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The association between *MTHFR* polymorphism, dietary methyl donors, and childhood asthma and atopy

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Abstract

Background: Studies investigating the genetic association of the C677T methylenetetrahydrofolate reductase (*MTHFR*) genotype and dietary methyl donors with asthma and atopy are limited, and have variable results.

Objective: To investigate the effect of dietary methyl donor intake on the risk of childhood asthma and atopy, based on the C677T polymorphism in the *MTHFR* gene.

Methods: This cross-sectional study included 2,333 elementary school children aged 6–8 years across Korea during 2005 and 2006, as part of the first Children's Health and Environmental Research survey. Genotyping for the *MTHFR* (rs1801133) polymorphism was performed using the TaqMan assay. Multivariable-adjusted logistic regression analysis was performed to determine a descriptive association between the dietary methyl donor intake, *MTHFR* polymorphism, and childhood asthma and atopy.

Results: Intake of dietary methyl donors like folates was significantly associated with a decreased risk of the wheezing symptom, in the past 12 months, and "ever asthma" diagnosis, respectively. Vitamin B_6 intake was also associated with a decreased atopy risk. The T allele of the *MTHFR* (rs1801133) gene was significantly associated with a decreased risk of atopy. Increased intakes of folate, vitamin B_2, and vitamin B_6 were protective factors against atopy, especially in children with the T allele on the *MTHFR* gene, compared to those with lower intakes and the CC genotype.

Conclusion: High intakes of dietary methyl donors were associated with reduced risk of atopy and asthma symptoms. These may have additive effects related to the susceptibility alleles of the *MTHFR* gene. The clinical implications require evaluation.

Key words: Asthma, Atopy, Folate, Methyl donors, *MTHFR*, Polymorphism

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Introduction

Worldwide trends in asthma prevalence has shown an increase followed by a stable, high prevalence,[1–3] which might be partially explained by the hygiene hypothesis, air pollution, and nutrients.[4,5] Dietary changes can affect pathophysiology of asthma through epigenetic mechanisms (which may lead to heritable or postnatal changes in gene expressions without alterations in the DNA sequence), such as DNA methylation.[6] Folate is required for many biological processes, including biosynthesis of purines and pyrimidines, and production of amino acids. Folate is also needed for the methylation of deoxyuridylate to thymidylate during DNA synthesis, which is necessary for normal cell division. Dietary methyl donors, including folate in the form of 5-methyl-tetrahydrofolate, are required for the one-carbon metabolic pathway to produce S-adenosylmethionine, the universal methyl donor essential for the DNA methylation process, which is a key player in epigenetic control of gene expression. Thus, differential intake of these nutrients can lead to differences in DNA methylation, ultimately altering gene expression.[7,8]

In humans, dietary nutrients such as folate, vitamin B_2, and vitamin B_6 are sources of methyl donors for DNA methylation. It is yet to be verified whether these B group vitamins are risk factors or preventive factors for asthma and atopy.[7] A cross-sectional study of Australian adults showed that the dietary intake of folate was positively associated with having physician-diagnosed asthma, but not with current asthma, airway responsiveness, or atopy.[9] In another cross-sectional study, the serum folate level was inversely associated with wheeze or atopy but not with physician-diagnosed asthma in children and adults in the United States.[10] Similarly, a 10 ng/mL decrease in serum folate was found to be associated with 45% higher odds of asthma in Peruvian children.[11] In contrast, a recent cross-sectional study reported that high serum folate metabolites were associated with current asthma in adults, and positively associated with lung function in children and adults.[12] Therefore, the research findings on effects of folate on asthma remain controversial.

Studies investigating the association of genetic variants of genes in the methyl donor metabolic pathway with asthma and atopy have been limited to date.[13-15] The vast majority of studies investigating the roles of genetic associations have been performed on genetic variants in the methylenetetrahydrofolate reductase (*MTHFR*) gene because of the association of its common variant, C677T, with the reduced enzymatic activity.[13] *MTHFR* C677T polymorphism (C→T substitution at nucleotide 677) causes the defective enzyme to alter the methionine metabolism, and greatly increases the levels of homocysteine (Hcy) in blood and urine, which can cause various complications. Individuals who carry two copies of this variant, homozygous (TT), tend to have higher Hcy levels and lower serum folate levels compared to controls.[13] The results of studies investigating the genetic association of the C677T *MTHFR* genotype with asthma and atopy have been variable. A large birth cohort study of British children and their mothers found no significant association between the C677T mutation and atopy or asthma.[14] In contrast, among 6,784 Danish adults, low serum folate levels and the TT genotype of the *MTHFR* C677T polymorphism were significantly associated with an increased risk of doctor-diagnosed asthma and attacks of shortness of breath.[15] However, no study has verified asthma, and atopy by implementing objective indicators such as bronchial hyper-responsiveness (BHR) measurements, and skin prick tests (SPTs) for children in the general population.

In this cross-sectional study, we investigated the relationship between dietary methyl donor intake and the risks of atopy, bronchial hyperresponsiveness, and asthma related to *MTHFR* polymorphism in school-aged children.

Methods

Study population

The study population consisted of primary school-going children aged 6–8 years across Korea, between 2005 and 2006, as part of the first Children's Health and Environmental Research study of the nationwide general population.[16] Of the 2,899 recruited children, 195 with missing data on all the three, age, sex, and diagnosis questionnaires, and 371 with missing data on the food frequency questionnaire (FFQ) were excluded, along with those whose total energy intake was < 500 or > 5,000 kcal per day. Thus, the final study population included 2,333 children (mean ± SD age 7.56 ± 1.00 years), and a total of 930 individuals underwent genotyping for *MTHFR* polymorphism (**Figure 1**).

