RESEARCH ARTICLE

Study of Five Pubertal Transition-Related Gene Polymorphisms as Risk Factors for Premature Coronary Artery Disease in a Chinese Han Population

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Abstract

Background

Recently, single nucleotide polymorphisms (SNPs) (DLK-rs10144321, SIX6-rs1254337, MKRN3-rs12148769, LIN28B-rs7759938, and KCNK9-rs1469039) were found to be strongly associated with age at menarche. Recent studies also suggested that age at menarche is a heritable trait and is associated with risks for obesity, type 2 diabetes mellitus (T2DM), cardiovascular disease, and all-cause mortality. Since an association between these five SNPs and premature coronary artery disease (CAD) has never been reported, we investigated whether these SNPs are associated with premature CAD and its severity in a Chinese Han population.

Methods

We enrolled 432 consecutive patients including 198 with premature CAD (<55 years in men and <65 years in women) and 234 controls. All subjects were genotyped for the five SNPs by the PCR-ligase detection reaction method. The associations between these SNPs and premature CAD and its severity were analyzed.

Results

The following genotypes were identified: GG, AG, and AA at rs10144321 and rs12148769; TT, AT, and AA at rs1254337; CC, CT, and TT at rs1469039; and TT and CT at rs7759938. Significant differences in genotype distribution frequencies at rs1254337 were found between controls and patients with premature CAD (P<0.05). No associations were found between the five SNPs and the severity of coronary lesions (all P>0.05). Compared with

controls, patients with premature CAD had a higher prevalence of T2DM and dyslipidemia, and the proportion of patients with T2DM rose significantly with an increase in the number of stenosed coronary vessels (all P<0.05). After adjustment for the clinical parameters in multivariable analysis, three factors were identified that significantly increased the risk of premature CAD: the AA genotype at rs1254337 (OR: 2.388, 95% CI: 1.190–4.792, P = 0.014), male gender (OR: 1.565, 95% CI: 1.012–2.420, P = 0.044), and T2DM (OR 2.252, 95% CI: 1.233–4.348, P = 0.015).

Conclusions

Among the five pubertal transition-related gene polymorphisms, we identified an association between rs1254337 and premature CAD in a Chinese Han population.

Background

The incidence of death from coronary artery disease (CAD) and acute myocardial infarction increased from 2002 to 2011 in China. In 2002, the mortality from CAD was 39.6 and 27.6 per 100,000 in urban and rural areas, respectively; and this increased to 96.0 and 75.7 per 100,000 in 2011 [1]. Genetic background plays an important role in the etiology of CAD [2–5], and CAD is a complex disease stemming from interactions between multiple genetic and environmental risk factors. Therefore, there is growing interest in finding novel genetic markers to improve risk stratification, and the early detection and treatment of high-risk patients has emerged as a key strategy in the prevention of cardiovascular disease (CVD) [6].

The prevalence of CVD is associated with socioeconomic prosperity, lifestyle changes, advancing age, and urbanization [7]. Patients with traditional risk factors, such as obesity, cigarette smoking, type 2 diabetes mellitus (T2DM), hyperlipidemia, and a family history of CAD, are at a higher risk for CAD, future cardiac death, and worse outcomes [1,8–10]. Furthermore, the morbidity and mortality from CAD in patients with premature CAD (males <55 years and females <65 years) may have a devastating impact on the families of these patients [11].

Age at menarche acts as a marker of puberty timing in females, and recent studies suggest that this age is a heritable trait, and early pubertal onset is associated with increased risks for elevated weight and/or body mass index (BMI), obesity, T2DM, CVD, cancer, and all-cause mortality [12–15]. More recently, Perry et al. [14] used genome-wide and custom-genotyping arrays in 182,416 women of European descent and found 123 signals at 106 genomic loci associated with age at menarche (P<5×10^{-8}). Among these interesting loci, five single nucleotide polymorphisms (SNPs) (DLK-rs10144321, SIX6-rs1254337, MKRN3-rs12148769, LIN28B-rs7759938, and KCNK9-rs1469039) were identified that belong to the forest plot of parent-of-origin-specific allelic associations at three imprinted menarche loci. Taken together, these novel findings suggest that these five SNPs might be associated with an increased risk of premature CAD and its severity. Genetic markers of CAD should facilitate the development of effective preventive strategies to attenuate the rapid rise in morbidity and mortality from CAD.

