FOLATE STATUS AND ASTHMA: AN EXAMINATION OF THE LITERATURE AND IMPLICATIONS FOR PRACTICE

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ABSTRACT

Asthma has a major public health impact in the United States and worldwide. Climbing asthma rates have triggered a search for environmental factors that could be contributing to the prevalence or severity of asthma. Among substantial changes over the past several decades has been a dramatic increase in folate intake, caused by widespread foodstuff fortification as well as maternal vitamin supplementation. Findings in a murine model suggest that folate supplementation affects offspring asthma risk. Cross-sectional studies in humans yield weak evidence of an inverse association between folate and asthma risk, in which lower folate is associated with greater odds of asthma. Birth cohort studies, on the other hand, provide weak evidence of a positive association between maternal folate supplementation and offspring asthma risk. Folate is a methyl donor that could theoretically alter asthma risk by contributing to the methylation of disease-modifying genes. Nevertheless, given the weak evidence for folate in modifying asthma risk, as well as the proven public health importance of folate in the prevention of neural tube defects, there is insufficient evidence to change current recommendations regarding folate supplementation during pregnancy. More clarity from well-designed, large observational studies, with careful measurement of folate status and asthma phenotypes, is necessary.

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PREFACE

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1.0 INTRODUCTION

In the United States (U.S.), the prevalence of asthma increased from 7.3% in 2001 to 8.4% in 2010, when approximately 25.7 million persons were estimated to have asthma[1]. Among other factors, altered eating habits (including nutrients such as vitamin D, vitamin E, or zinc) have been proposed as causes of increased childhood asthma[2-4]. A maternal diet enhanced with folate, for example, can theoretically lead to increased or reduced risk of offspring asthma by producing changes in DNA methylation[5]. Folate is a methyl donor that could alter asthma risk by contributing to methylation of disease genes in relevant tissues. Findings in murine models support this hypothesis[6] and encourage the exploration of an association between folate status and asthma or other allergic disease (i.e., atopy) in humans. In this review, I survey both gestational studies (evaluating the effect of maternal folate on offspring) and proband studies (evaluating the effect of folate status in children and adults). These studies represent the effect of folate status along a single temporal continuum, from fetal through adult exposures.

Studies of maternal folate status and the development of asthma or atopy have varied results. In some studies, increased maternal folate has been shown to increase risk of offspring wheezing[7-9], while in other studies increased maternal folate has been shown to decrease offspring wheezing risk[10] or have no effect whatsoever[11-13]. Nevertheless, a majority of gestational exposure studies find that increased maternal folate is associated with elevated risk of offspring wheezing, asthma, or other atopy, and there are plausible epigenetic mechanisms for these offspring risks[14,15].

On the other hand, folate status can have effects in patients at risk for asthma (i.e., proband effects) that differ from the gestational effects of maternal folate supplementation. While gestational exposure is identified as a possible risk factor for asthma, folate intake may in

fact protect children and adults from the development of asthma. Such an inverse relationship between folate status and markers of atopy is noted in several cross-sectional studies[16-18]. Proband studies of the MTHFR C677T polymorphism, which can be interpreted as a marker of low folate status[19], do not show uniform evidence of this inverse relationship[12,19-21], however.

Folate supplementation is proven to offer substantial protection against neural tube defects (NTD) in newborns[22]. Prior to sounding a warning regarding a link between folate and asthma, the quality and quantity of evidence must be considered. The public health challenge posed by folate involves balancing the known benefits regarding NTD, and unknown risks regarding asthma. Periconceptional folic acid administration is an ongoing public health issue, given that vulnerable groups (e.g., young and poorly educated) are less likely to adhere to standards regarding vitamin supplementation[23]. In a review of international studies, preconceptional folic acid use is noted to vary from 0.9% to 50%[24]. Given this poor adherence to guidelines at baseline, the premature announcement of heretofore unreported disease risk could be an irresponsible deterrent.

This review is a thorough examination of the existing evidence for association between folate status and asthma or atopy. Section 2 is a discussion of the primary animal model of gestational folate exposure, and section 3 is a review of human studies. Section 4 covers the complex issue of folate measurement as it impacts comparability of these studies. Section 5 deals with gene-by-environment interactions in folate metabolism, with a focus on MTHFR polymorphisms. In Section 6, I explore possible mechanisms for folate effects on asthma and other forms of atopy. Finally, Section 7 summarizes the public health significance of existing literature on folate and asthma.

