. Non-replication of genome-wide based associations between common variants in INSIG2 and PFKP and obesity in studies of 18,014 Danes. PLoS One 2008; 3: e2872.

[38] Campa D, Hüsing A, McKay JD, Sinilnikova O, Vogel U, Tjønneland A, Overvad K, Stegger J, Clavel-Chapelon F, Chabbert-Buffet N, Fagherazzi G, Trichopoulou A, Zylis D, Oustoglou E, Rohrmann S, Teucher B, Fisher E, Boeing H, Masala G, Krogh V, Sacerdote C, Panico S, Tumino R, Onland-Moret NC, van Gils CH, Bueno-de-Mesquita HB, Lund E, Chirlaque MD, Sala N, Quirós JR, Ardanaz E, Amiano P, Molina-Montes E, Hallmans G, Lenner P, Travis RC, Key TJ, Wareham N, Khaw KT, Rinaldi S, Slimani N, Chajes V, Siddiq A, Riboli E, Kaaks R, Canzian F. The INSIG2 rs7566605 polymorphism is not associated with body mass index and breast cancer risk. BMC Cancer 2010; 10: 563.

[39] Apalasamy YD, Moy FM, Rampal S, Bulgiba A, Mohamed Z. Genetic associations of the INSIG2 rs7566605 polymorphism with obesity-related metabolic traits in Malaysian Malays. Genetic Mol Res 2014; 13: 4904-4910.

[40] Liu X, Li Y, Wang L, Zhao Q, Lu X, Huang J, Fan Z, Gu D. The INSIG1 gene, not the INSIG2 gene, associated with coronary heart disease: tagSNPs and haplotype-based association study: The Beijing Atherosclerosis Study. Thromb Haemost 2008; 100: 886-892.

[41] Skelding KA, Gerhard GS, Vlachos H, Selzer F, Kelsey SF, Chu X, Erdman R, Williams DO, Kip KE. Association of an INSIG2 obesity allele with cardiovascular phenotypes is gender and age dependent. BMC Cardiovascular Disorders 2010; 10: 46.

[42] Yao YG, Kong QP, Wang CY, Zhu CL, Zhang YP. Different matrilineal contributions to genetic structure of ethnic groups in the silk road region in China. Mol Biol Evol 2004; 21: 2265-228.