However, to date, no information has been published on the potential association of these five SNPs with the risk of premature CAD. Therefore, the aim of the present study was to analyze, for the first time, whether these five SNPs are associated with premature CAD and its severity in a Chinese Han population.

Methods

Study subjects

We enrolled 432 unrelated patients of Han ethnicity who underwent elective coronary angiography (CAG) for suspicion of CAD from January 2008 to December 2010. The subjects included 198 patients (men aged 38~54 years and women aged 40~64 years) with premature CAD and 234 controls. Among these subjects, at least three generations had resided in China. CAD was diagnosed as stenosis $\geq$ 50% in at least one of the three main coronary arteries or major branches and a history of myocardial infarction defined by World Health Organization criteria, or a history of coronary balloon angioplasty, coronary stent implantation, or coronary artery bypass surgery. Premature CAD was defined as CAD diagnosed in males before 55 years old and in females before 65 years old. The mean duration of CAD in the 198 patients with premature CAD was 1.3 years. There were 234 participants without detectable coronary stenosis or elevated cardiac troponin I levels who served as controls. The exclusion criteria included the following: autoimmune disease, severe liver or kidney disease, a contraindication for heparin use, or a history of childhood adversity (physical abuse, sexual abuse, neglect, biological father absent from the home, other parent loss, parental mental illness, parental substance abuse, parental criminality, interparental violence, serious physical illness in childhood and family economic adversity). The Medical Research Ethics Committee of the Affiliated Zhongda Hospital of Southeast University reviewed and approved the present study. Written informed consent was obtained from each participant before the study began.

Elective coronary angiography

The methods used for coronary angiography (CAG) and grading the severity of coronary stenosis were described previously [3]. Based on the CAG results, the premature CAD patients were assigned to one of three groups according to the number of stenosed coronary arteries (1-, 2- and 3-vessel disease).

Data collection and definition of cardiovascular risk factors

A physical examination including anthropometric parameters and blood testing was performed on each subject by a qualified doctor using standard methods. Height assessment was accurate to 0.1 cm, weight was measured to an accuracy of 0.1 kg, and body mass index (BMI) was calculated as previously reported [5]. Each patient was evaluated for CAD risk factors, such as hypertension, T2DM, and smoking habits.

Plasma levels of fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (apo A1), apolipoprotein B (apo B), and lipoprotein (Lp)(a)were measured by standard enzymatic methods. TG levels were measured directly when >4.52 mmol/L (400 mg/dL). Dyslipidemia was defined as a TG level $\geq$1.70 mmol/L, TC $\geq$5.72 mmol/L, or HDL-C <0.90 mmol/L in males or <1.04 mmol/L in females.

DNA extraction and genotyping

DNA was extracted using a method that was described previously [3]. Genotyping for the five SNPs (rs10144321, rs1254337, rs12148769, rs7759938, and rs1469039) was performed by the modified method of polymerase chain reaction-ligase detection reaction as previously reported [16,17] using TaqMan genotyping assays on an ABI Prism 3730 Sequence Detection System (Applied Biosystems, Foster City, CA).

Statistical analyses

Statistical analyses were done using SPSS 19.0 software (SPSS, Inc, Chicago, IL). A Student's t test, one-way ANOVA, or chi-square test was used as appropriate. The $\chi 2$ goodness of fit for the Hardy-Weinberg equilibrium of the five SNPs among premature CAD cases and controls were calculated separately. Multivariable logistic regression analyses were used to calculate odds ratios (OR) [with 95% confidence intervals (CI)]. A chi-square test was used to compare the distribution frequencies of genotypes at the five SNPs between controls and patients with premature CAD, or among different groups of CAD patients according to the number of stenosed coronary arteries. All P-values were two-tailed with a significance level of 0.05.

