

## B vitamin treatments modify the risk of myocardial infarction associated with a MTHFD1 polymorphism in patients with stable angina pectoris



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### KEYWORDS

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**Abstract** *Background:* Metylenetetrahydrofolate dehydrogenase (MTHFD1) catalyzes three sequential reactions that metabolize derivatives of tetrahydrofolate (THF) in folate-dependent one-carbon metabolism. Impaired MTHFD1 flux has been linked to disturbed lipid metabolism and oxidative stress. However, limited information is available on its relation to the development of atherothrombotic cardiovascular disease.

*Methods and results:* We explored the association between a MTHFD1 polymorphism (*rs1076991 C > T*) and acute myocardial infarction (AMI), and potential effect modifications by folic acid/B12 and/or vitamin B6 treatment in suspected stable angina pectoris patients ( $n = 2381$ ) participating in the randomized Western Norway B Vitamin Intervention Trial (WENBIT). During the median follow-up of 4.9 years 204 participants (8.6%) suffered an AMI. After adjusting for established CVD risk factors, the MTHFD1 polymorphism was significantly associated with AMI (HR: 1.49; 95% CI, 1.23–1.81). A similar association was observed among patients allocated to treatment with vitamin B6 alone (HR: 1.53; 95% CI, 1.01–2.31), and an even stronger relationship was seen in patients treated with both vitamin B6 and folic acid/B12 (HR: 2.35; 95% CI, 1.55–3.57). However, no risk association between the MTHFD1 polymorphism and AMI was seen in patients treated with placebo (HR: 1.29; 95% CI, 0.86–1.93) or folic acid/B12 (1.17; 95% CI, 0.83–1.65).

*Conclusion:* A common and functional MTHFD1 polymorphism is associated with increased risk of AMI, although the risk seems to be dependent on specific B vitamin treatment. Further studies are warranted to elucidate the possible mechanisms, also in order to explore potential effect modifications by nutritional factors.

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treatments on the risk for serious cardiovascular events and mortality [4]. In specific, WENBIT had a  $2 \times 2$  factorial design in which participants with suspected or verified coronary artery disease (CAD), or aortic stenosis, were randomized to receive a daily capsule containing one of the following: 1) folic acid 0.8 mg plus vitamin B12 (cyanocobalamin) 0.4 mg plus vitamin B6 (pyridoxine hydrochloride) 40 mg; 2) folic acid plus vitamin B12; 3) vitamin B6; 4) placebo. In total, there were 3090 patients enrolled in WENBIT study. Among them, 2584 patients underwent coronary angiography for stable angina pectoris (SAP), 461 for acute coronary syndromes (ACS) and 45 for aortic valve stenosis at Haukeland University Hospital, Bergen, Norway or Stavanger University Hospital, Stavanger, Norway. The current study included patients with SAP only. Participants without successful genotyping ( $n = 203$ ) were excluded, leaving a total of 2381 participants for the final analyses.

The study protocol was in accordance with the principles of the Declaration of Helsinki, and was approved by the Regional Committees for Medical and Health Research Ethics, the Norwegian Medicines Agency and the Norwegian Data Inspectorate. All subjects were informed and agreed to participate in extended follow-up including genetic studies.

### Baseline data

Smoking status and the extent of CAD at angiography were defined as previously described [15]. Obesity was defined as body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>. Diabetes mellitus was classified by self-reports or by glucose criteria (fasting plasma glucose  $\geq 7.0$  mmol/L; or random plasma glucose  $\geq 11.1$  mmol/L measured at baseline of the study).

### Follow-up and clinical endpoints

The primary outcome for the present study was fatal or non-fatal AMI. Study subjects were followed from enrollment until the onset of AMI, or until the end of 2006. Details on the collection and classification of clinical endpoints have been described previously [16].

### Genotyping and biochemical analysis

Clinical information and blood samples were obtained at baseline before or immediately after coronary angiography and the blood samples were stored at  $-80$  °C. Earlier reports have described the biochemical analyses for relevant clinical indices [15,16]. Besides, genotyping of MTHFD1 *rs1076991* polymorphism was performed by MALDI-TOF mass spectrometry [17].

