

Methylenetetrahydrofolate Dehydrogenase 1 Polymorphisms Modify the Associations of Plasma Glycine and Serine With Risk of Acute Myocardial Infarction in Patients With Stable Angina Pectoris in WENBIT (Western Norway B Vitamin Intervention Trial)

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Background—Serine and glycine interconversion and methylenetetrahydrofolate dehydrogenase 1 (MTHFD1)-mediated 1-carbon transfer are the major sources of methyl groups for 1-carbon metabolism. Recently, plasma glycine and a common polymorphism in MTHFD1 have been associated with risk of acute myocardial infarction (AMI). It is, therefore, of interest to explore if these 2 pathways interact in relation to AMI.

Methods and Results—A total of 2571 participants in the WENBIT (Western Norway B Vitamin Intervention Trial) undergoing coronary angiography for stable angina pectoris were studied. Associations of plasma serine and glycine concentrations with risk of AMI across 2 common and functional MTHFD1 polymorphisms (*rs2236225* and *rs1076991*) were explored in Cox regression models. During a median follow-up of 4.7 years, 212 patients (8.2%) experienced an AMI. In age- and sex-adjusted analyses, plasma glycine ($P < 0.01$), but not serine ($P = 0.52$), showed an overall association with AMI. However, interactions of MTHFD1 *rs2236225* polymorphism with both plasma serine and glycine were observed ($P_{\text{interaction}} = 0.03$ for both). Low plasma serine and glycine were associated with an increased risk of AMI among patients carrying the *rs2236225* minor A allele. Similarly, low plasma glycine showed stronger risk relationship with AMI in the *rs1076991* CC genotype carriers but weaker associations in patients carrying the minor T allele ($P_{\text{interaction}} = 0.02$).

Conclusions—Our results showed that 2 common and functional polymorphisms in the *MTHFD1* gene modulate the risk associations of plasma serine and glycine with AMI. These findings emphasize the possible role of the MTHFD1 in regulating serine and glycine metabolism in relation to atherosclerotic complications.

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Key Words: association study ■ coronary artery disease ■ glycine ■ MTHFD1 ■ myocardial infarction ■ one-carbon metabolism ■ serine

The 1-carbon metabolism is essential in nucleic acid and protein biosynthesis, epigenetic regulation, and amino acid and lipid metabolism.^{1,2} Evidence also suggests the involvement of 1-carbon metabolism in the developments of atherosclerosis and cardiovascular disease.^{3,4}

Clinical Perspective on p 547

One-carbon units required for DNA biosynthesis and homocysteine remethylation are obtained both from formate⁵ through the action of the trifunctional methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) and from the interconversion of serine and glycine catalyzed by serine

hydroxymethyltransferase (Figure 1). The 5,10-methylenetetrahydrofolate exists at a branch point between these 2 sources of 1-carbon units⁶ and connects serine and glycine metabolism with MTHFD1 activities.

Glycine is a precursor for the synthesis of purines, proteins, glucose, and glutathione and has been suggested to modulate inflammation.⁷ We recently observed an inverse association between plasma glycine and risk of acute myocardial infarction (AMI) in patients with suspected stable angina pectoris (SAP).⁸ Furthermore, a polymorphism in the *MTHFD1* gene was associated with increased risk of AMI in the same population.⁹ Given the metabolic connection between the 2 pathways,

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it is of interest to explore whether these 2 sources of 1-carbon units interact as predictors of prognosis among patients with established coronary artery disease.

To test this hypothesis, we selected 2 putative functional polymorphisms in MTHFD1, 1958G>A (*rs2236225*) and 105C>T (*rs1076991*). The minor A allele of *rs2236225* has been shown to decrease de novo purine synthesis rate,¹⁰ while the minor T allele of *rs1076991* has been associated with nearly 70% decrease of MTHFD1 promoter activity.¹¹ We, therefore, assessed whether associations of plasma serine and glycine with risk of AMI were influenced by the MTHFD1 polymorphisms among SAP patients included in the WENBIT (Western Norway B Vitamin Intervention Trial).

