Study of the association of 17 lipid-related gene polymorphisms with coronary heart disease

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Abstract

Objective: Blood lipids are well-known risk factors for coronary heart disease (CHD). The aim of this study was to explore the association between 17 lipid-related gene polymorphisms and CHD.

Methods: The current study examined with 784 CHD cases and 739 non-CHD controls. Genotyping was performed on the MassARRAY iPLEX® assay platform.

Results: Our analyses revealed a significant association of *APOE* rs7259620 with CHD (genotype: χ^2=6.353, df=2, p=0.042; allele: χ^2=5.05, df=1, p=0.025; recessive model: χ^2=5.57, df=1, p=0.018). A further gender-based subgroup analysis revealed significant associations of *APOE* rs7259620 and *PPAP2B* rs72664392 with CHD in males (genotype: χ^2=8.379, df=2, p=0.015; allele: χ^2=5.190, df=1, p=0.023; recessive model: χ^2=19.3, df=1, p<0.0001) and females (genotype: χ^2=9.878, df=2, p=0.007), respectively. Subsequent breakdown analysis by age showed that *CETP* rs4783961, *MLXIPL* rs35493868, and *PON2* rs12704796 were significantly associated with CHD among individuals younger than 55 years of age (*CETP* rs4783961: χ^2=8.966, df=1, p=0.011 by genotype; *MLXIPL* rs35493868: χ^2=4.87, df=1, p=0.027 by allele; χ^2=4.88, df=1, p=0.027 by dominant model; *PON2* rs12704796: χ^2=6.511, df=2, p=0.039 by genotype; χ^2=6.210, df=1, p=0.013 by allele; χ^2=5.03, df=1, p=0.025 by dominant model). Significant allelic association was observed between *LEPR* rs656451 and CHD among individuals older than 65 years of age (χ^2=4.410, df=1, p=0.036).

Conclusion: Our study revealed significant associations of *APOE, PPAP2B, CETP, MLXIPL, PON2,* and *LEPR* gene polymorphisms with CHD among the Han Chinese. *(Anatol J Cardiol 2018; 19: 00-00)*

Keywords: coronary heart disease, *APOE, PPAP2B, CETP, MLXIPL, PON2, LEPR*

Introduction

Coronary heart disease (CHD) is characterized by atherosclerosis, which leads to vascular stenosis and occlusion. Dyslipidemia is known as a risk factor for CHD (1). Blood lipids have been reported to predict the risk of CHD (2, 3), which encouraged us to examine the association of lipid-related gene polymorphisms with CHD (4).

In this study, we selected seven adipocytokine signaling pathway genes, including three peroxisome proliferator-activated receptor (PPAR) signaling pathway genes [angiopoietin-like 4 (*ANGPTL4*), adiponectin (*ADIPOQ*), and apolipoprotein A-V (APOA5)], leptin (*LEP*), leptin receptor (*LEPR*), adiponectin receptor 1 (*ADIPOR1*), and 5'-AMP-activated protein kinase subunit gamma-1 (*PRKAG1*). PPAR or adipocytokine signaling pathway genes have been reported to be significantly upregulated in ruptured plaques (5). Of the remaining lipid-related genes, angiopoietin-like 3 (ANGPTL3) is a member of the angiopoietin-like protein family, which can regulate the activity of lipoprotein lipase in the lipolytic processing of triglyceride (TG)-rich lipoproteins (6). Apolipoprotein E (APOE) regulates plasma low-density lipoprotein (LDL) levels (7-9). Paraoxonase 2 (PON2) and paraoxonase 3 (PON3) are antioxidants against atherosclerosis (10). Very-low-density-lipoprotein receptor (VLDLR) can affect the metabolism of VLDL-TGs, which are associated with CHD (11). MLX-interacting protein-like (MLXIPL) gene encodes carbohydrate response element-binding protein that has been found to be significantly associated with CHD (12). Scavenger receptor class B type 1 (SCARB1) can regulate the levels of high-density lipoprotein cholesterol (HDL-C) and thus might influence CHD incidence (13). Cholesteryl ester transfer protein (CETP) has been shown to increase the risk of CHD by disrupting the balance of

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HDL-C and LDL-cholesterol levels in the plasma (14). Proprotein convertase subtilisin/kexin type 9 (PCSK9) can reduce blood cholesterol levels and is associated with CHD (15). Phosphatidic acid phosphatase type 2B (PPAP2B), which catalyzes phosphoric acid hydrolysis and thus contributes to glycerophospholipid and triacylglycerol syntheses, has been shown to be associated with CHD (16, 17).

On the basis of previous studies, the aim of this study was to assess the association of 17 lipid-related gene polymorphisms with CHD in the Han Chinese.

Methods

Sample collection

A total of 784 CHD cases and 739 non-CHD controls were enrolled in the current study. The enrolled cases were classified on the basis of the findings of standardized coronary angiography according to the Seldinger's method (18). The classification details have been reported in our previous studies (19-21). The study protocol was approved by the ethical committees of Ningbo First Hospital and Ningbo University. Written informed consent was obtained from all the participants.

Single nucleotide polymorphism (SNP) genotyping

DNA extraction and quantification were performed as previously described (22, 23). Genotyping was performed on the MassARRAY iPLEX® assay platform (Sequenom, San Diego, CA, USA). The primer sequences and the details of the selected SNPs are presented in Table 1.

