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Case Report

Widely Association Analysis and Identification New Early-Onset Factor for Prediction of Coronary Heart Disease

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Abstract

Hemorheological indexes, living habits, medical history and genetics factor are primary risk factors in Coronary Heart Disease (CHD). In the present study the relation of all factors to the severity of CHD was examined. The data of 282 patients (mean age: 60±9 years) diagnosed with CHD and 229 healthy controls (mean age: 59±7 years) from Wenzhou Medical University were analyzed. All detected hemorheological indexes (except for total bilirubin, fasting blood, uric acid and triglyceride) were determined associated with CHD ($P<0.01$). The factors of smoking, hypertension, diabetes and gout reached statistical significant level ($P<0.05$) in logistic regression analyses with CHD and four events reveal more than 99% accuracy for CHD prediction. The ten selected SNPs have not found closely related to CHD in Wenzhou people, but found that rs3782886, rs671, 4977574 and rs2383206, four SNPs locus can affect the uric acid content and rs1842896 can affect the LDL-CH concentration in blood and thus indirectly contribute to the incidence of CHD. These results indicate that these detected hemorheological indexes, living habits, medical history and genetics factor may have the possibility as an adjunct to early warning of CHD.

Keywords: Coronary heart disease; Hemorheological indexes; Living habits; Medical history; SNP

Abreviations: ALT: Alanine Transaminase; AST: Aspertate Amino Transferase; FBG: Fasting Blood-Glucose; SCr: Serum Creatinine; UA: Uric Acid; T-CH: Total Cholesterol; HDL-CH: High Density Lipoprotein Cholesterol; LDL-CH: Low Density Lipoprotein Cholesterol; WBC: White Blood Cell; Hb: Hemoglobin; PLT: Blood Platelet; HVR4: Hypervariable Region 4; EDTA: Ethylene Di Amine Tetra Acetic Acid

Introduction

Coronary Heart Disease (CHD), behind Stroke is the second leading cause of death of Chinese population, but the trend of number of death increase caused by CHD is significant in recently years [1,2]. It is a complex multifactorial disease in which habits, health states and genetic factors have a significant function in inducing [3-5]. Among these inducing factors, genetic factors are more significantly than other and known to influence the risk of CHD [6]. Abnormal physiobiochemic and hemorheological indexes, particularly, caused by genetic factor are important elements for the risk of CHD [7,8]. These genetic and other factors are associated with CHD and provides a possible for prediction of CHD in early onset. In previously study, some studies on the association with CHD have identified a few risk factors could be used as an index for prediction of CHD. The genetic factor, such as SNP, was paid lots of attention by researchers from medical area.

Plasminogen activator inhibitor type 1, angiotensin-converting enzyme, High-Density lipoprotein Cholesterol (HDL-C), Renin-Angiotensin-Aldosterone (RAAS) system gene and so on genetic factors have been found to have a significant associated relationship with CHD [9-11]. In addition, due to the advancement of genetic marker identification techniques, a large number of non-genotypic markers have also been found that have a significant association relationship with CHD and can be used to predict CHD in early onset [3,12,13]. Genetic factors, however, are not the only factor that causes and can predict to CHD, individual status and history of morbidity is also an important factor. For instance, decreasing physical activity and increasing of BMI (Body Mass Index) have significant effect on CHD [14], and family history of diabetes have influence the risk of CHD [15] that were investigated by medical researchers in previously studies.

Although a number of genetic and physiological factors have been found to have significant synergistic effects with CHD, the synergistic effect of the same factor in different populations is not necessarily the same. The coagulation factor VII gene Hypervariable Region 4 (HVR4) is one of them [16], and an allele on this gene revealed differential association with CHD in divergent ethnic groups. Therefore, when study on exploring the factors of

CHD and the prediction in early onset, the difference of population should not be to directly flow the previous study. In this study, we took advantage of rich longitudinal data on CHD incident events in the Affiliated Hospital of Wenzhou Medical University study and performed a SNaP Shot of incident CHD events in Chinese Han population of Wenzhou to identify potential factors for prediction of CHD in early onset.

