Clinical Trial/Experimental Study

Medicine®

OPEN

Association of *BAX* hypermethylation with coronary heart disease is specific to individuals aged over 70

Limei Zhang, MD[a], Huihui Ji, MSc[b], Yi Huang, MD[b], Haochang Hu, MBBS[b], Bin Li, MBBS[b], Yong Yang, MBBS[b], Hang Yu, MBBS[b], Xiaoying Chen, MSc[b], Wenxia Li, MBBS[b], Fang Liu, MBBS[b], Shi Wang, MD[a], Chunming Wang, MD[a], Ke Chen, MD[a], Yingchun Bao, MD[a], Haibo Liu, MD[c,*], Shiwei Duan, PhD[b,*]

Abstract

Introduction: As a member of B-cell lymphoma-2 (BCL-2) gene family, BCL-2 associated X (*BAX*) is important for cell apoptosis. In this work, we investigated the association of *BAX* promoter DNA methylation with coronary heart disease (CHD) in Han Chinese.

Methods: A SYBR green-based quantitative methylation specific PCR (qMSP) was used to test *BAX* methylation levels in 959 CHD cases and 514 controls.

Results: Although *BAX* methylation was not associated with CHD in the total samples, further breakdown analysis by age showed that *BAX* hypermethylation was significantly associated with CHD for individuals aged over 70 (median percentage of methylation ratio [PMR], 10.70% in cases versus (vs) 2.25% in controls, $P = .046$). Moreover, *BAX* methylation was associated with smoking and lipoprotein A (Lp(a)) for individuals aged over 70 (CHD: smoking $P = .012$, Lp(a) $P = .001$; non-CHD: smoking $P = .051$, Lp(a) $P = .004$). Further analysis of Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) data showed *BAX* expression was upregulated by 5-aza-2'-deoxycytidine demethylation agent (fold = 1.66, $P = .038$) and inversely correlated with *BAX* methylation ($r = -0.428$, $P = 8E\text{-}05$).

Conclusions: Our study supported that *BAX* hypermethylation might contribute to CHD risk via downregulation of *BAX* expression for individuals aged over 70.

Abbreviations: 5'-AZA = 5'-aza-2'-deoxycytidine, ApoA1 = apolipoprotein A1, ApoB = apolipoprotein B, ApoE = apolipoprotein E, BAX = BCL-2 associated X, BCL-2 = B-cell lymphoma-2, CHD = coronary heart disease, GEO = Gene Expression Omnibus, HDL = high density lipoprotein, LDL = low density lipoprotein, Lp (a) = lipoprotein A, NCBI = National Center for Biotechnology Information, PMRs = percentage of methylation ratios, qMSP = quantitative methylation specific PCR, TC = total cholesterol, TCGA = The Cancer Genome Atlas.

Keywords: age, *BAX*, coronary heart disease, DNA methylation, Lp(a), smoking

1. Introduction

Nearly 7 million annual deaths are attributable to coronary heart disease (CHD).[1] The CHD is characterized by the stenosis or occlusion of the coronary artery.[2] The CHD is a complex disease accompanied by several complications such as elevated cholesterol levels and cigarette smoking.[3]

The DNA methylation is a crucial epigenetic marker regulating gene expression.[4] It often occurs at a CpG site where a cytosine is directly followed by a guanine in the DNA sequence. Hypermethylation of gene promoter could induce transcriptional silencing[5,6] and often involve in the development of diseases.[7]

The B-cell lymphoma-2 (BCL-2) family proteins are famous for the capacity for the regulation of programmed cell death.[8] The BCL-2 can prevent cell apoptosis and its over-expression may promote cancer cell survival.[9] Human BCL-2 associated X (*BAX*) is the 1st death-promoting member in BCL-2 family.[10] The *BAX* can suppress cell apoptosis process.[8] The expression level of *BAX* was important for heart disease including CHD. Overexpressed *BAX* was found to accelerate myocyte apoptosis during ischemia and reperfusion.[11,12]

Although no association of *BAX* methylation and CHD was discovered between 205 CHD patients and matched controls in an early study,[13] we carried out the present study among 959

Editor: Ovidiu Constantin Baltatu.

LZ, HJ and YH these authors contributed equally to this work.

The research was supported by the grants from the National Natural Science Foundation of China (81371469 and 81400263), Ningbo City Medical Science and Technology projects (2014A20), and K. C. Wong Magna Fund in Ningbo University.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

[a] Department of Cardiology, Yinzhou People's Hospital, Ningbo University, Ningbo, [b] Medical Genetics Center, School of Medicine, Ningbo University, Ningbo, [c] Department of Cardiovascular Medicine, East Hospital, Tongji University School of Medicine, Shanghai, China.

[*] Correspondence: Shiwei Duan, Medical Genetics Center, School of Medicine, Ningbo University, No.818 Fenghua Road, Ningbo 315000, China. (e-mail: duanshiwei@nbu.edu.cn), Haibo Liu, Department of Cardiology, East Hospital, Tongji University School of Medicine, 150 Jimo Road, Pudong New Area, Shanghai 200032, China. (e-mail: liuhaibo1219@hotmail.com).