Statistical analysis

Genotype and allele distributions were compared between the two groups using the Chi-squared test. Differences were

Table 1. The primer sequences and the details of the selected SNPs*

Gene SNP	Primer sequences
PCSK9 rs2479409	1st_ primer: ACGTTGGATGGTGCCTACCATAGAATTCTG;
	2nd_primer: ACGTTGGATGGCCTACATGCATTTCAAGGG;
	Extend primer: tTTCAGGTTTTAAGTTTGCAAAGA;
PPAP2B rs72664392	1st_primer: ACGTTGGATGCATTTATTGTCCACTGTGCC;
	2nd_primer: ACGTTGGATGAAGGGCCTTCCCTTGTATCT;
	Extend primer: tcTCTTCTATAGTGCCTAGCA;
ANGPTL3 rs11207997	1st_primer: ACGTTGGATGTATGTACTATAATTACCCC;
	2nd_primer: ACGTTGGATGAAAAGCCGGCTCTAGCTGTC;
	Extend primer: tcctCATGGATTAGTCTCCTCATCT;
LEPR rs6656451	1st_primer: ACGTTGGATGCAATTACCATCAGCGCTGGG;
	2nd_primer: ACGTTGGATGGAGAATGTCCACTACGCTTC;
	Extend primer: TCATTCTTTCTCCCTTACC;
ADIPOR1 rs7523903	1st_primer: ACGTTGGATGTACAAAGTGCAGCTGGGAAG;
	2nd_primer: ACGTTGGATGTGCCCAGGCTGTCAAAAATG;
	Extend primer: GGTTGAGAAAGATTCAGAAAG;
ADIPOQ rs266729	1st_primer: ACGTTGGATGATGTGTGGCTTGCAAGAACC;
	2nd_primer: CACGCTCATGTTTTGTTTTTGAAG;
	Extend primer: CACGCTCATGTTTTGTTTTTGAAG;
MLXIPL rs35493868	1st_primer: ACGTTGGATGTCAAGCGATTCTCCCACTTC;
	2nd_primer: ACGTTGGATGCCCTGTCTCTACCAAACATA;
	Extend primer: GCATGTAGTCCTAGCTACT;
PON3 rs11770903	1st_primer: ACGTTGGATGAGGAAAAGACAGGAAACGGG;
	2nd_primer: ACGTTGGATGCTAGAAGAAAGAGGGCCTAC;
	Extend primer: caCTACCCTGCCAAGGAAA;
PON2 rs12704796	1st_primer: ACGTTGGATGTGAGAGCAGTCTGAGCTTTG;
	2nd_primer: ACGTTGGATGTCCCCAGGCATGGGTATTG;
	Extend primer: GAAGTCCCATACTCATGT;

Table 1. Cont.

Gene SNP	Primer sequences
LEP rs13228377	1st_primer: ACGTTGGATGAAACCCATAACATAAAGCGG;
	2nd_primer: ACGTTGGATGTTTGGGCATTACCAAACCCG;
	Extend primer: atTGGCAGGCTCGGTTCACC;
VLDLR rs7852409	1st_primer: ACGTTGGATGGGCGACCGCTGGTTGGCTC;
	2nd_primer: ACGTTGGATGCCCTGGATCAGGAAATTAGG;
	Extend primer: gggtAAATTAGGACAGGCACC;
APOA5 rs10750097	1st_primer: ACGTTGGATGGGATAGGCTATTTCAAGCAG;
	2nd_primer: ACGTTGGATGCCTGACTCATTTCCAGTCTC;
	Extend primer: CTGCCACATAAAACCAC;
PRKAG1 rs2293446	1st_primer: ACGTTGGATGCATGACCCTCGCCGTCAGC;
	2nd_primer: ACGTTGGATGAGGCAAGGAACCCACCCTTC;
	Extend primer: cacctCCCTCCCCCGGGTCCTC;
SCARB1 rs59358115	1st_primer: ACGTTGGATGTGTGCAGGGTGTATGGAGG;
	2nd_primer: ACGTTGGATGACCTTCTAGACCCTCATCTC;
	Extend primer: TCCCTGGAAGAAGCCCC;
CETP rs4783961	1st_primer: ACGTTGGATGCTTTGGTATTGGAGCAGGTG;
	2nd_primer: ACGTTGGATGGCCAAGGAAACATGAGTCGG;
	Extend primer: GGTCCTGCCCTAGTCC;
ANGPTL4 rs4076317	1st_primer: ACGTTGGATGGCCCGAGGACGGTTTTTATA;
	2nd_primer: ACGTTGGATGACCCCGCCTCCAAGACTCCT;
	Extend primer: aataCAAGACTCCTCCGCCCACTC;
APOE rs7259620	1st_primer: ACGTTGGATGAATGAGTCCCAGTCTCTCCC;
	2nd_primer: ACGTTGGATGTTTCAGAGGAGAAACCCGTG;
	Extend primer: GGTTCAGCAGCAAGA;

**PCSK9* - proprotein convertase subtilisin/kexin type 9; *PPAP2B* - phosphatidic acid phosphatase type 2B; *ANGPTL3* - angiopoietin-like 3; *LEPR* - leptin receptor; *ADIPOR1* - adiponectin receptor 1; *ADIPOQ* - adiponectin; *MLXIPL* - *MLX*-interacting protein-like; *PON3* - paraoxonase 3; *PON2* - paraoxonase 2; *LEP* - leptin; *VLDLR* - very-low-density-lipoprotein receptor; *APOA5* - apolipoprotein A-V; *PRKAG1* - 5′-AMP-activated protein kinase subunit gamma-1; *SCARB1* - scavenger receptor class B type 1; *CETP* - cholesteryl ester transfer protein; *ANGPTL4* - angiopoietin-like 4; *APOE* - apolipoprotein E

determined by odds ratios (ORs) and 95% confidence intervals (CIs). Hardy-Weinberg equilibrium (HWE) test was used to assess the consistency of genotypic distribution in the controls. A two-tailed $p<0.05$ was considered significant. A power analysis was performed using the Power and Sample Size Calculation software (v3.1.2, Nashville, USA).

