MTHFR: folic acid 7204/7205

677CC = homozygous for MTHFR gene variant with normal activity, 677CT = heterozygous for MTHFR gene variant with reduced activity, 677TT = homozygous for MTHFR gene variant with reduced activity, 1298AA = homozygous for MTHFR gene variant with normal activity, 1298AC = heterozygous for MTHFR gene variant with slightly reduced activity, 1298CC = homozygous for MTHFR gene variant with slightly reduced activity, BMI = body-mass index, CI = confidence interval, HR = hazard ratio, M = molar (mol/L), MTHFR = methylenetetrahydrofolate reductase, NS = non-significant, OR = odds ratio, OR_{adj} = adjusted odds ratio, S = significant

Brief summary and justification of choices:
Folic acid is converted to tetrahydrofolate by the enzyme dihydrofolate reductase (DHFR). Tetrahydrofolate is required for the synthesis of purine nucleotides and following conversion to 5,10-methylenetetrahydrofolate, it is also required for the synthesis of thymidine nucleotides by thymidylate synthase.
The enzyme MTHFR converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. 5-methyltetrahydrofolate is required for the methylation of homocysteine to methionine and is converted to tetrahydrofolate in this process. Methionine is required for protein synthesis and DNA methylation. Reduced activity of the enzyme MTHFR results in decreased intracellular tetrahydrofolate concentrations.
Because MTHFR is involved in folate metabolism, gene variants that result in reduced MTHFR enzyme activity might influence the effect of folates like folic acid.
Gene variant 677C>T:
Studies showed that the effect of therapy with folic acid either was not changed (Jin 2018 and Huo 2015) or was increased (Huang 2018 and Colson 2017) in patients with the 677T variant that results in reduced MTHFR activity. Patients with this variant had lower baseline folate concentrations and higher baseline homocysteine concentrations than patients without this variant (Jin 2018, Huang 2018, Colson 2017, and Crider 2011). Folic acid therapy partially corrected for this (Huang 2018, Colson 2017, and Crider 2011). Although this correction is only partial, there are no indications for adverse clinical effects for patients with the 677T variant after folic acid treatment. Despite indications for inadequate folic acid supplementation in the majority of pregnant women, Kondo 2014 found no increased risk for offspring with neural tube defects in women with the 677T variant. In addition, Crider 2011 reported periconceptional supplementation with folic acid 0.4 mg/day to decrease the incidence of neural tube defects with up to 85% in a population in which 35% had the 677TT genotype and 48% the 677CT genotype. Huo 2015 showed no difference between 677C>T genotypes in the decrease in stroke risk in hypertensive patients by treatment with folic acid 0.8 mg/day for 4.5 years. The meta-analysis of Shao 2017 showed the risk for methotrexate toxicity in studies with folic acid co-medication to be increased for patients with the 677T variant. In addition, this risk was numerically more increased in studies with 100% folic acid co-medication than in all studies together (so with full, with partial or without folic acid co-medication). However, Shao 2017 did not show whether this risk was significantly more increased in studies with folic acid co-medication than in studies without folic acid co-medication, and even if they would have, it would not have been clear whether this increase would have been due to a diminished effectiveness of folic acid in reducing methotrexate toxicity or to an increased effect on the toxicity of the folate antagonist methotrexate in studies with folic acid co-medication. Folic acid is added to methotrexate to suppress methotrexate toxicity, so used especially in regimens expected to give high methotrexate toxicity. In addition, none of the studies reported an increase in adverse events of folic acid treatment in subjects with the 677T variant. Den Dekker 2018 found some differences in respiratory parameters of children aged 10 years of mothers with or without the 677T variant using folic acid supplementation during pregnancy, but no difference in current asthma. For this reason, the KNMP Pharmacogenetics Working Group concludes that there is a MTHFR-folic acid interaction, but that there is insufficient evidence for a clinical effect that makes therapy adjustment useful (yes/no-interactions).
You can find an overview of the clinical and kinetic effects per genotype in the background information text of the gene-drug interactions in the KNMP Kennisbank. You may also have access to this background information text via your pharmacy of physician electronic decision support system.
Other gene variants:
For gene variant 1298A>C, Du 2018 showed a decreased risk of failure of treatment with folic acid 5 mg/day for 90 days for hyperhomocysteinaemia (fasting total plasma homocysteine concentration ≥ 15 µM) for carriers of a 1298C variant. The direction of this effect was opposite to the increased risk Du 2018 found for carriers of a 677T variant, while the effect of both variants on MTHFR is reported to be a reduction of activity. The authors indicated that there was a strong linkage disequilibrium between 677C>T and 1298A>C with 99.5% of alleles with a 677T-variant having a 1298A-variant. In addition, the reduction in the risk for treatment failure was similar for alleles with a 677C-variant