Methods

Study Population

The rationale of WENBIT (ClinicalTrials.gov number: NCT00354081) has been described in detail elsewhere.¹² Briefly, WENBIT had a 2×2 factorial design, in which participants with suspected or verified coronary artery disease, or aortic stenosis were randomized to receive a daily capsule containing one of the following: (1) folic acid 0.8 mg+vitamin B12 (cyanocobalamin) 0.4 mg+vitamin B6 (pyridoxine hydrochloride) 40 mg; (2) folic acid+vitamin B12; (3) vitamin B6; and (4) placebo. A total of 3090 patients were enrolled during 2000 to 2004. Of these, 2584 patients underwent coronary angiography for suspected SAP, 461 for acute coronary syndromes, and 45 for aortic valve stenosis at Haukeland University Hospital, Bergen, Norway, or Stavanger University Hospital, Stavanger, Norway. The current study includes participants with SAP only. Participants without successful genotyping (n=13) were excluded, leaving a total of 2571 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 Committee 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 Information and Clinical End Points

Clinical information and blood samples were collected at baseline coronary angiography. Patients delivering blood samples ≥8 hours since last meal were defined as fasting. Obesity was defined as body mass index ≥30 kg/m². Diabetes mellitus was classified by self-reports, glucose measurements (fasting plasma glucose ≥7.0 mmol/L or nonfasting plasma glucose ≥11.1 mmol/L), or by single measurement of glycohemoglobin ≥6.5% according to the American Diabetes Association guidelines.¹³ Classifications of smoking and the extent of coronary artery disease at angiography have been described previously.¹⁴

Study subjects were followed from enrollment until having an AMI or until the end of 2006. Information on clinical events was collected from the Cause of Death Registry at Statistics Norway and from the Western Norway Cardiovascular Registry, which contains all cardiovascular disease diagnoses from the patient administrative systems at the hospitals in Western Norway. AMI (including both fatal and nonfatal events) was classified according to the revised European criteria published in 2000.¹⁵

Genotyping and Biochemical Analyses

Routine biochemical analyses were performed in fresh blood samples at 2 hospitals as described previously.¹⁶ Study-specific blood samples were stored at -80°C until later analyzed at Bevital A/S, Norway (<http://www.bevital.no>). Plasma serine and glycine were determined by gas chromatography–tandem mass spectrometry.¹⁷ MTHFD1 *rs2236225* and *rs1076991* polymorphisms were genotyped by matrix-assisted laser desorption/ionization mass spectrometry.¹⁸

Statistical Analyses

Continuous variables that were not normally distributed were log-transformed prior to statistical analyses. Hardy–Weinberg equilibrium and minor allele frequencies of MTHFD1 *rs2236225* and *rs1076991* polymorphisms were calculated. Baseline categorical variables are reported as frequencies (percentages), while continuous variables are presented as medians (interquartile ranges).

The baseline characteristics were first tested for linear trends across MTHFD1 polymorphisms in univariate logistic regression and linear median regression models for categorical and continuous variables, respectively. Associations of plasma serine and glycine