Results

As shown in the Table 2, 17 SNPs were detected in the upstream regions of lipid-related genes. *LEPR* rs6656451 was located in the upstream region of a transcript isoform. Among the tested SNPs, *APOE* rs7259620 was significantly associated with CHD [genotype p=0.042 (df=2), allele p=0.025 (df=1), OR (95% CI)=1.196 (1.023-1.398); recessive model (GG+GA versus AA) p=0.018, df=1, OR (95% CI)=1.54(1.07-2.21)]. *PON2* rs12704796, *ADIPOQ* rs266729, *VLDLR* rs7852409, and *PPAP2B* rs72664392 were excluded from further analyses since their genotypic distributions did not meet HWE in the controls (data not shown). In addition, the association of the remaining 12 SNPs with CHD could not be evaluated in the total samples ($p>0.05$).

Further, subgroup analyses by gender were performed. *PON2* rs12704796 and *ADIPOR1* rs7523903 were excluded from the analyses since they did not meet HWE in the male subgroup; *ADIPOQ* rs266729 and *ADIPOR1* rs7523903 were excluded since they did not meet HWE in the female subgroup. *APOE* rs7259620 was significantly associated with CHD only in males [$\chi^2=8.397$, df=2, p=0.015 by genotype; $\chi^2=5.190$, df=1, p=0.023 by allele; $\chi^2=19.3$, df=1, $p<0.0001$ by recessive model (GG + GA versus AA), Table 3]. In addition, *PPAP2B* rs72664392 showed a genotype-level association with CHD in females ($\chi^2=9.878$, df=2, p=0.007, Table 3).

Age-based subgroup analyses revealed that *CETP* rs4783961, *MLXIPL* rs35493868, and *PON2* rs12704796 were significantly as-

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Table 2. Comparison of genotype and allele frequencies of genes between CHD cases and non-CHD controls*

Gene (SNP, allele)	Genotype counts (Cases vs. Controls)	Genotype (χ^2, *P*)	Allele (χ^2, *P*)	OR (95% CI)
APOE (rs7259620, G/A)	406/322/55 vs. 353/308/77	6.353, 0.042	5.05, 0.025	1.196 (1.023-1.398)
CETP (rs4783961, G/A)	467/281/29 vs. 452/240/39	N.S.	N.S.	N.S.
MLXIPL (rs35493868, G/C)	579/168/10 vs. 587/135/7	N.S.	N.S.	N.S.
ADIPOR1 (rs7523903, G/C)	474/277/25 vs. 448/250/33	N.S.	N.S.	N.S.
APOA5 (rs10750097, G/A)	235/378/171 vs. 237/355/146	N.S.	N.S.	N.S.
PCSK9 (rs2479409, G/A)	392/326/66 vs. 358/316/63	N.S.	N.S.	N.S.
SCARB1 (rs59358115, G/A)	583/186/15 vs. 546/178/15	N.S.	N.S.	N.S.
PRKAG1 (rs2293446, G/A)	270/362/144 vs. 279/331/121	N.S.	N.S.	N.S.
PON3 (rs11770903, A/G)	541/218/24 vs. 525/197/16	N.S.	N.S.	N.S.
LEP (rs13228377, A/G)	450/296/38 vs. 416/279/43	N.S.	N.S.	N.S.
ANGPTL4 (rs4076317, C/G)	394/322/58 vs. 361/315/54	N.S.	N.S.	N.S.
LEPR (rs6656451, C/T)	678/93/5 vs. 649/80/1	N.S.	N.S.	N.S.
ANGPTL3 (rs11207997, C/T)	445/291/37 vs. 443/244/41	N.S.	N.S.	N.S.
PON2 (rs12704796, G/A)	305/366/113 vs. 249/388/101	HWD in controls	N.A.	N.A.
ADIPOQ (rs266729, C/G)	399/316/61 vs. 401/263/67	HWD in controls	N.A.	N.A.
VLDLR (rs7852409, C/G)	546/208/29 vs. 513/190/30	HWD in controls	N.A.	N.A.
PPAP2B (rs72664392, T/C)	583/188/11 vs. 567/150/21	HWD in controls	N.A.	N.A.

**PCSK9* - proprotein convertase subtilisin/kexin type 9; *PPAP2B* - phosphatidic acid phosphatase type 2B; *ANGPTL3* - angiopoietin-like 3; *LEPR* - leptin receptor; *ADIPOR1* - adiponectin receptor 1; *ADIPOQ* - adiponectin; *MLXIPL* - MLX-interacting protein-like; *PON3* - paraoxonase 3; *PON2* - paraoxonase 2; *LEP* - leptin; *VLDLR* - very-low-density-lipoprotein receptor; *APOA5* - apolipoprotein A-V; *PRKAG1* - 5'-AMP-activated protein kinase subunit gamma-1; *SCARB1* - scavenger receptor class B type 1; *CETP* - cholesteryl ester transfer protein; *ANGPTL4* - angiopoietin-like 4; *APOE* - apolipoprotein E. Genotypic distributions of *PON2* rs12704796, *ADIPOQ* rs266729, *VLDLR* rs7852409, and *PPAP2B* rs72664392 did not meet HWE in the controls. N.S. - not significant; N.A. - not analyzed; HWD in controls: did not meet HWE in the controls; 95% CI - 95% confidence interval; OR - odds ratio. *APOE* (rs7259620, G/A) was significant in recessive model [GG+GA vs. AA, χ^2=5.57, *P*=0.018, OR (95% CI)=1.54(1.07-2.21)].

sociated with CHD among participants younger than 55 years of age (*CETP* rs4783961: χ^2=8.966, df=2, p=0.011 by genotype; *MLXIPL* rs35493868: χ^2=4.870, p=0.027 by allele; χ^2=4.88, df=1, p=0.027 by dominant model; *PON2* rs12704796: χ^2=6.511, df=2, p=0.039 by genotype; χ^2=6.210, df=1, p=0.013 by allele, χ^2=5.03, df=1, p=0.025 by dominant model, Table 4). In addition, *LEPR* rs656451 was associated with CHD in participants older than 65 years of age (χ^2=4.410, df=1, p=0.036 by allele, Table 4). No other SNPs were associated with CHD in the age-based subgroup analyses.