Medicine (2019) 98:4(e14130)

Received: 4 December 2017 / Received in final form: 27 September 2018 / Accepted: 13 November 2018

http://dx.doi.org/10.1097/MD.0000000000014130

CHD cases and 514 controls to assess the relationship between *BAX* methylation and CHD.

2. Materials and methods

2.1. Ethics statement

Institutional review ethics board approval was obtained from Yinzhou Peoples Hospital and Ningbo No.1 Hospital. All the individuals were formally informed, and the written informed consents were obtained from all the participants or their guardians.

2.2. Patient selection

The present study enrolled Chinese patients with CHD from Yinzhou Peoples Hospital and Ningbo No.1 Hospital. The patients were diagnosed with the angiographic evidence that coronary artery stenosis was greater than 50% or a history of prior angioplasty or coronary artery bypass surgery. The inclusion criteria were described previously.[14,15] The peripheral blood samples were drawn from 959 CHD (635 males and 324 females, median age: 62 years) patients and 514 healthy controls (291 males and 223 females, median age: 60 years) for DNA methylation assay. About 50% and 18% of participators were accompanied by hypertension and diabetes, respectively. Among them, about 33% of participators were smokers. The clinical indexes including low density lipoprotein (LDL), total cholesterol (TC), high density lipoprotein (HDL), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), apolipoprotein E (ApoE) and lipoprotein A (Lp(a)) were measured using standard protocols at the time of collecting DNA samples. Blood samples were stored at -80°C with EDTA anticoagulant tube.

2.3. DNA extraction, bisulfite conversion and quantitative methylation specific PCR (qMSP)

The procedures of DNA extraction from peripheral blood and the subsequent bisulfite conversion were the same as previously described.[16] The *BAX* methylation was measured by qMSP, and the percentage of methylation ratios (PMRs) was applied to represent gene methylation levels.[17,18] The details of qMSP were available in our previous publications.[19–22] The forward and reverse primer sequences of *BAX* were 5'-GAAGGTATTA-GAGTTGCGATT-3' and 5'-CCAATAAACATCTCCCGATAA-3', respectively. The forward and reverse primer sequences of *ACTB* were 5'-TGGTGATGGAGGAGGTTTAGTAAGT-3' and 5'-AACCAATAAAACCTACTCCTCCCTTAA-3', respectively.

2.4. Data-mining of the online datasets

The Gene Expression Omnibus (GEO) data sets from the National Center for Biotechnology Information (NCBI) was used to browse for gene expression microarrays data. The cells were collected before and after 5'-aza-2'-deoxycytidine (5'-AZA) treatment, respectively. The *BAX* expression values in cells were acquired from an Illumina HumanRef-8 v3.0 expression beadchip. The detailed data was under accession No. GSE38823 in GEO data sets.[23] The *BAX* methylation data and its mRNA expression values were retrieved from (The Cancer Genome Atlas) TCGA database.

2.5. Statistical analysis

A P value $< .05$ was considered to be statistically significant. Categorical data were shown as number and percentages, and then analyzed using Pearson Chi-squared or Fisher's exact test (when expected value of any cell is less than 5). Nonparametric test was used to compare the differences of *BAX* methylation between CHD cases and normal controls. The correlations between clinical indexes and *BAX* methylation were examined by the Spearman correlation.

3. Results

A fragment from CpG island of *BAX* promoter was measured in this study (Fig. 1). However, there was no significant difference of *BAX* methylation between CHD cases and controls ($P = .384$, Table 1). As shown in Table 1, a significant association was found between age and CHD [OR (95% CI) = 1.040 (1.025–1.055), $P =$ 7E-8]. Moreover, our results suggested that smoking and diabetes could increase the risk of CHD [smoking, $P = .026$, OR (95% CI) = 1.453 (1.046–2.020); diabetes, $P = .002$, OR (95% CI) = 1.822 (1.256–2.643)]. Besides, there were significant associations between CHD and the levels of biochemical indexes including LDL, triglyceride and Lp(a) [LDL, OR (95% CI) = 1.727 (1.171–2.547), $P = .006$; triglyceride, OR (95% CI) = 1.291 (1.084–1.538), $P = .004$; Lp(a), OR (95% CI) = 0.998 (0.997–0.999), $P = .001$]. The associations between *BAX* methylation and clinical indexes were also analyzed (Supplemental Table 1, http://links.lww.com/MD/C778).