Results

Analyses of baseline characteristics in the premature CAD and control groups (Table 1)

Patients with premature CAD had higher concentrations of TG and apo B, and a higher prevalence of T2DM and dyslipidemia compared with controls (all $P<0.05$). However, the mean values of age, BMI, FBS, TC, LDL-C, HDL-C, apo A1 and Lp(a) did not differ significantly between the two groups. Furthermore, there were no significant differences in the distribution of gender and smokers between the two groups (all $P>0.05$).

Comparison of the clinical characteristics among the control and CAD patients grouped according to the number of diseased coronary arteries (Table 2)

When all participants were divided into four groups that included controls and premature CAD patients with 1-, 2-, and 3-vessel disease, the proportion of patients with T2DM rose

Table 1. Baseline data of the study population.

	Controls	Premature CAD	*P*
Numbers, n	234	198	
Sex			
male, n (%)	105 (44.87)	104 (52.52)	0.113
female, n (%)	129 (55.13)	94 (47.48)	0.113
Age, years	52.34±5.60	52.22±5.95	0.741
Hypertension, n (%)	135 (57.69)	125 (63.13)	0.250
Type 2 diabetes mellitus, n (%)	25 (10.68)	39 (19.70)	0.009
Dyslipidemia, n (%)	67 (28.63)	77 (38.89)	0.024
Smokers, n (%)	68 (29.06)	54 (27.27)	0.681
BMI (kg/m^2)	24.48±3.17	25.38±4.11	0.847
Fasting blood sugar (mmol/L)	5.28±1.33	5.54±1.55	0.110
TC (mmol/L)	3.71±1.67	3.78±1.49	0.685
TG (mmol/L)	2.27±1.52	2.69±1.74	0.026
LDL-C (mmol/L)	2.74±0.73	2.83±0.75	0.293
HDL-C (mmol/L)	1.17±0.26	1.16±0.28	0.836
Apo A1 (g/L)	1.18±0.24	1.16±0.21	0.350
Apo B (g/L)	0.84±0.27	0.92±0.27	0.010
Lp(a) (mg/dL)	197.35±140.41	216.66±161.98	0.498

Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; BMI, body mass index; CAD, coronary artery disease; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Lp(a), lipoprotein(a); TC, total cholesterol; TG, triglyceride.

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Table 2. Comparison of the clinical characteristics among the controls and CAD patients grouped according to the number of diseased coronary arteries.

Variants	Controls (n = 234)	Premature CAD (n = 198), number of vessels involved			P values
		one (n = 105)	two (n = 46)	three (n = 47)	
Sex (male), n	105	51	25	28	0.244
Hypertension, n	135	63	29	33	0.434
Type 2 diabetes mellitus, n	25	18	9	12	0.033
Dyslipidemia, n	67	41	15	21	0.086
Smokers, n	68	23	16	15	0.328

P is the significance level of comparison of clinical characteristics among 4 groups by a chi-square test. CAD, coronary artery disease.

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significantly with the increment in the number of stenosed coronary vessels ($P = 0.033$). There were no significant differences in the distribution of gender, hypertension, dyslipidemia, and smokers among the four groups (all $P>0.05$) (Table 2).

Polymorphism frequency distribution between the premature CAD and control groups (Table 3)

The following genotypes were identified: GG, AG, and AA at rs10144321 and rs12148769; TT, AT, and AA at rs1254337; CC, CT, and TT at rs1469039; TT and CT at rs7759938; and CC, CG, and GG at rs4808793. No deviation from the Hardy-Weinberg equilibrium was detected in the control and premature CAD groups. Table 3 shows the results of evaluation of the frequencies of genotypes for the five polymorphisms between the control and premature CAD groups.

Significant differences were found in the genotype distribution frequencies at rs1254337 between controls and patients with premature CAD ($P = 0.019$). Binary logistic regression analysis revealed an association between rs1254337 and premature CAD. Compared with the TT genotype, patients with the AA genotype had a higher risk of premature CAD (OR: 2.296, 95% CI: 1.188–4.438, $P = 0.013$). However, there were no associations between premature CAD and the frequencies of the genotypes at rs10144321, rs12148769, rs7759938, and rs1469039.

