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The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort

R. Granell, J. Heron, S. Lewis, G. D. Smith, J. A. C. Sterne and J. Henderson
ALSPAC, Department of Social Medicine, University of Bristol, Bristol, UK

Clinical and Experimental Allergy

Summary

Background A recent study suggested a link between folate metabolism and atopy, based on a positive association between a common polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene and allergic sensitization in Danish adults.

Objective We investigated the associations between MTHFR C677T and allergy or atopy in a large, population-based birth cohort of children and their mothers, the Avon Longitudinal Study of Parents and Children (ALSPAC). We also looked for evidence of a pre-natal effect of maternal folate metabolism on subsequent atopic disease in the offspring.

Methods Mothers were recruited in pregnancy and the children followed from birth. Atopy in the child was assessed at 7–8 years of age by skin prick tests to common allergens. Asthma was defined as a physician diagnosis and current symptoms at 71/2 years of age. Asthma and allergy status of the mothers were obtained from self-completion questionnaires.

Results Data on MTHFR C677T genotype and allergy were available for 5364 children and on allergy and/or asthma for 7356 mothers. In children, the prevalence of atopy was 20.0% and asthma 10.0% whereas in mothers, the prevalence of self-reported allergy was 42.7% and asthma 11.5%. Atopy in the child was associated with male gender ($P < 0.001$), less tobacco smoke exposure and higher maternal education. MTHFR C677T genotype was not associated with social factors or dietary folate intake. We found no evidence of associations between the MTHFR C677T variant allele and atopy, allergy or asthma in mothers or children. There was no evidence to support an effect of maternal MTHFR C677T genotype on atopy in the offspring.

Conclusion The results of this study do not support the hypothesis that impaired folate metabolism is associated with allergy in adults or children in this population.

Correspondence:
Dr John Henderson, ALSPAC, Department of Social Medicine, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ, UK.
E-mail: a.j.henderson@bris.ac.uk

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Introduction

Atopy is a condition characterized by an individual tendency to develop IgE-mediated responses to environmental allergens and manifests as allergic inflammation in target organs, including the skin, nose and airway. A range of environmental exposures during critical periods of development, including early life, has been proposed to explain recent observed increases in the prevalence of atopic diseases, particularly in industrialized countries [1] but there is still no clear consensus on the exposures responsible for the rise in allergy during the latter half of the 20th century. A recent report suggested a link between folate metabolism and atopy, based on a positive association between a common polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene and allergic sensitization in Danish adults [2]. Folate is an essential vitamin cofactor and deficiency or impaired metabolism, associated with raised plasma homocysteine concentration, has been associated with activity of other inflammatory conditions, such as arthritis [3], and isolated folate deficiency has been associated with alterations of both cell-mediated and humoral immunity in human and animal studies [4].

Homozygosity for the T allele of the single-nucleotide polymorphism, C677T [5] of the MTHFR gene is associated with reduced enzyme activity, hyperhomocysteinaemia [6] and alterations of red blood cell folate distribution [7]. Unlike folate intake, genotype is not likely to be influenced by social, behavioural or lifestyle variables. Therefore, using the principles of Mendelian randomization [8, 9], associations between the functional polymorphism of MTHFR C677T and atopic outcomes are unlikely to be confounded by social or behavioural factors and are more likely to be representative of a causal relationship. Therefore, we investigated the associations between MTHFR C677T and atopy in a large, population-based cohort of children and their mothers, the Avon Longitudinal Study of Parents and Children (ALSPAC), to confirm the observations of Husemoen et al. [2] and to look for evidence of a pre-natal effect of maternal folate metabolism on subsequent atopic disease in their offspring.

Materials and methods

The ALSPAC is a longitudinal, population-based birth cohort study that recruited 14 541 pregnant women residing in Avon, UK, with expected dates of delivery between 1 April 1991 and 31 December 1992. There were 14 062 liveborn children. The study protocol has been described previously [10, 11] and further details are on the ALSPAC website: http://www.alspac.bris.ac.uk. Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

Data were collected using self-completion questionnaires sent to the mothers during pregnancy and approximately annually since the birth of the child. Hands-on assessments were carried out at annual research clinics from the age of 7 years. DNA was extracted from cord blood samples and venous blood collected at 7 years of age.