### Statistical analysis

Baseline categorical variables were summarized as percentages and continuous variables were presented as medians (interquartile range (IQR)). Hardy-Weinberg equilibrium (HWE) and MAF of MTHFD1 *rs1076991*

polymorphism were also calculated. Baseline variables across MTHFD1 *rs1076991* genotypes (CC, CT and TT) were assessed by un-adjusted median linear regression for continuous and logistic regression or Kruskal–Wallis rank sum test for categorical variables.

The risk association between the MTHFD1 polymorphism and AMI was tested in two Cox regression models: A simple model was adjusted for age (continuous) and gender (male/female); a multivariate model was further adjusted for established CAD risk factors including smoking status (yes/no), obesity (yes/no), hypertension (yes/no) and diabetes mellitus (yes/no). Additional adjustments for baseline serum apoA-1, apoB, and angiographic signs of coronary artery disease (CAD) had minor influences on the estimates and were therefore not included in the models. An additive genetic model was used in all survival analyses, in which we assumed a linear risk relationship among MTHFD1 *rs1076991* CC, CT, TT genotypes. This is equivalent to a comparison of the T-allele versus the C-allele. Proportional hazards assumptions were examined using the Schoenfeld and scaled Schoenfeld residuals. Interactions between vitamin B treatments and the MTHFD1 polymorphism on the outcome were evaluated by adding interaction product terms in the multivariate Cox models.

The statistical analyses were performed in R (R version: 3.1.0) using packages “genetics” and “survival” [18]. All reported  $P$  values were two-sided, and  $P < 0.05$  was considered statistically significant.

## Results

### Baseline characteristics

Of the 2381 SAP participants included, 1888 (79.3%) were males. The median (IQR) age of the population was 62 (48–76) years. Most baseline characteristics in the study did not differ according to the MTHFD1 *rs1076991* genotypes ( $P \geq 0.08$ , Table 1). However, diabetes mellitus was inversely associated with the number of T-allele ( $P = 0.02$ ). We did not observe any association between biomarkers for OCM and the MTHFD1 polymorphism at baseline ( $P \geq 0.25$ ). The observed MAF of the MTHFD1 polymorphism was 42.7% and did not deviate from Hardy-Weinberg equilibrium ( $P = 0.31$ ).

### The MTHFD1 polymorphism and AMI risk

During a median (IQR) follow-up time of 4.9 (2.8–7.0) years, 204 participants (8.6%) suffered an AMI. Kaplan–Meier curves showed a significant association between the MTHFD1 polymorphism and AMI occurrence ( $P_{\log\text{-rank}} = 3.5 \times 10^{-4}$ ) (Fig. 2). After adjusting for age and gender, the minor T-allele was linearly associated with AMI (HR: 1.46; 95% CI, 1.20–1.76), which remained significant after multivariate adjustment (HR: 1.49; 95% CI, 1.23–1.81). Notably, similar results were also observed in the complete WENBIT population (data not shown).

