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ORIGINAL INVESTIGATION

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A common insertion/deletion polymorphism of the thymidylate synthase (*TYMS*) gene is a determinant of red blood cell folate and homocysteine concentrations

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Abstract Substantial evidence suggests that a low folate/high homocysteine phenotype is pathogenic. We analyzed the impact of the thymidylate synthase (*TYMS*) 3′UTR ins/del polymorphism on folate and homocysteine levels and assessed the relationship between the *TYMS* 3′UTR ins/del polymorphism and key genetic and lifestyle variables. Among non-smokers only, the *TYMS* 3′UTR ins/del polymorphism was significantly associated with red blood cell folate (RBC folate; $P = 0.002$) and homocysteine ($P = 0.03$) concentrations. Median RBC folate concentration was much higher for *TYMS* 3′UTR del/del subjects (434 μg/l) compared with either ins/ins (282 μg/l) or ins/del (298 μg/l) subjects. The median homocysteine concentration for del/del homozygotes was considerably lower compared with either ins/ins homozygotes or ins/del heterozygotes. A possible additive effect for the impact of the *TYMS* 3′UTR del/del and *MTHFR* 677CC genotypes on RBC folate concentration was also observed. Our findings suggest that the *TYMS* 3′UTR del/del genotype is a significant determinant of elevated RBC folate concentration in a non-smoking population of northwestern European adults and that this genotype confers protection against diseases for which a low folate/high homocysteine phenotype appears to be an etiologic component.

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Introduction

Folate functions primarily in the transfer and processing of one-carbon units, reactions that are essential for the remethylation of homocysteine to form methionine, for the synthesis of thymidylate and purines, and for the provision of methyl groups necessary for various methylation reactions. A low folate/high homocysteine phenotype is widely acknowledged to be associated with an increased risk of developing a broad range of medical conditions, such as cardiovascular disease, complications in pregnancy including neural tube defects (NTDs), certain malignancies, and Alzheimer's disease (Lucock 2000). However, whether low folate, high homocysteine, or a combination of both is pathogenic is still much debated.

Thymidylate synthase (TYMS), a key enzyme in the folate/homocysteine metabolic pathway, catalyzes the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate (dTMP), thereby providing the sole intracellular de novo source of dTMP (Liu et al. 2002; Fig. 1). This reaction is essential for pyrimidine biosynthesis and thus for DNA replication and repair. 5,10-Methylenetetrahydrofolate (5,10-methyleneTHF), an essential component of the aforementioned pathway, is a common cofactor for the enzymes TYMS and 5,10-methylenetetrahydrofolate reductase (MTHFR). When folate status is suboptimal, limiting levels of

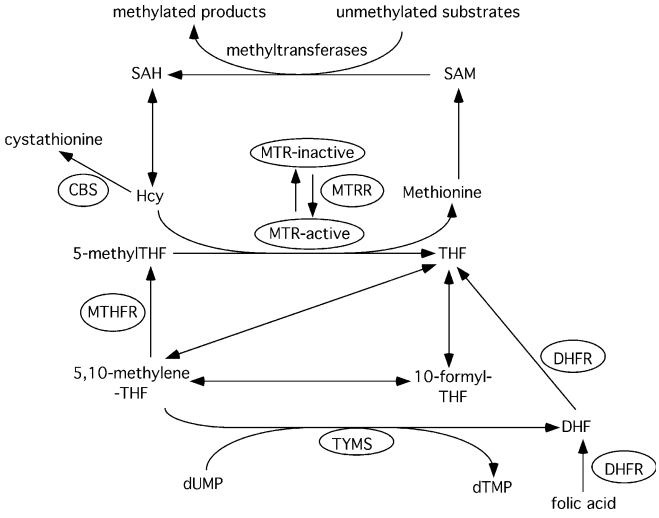

Fig. 1 Schematic representation of homocysteine/folate metabolism (*CBS* cystathionine *β*-synthase, *DHF* dihydrofolate, *DHFR* dihydrofolate reductase, *dTMP* deoxythymidine monophosphate, *dUMP* deoxyuridine monophosphate, *Hcy* homocysteine, *MTHFR* 5,10-methylenetetrahydrofolate reductase, *MTR* methionine synthase, *MTRR* methionine synthase reductase, *SAH* S-adenosylhomocysteine, *SAM* S-adenosylmethionine, *THF* tetrahydrofolate, *TYMS* thymidylate synthase)

