

## CLINICAL STUDY

# The methylenetetrahydrofolate reductase C677T genotype and the risk of obesity in three large population-based cohorts

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## Abstract

**Objective:** Epidemiological studies have shown that low folate levels are associated with a high body mass index (BMI). These findings have potentially important health implications and warrant further investigation to determine whether a causal relationship exists and the direction of this relationship. The methylenetetrahydrofolate reductase (*MTHFR*) C677T *TT* genotype is associated with reduced folate availability and may be a surrogate for measuring folate levels. We sought to determine whether *MTHFR* C677T genotype was associated with obesity.

**Design:** We carried out our study on four populations from three longitudinal studies based in the UK and Denmark in which DNA for genotyping was obtained along with measures of obesity.

**Methods:** Our subjects were taken from the British Women's Heart and Health Study (BWHHS), the Avon Longitudinal Study of Parents and Children (two populations: mothers and children) and the Copenhagen City Heart Study. We performed analyses separately by population, and then carried out a meta-analysis, combining similar populations.

**Results:** Initial findings in the BWHHS suggested that the *TT* genotype may be associated with an increased risk of obesity BMI  $\geq 30$ , however, no association was found with BMI or central adiposity in this cohort. This genotype was not associated with obesity in our other cohorts.

**Conclusions:** Our results suggest that the initial positive finding with obesity in the BWHHS was a chance finding. Our findings do not support a causal effect of low folate on obesity.

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## Introduction

Recent findings that low folate levels are associated with a high body mass index (BMI) (1–3) have potentially important health implications. The apparent association warrants further investigation to determine whether a causal relationship exists as the current obesity epidemic is a major public health issue, affecting the whole population irrespective of age, gender and ethnic group. Folate supplementation of commonly consumed foods (e.g. flour), currently being considered in the UK (Scientific Advisory Committee on Nutrition. Folate and Disease Prevention, TSO, London, 2006), could be a potential population level intervention to reverse the obesity epidemic if low folate levels are indeed causally related to obesity.

Lower serum folate levels have been found to be strongly associated with increased BMI in women of childbearing age in two waves of the National Health and Nutrition Examination Survey, each 10 kg/m<sup>2</sup>

increase in BMI was associated with a 15.6% decrease in serum folate ( $P < 0.001$ ), an association that persisted even after controlling for age, ethnicity, folate intake and red blood cell folate (1). A further large cross-sectional study of women in prenatal care replicated the finding of an association between low serum folate levels and obesity (2). Also, a small case-control study found much higher plasma homocysteine, a biomarker of low folate levels, among obese children and adolescents compared with non-obese controls (3). Further, indirect evidence for an association between low folate and obesity is that high pre-pregnancy BMI has been consistently found to be associated with neural tube defects (4, 5), which are caused by low perinatal folate levels (6).

It is possible that lower levels of serum folate are observed among heavier women, simply because their requirements are greater (1). Another plausible explanation for the association between low folate status and obesity could be confounding by dietary habits, since it

is likely that those individuals who have a high-energy diet will eat less fruit, vegetables and cereals and thus have a lower folate intake. However, we have observed an association between the *MTHFR* C677T TT genotype, which is associated with reduced folate availability (7), and obesity in the British Women's Heart and Health Study (BWHHS) (8). Clearly, this genotype could not have been altered by adult BMI, and it is not subject to confounding by lifestyle factors (9, 10). Thus, our *MTHFR* genotype–obesity findings suggest that folate levels may be causally related to greater BMI and obesity.

A potential mechanism by which folate could influence body mass and obesity is via epigenetic control of gene expression. Methylation of cytosines in CpG dinucleotides is an important epigenetic modification, which affects gene expression and thus cellular function. To a certain extent, methylation patterns can be controlled by environmental factors such as intake of dietary folate, which is an important donor of methyl groups required for methylation. Folate depletion in humans has been observed to diminish genomic DNA methylation (11, 12). The hypothesis that epigenetic changes including methylation are linked to adult obesity is supported by the observation that in humans several genes have been shown to exhibit changes in expression that correlated closely with BMI and/or waist/hip ratio (13). Further, obesity is one of the symptoms of Prader–Willi syndrome that is caused by irregular DNA methylation patterns in a given region on chromosome 15q (14).