Since age was one of the most influential factors on alteration of DNA methylation,[24] we further performed a breakdown analysis by age. As shown in Table 2, *BAX* hypermethylation was significantly associated with CHD among individuals aged over 70 (median PMR, 10.70% versus 2.25%, $P = .046$). For individuals aged over 70, *BAX* hypermethylation was found to be associated with smoking which was a risk factor of CHD (CHD: $P = .012$; non-CHD: $P = .051$, Table 3). Moreover, an inverse association was observed between *BAX* methylation and triglyceride in CHD cases aged over 70 ($r = -0.153$, $P = .040$, Table 4). And among individuals aged over 70, *BAX* methylation was inversely related to Lp(a) (cases, $r = -0.266$, $P = .001$; $r = -0.411$, $P = .004$, Table 4). Therefore, our results indicated that the association of *BAX* hypermethylation with CHD was specific to individuals aged over 70.

As shown in Table 5, smoking was significantly associated with BAX methylation in individuals aged over 70 and all subjects (over 70, $r = 0.228$, $P = .001$, total, $r = 0.056$, $P = .045$). Triglyceride was related to BAX methylation in aged 50–59 ($r = -0.115$, $P = .022$). Besides, there were significant associations between Lp(a) and BAX methylation in aged 50–59, 60–69, ≥ 70, and all subjects, respectively (50–59, $r = -0.261$, $P =$ 6.E-07, 60–69, $r = -0.253$, $P =$ 2.E-08, ≥ 70, $r = -0.384$, $P =$ 2.E-08, total, $r = -0.274$, $P =$ 2.E-22).

The GEO data analysis showed a significantly higher level of *BAX* in cells after 5'-AZA demethylation agent treatment (fold = 1.66, $P = .038$, Fig. 2). The TCGA data analysis indicated *BAX* methylation was inversely associated with *BAX* expression ($r = -0.428$, $P =$ 8E-5, Fig. 3). Therefore, we hypothesized that *BAX* hypermethylation might contribute to CHD among individuals aged over 70 via its downregulation of *BAX* expression.

4. Discussion

Our results demonstrated the association of *BAX* hypermethylation with CHD was specific to individuals aged over 70. Among individuals aged over 70, *BAX* hypermethylation was associated with smoking and lower plasma Lp(a) level. The *BAX* expression