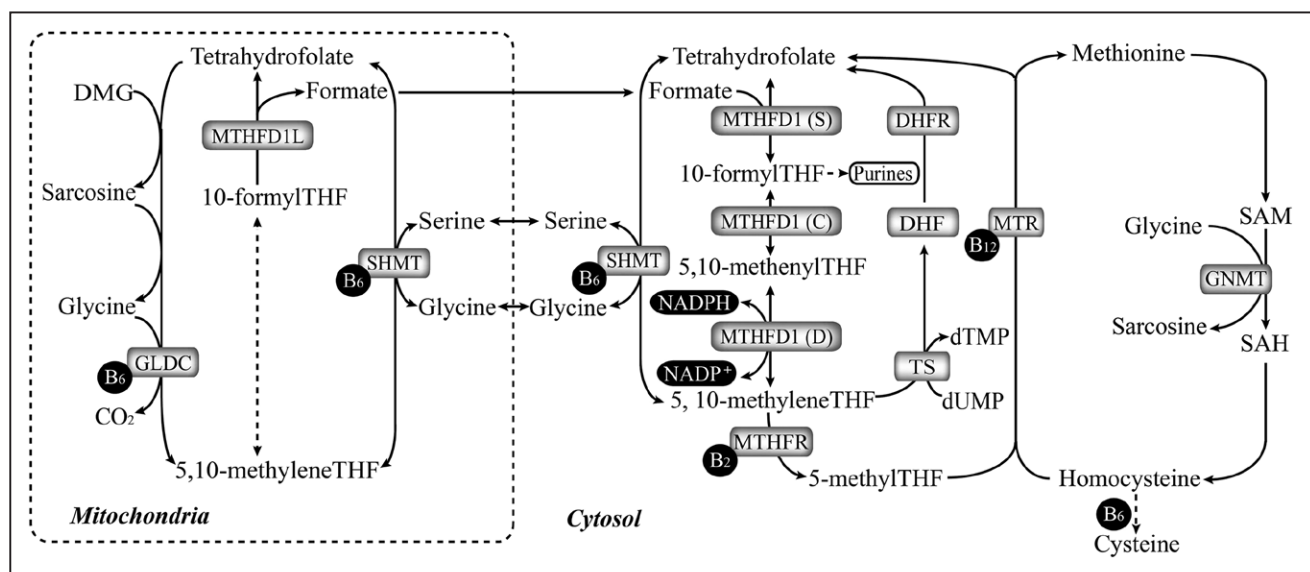


Figure 1. The role of MTHFD1 in the folate-mediated 1-carbon metabolism (1CM). MTHFD1 encodes a protein with 3 distinct activities, 10-formyltetrahydrofolate synthetase (S), methenyltetrahydrofolate cyclohydrolase (C), and methylenetetrahydrofolate dehydrogenase (D). Those tetrahydrofolate derivatives formed by MTHFD1 are involved in DNA synthesis and homocysteine remethylation. DHF indicates dihydrofolate; DHFR, dihydrofolate reductase; DMG, dimethylglycine; GLDC, glycine decarboxylase; GNMT, glycine N-methyltransferase; MTHFD1, methylenetetrahydrofolate dehydrogenase 1; MTHFR, methylenetetrahydrofolate reductase; MTR, 5-methyltetrahydrofolate-homocysteine methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase.

Table 1. Baseline Characteristics According to MTHFD1 Polymorphisms in Patients With Stable Angina Pectoris in WENBIT

| | <i>rs2236225</i> | | | <i>P_{trend}</i> [*] | <i>rs1076991</i> | | | <i>P_{trend}</i> [*] |
|-------------------------------|------------------|--------------|--------------|---------------------------------------|------------------|--------------|--------------|---------------------------------------|
| | GG (n=652) | GA (n=1202) | AA (n=572) | | CC (n=794) | CT (n=1141) | TT (n=446) | |
| Age, y | 62 (13) | 62 (14) | 62 (15) | 0.67 | 62 (14) | 62 (14) | 62 (15) | 0.77 |
| Male sex, n (%) | 520 (79.8) | 942 (78.4) | 465 (81.3) | 0.55 | 626 (78.8) | 909 (79.7) | 353 (79.1) | 0.83 |
| Current smoking, n (%) | 215 (32.8) | 387 (32.2) | 181 (31.6) | 0.62 | 231 (29.1) | 391 (34.3) | 147 (33.0) | 0.08 |
| Obesity, n (%) | 122 (18.7) | 231 (19.2) | 97 (17.0) | 0.46 | 167 (21.0) | 198 (17.4) | 80 (17.9) | 0.10 |
| Hypertension, n (%) | 296 (45.4) | 569 (47.3) | 278 (48.6) | 0.26 | 390 (49.1) | 533 (46.7) | 203 (45.5) | 0.19 |
| Diabetes mellitus, n (%) | 229 (35.1) | 437 (36.4) | 201 (35.1) | 0.98 | 302 (38.0) | 403 (35.3) | 145 (32.5) | 0.06 |
| Previous MI, n (%) | 285 (43.7) | 540 (44.9) | 253 (44.2) | 0.84 | 346 (43.6) | 521 (45.7) | 194 (43.5) | 0.86 |
| Serum CRP, mg/L | 1.69 (2.37) | 1.79 (2.88) | 1.78 (2.53) | 0.19 | 1.79 (2.91) | 1.70 (2.51) | 1.93 (2.65) | 0.74 |
| Serum lipid parameters | | | | | | | | |
| ApoA-1, mg/dL | 1.27 (0.33) | 1.26 (0.34) | 1.26 (0.33) | 0.99 | 1.27 (0.34) | 1.25 (0.32) | 1.27 (0.36) | 0.40 |
| ApoB, mg/dL | 0.85 (0.29) | 0.85 (0.32) | 0.83 (0.30) | 0.14 | 0.83 (0.30) | 0.85 (0.31) | 0.85 (0.27) | 0.99 |
| Triglycerides, mmol/L | 1.52 (1.02) | 1.53 (1.04) | 1.60 (1.20) | 0.27 | 1.60 (1.16) | 1.50 (1.08) | 1.51 (0.93) | 0.31 |
| Total cholesterol, mmol/L | 4.90 (1.40) | 4.90 (1.40) | 4.90 (1.60) | 0.72 | 4.90 (1.48) | 5.0 (1.40) | 4.80 (1.30) | 0.50 |
| LDL cholesterol, mmol/L | 2.90 (1.20) | 2.90 (1.21) | 2.80 (1.30) | 0.26 | 2.86 (1.22) | 2.90 (1.35) | 2.90 (1.20) | 0.74 |
| HDL cholesterol, mmol/L | 1.20 (0.42) | 1.20 (0.40) | 1.20 (0.50) | 0.79 | 1.20 (0.49) | 1.20 (0.40) | 1.20 (0.40) | 0.17 |
| Statins at discharge, n (%) | 574 (88.0) | 1595 (80.5) | 500 (87.4) | 0.77 | 794 (88.9) | 1009 (88.4) | 392 (87.9) | 0.58 |
| Plasma serine, μmol/L | 95.1 (26.7) | 92.9 (25.9) | 92.0 (29.0) | 0.13 | 92.3 (24.7) | 93.3 (27.0) | 93.2 (26.4) | 0.59 |
| Plasma glycine, μmol/L | 197.3 (53.6) | 198.8 (59.0) | 197.2 (56.3) | 0.78 | 196.6 (54.2) | 198.6 (58.8) | 199.8 (58.7) | 0.09 |