1.1 FOLATE METABOLISM

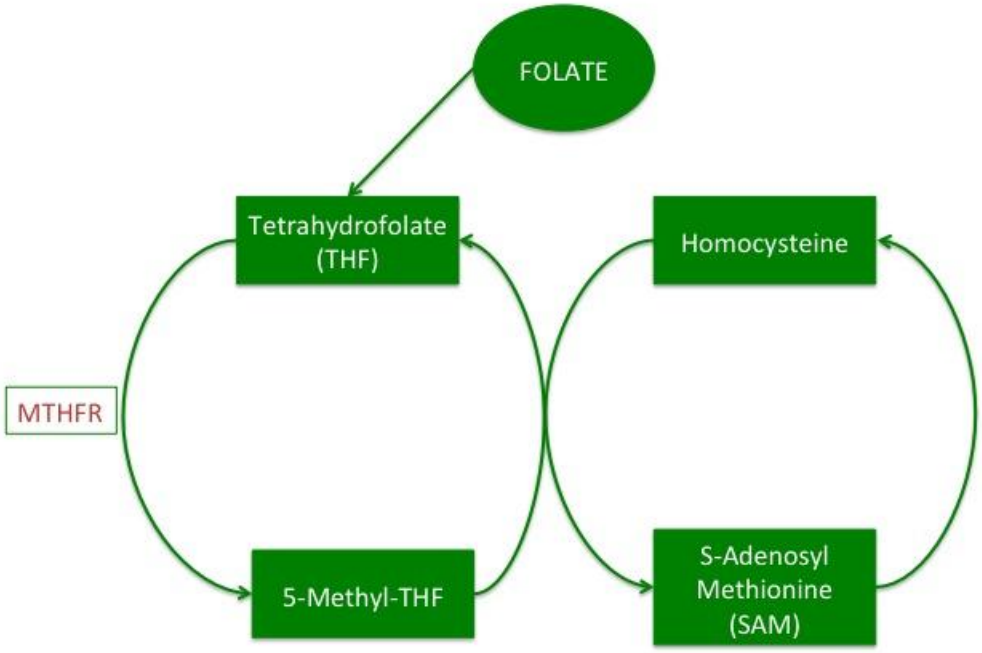

Figure 1. Folate metabolism

Folic acid is a synthetic form of folate used in fortification and supplementation. Absorption of dietary folates occurs in the proximal small intestine. Folic acid is among the monoglutamate forms of folate that can be easily absorbed. Polyglutamates, including natural food folates, need to be hydrolyzed prior to absorption, and bioavailability of dietary folates is estimated as approximately half that of folic acid[25]. Precise Dietary Folate Equivalents (DFEs) that account for differences in bioequivalence between folic acid and dietary folates are difficult to establish[26]. Folate absorption is saturable, and as intake increases, unreduced folic acid can appear in the circulation[27].

Folate is essential to synthesize purines and pyramidines, as well as to metabolize amino acids, but is also needed for the formation of S-adenosylmethionine (SAM). SAM has a role in

the methylation pathways of phospholipids, RNA, and DNA[28]. Folate metabolism also generates essential and nonessential amino acids, including serine, glycine, cysteine, and methionine.

The tetrahydrofolate (THF) molecule is a methyl acceptor that is converted to methylene-THF. The subsequent conversion of this metabolite to 5-methyl-THF is catalyzed by methylene tetrahydrofolate reductase (MTHFR) in the rate-limiting step of folate metabolism. During this process, a methyl group is transferred to homocysteine, resulting in the production of methionine[29] that regenerates SAM (summarized in Figure 1).

A C to T substitution at position 677 is a common missense mutation in the MTHFR gene. This mutated gene product has reduced enzymatic function[30]. Due to the impairment, the MTHFR C677T polymorphism is identified as a genetic marker of decreased serum folate[19]. Moreover, via this interaction with folate status, the MTHFR C677T polymorphism also can affect DNA methylation status[31].

2.0 ANIMAL MODELS

Hollingsworth et al. report the primary animal study assessing folate status and the development of respiratory disease[6]. Using a murine model, they supplement the maternal diet with methyl donors, including folic acid. Offspring of these mice (the F1 generation) are more likely to have evidence of airway hyperreactivity, lung lavage eosinophilia and IL-13, as well as elevated serum IgE. Some of these traits are subsequently transmitted to the F2 generation.