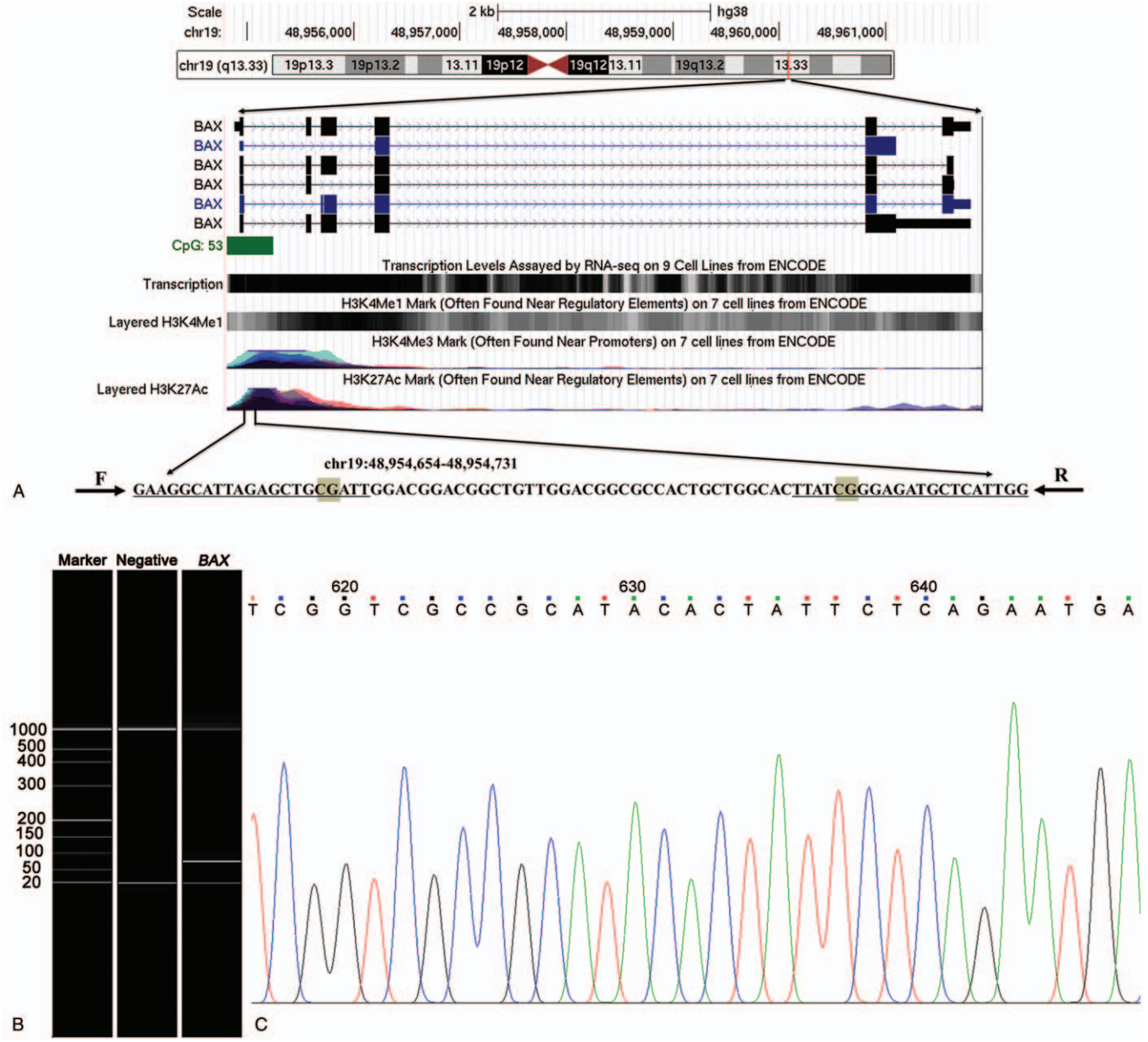

Figure 1. The target sequence in *BAX* methylation assay. The 2 tested CpG dinucleotides in *BAX* promoter. F and R were forward primer and reverse primer, respectively. Verification of the qMSP product length by capillary electrophoresis; Sequencing validation. BAX = BCL-2 associated X, qMSP = quantitative methylation specific PCR.

was induced by methylation inhibitor and further online data mining found that *BAX* expression was inversely related to its methylation.

The *BAX* was considered to be the most important apoptosis-inhibition gene.[25–27] Abnormal *BAX* expression was shown to be associated with heart disease.[28,29] Our study suggested that *BAX* methylation was significantly higher in CHD patients over 70. Moreover, further analyses of GEO and TCGA data showed that *BAX* hypermethylation would suppress its RNA expression. Our study showed that the *BAX* hypermethylation might contribute to CHD among individuals aged over 70 via its downregulation of *BAX* expression. Further studies were needed to discover the role of the *BAX* methylation in CHD.

The *BAX* is an important player in apoptosis and encodes a variety of transcripts. The *BAX* is associated with developmental, cancer and age-related changes in apoptosis.[27] Inactivation of *BAX* in mice prolongs fertility potential and minimizes age-related health problems.[30] It is unclear how these processes are related to human aging, but the role of *BAX* in human aging is possible. In the present study, *BAX* methylation was significantly decreased in Non-CHD individuals aged over 70 while the CHD group in the same age seems to keep the methylation level. Previous studies have shown that statins can promote epigenetic-based control in CVD prevention through histone modifications.[31] In addition, atorvastatin inhibits neointimal formation by inducing p16 expression by inducing DNA methylation in the p16 promoter region.[32] Thus, the decreased *BAX* methylation in the CHD group may be rescued by drug therapy or other treatment-related factors. Further research was needed to confirm this hypothesis.

Table 1

Multiple logistic regression analyses between phenotypes and coronary heart disease (CHD)*.

	β	S.E.	Odds ratio	95% CI	*P*
BAX methylation (%)	0.000	0.000	1.000	1.000–1.001	.387
Age, y	0.039	0.007	1.040	1.025–1.055	**7E-8**
Gender	0.265	0.163	1.303	0.947–1.792	.104
Smoking	0.374	0.168	1.453	1.046–2.020	**.026**
Hypertension	0.152	0.137	1.164	0.890–1.522	.267
Diabetes	0.600	0.190	1.822	1.256–2.643	**.002**
LDL (mmol/L)	0.546	0.198	1.727	1.171–2.547	**.006**
TC (mmol/L)	-0.240	0.166	0.786	0.568–1.089	.148
HDL(mmol/L)	0.024	0.061	1.024	0.908–1.155	.701
Triglyceride (mmol/L)	0.256	0.089	1.291	1.084–1.538	**.004**
ApoA1(mmol/L)	-0.177	0.199	0.838	0.567–1.239	.376
ApoB(mmol/L)	-0.442	0.333	0.643	0.335–1.234	.184
ApoE(mmol/L)	-0.012	0.011	0.988	0.966–1.010	.275
Lp(a) (mmol/L)	-0.002	0.001	0.998	0.997–0.999	**.001**

* *P* value less than or equal to .05 was in bold.

ApoA1 = apolipoprotein A, ApoB = apolipoprotein B, ApoE = apolipoprotein E, CHD = coronary heart disease, HDL = high density lipoprotein, LDL = low density lipoprotein, Lp(a) = lipoprotein A, TC = total cholesterol

Table 2

The comparisons of *BAX* methylation between coronary heart disease (CHD) and non-CHD by age*.

	CHD		Non-CHD		
Age, y	**Number**	**(Median [quartile range])**	**Number**	**(Median [quartile range])**	***P***
40–49	85	13.40 (1.44, 25.85)	79	15.70 (4.79, 32.30)	.231
50–59	257	16.50 (3.59, 33.80)	157	11.80 (2.74, 27.45)	.165
60–69	387	14.90 (3.90, 30.20)	198	13.45 (1.93, 27.60)	.308
70+	187	10.70 (1.10, 29.30)	57	2.25 (0.82, 18.20)	**.046**

* *P* value less than or equal to .05 was in bold; the percentage of methylated reference (PMR) was represented by median (percentile range). CHD = coronary heart disease.