5,10-methyleneTHF will either (1) restrict the production of 5-methyltetrahydrofolate (5-methylTHF), thereby impeding homocysteine remethylation to methionine and resulting in elevated plasma homocysteine concentrations, or (2) hinder pyrimidine biosynthesis, thus affecting processes such as cell proliferation that are dependent on nucleic acids. The phenotypic impact of functional variants of MTHFR on homocysteine concentration has been the focus of numerous studies, and it is generally accepted that, under low folate conditions, homozygosity for the T allele of the *MTHFR* C677T polymorphism, resulting in a biochemically thermolabile form of the enzyme, is associated with raised homocysteine concentrations (Frosst et al. 1995). Results from a study conducted by Trinh et al. (2002) suggest that TYMS and MTHFR compete for the available supply of 5,10-methyleneTHF. Therefore, functional variants of TYMS may differentially impact the levels of 5,10-methyleneTHF available to MTHFR, thereby indirectly affecting both 5-methylTHF and plasma homocysteine levels.

To date, two common polymorphisms located within the untranslated regions (UTRs) of the *TYMS* gene and thought to influence enzyme activity have been reported. A 28-bp tandem repeat polymorphism in the 5′UTR is most frequently present in either two or three copies (Horie et al. 1995). Recently, functional studies have indicated that the mRNA specified by the three-repeat (3R) allele is translated more efficiently than that specified by the two-repeat (2R) allele (Kawakami et al. 2001; Ulrich et al. 2002), although the mechanism underlying this difference remains unclear. Furthermore, Trinh et al. (2002) have reported that the 3R3R genotype is associated with decreased serum folate and elevated homocysteine in middle-aged Singapore Chinese subjects. However, we have subsequently found no such association in a cohort of healthy young northwestern European men and women (Brown et al. 2004a). The other *TYMS* polymorphism, a 6-bp insertion/deletion (ins/del) located at bp 1494 in the region specifying the mRNA 3′UTR (Ulrich et al. 2000), has been proposed to affect mRNA stability and translation (Chu and Dolnick 2002). Recently, Volcik and coworkers (2003) have conducted a study to evaluate whether the *TYMS* 5′UTR and 3′UTR polymorphisms are risk factors for spina bifida, an NTD known to be potentiated by low folate status. In non-Hispanic whites, the homozygous 3′UTR ins/ins genotype is associated with a greater than three-fold increased risk for spina bifida, a figure that is even higher (greater than four-fold) for the composite ins/ins plus 5′UTR 2R2R genotype.

To examine further the phenotypic impact of variation in the *TYMS* gene, we performed a genetic analysis of the recently identified 3′UTR ins/del polymorphism in healthy young northwestern European adults. Our primary aim was to determine whether there was any association between genotype and plasma homocysteine, RBC folate or serum folate concentration; our secondary aim was to identify lifestyle and genetic factors that could interact with the 3′UTR ins/del polymorphism to elicit a potentially pathogenic folate/homocysteine phenotype. We have previously demonstrated that there is a strong interaction between the *MTHFR* 677TT genotype and smoking that greatly increases the risk of elevated homocysteine in the context of low serum folate in the same population (Brown et al. 2004b). Therefore, a particular focus of this study was an assessment of the impact of *TYMS* genotype on folate/homocysteine phenotype in the context of smoking behavior and the *MTHFR* C677T polymorphism.

Materials and methods

Study population

Study subjects ($n = 444$) were drawn from the Young Hearts Project, an ongoing prospective study designed to monitor the prevalence of coronary disease risk factors over time in young subjects from Northern Ireland (Boreham et al. 1997, 1999; Kluijtmans et al. 2003). Ethical approval was granted by the Research Ethics Committee, Queen's University Belfast, and all subjects provided written informed consent. The biochemical data used for the analyses reported here were acquired at the third screening visit, at which time the subjects were between 20 and 26 years old. At this visit, subjects were also classified as current cigarette smokers or current non-smokers. The 36 former smokers in the population were classified as current non-smokers.