Further analysis showed there were no significant differences among the three genotypes at rs1254337 and the average age, or the mean values of FBS, TC, TG, LDL-C, HDL-C, apo A1, apo B, LP(a), and BMI. In addition, there were no significant differences in the distribution of gender, hypertension, T2DM, dyslipidemia, and smokers among the three genotypes at rs1254337 (all $P>0.05$).

Distribution of genotypes at the five SNPs in CAD patients grouped according to the severity of coronary lesions (Table 4)

When all patients with premature CAD were grouped according to the number of stenosed vessels (1-, 2-, and 3-vessel disease), there were no significant differences in the distribution of the genotypes at the five SNPs among the three groups (all $P>0.05$) (Table 4).

Multivariable logistic regression analysis of risk factors and different genotypes for the occurrence of premature CAD (Table 5)

Table 5 shows that after adjustment for the clinical parameters (smoking, hypertension, dyslipidemia, and continuous variables), three factors were identified that significantly increased the risk of premature CAD: the AA genotype at rs1254337 (OR: 2.388, 95% CI: 1.190–4.792,

Table 3. Genotype distributions at the five SNPs in the control and premature CAD groups.

	Control, n(%)	Premature CAD, n(%)	OR (95% CI)
rs10144321			
GG	73(31.2) *	62 (31.3)*	1.0 (reference)
AG	104(44.4)	100(50.5)	0.883 (0.571–1.366)
AA	57 (24.4)	36 (18.2)	1.345 (0.786–2.301)
P	0.255		
rs1254337			
TT	124(53.0)	81 (40.9)	1.0 (reference)
AT	92 (39.3)	90 (45.5)	1.498 (1.000–2.242)
AA	18 (7.7)	27 (13.6)	2.296 (1.188–4.438)#
P	0.019		
rs12148769			
GG	94 (40.2)	84 (42.4	1.0 (reference)
AG	114 (48.7)	94 (47.5)	1.084 (0.725–1.619)
AA	26 (11.1)	20 (10.1)	1.162 (0.605–2.232)
P	0.874		
rs7759938			
TT	227 (97.0)	185 (93.4)	1.0 (reference)
CT	7 (3.0)	13 (6.6)	2.279(0.891–5.829)
P	0.078		
rs1469039			
CC	102 (43.6)	99 (50.0)	1.0 (reference)
CT	105 (44.9)	87 (43.9)	1.864(0.892–3.896)
TT	27 (11.5)	12 (6.1)	1.184(0.748–1.550)
P	0.104		

*Number of individuals with percentage in parentheses. CAD, coronary artery disease; OR, odds ratio; CI, confidence intervals. The chi-square test and likelihood ratio test were used to analyze the genotypes. *P* is the level of significance for the premature CAD group compared with the control group.
P = 0.013 compared with the TT genotype.

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P = 0.014), male gender (OR: 1.565, 95% CI: 1.012–2.420, *P* = 0.044), and T2DM (OR 2.252, 95% CI: 1.233–4.348, *P* = 0.015).

Discussion

This is the first case-control study assessing the relationship among five pubertal transition-related gene SNPs (rs10144321, rs1254337, rs12148769, rs7759938, and rs1469039), and the development and severity of premature CAD. The current study for the first time demonstrates an association between rs1254337 and premature CAD in a Chinese Han population. Compared with rs1254337 TT genotype carriers, the AA genotype carriers had a 1.388-fold higher risk of developing premature CAD. However, the current study was unable to provide supportive genetic prediction values for the other 4 SNPs and premature CAD.

Age at menarche acts as a marker of timing of puberty in females, and recent studies suggest that it is a heritable trait, and early pubertal onset is associated with an increased risk of obesity, T2DM, CVD, cancer, and all-cause mortality [12–15]. Pathway analyses implicated nuclear hormone receptors, particularly retinoic acid andγ-aminobutyric acid-B2 receptor signaling, as potential novel mechanisms that regulate pubertal timing in humans [14]. The presence of

Table 4. Distribution of genotypes at the five SNPs in CAD patients grouped according to the severity of coronary lesions.