Discussion

In the present study, we examined the association of 17 lipid-related SNPs with CHD among 784 CHD cases and 739 non-CHD controls. We identified a male-specific association of *APOE* rs7259620 with CHD. Meanwhile, we also found a significant association of *PON2* rs12704796 with CHD among participants younger than 55 years of age. On the genotypic level, we identify a significant association of CHD with *PPAP2B* rs72664392 in females and *CETP* rs4783961 in participants younger than 55 years of age. On the allelic level, we identified a significant association of CHD with *MLXIPL* rs35493868 in participants younger than 55 years of age and *LEPR* rs6656451 in participants older than 65 years of age.

Previous studies have indicated that *APOE* is significantly associated with CHD. *APOE ε2* was shown to reduce the risk of CHD by 20% (24), whereas ε4 was shown to increase the risk of CHD by approximately 42% compared with ε3/ε3 genotype (25). Epidemiological evidence has shown that males are at a higher risk of CHD than females worldwide (26). Gender disparity has been found in APOE-related cardiovascular disease (27). In the previous studies, we have shown that CHD risk was gender-dependent in the Han Chinese and that *APOE* rs4420638 polymorphism was significantly associated with increased CHD risk in male Han Chinese (28). This observation might be explained by the differences of hormonal profiles, smoking status, alcohol-drinking, occupation, and dietary habits between males and females (29, 30). In the present study, we also identified a novel genetic variant of *APOE* associated with CHD in males.

PPAR2B is a negative regulator of inflammatory cytokines, leucocyte adhesion, cell survival, and migration in human primary aortic endothelial cells (31), suggesting that PPAR2B can protect blood vessel against inflammation (32). Mechanosensitive PPAP2B plays a critical role in promoting anti-inflammatory phenotype and maintaining the vascular integrity of endothelial monolayer under atheroprotective flow (33). However, discrepancies exist regarding the association of *PPAP2B* with CHD (34). *PPAP2B* rs1759752 is associated with increased CHD risk in males, while *PPAP2B* rs12566304 is

Table 3. Comparison of genotype and allele frequencies of genes between CHD cases and non-CHD controls by gender

Group	Gene (SNP, allele)	Genotype counts (Cases vs. Controls)	Genotype (χ^2, *P*)	Allele (χ^2, *P*)	OR (95% CI)
Male					
	APOE (rs7259620, G/A)	283/217/37 vs. 199/171/51	8.397, 0.015	5.190, 0.023	1.258 (1.032-1.533)
	CETP (rs4783961, G/A)	322/195/17 vs. 252/144/23	N.S.	N.S.	N.S.
	MLXIPL (rs35493868, G/C)	410/113/9 vs. 345/70/3	N.S.	N.S.	N.S.
	APOA5 (rs10750097, G/A)	163/261/114 vs. 145/194/82	N.S.	N.S.	N.S.
	PCSK9 (rs2479409, G/A)	273/227/38 vs. 207/173/40	N.S.	N.S.	N.S.
	SCARB1 (rs59358115, G/A)	404/123/11 vs. 310/104/7	N.S.	N.S.	N.S.
	PRKAG1 (rs2293446, G/A)	186/250/97 vs. 152/196/71	N.S.	N.S.	N.S.
	PON3 (rs11770903, A/G)	374/148/15 vs. 298/114/9	N.S.	N.S.	N.S.
	LEP (rs13228377, A/G)	315/196/27 vs. 243/152/26	N.S.	N.S.	N.S.
	ANGPTL4 (rs4076317, C/G)	259/234/39 vs. 218/171/29	N.S.	N.S.	N.S.
	LEPR (rs6656451, C/T)	463/67/3 vs. 372/45/1	N.S.	N.S.	N.S.
	ANGPTL3 (rs11207997, C/T)	311/196/24 vs. 253/138/26	N.S.	N.S.	N.S.
	VLDLR (rs7852409, C/G)	379/139/20 vs. 295/105/17	N.S.	N.S.	N.S.
	PPAP2B (rs72664392, T/C)	410/117/9 vs. 326/86/9	N.S.	N.S.	N.S.
	PON2 (rs12704796, G/A)	217/242/79 vs. 147/221/52	HWD in controls	N.A.	N.A.
	ADIPOQ (rs266729, C/G)	261/226/46 vs. 227/155/37	N.S.	N.S.	N.S.
	ADIPOR1 (rs7523903, C/G)	331/185/18 vs. 251/156/12	HWD in controls	N.A.	N.A.
Female					
	APOE (rs7259620, G/A)	123/105/18 vs. 154/137/26	N.S.	N.S.	N.S.
	CETP (rs4783961, G/A)	145/86/12 vs. 200/96/16	N.S.	N.S.	N.S.
	MLXIPL (rs35493868, G/C)	187/55/1 vs. 242/65/4	N.S.	N.S.	N.S.
	APOA5 (rs10750097, G/A)	72/117/57 vs. 91/161/64	N.S.	N.S.	N.S.
	PCSK9 (rs2479409, G/A)	119/99/28 vs. 151/143/23	N.S.	N.S.	N.S.
	SCARB1 (rs59358115, G/A)	179/63/4 vs. 236/74/8	N.S.	N.S.	N.S.
	PRKAG1 (rs2293446, G/A)	84/112/47 vs. 127/135/50	N.S.	N.S.	N.S.
	PON3 (rs11770903, A/G)	167/70/9 vs. 227/83/7	N.S.	N.S.	N.S.
	LEP (rs13228377, A/G)	135/100/11 vs. 173/127/17	N.S.	N.S.	N.S.
	ANGPTL4 (rs4076317, C/G)	1135/88/19 vs. 143/144/25	N.S.	N.S.	N.S.
	LEPR (rs6656451, C/T)	215/26/2 vs. 277/35/0	N.S.	N.S.	N.S.
	ANGPTL3 (rs11207997, C/T)	134/95/13 vs. 190/106/15	N.S.	N.S.	N.S.
	VLDLR (rs7852409, C/G)	167/69/9 vs. 218/85/13	N.S.	N.S.	N.S.
	PPAP2B (rs72664392, T/C)	241/64/12 vs. 173/71/2	9.878, 0.007	N.S.	N.S.
	PON2 (rs12704796, G/A)	88/124/34 vs. 102/167/49	N.S.	N.S.	N.S.
	ADIPOQ (rs266729, C/G)	138/90/15 vs. 174/108/30	HWD in controls	N.A.	N.A.
	ADIPOR1 (rs7523903, C/G)	143/92/7 vs. 197/94/21	HWD in controls	N.A.	N.A.