Determination of homocysteine, folate, and vitamin B_{12}

Blood samples were collected from fasted subjects for determination of biochemical parameters and for DNA extraction. Homocysteine concentrations were measured by an established high performance liquid chromatography method (Ubbink et al. 1991). Serum folate and vitamin B_{12} concentrations were determined by time-resolved immunofluorescence on an AutoDelfia analyzer (Wallac, UK). RBC folate concentrations were determined by a microbiological assay as previously described (Molloy and Scott 1997). RBC folate concentrations are expressed as micrograms per liter of packed red blood cells.

Genetic analysis

The *TYMS* 3′UTR region containing the ins/del polymorphism was amplified by the polymerase chain reaction (PCR) in a 25-μl volume that contained 50 ng genomic DNA, 200 nM forward (5′-CAAATCTGAGGGAGCTGAGT-3′) and reverse (5′-CAGATAAGTGGCAGTACAGA-3′) primers, 200 μM dNTPs, 10× PCR buffer (Qiagen), 1.5 mM $MgCl_2$, and 1.5 U Hotstar *Taq* DNA polymerase (Qiagen). Thermocycling conditions consisted of 15 min at 95°C, followed by 35 cycles of 94°C for 40 s, 58°C for 40 s, and 72°C for 40 s, with a final extension step of 72°C for 5 min. The 148-bp PCR product was digested with 2 U *Dra*I for 3 h at 37°C. PCR products were separated on 10% non-denaturing polyacrylamide gels, run at 150 V for 1 h, and visualized by ethidium bromide staining. The insertion allele yielded products of 60 bp and 88 bp, whereas the deletion allele resulted in a product of 148 bp. The *MTHFR* C677T genotypes in this study population and the genotyping method used have previously been reported (Kluijtmans et al. 2003).

Statistical methods

Distributions of homocysteine, serum folate, RBC folate, and vitamin B_{12} were positively skewed, even after logarithmic transformation. Therefore, all analyses were performed by using untransformed ranked data. The Hardy-Weinberg equilibrium for the *TYMS* 3′UTR genotypes was assessed by the χ^2 test. Differences between groups for serum folate, RBC folate, homocysteine, and vitamin B_{12} were assessed by the Kruskal-Wallis test or the Wilcoxon Rank Sum test. Where appropriate, *P*-values for pairwise differences were corrected for multiple comparisons by the Bonferroni method. Odds ratios (ORs) for *TYMS* 3′UTR del/del homozygotes relative to ins/ins homozygotes in non-smokers were estimated by logistic regression analysis after stratification by RBC folate concentrations.

A model for RBC folate was developed to identify factors interacting with the *TYMS* 3′UTR ins/del polymorphism in the entire population by using generalized linear regression. A log-link function and gamma error structure were employed to account for the rightward skew of the data. Main effects were evaluated individually and were then added into the model based upon their degree of significance in the χ^2 test for each, with the most significant effect being added first. The likelihood ratio test (LRT) was used to determine significance after adjustment for the impact of additional main effects. Serum folate, sex, age, vitamin supplement use, smoking status, and the *TYMS* 3′UTR ins/del, *TYMS* 5′UTR2R/3R, and *MTHFR* C677T genotypes were considered as main effects. The model containing all significant main effects was considered the base model. Interactions between *TYMS* 3′UTR ins/del genotype and smoking status, serum folate, sex, vitamin supplementation use, *TYMS* 5′UTR 2R/3R genotype, or *MTHFR* C677T genotype, between serum folate and smoking status, sex, or *MTHFR* C677T genotype, and between *MTHFR* C677T genotype and smoking status were each tested by using the LRT to determine whether the base model could be significantly improved. The final model including all significant interaction terms was then compared with the same model without any terms including *TYMS* 3′UTR genotype by the LRT. The proportion of RBC folate variation that could be explained by the final models with and without *TYMS* 3′UTR terms was estimated by Efron's pseudo-R^2 statistic (Hardin and Hilbe 2001).