**Table 1** Baseline characteristics of the WENBIT cohort according to the MTHFD1 promoter polymorphism.

	CC (N = 794)	CT (N = 1141)	TT (N = 446)	Total (N = 2381)	P for trend
<i>General characteristics</i>					
Age (year)	62 ± 14	62 ± 14	62 ± 15	62 ± 14	0.71
Gender (male, %)	626 (79)	909 (80)	353 (79)	1888 (79)	0.83
Body mass index (kg/m <sup>2</sup> )	26.6 ± 4.6	26.4 ± 4.6	26.4 ± 4.6	26.5 ± 4.6	0.16
<i>Cardiovascular history and risk factors, n (%)</i>					
Prior MI	346 (43.6)	521 (45.7)	194 (43.5)	1061 (44.5)	0.86
Prior PCI	167 (21.0)	255 (22.3)	99 (22.2)	521 (21.9)	0.57
Prior CABG	105 (13.2)	165 (14.5)	60 (13.5)	330 (13.9)	0.79
Hypertension	390 (49.1)	533 (46.7)	203 (45.6)	1126 (47.3)	0.19
Diabetes mellitus	132 (16.6)	162 (14.2)	52 (11.7)	346 (14.5)	0.02
Current smoking	209 (26.3)	357 (31.3)	133 (29.8)	699 (29.4)	0.08
<i>Angiographic evidence of CAD</i>					
No significant CAD	87 (11.0)	133 (11.7)	48 (10.8)	268 (11.3)	0.80
Single vessel disease	234 (29.5)	309 (27.1)	127 (28.5)	670 (28.1)	
Double vessel disease	210 (26.4)	297 (26.0)	127 (28.5)	634 (26.6)	
Triple vessel disease	263 (33.1)	402 (35.2)	144 (32.2)	263 (34.0)	
<i>Biochemistry parameters</i>					
ApoB/ApoA1 ratio	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)	0.60
Estimated GFR (mL/min/1.73 m <sup>2</sup> )	92 (17)	92 (19)	92 (20)	92 (18)	0.42
Serum CRP (mg/L)	1.8 (2.9)	1.7 (2.5)	1.9 (2.7)	1.8 (2.7)	0.74
Serum Creatinine (μmol/L)	90 (16)	91 (17)	90 (17)	90 (17)	0.18
<i>Treatment following baseline coronary angiography, n (%)</i>					
No or medications only	275 (34.6)	378 (33.1)	163 (36.5)	816 (34.3)	0.62
Percutaneous coronary intervention	300 (37.8)	462 (40.5)	169 (37.9)	931 (39.1)	
Coronary artery bypass grafting	193 (24.3)	265 (23.2)	101 (22.6)	559 (23.5)	
<i>Medication at discharge, n (%)</i>					
Aspirin	713 (89.8)	1024 (89.7)	407 (91.3)	2144 (90.0)	0.48
Statins	706 (89.0)	1009 (88.4)	392 (87.9)	2107 (88.5)	0.58
Beta blockers	614 (77.3)	884 (77.5)	358 (80.2)	1856 (78.0)	0.64
ACEIs	178 (22.4)	224 (19.6)	96 (21.5)	498 (20.9)	0.51
Loop diuretics	87 (11.0)	110 (9.6)	40 (9.0)	233 (9.8)	0.23
<i>Biomarkers for OCM</i>					
Plasma homocysteine (μmol/L)	10.2 (3.5)	10.2 (3.7)	10.4 (3.6)	10.2 (3.6)	0.76
Plasma PLP (nmol/L)	40.4 (27.2)	39.0 (26.1)	40.5 (27.5)	40.0 (26.9)	0.89
Serum folate (nmol/L)	9.8 (7.4)	9.9 (7.0)	10.0 (7.4)	9.9 (7.2)	0.24
Serum cobalamin (μmol/L)	331 (160)	341 (173)	333 (172)	337 (170)	0.79
Serum MMA (μmol/L)	0.16 (0.06)	0.16 (0.07)	0.16 (0.07)	0.16 (0.07)	0.25

Abbreviations: ACEI, angiotensin-converting-enzyme inhibitor; Apo, apolipoprotein; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CRP, C-reactive protein; GFR, glomerular filtration rate; MMA, methylmalonic acid; OCM, one carbon metabolism; PCI, percutaneous coronary intervention; PLP, pyridoxal phosphate.

### The effect modifications of B vitamin treatment

Table 2 describes the risk associations between the MTHFD1 rs1076991 and AMI according to WENBIT treatment allocation. Among patients treated with placebo or folic acid/vitamin B12, we found similar trends towards positive associations between the number of T-allele and AMI risk in the multivariate models (HR: 1.29; 95% CI, 0.86–1.93 and 1.17; 95% CI, 0.83–1.65, respectively;  $P_{\text{int}} = 0.76$ ). A significant association was observed among patients allocated to vitamin B6 treatment (HR: 1.53; 95%