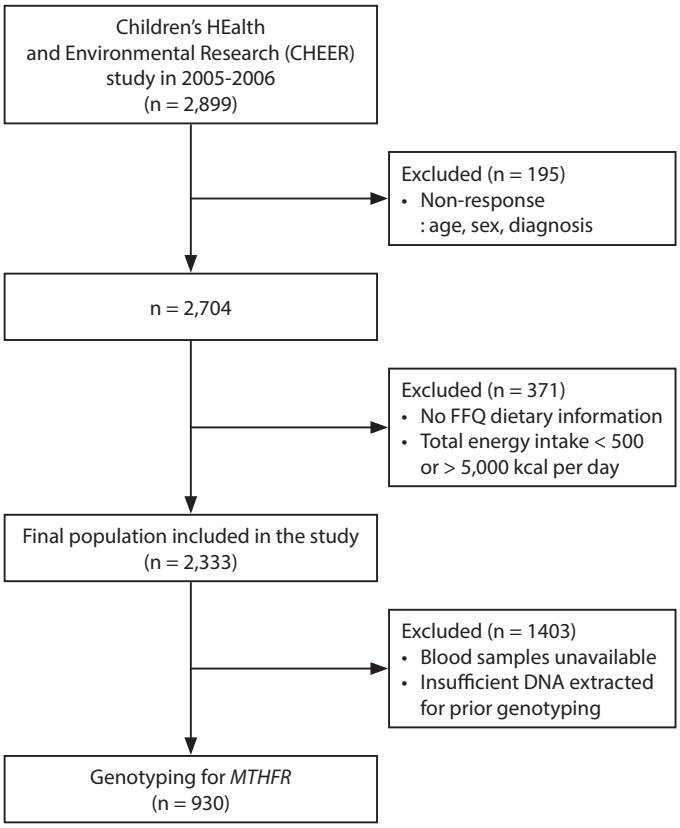

Figure 1. Flow chart of study participants.

The study protocols were reviewed and approved by the Institutional Review Board of the University of Ulsan College of Medicine (IRB no. 2006-0081), and written consent was obtained from the parents or guardians of the participants.

Phenotype definitions

Four dichotomous phenotypes were defined a priori for the analysis: (1) asthma, defined as doctor-diagnosed asthma and a positive response to the question "Has your child ever been diagnosed with asthma by a physician?" in the survey. Because the asthma diagnosis used in this study was ever-diagnosed asthma, it included all those diagnosed with asthma in the past, and current asthma referred to asthma symptoms, in the past 12 months; (2) atopy, defined as any SPT wheal that was at least 3 mm greater than the negative control for any allergen test; (3) atopic asthma, defined as diagnoses of both asthma and atopy; and (4) BHR, assessed using the methacholine-challenge test, where a positive response was defined as provocative concentration (PC_{20}) causing a 20% decrease in forced expiratory volume in 1 s (FEV_1), and BHR was defined as a $PC_{20} \leq 8$ mg/mL.

Questionnaire-based survey

The International Studies of Asthma Allergic diseases in Childhood (ISAAC) questionnaire has been validated in Korean children and was used to evaluate the presence of allergic diseases (including asthma) and risk factors for allergic diseases in each survey.[16] A participant was deemed to have had asthma at enrollment if there was an affirmative response to the question in the ISAAC questionnaire, "Has your child ever been diagnosed with asthma by a physician?". The presence of asthma symptoms in the last 12 months was assessed through the question, "Has your child ever had symptoms of asthma such as wheezing or whistling in the chest during the last 12 months?"

Dietary assessment

We assessed dietary intake through a validated semi-quantitative food frequency questionnaire (FFQ) commonly used in other studies.[16] The questionnaire has previously been validated and includes 113 food items with 9 non-overlapping intake frequencies over the preceding year (ranging from "rarely eaten" to "eaten more than three times per day") and 3 portion sizes (small, average, or large). Using the Computer-Aided Nutritional Analysis Program III (CAN PRO III) developed by the Korean Nutrition Society, the amount of each food item included in the FFQ was converted into grams, from which the daily nutrient intake was calculated. The dietary intakes of micronutrients (B group vitamins and folic acid) were analyzed.

Skin prick tests (SPTs)

SPTs were performed for the 14 most common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, dog dander, cat epithelium, cockroaches, *Alternaria alternate*, *Aspergillus fumigatus*, grass pollen mixture, tree mixture I, tree mixture II, ragweed, mugwort, alder, and oak), and 4 food allergens (peanuts, egg whites, cow's milk, and soybeans). Indoor allergens included *D. pteronyssinus*, *D. farina*, cockroaches, dog dander, and cat epithelium. Outdoor allergens included grass mixture, tree mixture I, tree mixture II, ragweed, mugwort, alder, oak, *A. alternate*, and *A. fumigatus*. Histamine (10 mg/mL) was used as the positive control, and normal saline was used as the negative control. A mean wheal size of ≥ 3 mm when measured after 15 min and wheals caused by histamine were considered positive. Atopy was defined as at least one positive test result to any of the 18 allergens on the SPT.