**PCSK9* - proprotein convertase subtilisin/kexin type 9; *PPAP2B* - phosphatidic acid phosphatase type 2B; *ANGPTL3* - angiopoietin-like 3; *LEPR* - leptin receptor; *ADIPOR1* - adiponectin receptor 1; *ADIPOQ* - adiponectin; *MLXIPL* - MLX-interacting protein-like; *PON3* - paraoxonase 3; *PON2* - paraoxonase 2; *LEP* - leptin; *VLDLR* - very-low-density-lipoprotein receptor; *APOA5* - apolipoprotein A-V; *PRKAG1* - 5'-AMP-activated protein kinase subunit gamma-1; *SCARB1* - scavenger receptor class B type 1; *CETP* - cholesteryl ester transfer protein; *ANGPTL4* - angiopoietin-like 4; *APOE* - apolipoprotein E. Genotypic distributions of *PON2* rs12704796 in males, *ADIPOQ* rs266729 in females, and *ADIPOR1* rs7523903 did not meet HWE in the male controls, female controls, and both male and female controls, respectively. N.S. - not significant; N.A. - not analyzed; HWD in controls: did not meet HWE in the controls; 95% CI - 95% confidence interval; OR - odds ratio. *APOE* (rs7259620, G/A) was significant in males under recessive model [GG+GA vs AA, χ^2=19.3, *P*<0.0001, OR (95% CI)=2.65 (1.69-4.15)].

associated with a decreased CHD risk in females (34). Other studies have shown that *PPAP2B* rs17114036-A is associated with CHD (35, 36). In contrast, *PPAP2B* rs17114036 is not associated with CHD after adjustments for gender (16, 35). Here, we identified a novel

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Table 4. Comparison of genotype and allele frequencies of genes between CHD cases and non-CHD controls by age*