Variables are given in medians (IQR) or counts (percentages). Apo indicates apolipoprotein; CRP, C-reactive protein; GFR, glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; and MI, myocardial infarction.

*Unadjusted *P* for trend.

with MTHFD1 polymorphisms were additionally tested after adjustment for age (continuous), sex (male/female), and fasting status (yes/no). Associations between serum lipid parameters and MTHFD1 polymorphisms were tested after adjusting for age (continuous), sex (male/female), and statin treatment (yes/no). An additive genetic model was used in all analyses, in which each additional copy of the mutant allele increases the response by the same amount.

Cox regression models were used to estimate hazard ratios of plasma serine or glycine with risk of AMI in overall population and pre-specified subgroups defined by the MTHFD1 genotypes. All hazard ratios were reported as per 1 SD increment. A simple model (model I) was adjusted for age (continuous) and sex (male/female), whereas a multivariate model (model II) additionally included fasting status (yes/no), smoking (yes/no), obesity (yes/no), hypertension (yes/no), diabetes mellitus (yes/no), extent of coronary artery disease at angiography (ordinal, 1-, 2- or 3-vessel disease), estimated glomerular filtration rate (continuous), apolipoprotein B, low-density lipoprotein cholesterol and triglycerides (all continuous), statin treatment (yes/no), and WENBIT intervention treatment with vitamin B6 (yes/no) and folic acid+B12 (yes/no). Additional adjustment of previous myocardial infarction history did not affect risk estimates and was excluded in the final model (data not shown). The assumption of proportionality was examined by the Schoenfeld and scaled Schoenfeld residuals.

Nonlinear dose–response relationship of plasma serine or glycine with risk of incident AMI was visualized by generalized additive model plots for both simple and multivariate models. Potential effect modifications of 2 MTHFD1 polymorphisms (continuous) on plasma serine or glycine (continuous) were explored separately by including an interaction product in Cox model, which yielded 4 comparisons under simple adjustment and 4 under multivariate adjustment. As such, the 4 interaction tests under each adjustment were examined separately at a significant false discovery rate ≤0.05 to address multiple hypothesis testing.

The statistical analyses were performed with R (R Core Team, Vienna, Austria; version 3.2.2; using packages stats, genetics, Hmisc,

MASS, lm.beta, survival, and visreg). All reported *P* values are 2-sided, and *P*<0.05 was considered significant.

Results

Baseline Characteristics According to MTHFD1 Polymorphisms

MTHFD1 *rs2236225* and *rs1076991* were found to be in Hardy–Weinberg equilibrium (*P*=0.71 and *P*=0.31, respectively), and their minor allele frequencies were 0.48 and 0.43, respectively. We did not observe linkage disequilibrium between the 2 polymorphisms (*r*²=0.03, *D'*=0.22).

Demographics are summarized in Table 1. The median (interquartile ranges) age for the 2571 patients at baseline was 62 (14) years, and 79.7% were males. Of the total population, 32.0% were current smokers, 18.5% were obese, 47.2% had hypertension, 36.1% were diagnosed with diabetes mellitus, and 44.5% had experienced at least 1 previous AMI.

