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Dietary vegetable intake is inversely associated with ATP-binding cassette protein A1 (*ABCA1*) DNA methylation levels among Japanese women

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ABSTRACT

Objective: Dietary intake of vegetables is one of the key lifestyle factors associated with preventing cancer and cardiovascular disease (CVD). Although previous studies have provided evidence that dietary factors can alter global DNA methylation levels in humans, little work has been done on dietary factors influencing methylation levels of specific genes associated with CVD. The aim of this study was to examine whether dietary intake of vegetables was associated with adenosine triphosphate–binding membrane cassette transporter A1 (*ABCA1*) DNA methylation levels in leukocytes in a Japanese population.
Methods: This cross-sectional study included 279 Japanese adults (125 men, 154 women) without any clinical history of cancer, stroke, or ischemic heart disease. *ABCA1* DNA methylation levels in leukocytes were measured using a pyrosequencing method. Information on dietary vegetable intake was obtained from the validated food frequency questionnaire.
Results: Mean *ABCA1* DNA methylation levels in men and women were 35.6% ± 6.5% and 36.9% ± 6.7%, respectively. In women, multivariable linear regression analysis showed that the group with the highest dietary vegetable intake (carrot, broccoli, pumpkin, and all vegetables) showed significantly lower levels of *ABCA1* DNA methylation than the lowest intake group ($P = 0.04$, <0.001, 0.001, and 0.02, respectively). No significant association was observed between dietary intake of vegetables and DNA methylation levels in men.
Conclusions: High dietary intake of vegetables was associated with decreased *ABCA1* DNA methylation levels in Japanese women. This may contribute to a better understanding of the protective effects of dietary vegetable intake on CVD.

Introduction

Dietary intake of vegetables is one of the key lifestyle factors associated with preventing cancer and cardiovascular disease (CVD) [1,2]. Emerging evidence has suggested that dietary factors alter global DNA methylation levels in humans, suggesting that protective roles of nutrients may be mediated by epigenetic alterations in the human genome [3–5]. However, to our knowledge, little work has focused on dietary vegetable intake influencing DNA methylation levels of specific genes associated with CVD [6].

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Adenosine triphosphate (ATP)–binding membrane cassette transporter A1 (ABCA1) is a membrane transporter that plays an important role in the transfer of lipids from cells to lipid-poor apolipoprotein A-I (apo A-I), which modulates the concentration of plasma high-density lipoprotein cholesterol (HDL-C) in humans [7]. Previous seminal studies have identified that mutations in the gene encoding *ABCA1* are responsible for Tangier disease (OMIM 205400) [8] and familial hypoalphalipoproteinemia (OMIM 604091) [9]. These two genetic disorders are characterized by significantly decreased plasma HDL-C concentrations and increased risks for coronary artery disease (CAD) [10,11].

More recently, epigenetic changes in the *ABCA1* gene have been investigated [12,13]. The field of epigenetics researches heritable modifications that do not involve changes to the primary DNA sequence [14]. DNA methylation is one of the better understood epigenetic modifications and is considered the most stable epigenetic marker [15]. A number of lines of evidence have been accumulated regarding the associations of DNA methylation levels with genes, various diseases, clinical profiles, and levels of expression in humans [16–18]. As with other genes, previous studies revealed that *ABCA1* DNA methylation levels might be associated with plasma HDL-C concentrations and the risk for CAD [19,20]. However, few studies have investigated lifestyle factors influencing *ABCA1* DNA methylation levels. The aim of this study was to examine whether dietary intake of vegetables was associated with *ABCA1* DNA methylation levels in a Japanese population.

Materials and methods

Study participants

Participants enrolled in this cross-sectional study were composed of 525 Japanese individuals. All participants took part in a community-based health examination in Yakumo Town, Hokkaido, in the northern part of Japan, at the end of August 2015. Of the participants, 48 individuals who could not complete the questionnaire in this research were excluded. We also excluded 36 participants undergoing treatment for cancers, myocardial infarction, and cerebrovascular disease. After excluding 162 individuals who did not have the *ABCA1* DNA methylation levels measured, final analysis was performed in the data set for 279 individuals (125 men, 154 women). All participants provided written informed consent. The protocol of this study was approved by the Ethics Review Committee at Fujita Health University.