CI, 1.01–2.31), which, however, was not statistically different from placebo treatment ( $P_{\text{int}} = 0.59$ ). Notably, we observed a more profound association between the polymorphism and AMI among patients allocated to the combined vitamin B6 and folic acid/B12 treatment (HR: 2.35; 95% CI, 1.55–3.57;  $P_{\text{int}} = 0.047$  vs. placebo). This interaction seemed to be introduced by a shift from a lower to higher risk of the combined B vitamin treatment according to the number of T-allele (HR among the CC homozygotes: 0.38; 95% CI, 0.12–1.23; CT heterozygotes: 1.14; 95% CI, 0.68–1.93; and TT homozygotes: 1.98; 95% CI, 0.86–4.55).

## Discussion

### Main findings

A common and functional MTHFD1 polymorphism is associated with increased risk of AMI among the SAP patients in WENBIT. We furthermore observed a significant interaction between combined treatment of folic acid/vitamin B12 and vitamin B6 with the MTHFD1 polymorphism on risk of AMI occurrence.

### Strengths and limitations

The MTHFD1 *rs1076991* is a common polymorphism, which ensures sufficient power to detect underlying gene–disease associations. The large sample size and long-term follow-up are strengths of the current study. To decrease the probability of type I errors by multiple testing, we did not assess the existence of other genetic variants besides the prime candidate SNP in the region of linkage disequilibrium (LD) at the MTHFD1 locus. Based on HapMap data, several SNPs in this region are in strong LD with MTHFD1 *rs1076991* and may therefore represent the underlying causes of the observed association (Supplementary table 1 and figure 1). Also, our results should be validated in independent populations, preferably with other clinical and baseline characteristics than the present cohort.

In the current investigation, we excluded patients with an acute coronary syndrome to avoid the influence of acute inflammation on SNP–treatment interactions. Notably, several previous studies have shown that acute coronary syndrome is associated with inflammatory

response [19], which affects biomarkers of B vitamin status [20]. Thus, the current results are primarily relevant for patients with SAP only.

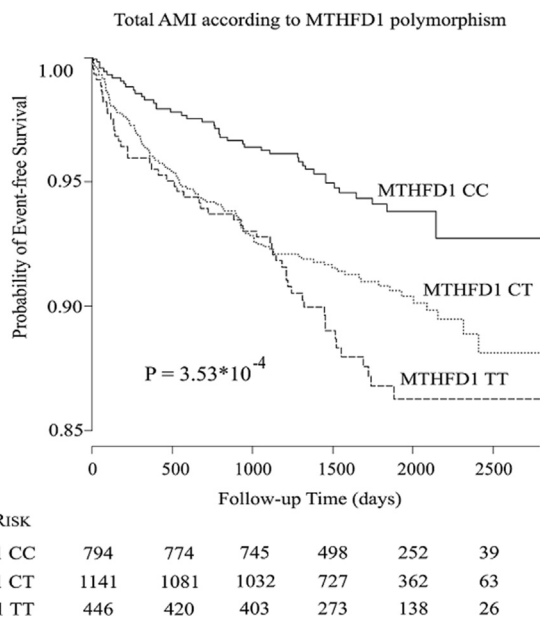
### The cytosolic MTHFD1, diseases and biomarkers

Production of cytosolic C1-THF synthase (10-formyl-THF, 5,10-methenyl-THF and 5,10 methylene-THF) are involved in *de novo* purine and thymidylate synthesis, as well as in homocysteine remethylation. However, we did not observe any association of the MTHFD1 *rs1076991* polymorphism with plasma homocysteine levels, which concurs with results from another study [21]. Population studies have shown associations between MTHFD1 polymorphisms and neural tube defects [22] and gastric cancer [23]. One study showed strong association between MTHFD1 *rs1076991* and B-cell acute lymphoblastic leukemia [24]. To the best of our knowledge, the current study is among the first to focus on the promoter polymorphism of MTHFD1 with AMI occurrence in SAP patients.