Group	Gene (SNP, allele)	Genotype counts (Cases vs. Controls)	Genotype (χ^2, *P*)	Allele (χ^2, *P*)	OR (95% CI)
≤55					
	CETP (rs4783961, G/A)	99/76/4 vs. 147/73/16	8.966, 0.011	N.S.	N.S.
	APOA5 (rs10750097, G/A)	55/82/43 vs. 80/112/47	N.S.	N.S.	N.S.
	PCSK9 (rs2479409, G/A)	84/77/19 vs. 121/99/19	N.S.	N.S.	N.S.
	SCARB1 (rs59358115, G/A)	131/46/3 vs. 177/58/4	N.S.	N.S.	N.S.
	PRKAG1 (rs2293446, G/A)	64/78/37 vs. 88/109/39	N.S.	N.S.	N.S.
	PON3 (rs11770903, A/G)	129/45/5 vs. 173/58/8	N.S.	N.S.	N.S.
	MLXIPL (rs35493868, G/C)	131/45/3 vs. 194/40/2	N.S.	4.87, 0.027	0.619 (0.403-0.951)
	ADIPOR1 (rs7523903, G/C)	112/60/7 vs. 133/94/9	N.S.	N.S.	N.S.
	LEP (rs13228377, A/G)	102/68/10 vs. 131/94/14	N.S.	N.S.	N.S.
	VLDLR (rs7852409, C/G)	116/53/11 vs. 165/61/11	N.S.	N.S.	N.S.
	ANGPTL4 (rs4076317, C/G)	96/67/15 vs. 123/92/21	N.S.	N.S.	N.S.
	LEPR (rs6656451, C/T)	149/29/0 vs. 213/22/1	N.S.	N.S.	N.S.
	ANGPTL3 (rs11207997, C/T)	94/73/11 vs. 144/81/11	N.S.	N.S.	N.S.
	PON2 (rs12704796, G/A)	74/86/20 vs. 73/124/42	6.511, 0.039	6.210, 0.013	1.431 (1.079–1.879)
	APOE (rs7259620, G/A)	88/80/11 vs. 121/88/30	HWD in controls	N.A.	N.A.
	ADIPOQ (rs266729, C/G)	83/71/24 vs. 138/76/22	HWD in controls	N.A.	N.A.
	PPAP2B (rs72664392, T/C)	129/47/3 vs. 187/44/8	HWD in controls	N.A.	N.A.
55-65					
	CETP (rs4783961, G/A)	164/95/11 vs. 160/92/11	N.S.	N.S.	N.S.
	APOA5 (rs10750097, G/A)	82/134/55 vs. 82/137/49	N.S.	N.S.	N.S.
	PCSK9 (rs2479409, G/A)	139/118/14 vs. 122/123/23	N.S.	N.S.	N.S.
	*SCARB*1 (rs59358115, G/A)	214/54/3 vs. 198/64/7	N.S.	N.S.	N.S.
	PRKAG1 (rs2293446, G/A)	91/126/53 vs. 94/126/44	N.S.	N.S.	N.S.
	PON3 (rs11770903, A/G)	190/70/11 vs. 189/76/3	N.S.	N.S.	N.S.
	MLXIPL (rs35493868, G/C)	222/44/3 vs. 214/46/2	N.S.	N.S.	N.S.
	ADIPOR1 (rs7523903, G/C)	169/92/9 vs. 171/82/10	N.S.	N.S.	N.S.
	LEP (rs13228377, A/G)	156/96/19 vs. 147/106/15	N.S.	N.S.	N.S.
	VLDLR (rs7852409, C/G)	196/65/10 vs. 193/64/9	N.S.	N.S.	N.S.
	ANGPTL4 (rs4076317, C/G)	129/116/24 vs. 127/119/17	N.S.	N.S.	N.S.
	LEPR (rs6656451, C/T)	240/29/1 vs. 222/41/0	N.S.	N.S.	N.S.
	ANGPTL3 (rs11207997, C/T)	158/100/10 vs. 157/92/13	N.S.	N.S.	N.S.
	PON2 (rs12704796, G/A)	97/130/44 vs. 88/144/37	HWD in controls	N.A.	N.A.
	APOE (rs7259620, G/A)	140/106/25 vs. 125/112/31	N.S.	N.S.	N.S.
	ADIPOQ (rs266729, C/G)	138/115/17 vs. 151/88/24	HWD in controls	N.A.	N.A.
	PPAP2B (rs72664392, T/C)	198/68/4 vs. 203/55/10	HWD in controls	N.A.	N.A.
≥65					
	CETP (rs4783961, G/A)	204/110/14 vs. 145/75/12	N.S.	N.S.	N.S.
	APOA5 (rs10750097, G/A)	98/162/73 vs. 75/106/50	N.S.	N.S.	N.S.
	*PCSK*9 (rs2479409, G/A)	169/131/33 vs. 115/94/21	N.S.	N.S.	N.S.
	SCARB1 (rs59358115, G/A)	238/86/9 vs. 171/56/4	N.S.	N.S.	N.S.
	PRKAG1 (rs2293446, G/A)	115/158/54 vs. 97/96/38	N.S.	N.S.	N.S.

Table 4. Cont.

Group	Gene (SNP, allele)	Genotype counts (Cases vs. Controls)	Genotype (χ^2, *P*)	Allele (χ^2, *P*)	OR (95% CI)
	PON3 (rs11770903, A/G)	222/103/8 vs. 163/63/5	N.S.	N.S.	N.S.
	MLXIPL (rs35493868, G/C)	244/79/4 vs. 179/49/3	N.S.	N.S.	N.S.
	ADIPOR1 (rs7523903, G/C)	193/125/9 vs. 144/74/14	N.S.	N.S.	N.S.
	LEP (rs13228377, A/G)	192/132/9 vs. 138/79/14	N.S.	N.S.	N.S.
	VLDLR (rs7852409, C/G)	234/90/8 vs. 155/65/10	N.S.	N.S.	N.S.
	ANGPTL4 (rs4076317, C/G)	169/139/19 vs. 111/104/16	N.S.	N.S.	N.S.
	LEPR (rs6656451, C/T)	289/35/4 vs. 214/17/0	N.S.	4.41, 0.036	0.545 (0.307-0.968)
	ANGPTL3 (rs11207997, C/T)	193/118/16 vs. 142/71/17	N.S.	N.S.	N.S.
	*PON*2 (rs12704796, G/A)	134/150/49 vs. 88/120/22	HWD in controls	N.A.	N.A.
	*APO*E (rs7259620, G/A)	178/136/19 vs. 107/108/16	N.S.	N.S.	N.S.
	*ADIPO*Q (rs266729, C/G)	178/130/20 vs. 112/99/21	N.S.	N.S.	N.S.
	PPAP2B (rs72664392, T/C)	256/73/4 vs. 177/51/3	N.S.	N.S.	N.S.

**PCSK9* - proprotein convertase subtilisin/kexin type 9; *PPAP2B* - phosphatidic acid phosphatase type 2B; *ANGPTL3* - angiopoietin-like 3; *LEPR* - leptin receptor; *ADIPOR1* - adiponectin receptor 1; *ADIPOQ* - adiponectin; *MLXIPL* - MLX-interacting protein-like; *PON3* - paraoxonase 3; *PON2* - paraoxonase 2; *LEP* - leptin; *VLDLR* - very-low-density-lipoprotein receptor; *APOA5* - apolipoprotein A-V; *PRKAG1* - 5'-AMP-activated protein kinase subunit gamma-1; *SCARB1* - scavenger receptor class B type 1; *CETP* - cholesteryl ester transfer protein; *ANGPTL4* - angiopoietin-like 4; *APOE* - apolipoprotein E. N.S. - not significant; N.A. - not analyzed; HWD in controls: did not meet HWE in the controls; 95% CI - 95% confidence interval; OR - odds ratio. *MLXIPL* (rs35493868, G/C) was significant in dominant model [age ≤55 GG vs GC + CC, χ^2=4.88, *P*=0.027, OR (95% CI)=0.59 (0.37–0.95)]. *PON2* (rs12704796, G/A) was significant in dominant model [age ≤55 GG vs GA + AA, χ^2=5.03, *P*= 0.025, OR (95% CI)=1.59 (1.06–2.38)]

polymorphism (rs72664392) in *PPAP2B* promoter associated with CHD in females. This finding could be partly explained by the particular genetic background.