Assessment of atopy

The child's atopic status was determined by skin prick test (SPT) responses to a panel of allergens at 7–8 years of age. Briefly, the skin of the anterior surface of the left forearm was cleaned and allergen extracts applied. A new, sterile lancet was used to prick the skin through each allergen. The allergens were blotted off after 5 min and the response measured after 15 min as the mean diameter of the resulting weal (the mean of the maximal diameter and its perpendicular). SPTs were performed to house dust mite (HDM) (*Dermatophagoides pteronyssinus*), mixed grass, cat, peanut, mixed nut and egg white (ALK Abelló, Hoersholm, Denmark) and to positive (1% histamine) and negative (diluent) controls in all subjects. Supplementary panels of animals, foodstuffs and aeroallergens were also used on a quasi-random basis in addition to the core panel above. A positive response was defined as a mean weal diameter $\geqslant 2$ mm. We have previously reported in this population that a positive-SPT to one or more of cat, grass or HDM identifies $>95\%$ of children with any positive SPT [12], so this definition of atopy was used in subsequent analyses.

Assessment of asthma and atopic asthma

Asthma in children was defined as a positive response to a question at 91 months (7 1/2 years), 'Has a doctor ever told you that your child had asthma?' together with a positive response to a question about wheeze during the previous 12 months. Atopic asthma was defined similarly with the addition of any positive SPT response and non-atopic asthma was characterized by the absence of a positive skin test response at age 7 years.

History of asthma and/or allergy in the mother was obtained from a self-completion questionnaire administered during pregnancy.

Assessment of folate intake

Dietary folate intake (μg/day) of mothers at 32 weeks of pregnancy (excluding supplements) and of children at 3 and 7 years of age were estimated from individual 3-day food frequency questionnaires as described previously [13–15]. Additionally, the reported use of folate supplements by the mother was obtained from self-completion questionnaires administered at 18 and 32 weeks of pregnancy.

Determination of methylenetetrahydrofolate reductase (C677T) genotype

MTHFR C677T genotype was determined by K-Biosciences Ltd (Hoddesdon, Herts, UK; www.kbioscience.co.uk), who use their own form of competitive allele-specific PCR system (KASPar) and Taqman™, for SNP analysis.

Possible confounding variables

The child's exposure to environmental tobacco smoke was obtained from maternal questionnaires, which asked about her personal smoking habit during and after pregnancy. Maternal educational attainment [GCE ordinary level (equivalent to school leaving certificate at age 16 years) or lower; GCE advanced level or higher education] and employment (manual, non-manual) were obtained from self-completion questionnaires during pregnancy and mother's age at delivery was obtained from clinical records.

Statistical analysis

The analyses were limited to individuals (children or mothers) with complete data on atopy and MTHFR C677T genotype. Thirteen child–mother pairs (0.2%) were excluded due to incompatibility of the genotyping (CC child and TT mother or TT child and CC mother), subsequently found to be due to the mismatching of mother–child samples.

We first analysed relationships between allergy and atopy, respectively, in the mothers and children with dietary determinants of folate status and possible confounders, including maternal age, mothers' smoking during and after pregnancy, mothers' education and social class, and the gender of the child. To check whether that genotype was random with respect to environmental and social variables [9], we analysed the associations of these confounding variables with maternal and child MTHFR C677T genotype using *F*-tests for continuous data and χ^2-tests for categorical data across the three genotype groups (CC, CT and TT).

The associations between MTHFR C677T and atopic outcomes were analysed independently for maternal and child cohorts using χ^2-tests. For children, the outcomes considered were atopy, asthma and atopic asthma. A Wald test was performed after multinomial logistic regression to compare the atopic asthmatic vs. the non-atopic asthmatic children. For mothers, the outcomes considered were self-reported allergy or asthma. We also examined possible interactions between folate intake and genotype in mothers and children on the respective outcomes of self-reported allergy and objectively measured atopy using multivariable analyses with adjustment for folate intake or supplementation as appropriate and for possible confounding variables.

Finally, the effect of maternal MTHFR C677T genotype on children's atopy was considered by analysing the relationship between childhood atopy and maternal genotype, stratified by the child's genotype. All analyses were carried out using Stata 9.0 (Stata Corp, College Station, TX, USA).