The investigators also seek to distinguish between gestational effects (on the F1 generation), and the proband effects of childhood and adult methyl donor exposure. Probands exposed to methyl donors only during lactation or adulthood do not develop significant

differences in markers of allergic airway disease. Therefore, the investigators note that methyl donor exposure appears to have pro-atopic effects during gestation only[6].

To identify whether epigenetic changes account for these findings, DNA methylation in lung tissue in F1 mice that undergo high-methyl-donor gestation is compared with methylation in F1 mice that undergo low-methyl-donor gestation. Significant differences in methylation are observed, with 82 loci showing significant differences and representing potential candidate genes in the development of allergic airway disease[6].

The hypothesized effect of this DNA methylation, decreased transcription in splenocytes of mice gestated under a high-methyl-donor diet, is reversible. Application of a demethylating agent in vitro produces a significant increase in Runx3 mRNA. Runx3 is a gene that negatively regulates allergic airway disease.[6] This animal study provides some of the most compelling data suggesting a connection between folate and asthma, as mediated by methylation.

3.0 EPIDEMIOLOGY OF FOLATE AND ASTHMA OR ATOPY

Murine model findings prompted epidemiologic study of the relationship between folate status and asthma or atopy. These studies (summarized in Tables 1 and 2) examine many temporal relationships between folate status and asthma or atopy, have variable sample sizes, and have varying classification of wheezing and folate status. Selection bias could affect many of these studies[32] since they either involve a subset of participants from a larger recruitment or represent a significant loss of participants over time[9-11,13,17,18,33-37]. These human studies evaluate the effects of fetal exposures (i.e., due to maternal supplementation), as well as of childhood and adult exposures, as measured by proband folate intake or serum folate status.

Both folate status and the effect of folate may vary between males and females, as well as among racial and ethnic groups. A cross-sectional study based on NHANES data[16] notes an inverse relationship between folate status and wheezing among non-Hispanic white and black subjects. Among Hispanic subjects, however, the investigators find an association between serum folate and wheezing. Hispanic subjects and non-Hispanic black subjects are noted to have significantly lower folate levels.

NHANES data[38] also is reported to show higher folate levels among older women as compared to men. In a Norwegian study of dietary intake in adolescents with asthma[33], based on food questionnaire data, girls are reported to have a lower dietary intake of folate as compared to boys.

3.1 CROSS-SECTIONAL STUDIES

There are seven cross-sectional studies of folate and asthma or atopy[16-19,33-35] (summarized in Table 1). In the cross-sectional data from the National Health and Nutrition Examination Survey (NHANES)[16], higher serum levels of folate are associated with lower rates of atopy and wheezing [for wheezing, adjusted odds ratio (aOR) for highest vs. lowest quintile = 0.6, 95% confidence interval (CI) 0.4-0.8]. Atopy is measured with both serum specific and total IgE levels. Gestational exposure is not assessed in this study.

In a case-control study of Egyptian adults[17], subjects with asthma and positive skin prick testing are noted to have significantly lower serum folate levels (9.1 ng/mL vs. 11.3 ng/mL among non-atopic asthma patients, p=0.001). In these atopic asthmatic patients, folate levels are inversely correlated with serum total IgE. Folate status does not correlate directly with asthma, but rather with markers of atopy in atopic asthmatic patients. Another case-control study in

England[18] identifies a direct association between low folate, as measured by food frequency questionnaire, and a diagnosis of asthma [aOR for each quintile increase = 0.89, 95% CI 0.81-0.98]. In a large study of Danish adults, low serum folate is significantly associated with asthma diagnosis (aOR for lowest vs. highest quartile = 1.37, 95% CI 1.05-1.79). Despite this evidence for an inverse association between folate and asthma, one large Australian study shows evidence of a positive association between dietary folate and asthma (OR per microgram = 2.2, 95% CI 1.2-3.9), and two smaller studies show no evidence for an association between folate status and asthma or atopy[33,35].

In summary, there is not clear evidence that low levels of folate cause asthma. Several observational studies, however, associate low folate status and higher rates of asthma or atopy[16-19]. This potential protective effect of folate supplementation against asthma or atopy in children and adults merits further evaluation.

