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DNA Methylation in *NFATC1* Gene Promoter is Related to Longevity in Chinese Population

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Abstract

Background: *NFATC1* gene is associated with age-related complex traits, including longevity, when it overexpresses. DNA methylation in the *NFATC1* gene promoter can downregulate the gene expression. This study aims to examine the association between DNA methylation profile in the *NFATC1* promoter and family history of longevity in Chinese population.

Methods: We recruited 87 individuals in Bama County, Guangxi in China who were grouped into cases with family history of longevity (n=59, mean age=75.6 years) and controls without family history of longevity (n=28, mean age=57.5 years). The DNA methylation profile in the *NFATC1* gene promoter was analyzed by pyro sequencing approach.

Results: DNA methylation levels were significantly, positively correlated with age at the sites of 77157888, 77157891, and 77157898 and significantly, negatively correlated with age at the site 77157909. The methylation levels at chromosomal position 77157891 were significantly higher in the case group (β value=0.943) than in the control group (β value=0.911) with p=0.02 for the difference, adjusting for age, gender, smoking, alcohol use and cardiovascular risk factors. DNA methylation levels at site 77157909 were significantly lower in females than in males (p=0.02).

Conclusions: The findings of this study suggest that the DNA methylation profile in *NFATC1* gene promoter is associated with family history of longevity in the Chinese population.

Keywords: DNA methylation; *NFATC1* gene; Longevity; Case-control study

Introduction

Human lifespan is the outcome of complex interactions of environmental exposures, personal lifestyles and behaviors, and multiple genetic factors [1-3]. A number of longevity-related gene variants have been identified by genome-wide association studies during the past decade [4,5]. DNA methylation, a form of epigenetic modification that controls gene expression, plays an essential role in ageing process and longevity [6]. Genome-wide DNA methylation levels declined with ageing in humans although DNA methylation levels at specific CpG sites showed different trends in change over time [7,8]. DNA methylation contributes to transcription silencing when it occurs surrounding the transcription start site [9,10]. Recent studies indicated that some disease-associated genes were depressed after being methylated, resulting in lower incidence of age-related diseases [11,12]. This implies that individuals who carry hyper-methylated disease-associated genes could stay in better health status.

Our longevity study in Bama County population of Guangxi in China has found that longevity was associated with higher global DNA methylation levels as well as in *NFATC1* gene [13,14]. It was reported that *NFATC1* was highly expressed in hypertension, carcinomas, osteoporosis, and other age-associated diseases [15-18]. It has been shown in an animal study that the hair follicle stem cells of aged mice could maintain cell activity, generate hair and make the hair stay in youth when *NFATC1* was inhibited [19]. The bone resorption activity was suppressed, and the age-related osteoporosis was delayed to occur if the *NFATC1* gene was down-regulated [20]. These findings suggest that *NFATC1* gene is important in ageing process and the development of age-related disease.

This study aims to examine the association between DNA methylation profile in the *NFATC1* gene promoter region and family history of longevity in a case-control study conducted in Bama County of Guangxi, China.

Materials and Methods

Study subjects

Cases (n=59, 29 males, 30 females, mean age=75.6 years, including 28 longevity (mean age=94.3 ± 4.1) and 31 their offspring (mean age=58.7 ± 17.8)) and controls (n=28, 14 males, 14 females, mean age=57.5 years) were recruited in a case control study conducted in Bama County in Guangxi, China. The cases were defined as those who had grandparents and/or parents who lived beyond 90 years (positive family history of longevity); controls were defined as those who did not have any grandparents or parents who lived beyond 90 years (negative family history of longevity). Gender was the matching variable. Patients of infectious diseases, carcinomas or using the immune inhibition drugs at interview were excluded. General examinations included weight, height, blood pressure, blood lipids and questionnaire survey on smoking and alcohol use.

All subjects gave a written informed consent. Study protocols were approved by the Institutional Review Board of Guangxi Medical University.