All statistical analyses were carried out by using SAS version 8.2. Tests were two-tailed and *P*-values of < 0.05 (or Bonferroni-adjusted values where appropriate) were considered statistically significant.

Results

Population characteristics

Population characteristics, including *TYMS* 3′UTR ins/del genotype frequencies and RBC folate, serum folate, homocysteine, and vitamin B_{12} concentrations for the population as a whole and after stratification by smoking status, are presented in Table 1. RBC folate, serum folate, and vitamin B_{12} concentrations were significantly higher in non-smokers than in smokers ($P < 0.05$). Median homocysteine concentrations were lower in non-smokers than in smokers, but the difference was not statistically significant. Genotypes in the population as a whole and in the subset of non-smokers were in Hardy-Weinberg equilibrium ($P = 0.38$ and 0.15, respectively).

Biochemical parameters and genotype

Biochemical parameters presented by *TYMS* 3′UTR ins/del genotype for the entire population and after

Table 1 Population characteristics (*n* number of individuals, *RBCs* red blood cells). Concentrations are expressed as median (the interquartile range is given in *square brackets*).

Characteristic	All	Non-smokers	Smokers
n (%)	444[a]	275 (62.8)	163 (37.2)
Men (%)	228 (51.8)	143 (63.3)	83 (36.7)
Women (%)	212 (48.2)	132 (67.7)	80 (32.3)
TYMS 3′UTR genotype			
ins/ins	197 (44.4)	123 (63.4)	71 (36.6)
ins/del	207 (46.6)	132 (64.4)	73 (35.6)
del/del	40 (9.01)	20 (51.3)	19 (48.7)
Red cell folate[b] (μg/l RBCs)	284 [211–367] (365)	295 [224–384] (231)	255 [204–345] (131)
Folate[b] (nmol/l)	13.0 [9.5–18.8] (349)	14.1 [10.1–20.1] (218)	11.3 [8.7–15.5] (128)
Homocysteine (μmol/l)	8.9 [7.5–11.0] (399)	8.7 [7.5–10.5] (246)	9.5 [7.5–11.7] (150)
Vitamin B_{12}^{b} (pmol/l)	267 [196–339] (350)	279 [201–357] (218)	234 [179–316] (129)

[a]Sex was missing for four subjects; smoking status was missing for six subjects
[b]$P < 0.05$ for smokers compared with non-smokers

stratification by smoking status are presented in Table 2. For the population as a whole, there was no statistically significant association between genotype and any of the biochemical parameters, although a trend toward higher RBC folate concentrations in del/del homozygotes was observed.

Among non-smokers, *TYMS* 3′UTR ins/del genotype was significantly associated with RBC folate ($P = 0.002$) and homocysteine ($P = 0.03$) concentrations. There was also a trend toward higher serum folate levels in del/del homozygotes. After stratification of non-smokers by gender, no apparent differences were noted between males and females with respect to the relative strengths of the above associations (not shown). Among smokers, no significant associations between *TYMS* 3′UTR ins/del genotype and any of the biochemical parameters were observed.

RBC folate and homocysteine concentrations by TYMS 3′UTR ins/del genotype among non-smoking subjects, subdivided into RBC folate quartiles

The strength of the association of the *TYMS* 3′UTR ins/del polymorphism with RBC folate and homocysteine concentrations among non-smokers was assessed across the RBC folate distribution by quartile. Significant associations between genotype and both RBC folate ($P = 0.004$) and homocysteine ($P = 0.02$) levels were observed only in quartile 4 (highest RBC folate). The median RBC folate concentration was much higher for *TYMS* 3′UTR del/del homozygotes in this quartile compared with either ins/del heterozygotes or ins/ins homozygotes, and as expected, the homocysteine concentration for the del/del genotype was considerably lower (Table 3).