Variants	Genotypes	Number of vessels involved			P values
		One	Two	Three	
rs10144321	GG, n	33	16	13	
	AG, n	54	21	25	0.937
	AA, n	18	9	9	
rs1254337	TT, n	40	19	22	
	AT, n	49	20	21	0.759
	AA, n	16	7	4	
rs12148769	GG, n	48	18	18	
	AG, n	48	21	25	0.635
	AA, n	9	7	4	
rs7759938	TT, n	97	44	44	0.755
	CT, n	8	2	3	
rs1469039	CC, n	51	22	26	
	CT, n	47	21	19	0.930
	TT, n	7	3	2	

P is the significance level of comparison among three different groups according to the number of coronary arteries involved in patients with premature CAD. SNPs, single nucleotide polymorphisms.

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menarche age-raising alleles at rs1254337 was associated with lower adult BMI and taller adult height [14]. Epidemiological evidence suggests that early menarche (onset of menses ≤ 11 years) has increased in prevalence in recent birth cohorts and is associated with multiple unfavorable medical and mental health outcomes in adulthood. Available data support the concept that childhood adversities occurring prior to menarche contribute to early menarche. Childhood adversity predicts central obesity beyond more contemporary, modifiable risk factors [18]. In particular, childhood sexual abuse is the adversity most strongly associated with early menarche [19]. In the present study, we excluded participants who experienced childhood adversities or abuse to minimize the potential influence of these variables on the occurrence of premature CAD in adults.

In the present study, patients with premature CAD had higher concentrations of TG and apo B, and a higher prevalence of T2DM and dyslipidemia compared with controls. Furthermore, the proportion of patients with T2DM rose significantly with an increase in the number of stenosed coronary vessels. Hypercholesterolemia and T2DM are well-known risk factors for CAD, and investigators used to believe that familial combined hyperlipidemia (FCHL) carried

Table 5. Multivariable logistic regression analysis of cardiovascular risk factors and different genotypes for the occurrence of premature CAD.

Variants		B	P	OR	95% CI
rs1254337	TT		0.000	1.00 (reference)	
	AT	0.310	0.156	1.364	0.888–2.093
	AA	0.870	0.014	2.388	1.190–4.792
Sex, male		0.448	0.044	1.565	1.012–2.420
Type 2 diabetes mellitus		0.843	0.015	2.252	1.233~4.348

See Table 3 for abbreviations.

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a greater risk of premature CAD than familial hypertriglyceridemia (FHTG). However, recent clinical studies [20,21] have shown that FCHL and FHTG are more alike than dissimilar, and the risk of CAD in FCHL and FHTG is strongly related to the presence of metabolic syndrome. Elevated TG concentrations (as a feature of metabolic syndrome) are associated with an increased risk of premature CAD, and the association between the plasma TG levels and premature familial CAD is strong, graded, and independent. Apo B levels improve risk assessment of future CAD events above and beyond LDL-C or non-HDL-C levels [22], and non-HDL-C and apo B levels are each associated with the risk of future major cardiovascular events [23]. In conjunction with available data, these findings suggest that the hypertriglyceridemia is not benign and warrants aggressive treatment.

Based on our assessment of the relationships between these five pubertal transition-related gene polymorphisms and premature CAD and its severity, we found a significant difference in the genotype distribution frequencies at rs1254337 between controls and premature CAD patients. Furthermore, binary logistic regression analysis revealed an association of the rs1254337 polymorphism with premature CAD; patients with the AA genotype had a 1.388-fold higher risk of premature CAD compared with GG genotype carriers. Moreover, multivariable logistic regression analysis showed that the association between the AA genotype and premature CAD was independent of other clinical variables. However, we could not find any significant relationships between rs1254337 and the clinical and metabolic characteristics of our study subjects. Also, we did not detect any significant differences in the genotype frequency distributions of these five SNPs among CAD patients with 1-, 2-, and 3-vessel disease. In addition, there were no significant differences in the frequencies of genotypes at rs10144321, rs12148769, rs7759938, and rs1469039 between controls and patients with premature CAD.