Table 3

The comparisons of BCL-2 associated X (*BAX*) methylation between smoking and non-smoking among individuals aged over 70*.

	Smoking (median [quartile range])	Non-smoking (median [quartile range])	*P*
CHD	21.20 (7.78, 49.45)	10.60 (1.15, 25.90)	**.012**
non-CHD	18.10 (8.84, 24.45)	1.93 (0.91, 18.00)	.051

* *P* value less than or equal to .05 was in bold; the percentage of methylated reference (PMR) was represented by median (quartile range). BAX = BCL-2 associated X, CHD = coronary heart disease.

Ageing is a risk factor for multiple chronic diseases.[33] Ageing impacts all organ systems leading to decreased functionality and eventual death.[34] Accumulating evidence has shown that DNA methylation changes are associated with ageing and its related phenotypes.[24,35–38] Our study found that *BAX* methylation was associated with CHD only among individuals aged over 70, adding a new ageing-related clue of CHD.

Table 4

Associations of BCL-2 associated X (*BAX*) methylation and biochemical indexes among individuals aged over 70*.

***BAX* methylation**	**LDL (mmol/L)**	**Triglyceride (mmol/L)**	**Lp(a) (mmol/L)**
CHD			
r	0.101	−0.153	−0.266
P	.177	**.040**	**.001**
Non-CHD			
r	0.176	0.147	−0.411
P	.203	.288	**.004**

* *P* value less than or equal to 0.05 was in bold; Spearman test was used to detect the relationship between *BAX* methylation and clinical indexes. BAX = BCL-2 associated X, CHD = coronary heart disease, LDL = low density lipoprotein, Lp(a) = lipoprotein A.

Tobacco smoking was associated with significant modifications of gene methylation.[39–41] Tobacco smoking was shown to increase the risk of cardiovascular disease.[42,43] A mice experiment showed *BAX* expression could be induced by exposure to smoking in oocytes.[8] Furthermore, ovarian damage caused by smoking could prevented by *BAX* inactivation.[44] Our study found that *BAX* methylation was associated with smoking among individuals aged over 70. The *BAX* methylation might be involved in the regulation of cell apoptosis caused by smoking.

In the recent years, the relationship between lipid metabolism and CHD have been discovered by previous studies.[45,46] The ratio of TG/HDL-C was proportional to the severity degree of CHD.[47] The *BAX* methylation level would deceased by oxidized LDL and then lead to cell apoptosis. Inconsistent with a previous study,[48] our results showed *BAX* methylation was inversely related to plasma Lp(a), suggesting that *BAX* hypermehtylation correlated with a lower level of plasma Lp(a), a protective factor of CHD shown in the present study. Further studies covering

Table 5

Associations of BAX = BCL-2 associated X (*BAX*) methylation and biochemical indexes among individuals*.

BAX methylation		Age, y	LDL (mmol/L)	TC (mmol/L)	HDL(mmol/L)	Triglyceride (mmol/L)	ApoA1(mmol/L)	ApoB(mmol/L)	ApoE(mmol/L)	Lp(a) (mmol/L)
40–49	r	−0.241	0.034	0.010	−0.152	−0.072	−0.296	−0.153	0.022	−0.111
	P	**.002**	.680	.905	.062	.378	**3.E-04**	.065	.800	.200
50–59	r	−0.108	0.042	0.005	−0.062	−0.115	−0.121	−0.090	−0.053	−0.261
	P	**.028**	.399	.926	.215	.022	**.017**	.078	.327	**6.E-07**
60–69	r	−0.052	−0.016	−0.016	−0.086	0.037	−0.146	−0.126	0.006	−0.253
	P	.213	.706	.706	**.042**	.389	**.001**	**.003**	.901	**2.E-08**
70+	r	−0.189	0.116	0.075	−0.043	−0.088	−0.140	−0.015	−0.064	−0.384
	P	**.003**	.077	.254	.508	.179	**.033**	.818	.382	**2.E-08**
Total	r	−0.117	0.041	0.014	−0.084	−0.031	−0.161	−0.096	−0.031	−0.274
	P	**7.E-06**	.123	.610	**.002**	.252	**3.E-09**	**4.E-04**	.293	**2.E-22**

* *P* value less than or equal to .05 was in bold, Spearman test was used to detect the relationship between *BAX* methylation and clinical indexes. BAX = BCL-2 associated X, LDL = low density lipoprotein, Lp(a) = lipoprotein A.