In univariate analyses, we did not observe significant trends of plasma serine, glycine, or other baseline characteristics across MTHFD1 *rs2236225* and *rs1076991* polymorphisms. The number of minor A allele of *rs2236225* polymorphism was marginally associated with lower apolipoprotein B after adjusting for age, sex, and statin treatment (*P_{trend}*=0.06). However, in nonstatin users (n=292), no such trend was found (*P_{trend}*=0.99). After adjustment for age, sex, and fasting status, there was a trend toward an inverse association between plasma glycine and the number of minor T allele of *rs1076991* polymorphism (*P_{trend}*=0.08).

Table 2. Overall Risk Associations Per 1 SD of Plasma Serine and Glycine on Acute Myocardial Infarction

| | Hazard Ratio | 95% CI | P Value |
|------------------|--------------|-----------|---------|
| Model I* | | | |
| Serine | 0.95 | 0.83–1.10 | 0.52 |
| Glycine | 0.83 | 0.72–0.95 | <0.01 |
| Model II† | | | |
| Serine | 0.99 | 0.86–1.15 | 0.89 |
| Glycine | 0.83 | 0.71–0.97 | 0.02 |

CAD indicates coronary artery disease; CI, confidence interval; GFR, glomerular filtration rate; LDL, low-density lipoprotein; and WENBIT, Western Norway B Vitamin Intervention Trial.

*Adjusted for age and sex.

†Adjusted for age, sex, fasting status, smoking, obesity, hypertension, diabetes mellitus, extent of CAD, eGFR, apolipoprotein B, LDL, total cholesterol, statin treatment, WENBIT treatment with vitamin B6, and folic acid plus vitamin B12.

Associations of Plasma Serine and Glycine With Risk of AMI According to MTHFD1 Polymorphisms

During a median (interquartile ranges) follow-up time of 4.7 (2.5–6.8) years, 212 patients (8.2%) experienced an AMI (62 fatal events and 150 nonfatal events). Plasma serine showed no overall association with AMI (Table 2 and Figure 2). In line with our previous findings,⁸ there was an inverse relationship between plasma glycine and AMI, which was not attenuated by multivariate adjustment (Table 2 and Figure 2).

The associations of plasma serine and glycine levels with AMI according to MTHFD1 *rs2236225* polymorphism are summarized in Table 3. After adjusting for age and sex, we observed an interaction between plasma serine and *rs2236225* ($P_{\text{interaction}}=0.03$), which remained significant after adjusting for multiple comparison (false discovery rate =0.04). Plasma serine was inversely associated with AMI among patients carrying the minor A allele but not among patients with the GG genotype at *rs2236225*. A similar interaction was also observed between plasma glycine and *rs2236225* ($P_{\text{interaction}}=0.03$; false discovery rate =0.04). The risk estimates were essentially similar after multivariate adjustment.

We observed an effect modification of the *rs1076991* polymorphism on plasma glycine ($P_{\text{interaction}}=0.02$; Table 4). Plasma glycine showed a stronger association with AMI in patients with the *rs1076991* CC genotype but weaker associations among the *rs1076991* minor T allele carriers. The interaction remained significant after multivariate adjustment ($P_{\text{interaction}}=0.04$). Similar results were also seen even after accounting for multiple comparisons (Table 4). On the other hand, however, the association between serine and AMI were not significantly modified by *rs1076991* polymorphism.

Discussion

Main Findings

In the current study, we observed no overall significant association between plasma serine and AMI, whereas the inverse relationship between plasma glycine and AMI is in line with our previous findings.⁸ Notably, however, the risk associations