Bronchial hyper-responsiveness (BHR)

Spirometry was performed according to the American Thoracic Society/European Respiratory Society guidelines using a portable microspirometer (Microspiro HI-298, Chest Corporation, Tokyo, Japan). The FEV_1, forced vital capacity (FVC), and forced expiratory flow at 25%–75% of the FVC ($FEF_{25-75\%}$) were measured. The methacholine bronchial challenge test was performed to assess BHR; for this, the tidal inhalation of doubling doses of methacholine ranging from 0.625 to 25 mg/mL (0.625, 1.25, 2.5, 5, 10, and 25 mg/mL) were used. The cumulative methacholine dose that caused PC_{20} was calculated, according to American Thoracic Society recommendations. Details of the BHR measurements are provided elsewhere.[16] Participants were considered to have BHR to methacholine when their methacholine PC_{20} was ≤ 8 mg/mL.

MTHFR polymorphism

Genomic DNA was extracted from peripheral venous blood samples using the Gentra Puregene® Blood kit (Qiagen, Germantown, MD, USA). The genotyping of *MTHFR* polymorphism (rs1801133) was screened using the TaqMan fluorogenic 5′ nuclease assay (ABI, Foster City, CA, USA). The final volume of the PCR was 5 μL, which contained 10 ng of genomic DNA and 2.5 μL of the TaqMan Universal polymerase chain reaction master mix, along with 0.13 μL of the assay mix (Assay ID C_1202883_20). The endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI). Duplicate samples and negative controls were included to ensure accuracy of the genotyping.

Statistical analysis

The nutrients were adjusted considering the total energy intake using the residual method.[17] The adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were obtained using logistic regression analysis according to the tertile of the dietary factors under investigation, with two low tertiles as references. We compared the higher intake group with two lower tertiles, but not the lowest group, as a reference to determine a descriptive association between the dietary methyl donor uptake, *MTHFR* polymorphism, childhood asthma, and atopy. The most common *MTHFR* genotype (CC) was used as a reference genotype (wild type). Because of their low prevalence, the variant genotypes CT and TT were combined and considered as a "variant" genotype. We also calculated the correlation coefficients between the unadjusted

intakes of methyl donors and other nutrients, including vitamin C, vitamin E, and n-3/n-6 fatty acids (**Table S1**). To determine Pearson correlation coefficients, the logarithms of the dietary variables were taken rather than their original values. Statistical analysis was performed using SAS for Windows (version 9.2; SAS Institute, Cary, NC, USA). A multiple logistic regression analysis was performed by adjusting for key covariates such as age, sex, body mass index, parental history of allergic disease, maternal education, log-transformed total energy intake, and monthly household income. A *P*-value of < 0.05 was considered statistically significant.

Results

Participants' characteristics

The characteristics of the study population are listed in **Table 1**. There was a parental history of allergic diseases in 31.85% of the participants. The prevalence of children with an ever asthma diagnosis was 10.98%, with a value of 12.16% for children that had experienced the wheezing symptom in the past 12 months (**Table 1**). The prevalence values for BHR and atopy in children were 14.90% and 27.87%, respectively.

The daily intake of methyl donors (folate, Vitamin B_2 and B_6) are listed in **Table 1**. The average intake of total calories was within the dietary reference intake (DRI) range for Koreans, the average folate intake was below the DRI value, while the dietary intakes of vitamin B_2 and vitamin B_6 were slightly above the DRI values.

Table 1. General characteristics of the participants.

	N[a] = 2333	%	Non-atopy	%	Atopy	%	Meeting % KDRI
Age (y),	7.56 ± 1.00		7.73 ± 1.25		7.78 ± 1.30		
Sex (male)	1199/2333	51.4	430/906	47.8	201/350	57.4	
BMI (kg/m²)	16.79 ± 2.48		16.74 ± 2.54		16.96 ± 2.60		
Parental history of allergic diseases	730/2292	31.9	264/885	29.8	122/346	35.3	
Maternal education							
Low (≤ high school)	1315/2237	58.8	512/854	60.0	192/331	58.0	
High	922/2237	41.2	342/854	40.0	139/331	42.0	
Household income (10,000 won)							
≤ 199	687/2297	29.9	296/893	33.2	99/341	29.0	
200–299	797/2297	34.7	290/893	32.5	117/341	34.3	
≥ 300	813/2297	35.4	307/893	34.4	125/341	36.7	
Passive smoking (yes)	1025/2277	45.0	408/882	46.3	151/334	45.2	
Measurements							
PC_{20} ≤ 8 mg/dL	181/1215	14.9	105/880	11.9	76/334	22.8	
Atopy	350/1256	27.9			350/350	100.0	
Prevalence							
Wheezing symptom in the past 12 months	282/2320	12.2	91/900	10.1			
Asthma diagnosis, ever	254/2314	11.0	66/897	7.4			
Atopic wheezing symptom in the past 12 months	66/1250	5.3			66/350	18.9	
Atopic asthma diagnosis, ever	54/1246	4.3			54/349	15.5	

Table 1. (Continued)

	N[a] = 2333	%	Non-atopy	%	Atopy	%	Meeting % KDRI
***MTHFR* (rs1801133)**							
CC	297/930	31.9	99/364	27.2	51/126	40.5	
CT	469/930	50.4	199/364	54.7	52/126	41.3	
TT	164/930	17.6	66/364	18.1	23/126	18.3	
Hardy-Weinberg Equilibrium *P*-value	0.3657		0.0506		0.1392		
Nutrients							
Total energy intake (kcal/day)[b]	1688.24 ± 717.39						1500–1700
Folate (μg DFE/day)[c]	185.88 ± 65.78		184.67 ± 66.30		185.95 ± 69.35		220
Vitamin B_2 (mg/day)	1.20 ± 0.35		1.19 ± 0.37		1.20 ± 0.36		0.8–0.9
Vitamin B_6 (mg/day)	1.31 ± 0.33		1.31 ± 0.34		1.29 ± 0.33		0.9