Table 1. Cross-sectional studies: evidence for association between folate status and asthma or atopy

Evidence for association between folate status and asthma or atopy	Reference	Summary	Limitations
Inverse association			
	Farres et al.[17]	Study of adults (n=180). Among subjects with asthma, serum folate inversely associated with IgE.	Small sample size and lack of dietary intake information.
	Matsui and Matsui[16]	Study of children and adults, 2-85 years old (n=8,083). Serum folate inversely associated with wheezing.	Broad age inclusion and lack of dietary intake information.
	Patel et al.[18]	Study of adults, 45-75 years old (n=1,030). Dietary folate intake inversely associated	Lack of nutritional supplement information and lack of folate levels.

Table 1 continued

		with asthma.	
	Thuesen et al.[19]	Study of adults, 30-60 years old (n=6,784). Serum folate inversely associated with asthma.	Lack of information regarding nutritional supplements and dietary intake.
Positive association			
	Woods et al.[34]	Study of adults, 20-44 years old (n=1,601). Dietary folate intake associated with asthma.	Lack of folate levels.
No association			
	Bueso et al.[33]	Study of children, 13-14 years old (n=169). No significant association between folate intake and asthma.	Small sample size and lack of folate levels.
	Shaheen et al.[35]	Study of children, 2-4 years old (n=40). No significant association between serum folate and atopic dermatitis.	Small sample size and lack of dietary intake information.

3.2 LONGITUDINAL STUDIES

There are thirteen longitudinal studies of folate and asthma or atopy (summarized in Table 2). Only two of these studies are designed to identify associations between a child's folate status and risk of asthma or atopic disease. In one small U.S. cohort, children with higher serum folate levels are more likely to have allergic sensitization between 3-9 years of age (rate of sensitization to seasonal aeroallergens among high vs. low folate cluster = 21% vs. 44%, $p = .03$)[15]. In another small study by the same group, children with second-quartile folate levels have a higher IgE than children with first-quartile folate levels (295 vs. 114 kU/L, $p <= .01$)[39]. These studies are limited by potential selection bias and small sample size.

The remainder of these longitudinal studies are birth cohorts in which maternal folate status during gestation is assessed in addition to offspring asthma. Such birth cohort studies are

designed to identify whether folic acid supplementation contributes to increased risk of offspring asthma or atopy. Five of these studies use questionnaire data to evaluate maternal folate status[9,11-13,37]. In a cohort study from the Netherlands[11], prenatal folic acid supplementation is associated with an increased risk of wheezing in children at age 1 year (prevalence ratio = 1.20, 95% CI 1.04-1.39). Participants completed yearly questionnaires regarding respiratory disease and allergic symptoms. Lab testing at the end of this period included serum specific IgE testing as well as a methacholine challenge test. An increased risk of wheeze is observed at age one year in the children of mothers who use folic acid supplements during pregnancy, but no other associations are noted. In this study, however, maternal folate supplementation and most wheezing outcomes are self-reported.

In a smaller Australian prospective birth cohort[9], increased folate intake is associated with increased risk of physician-diagnosed asthma in offspring at age 3.5 years (RR = 1.26, 95% CI 1.08-1.43), as well as with persistent asthma. Significant association is not present on reexamination of offspring at age 5.5 years. Self-reported supplementation and food frequency questionnaires account for folate intake, and almost one-quarter of the children are not followed throughout the study period. Other birth cohorts involving maternal questionnaire data show no significant association between folate and asthma[13,37] or atopy[12].

Three birth cohorts reflect the attempt to identify associations between early childhood wheezing and maternal folate intake data[8,36,40]. Haberg et al. have conducted multiple studies in Norwegian children in which folate supplementation during pregnancy is shown to be associated with increased risk of wheezing in early childhood[7,8]. The first study is a large cohort employing supplementation data, and the second is a nested case-control study using maternal plasma folate data. In their first study[8] [adjusted relative risk (aRR) for offspring wheezing = 1.06, 95% CI

1.03-1.10], the maternal folate supplementation is self-reported, and in both studies the wheezing outcomes is primarily self-reported. In the later study (aOR for offspring asthma at age 3 years for highest vs. lowest quintile = 1.66, 95% CI 1.16-2.37)[7], wheezing is assessed both by positive response to a maternal questionnaire, and by the use of asthma therapy within the preceding year. The investigators also find a correlation between reported maternal folate supplementation and maternal serum folate levels. Two other studies of early childhood wheezing involving maternal folate intake data[36,40] show no effect of maternal folate on asthma.