Data collection

Self-administered questionnaires were predistributed to applicants beforehand. At the health examination site, well-trained public health nurses collected and confirmed missing data in interviews. Information for dietary intake frequency was assessed using a validated short food frequency questionnaire (FFQ) with 47 food items [21], of which five vegetable items (carrot, broccoli, pumpkin, other green leafy vegetables, and other green and yellow vegetables) were analyzed in the present study. Based on the frequency of dietary vegetable intake, study participants were divided into four groups (≤1–3/mo; 1–2/wk; 3–4/wk; or >5–6/wk). To calculate a summed intake frequency of these five vegetables, the following values were assigned in each vegetable: 1 for ≤1 to 3/mo; 2 for 1 to 2/wk; 3 for 3 to 4/wk; and 4 for >5 to 6/wk, and then summed per participant. Based on the summed intake frequency, we divided participants into quartiles. The following information regarding individuals' health condition and clinical history were collected:

- Smoking status (current, ever, or never);
- Alcohol consumption (current, ever, or never);
- Exercise habit (almost none, 1–2 h/wk, 3–4 h/wk, or ≥5 h/wk);
- Medical history of cancer, stroke, and ischemic heart disease (yes or no).

During the health examination, fasting blood samples were collected from each participant and then centrifuged within 1 h of sampling. Biochemical analyses of collected blood specimens were performed in the laboratory of Yakumo General Hospital.

Measurement of ABCA1 *DNA methylation levels*

DNA was extracted from buffy coat samples treated with a lysis solution using the NucleoSpin Tissue kit (Takara, Japan). The gold standard pyrosequencing technology was used to determine base-specific cytosine methylation levels. According to the protocol from the manufacturer, extracted DNA was treated with sodium bisulfite using the EpiTech Fast DNA bisulfite kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was performed using the Takara EpiTaq HS (for bisulfite-treated DNA). The sequencing primers used in PCR are described in Supplementary Figure 1. After PCR amplification, we measured eight CpG dinucleotides upstream from the first exon of the *ABCA1* gene using the PyroMark Q24 Advanced (Qiagen) with sequence primers as measured in previous studies [18,19]. Levels of *ABCA1* DNA methylation in each CpG are expressed as the percentage of methylated cytosine. The mean value of *ABCA1* DNA methylation levels at eight CpGs was calculated for each participant and used in the statistical analyses.

Statistical analysis

Continuous variables with a normal distribution are expressed as mean ± SD, whereas triacylglycerols (TGs) are presented as the median (interquartile range) because of the log-normal distribution. Analysis of variance (ANOVA) was used to compare means of *ABCA1* DNA methylation levels across four groups of dietary vegetable intake. Tukey's honestly significant difference test was used as a post hoc test of the ANOVA. We used multivariable linear regression analysis to examine associations between dietary vegetable intake and *ABCA1* DNA methylation levels after adjusting for potential confounding factors such as age, smoking status, alcohol consumption status, habitual exercise, and neutrophil percentage. $P < 0.05$ was considered statistically significant, and all tests were two-tailed. All statistical analyses were performed using R version 3.5.1 statistical software (R foundation, Vienna, Austria).