Results

A total of 5364 children (2748 males) had complete data on atopy and MTHFR C677T genotype and 3940 children had data on asthma and genotype. The characteristics of the mothers and children included in the analyses are shown in Table 1. Children's mean dietary intake of folate increased from 159 μg/day at 3 years to 213 mg/day at 7 years of age. Maternal mean folate intake in late pregnancy was 256 μg/day and a higher proportion of mothers reported taking folate supplements in late (30.1%) than early (12.1%) pregnancy. Around 57% of mothers were in the lower educational attainment category, 26.6% reported smoking during pregnancy and 18.5% of children were exposed to environmental tobacco smoke.

The prevalence of atopy in the children included in this study was 20.0%, compared with 20.7% in the total population ($n = 6518$) tested for atopy [12]. The prevalence of asthma was 10.0%. There were 7356 mothers [mean (SD) age at delivery 28.4 (4.7) years] with self-reported allergy and MTHFR data. The prevalence of reported allergy and asthma was 42.7% and 11.5% respectively, in these women.

The distribution of MTHFR C677T genotypes in the children was 45.0% CC homozygotes, 44.2% CT heterozygotes and 10.8% TT homozygotes and in the mothers it was 44.8% CC, 44.1% CT and 11.1% TT. The overall frequency of the T allele was 33% and neither children's nor mothers' genotype distributions deviated significantly from Hardy–Weinberg equilibrium.

Table 1 shows the prevalence of atopy in children and self-reported allergy in mothers according to dietary folate intake, child's sex and maternal social factors. Atopy in the child was not associated with their dietary folate intake or with the mother's intake during pregnancy. More boys than girls had atopy at 7 years ($P < 0.001$) and atopy in children was negatively associated with exposure to pre-natal and post-natal tobacco smoke. Mothers of atopic children were more likely to be in non-manual occupations compared with mothers of non-atopic children.

Maternal self-reported allergy was positively associated with maternal dietary folate and folate supplementation during pregnancy and was negatively associated with low educational attainment, although not with employment status. Analysis of mean difference in dietary folate intake according to maternal social factors demonstrated that mothers with low educational attainment had a lower mean [95% confidence interval (CI)] daily folate intake [−28 μg/day (−31.5, −24.6)] than more highly educated mothers and those in manual occupations had a lower intake [−14 μg/day (−18.7, −9.4)] than non-manual workers.

Table 2 shows associations of atopy and self-reported allergy with the MTHFR C677T genotype in children and mothers, respectively. There was no evidence to support an effect of MTHFR C677T genotype on atopy or asthma in either population. When we stratified asthma by atopic status, there was some evidence that the prevalence of asthma subtypes varied by genotype (Wald's test, $P = 0.048$), with higher prevalence in heterozygotes than in either of the two homozygote groups.

MTHFR C677T genotype was not consistently associated with any of the dietary or social variables considered. There was no evidence that maternal folate intake ($P = 0.89$) and use of folate supplements at 18 weeks ($P = 0.48$) or at 32 weeks of gestation ($P = 0.37$) differed

Table 1. Characteristics of children and mothers cohorts according to the presence of allergy