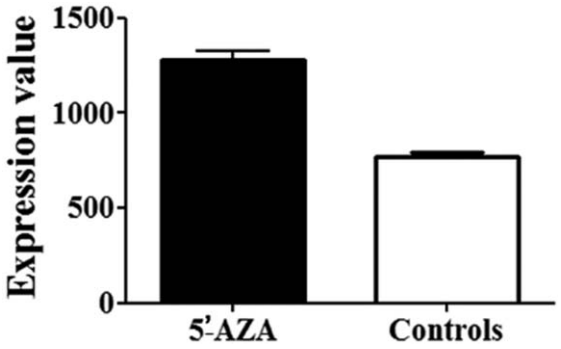

Figure 2. Demethyaltion agent significantly increased *BAX* expression. 5'-AZA denoted the cells treated by 5'-AZA. Controls denoted the cells without the treatment. Y-axis was the level of *BAX* expression. The detailed data was retrieved from accession No.GSE38823 in GEO database. BAX = BCL-2 associated X, 5'-AZA = 5'-aza-2'-deoxycytidine, GEO = Gene Expression Omnibus.

large samples should be done to explore the detailed relationship between CHD and Lp(a).

A recent study showed that the levels of TC, LDL, non-HDL and ApoB were significantly higher in patients with myocardial infarction than in the control group, and these lipid levels showed a downward trend with age (27737874). Meanwhile, the levels of ApoA1 and HDL in patients with myocardial infarction were significantly lower than those in the control group, and the levels of both increased significantly with age (27737874). In high-fat-fed mice, lipid accumulation in the hippocampal CA3 region was observed, and plasma lipid levels (including triglycerides, TC, LDL, and HDL) were significantly increased, accompanied by an increase in *BAX* expression (28000893). Our study found that *BAX* methylation levels were inversely correlated with age, ApoA1, and Lp(a) in different age groups. Taken together, we hypothesized that changes in *BAX* methylation levels were likely to have an effect on the pathogenesis of CHD under the combined effects of age and lipid levels.

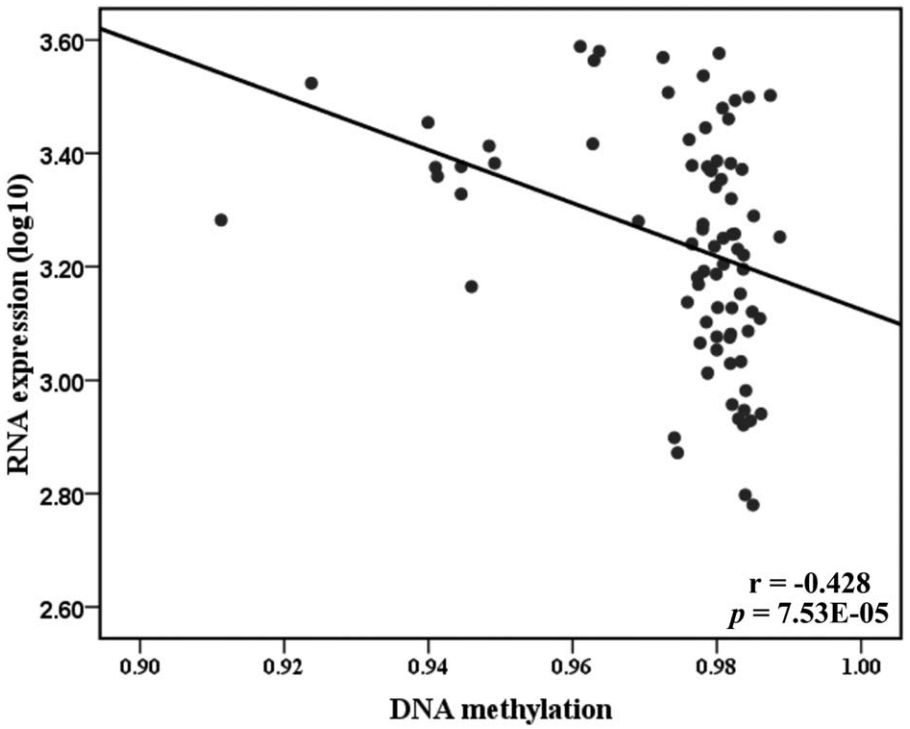

Figure 3. The *BAX* methylation inversely correlated with *BAX* expression. Pearson correlation were performed for the correlation test. The *BAX* methylation and its mRNA expression values were retrieved from TCGA database. BAX = BCL-2 associated X, TCGA = The Cancer Genome Atlas.

There are some limitations in the present study. Firstly, since our findings from a case-control study are just correlative and possibly not causal, the case-effect relationship between *BAX* methylation and CHD remains unclear. Therefore, a more convincing method such as cohort study is needed in the future. Secondly, only 2 CpG sites from CpG island has been selected to represent the whole gene of *BAX*. Thirdly, we only have measured *BAX* methylation in peripheral blood samples. However, the DNA methylation profile may vary among different tissues more or less. Further studies are needed to confirm our findings in other tissues.