Three of the birth cohorts, including the nested Haberg study[7], evaluate maternal serum or red blood cell folate measurements[10,14]. In a Netherlands cohort study[10], higher levels of maternal folate are associated with a slightly decreased risk of asthma (i.e., an inverse relationship) in children age 7 to 8 years (p-value for trend across quintiles = .05). In this study, however, more than a quarter of the children are not followed up to age 7, and less than a third of the mothers have laboratory folate measurements. While a Dutch cohort shows no evidence of association between maternal folate and asthma, there is a significant positive association between first-trimester maternal serum folate and offspring eczema (aOR = 1.18, 95% CI 1.05-1.33)[14].

Among these birth cohorts, there are differences not only in measurement of folate status, whether self-reported or assessed in a laboratory, but also in the characterization of wheezing. Self-reported outcomes, in which parents note that their children wheeze, for example, are used in most of the studies[7,8,11,36,40], but physician-diagnosed asthma is employed frequently as well[9,10,13,37]. Birth cohorts evaluating maternal supplementation and early childhood (under age 5) asthma or atopy include some evidence of positive association[7-9,14] or no effect[36,40]. Such studies, however, are limited both by short duration of follow-up as well as by the difficulty of

identifying asthma phenotypes in early childhood[32]. On the other hand, the birth cohorts involving asthma or atopy among older children—and its association with maternal folate questionnaire data[9,11-13,37]—are limited by substantial loss to follow-up (up to 62%)[32]. While many studies of folate incorporate pulmonary function data[10,19,39], including some with methacholine challenge, there are no significant objective findings.

In summary, five of the birth cohort studies show no evidence for association between maternal folate status during gestation and offspring asthma or atopy. Nevertheless, five birth cohorts[7-9,11,14], including the largest such study to date[8], offer weak evidence of a positive association between maternal folate status and offspring asthma or atopy. The effect of gestational supplementation warrants further consideration in large, well-designed observational studies.

Table 2. Longitudinal studies: evidence for association between folate status and asthma or atopy

Evidence for association between folate status and asthma or atopy	Reference	Summary	Limitations
Inverse association			
	Magdelijns et al.[10]	Study of mothers and children, up to 7 years old (n=2,834). Maternal intracellular folate inversely associated with offspring asthma.	Lack of information regarding maternal dietary intake.
Positive association			
	Bekkers et al.[11]	Study of mothers and children, up to 8 years old (n=3,786). Maternal folate supplementation associated with offspring wheezing.	Lack of information regarding maternal dietary intake or folate levels.
	Haberg et al.[8]	Study of mothers and children, up to 18 months old	Lack of folate levels and limited discrimination

Table 2 Continued

		(n=32,077). First-trimester maternal supplementation associated with offspring asthma.	between early childhood wheezing and asthma.
	Haberg et al.[7]	Study of mothers and children, up to 3 years old (n=1,962). Second-trimester maternal serum folate associated with offspring wheezing.	Lack of information regarding dietary intake and limited discrimination between early childhood wheezing and asthma.
	Kiefte-de Jong et al.[14]	Study of mothers and children, up to 4 years old (n=8,742). First-trimester maternal serum folate associated with offspring atopic dermatitis.	Limited discrimination between early childhood wheezing and asthma.
	Lin et al.[39]	Study of children, 5-17 years old (n=144). Compared to first quartile, second quartile serum folate associated with IgE.	Small sample size and lack of information regarding dietary intake.
	Okupa et al.[15]	Study of children, 2-9 years old (n=138). Serum folate levels associated with allergic sensitization.	Small sample size and lack of information regarding dietary intake.
	Whitrow et al.[9]	Study of mothers and children, up to 5.5 years old (n=423). Maternal folate supplementation associated with offspring asthma.	Lack of folate levels.
No association			
	Granell et al.[12]	Study of mothers and children, up to 7-8 years old (n=5,364). No significant association between third-trimester maternal supplementation and offspring atopy.	Limited information regarding maternal dietary intake and lack of folate levels.
	Litonjua et al.[40]	Study of mothers and children, up to 2 years old (n=1,290). No	Lack of information regarding maternal folate levels and