Laboratory analysis

Biological specimen: 2 ml peripheral blood was collected for testing lipids, and another 2 ml peripheral blood was used for extracting DNA.

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Designing for amplification primers and sequencing primer: The *NFATC1* DNA sequence was downloaded from the NCBI web (https://www.ncbi.nlm.nih.gov/), the promoter region, transcription start site (TSS) and CpG islands were searched and located online. (http://epd.vital-it.ch/cgibin/get_doc?db=hgEpdNew&format=genome&entry=*NFATC1*_2, http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi). The amplification primers (forward: GGTTTGTGTGTATTAAGGATTAGATTATAT, reward: ATCTTTCCAAATTCAAATCTCTATACC) and sequencing primer (s: AGTTTTTATAAAATATATTTATG) were designed based on the promoter region with PyroMark Assay Design 2.0 software. A 262bp fragment (-2483bp~-2222bp) which covered one CpG island (-2436~-2335bp) with 6 CpG sites (chromosomal positions were 77157888, 77157891, 77157898, 77157901, 77157905, 77157909) was amplified and sequenced. The +1 represented the TSS, and the CpG sites in the promoter region located in the upstream of TSS.

Pyrosequencing: Fifty nanograms per microliter (no more than 40 μl in total) DNA was transformed by EpiTect Bisulfite Kit (Qiagen Company, German, NO.59104) to change all the unmethylation C to U and then to T in the following PCR amplification (Qiagen Company, German, NO.978703). The amplification primers and sequence primer were synthesized by Beijing Genomics Institution(China). The amplification fragment was sequenced by PyroMark Q96 ID (German) with PyroMark Gold Q96 Reagents (Qiagen Company, German, NO. 972804). The operating was firmly performed by the manuals.

Variables	Case	Control	p-values
N	59	28	
Age (years)	75.6(22.2)	57.5(19.2)	<0.001
Males/females	29/30	14/14	0.92
Smokers	8/51	7/21	0.19
Drinkers	15/44	8/20	0.76
Systolic BP (mmHg)	124.6(15.4)	127.8(15.3)	0.06
Diastolic BP (mmHg)	78.8(7.8)	81.8(9.2)	0.04
TG (mmol/L)	1.16(0.49)	1.69(1.42)	0.05
HDL-C (mmol/L)	1.12(0.27)	1.23(0.29)	0.06
LDL-C (mmol/L)	2.59(0.88)	3.06(0.73)	<0.001
Continuous variables are presented as means (SD). BP: Blood Pressure; TG: Triglycerides; HDL-C: High-density Lipoprotein Cholesterol; LDL-C: Low-density Lipoprotein Cholesterol			

Table 1: Characteristics of the participants of case and control groups.