Material and Methods

Data collection and phenotype analyses

From November 2015 to June 2016,281 patients were identified with CHD that included 120 patients with Acute Myocardial Infarction (AMI), 2 patients with old myocardial infarction, 159 patients with angina pectoris from the Affiliated Hospital of Wenzhou Medical University were enrolled in our study. There are 229 cases for healthy control group collected through regular health screening of people with no history of CHD, diabetes mellitus, or dyslipidemia. All patients were identified according to the 1999 World Health Organization diagnostic criteria for CHD. Coronary angiography was performed in all patients. Background information included sex, BMI, age, whether or not smoking and drinking, and the history of hypertension, diabetes and gout of all investigators, patients and control cases, were collected as far as possible with diagnosing CHD and medical test. The study was approved by the ethics committee at the Wenzhou Medical University.

Hemorheological indexes and genotype analyses

For each patients and control case, 2ml and 5ml of peripheral fasting blood were collected into Ethylene Di Amine Tetra Acetic Acid (EDTA)-coated tubes and stored at -20 ℃ and used for hemorheological indexes testing and genotype analyses for each SNP. Within 1 h, the blood for hemorheological indexes was test by using the Abbott Architect i2000SR, Johnson and Vitus 5600 and Sysmex XE-5000 to analyze the indexes of TBiL (Total Bilirubin), ALT(Alanine Transaminase), AST(Aspertate Amino Transferase), FBG (Fasting Blood-Glucose), SCr (Serum Creatinine), UA (Uric Acid), T-CH(Total Cholesterol), TG (Triglyceride), HDL-CH(High Density Lipoprotein Cholesterol), LDL-CH(Low Density Lipoprotein Cholesterol), WBC(White Blood Cell), Hb (Hemoglobin) and PLT (Blood Platelet), and recorded all the corresponding data.

DNA extraction

The total DNA of each sample was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA). The DNA concentration of each sample was detected on a spectrophotometer (NanoVue; GE Healthcare, Piscataway, NJ, USA) and diluted to a working concentration of 5-10ng/μl.

Genotype analyses

In this study, ten SNP locus were selected as candidate genes for prediction of CHD, and named rs2123536, rs1842896, rs671, rs3782886, rs1333049, rs4977574, rs944797, rs2383207, rs2383206, rs7136259, respectively. Among these SNP, seven SNP from three genes are BRAP (BRCA1 associated protein), ALDH2 (acetaldehyde dehydrogenase 2) and CDKN2B-AS1. Polymorphisms of each locus were detected by a highly sensitive ligase detection reaction using the primers showed in Table 1. A 20μL PCR reaction was generated consisting of DNA (2μl), 4 ul Primer Mix (content same molecular mass of ten pair primer), 3mM Mg^{2+} (1.6μl), 10μl ExTaq (Takara, Japan) 2mM dNTP (2μl), and 10mM of each primer (4μl). The multiplex PCR reaction conditions were set as follows: 95 ℃ for 5min; 40 cycles of 10s at 95 ℃, 40s at 50 ℃, and 20s at 72 ℃, with the final step consisting of 7min at 72 ℃. After PCR reaction, 5U SAP enzyme and 2U Exonuclease I enzyme were added to 15μl of PCR product, incubated at 37 ℃ for 1 hour and then 75 ℃ for 15 min to purify PCR product. Then, 5μl SNaPshot Multiplex Kit (ABI, USA), 2ul purified multiplex PCR product and 1ul extension primer mixture were put together, and the mixture was conducted the extended reaction as follows procedure: 95 ℃ for 10s, 25 cycles of 10 s at 95 ℃, 5 s at 50 ℃, and 30sec at 60 ℃, with the final step consisting of 30s at 60 ℃. After extended reaction, the per 10μl product was added 1U SAP enzyme, incubated at 37 ℃ for 1 hour and then 85 ℃ for 15min to purify extended product. Take 1μl of the purified extended product was performed sequencing on ABI3730XL and analyzing the genotype of each locus of each sample by Gene Mapper 4.0 Applied Biosystems Co., Ltd., USA.

Table 1: The primers of ten locus used in this study.