In summary, our research found that *BAX* hypermethylation might contribute to CHD among individuals aged over 70 years.

Author contributions

The experiment was designed by HL and SD. The patients' information was collected by LZ, SW, CW, YB, YH and KC. The experiment was performed by BL, YY, HY and XC. The data was analyzed by WL and FL. The draft was written by HJ and HH.
Conceptualization: Haibo Liu, Shiwei Duan.
Data curation: Limei Zhang, Yi Huang, Bin Li, Yong Yang, Yu Huang, Wenxia Li, Chunming Wang, Ke Chen.
Formal analysis: Yong Yang, Yu Huang, Xiaoying Chen, Fang Liu, Yingchun Bao.
Investigation: Bin Li, Xiaoying Chen, Wenxia Li, Haibo Liu.
Methodology: Haochang Hu, Yong Yang, Xiaoying Chen, Shi Wang, Chunming Wang, Yingchun Bao.
Project administration: Yingchun Bao.
Resources: Shi Wang, Chunming Wang.
Software: Fang Liu.
Writing – original draft: Huihui Ji, Haochang Hu.
Writing – review & editing: Shiwei Duan.
Shiwei Duan orcid: 0000-0001-7682-2877.

References

[1] Sharma P, Garg G, Kumar A, et al. Genome wide DNA methylation profiling for epigenetic alteration in coronary artery disease patients. Gene 2014;541:31–40.
[2] Roberts ET, Horne A, Martin SS, et al. Cost-effectiveness of coronary artery calcium testing for coronary heart and cardiovascular disease risk prediction to guide statin allocation: the Multi-Ethnic Study of Atherosclerosis (MESA). PLoS One 2015;10:e0116377.
[3] Wong ND. Epidemiological studies of CHD and the evolution of preventive cardiology. Nat Rev Cardiol 2014;11:276–89.
[4] Calicchio R, Doridot L, Miralles F, et al. DNA methylation, an epigenetic mode of gene expression regulation in reproductive science. Curr Pharm Des 2014;20:1726–50.
[5] Dozmorov MG. Polycomb repressive complex 2 epigenomic signature defines age-associated hypermethylation and gene expression changes. Epigenetics 2015;10:484–95.
[6] Peng R, Liu H, Peng H, et al. Promoter hypermethylation of let-7a-3 is relevant to its down-expression in diabetic nephropathy by targeting UHRF1. Gene 2015;570:57–63.
[7] Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol 2010;28:1057–68.
[8] Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993;74:609–19.
[9] Pan W, Yang J, Wei J, et al. Functional BCL-2 regulatory genetic variants contribute to susceptibility of esophageal squamous cell carcinoma. Sci Rep 2015;5:11833.
[10] Wei MC, Zong WX, Cheng EH, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 2001;292:727–30.
[11] Babu PP, Suzuki G, Ono Y, et al. Attenuation of ischemia and/or reperfusion injury during myocardial infarction using mild hypothermia in rats: an immunohistochemical study of Bcl-2, Bax, Bak and TUNEL. Pathol Int 2004;54:896–903.
[12] Liu T, Zhou Y, Wang JY, et al. Coronary microembolization induces cardiomyocyte apoptosis in swine by activating the LOX-1-dependent mitochondrial pathway and Caspase-8-dependent pathway. J Cardiovasc Pharmacol Ther 2016;21:209–18.
[13] Zhuang J, Peng W, Li H, et al. Methylation of p15INK4b and expression of ANRIL on chromosome 9p21 are associated with coronary artery disease. PLoS One 2012;7:e47193.
[14] Huang Y, Ye HD, Gao X, 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.
[15] Huang Y, Ye H, Hong Q, et al. Association of CDKN2BAS polymorphism rs4977574 with coronary heart disease: a case-control study and a meta-analysis. Int J Mol Sci 2014;15:17478–92.
[16] Hong Q, Chen X, Ye H, et al. Association between the methylation status of the MGMT promoter in bone marrow specimens and chemotherapy outcomes of patients with acute myeloid leukemia. Oncol Lett 2016;11:2851–6.
[17] Yang B, Du Z, Gao YT, et al. Methylation of Dickkopf-3 as a prognostic factor in cirrhosis-related hepatocellular carcinoma. World J Gastroenterol 2010;16:755–63.
[18] Chen X, Yang Y, Liu J, et al. NDRG4 hypermethylation is a potential biomarker for diagnosis and prognosis of gastric cancer in Chinese population. Oncotarget 2017;8:8105–19.
[19] Chen R, Hong Q, Jiang J, et al. AGTR1 promoter hypermethylation in lung squamous cell carcinoma but not in lung adenocarcinoma. Oncol Lett 2017;14:4989–94.
[20] Li B, Chen X, Jiang Y, et al. CCL2 promoter hypomethylation is associated with gout risk in Chinese Han male population. Immunol Lett 2017;190:15–9.
[21] Yang Y, Chen X, Hu H, et al. Elevated UMOD methylation level in peripheral blood is associated with gout risk. Sci Rep 2017;7:11196.
[22] Hu H, Chen X, Wang C, et al. The role of TFPI2 hypermethylation in the detection of gastric and colorectal cancer. Oncotarget 2017;8:84054–65.
[23] Lee CH, Wong TS, Chan JY, et al. Epigenetic regulation of the X-linked tumour suppressors BEX1 and LDOC1 in oral squamous cell carcinoma. J Pathol 2013;230:298–309.
[24] Teschendorff AE, Menon U, Gentry-Maharaj A, et al. Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. Genome Res 2010;20:440–6.
[25] Nunez G, Clarke MF. The Bcl-2 family of proteins: regulators of cell death and survival. Trends Cell Biol 1994;4:399–403.
[26] White E. Life death and the pursuit of apoptosis. Genes Dev 1996;10:1–5.
[27] Vekrellis K, McCarthy MJ, Watson A, et al. Bax promotes neuronal cell death and is downregulated during the development of the nervous system. Development 1997;124:1239–49.
[28] Schoier J, Hogdahl M, Soderlund G, et al. Chlamydia (Chlamydophila) pneumoniae-induced cell death in human coronary artery endothelial cells is caspase-independent and accompanied by subcellular translocations of Bax and apoptosis-inducing factor. FEMS Immunol Med Microbiol 2006;47:207–16.
[29] Ma S, Zhang H, Sun W, et al. Hyperhomocysteinemia induces cardiac injury by up-regulation of p53-dependent Noxa and Bax expression through the p53 DNA methylation in ApoE(-/-) mice. Acta Biochim Biophys Sin 2013;45:391–400.
[30] Perez GI, Jurisicova A, Wise L, et al. Absence of the proapoptotic Bax protein extends fertility and alleviates age-related health complications in female mice. Proc Natl Acad Sci U S A 2007;104:5229–34.
[31] Schiano C, Vietri MT, Grimaldi V, et al. Epigenetic-related therapeutic challenges in cardiovascular disease. Trends Pharmacol Sci 2015;36:226–35.
[32] Zhu B, Gong Y, Yan G, et al. Atorvastatin treatment modulates p16 promoter methylation to regulate p16 expression. FEBS J 2017;284:1868–81.
[33] Prasad S, Sung B, Aggarwal BB. Age-associated chronic diseases require age-old medicine: role of chronic inflammation. Prev Med 2012;54 (Suppl):S29–37.
[34] Valdes AM, Glass D, Spector TD. Omics technologies and the study of human ageing. Nat Rev Genet 2013;14:601–7.
[35] Alisch RS, Barwick BG, Chopra P, et al. Age-associated DNA methylation in pediatric populations. Genome Res 2012;22:623–32.
[36] Florath I, Butterbach K, Muller H, et al. Cross-sectional and longitudinal changes in DNA methylation with age: an epigenome-wide analysis revealing over 60 novel age-associated CpG sites. Hum Mol Genet 2014;23:1186–201.