Preliminary analysis has shown that the *MTHFR* C677T TT genotype was associated with an ~20% increase in the prevalence of obesity in the BWHHS, although only very small differences in BMI were observed by genotype (8). Since initial positive genotype–phenotype associations frequently fail to replicate (15), we sought to examine the association of *MTHFR* C677T genotype with BMI and obesity in two further population-based cohorts and to present a full analysis in the BWHHS.

## Methods

### Study population

We examined the association between the *MTHFR* genotype in four distinct populations within three cohort studies, namely the BWHHS, the Avon Longitudinal Study of Parents and Children (two populations: mothers and children) and the Copenhagen City Heart Study (CCHS).

**The British Women's Heart and Health Study (BWHHS)** Between 1999 and 2001 4286 women aged 60–79 years, who were randomly selected from 23 British towns were interviewed, examined, completed

medical questionnaires and had detailed reviews of their medical records. Of 4286 women who participated in the BWHHS, 3938 (92%) had complete data on all anthropometric measurements and of these 3438 (87%) provided consent for genetic testing and had adequate *MTHFR* genotype assays. Twenty women were excluded because they had non-white ethnicity, leaving a final sample of 3416.

**The Avon Longitudinal Study of Parents and Children (ALSPAC)** A population-based prospective study investigating factors that affect the health and development of children and their parents. Pregnant women living in Bristol, England, who had an expected date of delivery between April 1991 and December 1992, were eligible. A total of 14 541 women enrolled in the study. Extensive data have been collected on the children and their mothers from pregnancy onwards by questionnaire, abstraction from medical notes, record linkage and by attendance at research clinics. Of 8128 women with genotype data, information on pre-pregnancy BMI was available for 6952 (86%), 461 (7%) women were excluded due to non-white or unknown ethnicity, leaving a final sample of 6491 women. Of 8782 children with genotype data, information on BMI at around age 9 was available for 5619 (64%), a further 489 (9%) children were excluded due to non-white or unknown ethnicity, leaving a final sample of 5130 children.

**Copenhagen City Heart Study (CCHS)** A prospective cardiovascular study of individuals selected from the Central-Population-Register Code designed to reflect the adult Danish general population, including both men and women. Those invited were stratified into 5-year age groups from 20 to 95 years, with main emphasis placed on the 35- to 70-year olds. In the CCHS, 9252 individuals were available for genotyping and 9238 received a final genotype, 9173 individuals of those genotyped had data on all measures of adiposity and so were included in the final analysis. The cohort is very ethnically homogenous so no participants were excluded on the basis of ethnicity. The Danish Ethics Committees for the cities of Copenhagen and Frederiksberg approved the study. Informed consent was obtained from participants.

### Measurement of weight at height

Weight and height were measured using standard procedures and were used to calculate BMI; in all studies obesity was defined using the WHO standard threshold of 30 kg/m<sup>2</sup> for adults. For the ALSPAC children, the following cut-offs were used; males >23.39 kg/m<sup>2</sup> and

**Table 1** Distribution of methylenetetrahydrofolate reductase (*MTHFR*) genotype among the cohorts in this study.

	CC	CT	TT	T-allele frequency	P for Hardy–Weinberg
BWHHS	1525 (44.7%)	1496 (43.7%)	395 (11.6%)	0.33	0.28
CCHS	4400 (48.0%)	3930 (42.8%)	843 (9.2%)	0.31	0.42
ALSPAC mothers	2870 (44.2%)	2868 (44.1%)	753 (11.6%)	0.34	0.37
ALSPAC boys	1170 (45.2%)	1138 (43.9%)	282 (10.9%)	0.33	0.83
ALSPAC girls	1100 (43.3%)	1145 (45.1%)	295 (11.6%)	0.34	0.91

females  $> 23.46 \text{ kg/m}^2$  (16). In the ALSPAC mothers, self-reported pre-pregnancy weight and waist and hip circumference were used, which was obtained at the 18-week antenatal clinic. In the ALSPAC children, weight and height at around age 9 years measured by trained nurses at the clinic were used in this analysis. In the BWHHS, all measurements were undertaken at the baseline clinical examination and were measured in the clinic by trained nurses. In the CCHS, the participants were interviewed at baseline at Rigshospitalet, Copenhagen University Hospital and weight and height were measured.