BMI, body mass index; *MTHFR*; methylenetetrahydrofolate reductase; DFE, dietary folic acid equivalent; KDRI, Korean Dietary Reference Intake;
[a]Mean ± SD or N (%)
[b]Energy (kcal/day) for 6–8-year-old male/female
[c]Folate (μg DFE/day) for 6–8-year-old male/female

Relationships between dietary methyl donor intakes and risks of asthma and atopy

A high dietary folate intake was a protective factor against the wheezing symptom in the past 12 months (aOR, 0.74; 95%CI, 0.55–1.00) and ever asthma diagnosis (aOR, 0.73; 95%CI, 0.53–0.99) (**Table 2**). A high intake of vitamin B_6 was associated with a reduced risk of atopy (aOR, 0.74; 95%CI, 0.56–0.99).

The amount of high, medium, and low intake of folate were 119.76 ± 24.01, 179.85 ± 15.88, and 257.95 ± 50.50, respectively (**Table S2**). In an analysis that did not combine the lower two tertiles, when the higher intake was compared with the two other tertiles, a high folate intake still showed a protective effect against the wheezing symptom in the past 12 months and asthma diagnosis, although it was not significant (**Table S3**).

Relationships between MTHFR polymorphism and risks of asthma and atopy

The CT + TT genotypes of the *MTHFR* C677T polymorphism were associated with a low prevalence of atopy compared to the CC genotype (aOR, 0.57; 95%CI, 0.36–0.89) (**Table 2**). However, the *MTHFR* C677T genotype was not significantly associated with asthma symptoms, ever asthma diagnosis, or BHR.

Effects of dietary methyl donor intakes on asthma and atopy risks according to MTHFR polymorphism

Children with the T allele who had high intakes of folate, vitamin B_2, and vitamin B_6 were less likely to have atopy than children with the CC genotype who had low intakes of folate, vitamin B_2, and vitamin B_6 (aOR, 0.48; 95%CI, 0.25–0.93, aOR, 0.50; 95%CI, 0.26–0.95, and aOR, 0.40; 95%CI, 0.21–0.76, respectively) (**Table 3**). However, no such associations were found between asthma symptoms, ever asthma diagnosis, and BHR.

Effects of dietary methyl donor intakes on risks of atopic asthma and atopic BHR according to MTHFR polymorphism

High intakes of folate, vitamin B_2, and vitamin B_6 were also protective factors against the atopic wheezing symptom in the past 12 months (aOR, 0.17; 95%CI, 0.04–0.80, aOR, 0.26; 95%CI, 0.07–1.01, and aOR, 0.26; 95%CI, 0.07–0.99, respectively), especially for the CT or TT genotype at nucleotide 677T *MTHFR* compared to those with low intakes and CC at this position (**Table S4**). However, these effects were not found for the ever asthma diagnosis with atopy and atopic BHR.

Table 2. aOR and 95%CIs for asthma and atopy according to intake of dietary methyl donors and *MTHFR* polymorphism.

	Wheezing symptom in the past 12 months			Asthma diagnosis, ever			BHR (PC_{20} ≤ 8 mg/dL)			Atopy		
	N	aOR	95%CI	N	aOR	95%CI	N	aOR	95%CI	N	aOR	95%CI
Folate												
Low	185/1213	1.00		170/1226	1.00		112/626	1.00		209/552	1.00	
High	68/633	0.74	(0.55–1.00)	63/637	0.73	(0.53–0.99)	54/298	1.02	(0.72–1.46)	102/267	1.01	(0.76–1.34)
P	0.0479*			0.0414*			0.9104			0.9321		
Vitamin B_2												
Low	162/1229	1.00		149/1239	1.00		108/618	1.00		201/551	1.00	
High	91/617	1.11	(0.84–1.46)	84/624	1.08	(0.81–1.45)	58/306	1.09	(0.76–1.55)	110/268	1.05	(0.79–1.39)
P	0.4858			0.5870			0.6432			0.7280		
Vitamin B_6												
Low	173/1218	1.00		157/1231	1.00		119/614	1.00		224/534	1.00	
High	80/628	0.93	(0.70–1.23)	76/632	0.96	(0.72–1.29)	47/310	0.79	(0.54–1.14)	87/285	0.74	(0.56–0.99)
P	0.6037			0.7860			0.1985			0.0441*		
***MTHFR* variant**												
CC	34/246	1.00		27/252	1.00		22/118	1.00		47/95	1.00	
CT+TT	81/516	1.19	(0.77–1.84)	75/521	1.42	(0.89–2.28)	44/266	0.90	(0.51–1.58)	71/251	0.57	(0.36–0.89)
P	0.4417			0.1445			0.7124			0.0127*		

Adjusted for age, sex, BMI (continuous), parental history of asthma/allergic rhinitis/atopic dermatitis, environmental tobacco smoke (ETS), maternal education, household income, log-transformed energy intake.
*Significantly different at $P < 0.05$.
aOR: adjusted odds ratio, BHR: bronchial hyperresponsive, BMI: body mass index, CI: confidence interval, *MTHFR*: methylenetetrahydrofolate reductase.

Table 3. Effects of gene–environment interaction between *MTHFR* polymorphism and intakes of dietary methyl donors on the risks of asthma and atopy.