Statistical analysis

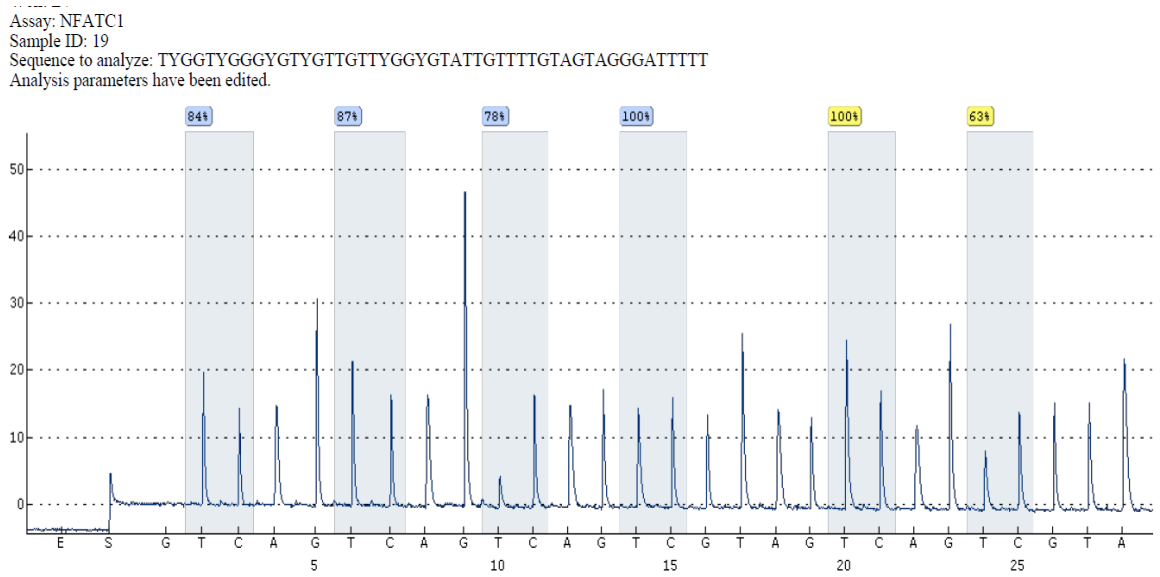
The data were analyzed by SPSS 16.0 statistical software. Generalized linear models (GLMs) were used to test the difference in continuous variables between case and control groups. Chi-square test was used to compare the distributions of gender, smokers and drinkers between case and control groups. The association between DNA methylation levels and family history of longevity was examined using GLMs, adjusting for age, gender, smoking, alcohol use, blood pressure, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). It was considered statistically significant at $p<0.05$ in a two-sided test.

Results

Table 1 shows characteristics of the study participants of case and control groups. The mean levels of continuous variables were compared between case and control groups, adjusting for age (except age itself) and gender. There were significant differences in age (case > control), DBP and LDL-C (case < control) between cases and controls. SBP, TG and HDL-C showed marginally significant differences between cases and controls.

Figure 1 shows Signal intensity at 6 CpG sites in the target sequence of the *NFATC1* gene by pyrogram analysis of one subject. The grey columns represent DNA methylation levels at 6 CpG sites of 77157888, 77157891, 77157898, 77157901, 77157905, and 77157909. Two CpG sites were fully methylated (100%) at chromosomal positions 77157901 and 77157905 in all participants. The DNA methylation levels at the remaining 4 CpG sites were used for the analyses of the case-control study.

Table 2 presents DNA methylation levels at four CpG sites in case and control groups. The association between DNA methylation levels



The y-axis represents the signal intensity in arbitrary units. The x-axis shows the nucleotide dispensation order. Grey shaded areas shows the location of CpG sites of 77157888, 77157891, 77157898, 77157901, 77157905 and 77157909. Percentage of DNA methylation is indicated above each CpG site.

Figure 1: Signal intensity at 6 CpG sites in the target sequence of the *NFATC1* gene by pyrogram analysis of one subject.

CpG sites*	case	control	p-values
77157888	0.918 (0.046)	0.900(0.048)	0.59
77157891	0.943(0.043)	0.911(0.035)	0.02
77157898	0.836(0.031)	0.822(0.026)	0.17
77157909†	0.561(0.043)	0.579(0.034)	0.30

*, The CpG site information is based on NCBI human reference genome GRCh 37. p13 primary assembly.
DNA methylation levels are presented as means (SD).
†, Significantly different between female and male offspring (p=0.02).

Table 2: DNA methylation levels at four CpG sites in case and control groups.