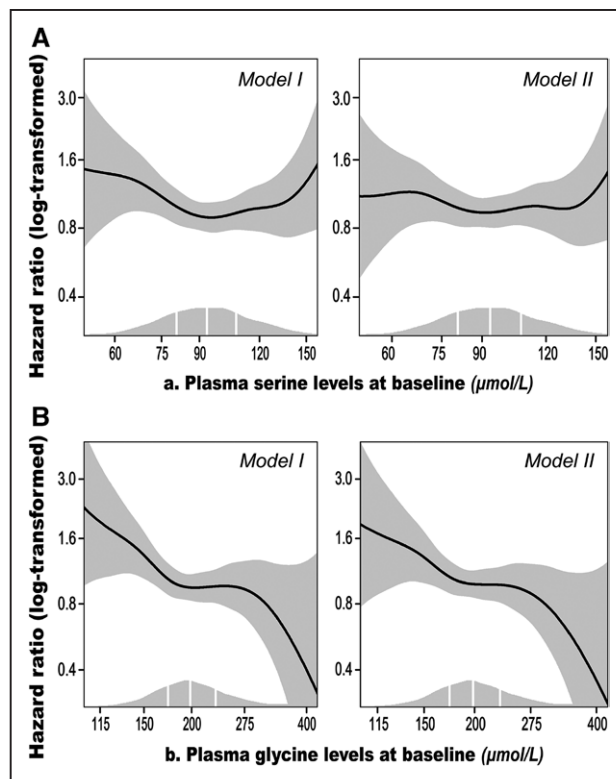


Figure 2. Dose–response associations of (log-transformed) plasma serine and glycine with risk of acute myocardial infarction in patients with stable angina pectoris in WENBIT (Western Norway B Vitamin Intervention Trial). Generalized additive regressions are used with the adjustment for age and sex in model I, whereas model II is additionally adjusted for fasting status, smoking, obesity, hypertension, diabetes mellitus, extent of coronary artery disease (CAD), estimated glomerular filtration rate (eGFR), apolipoprotein B, low-density lipoprotein (LDL), total cholesterol, statin treatment, and WENBIT treatment with vitamin B6 and folic acid+B12. The solid lines and the shaded areas represent hazard ratios of plasma serine (A) and glycine (B) and their 95% confidence interval (CI), respectively. The areas under the curve along the X axes represent the distributions of the plasma serine (A) and glycine (B) concentrations ($\mu\text{mol/L}$). The vertical white lines denote the 25th, 50th, and 75th percentiles of plasma serine (A) and glycine (B), respectively.

for plasma serine and glycine with AMI were modified by the *rs2236225* and *rs1076991* polymorphisms in the MTHFD1 gene. More specifically, low plasma serine and glycine were associated with an increased risk of AMI only among patients carrying the *rs2236225* minor A allele. Similarly, low plasma glycine showed stronger risk relationship with AMI among patients with the *rs1076991* CC genotype as compared with those with the minor T allele carriers.

MTHFD1 Polymorphisms, Serine, Glycine, and Cardiovascular Disease

Both experimental^{19,20} and observational²¹ studies have suggested a link between the MTHFD1 *rs2236225* polymorphism and choline deficiency, which is associated with impaired hepatic very low-density lipoprotein secretion and fatty liver disease.^{22,23} Based on the current study population, we have shown increased risk of AMI in patients carrying the MTHFD1 *rs1076991* polymorphism when they were treated with B vitamins.⁹ In addition, higher plasma glycine is associated with

Table 3. Risk Estimates of Plasma Serine and Glycine Per 1 SD on Acute Myocardial Infarction According to MTHFD1 rs2236225 Polymorphism

| | rs2236225 (Events/Total) | | | P _{int} | FDR |
|------------------|--------------------------|------------------|------------------|------------------|------|
| | GG (56/652) | GA (99/1202) | AA (46/572) | | |
| Model I* | | | | | |
| Serine | 1.13 (0.87–1.47) | 0.93 (0.75–1.15) | 0.75 (0.56–1.01) | 0.03 | 0.04 |
| Glycine | 1.12 (0.86–1.45) | 0.71 (0.57–0.87) | 0.73 (0.53–1.02) | 0.03 | 0.04 |
| Model II† | | | | | |
| Serine | 1.17 (0.88–1.55) | 1.03 (0.83–1.28) | 0.74 (0.55–0.98) | 0.03 | 0.05 |
| Glycine | 1.01 (0.76–1.33) | 0.79 (0.63–0.99) | 0.71 (0.50–1.01) | 0.03 | 0.05 |

The risk associations are presented as hazard ratio per 1 SD of increment (95% confidence interval). CAD indicates coronary artery disease; FDR, false discovery rate; GFR, glomerular filtration rate; LDL, low-density lipoprotein; and WENBIT, Western Norway B Vitamin Intervention Trial.

*Adjusted for age and sex.