		Wheezing symptom in the past 12 months			Asthma diagnosis, ever			BHR ($PC_{20} \leq 8$ mg/dl)			Atopy		
		N	aOR	(95%CI)	N	aOR	(95%CI)	N	aOR	(95%CI)	N	aOR	(95%CI)
Folate	***MTHFR***												
Low	CC	26/162	1		21/166	1		17/83	1		31/70	1	
High	CC	8/84	0.58	(0.25–1.36)	6/86	0.50	(0.19–1.31)	5/35	0.68	(0.23–2.02)	16/25	1.47	(0.68–3.18)
Low	CT + TT	61/332	1.18	(0.71–1.97)	55/337	1.31	(0.76–2.27)	29/177	0.81	(0.42–1.59)	52/161	0.73	(0.43–1.24)
High	CT + TT	20/184	0.72	(0.38–1.35)	20/184	0.90	(0.47–1.74)	15/89	0.81	(0.38–1.75)	19/90	0.48	(0.25–0.93)
P for trend		0.7736			0.6438			0.6127			0.0136*		
P for interaction		0.9335			0.5856			0.5660			0.1090		
Vitamin B_2	***MTHFR***												
Low	CC	23/166	1		19/169	1		17/78	1		31/64	1	
High	CC	11/80	1.01	(0.46–2.22)	8/83	0.86	(0.36–2.08)	5/40	0.57	(0.19–1.69)	16/31	1.14	(0.53–2.47)
Low	CT+TT	48/329	1.10	(0.64–1.90)	44/332	1.25	(0.70–2.23)	29/170	0.78	(0.40–1.52)	48/159	0.65	(0.38–1.13)
High	CT+TT	33/187	1.35	(0.75–2.43)	31/189	1.55	(0.83–2.88)	15/96	0.74	(0.34–1.61)	23/92	0.50	(0.26–0.95)
P for trend		0.3171			0.1206			0.5253			0.0138*		
P for interaction		0.6845			0.4862			0.4237			0.4156		
Vitamin B_6	***MTHFR***												
Low	CC	27/163	1		19/170	1		19/80	1		34/65	1	
High	CC	7/83	0.49	(0.20–1.19)	8/82	0.86	(0.36–2.07)	3/38	0.30	(0.08–1.08)	13/30	0.88	(0.40–1.93)
Low	CT+TT	52/328	0.98	(0.59–1.64)	53/326	1.52	(0.87–2.68)	30/171	0.73	(0.38–1.39)	52/158	0.64	(0.37–1.08)
High	CT+TT	29/188	0.97	(0.55–1.73)	22/195	1.08	(0.56–2.07)	14/95	0.60	(0.28–1.28)	19/93	0.40	(0.21–0.76)
P for trend		0.7774			0.4572			0.3104			0.0042*		
P for interaction		0.1753			0.7092			0.1734			0.4934		

Adjusted for age, sex, BMI (continuous), parental history of asthma/allergic rhinitis/atopic dermatitis, environmental tobacco smoke (ETS), maternal education, household income, log-transformed energy intake.
*Significantly different at $P < 0.05$.
aOR: adjusted odds ratio, BHR: bronchial hyperresponsive, BMI: body mass index, CI: confidence interval, *MTHFR*: methylenetetrahydrofolate reductase.

Discussion

We investigated the relationships between dietary methyl donor intakes and childhood asthma and atopy related to the *MTHFR* C677T polymorphism in Korean school-aged children in nationwide general population. This study had three major findings: 1) high dietary methyl donor intakes were associated with reduced risks of asthma symptoms and ever asthma diagnosis; 2) the *MTHFR* C677T polymorphism was associated with a decreased risk of atopy, and 3) a combination of the genetic polymorphism *MTHFR* and intakes of high methyl donors, including folate and vitamin B_6, was associated with atopy and atopic asthma. Therefore, we suggest that high folate and vitamin B_6 intakes and *MTHFR* genetic susceptibility may have an additive effect on pre-existing atopic asthma in school-aged children.

MTHFR is considered a key enzyme in one-carbon metabolism because it irreversibly converts 5,10-methylenetetrahydrofolate, which serves as the methyl donor of methionine, a precursor of S-adenosylmethionine (SAM), to 5-methyltetrahydrofolate. Mutation of the *MTHFR* gene which causes the C677T polymorphism is located at exon 4 which results in the conversion of valine to alanine at codon 222, a common polymorphism that reduces the activity of this enzyme. This A222V conversion affects the N-terminal catalytic region of the 656-amino acid *MTHFR* protein.[18] In individuals with the homozygous TT genotype, the inability to convert Hcy to methionine is known to result from the decreased *MTHFR* enzymatic activity, and results in increased plasma Hcy levels. The association between atopy and the *MTHFR* C677T polymorphism was established in a study evaluating factors affecting atopy and folate metabolism in adults.[19] They found that inhibition of the re-methylation cycle due to decreased folic acid and changes in TH1/TH2 equilibrium due to low antioxidant capacity may be associated with atopy development.[7,8,20]