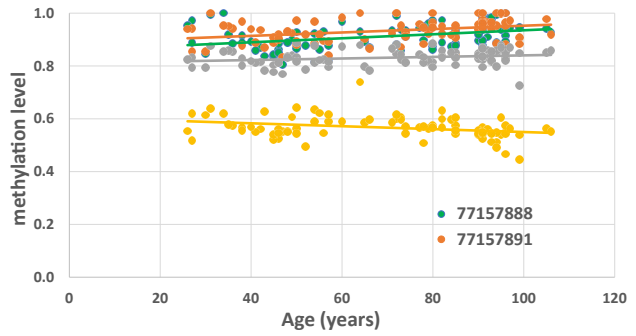


Figure 2: The correlation between DNA methylation levels at four CpG sites with age.

and family history of longevity was examined using GLMs, adjusting for age, gender, smoking, alcohol use, blood pressure, LDL-C, HDL-C, and TG. The DNA methylation level in the position 77157891 was significantly higher in cases than in controls. DNA methylation levels in the positions 77157888, 77157898 and 77157909 did not differ significantly between case and control groups. DNA methylation levels at site 77157909 were significantly lower in females than in males (p=0.02).

Figure 2 presents the correlation between the methylation levels at 4 CpG sites and age. The DNA methylation levels were positively, significantly correlated with age in the positions of 77157888, 77157891, 77157898 and negatively, significantly correlated with age in the position 77157909.

Discussion

Cardiovascular disease and age-related chronic diseases are considered as the leading causes of death and disabilities worldwide [21]. Elevated blood pressure and lipids are important cardiovascular risk factors [22-24]. In this study, the family members who have a family history of longevity tend to have lower levels of blood pressure and lipids, suggesting a genetic background of cardiovascular disease which protects the offspring from suffering cardiovascular diseases and thus leads to a longer lifespan. A study in Chinese population reported the association of blood lipids and genetic variants with longevity in Guangxi, China [25]. Another large-scale study in Chinese population also found that family history of longevity was associated with lower prevalence rates of chronic diseases, including cardiovascular disease [26].

The *NFATC1* gene codes one of the important nuclear factors of activated T-cell proteins. This gene has two promoters (P1 and P2) which autoregulate gene transcription. P1 is always hyper-methylated [27]. The sequence we analysed in this study is located in the P1 promoter region. The positions of 77157888 and 77157898 are also the SNP sites of rs115468573(A>C) and rs557438298(C>G), respectively (https://www.ncbi.nlm.nih.gov/snp). Although the function of the two SNP sites is not yet clear, studies indicate that the CpG-SNP conjoint may increase DNA methylation [28,29], suggesting that the SNP on the C of CpG dinucleotide would elevate DNA methylation levels in the *NFATC1* gene promoter and downregulate the gene expression.

Some age-related diseases such as type-2 diabetes, cardiovascular disease, stroke and Alzheimer's disease would be delayed to occur and facilitate people to reach longevity if the corresponding key genes were methylated [30]. *NFATC1* is such a disease-related gene as the literature describes. Hyper-methylation may block transcription factors from binding to the DNA promoter regions, result in expression downregulation and delay the onset of the *NFATC1*-induced diseases [27]. We have shown in the current study that DNA methylation level at site 77157891 was higher in individuals who had family history of longevity than those who did not have family history of longevity. Parents and their offspring, the first-degree relatives, share 50% of gene alleles identical by descent.

Conclusion

The findings from this study suggest that hyper-methylation in the *NFATC1* gene might contribute to longevity in Chinese population. Further investigation is needed regarding the influence of gene variants in the *NFATC1* gene promoter region on DNA methylation levels at site 77157891. Of note, DNA methylation levels significantly decreased at site 77157909 and significantly increased at other 3 sites with increasing age in the current study. These age-related trends were consistent with differences in the DNA methylation levels between groups with and without family history of longevity in this case-control study. In addition, we found that DNA methylation levels at site 77157909 were significantly lower in females than in males among the offspring. Zhang et al. reported that females had a longer lifespan than males in a Chinese population from the same province in China [31]. Taken together, the age and gender-related trends and the difference between cases and controls in DNA methylation levels at the site 77157909 noted in the current study suggest that epigenetic modification in this position of the *NFATC1* gene might be associated with a longer lifespan.

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