Table 2 Continued

	significant association between first- or second-trimester maternal folate intake and offspring wheezing.	limited discrimination between early childhood wheezing and asthma.
Martinussen et al.[37]	Study of mothers and children, up to 6 years old (n=1,499). No significant association between first-trimester maternal folate supplementation and offspring asthma.	Limited information regarding maternal dietary intake and lack of folate levels.
Miyake et al.[36]	Study of mothers and children, up to 24 months old (n=763). No significant association between maternal folate intake and offspring wheezing.	Limited information regarding maternal dietary intake and lack of folate levels, as well as limited discrimination between early childhood wheezing and asthma.
Nwaru et al.[13]	Study of mothers and children, up to 5 years old (n=2,441). No significant association between third-trimester maternal folate intake and offspring asthma.	Limited information regarding maternal dietary intake and lack of folate levels.

4.0 FOLATE MEASUREMENT

Low serum folate is typically identified as less than 3 ng/mL[38]. Red blood cell folate (normal >140ng/mL) is understood to reflect levels of stored folates[41]. In the U.S., patterns of folate deficiency changed dramatically after the mandatory folic acid food fortification program was instituted from 1996-1998. This program was undertaken to reduce the risk of neural tube defects (NTD), and NTD incidence dropped significantly following folic acid fortification[42]. The United States National Health and Nutrition Examination Survey (NHANES) data from 2010 indicates

that the prevalence of low serum folate decreased from 24% to <=1% since fortification[43]. In countries where fortification is inadequate, NTD rates can remain unchanged[44].

There is substantial variability among the asthma and atopy studies discussed above as to when and how folate is measured. As noted, many of the studies use maternal self-reported folate supplementation and food frequency questionnaires[8,9,11,13,37,40], with no laboratory measurement of folate. Moreover, in one study[36], supplement use is ignored. Among studies that involve laboratory measurement, some use serum folate values[7] and others use intracellular folate levels[10], which can more accurately reflect folic acid stores over preceding months[41].

Folate measurements are variably effective in different subjects. If 5-methyl-THF predominates as the one-carbon form of folate in serum and red blood cells, conventional assays can be sufficient. On the other hand, in people with the common MTHFR C677T polymorphism, for example, a variety of other one-carbon folate forms are present[45] that may go underestimated. Other approaches, including mass spectrometry, can more effectively assess the presence of both folate metabolites and unmetabolized folic acid[46].

These studies come to varied conclusions regarding the timing of maternal folate supplementation. Haberg et al. note increased wheezing in children born to mothers who supplement folate in the first trimester[8] and in the second trimester[7]. Whitrow et al. note a similar association in children born to mothers who supplement folate in the third trimester[9], but not in early pregnancy. These authors suggest that a second trimester effect of maternal folate on asthma could be mediated by the increase in amniotic IgE characteristic of gestation weeks 16-18[47]. This positive association is not noted consistently, however. The Martinussen et al.[37] prospective cohort study, for example, is designed to evaluate the effect of first-trimester folate supplementation, and no increased wheezing in offspring is observed.

Folate intake can be difficult to assess with food frequency questionnaires[19]. Folates in foods have poor stability and incomplete bioavailability[48]. By comparison, folic acid supplementation is relatively stable and bioavailable. Food frequency questionnaires have been specially modified for use in pregnancy methyl-donor studies[49].

In Bueso et al.[33], participants complete a four-day food diary. They are given a video with instructions regarding how to fill out the 18-page form. An accompanying photographic booklet helps these teenage participants to identify portion size. A similar food frequency questionnaire, validated for use in a Japanese population, is used in the Miyake et al.[36] prospective study. Validated food frequency questionnaires are also employed in Whitrow et al.[9], but multiple commentators suggest that these estimates are incorrect[50,51] because they take into account only free folic acid and do not include accurate fortification data.

Multiple quality cohort studies[8,11,16,37] focus only on the presence of supplements and do not account for maternal dietary intake. High serum folate levels are commonly associated with supplement use[52], and it is reasonable to question the relevance of enriched cereal-grain product consumption in studies of adverse folate effects. Nevertheless, the complexity of evaluating food diaries and questionnaires, as well as measuring folate in the laboratory, provides one indication of why consistent findings in studies of folate and asthma are difficult to achieve.

A causative pattern of folate supplementation triggering offspring wheezing may not stop at birth. This positive relationship could persist through a critical period of early childhood during which high folate levels would continue to be associated with allergic sensitization[15]. In later childhood, however, folate could change from a causative factor in childhood wheezing to a protective one.