### Genotyping

DNA was extracted by salting out procedure (17). Genotyping in the ALSPAC and the BWHHS was undertaken by KBioscience Ltd ([www.kbioscience.co.uk](http://www.kbioscience.co.uk)), who use their own form of competitive allele-specific PCR system (KASPar) and Taqman (Applied Biosystem, Foster City, CA, USA) for SNP analysis. In the CCHS, the *MTHFR C677T* polymorphism was genotyped using restriction enzymes digesting the PCR product, which were then separated on a gel (18). Two independent investigators confirmed each genotype.

**Table 2** Distribution of indicators of adiposity by methylenetetrahydrofolate reductase (*MTHFR*) genotype.

Outcome variables	Number (%) for dichotomous variables or mean (s.d.) for continuous variables by genotype			P
	CC	CT	TT	
<i>Obese (&gt; 30 kg/m<sup>2</sup>)</i>	<i>N (%)</i>	<i>N (%)</i>	<i>N (%)</i>	
BWHHS	360/1525 (23.6)	410/1496 (27.5)	112/395 (28.4)	0.02
CCHS females	315/2454 (12.8)	308/2145 (14.4)	57/465 (12.3)	0.24
CCHS males	273/1946 (14.0)	266/1785 (14.9)	50/378 (13.2)	0.61
CCHS ALL	588/4400 (13.4)	574/3930 (14.6)	107/843 (12.7)	0.16
ALSPAC mothers	163/2870 (5.7)	155/2868 (5.4)	38/753 (5.0)	0.77
ALSPAC girls <sup>a</sup>	53/1100 (4.8)	51/1145 (4.4)	18/295 (6.1)	0.50
ALSPAC boys <sup>a</sup>	62/1170 (5.3)	42/1138 (3.7)	7/282 (2.5)	0.05
ALSPAC children ALL	115/2270 (5.1)	93/2283 (4.1)	25/577 (4.3)	0.27
<i>Body mass index (kg/m<sup>2</sup>)</i>	<i>Mean (s.d.)</i>	<i>Mean (s.d.)</i>	<i>Mean (s.d.)</i>	
BWHHS	27.4 (4.7) N=1525	27.7 (5.2) N=1496	27.8 (5.2) N=395	0.14
CCHS females	25.0 (4.5) N=2454	25.2 (4.6) N=2145	24.9 (4.5) N=465	0.13
CCHS males	26.0 (3.9) N=1946	26.0 (4.0) N=1785	25.9 (3.9) N=378	0.90
CCHS ALL	25.4 (4.3) N=4400	25.6 (4.4) N=3930	25 (4.3) N=843	0.18
ALSPAC mothers	23.0 (3.9) N=2870	23.0 (3.8) N=2868	22.9 (3.7) N=753	0.64
ALSPAC girls	17.8 (2.9) N=1100	17.8 (2.9) N=1145	17.9 (3.1) N=295	0.86
ALSPAC boys	17.6 (2.9) N=1170	17.3 (2.5) N=1138	17.3 (2.7) N=282	0.06
ALSPAC children ALL	17.7 (2.9) N=2270	17.6 (2.7) N=2283	17.6 (2.9) N=577	0.50
<i>Waist–hip ratio</i>				
BWHHS	0.82 (0.07) N=1525	0.82 (0.07) N=1496	0.82 (0.07) N=395	0.27
CCHS females	0.83 (0.08) N=2454	0.83 (0.08) N=2145	0.83 (0.08) N=465	0.96
CCHS males	0.94 (0.08) N=1946	0.95 (0.08) N=1785	0.95 (0.07) N=378	0.57
CCHS ALL	0.88 (0.10) N=4400	0.88 (0.10) N=3930	0.88 (0.10) N=843	0.59
ALSPAC mothers	0.74 (0.07) N=1634	0.74 (0.06) N=1615	0.74 (0.07) N=407	0.45
<i>Waist circumference (cm)</i>				
BWHHS	85.7 (11.6) N=1525	86.5 (12.5) N=1496	86.5 (12.7) N=395	0.15
CCHS females	99.5 (9.5) N=2454	99.9 (9.8) N=2145	99.1 (9.3) N=465	0.18
CCHS males	100.2 (7.7) N=1946	100.0 (7.4) N=1785	99.9 (7.5) N=378	0.50
CCHS ALL	99.8 (8.7) N=4400	99.9 (8.8) N=3930	99.5 (8.5) N=843	0.36
ALSPAC mothers	68.6 (7.2) N=1891	68.3 (6.8) N=1830	68.3 (7.0) N=491	0.40