Aging is a pivotal risk factor for CHD (37, 38). The incidence of CHD in people younger than 40 years of age is 0.6%, and it increases two-fold or more with every 10-year increase in age (39). High adiponectin concentration has been shown to be associated with a lower risk of CHD in people younger 65 years of age (40). In people younger than 55 years of age, *PON2* rs12704796-A has been shown to increase the risk of CHD by 43.1%, whereas *MLXIPL* rs35493868-G has been shown to reduce the risk of CHD by 38.1%. In addition, *LEPR* rs6656451-T has been reported to reduce the risk of CHD by 45.5% among people older than 65 years of age.

Study limitations

Our results did not demonstrate a significant association of 11 of the tested SNPs with CHD. A power analysis revealed that these SNPs showed a minimal or moderate power to detect a significant association in the current study (power=0.074-0.425). In addition, several SNPs did not present reliable association results in gender- and age-based subgroup analyses since their genotype distributions did not meet HWE in the controls. Future association study of these SNPs with CHD is warranted in other cohorts.

Conclusion

Our study demonstrated the gender- or age-dependent association of six SNPs (*APOE* rs7259620, *PPAP2B* rs72664392, *CETP* rs4783961, *PON2* rs12704796, *MLXIPL* rs35493868, and *LEPR* rs6656451) CHD in Han Chinese population. However, future replication is required to validate our findings.

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References

1. Berkinbayev S, Rysuly M, Mussayev A, Blum K, Baitasova N, Mussagaliyeva A, et al. Apolipoprotein Gene Polymorphisms (APOB, APOC111, APOE) in the Development of Coronary Heart Disease in Ethnic Groups of Kazakhstan. J Genet Syndr Gene Ther 2014; 5: 216.
2. Goldbourt U, Yaari S, Medalie JH. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality. A 21-year follow-up of 8000 men. Arterioscler Thromb Vasc Biol 1997; 17: 107-13.
3. Daoud MS, Ataya FS, Fouad D, Alhazzani A, Shehata AI, Al-Jafari AA. Associations of three lipoprotein lipase gene polymorphisms, lipid profiles and coronary artery disease. Biomed Rep 2013; 1: 573-82.
4. Farmakiotis D, Chien KS, Shum TC, Rodriguez-Barradas M, Musher DM. Photo quiz. To scan or not to scan?. Clin Infect Dis 2013; 56: 1003, 1052-3.
5. Lee K, Santibanez-Koref M, Polvikoski T, Birchall D, Mendelow AD, Keavney B. Increased expression of fatty acid binding protein 4 and

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DOI:10.14744/AnatolJCardiol.2018.23682