The role of the *MTHFR* genotypes can be changed by various biological exposures, including allergens, endotoxins, and different dietary conditions, which are significantly associated with the development of allergic sensitization and recurrent wheezing.[21] Although there is no significant association between the *MTHFR* polymorphisms, and atopy or asthma when the genotype is not classified,[14] some studies have shown that the TT genotype of the *MTHFR* C677T polymorphism is associated with an increased risk of physician-diagnosed asthma and atopy.[15,21] The *MTHFR* C677T genotype is also associated with folic acid concentration because the CC genotype is associated with higher plasma folate concentrations than those for the CT and TT genotypes, which yield lower plasma folate and higher total Hcy levels.[22,23] Of note, the plasma folate level is more affected by the folate intake in the TT genotype than in the CC or CT genotype.[24] When participants with the TT genotype use folate or vitamin B supplementation, they demonstrate increased folate concentrations and decreased Hcy levels, suggesting that genetic polymorphisms of folate-metabolizing enzymes can be important modulators of Hcy levels, specifically in individuals with the TT genotype.[23,25–27] Thus, for individuals with the TT genotype, folate intervention based on personalized nutrition could be effective. Although the underlying mechanisms remain unknown, the results of the present study suggest that the CT or TT genotypes of the *MTHFR* C677T polymorphism, particularly when exposed to high methyl donors, may be associated with a reduced risk of atopic asthma in school-aged children. Further large-scale studies are needed to confirm these results.

Folate is involved in DNA methylation through the formation of SAM and Hcy metabolism, thereby influencing the pathogenesis of asthma.[28] Folate is naturally present in a wide variety of foods, including dark green leafy vegetables, legumes, and some fruits such as oranges.[29] Folate deficiency can lead to significant health problems, including increased susceptibility to infection and several disorders characterized by the enhanced activation of the cellular immune system, such as Alzheimer's disease, rheumatoid arthritis, cardiovascular disease, malignancies, birth defects, and pregnancy complications.[26] Furthermore, impaired folate metabolism may be related to the development of atopy.[15,21] Maternal folate supplementation, and specifically its timing, during pregnancy is an important determinant of childhood asthma.[30,31] Reportedly, low serum folate levels partially influence asthma in children,[10,11] and high serum folate metabolites have been positively associated with lung function in children.[12] However, there is insufficient evidence to support the association between dietary folate intake and childhood allergic disease.

The rationality of the conclusions from these previous studies would be greater if the sample sizes of the individual studies were larger. In addition, the *MTHFR* C677T polymorphism likely affects the role of *MTHFR* in the metabolism of Hcy as discussed in previous section. Furthermore, the diversity of the disease expression in different ethnic groups or populations has indicated the importance of considering the *MTHFR* C677T gene polymorphism for explanation of geographic factors in disease-association analysis. Further investigation is therefore needed to determine the cause of the conflicting results. In our study, high dietary folate intake was associated with a decreased risk of asthma symptoms and atopy in school-aged children. In addition, the intake of high folate and vitamin B_6 with the *MTHFR* polymorphism was a protective factor against atopic asthma. In line with the findings of previous studies, our results support the previous hypothesis suggesting an association between low dietary folate intake and the development of asthma and atopy.

To date, there have been few studies investigating the association between genetic variants in genes of the methyl donor metabolic pathway with asthma and atopy. There are possible explanations for the mechanism of the association between the intake of methyl donors and allergic diseases. A recent subcohort study showed that phosphatidylcholine intake was associated with asthma-related health outcomes, including lung inflammation and pulmonary function in children, as measured by FEV_1.[32] Specifically, the target gene interferon-gamma may provide more evidence

for the mechanistic pathway that links dietary intake and asthma-related health.[32,33]

A strength of this study was its large sample size, recruited from the general, nationwide population. In particular, it is deemed to be of considerable significance because no other study has measured BHR or SPTs in children from a general, nationwide population; instead they have been mostly comprised of case-control studies. Therefore, the results of this study are considered to be more generalizable. Another strength includes the use of the validated ISAAC questionnaire with a relatively high response rate (95%) and low proportion of missing values. We further identified associations between dietary methyl donors and the risks of asthma and atopy, with *MTHFR* polymorphism possibly providing an additive effect. A better understanding of such subgroup analyses, which are stratified by dietary factor and genotype, will allow for more targeted assessments of the combined effect of the gene-environment interaction between nutrient status and single-nucleotide polymorphisms, which has been associated with childhood asthma and atopy.

This study had certain limitations. The cross-sectional study design did not permit us to establish a causal relationship between the questionnaire-based dietary methyl donor intake assessment and development of atopy and asthma. Moreover, to propose descriptive associations between the dietary methyl donor intakes, *MTHFR* polymorphism, and childhood asthma and atopy, we compared the higher intake group with two lower tertiles, rather than the lowest group, as a reference. Because no convincing statistical evidence for gene and environment interaction has been observed, well-designed prospective studies with additional information on other environmental factors that may be concomitantly associated with RBC folate, serum B group vitamins, plasma Hcy, and the methylated DNA level are needed to confirm the current findings.

Based on the *MTHFR* polymorphism in school-aged children, these findings provide a plausible explanation for the relationship between methyl donor intake and the development of atopy and asthma. We confirmed previously observed associations between the nutrient status and single-nucleotide polymorphisms in the genes involved in the uptake or metabolism of these nutrients and atopy, as well as atopic asthma. In addition, we found that a high intake of methyl donors was negatively associated with atopy and existing atopic asthma, with possible additive effects by *MTHFR* polymorphisms in school-aged children. The clinical implications of these findings require further evaluation.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Author contributions

- SJH designed the research.
- YJC, SOK, and MJK analyzed the data.
- YJC and SYL wrote the paper.
- SYL, JHS, JY, HJC, SJ, and SJH conceptualized the original study and provided an essential database.
- SJH had the primary responsibility for the final content.
- All the authors have read and approved the final manuscript.