5.0 FOLATE GENE-BY-ENVIRONMENT INTERACTIONS

The methylenetetrahydrofoate reductase (MTHFR) gene is frequently implicated in studies of folate status and asthma. The common homozygous C677T mutation in the MTHFR gene is reported as a cause of abnormal folate metabolism[45]. The T allele has an allele frequency of more than one-third in multiple reports[30,53]. Individuals with the TT genotype have consistently lower serum folate than other genotypes, and some authors suggest that this genotype can be used as a marker of low folate status[19].

In a large English cohort study including 5,364 children[12], atopy is assessed in children ages 7 to 8 by skin prick testing, and the children are assessed for the presence of physician-diagnosed asthma or current symptoms. The MTHFR C677T variant is not noted to have an association with asthma or allergy in these children, even after adjustment for dietary folate intake.

Likewise, a cross-sectional study of Danish adolescents and adults[20] does not find an association between the C677T polymorphism and either subjective or objective indicators of atopy. Lab data from this study includes serum specific IgE, skin prick testing, and methacholine challenge. In a subsequent cross-sectional study with longitudinal follow-up by the same authors[19], however, there is an association between doctor-diagnosed asthma and both folate status and C677T polymorphism (TT vs. CC genotype aOR = 1.52, 95% CI 1.12-2.06). They also note that individuals with both TT genotype and low estimated dietary folate intake are even more likely to have atopy when compared with those with higher folate intake. A Chinese case-control study of 1,682 adults likewise finds greater prevalence of the TT genotype among atopic asthmatic patients as compared with controls[54].

In a separate cross-sectional study of 1,482 Danish adults[21], Husemoen et al. identify the MTHFR C677T variant genotype and assess serum specific IgE levels for inhalant allergens. The MTHFR TT homozygotes have a significantly higher risk for atopy than either heterozygotes (CT) or normal (CC) individuals (aOR 1.76, 95% CI 1.19-2.60). This study does not assess the presence of asthma, however.

A cohort study of Spanish children at ages 4 to 6 years[55] identifies hypomethylation of the arachidonate 12-lipoxygenase (ALOX12) gene as being associated with increased risk of persistent wheezing. The investigators collect data regarding maternal folate supplementation during pregnancy, but no association between gestational folate supplementation and ALOX12 hypomethylation is identified.

While there is a possible association between folate and asthma, there is not a genetic marker of folate status that consistently is associated with asthma or atopy. There is good evidence for association between MTHFR genotype and folate status[19], but the interpretation of MTHFR asthma studies is limited due to possible selection bias, as well as insufficient identification of the asthma phenotype[32]. Individuals with genetic polymorphisms may have a different distribution of folate metabolites[46], and a complete laboratory folate evaluation could yield more consistent results.

6.0 MECHANISMS OF FOLATE EFFECTS ON ASTHMA OR ATOPY

The primary mechanistic explanation for folate effects in asthma or atopy is DNA methylation. Epigenetic change could also account for heritable disease risk. Synergistic effects associated with varied diets high in fruits and vegetables[56], however, could also account for the perceived effects of folate status.

6.1 MATERNAL SUPPLEMENTATION AND METHYLATION

Methylation patterns can affect genetic expression broadly, but they are also implicated in specific patterns of cytokine production in the asthma phenotype[57]. T lymphocytes mature into Th1 and Th2 lineages that generate cellular or humoral immune response. Th2 cells, which are associated with distinct methylation patterns[58], produce cytokines implicated in asthma and other atopic response.

At the 5' end of a gene, there is a higher prevalence of cytidine phosphate guanosine (CpG) sequences, called CpG islands[59]. Methylation of CpG islands in promoter regions can silence transcription[60]. Approximately 88% of active promoter regions are associated with CpG islands and can thereby theoretically be governed by methylation[61]. FoxP3 is a transcription factor regulating development of Treg cells, which modulate the immune response. FoxP3 expression and Treg cell development are noted to vary with methylation patterns[62].