	Locus	Forward Primer Sequence	Reverse Primer Sequence
Primer for PCR Reaction	rs1333049	TCACTACCCTACTGTCATTCCTC	CTTGCTTACCTCTGCGAGTG
	rs2383207	GAGAACTCAAAGATACTTAGCCCT	TTTTCAACAAGACATAACTAATAGC
	rs7136259	ATGCCACATAGCTTAGGGGC	GAAATGCGTTGCAATTTCGT
	rs2123536	GCCTCACGAGACAATTTTGC	GGGTTCCAAACTGCGAAA
	rs1842896	ATACAGTTACTTGCCTCAGGTTG	CATGATAACTAGTGCTTTTAAGGG
	rs3782886	GTTCAACTCAATAGAAGATGACG	TATAGGAACAATTCACACTTACATC
	rs944797	ATGACCTCTTTAGTTTGGTTCTACT	GCGTCCTGGCATAAGTGTTAG
	rs2383206	CTATCCTGGTTGCCCCTTC	CCATTTGGTGCTCATTGAAGAT
	rs671	GGAGCCCAGTCACCCTTTG	GGTCCCACACTCACAGTTTTC
	rs4977574	CATGTTTGCTTTCAGGGTACA	GCTGAAGGGACTCAATGAGAG

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Primer for Extended Reaction	rs1333049	TTCCTCATACTAACCATATGATCAACAGTT	
	rs2383207	TTTTTTTTTTTTTTACTCCTGTTCGGATCCCTTC	
	rs7136259	TTTTTTTTTTTCTAGGTTTGTGTAGTACACTGTGTGAAT	
	rs2123536	TTTTTTTTTTTTTTTTTTTTTCTCCCTGCCCCTTAGTCTTTCAC	
	rs1842896	TTTGATAACTAGTGCTTTTAAGGGAATG	
	rs3782886	TTTTTTTTTTTTGCCAGCTAGTGTATGTGGAAAG	
	rs944797	TTTTTTTTTTTTCTACTTATACTTTCAAGGAGGGAAGAC	
	rs2383206	TTTTTTTTTTTTTTCTGTCTTTTCCTTAGAAATGTTATTGTAGT	
	rs671	TTTTTTTTTTTTTTTTTTTTTTTTGGTCCCACACTCACAGTTTTCACTT	
	rs4977574	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGTACATCAAATGCATTC-TATAGC	

Statistics

A quantitative difference among the means of different group was evaluated by one-way ANOVA. Homogeneity of variances among groups and genotypes was evaluated using the Duncan test. Multivariable logistic regression and principal component analyses were conducted using R 3.0 for Windows (R team). When one of variable in test have miss value, the corresponding sample was ignored to perform.

Results

Relationship between age BMI with CHD

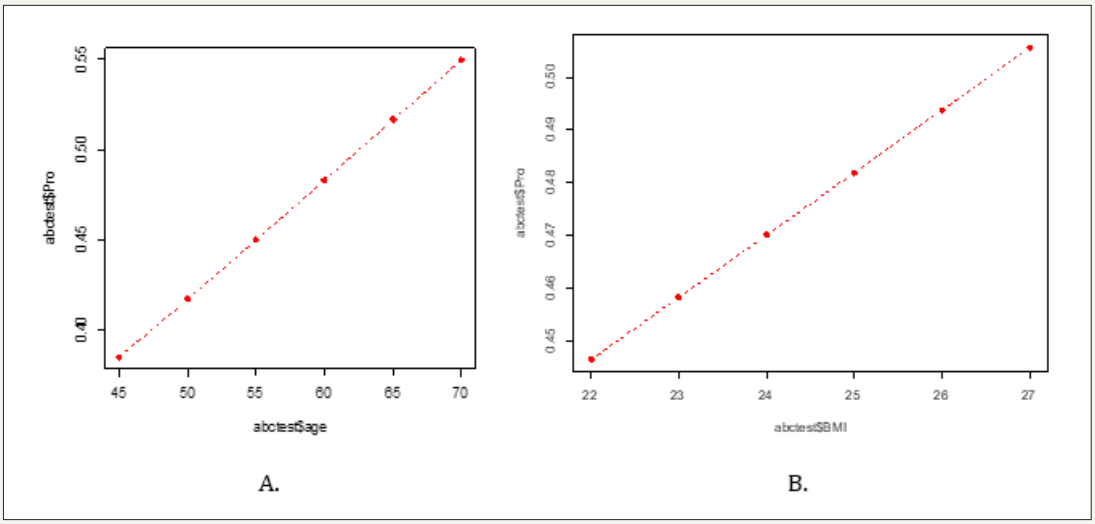

Figure 1: The impact of BMI and age on the probability of occurrence of CHD.

Table 2: The results of age, BMI one-way test between CHD patients and control group.