	Children's atopy				Mothers' self-reported asthma and/or allergy			
	Total ($N = 5364$)	Yes ($N = 1075$)	No ($N = 4289$)	P-value*	Total ($N = 7356$)	Yes ($N = 3308$)	No ($N = 4048$)	P-value*
Continuous variables								
Age [mean (SD)]	–	–	–	–	28.4 (4.7)	28.5 (4.8)	28.2 (4.7)	0.007
Child's daily folate intake (μg/day) at 3 years [mean (SD)]	158.8 (40.8)	157.8 (42.7)	159.2 (40.4)	0.36	–	–	–	–
Child's daily folate intake (μg/day) at 7 years [mean (SD)]	212.8 (52.1)	211.2 (51.0)	213.2 (52.4)	0.29	–	–	–	–
Mother's daily folate intake (μg/day) during pregnancy [32 weeks gestation, mean (SD)]	255.8 (70.1)	255.6 (70.0)	255.8 (70.1)	0.91	249.6 (71.7)	251.8 (72.4)	247.8 (71.1)	0.02
Binary variables				P-value**				P-value**
Mother took folate supplement during pregnancy [18 weeks, N (%)]	631 (12.1)	126 (11.9)	505 (12.1)	0.89	790 (11)	395 (12.2)	395 (10.0)	0.003
Mother took folate supplement during pregnancy [32 weeks, N (%)]	1521 (30.1)	331 (32.5)	1190 (29.5)	0.07	2008 (29.2)	949 (30.5)	1059 (28.0)	0.02
Child's gender [number of males (%)]	2748 (51.2)	651 (60.6)	2097 (48.9)	<0.001	–	–	–	–
Mother smoked during pregnancy [N (%)]	1402 (26.6)	257 (24.3)	1145 (27.2)	0.05	2262 (31.3)	980 (30.1)	1282 (32.4)	0.04
Child's post-natal tobacco smoke exposure [N (%)]	915 (18.5)	163 (16.3)	752 (19.1)	0.04	1432 (22.2)	625 (21.3)	807 (22.9)	0.14
Mothers with GCE O-level education or lower [N (%)]†	2923 (56.6)	558 (53.7)	2365 (57.3)	0.04	4453 (63.3)	1869 (58.7)	2584 (67.1)	<0.001
Mothers with manual job [N (%)]	733 (16.3)	121 (13.2)	612 (17.1)	0.005	1097 (18.7)	483 (18.1)	614 (19.2)	0.28

†Compared with GCE A-level or higher education.
*P-value for T-test.
**P-value for χ^2-test.

Table 2. Prevalence of childhood atopy and asthma and maternal self-reported allergy and/or asthma by MTHFR genotype

	Child's MTHFR			
Childhood outcomes	CC ($N = 2416$)	CT ($N = 2639$)	TT ($N = 579$)	P-value*
Atopy [N (%)]	483 (20.0)	488 (20.6)	104 (18.0)	0.36
Asthma [N (%)]	178 (9.9)	171 (10.0)	44 (10.4)	0.94
Atopic asthma [N (%)]	90 (5.0)	104 (6.1)	19 (4.5)	0.048
	Mother's MTHFR			
Mothers' self-reported outcomes	CC ($N = 3295$)	CT ($N = 3245$)	TT ($N = 816$)	P-value*
Asthma and/or allergy [N(%)]	1488 (45.2)	1449 (44.7)	371 (45.5)	0.88
Asthma [N (%)]	386 (11.6)	365 (11.1)	92 (11.2)	0.80
Allergy [N (%)]	1423 (43.1)	1363 (41.8)	349 (42.7)	0.53

*P-value for χ^2-test.
MTHFR, methylenetetrahydrofolate reductase.

across maternal C677T genotypes. The same was true for children's dietary folate intake at 3 years ($P = 0.41$) and 7 years ($P = 0.21$) with regard to the child's genotype. Maternal dietary intake of folate at 32 weeks of gestation was higher when the child had CT or TT compared with the CC genotype ($P = 0.045$). None of the other variables considered was consistently associated with either maternal or child's genotype.

Table 3. Adjusted associations of dietary and supplemented folate intake with atopy in children and self-reported allergy in mothers and interactions with MTHFR genotype