Table 2 Associations between *TYMS* 3′UTR genotype and biochemical parameters (*n* number of individuals, *RBCs* red blood cells)

Biochemical parameter	Subset	Median [interquartile range] (*n*)			*P*-value
		ins/ins	ins/del	del/del	
Red cell folate (μg/l RBCs)	All	282 [211–337] (165)	281 [211–378] (169)	312 [223–434] (31)	0.15
	Non-smokers	282 [212–340] (110)	298 [226–397] (105)	434 [302–613] (16)	0.002[a]
	Smokers	283 [207–337] (53)	241 [187–363] (64)	271 [189–307] (14)	0.49
Folate (nmol/l)	All	12.6 [9.4–18.3] (155)	12.7 [9.4–19.1] (159)	13.6 [9.7–17.0] (35)	0.69
	Non-smokers	13.1 [9.7–19.1] (101)	14.4 [10.7–21.1] (100)	17.0 [12.5–25.2] (17)	0.18
	Smokers	12.2 [9.1–15.5] (52)	10.4 [8.1–17.1] (59)	13.2 [9.7–15.1] (17)	0.61
Homocysteine (μmol/l)	All	9.0 [7.6–11.1] (174)	8.8 [7.5–11.1] (187)	8.4 [6.9–10.5] (38)	0.39
	Non-smokers	8.9 [7.6–10.6] (111)	8.6 [7.5–10.7] (117)	7.4 [6.3–9.6] (18)	0.03[b]
	Smokers	9.0 [7.3–11.5] (61)	9.6 [7.6–12.4] (70)	9.8 [8.1–10.9] (19)	0.57
Vitamin B_{12} (pmol/l)	All	255 [186–319] (156)	280 [203–347] (159)	267 [191–370] (35)	0.16
	Non-smokers	270 [195–341] (101)	286 [210–360] (100)	262 [183–377] (17)	0.46
	Smokers	219 [165–285] (53)	250 [180–336] (59)	283 [226–353] (17)	0.13

[a]Bonferroni-corrected $P = 0.002$ for del/del versus ins/ins
[b]Bonferroni-corrected $P = 0.02$ for ins/ins versus del/del

Table 3 RBC folate concentrations by *TYMS* 3′UTR genotype among non-smokers in subjects with low and high RBC folate (*n* number of subjects, *RBCs* red blood cells)

Quartile RBC folate	Parameter	Median [Interquartile range] (*n*)			*P*-value
		ins/ins	ins/del	del/del	
4	RBC folate (μg/l RBCs)	415 [393–448] (23)	453 [410–486] (33)	572 [437–809] (8)	0.004[a]
	Homocysteine (μmol/l)	7.8 [6.6–9.2] (23)	7.6 [7.3–8.6] (33)	6.1 [5.1–7.0] (8)	0.02[b]
3	RBC folate	315 [299–333] (31)	325 [306–342] (25)	323 [302–333] (4)	0.58
	Homocysteine	8.3 [7.5–9.4] (31)	8.5 [7.4–9.4] (25)	8.3 [7.9–9.6] (4)	0.81
2	RBC folate	251 [226–269] (30)	247 [229–259] (25)	244 [214–275] (2)	0.99
	Homocysteine	9.3 [7.7–10.5] (30)	9.4 [7.1–10.7] (25)	9.2 [7.0–11.3] (2)	0.96
1	RBC folate	181 [147–196] (26)	180 [154–201] (22)	179 (1)	0.94
	Homocysteine	11.8 [10.1–16.8] (24)	10.4 [8.4–12.9] (19)	6.9 (1)	0.07

[a]Bonferroni-corrected $P = 0.005$ for del/del versus ins/ins and 0.04 for del/del versus ins/del
[b]Bonferroni-corrected $P = 0.01$ for del/del versus ins/del

Examination of the distribution of del/del and ins/ins homozygotes in each of the RBC folate quartiles revealed that more than half of the former were in quartile 4. As would be predicted from the results outlined above, the del/del homozygotes in quartile 4 included those with the highest RBC folate concentrations. The impact of the del/del genotype on phenotype was illustrated by the finding that non-smokers with this genotype had a more than ten-fold increased chance of being in the top 10% of the RBC folate distribution relative to non-smokers with the ins/ins genotype (OR = 10.4, 95% confidence interval [CI] = 2.8–38.3; Table 4).