Group	N	Age		BMI	
		Mean	Std	Mean	Std
Patient	282	60.19024	8.821092	25.06625	3.344074
Control	229	58.64126	6.834366	24.43379	4.253612
P value for one-way Test		0.04426		0.08672	

Through theone-waytest, it was found that both the age and BMI of CHD patients were increased compared with the control group. The mean age of the CHD group was over 60 years old, which was significantly higher than that of the control group (P<0.05), but there was no significant difference in body mass index between the two groups. The logistic regression analysis showed that the estimated regression model of age and BMI in patient population was reached the statistical significance (Table 2). The risk of occurrence of CHD was increased by about 3% per 5 years from 45 to 70, and each unit increase in BMI the risk of CHD was increased more than one percent. Therefore, the impaction of age is greater than BMI according to the results (Figure 1).

Relationship between habits, medical history with CHD

Based on chi-square test of profile habits and medical history between groups, it was found that there were significant differences in smoking, drink, hypertension, hyperlipidemia, diabetes and gout (Table 3). The regression coefficient of four indicators of smoking, hypertension, diabetes and gout reached statistical significant level ($P<0.05$) by further logistic regression analysis. Through the model to predict the impact of four indicators on the probability of CHD, found that any three indicators at the same time in a person when the risk of CHD will reach more than 95%, and four indicators also have the risk of CHD up to more than 99%.

Table 3: The results of habits, medical history chi-square test between CHD patients and control group.

Group	Smoking		Drunk		Hypertension		Hyperlipidemia	
	0	1	0	1	0	1	0	1
Control	0.8061	0.1938	0.8942	0.1057	0.6855	0.3144	0.8384	0.1615
Patient	0.539	0.4609	0.7553	0.2446	0.312	0.6879	0.7482	0.2517
P value for Chi-test	4.90E-10		8.96E-05		< 2.2e-16		0.01756	
Group	Diabetes		Gout		Stroke			
CHD	0	1	0	1	0	1	0	1
Control	0.9388	0.0611	0.9868	0.0131	0.9912	0.0087		
Patient	0.7056	0.2907	0.9397	0.0603	0.9787	0.0212		
P value for Chi-test	1.90E-10		0.01222		0.4368			

Hemorheological indexes impacted on CHD

Table 4: The results of hemorheological indexes One-way test between CHD patients and control group.

Indexes	Control	Case	One-Way Test P value	Notes
TBiL	11.64±5.092	11.76±5.079	0.444	
ALT	26.46±19.813	38.8±53.841	0.000147	**
AST	23.85±12.338	77.79±128.609	2.08E-10	***
FBG	6.05±3.385	6.27±3.363	0.1348	
SCr	62.53±14.792	74.8±32.945	9.54E-09	***
UA	334.18±77.945	358.14±113.9	0.05986	.
T-CH	5.38±1.089	6.73±33.106	9.69E-13	***
TG	1.72±1.242	2.42±9.137	0.05116	.
HDL-CH	1.32±0.34	1.05±0.269	2.20E-16	***
LDL-CH	3.08±0.915	2.81±0.997	9.51E-05	***
WBC	6.28±1.626	7.89±3.113	7.46E-09	***
Hb	142.93±17.31	134.73±15.923	4.23E-09	***
PLT	229.59±55.644	221.66±66.283	3.73E-02	**

Table 5: The results of principal component analysis with significant different hemorheological indexes between CHD patients and control group.

		Parameter	Std	Z Value	P Value	Notes
Before Reduce	Intercept	0.4627	0.1278	3.62	0.00029	***
	RC1	1.8639	0.2516	7.41	1.30E-13	***
	RC2	0.0988	0.1196	0.83	0.40865	
	RC3	0.9545	0.1498	6.37	1.90E-10	***
	RC4	-1.0664	0.147	-7.25	4.10E-13	***
After Reduce	Intercept	0.4647	0.1283	3.623	0.000292	***
	RC1	1.8403	0.2504	7.35	1.98E-13	***
	RC3	0.9455	0.1499	6.307	2.84E-10	***
	RC4	-1.0629	0.1469	-7.236	4.61E-13	***

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Statistics significant level (P<0.05) or extremely significant (P<0.01), except for TBiL, FBG, UA and TG, were found by one-way test of two group in all other hemorheological indexes (Table 4). According to the two-two correlation coefficient and matrix of the constructed hemorheological indexes, the correlation between most indexes is horizontal or vertical. In addition, the distribution of hemorheological indexes in the patients population and the control population showed a significant difference between the two groups, mainly in the control group, the relative correlation between the two groups was relatively scattered, and the risk of the population there are a number of significant correlation between the hemorheological indexes (Figure 1). Through the principal component analysis, it is found that all the hemorheological indexes with significant difference between CHD patients and control in one-way test can be attributed to the representative of the four principal components with cumulative contribution of 100%, of which the cumulative contribution of the first three principal components is more than 82%.Using the logistics regression model to analyze the four principal components and risk of CHD found that the second principal component and the risk of CHD did not exist significantly affect. The parameters of the regression model after RC2 removal are as showed in Table 5.