	Childhood atopy [OR (95% CI)]				
Continuous variables	Overall ($N = 3747$)	MTHFR CC ($N = 1663$)	MTHFR CT ($N = 1658$)	MTHFR TT ($N = 426$)	P_interaction*
Children folate intake at 3 years (per 100 μg/day)†	0.86 (0.71, 1.05)	0.86 (0.64,1.16)	0.84 (0.63,1.13)	0.88 (0.49,1.61)	0.98
Children folate intake at 7 years (per 100 μg/day)†	0.91 (0.78, 1.07)	0.84 (0.66, 1.08)	0.98 (0.78, 1.23)	0.95 (0.58, 1.56)	0.70
Mothers folate intake at 32 weeks (per 100 μg/day)‡	0.98 (0.88, 1.10)	0.87 (0.73, 1.03)	1.11 (0.94, 1.30)	0.98 (0.69, 1.39)	0.22
Binary variables	Overall ($N = 4176$)	MTHFR CC ($N = 1853$)	MTHFR CT ($N = 1856$)	MTHFR TT ($N = 467$)	P_interaction*
Mother took supplement folate at 18 weeks§	0.99 (0.78, 1.25)	0.75 (0.51, 1.09)	1.31 (0.95, 1.82)	0.78 (0.35, 1.75)	0.08
Mother took supplement folate at 32 weeks§	1.15 (0.98, 1.35)	0.91 (0.71, 1.18)	1.35 (1.07, 1.71)	1.44 (0.88, 2.37)	0.05
	Mothers' self-reported allergy [OR (95% CI)]				
Continuous variables	Overall ($N = 5259$)	MTHFR CC ($N = 2348$)	MTHFR CT ($N = 2319$)	MTHFR TT ($N = 592$)	P_interaction*
Mothers folate intake at 32 weeks (per 100 μg/day)‡	1.03 (0.95, 1.12)	0.97 (0.86, 1.09)	1.04 (0.92, 1.17)	1.22 (0.97, 1.54)	0.31
Binary variables	Overall ($N = 5250$)	MTHFR CC ($N = 2344$)	MTHFR CT ($N = 2319$)	MTHFR TT ($N = 587$)	P_interaction*
Mother took supplement folate at 18 weeks§	1.16 (0.97, 1.37)	1.21 (0.94, 1.56)	1.03 (0.79, 1.33)	1.65 (0.93, 2.93)	0.30
Mother took supplement folate at 32 weeks§	1.09 (0.96, 1.22)	1.23 (1.03, 1.46)	1.01 (0.84, 1.21)	0.86 (0.60, 1.23)	0.13

†Adjusted for exposure to pre-natal and post-natal smoking, maternal education and social class.
‡Adjusted for mothers' folate supplementation at 32 weeks, pre-natal and post-natal smoking, maternal education and social class.
§Adjusted for mothers folate intake at 32 weeks, pre-natal and post-natal smoking, maternal education and social class.
OR, odds ratio; CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.

Adjusted associations between folate intake and supplementation and interactions with the MTHFR genotype on self-reported allergy or atopy are shown in Table 3. Adjustment for confounding had only a small attenuating effect on the effect estimates. Children's estimated dietary folate did not show evidence of interaction with their MTHFR C677T genotype on the risk of atopy. However, there was some evidence of an interaction between maternal folate supplementation at 32 weeks of gestation and the child's genotype on the association with childhood atopy. This finding was not supported by strong evidence of a similar interaction between the child's genotype and maternal dietary folate at 32 weeks gestation or folate supplementation in earlier pregnancy on atopy risk. In mothers, there was also some evidence that increased folate intake and folate supplementation at 18 weeks but not 32 weeks of gestation interacted with the MTHFR TT genotype to increase the risk of allergy in these women.

We also investigated the possibility of an interaction between the effects of maternal smoking and the MTHFR C677T genotype on the risk of atopy in children or self-reported allergy in the mothers (Table 4). This suggested an increased risk of atopy in children in association with the CT genotype and pre-natal tobacco smoke exposure and a decreased risk in association with the TT genotype and post-natal tobacco smoke exposure, but there was no strong evidence for an overall interaction between maternal smoking and the child's MTHFR genotype on the risk of childhood atopy [P(interaction) = 0.16].

Table 5 shows associations between children's atopy and maternal genotype, according to the child's genotype. Children who were homozygous for the wild-type allele (CC) had a greater prevalence of atopy if their mother was also homozygous (CC) compared with heterozygous (CT) mothers. This does not support the hypothesis that the variant C677T allele in the mother is a risk factor for atopy in the child. To further investigate this finding, we stratified the analysis by maternal allergic status and found that this pattern was also seen in the children of mothers with no self-reported allergy (Child CC/Mother CC 21.1%, Child CC/Mother CT 13.1%; $P = 0.006$) and not

Table 4. Interactions between maternal smoking status and MTHFR genotype on risk of atopy in children or self-reported allergy in mothers