The higher RBC folate levels seen in del/del non-smokers could not be attributed to vitamin supplement use, since there were no significant differences in the numbers of supplement users between the genotype classes in non-smokers as a whole or in the subset of non-smokers in the highest RBC folate quartile ($P = 0.15$ and 0.18, respectively).

Interaction of TYMS 3′UTR ins/del with TYMS 5′UTR 2R/3R and MTHFR C677T

The *TYMS* 3′UTR polymorphism was in significant linkage disequilibrium with the 5′UTR polymorphism ($P < 0.0001$), such that the del allele of the former was disproportionately associated with the 3R allele of the latter. However, when the *TYMS* 3′UTR genotype was stratified by *TYMS* 5′UTR genotype in non-smokers, no significant associations between any of the composite genotypes and either RBC folate or homocysteine were observed. The number of subjects in the study population with composite *TYMS* 3′UTR del plus 5′UTR 3R alleles may have been too small to generate an association between the 5′UTR 3R variant and a high folate/low homocysteine phenotype. An alternative explanation may be that the 3R allele, which is associated with high TYMS protein concentration and absolute activity in vivo (Kawakami et al. 1999), mandates a more efficient utilization of folate levels associated with the *TYMS* 3′UTR del allele, and that these opposing effects on folate/homocysteine phenotype neutralize each other when the respective variants are in cis.

Table 4 Odds ratios for elevated RBC folate in non-smokers conferred by *TYMS* del/del genotype relative to the ins/ins genotype (*OR* odds ratio, *95% CI* 95% confidence interval)

Stratum	*TYMS* del/del relative to ins/ins		
	OR	95% CI	*P*-value
Top 10%	10.4	2.8–38.3	< 0.0001
Top 25%	4.8	1.6–14.5	0.002
Top 50%	4.8	1.3–17.9	0.01

As the *MTHFR* C677T polymorphism is a strong determinant of homocysteine and serum folate concentrations (Harmon et al. 1996; Jacques et al. 1996), and of RBC folate concentrations (Molloy et al. 1997) we examined the impact of the *TYMS* 3′UTR ins/del polymorphism on these phenotypic variables after stratification of the study population by *MTHFR* genotype. There was a statistically significant association between the *TYMS* 3′UTR del/del genotype and elevated RBC folate levels in the *MTHFR* 677CC subgroup ($P = 0.03$), i.e., those with the del/del genotype had RBC folate levels approximately 125 μg/l higher than those of their ins/del and ins/ins peers. A quantitatively comparable increment in RBC folate levels in del/del homozygotes relative to the ins/del heterozygotes and ins/ins homozygotes was also observed in the *MTHFR* 677TT subgroup; however, this trend did not reach statistical significance, possibly because of the small numbers of subjects. There was no such association in the *MTHFR* 677CT subgroup, although a statistically significant association between the *TYMS* 3′UTR del/del genotype and homocysteine was seen. These findings suggest that the effect of the *TYMS* 3′UTR del/del and *MTHFR* 677CC genotypes on RBC folate concentration may be additive.

Table 5 RBC folate and homocysteine concentrations by *TYMS* 3′UTR genotype stratified by *MTHFR* C677T genotype among non-smokers (*n* number of individuals, *RBC* red blood cell)

MTHFR C677T genotype	Parameter	Median [interquartile range] (*n*)			P^a-value
		ins/ins	ins/del	del/del	
CC	RBC folate (μg/l RBCs)	308 [261–362] (48)	319 [226–410] (46)	439 [320–848] (7)	0.03[b]
	Homocysteine (μmol/l)	8.7 [7.5–10.1] (48)	8.8 [7.5–10.4] (51)	8.6 [5.1–10.5] (9)	0.73
CT	RBC folate	251 [194–316] (51)	298 [228–376] (48)	277 [196–555] (4)	0.11
	Homocysteine	9.2 [7.8–10.7] (52)	8.4 [7.0–10.2] (51)	7.2 [7.0–7.5] (5)	0.01[b]
TT	RBC folate	279 [176–329] (11)	248 [201–482] (11)	380 [305–518] (4)	0.17
	Homocysteine	7.8 [7.2–10.6] (11)	10.4 [8.1–13.4] (15)	8.0 [5.9–10.6] (3)	0.33