[37] Bell JT, Tsai PC, Yang TP, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. PLoS Genet 2012;8:e1002629.
[38] Jung M, Pfeifer GP. Aging and DNA methylation. BMC Biol 2015; 13:7.
[39] Breitling LP, Yang R, Korn B, et al. Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. Am J Hum Genet 2011;88:450–7.
[40] Shenker NS, Polidoro S, van Veldhoven K, et al. Epigenome-wide association study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) identifies novel genetic loci associated with smoking. Hum Mol Genet 2013;22:843–51.
[41] Huang T, Chen X, Hong Q, et al. Meta-analyses of gene methylation and smoking behavior in non-small cell lung cancer patients. Sci Rep 2015;5:8897.
[42] Kannel WB, D'Agostino RB, Belanger AJ. Fibrinogen, cigarette smoking, and risk of cardiovascular disease: insights from the Framingham Study. Am Heart J 1987;113:1006–10.
[43] Power C, Atherton K, Thomas C. Maternal smoking in pregnancy, adult adiposity and other risk factors for cardiovascular disease. Atherosclerosis 2010;211:643–8.
[44] Matikainen T, Perez GI, Jurisicova A, et al. Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat Genet 2001;28:355–60.
[45] Mathes P, Thiery J. The role of lipid metabolism in the prevention of coronary heart disease. Z Kardiol 2005;94(Suppl 3): III/43-55.
[46] Cambien F. Coronary heart disease and polymorphisms in genes affecting lipid metabolism and inflammation. Curr Atheroscler Rep 2005;7:188–95.
[47] Yunke Z, Guoping L, Zhenyue C. Triglyceride-to-HDL cholesterol ratio. Predictive value for CHD severity and new-onset heart failure. Herz 2014;39:105–10.
[48] Hopewell JC, Seedorf U, Farrall M, et al. Impact of lipoprotein(a) levels and apolipoprotein(a) isoform size on risk of coronary heart disease. J Intern Med 2014;276:260–8.