DNA methylation has an epigenetic role in the development of a Th2 phenotype[5,63]. Folate, like dietary methionine and choline, is identified as a methyl donor that leads to the methylation of DNA[64]. A methyl donor can alter disease risk by methylating, and thereby downregulating, genes that govern the development of an atopic phenotype. Prescott and Clifton[63] identify folate as being the current most notable candidate for epigenetic determination of asthma phenotype. The Runx3 transcription factor implicated in the Hollingsworth study has a corresponding binding site in the FoxP3 promoter region, and Runx3 and FoxP3 are identified jointly as important to the development of the Treg lineage[65].

6.2 FOLATE STATUS AND IMMUNE SYSTEM FUNCTION

Folate is suggested to modulate immune response and provide resistance to infection. Folate deficiency decreases numbers of circulating T lymphocytes[66] and impairs natural killer (NK) cell-mediated cytotoxicity[67]. A mother who continues to take supplements after the birth of her child could be less likely to develop respiratory illness herself, and therefore less likely to have children who wheeze[40].

Folate status can also be a marker of nutritional status[15]. Many vitamins and trace elements are studied for their effect on immune function. Children with low folate could have multiple relative nutritional deficiencies that cumulatively would put them at risk for wheezing illness. The cross-sectional Australian study of 1,601 young adults[34] finds that apple and pear consumption are protective for asthma (aOR 0.83, 95% CI 0.71-0.98), and people consuming fewer dietary antioxidants are noted to have increased bronchial reactivity[68].

6.3 FOLATE TOXICITY AND IMMUNE REGULATION

The synthetic folic acid found in supplements is absorbed differently than dietary folates, and intestinal folic acid transport is saturable[69]. Excess intake can result in circulating unmetabolized folic acid[70]. The effects of unmetabolized folic acid are unknown, but some investigators have proposed that the circulating folic acid can cause immune dysregulation, including reduced natural killer cell cytotoxicity[71].

7.0 SUMMARY AND FUTURE DIRECTIONS

Several studies of maternal folate supplementation find an association with increased risk of wheezing, asthma, or other markers of atopy in offspring[7-9,11]. The murine model study suggests

the same association, and even identifies reversibility of DNA methylation in atopy candidate genes[6]. Differential methylation is the most promising mechanism to explain the effect of folate on the development of asthma.

Higher serum levels of folate in a patient at risk for asthma or atopy may be protective[16-19], but studies of low folate status in patients with MTHFR polymorphism have shown varied results[12,21]. Approaches to the assessment of folate status vary widely, and there are multiple challenges that complicate the accurate recording of dietary folate intake.

Most of these conclusions are drawn from observational studies. There is established benefit of maternal folate supplementation in preventing NTD, and changes to folate intake cannot be recommended on the basis of available literature. There are not randomized clinical trials investigating the effects of differing levels of folate supplementation[72]. Moreover, effective accounting for folate levels will entail joint study of intake, supplementation, and serum levels. The value of employing red blood cell folate in these studies is not yet known, and serum measurements should include multiple folate species (e.g., 5-methyl-THF, 5-formyl-THF, and folic acid) that comprise total folate in serum[73].

7.1 PUBLIC HEALTH RECOMMENDATIONS

Several studies suggest a protective effect of folate in children and adults that is the opposite of gestational exposure, and effects on all these groups warrant further investigation. Large observational studies would be needed prior to the launch of clinical trials assessing safety, dosing, and delivery of folate in asthma treatment. In addition, recommendations could vary based on timing, sex, race, and ethnicity, and these factors will need to be explored in future studies.

In evaluating the impact of the murine model study, Miller[74] speculates about a connection between folate supplementation and increasing asthma prevalence, but cautions against drawing conclusions from an animal model. Moreover, the primary increase in asthma prevalence in the United States occurred prior to the mandatory folic acid supplementation of foods[75].

Some investigators suggest that there could be an optimal time for folic acid supplementation, in which neuroprotective effects are maintained but atopy risks are minimized[14,76]. The decision-making process governing folate supplementation could differ depending on the patient population[50]. In populations at lower risk for asthma, for example, maternal folate supplementation could be even more strongly encouraged in order to limit risks of spina bifida and pregnancy-associated anemia.

Ultimately, public health recommendations directed toward asthma—or any other perceived supplementation risk—will depend on our thorough understanding of how folate reduces NTD. Some women who take folic acid still have offspring with NTD, which has prompted a search for other nutritional or genetic factors underlying NTD[77]. The delineation of the dose-response relationship between folate and NTD will be needed to guide future public health recommendations[72] for other disease processes such as asthma.

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