The RC1 and RC3 showed a linear increase in the risk of CHD in the regression model, but the RC4 showed negative correlation with the risk of CHD. According to this corrected regression model, the results showed that RC1 had the greatest influence on the risk of CHD, while the three greatest coefficient of indexes in RC1 were AST, ALT and WBC, respectively. The contribution of Hb to RC4 was the greatest in all biochemical indexes, indicating that Hb too much to reduce the risk of CHD have a certain inhibitory effect.

Association analyses between SNP and CHD, hemorheological indexes

Each SNP genotype was scored as Myocardial Infarction Genetics Consortium published in previously [3]. In briefly, the score was composed of allelic dosage (assignment 0, 1, or 2 for three genotypes in each SNP locus), weighted by the effect size of that allele on the patient population. Based on the score of SNPs, ten SNP loci were not significantly different between the control and the group of CHD (Table 6). All the hemorheological indexes were analyzed by one-way ANOVA in each SNP. The results showed that rs3782886, rs671, 4977574 and rs2383206 had significant effect on UA, and the significant association between rs1842896 and LDL-CH was found too (Table 7 & 7a).

Table 6: The results of association between the ten SNPs and CHD.

	Parameter	Std	Z Value	P Value
(Intercept)	0.24093	0.87853	0.274	0.784
rs1333049	0.05681	1.12312	0.051	0.96
rs2383207	1.2796	2.35053	0.544	0.586
rs7136259	0.46537	0.56943	0.817	0.414
rs2123536	0.29642	0.49053	0.604	0.546
rs1842896	0.24934	0.25066	0.995	0.32
rs3782886	-0.44866	0.47246	-0.95	0.342
rs944797	-3.09208	3.97716	-0.777	0.437
rs2383206	0.12608	0.9751	0.129	0.897
rs671	-1.00243	1.03217	-0.971	0.331
rs4977574	0.07648	0.93492	0.082	0.935

Table 7

SNP		FBG			UA			T.CH		
	Genotype	0	1	2	0	1	2	0	1	2
rs1333049	Mean	6.43	5.85	6.45	328.63	348.82	355.08	9.05	4.96	5.08
	Std	4.792	1.563	3.935	92.095	106.964	92.569	46.234	1.254	1.204
	F value	1.924			2.645			1.441		
	P value	0.147			0.072			0.238		
rs2383207	Mean	6.3	5.82	6.9	347.04	347.31	328.7	5.04	7.59	4.89
	Std	3.275	1.596	6.972	96.95	107.873	80.564	1.223	37.058	1.263
	F value	2.405			0.823			0.73		
	P value	0.091			0.44			0.482		
rs7136259	Mean	6.55	6.21	5.97	351.64	349.65	336.87	5.3	6.94	4.9
	Std	2.004	3.1	3.963	95.676	100.347	100.237	1.259	31.864	1.205
	F value	0.654			1.007			0.442		
	P value	0.52			0.366			0.643		
rs2123536	Mean	6.34	5.98	6.22	335.51	357.05	335.86	4.86	7.46	4.97
	Std	4.763	1.639	2.14	87.806	108.207	103.407	1.181	34.969	1.349
	F value	0.642			2.923			0.754		
	P value	0.527			0.055			0.471		