<sup>a</sup> $> 23.39 \text{ kg/m}^2$  males and  $> 23.46 \text{ kg/m}^2$  females.

P values are for evidence of heterogeneity across categories (i.e. two degrees of freedom).

### Statistical analysis

Assumptions of Hardy–Weinberg equilibrium were formally tested using a likelihood ratio test. Prevalence (for dichotomous variables) and means (for continuous variables) for obesity and other anthropometric measures were calculated by genotype. One-way ANOVA was used for testing differences between genotypes for continuous variables and  $\chi^2$  tests were used for testing differences between genotypes for dichotomous variables. Pearson  $\chi^2$  tests were used to test for Hardy–Weinberg equilibrium.

Unadjusted odds ratios were calculated for prevalence of obesity among *TT* versus *CC* genotypes, and these data were used to carry out a formal meta-analysis. Since the different studies were carried out on diverse age groups, we carried out three analyses for adults (CCHS and BWHHS), young women (ALSPAC mothers) and children (ALSPAC children). Because the results for the different studies and in different sexes in the ALSPAC were heterogeneous, we undertook random effects meta-analyses.

### Results

We examined the association between obesity and other anthropometric measures and *MTHFR C677T* genotype in four population-based cohorts totalling 24 210 individuals from the United Kingdom and Denmark. The mean age of participants in the CCHS was 57.6

(s.d. = 15.2) years (range 21–93) at the time that adiposity was measured. The mean age of women at early pregnancy and the time of recruitment into ALSPAC were 28.5 (s.d. = 4.7) years (range 15–44), the age of children in the ALSPAC when BMI was measured was 9.9 (s.d. = 0.3) years (range 8.8–11.8). The mean age of participants in the BWHHS was 68.8 (s.d. = 5.5) years (range 59–80). The distribution of age did not differ by genotype in any cohort. T-allele frequencies ranged from 0.31 to 0.34 (Table 1), genotypes were in Hardy–Weinberg equilibrium in all populations.

We found an association between genotype and prevalence of obesity (BMI > 30 kg/m<sup>2</sup>) in the BWHHS, which was not replicated in the other cohorts (Table 2, and Fig. 1). In the BWHHS, as in the ALSPAC and CCHS, *MTHFR C677T* genotype was not associated with BMI, waist circumference or waist–hip ratio (all analysed as continuous variables) in adults. In the ALSPAC boys, there was weak evidence that the *CT* and *TT* genotypes were associated with a reduction in BMI relative to the *CC* genotype, and the risk of obesity appeared to reduce in a dose–response manner with the number of T-alleles. Our meta-analysis found an overall odds ratio for obesity in *TT* versus *CC* adults of 1.06 (95% CI 0.86–1.32). There was no evidence that *MTHFR C677T* was associated with social class, smoking, alcohol intake or physical activity factors that may confound an association between folate intake and obesity (data available from authors).