	Risk of children's atopy [OR (95% CI)]		
Smoking status	MTHFR CT	MTHFR TT	*P*_interaction
Crude (*N* = 4892)	1.07 (0.92, 1.24)	0.92 (0.72, 1.17)	
Adjusted (*N* = 4892)*	1.06 (0.92, 1.23)	0.91 (0.72, 1.16)	
Non-exposed to maternal smoking (*N* = 3595)	1.02 (0.86, 1.21)	1.01 (0.77, 1.32)	
Exposed to pre-natal maternal smoking only (*N* = 399)	1.60 (0.94, 2.71)	0.81 (0.29,2.26)	
Exposed to post-natal maternal smoking (*N* = 898)	1.07 (0.75, 1.53)	0.54 (0.27,1.08)	0.16
	Risk of mothers' self-reported allergy [OR (95% CI)]		
	MTHFR CT	MTHFR TT	*P*_interaction
Crude (*N* = 7224)	0.98 (0.88, 1.08)	1.02 (0.88, 1.2)	
Adjusted (*N* = 7224)†	0.97 (0.88, 1.07)	1.03 (0.88, 1.2)	
Smoker (*N* = 2262)	1.05 (0.88, 1.25)	1.05 (0.8, 1.38)	
Non-smoker (*N* = 4962)	0.94 (0.84, 1.06)	1.02 (0.84, 1.23)	0.60

*Adjusted for maternal smoking status (0 = none, 1 = pre-natal only, 2 = post-natal independently of pre-natal) reference group is CC.
†Adjusted for maternal smoking status based on pre-natal report reference group is CC.
OR, odds ratio; CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.

Table 5. Prevalence of childhood atopy by maternal genotype; stratified by child's genotype

	Mothers' MTHFR			
	CC	CT	TT	*P*-value*
	(*N* = 1003)	(*N* = 531)		
Children's atopy/ CC children [*N* (%)]	225 (22.4) (*N* = 488)	85 (16.0) (*N* = 774)	– (*N* = 248)	0.003
Children's atopy/ CT children [*N* (%)]	105 (21.5)	169 (21.8) (*N* = 221)	57 (23.0) (*N* = 128)	0.90
Children's atopy/ TT children [*N* (%)]	–	38 (17.2)	21 (16.4)	0.85

*P-value for χ^2-test.
MTHFR, methylenetetrahydrofolate reductase.

in children of those with a reported history of allergy (data supplement available on request). However, there was no strong evidence for an interaction between maternal history of allergy and child's genotype on the association with atopy in children (CC children, *P* (interaction) = 0.24; CT children, *P* (interaction) = 0.74; TT children, *P* (interaction) = 0.84].

Discussion

We have shown in a large, population-based birth cohort that a common functional polymorphism of the MTHFR gene (C677T) is not associated with self-reported allergy or asthma in adult women or atopy in their children, in contrast to previous studies that have reported associations between homozygosity for the minor allele (TT) and atopy [2] or atopic asthma [16]. Additionally, because we had access to data from a large number of mother–child pairs, we were able to investigate the possible effect of maternal MTHFR genotype on atopy in their offspring. This also demonstrated a lack of association between homozygous TT mothers and atopy in their children. These data suggest that a folate-deficient state associated with MTHFR C677T in this contemporary population of mothers and children is not linked to the development of allergy. Although we did find an interaction between maternal folate supplementation at 32 weeks and the child's MTHFR C677T genotype in association with childhood atopy, the direction of this association opposed the prior hypothesis that folate deficiency was a risk factor for atopy.

It has been suggested that dietary folate intake may be suboptimal in contemporary European populations [17]. Folate is an essential co-factor for one-carbon transfers, necessary for protein and DNA methylation and for the synthesis of nucleotides [18]. Folate deficiency has been suggested as a risk factor for adverse health outcomes, including neural tube defects [19], cardiovascular diseases [20] and some forms of cancers [21] but there is uncertainty about the relative contributions of reduced folate status and increased plasma homocysteine levels to these outcomes and randomized-controlled studies have not supported a beneficial effect of folate supplementation on cardiovascular disease [22]. MTHFR C677T is a functional polymorphism that leads to increased plasma homocysteine and reduced folate concentrations in subjects homozygous for the minor T allele [23]. Studies of this genotype, particularly in the presence of low dietary folate status, have suggested associations with coronary heart disease [24], although a more recent meta-analysis