leptin in resident macrophages characterises atherosclerotic plaque rupture. Atherosclerosis 2013; 226: 74-81.
6. Lichtenstein L, Kersten S. Modulation of plasma TG lipolysis by Angiopoietin-like proteins and GPIHBP1. Biochim Biophys Acta 2010; 1801: 415-20.
7. Kessentini Y, Ben Ahmed A, Al-Juaid SS, Mhiri T, Elaoud Z. Crystal structure and vibrational spectral studies of a new organic-inorganic crystal: 4-Benzylpiperidinium trioxonitrate. Spectrochim Acta A Mol Biomol Spectrosc 2014; 129: 478-83.
8. Zurnic I, Djuric T, Koncar I, Stankovic A, Dincic D, Zivkovic M. Apolipoprotein E gene polymorphisms as risk factors for carotid atherosclerosis. Vojnosanit Pregl 2014; 71: 362-7.
9. Yu Y, Xue L, Zhao CY. [Study on polymorphism in the apolipoprotein A5 gene in patients with premature coronary heart disease]. Beijing Da Xue Xue Bao Yi Xue Ban 2007; 39: 576-80.
10. Su SY, Chen JH, Huang JF, Wang XL, Zhao JG, Shen Y, et al. Paraoxonase gene cluster variations associated with coronary heart disease in Chinese Han women. Chin Med J (Engl) 2005; 118: 1167-74.
11. MacDougall ED, Kramer F, Polinsky P, Barnhart S, Askari B, Johansson F, et al. Aggressive very low-density lipoprotein (VLDL) and LDL lowering by gene transfer of the VLDL receptor combined with a low-fat diet regimen induces regression and reduces macrophage content in advanced atherosclerotic lesions in LDL receptor-deficient mice. Am J Pathol 2006; 168: 2064-73.
12. Guo S, Zheng F, Qiu X, Yang N. ChREBP gene polymorphisms are associated with coronary artery disease in Han population of Hubei province. Clin Chim Acta 2011; 412: 1854-60.
13. Stanislovaitiene D, Lesauskaite V, Zaliuniene D, Smalinskiene A, Gustiene O, Zaliaduonyte-Peksiene D, et al. SCARB1 single nucleotide polymorphism (rs5888) is associated with serum lipid profile and myocardial infarction in an age- and gender-dependent manner. Lipids Health Dis 2013; 12: 24.
14. Raposo HF, Patrício PR, Simões MC, Oliveira HC. Fibrates and fish oil, but not corn oil, up-regulate the expression of the cholesteryl ester transfer protein (CETP) gene. J Nutr Biochem 2014; 25: 669-74.
15. Wu NQ, Li JJ. PCSK9 gene mutations and low-density lipoprotein cholesterol. Clin Chim Acta 2014; 431: 148-53.
16. López-Mejías R, Genre F, García-Bermúdez M, Ubilla B, Castañeda S, Llorca J, et al. Lack of association between ABO, PPAP2B, ADAMST7, PIK3CG, and EDNRA and carotid intima-media thickness, carotid plaques, and cardiovascular disease in patients with rheumatoid arthritis. Mediators Inflamm 2014; 2014: 756279.
17. Escalante-Alcalde D, Sanchez-Sanchez R, Stewart CL. Generation of a conditional Ppap2b/Lpp3 null allele. Genesis 2007; 45: 465-9.
18. Zhang L, Yuan F, Liu P, Fei L, Huang Y, Xu L, et al. Association between PCSK9 and LDLR gene polymorphisms with coronary heart disease: case-control study and meta-analysis. Clin Biochem 2013; 46: 727-32.
19. Xu L, Chen X, Ye H, Hong Q, Xu M, Duan S. Association of four CpG-SNPs in the vascular-related genes with coronary heart disease. Biomed Pharmacother 2015; 70: 80-3.
20. Ye H, Zhou A, Hong Q, Tang L, Xu X, Xin Y, et al. Positive Association between APOA5 rs662799 Polymorphism and Coronary Heart Disease: A Case-Control Study and Meta-Analysis. PLoS One 2015; 10: e0135683.
21. Ye H, Zhou A, Hong Q, Chen X, Xin Y, Tang L, et al. Association of seven thrombotic pathway gene CpG-SNPs with coronary heart disease. Biomed Pharmacother 2015; 72: 98-102.
22. Jiang D, Wang Y, Shen Y, Xu Y, Zhu H, Wang J, et al. Estrogen and promoter methylation in the regulation of PLA2G7 transcription. Gene 2016; 591: 262-7.
23. Ye H, Hong Q, Li Y, Xu X, Huang YI, Xu L, et al. A lack of association between the IKZF2 rs12619285 polymorphism and coronary heart disease. Exp Ther Med 2015; 9: 1309-13.
24. Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA 2007; 298: 1300-11.
25. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med 2004; 141: 137-47.
26. Schwann TA, Tatoulis J, Puskas J, Bonnell M, Taggart D, Kurlansky P, et al. Worldwide Trends in Multi-arterial Coronary Artery Bypass Grafting Surgery 2004-2014: A Tale of 2 Continents. Semin Thorac Cardiovasc Surg 2017; 29: 273-80.
27. Ordovas JM. Gender, a significant factor in the cross talk between genes, environment, and health. Gend Med 2007; 4 Suppl B: S111-22.
28. Huang Y, Ye HD, Gao X, Nie S, Hong QX, Ji HH, et al. Significant interaction of APOE rs4420638 polymorphism with HDL-C and APOA-I levels in coronary heart disease in Han Chinese men. Genet Mol Res 2015; 14: 13414-24.
29. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2224-60.
30. Chouinard-Watkins R, Plourde M. Fatty acid metabolism in carriers of apolipoprotein E epsilon 4 allele: is it contributing to higher risk of cognitive decline and coronary heart disease? Nutrients 2014; 6: 4452-71.
31. Touat-Hamici Z, Weidmann H, Blum Y, Proust C, Durand H, Iannacci F, et al. Role of lipid phosphate phosphatase 3 in human aortic endothelial cell function. Cardiovasc Res 2016; 112: 702-13.
32. Panchatcharam M, Miriyala S, Salous A, Wheeler J, Dong A, Mueller P, et al. Lipid phosphate phosphatase 3 negatively regulates smooth muscle cell phenotypic modulation to limit intimal hyperplasia. Arterioscler Thromb Vasc Biol 2013; 33: 52-9.
33. Wu C, Huang RT, Kuo CH, Kumar S, Kim CW, Lin YC, et al. Mechanosensitive PPAP2B Regulates Endothelial Responses to Atherorelevant Hemodynamic Forces. Circ Res 2015; 117: e41-e53.
34. Sun YX, Gao CY, Lu Y, Fu X, Jia JG, Zhao YJ, et al. Association between PPAP2B gene polymorphisms and coronary heart disease susceptibility in Chinese Han males and females. Oncotarget 2017; 8: 13166-73.
35. Schunkert H, König IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011; 43: 333-8.
36. Ren H, Panchatcharam M, Mueller P, Escalante-Alcalde D, Morris AJ, Smyth SS. Lipid phosphate phosphatase (LPP3) and vascular development. Biochim Biophys Acta 2013; 1831: 126-32.
37. Rai M, Baker WL, Parker MW, Heller GV. Meta-analysis of optimal risk stratification in patients >65 years of age. Am J Cardiol 2012; 110: 1092-9.
38. Petoumenos K, Worm SW. HIV infection, aging and cardiovascular disease: epidemiology and prevention. Sex Health 2011; 8: 465-73.
39. Yan YL, Qiu B, Hu LJ, Jing XD, Liu YJ, Deng SB, et al. Efficacy and safety evaluation of intensive statin therapy in older patients with coronary heart disease: a systematic review and meta-analysis. Eur J Clin Pharmacol 2013; 69: 2001-9.
40. Zhang H, Mo X, Hao Y, Huang J, Lu X, Cao J, et al. Adiponectin levels and risk of coronary heart disease: a meta-analysis of prospective studies. Am J Med Sci 2013; 345: 455-61.