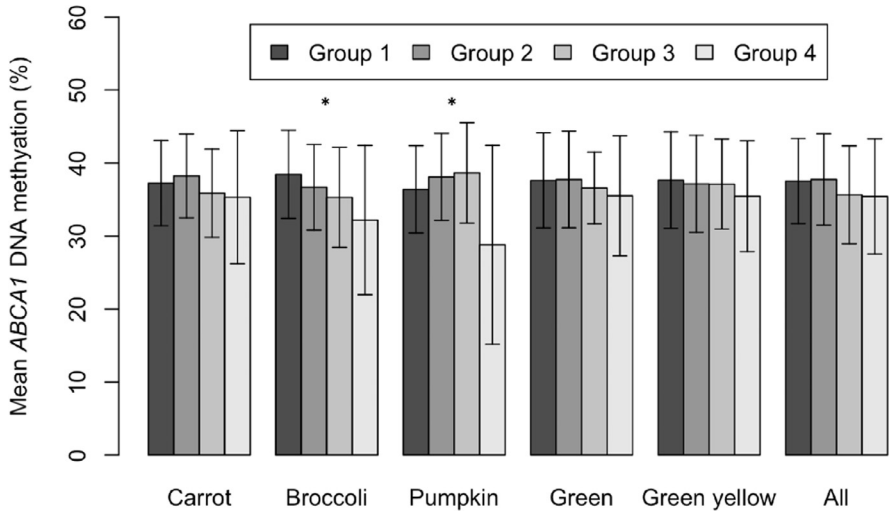

Fig. 1. Differences in *ABCA1* DNA methylation among four groups of dietary vegetable intake. *P*-values represent the results of a comparison between the highest intake group and the lowest intake group (*$P < 0.05$). Error bars represent standard error of the mean.

Results

Basic characteristics of study subjects are shown in Table 1. Mean *ABCA1* DNA methylation levels in men and women were 35.6% ± 6.5% and 36.9% ± 6.7%, respectively. For lifestyle factors, dietary fat intake, habitual smoking, and alcohol intake differed between the sexes (all $P < 0.001$), but these factors were not correlated with *ABCA1* DNA methylation levels both in men ($P = 0.83$, 0.85, and 0.57) and women ($P = 0.10$, 0.51, and 0.63).

Association between dietary vegetable intake and ABCA1 *DNA methylation levels*

The highest intake group in two vegetables (broccoli and pumpkin) showed lower mean *ABCA1* DNA methylation levels compared with the lowest intake group in women (Fig. 1). After adjusting for potential confounders, significant associations between mean *ABCA1* DNA methylation levels and dietary intake frequencies of some vegetables (carrot, broccoli, pumpkin, and all vegetables) were observed in women (highest intake group versus lowest intake group, $P = 0.04$, <0.001, 0.001, and 0.02, respectively; Table 2). Nominal significant associations also were observed between mean *ABCA1* DNA methylation and dietary intake frequencies of other green leafy vegetables and other green and yellow vegetables (highest intake group versus lowest intake group, $P = 0.05$ and 0.07). However, no significant associations with mean *ABCA1* DNA methylation level were seen for each dietary vegetable intake in men. Although we used mean *ABCA1* DNA methylation levels in our primary analysis, associations between DNA methylation levels at eight CpGs and dietary vegetable intake are shown in Supplementary Table 1.

Discussion

The present study evaluated the association between dietary vegetable intake and leukocyte *ABCA1* promoter DNA methylation levels in a general population. We identified significant inverse associations between dietary intake of several vegetables and leukocyte *ABCA1* promoter DNA methylation levels in Japanese women.

The effects of dietary factors on global DNA methylation levels have been a focus of recent research. In particular, the association between folic acid, a key vitamin in DNA methylation, and global DNA methylation has been well investigated [22–31]. Although several observational studies have identified that lower folic acid status is associated with decreased global DNA methylation levels in women [22–24], those findings have been controversial. Two interventional studies found that controlled folic acid depletion resulted in decreased global DNA methylation levels in older women [30,31]. With the exception of folic acid, Zhang et al. revealed a dietary pattern characterized by high intake of vegetables and fruit was associated with global DNA methylation [32]. Although these previous studies have highlighted beneficial effects of dietary factors on global DNA methylation, this appears to be the first study to elucidate that higher vegetable intake was associated with hypomethylation of a specific gene associated with lipid metabolism and CVD in a general population. Further work is expected to confirm and replicate this result in other populations.