### Effects of B vitamin treatments on the association of MTHFD1 with AMI

We found a particularly strong association between the MTHFD1 polymorphism and AMI in patients allocated to combined vitamin B6 and folic acid/B12 treatment. Concomitant high levels of folate and B6 resulted in a non-significant beneficial effect against AMI in the MTHFD1 CC homozygous, and showed a more adverse tendency in patients with MTHFD1 T-allele. Vitamin B6 intake is shown to be inversely related to hepatic and plasma glycine levels [25], and positively associated with adenosylmethionine (SAM) [26] due to decreased SAM consumption by GNMT. Accumulated SAM has been shown to cause hepatic lipid accumulation and other atherothrombotic changes, including overt dyslipidemia [27] and oxLDL-induced foam cell formation [28]. On the other hand, adequate folate status is crucial for balancing the transmethylation flux. Folate deficiency has been associated with elevated circulating formate levels, impaired nucleotide synthesis, DNA hypomethylation and hyperhomocysteinemia [29]. Recent evidence linked folate intake with elevated hepatic SAM [30], which is a known inhibitor to betaine–homocysteine methyltransferase [31], which is suggested to regulate liver lipids and to induce apoB expression [32]. Indeed, excess SAM has been associated with hepatic apoB mRNA expression and VLDL assembly [33]. Coupled with the evidence that MTHFD1 deficiency has also been associated with accumulated intercellular SAM [34], these findings may be indicative of a potential mechanism in which lipid accumulation is exacerbated by the combined treatment in those with MTHFD1 deficiency, which may further promote atherosclerosis. Nevertheless, the interaction of the MTHFD1 genotype with glycine, lipid and B6 metabolism should be further explored.

Genetic analysis may provide more insights to the underlying pathophysiology of atherosclerosis. The



**Figure 2** Kaplan–Meier estimates of AMI according to the MTHFD1 polymorphism.

**Table 2** HRs of AMI by MTHFD1 *rs1076991* polymorphism in WENBIT population and different treatment arms.

	Events/total	Simple model <sup>a</sup>			Multivariate model <sup>b</sup>			<i>P</i> <sub>int</sub> <sup>c</sup>
		HR	95% CI	<i>P</i> value	HR	95% CI	<i>P</i> value	
WENBIT	204/2381	1.46	1.20–1.76	$1.3 \times 10^{-4}$	1.49	1.23–1.81	$5.5 \times 10^{-5}$	
Placebo	48/601	1.27	0.85–1.90	0.24	1.29	0.86–1.93	0.22	Ref.
Vitamin B6	46/593	1.44	0.95–2.16	0.08	1.53	1.01–2.31	0.04	0.59
Folic acid/B12	61/598	1.19	0.85–1.67	0.31	1.17	0.83–1.65	0.38	0.76
B6 + Folic acid/B12	49/589	2.28	1.51–3.44	$9.0 \times 10^{-5}$	2.35	1.55–3.57	$6.3 \times 10^{-5}$	0.047

<sup>a</sup> Simple model was adjusted for age and gender.

<sup>b</sup> Multivariate model was adjusted further for smoking status, obesity, hypertension and diabetes mellitus.

<sup>c</sup> *P*<sub>int</sub> refers to the gene-treatment interaction in multivariate models which compared SNP association between placebo group and designated treatment groups.

modulation of AMI risk conferred by MTHFD1 polymorphism through allocation to vitamin B6 and folic acid/B12 in the current study may be interpreted in the context of personalized medicine. Since B-vitamin status may be correlated to other lifestyle factors like obesity or the intake of macronutrients, further studies are warranted to examine if the MTHFD1 genotype may influence the association between such lifestyle factors and atherogenesis, and if such association may be mediated partly by vitamin status.

## Conclusion

We demonstrate that B vitamin treatment may introduce a strong association between a common and functional MTHFD1 promoter polymorphism and AMI risk in patients with SAP. Our results may potentially provide insight into the conflicting results of randomized B vitamin intervention trials on cardiovascular disease. Further studies should evaluate if this genotype may modify the risk of chronic diseases affected by other dietary or lifestyle factors.

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## Disclosures

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## Appendix A. Supplementary material

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2015.12.009>.

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