Considering the known effects of lipid disorders on premature CAD [20–23], it was surprising that we did not detect any differences in the clinical or metabolic characteristics among the three genotypes at rs1254337 in our premature CAD patients. Furthermore, there was no association between this polymorphism and the severity of coronary lesions. Thus, we were not able to explain the mechanism responsible for the relationship between rs1254337 and premature CAD, despite the publication of a recent study that showed that earlier pubertal timing was associated with higher risks for angina, hypertension, and T2DM [24]. Therefore, it is still necessary to explore the pathophysiological mechanisms of pubertal transition-related gene polymorphisms involved in the progression of premature CAD as well as to establish reasonable prevention strategies to attenuate morbidity and mortality associated with premature CAD.

The present study has some strengths and limitations. First, our study sample is relatively small and this might have an impact on the statistical power. Second, this single-center, case-control study included only middle-aged subjects suspected of having premature CAD, so the participants did not represent the characteristics of the general population. The fact that all participants underwent elective CAG indicates that our results are reliable. Thirdly, since certain genotypes and alleles might be race-specific, it may not be possible extrapolate our results to other ethnic populations. Even so, the present study adds new information to this specific field and could serve as the basis for further studies.

Conclusions

In a sample of subjects from China, our findings for the first time suggest that a genetic variant at rs1254337 may exert an influence on the development of premature CAD. However, this finding needs to be further verified by prospective studies in a larger population with diverse ethnicities to elucidate possible mechanisms. Furthermore, it is too early to regard the pubertal transition-related gene rs1254337 as a causal factor of premature CAD.

Author Contributions

Conceived and designed the experiments: ZC. Performed the experiments: BC FYX. Analyzed the data: ZC. Contributed reagents/materials/analysis tools: CCT GSM LW. Wrote the paper: ZC.

References

1. Chen W, Gao R, Liu L, Zhu M, Wang W, Wang Y, et al. Report on cardiovascular diseases in China 2013. Chinese Circulation Journal. 2014; 29:487–491.

2. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007; 316:1488–1491. PMID: 17478681

3. Chen Z, Qian Q, Ma G, Wang J, Zhang X, Feng Y, et al. A common variant on chromosome 9p21 affects the risk of early-onset coronary artery disease. Mol Biol Rep. 2009; 36:889–893. doi: 10.1007/s11033-008-9259-7 PMID: 18459066

4. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. N Engl J Med. 2007; 357:443–453. PMID: 17634449

5. Chen Z, Zhang X, Ma G, Qian Q, Yao Y. Association study of four variants in KCNQ1 with type 2 diabetes mellitus and premature coronary artery disease in a Chinese population. Mol Biol Rep. 2010; 37:207–212. doi: 10.1007/s11033-009-9597-0 PMID: 19575309

6. Smith SC Jr. Screening for high-risk cardiovascular disease: a challenge for the guidelines. Arch Intern Med. 2010; 170:40–42. doi: 10.1001/archinternmed.2009.457 PMID: 20065197

7. Wang W, Hu SS, Kong LZ, Gao RL, Zhu ML, Wang WY, et al. Summary of report on cardiovascular diseases in China, 2012. Biomed Environ Sci. 2014; 27:552–558. doi: 10.3967/bes2014.085 PMID: 25073915

8. He J, Gu D, Wu X, Reynolds K, Duan X, Yao C, et al. Major causes of death among men and women in China. N Engl J Med. 2005; 353:1124–1134 PMID: 16162883

9. Yang ZJ, Liu J, Ge JP, Chen L, Zhao ZG, Yang WY; China National Diabetes and Metabolic Disorders Study Group. Prevalence of cardiovascular disease risk factor in the Chinese population: the 2007–2008 China National Diabetes and Metabolic Disorders Study. Eur Heart J. 2012; 33:213–20. doi: 10.1093/eurheartj/ehr205 PMID: 21719451