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rs1842896	Mean	70.04	68.52	343.44	59.09	49.08	34.6074	30.5369	6.34	5.91
	Std	31.101	18.148	97.362	105.845	97.035	50.72043	21.05044	4.158	1.551
	F value	1.895			0.054			0.501		
	P value	0.169			0.816			0.479		
rs3782886	Mean	6.3	5.93	6.31	384.31	327.5	352.83	4.87	7.72	5.03
	Std	1.985	1.703	4.264	146.042	88.849	97.913	1.312	37.899	1.276
	F value	0.718			6.772			0.795		
	P value	0.488			0.001			0.452		
rs944797	Mean	6.48	5.73	6.32	328.64	346.66	354.48	10.01	4.96	5.02
	Std	5.148	1.219	3.377	97.402	92.036	107.587	51.394	1.252	1.233
	F value	1.863			2.381			1.811		
	P value	0.156			0.094			0.165		
rs2383206	Mean	6.51	5.88	6.44	355.58	350.09	325.66	5.17	4.92	9.29
	Std	4.269	1.678	4.804	89.682	105.983	93.266	1.218	1.235	47.326
	F value	1.908			3.361			1.571		
	P value	0.15			0.035			0.209		
rs671	Mean	6.36	5.81	6.63	350.42	329.15	403.64	5.02	7.7	5.06
	Std	4.29	1.36	1.966	97.949	88.086	158.427	1.25	38	1.369
	F value	1.736			7.883			0.765		
	P value	0.177			<0.001			0.466		
rs4977574	Mean	5.98	6.07	6.52	355.85	352.73	319.77	5.08	4.95	9.3
	Std	1.587	3.026	4.906	94.661	110.641	73.59	1.202	1.253	47.325
	F value	0.944			5.564			1.574		
	P value	0.39			0.004			0.208		

Table 7(a): The results of association between the ten SNPs and hemorheological indexes.

SNP		TG			HDL.CH			LDL.CH		
	Genotype	0	1	2	0	1	2	0	1	2
rs1333049	Mean	1.68	2.46	1.82	1.19	1.15	1.18	2.97	2.89	2.98
	Std	1.056	9.436	1.39	0.328	0.347	0.315	1.027	0.978	1.002
	F value	0.758			0.716			0.432		
	P value	0.469			0.489			0.65		
rs2383207	Mean	1.83	2.57	1.47	1.17	1.16	1.23	2.96	2.93	2.87
	Std	1.342	10.219	0.691	0.329	0.332	0.361	1.003	0.979	1.04
	F value	0.976			0.896			0.187		
	P value	0.378			0.409			0.829		
rs7136259	Mean	1.98	1.8	2.54	1.16	1.17	1.18	3.16	2.96	2.85
	Std	1.604	1.188	10.847	0.275	0.326	0.36	1.028	1.053	0.885
	F value	0.699			0.13			2.035		
	P value	0.497			0.878			0.132		
rs2123536	Mean	1.72	1.87	4.04	1.18	1.17	1.15	2.83	3.05	2.89
	Std	1.103	1.165	18.07	0.333	0.318	0.389	0.929	1.027	1.069
	F value	3.372			0.113			2.758		
	P value	0.035			0.893			0.064		

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rs1842896	Mean	1.81	2.49	1.16	1.2	2.86	3.05	7.25	7.12	138.25
	Std	1.209	10.256	0.316	0.358	0.888	1.106	2.761	2.59	16.255
	F value	1.278			1.85			4.575		
	P value	0.259			0.174			0.033		
rs3782886	Mean	1.79	1.7	2.39	1.1	1.16	1.19	2.86	2.98	2.93
	Std	0.962	0.988	8.866	0.29	0.306	0.357	1.173	0.908	1.03
	F value	0.669			1.357			0.318		
	P value	0.512			0.258			0.728		
rs944797	Mean	1.57	2.85	1.8	1.21	1.15	1.16	2.98	2.88	2.96
	Std	0.783	11.823	1.258	0.347	0.329	0.328	1.01	0.989	0.996
	F value	1.52			1.259			0.397		
	P value	0.22			0.285			0.673		
rs2383206	Mean	1.77	2.41	1.67	1.2	1.14	1.21	3.05	2.87	2.99
	Std	1.347	8.988	1.069	0.319	0.33	0.353	0.987	0.98	1.027
	F value	0.696			2.141			1.493		
	P value	0.499			0.119			0.226		
rs671	Mean	2.36	1.71	1.85	1.19	1.15	1.1	2.92	2.95	2.97
	Std	8.741	0.996	1	0.353	0.311	0.286	1.009	0.911	1.269
	F value	0.577			1.346			0.077		
	P value	0.562			0.261			0.926		
rs4977574	Mean	1.71	2.46	1.63	1.19	1.14	1.22	3	2.89	3
	Std	1.301	9.023	1.033	0.318	0.332	0.348	0.98	0.985	1.029
	F value	0.898			2.698			0.766		
	P value	0.408			0.068			0.465		