having either a 1298A-variant or a 1298C-variant. So, the observed effect is most likely due to the linkage disequilibrium with 677C>T and not to 1298A>C itself. Therefore, it was decided that there was not enough evidence for an clinically significant effect of 1298A>C on MTHFR activity and thus, no cause for inclusion of this gene variant in the MTHFR pharmacogenetic interactions.
For gene variant 1781G>A, Du 2018 did not show an effect on the risk of failure of treatment with folic acid 5 mg/day for 90 days for hyperhomocysteinaemia (fasting total plasma homocysteine concentration ≥ 15 µM). Therefore, it was decided that there was not enough evidence for an effect of this gene variant on MTHFR activity and thus, no cause for inclusion of this gene variant in the MTHFR pharmacogenetic interactions.

The table below follows the KNMP nomenclature for MTHFR gene variants. The nomenclature used in the table below may therefore differ from the nomenclature used by the authors in the article.

<table>
<tr><th>Source</th><th>Code</th><th>Effect</th><th>Comments</th></tr>
<tr><td>ref. 1
Chen MY et al. Defining the plasma folate concentration associated with the red blood cell folate concentration threshold for optimal neural tube defects prevention: a population-based, randomized trial of folic acid supplementation.
Am J Clin Nutr 2019;109:1452-61.
PubMed PMID: 31005964.</td><td>4</td><td>941 women without anaemia and vitamin B12 deficiency received folic acid (0.1 mg/day (in 1 or 4 doses), 0.4 mg/day (in 1 or 4 doses), 4 mg once daily, or 4 mg once weekly) for 6 months. This concerns the same study as Crider 2011. 6 month data were available for 909 women.
To promote optimal neural tube defect risk reduction at the population level, WHO recently recommended that the population red blood cell folate concentrations should be above a threshold of 906 nM (400 ng/mL) in women of reproductive age. In contrast, the threshold for preventing megaloblastic anaemia is much lower (i.e., 305 nM).
The correlation coefficient was defined as the correlation coefficient between natural logarithm of red blood cell folate concentration and natural logarithm of plasma folate concentration.
677CC and 677CT were more prevalent in the studied group (women without anaemia and vitamin B12 deficiency) than in the excluded group with anaemia and/or vitamin B12 deficiency. In addition, the included women were more likely to be < 25 years of age than the excluded women.
The median plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nmol/L was estimated with a multivariate Bayesian model.