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Supplemental Materials

Supplementary Table S1. Pearson correlation coefficients between intakes of methyl donors and other nutrients.

	folate	Vit B_2	Vit B_6	Vit C	Vit E	n-3	n-6
folate	1	0.28304	0.66397	0.61955	0.52131	0.34862	0.40947
		< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
	2333	2333	2333	2331	2332	2332	2332
Vit B_2	0.28304	1	0.43261	0.34142	0.3236	0.30695	0.39964
	< .0001		< .0001	< .0001	< .0001	< .0001	< .0001
	2333	2333	2333	2331	2332	2332	2332
Vit B_6	0.66397	0.43261	1	0.68139	0.67168	0.4476	0.5286
	< .0001	< .0001		< .0001	< .0001	< .0001	< .0001
	2333	2333	2333	2331	2332	2332	2332
Vit C	0.61955	0.34142	0.68139	1	0.35567	0.19426	0.26069
	< .0001	< .0001	< .0001		< .0001	< .0001	< .0001
	2331	2331	2331	2331	2331	2331	2331
Vit E	0.52131	0.3236	0.67168	0.35567	1	0.79312	0.84358
	< .0001	< .0001	< .0001	< .0001		< .0001	< .0001
	2332	2332	2332	2331	2332	2332	2332
n-3	0.34862	0.30695	0.4476	0.19426	0.79312	1	0.76516
	< .0001	< .0001	< .0001	< .0001	< .0001		< .0001
	2332	2332	2332	2331	2332	2332	2332
n-6	0.40947	0.39964	0.5286	0.26069	0.84358	0.76516	1
	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	
	2332	2332	2332	2331	2332	2332	2332

Supplementary Table S2. The amount of high, medium, and low intake of folate.

	N	mean	SD
Folate (μg DFE/day)	**2333**	**185.88**	**65.78**
Low	777	119.76	24.01
Medium	778	179.85	15.88
High	778	257.95	50.50
Folate (μg DFE/day)	**2333**	**185.88**	**65.78**
Low	1555	149.82	36.29
High	778	257.95	50.50

Supplementary Table S3. Association between dietary methyl donors intake, *MTHFR* polymorphism, and childhood asthma and atopy.

	Wheezing symptoms in the past 12 months			Asthma diagnosis, ever		
	N	aOR	(95%CI)	N	aOR	(95%CI)
Folate						
Low	92/604	1.00		81/615	1.00	
Medium	93/609	1.02	(0.75–1.40)	89/611	1.12	(0.81–1.55)
High	68/633	0.75	(0.53–1.05)	63/637	0.77	(0.54–1.09)
Vitamin B_2						
Low	90/605	1.00		78/615	1.00	
Medium	72/624	0.78	(0.56–1.08)	71/624	0.90	(0.63–1.26)
High	91/617	0.98	(0.71–1.35)	84/624	1.03	(0.74–1.43)
Vitamin B_6						
Low	85/603	1.00		81/606	1.00	
Medium	88/615	1.05	(0.76–1.46)	76/625	0.94	(0.67–1.32)
High	80/628	0.95	(0.68–1.32)	76/632	0.93	(0.67–1.31)

	BHR (PC_{20} ≤ 8 mg/dl)			Atopy		
	N	aOR	(95%CI)	N	aOR	(95%CI)
Folate						
Low	58/306	1.00		98/279	1.00	
Medium	54/320	0.86	(0.58–1.30)	111/273	1.19	(0.86–1.65)
High	54/298	0.95	(0.63–1.43)	102/267	1.11	(0.80–1.54)
Vitamin B_2						
Low	56/310	1.00		100/279	1.00	
Medium	52/308	0.97	(0.64–1.47)	101/272	1.01	(0.72–1.40)
High	58/306	1.07	(0.71–1.61)	110/268	1.05	(0.76–1.46)
Vitamin B_6						
Low	63/315	1.00		110/281	1.00	
Medium	56/299	0.90	(0.60–1.34)	114/253	1.16	(0.85–1.60)
High	47/310	0.75	(0.49–1.13)	87/285	0.80	(0.58–1.11)

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Supplementary Table S4. The gene environment interaction between *MTHFR* polymorphism and intake of dietary methyl donors on the risk of atopic asthma.

		Atopic wheezing symptom in the past 12months			Atopic asthma diagnosis, ever			Atopic BHR ($PC_{20} \leq 8$ mg/dl)		
		N	aOR	(95%CI)	N	aOR	(95%CI)	N	aOR	(95%CI)
Folate	***MTHFR***									
Low	CC	11/90	1		8/93	1		10/90	1	
High	CC	2/39	0.51	(0.11–2.49)	1/40	0.32	(0.04–2.69)	1/39	0.22	(0.03–1.84)
Low	CT + TT	12/201	0.54	(0.23–1.31)	13/200	0.80	(0.31–2.05)	11/195	0.51	(0.21–1.27)
High	CT + TT	2/107	0.17	(0.04–0.80)	4/105	0.46	(0.13–1.62)	7/97	0.67	(0.24–1.88)
P for trend			0.0187*			0.3420			0.3864	
P for interaction			0.6579			0.6310			0.1365	
Vitamin B_2	***MTHFR***									
Low	CC	9/86	1		5/90	1		8/87	1	
High	CC	4/43	0.96	(0.26–3.57)	4/43	1.48	(0.35–6.28)	3/42	0.83	(0.20–3.46)
Low	CT+TT	11/196	0.61	(0.23–1.57)	12/195	1.14	(0.38–3.48)	13/186	0.80	(0.31–2.06)
High	CT+TT	3/112	0.26	(0.07–1.01)	5/110	0.75	(0.20–2.80)	5/106	0.52	(0.16–1.71)
P for trend			0.0392*			0.6307			0.3135	
P for interaction			0.3822			0.3790			0.7941	
Vitamin B_6	***MTHFR***									
Low	CC	10/89	1		6/93	1		10/89	1	
High	CC	3/40	0.75	(0.19–2.99)	3/40	1.17	(0.27–5.17)	1/40	0.20	(0.02–1.66)
Low	CT+TT	11/199	0.55	(0.22–1.37)	14/196	1.15	(0.42–3.20)	12/189	0.56	(0.23–1.38)
High	CT+TT	3/109	0.26	(0.07–0.99)	3/109	0.44	(0.10–1.84)	6/103	0.52	(0.18–1.51)
P for trend			0.0352*			0.3975			0.2579	
P for interaction			0.6239			0.2576			0.2006	