[a]Kruskal-Wallace test
[b]$P < 0.05$ for del/del versus ins/ins

Model

A generalized linear model for RBC folate concentration was constructed, both to ascertain whether the *TYMS* 3′UTR ins/del genotype remained a significant determinant of RBC folate levels after correction for other factors possibly affecting this variable and to assess possible interactions between *TYMS* 3′UTR ins/del genotype and other factors. The final model included serum folate, sex, and *MTHFR* C677T genotype as main effects and interactions between serum folate and smoking status, *TYMS* 3′UTR ins/del genotype and smoking status, *MTHFR* C677T genotype and serum folate, and *TYMS* 3′UTR ins/del genotype and serum folate, as significant determinants of RBC folate. The terms that included *TYMS* 3′UTR ins/del genotype contributed significantly to the final model (LRT $P < 0.0001$) and increased the proportion of the variation in RBC folate explained by the model from 33% to 52%. An interaction term for the *TYMS* 3′UTR ins/del and *MTHFR* C677T genotypes did not contribute significantly to the model.

Discussion

The data presented here indicate that the *TYMS* 3′UTR del/del genotype is a significant determinant of RBC folate concentrations in healthy young northwestern European men and women. This finding may be of particular relevance to spina bifida birth outcome, for which there is a well-established association between low maternal folate status and increased risk (Mills et al. 1995). Maternal periconceptional supplementation with folic acid is widely accepted to lower significantly (by as much as 70%) the risk of NTDs (MRC Vitamin Study Research Group 1991; Czeizel and Dudas 1992). Our data suggest that individuals who have the *TYMS* 3′UTR del/del genotype may be genetically predisposed to have a superior folate status. The consequent hypothesis that a maternal *TYMS* 3′UTR del/del genotype would protect against NTD birth outcome is consistent with a recent report in which an increased risk of spina bifida has been associated both with the ins/ins genotype alone (greater than three-fold risk) and with the composite 5′UTR 2R2R plus ins/ins genotype (greater than four-fold risk; Volcik et al. 2003).

As expected from the above, we have also observed that the *TYMS* 3′UTR del/del genotype is associated with lower homocysteine concentrations. Several studies have reported that a modest elevation in homocysteine is an independent and graded risk factor for cardiovascular disease (Refsum et al. 1998) and a number of other human pathologies (Lucock 2000). Nevertheless, whether low folate or high homocysteine (or a combination of both) is pathogenic remains contentious. Regardless of which variable is causative, our data suggest that having the *TYMS* 3′UTR del/del genotype, by virtue of its association with a "favorable" high folate/low homocysteine phenotype, should protect against diseases for which a low folate/high homocysteine phenotype is considered to be an etiologic component. However, this study indicates that smoking is a stronger determinant of both folate and homocysteine status than the TYMS genotype. It also shows that the potential benefit conferred by the del/del genotype is entirely eliminated by smoking. The apparent restriction of the capacity of the *TYMS* 3′UTR del/del genotype to mandate high RBC folate concentrations to non-smokers is not surprising given the well-documented association between smoking and a low folate/high homocysteine phenotype that is generally attributed to lower dietary intake, altered absorption, or increased oxidative catabolism of folate (Mansoor et al. 1997; De Bree et al. 2002).

In conclusion, we have demonstrated that a substantial proportion of Caucasians have a *TYMS* genotype that favors a high folate/low homocysteine phenotype. However, our data also indicate that this potential health advantage is not realized in smokers. Therefore, *TYMS* 3′UTR del/del homozygotes who stop smoking may not only eliminate a major source of environmental risk factors for many common diseases, but may also acquire a genetically mandated, protective high folate/low homocysteine phenotype.

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