10. Wang W, Hu SS, Kong LZ, Gao RL, Zhu ML, Wang WY, et al. Summary of report on cardiovascular diseases in China, 2012. Biomed Environ Sci. 2014; 27:552–8. doi: 10.3967/bes2014.085 PMID: 25073915

11. Klein LW, Nathan S. Coronary artery disease in young adults. J Am Coll Cardiol. 2003; 41:529–531. PMID: 12598060

12. Prentice P, Viner RM. Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. Int J Obes (Lond). 2013; 37:1036–1043.

13. Demerath EW, Liu CT, Franceschini N, Chen G, Palmer JR, Smith EN, et al. Genome-wide association study of age at menarche in African-American women. Hum Mol Genet. 2013; 22:3329–3346. doi: 10.1093/hmg/ddt181 PMID: 23599027

14. Perry JR, Day F, Elks CE, Sulem P, Thompson DJ, Ferreira T, et al. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. Nature. 2014; 514:92–97. doi: 10.1038/nature13545 PMID: 25231870

15. Tu W, Wagner EK, Eckert GJ, Yu Z, Hannon T, Pratt JH, et al. Associations between menarche-related genetic variants and pubertal growth in male and female adolescents. J Adolesc Health. 2015; 56:66–72. doi: 10.1016/j.jadohealth.2014.07.020 PMID: 25287989

16. Favis R, Day JP, Gerry NP, Phelan C, Narod S, Barany F. Universal DNA array detection of small insertions and deletions in BRCA1 and BRCA2. Nat Biotechnol. 2000; 18:561–564. PMID: 10802632

17. Xiao Z, Xiao J, Jiang Y, Zhang S, Yu M, Zhao J, et al. A novel method based on ligase detection reaction for low abundant YIDD mutants detection in hepatitis B virus. Hepatol Res. 2006; 34:150–155. PMID: 16500145

18. Davis CR, Dearing E, Usher N, Trifiletti S, Zaichenko L, Ollen E, et al. Detailed assessments of childhood adversity enhance prediction of central obesity independent of gender, race, adult psychosocial risk and health behaviors. Metabolism. 2014; 63:199–206. doi: 10.1016/j.metabol.2013.08.013 PMID: 24211017

19. Henrichs KL, McCauley HL, Miller E, Styne DM, Saito N, Breslau J. Early menarche and childhood adversities in a nationally representative sample. Int J Pediatr Endocrinol. 2014; 2014:14. doi: 10.1186/1687-9856-2014-14 PMID: 25089128

20. Hopkins PN, Heiss G, Ellison RC, Province MA, Pankow JS, Eckfeldt JH, et al. Coronary artery disease risk in familial combined hyperlipidemia and familial hypertriglyceridemia: a case-control comparison from the National Heart, Lung, and Blood Institute Family Heart Study. Circulation. 2003; 108:519–523. PMID: 12847072

21. Hopkins PN, Wu LL, Hunt SC, Brinton EA. Plasma triglycerides and type III hyperlipidemia are independently associated with premature familial coronary artery disease. J Am Coll Cardiol. 2005; 45:1003–1012. PMID: 15808755

22. Pencina MJ, D'Agostino RB, Zdrojewski T, Williams K, Thanassoulis G, Furberg CD, et al. Apolipoprotein B improves risk assessment of future coronary heart disease in the Framingham Heart Study beyond LDL-C and non-HDL-C. Eur J Prev Cardiol. 2015 Jan 29 [Epub ahead of print].

23. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, Nestel PJ, et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. JAMA. 2012; 307:1302–1309. doi: 10.1001/jama.2012.366 PMID: 22453571

24. Day FR, Elks CE, Murray A, Ong KK, Perry JR. Puberty timing associated with diabetes, cardiovascular disease and also diverse health outcomes in men and women: the UK Biobank study. Sci Rep. 2015; 5:11208. doi: 10.1038/srep11208 PMID: 26084728