Discussion

With the change of modern diet and the increase in the pressure of life, the incidence of CHD is rising, the research data show that if the myocardial infarction patients within one hour of onset of treatment, the mortality rate of up to about 30% [17,18], So early prediction, early prevention, effective control of the disease, improving the predictive ability of patients with CHD is a very significant study in medical area. In this study, Wenzhou Chinese patients with CHD were observed and found that the risk of CHD can reach more than 48% with age of 60 or more people, while the age per 5-year-old the risk of CHD will increase by more than 3% and more than 4% risk will reveal with BMI per one unit up on the basis of the average. The results demonstrated that Wenzhou Chinese have later than 10 year by previous reported age of onset in Chinese woman [19], which is also proved that the difference of population have divergent sensitive for CHD as previous study reported [18,20]. Wenzhou is a relatively economically developed city in China and the people have extra condition to pay attention on their healthy like developed country [21]. Therefore, it is not surprising that CHD is more later onset in the population of this area than the average of China in total. The better economic condition, however, will boast BMI increasing and then cause increasing risk of CHD particularly in developing country [22-24]. It demonstrated that both age and BMI have a certain supporting effect on the early prediction of CHD in Wenzhou Chinese, and suggested that when the person with age of 60 years or older, they need to strengthen the detection of CHD and improve the health status to prevent the occurrence of CHD, especially when the their BMI reaches 27 or more.

Moreover, bad habits, for instance smoking, is a strongest associated factor with CHD risk [24] what was also documented by our study. On the analysis of patients' medical history with CHD, the effect of hypertension and diabetes on CHD is consistent with previous studies [25,26], but stroke history have not associated with CHD, even though, they share similar risk factors [27]. In addition, this study also identified that gout and CHD has a significant association relationship. Gout is a crystal-related joint disease caused by the deposition of monosodium monostate, which is directly related to hyperuricemia caused by purine metabolic disorders and/or uric acid excretion. The function of hyperuricemia increase the risk of CHD events have verified by Kim and his working team [28]. Although these factors have a relatively close impact on the likelihood of CHD and its morbidity, the incidence of CHD can reach more than 90% when any three of smoking, hypertension, diabetes and gout occurring/occurred simultaneously in one person, and the incidence of CHD will reach more than 99%. So these four events as early warning of CHD has a high degree of credibility in Wenzhou people.

Previous study has identified some hemorheological parameter associated with CHD, such as hematocrit, fibrinogen level, plasma viscosity and apparent whole blood viscosity [29] and proved that hemorheological indexes have a certain significance for the occurrence of CHD, and also have a greater effect in the prediction of CHD. In this study, the seven indexes of ALT, AST, SCr, T-CH, HDL-CH, LDL-CH, WBC, Hb and PLT in patients with CHD were significantly higher than those in the control group ($P<0.01$), while the TBiL, FBG, UA and TG indexes in the patients group were not significantly different from those in the control group ($P>0.05$).In addition, through the principal component analysis and logistics model regression study found that the three top hemorheological indexes for increasing the risk of CHD were AST, UA and TG. In contrast the three top hemorheological indexes to reduce the risk of CHD has a significant function were PLT, HDL-CH and LDL-CH. AST and ALT have been verified that they can be used to predict the severity of CHD [30,31] what was documented in this study again in Wenzhou people. Although UA and TG do not directly affect the morbidity of CHD, but they have an indirect effect on CHD via improving the incidence of obesity and hyperuricemia [28,32,33]. Both HDL-CH and LDL-Ch are important factors that contribute to the regulation of the atherosclerotic process and have a significant reduction in the incidence of CHD [34,35]. Therefore, this study suggested that the early prediction of CHD should combine directly and indirectly hemorheological indexes that will be better to enhance the result for diagnosis, especially through the calculated value of principal component formula can gain greater accuracy than single index.

The ten selected SNPs have been proved the association between them with CHD in white people [3,36], but all of them have not found closely related to CHD in Wenzhou people. This result is sufficient to show the presence specific of CHD related SNP inspecificity population. Although the direct relationship between these SNPs and CHD was not detected, but found that rs3782886, rs671,4977574 and rs2383206, four SNPs locus can affect the UA content and rs1842896 can affect the LDL-CH concentration in blood and thus indirectly contribute to the incidence of CHD. However, the mechanism of found SNPs work on hemorheological indexes is not clearly in previous study.

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