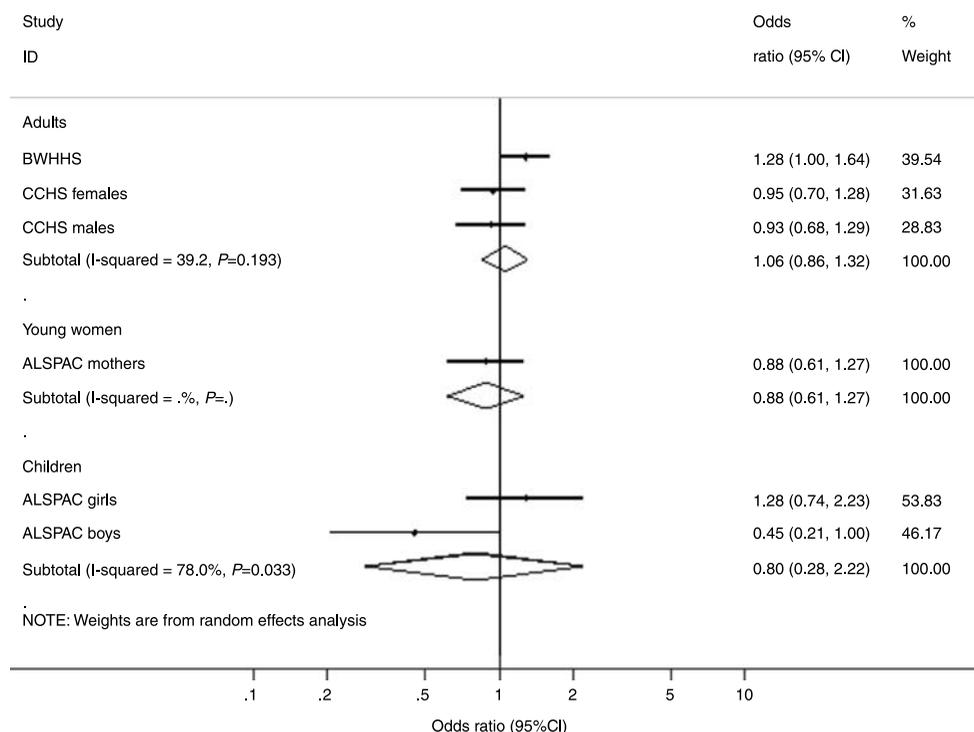


Figure 1 Forest plot showing risk of obesity amongst *MTHFR C677T TT* homozygotes versus *CC* homozygotes.

## Discussion

We found no evidence that the *TT* genotype of the *MTHFR* gene was associated with BMI or obesity in two out of three of our adult cohorts, which suggests that the initial positive finding with obesity in the BWHHS was a chance finding arising due to the large number of association tests that were performed between this genotype and phenotypes in the BWHHS. This emphasizes the need to replicate positive associations in other studies in order to guard against the problem of multiple testing. We found that among boys in the ALSPAC cohort, who were around age 9 at the time of measurement, the T-allele was associated with a reduction in the prevalence of obesity and a reduction in BMI. This most likely could also be a chance finding, but requires further investigation to clarify whether this is the case or whether there is in fact evidence of a protective effect of the T-allele in pre-adolescent boys.

We have used a genetic variant that influences circulating folate levels to test whether low folate availability is a risk factor for obesity. This analysis is not subject to reverse causation since an individual's genotype is determined at conception and cannot be determined by an individual's weight later in life. In addition, this genetic variant that affects the metabolism of folate, for instance, does not appear to be associated with other dietary and lifestyle factors, which are typically associated with dietary folate intake (8–10).

Our findings are consistent with a small study on obesity carried out in Spain (19) and also an Italian study, which found no association between this genotype and metabolic syndrome (20). These findings argue against a role for methylation in determining adult and childhood obesity, particularly as the *MTHFR* C677T *TT* homozygotes have been shown to exhibit a diminished level of DNA methylation compared with *CC* homozygotes (21). However, we are unable to shed any light on whether those who have a greater BMI also have a greater requirement for folate, this question requires further research. A recent family study has suggested that polymorphisms in this gene may be related to lean body mass rather than fat mass (22), whilst our study of BMI (which is a composite of the two) does not support these findings; in this analysis, we did not look at lean body mass separately as lean mass was not measured in all our populations, this hypothesis therefore requires further investigation.

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