Adjusted for age, sex, BMI (continuous), parental history of Asthma/Allergic Rhinitis/Atopic Dermatitis, Environmental Tobacco Smoke (ETS), maternal education, household income, log-transformed energy intake.
*Significantly different at $P < 0.05$.
aOR, adjusted odds ratio; BHR, bronchial hyperresponsive; BMI, body mass index; CI, confidence interval; *MTHFR*, methylenetetrahydrofolate reductase;

Supplementary Table S5. Summary of related studies on folate status, allergy outcomes, and *MTHFR* polymorphisms.

References	Country	Sample size	Age	Folic acid exposure	Key findings	Allergic outcome	C677T mutation in *MTHFR*
Granell[14]	UK	5,364	7-8 yr	Maternal folate intake in the third trimester of pregnancy	No significant association with	Atopy (≥ 1 positive skin test to allergens)	No associations
Thuesen[15]	Denmark	6,784	30-60 yr	Serum folate	Inversely associated with	Physician-diagnosed asthma	TT genotype
Husemoen[19]	Denmark	1,671	30-60 yr	Serum folate	Inversely associated with	Atopy	TT genotype
Zou[34]	China	433 asthmatic and 1,249 healthy			Significantly association with	Atopic asthma	TT genotype
Matsui and Matsui[10]	U.S.	8,083	2-85yr	Serum folate	Inversely associated with	Wheeze, total IgE, and atopy	
					No significant association with	Physician-diagnosed asthma	
Bueso[35]	Norway	169	13-14 yr	Dietary intake of folate	No significant association with	Physician-diagnosed asthma	
Farres[36]	Egypt	180		Serum folate	No significant association with	Asthma	
		Among subjects with asthma		Serum folate	Inversely associated with	Total IgE	
Woods[9]	Australia	1,601	20-44 yr	Dietary intake of folate	Significantly association with	Physician-diagnosed asthma ever	
					No significant association with	Current asthma, airway responsiveness, or atopy	
Shaheen[37]	India	40	2-4 yr	Serum folic acid	No significant association with	Atopic dermatitis	
Patel[38]	England	1,030	45-75	Dietary intake of folate	Significantly association with	Reduced odds of physician-diagnosed asthma	
Okupa[39]	U.S.	138	2-9 yr	Increased serum folate levels at or before age 6 yr	Significantly association with	Allergic sensitization	
					No significant association with	Total IgE, asthma, or wheezing at ages 6 or 9 yr	
Lin[40]	U.S.	144	5-17 yr	Serum folate	No significant association with	Fractional exhaled nitric oxide, degree of atopy, lung function, or hospitalizations for asthma	
				Folate level in the second quartile	Significantly association with	Increased total IgE	
Miyake[41]	Japan	763	16-24 mo	Maternal folate intake at any trimester of pregnancy	No significant association with	Wheeze or eczema	
Litonjua[42]	U.S.	1,290	2 yr	Maternal folate intake in the first and second trimesters of pregnancy	No significant association with	Wheeze or eczema	
Håberg[43]	Norway	32,077	18 mo	Folic acid supplementation in the first trimester of pregnancy	Significantly association with	Increased risk of wheeze, LRIs, and hospitalizations	

Supplementary Table S5. (Continued)

References	Country	Sample size	Age	Folic acid exposure	Key findings	Allergic outcome	C677T mutation in *MTHFR*
Nwaru[44]	Finland	2,441	5 yr	Maternal folate intake in the third trimester of pregnancy	No significant association with	Asthma	
Whitrow[45]	Australia	423	5.5 yr	Maternal folic acid supplementation in late pregnancy	Significantly association with	Increased risk of asthma at 3.5 yr	
					No significant association with	Increased risk of asthma at 5.5 yr	
Bekkers[46]	Netherland	3,786	8 yr	Folic acid supplementation during pregnancy	No significant association with	Asthma symptoms, wheeze, LRIs, or eczema	
					Inversely associated with	Airway responsiveness or allergic sensitization	
Magdelijns[47]	Netherland	2,834	7 yr	Maternal folate supplementation during pregnancy	No significant association with	Allergic sensitization at age 2 yr or asthma at age 6-7 yr	
				Maternal RBC folate level in the last trimester of pregnancy	Inversely associated with	Asthma at age 6-7 yr	
Kiefte-de Jong[48]	Netherland	8,742	4 yr	Maternal plasma folate level in the first trimester of pregnancy	Significantly association with	Increased odds of eczema	
					No significant association with	Asthma at age 4 yr	
Haberg[49]	Norway	1,962	3 yr	Maternal folate level in the second trimester of pregnancy	Significantly and linearly association with	Increased odds of asthma	

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