†Adjusted for age, sex, fasting status, smoking, obesity, hypertension, diabetes mellitus, extent of CAD, eGFR, apolipoprotein B, LDL, total cholesterol, statin treatment, WENBIT treatment with vitamin B6, and folic acid plus vitamin B12.

decreased risk of type 2 diabetes mellitus,²⁴ a favorable lipid profile and decreased risk of AMI, especially in patients with higher serum apolipoprotein B and low-density lipoprotein cholesterol levels.⁸ The association between serine status and cardiovascular end points has, to the best of our knowledge, not been evaluated previously in large-scale observational studies.

Potential Mechanisms

The underlying mechanisms for the effect modifications of MTHFD1 polymorphisms on the associations of plasma serine and glycine with AMI risk are yet to be elucidated. However, our findings may be related to the severity of MTHFD1 deficiency, the availability of serine and glycine for activated 1-carbon units, and the metabolic cross talk of these 2 interconnected pathways.

Indeed, serine and glycine status have been shown to influence the direction of the reversible MTHFD1 flux,²⁵ which may be associated with the production^{26,27} or consumption²⁸ of NADPH, a crucial reductant in fatty acid and cholesterol

synthesis. Notably, ≈80% of the NADPH produced by the MTHFD1 is used for fatty acid synthesis,²⁷ but there are no data linking this flux directly to cholesterol synthesis. In the current study, adjustment for apolipoprotein B, low-density lipoprotein cholesterol and triglycerides had negligible influence on the risk estimates of plasma serine and glycine, suggesting that cholesterol-related atherogenesis may not be relevant for the observed effect modifications by the MTHFD1 polymorphisms.

Findings on the effects of MTHFD1 on plasma serine and glycine status are inconsistent. An animal study revealed significant decrease of plasma serine levels in transgenic mice, which lacks MTHFD1 synthetase activity under folate-deficient diet.¹⁹ In contrast, studies with the same transgenic model found no alterations of plasma serine and glycine concentrations under folate- and choline-deficient diets.^{29,30} These latter findings are in agreement with our current observations showing no significant association of the MTHFD1 polymorphisms with plasma serine and glycine levels. Discrepancy of the evidence may arise from different dietary patterns

Table 4. Risk Estimates of Plasma Serine and Glycine Per 1 SD on Acute Myocardial Infarction According to MTHFD1 rs1076991 Polymorphism

| | rs1076991 (Events/Total) | | | P _{int} | FDR |
|------------------|--------------------------|------------------|------------------|------------------|------|
| | CC (45/794) | CT (106/1141) | TT (53/446) | | |
| Model I* | | | | | |
| Serine | 0.66 (0.49–0.91) | 1.04 (0.85–1.27) | 0.99 (0.75–1.31) | 0.12 | 0.12 |
| Glycine | 0.59 (0.43–0.83) | 0.82 (0.67–1.01) | 1.00 (0.77–1.31) | 0.02 | 0.04 |
| Model II† | | | | | |
| Serine | 0.73 (0.53–1.01) | 1.03 (0.84–1.26) | 1.06 (0.79–1.45) | 0.23 | 0.23 |
| Glycine | 0.67 (0.47–0.96) | 0.78 (0.62–0.96) | 1.07 (0.79–1.46) | 0.04 | 0.05 |

The risk associations are presented as hazard ratio per 1 SD of increment (95% confidence interval). CAD indicates coronary artery disease; FDR, false discovery rate; GFR, glomerular filtration rate; LDL, low-density lipoprotein; and WENBIT, Western Norway B Vitamin Intervention Trial.

*Adjusted for age and sex.

†Adjusted for age, sex, fasting status, smoking, obesity, hypertension, diabetes mellitus, extent of CAD, eGFR, apolipoprotein B, LDL, total cholesterol, statin treatment, WENBIT treatment with vitamin B6, and folic acid plus vitamin B12.

because both serine and glycine that can be obtained from the diet are associated with B vitamin status and act as 1-carbon sources.^{31,32}

The 2 MTHFD1 polymorphisms revealed opposite effects on modulating the risk estimates of plasma serine and glycine on AMI. The synthetase domain of MTHFD1 is responsible for the conversion of formate and tetrahydrofolate to 10-formyltetrahydrofolate for de novo purine synthesis. The cyclohydrolase activity of MTHFD1 catalyzes the interconversion of 10-formyltetrahydrofolate and 5,10-methylenetetrahydrofolate, which can be further reduced to 5,10-methylenetetrahydrofolate by the dehydrogenase activity (Figure 1). Specifically, the MTHFD1 rs2236225 polymorphism is located in the synthase domain and may result in inadequate 10-formyltetrahydrofolate production.¹⁰ Sufficient serine and glycine may, therefore, counteract low MTHFD1 flux and decreased purine synthesis and explain the stronger beneficial effects of serine and glycine in patients carrying the rs2236225 minor A allele.