has cast doubt on the causal inferences of this study [25], neural tube defect, cleft lip and palate, and psychiatric morbidity including schizophrenia [26] and depression [27]. Recently, Husemoen et al. [2] have reported an association between MTHFR C677T and allergy in adults, with some evidence of an interaction between dietary folate status and MTHFR TT genotype, although the precise mechanism of this association remains unclear. It has been reported that increased dietary folate can attenuate or negate the adverse effects of MTHFR C677T [28], hence it would be reasonable to predict that high folate intake might obscure a true relationship between the TT genotype and allergy. We did not have available plasma folate and homocysteine concentrations in our populations but, on the basis of estimated dietary folate intake of mothers and children, we found that folate supplementation by mothers during pregnancy was associated with an increased risk of atopy in children carrying the T allele and the increased dietary intake of folate in pregnancy was associated with a higher risk of self-reported allergy in mothers with the TT genotype. As these effects were in the opposite direction to the predicted effects of higher folate intake attenuating the increased risk associated with TT genotype, we believe they are likely to be chance findings. Another possibility is that folate supplementation has an independent association with increased prevalence of atopy. However, if true we would have expected such an effect to be stronger in association with the wild-type homozygote genotype.

In keeping with observations from other studies [29], we found relationships between dietary folate intake and a number of social and lifestyle variables (data available on request). However, as expected, there was no evidence of association between the MTHFR genotype and any of the social or behavioural variables studied. Therefore, the analyses reported here are not likely to have been confounded by environmental variables or modified by maternal folate intake or supplementation

Although our study is marked by a large sample size and a well-characterized population, there were some potential weaknesses that may have influenced our findings. The population that was available for analysis in this study was selected on the basis of the availability of outcome variables for children at 7 years. In common with the majority of longitudinal cohort studies, attrition of the original sample resulted in the study population having a lower proportion of women from lower socioeconomic categories, fewer women who reported smoking during pregnancy and an increased proportion of women with higher educational attainment. This may account for some differences between our study population and the Danish population reported by Husemoen et al. [2], such as the rates of reported smoking being higher in the latter. However, loss to follow-up is highly unlikely to be associated with genotype and so would not be expected to bias the primary analyses between genotype and outcomes in this study.

Allergic sensitization was objectively measured in the child population but we had to rely on maternal self-report to determine the allergic status of the mothers. The report is based on recall of a lifetime history of allergic diseases at a mean age of around 28 years and is therefore susceptible to recall bias and misclassification. The difference in allergy prevalence between the adult and child populations in this study suggests that allergy was over-reported by the mothers, although the overall prevalence (42.7%) does not greatly exceed the objectively measured prevalence of atopy in a contemporary adult male UK population [30]. Although misclassification of allergy outcome is likely in the maternal population, we believe that it is also likely to be random with respect to genotype and therefore not to have influenced the results shown. However, it is possible that the random misclassification of allergic status in mothers could have attenuated associations towards the null value. When mothers' allergic status was restricted to asthma, the prevalence was closer to the observed prevalence of atopy in the children and there remained no evidence of an association with the MTHFR genotype. We did not have objective measurements of maternal folate status based on blood samples in this study but there is good evidence that MTHFR C677T is a functional polymorphism that is linked to plasma homocysteine levels and modified by dietary folate status [31]. Although, a deficiency state associated with TT homozygosity might influence dietary behaviour, there was no evidence from our analyses that maternal folate intake varied by the MTHFR C677T genotype. This was true also for the study by Husemoen et al. [2], in which only plasma homocysteine measurements were strongly associated with the MTHFR C677T genotype. Therefore, we believe it is unlikely that we have overlooked a true positive association between folate metabolism and allergy in this study. The differences between our findings and those of Husemoen et al. are not readily explicable. Estimated dietary folate intakes in our study were lower than those reported by these authors. It is conceivable that dietary modifications during pregnancy attenuated pre-existing population differences in folate intake, thus obscuring an association between dietary folate and allergy in women in our study. Also, lifestyle and behavioural factors that may have modified associations between folate status and allergy, such as smoking status, differed between the two populations although we found no consistent evidence that any of the factors considered in this study interacted with the MTHFRC677T genotype to modify the risk of allergy in the direction proposed in the Danish population.

In summary, the results of the present study do not support a role for impaired folate metabolism associated with MTHFR C677T polymorphism in the aetiology of

allergy in a contemporary British cohort of adults and children. Additionally, we found no evidence that the maternal MTHFR C677T genotype was associated with increased risk of objectively measured atopy in children at the age of 7 years.

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