Notably, in this study, women with higher intake of several vegetables (carrot, broccoli, pumpkin, and all vegetables) showed decreased levels of *ABCA1* promoter DNA methylation. Carrot and pumpkin are known to be major sources of β-carotene, a powerful antioxidant [33]. These vegetables are categorized in top β-carotene–rich foods (raw carrot: 18.3 mg/100 g wet weight; canned pumpkin: 6.9 mg/100 g wet weight) [34,35]. Previous epidemiologic studies on β-carotene and carotenoids identified their protective roles in several chronic diseases and pathophysiologic conditions [36–40]. Regarding the effect of β-carotene intake on DNA methylation, an evaluation by Bollati et al. found that dietary intake of β-carotene was significantly associated with DNA methylation of an inflammatory gene among obese individuals [6]. Although little has been reported regarding the association of carotene intake and DNA methylation in a general population, dietary carotene intake was inversely associated with *ABCA1* DNA methylation in our data set. Therefore, we hypothesized that dietary intake of β-carotene–rich vegetables (carrots and pumpkin) may reduce *ABCA1* DNA methylation levels, resulting in increased levels of HDL-C and prevention of CVD. Broccoli has a range of health benefits, including being one of the best-known sources of vitamins C and E. In particular, broccoli contains 89.2 mg/100 g wet weight and is highly ranked in vitamin C-rich vegetables [35]. Vitamin C, as L-ascorbic acid, exists as the ascorbate anion under conditions of physiologic pH and plays a pivotal role in the ten-eleven translocation–mediated DNA demethylation process [41,42]. This function of ascorbate status in DNA demethylation has been confirmed in various cell types and animal models [43–45]. One conceivable hypothesis underlying our results is that higher broccoli intake may induce lower levels of *ABCA1* DNA methylation by regulating the ten-eleven translocation–mediated DNA demethylation process. Vitamin E is the collective name for a group of eight different compounds with antioxidant properties. The two main vitamers of vitamin E in the human diet are α- and γ-tocopherol, and vitamin E modulates cell signaling, proliferation, and gene expression [46]. Although a recent interventional study revealed that α-tocopherol intake affects microRNA (miR)-9-1 and miR-9-3 promoter gene DNA methylation [47], little information is available on the effects of vitamin E intake on DNA methylation level. Further studies are needed to examine whether dietary intake of micronutrients, especially vitamins, induces hypomethylation in the *ABCA1* gene promoter region.

ABCA1 is an important membrane regulating blood HDL-C concentrations. Mutations in this gene can cause Tangier disease, a

Table 1
Basic characteristics of study participants

	Men (n = 125)	Women (n = 154)
Age (y)	64 ± 9.2	62.4 ± 10.1
Body mass index (kg/m^2)	24.5 ± 2.8	23.1 ± 3.4
Blood glucose (mmol/L)	5.17 ± 0.78	4.86 ± 0.90
Triacylglycerols* (mmol/L)	1.20 (0.81–1.73)	0.90 (0.70–1.28)
Systolic blood pressure (mm Hg)	136.6 ± 21.3	128 ± 20.1
Diastolic blood pressure (mm Hg)	81.5 ± 14.8	71.8 ± 12.4
Mean *ABCA1* DNA methylation level (%)	35.6 ± 6.5	36.9 ± 6.7
Dietary fat intake* (mg/d)	40.9 (35.7–46.7)	44.5 (39.8–51.6)
Smoking status (%)		
Never	25 (20)	109 (70.8)
Ever	67 (53.6)	30 (19.5)
Current	33 (26.4)	15 (9.7)
Alcohol consumption (%)		
Never	33 (26.4)	104 (67.6)
Ever	4 (3.2)	2 (1.3)
Current	88 (70.4)	48 (31.2)
Exercise habits (%)		
Almost none	69 (55.2)	103 (66.9)
1–2 h/wk	29 (23.2)	28 (18.2)
3–4 h/wk	12 (9.6)	13 (8.4)
>5 h/wk	15 (12)	10 (6.5)
Medication users (%)		
Hypertension	36 (28.8)	39 (25.3)
Diabetes	10 (8)	8 (5.2)
Dyslipidemia	25 (20)	41 (26.6)

*Values are expressed as median (25th–75th percentiles).