On the contrary, the T-allele of rs1076991 polymorphism has been suggested to decrease the MTHFD1 promoter activity by nearly 70%,¹¹ which may reduce the MTHFD1 flux. Meanwhile, increased intracellular glycine was associated with impaired 5,10-methylenetetrahydrofolate, possibly because of reverse flux through serine hydroxymethyltransferase.²⁷ Therefore, the interaction observed between rs1076991 polymorphism and plasma glycine may be indicative of a role shift of MTHFD1 from sufficiency to deficiency in regulating purine synthesis and reverse flux between serine and glycine.

Strengths and Limitations

The MTHFD1 rs2236225 and rs1076991 are common polymorphisms.³³ Their high minor allele frequencies ensure adequate power to detect underlying risk associations. The relatively large sample size, long-term follow-up and detailed baseline clinical characteristics are also strengths of the study. In population studies, a single assessment of biomarker status may underestimate the true strength of long-term risk associations because of large variance, a phenomenon known as regression-dilution bias.³⁴ Notably, a previous study from a subgroup of the current population showed excellent within-person reproducibilities of plasma serine and glycine (intra-class correlation coefficients >0.75 for both biomarkers),³⁵ which allows for 1-exposure assessment for biomarker status and reduces the risk of regression-dilution bias.³⁶

Serine and glycine are involved in several metabolic pathways, which may be influenced by associated genetic and metabolic traits, as well as by dietary habits. We did not evaluate lifestyle or dietary determinants of plasma serine and glycine that might have influence on risk association studies. Hence, the possibility of residual confounding cannot be excluded. Notably, multivariate adjustment including vitamin B intervention treatments did not substantially influence the risk estimates. In addition, previous studies have shown that acute coronary syndrome is associated with a strong inflammatory response,³⁷ which affects vitamin B6 status³⁸ and may accordingly influence plasma glycine levels.³⁹ The current investigation, however, excluded patients with acute coronary syndrome to avoid the potential influence of acute inflammation. Furthermore, the current study was motivated by our

previous findings and should be regarded as hypothesis generating. As such, the nominally significant interactions should preferably be replicated in patients with different clinical characteristics, as well as in population-based cohorts.

Conclusion

Our results suggest that 2 common and functional polymorphisms in the MTHFD1 gene modulate the risk associations of plasma serine and glycine with AMI in patients with SAP. These findings emphasize the possible role of the trifunctional MTHFD1 in regulating serine and glycine metabolism in relation to atherosclerotic complications.

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Disclosures

None.

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CLINICAL PERSPECTIVE

One-carbon metabolism is essential in nucleic acid biosynthesis, epigenetic regulation, and amino acid and lipid metabolism. Evidence also implicates the involvement of 1-carbon metabolism in the development of atherosclerosis and cardiovascular disease. One-carbon units are obtained through the activity of the trifunctional methylenetetrahydrofolate dehydrogenase 1 and from the interconversion of intracellular serine and glycine. Both pathways share 5,10-methylenetetrahydrofolate as metabolic cross point and have been associated with acute myocardial infarction (AMI). It is, therefore, of interest to explore if these 2 pathways interact in relation to AMI. In the current study, we observed no overall significant association between plasma serine and AMI, whereas the inverse relationship between plasma glycine and AMI is in line with our previous findings. The risk associations for plasma serine and glycine with AMI were modified by the *rs2236225* and *rs1076991* polymorphisms in the *MTHFD1* gene. More specifically, low plasma serine and glycine were associated with an increased risk of AMI only among patients carrying the *rs2236225* minor A allele. In addition, low plasma glycine showed stronger risk relationship with AMI among patients with the *rs1076991* CC genotype, as compared with those carrying the minor T allele. These findings emphasize the possible role of the trifunctional methylenetetrahydrofolate dehydrogenase 1 in regulating serine and glycine metabolism in relation to atherosclerotic complications.

Methylenetetrahydrofolate Dehydrogenase 1 Polymorphisms Modify the Associations of Plasma Glycine and Serine With Risk of Acute Myocardial Infarction in Patients With Stable Angina Pectoris in WENBIT (Western Norway B Vitamin Intervention Trial)

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