Table 2
Multiple linear regression analysis for the associations between mean *ABCA1* DNA methylation levels and vegetable intake

		Men		Women	
Vegetables	Group	Standardized β	*P*-value	Standardized β	*P*-value
Carrot	2	−0.191	0.06	−0.053	0.63
	3	−0.054	0.58	−0.169	0.12
	4	−0.098	0.32	**−0.218**	**0.04**
Broccoli	2	−0.084	0.39	−0.147	0.09
	3	**−0.220**	**0.02**	**−0.178**	**0.04**
	4	−0.125	0.20	**−0.295**	**<0.001**
Pumpkin	2	−0.070	0.46	0.055	0.52
	3	0.132	0.15	0.075	0.36
	4	−0.012	0.89	**−0.276**	**0.001**
Other green vegetables	2	0.126	0.25	−0.035	0.80
	3	−0.081	0.46	−0.144	0.28
	4	0.121	0.27	−0.259	0.05
Other green and yellow vegetables	2	−0.033	0.76	−0.091	0.46
	3	−0.053	0.63	−0.122	0.34
	4	0.009	0.93	−0.220	0.07
All vegetables	2	−0.192	0.08	−0.082	0.41
	3	−0.038	0.72	−0.172	0.07
	4	−0.212	0.06	**−0.241**	**0.02**

Bold values indicate a statistically significant difference with a $P < 0.05$.
Adjusted for age, smoking status, alcohol consumption status, habitual exercise, and neutrophil percentage.

disease characterized by reduced HDL-C levels [8]. Regarding *ABCA1* DNA methylation, Tobi et al. first showed that *ABCA1* promoter DNA methylation levels are associated with aging and prenatal exposure to famine decades ago [12]. Houde et al. found that *ABCA1* DNA methylation levels were inversely associated with *ABCA1* gene expression levels [48]. The same group subsequently observed that alterations in DNA methylation at the *ABCA1* promoter gene locus were associated with circulating lipid profiles and increased risk in CAD in both a general population and patients with familial hypercholesterolemia patients [19,20]. As reported in the previous studies, we also observed a significant association between leukocyte *ABCA1* DNA methylation and HDL-C in women (standardized $\beta = -0.251$; $P = 0.01$), but not in men (standardized $\beta = -0.121$; $P = 0.24$). Taking these previous studies into consideration, we hypothesized that greater dietary intake of vegetables might induce decreased levels of *ABCA1* DNA methylation, resulting in increased blood HDL levels and decreased risk for CVD.

In the present study, despite finding no significant difference in *ABCA1* DNA methylation levels between sexes, we observed a discrepancy in the results between men and women. Previous nutritional studies have reported that food intake as estimated by the FFQ differed between men and women in some vegetables [49,50]. Although male and female participants in this study had conceivably completed the FFQ differently, sex differences in associations between *ABCA1* DNA methylation and dietary vegetable intake remain to be investigated in further detail.

One limitation of this study related to the effect of prescribed medications on DNA methylation. Statins are the most common drug in the world and are used to decrease blood cholesterol levels. A previous study showed that statins may affect the epigenome through inhibition of DNA methyltransferase activity [51]. Although medication use for dyslipidemia was more frequent in women than in men, this sex difference was not significant ($P = 0.41$). Therefore, it was unlikely that medication for dyslipidemia was a confounding factor of the association between *ABCA1* DNA methylation levels and dietary vegetable intake. We should mention additional nutrients from dietary supplements. Information about the usage of dietary supplements was obtained from the self-administered questionnaire, although we did not obtain detailed information (e.g., dosage, frequency, and name of product). After including use of supplements as a confounding factor, significant associations between *ABCA1* DNA methylation levels and dietary vegetable intake remained (Supplementary Table 2).

Conclusion

Results from the present study showed that dietary intake of several vegetables was significantly associated with mean *ABCA1* DNA methylation levels in leukocytes among Japanese women. The present results may provide insights into a possible epigenetic link between preventive effects of dietary vegetable intake on CVD.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.nut.2019.02.010.

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