Genotyping:
- 163x 677CC
- 448x 677CT
- 330x 677TT

Results:
<table>
<tr><td colspan="5">Results compared to 677CC:</td></tr>
<tr><td colspan="2"></td><td>677TT</td><td>677CT</td><td>value for 677CC</td></tr>
<tr><td rowspan="3">correlation coefficient between plasma and red blood cell folate concentrations</td><td>pretreatment</td><td>NS</td><td>NS</td><td>0.45</td></tr>
<tr><td>6 months</td><td>NS</td><td>NS</td><td>0.60</td></tr>
<tr><td colspan="4">The authors indicate that the correlation increases with folic acid treatment, because the variance decreases when the population is homogenised by 6 months of folic acid treatment and much higher concentrations, where the same absolute difference is a much smaller percentage change.</td></tr>
<tr><td colspan="2" rowspan="2">estimated median plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nM based on both pretreatment and month 6 data</td><td>+ 3.1 (95% CI: 0.2 - 5.9) (S)</td><td>NS</td><td>23.2 nM</td></tr>
<tr><td colspan="3">For 677TT, the median red blood cell folate concentration after supplementation was 99% of the 906 nmol/L threshold.</td></tr>
<tr><td>677TT: AA</td><td colspan="2">estimated median plasma folate concentrations corres-</td><td>NS</td><td>NS</td><td>24.9 nM</td></tr>
</table>
</td><td>Authors' conclusion:
''The relation between red blood cell and plasma folate concentrations was modified by BMI and genotype and substantially by low plasma vitamin B-12. This suggests that the threshold of 25.5 nmol/L for optimal neural tube defect prevention may be appropriate in populations with similar characteristics, but it should not be used in vitamin B-12 insufficient populations.''</td></tr>
</table>

<table>
<tr><td>ref. 1, continuation</td><td>677CT: AA</td><td>ponding to the red blood cell folate concentration of 906 nM at month 6</td><td></td><td></td><td></td><td></td></tr>
<tr><td>ref. 2
Jin H et al.
An evidence-based approach to globally assess the covariate-dependent effect of the MTHFR single nucleotide polymorphism rs1801133 on blood homocysteine: a systematic review and meta-analysis.
Am J Clin Nutr 2018;107:817-25.
PubMed PMID: 29722849.</td><td>4

677TT: AA

677CT: AA</td><td colspan="4">Meta-analysis of the effect of 677C>T on total plasma or serum homocysteine and meta-regression analysis of the effect of high folic acid intake. Of the approximately 190 experimental groups with folic acid intake data, approximately 70 had high folic acid intake, corresponding to approximately 25000 subjects. High folic acid intake was defined as intake in mandatory food fortified countries, folic acid supplement intake or high folic acid intake according to the study. Low folic acid intake was defined as no supplementation or food fortification.
Meta-analyses were performed with a random-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and data exaction was standardised.
Risk-of-bias of the included observations was analysed by using the Item Bank on Risk of Bias and Precision of Observational Studies. Selection bias resulting from the participants' deviation from the general population (e.g. sampling from hospital patients and the healthy worker's bias), detection bias resulting from assay techniques, and the selective outcome reporting were also considered.
Heterogeneity between the studies in the meta-analyses was high and there were indications for a slight publication bias toward a total homocysteine-elevating effect for 677TT, but not for 677CT.

Genotyping (based on subjects with and without folic acid intake):
- 44% 677CC
- 43% 677CT
- 13% 677TT

Results:
<table>
<tr><td colspan="3">Results compared to 677CC:</td></tr>
<tr><td></td><td>677TT</td><td>677CT</td></tr>
<tr><td>difference in total homocysteine for subjects with and without folic acid intake data</td><td>+ 1.29 µM (S)</td><td>+ 1.07 µM (S)</td></tr>
<tr><td>modification of the effect by high folic acid intake</td><td>- 1.10 µM (NS for high compared to low intake, but S for high versus mixed versus unknown versus low intake)</td><td>NS</td></tr>
<tr><td colspan="3">The authors indicate that despite an increase in plasma or serum total homocysteine concentrations in the majority of 677TT and 677CT, the majority of the subjects with low or high folic acid intake did not have hyperhomocysteinaemia (total homocysteine ≥ 13 µM). Modelling showed hyperhomocysteinaemia to be present in 2.59% of 667CT and in 19.1% of 667TT.</td></tr>
</table></td><td>Authors' conclusion:
''The effect of rs1801133 (677 C>T) on total homocysteine exhibits significant variability that can be attributed to effect modification as well as confounding by these covariates (sex, age, race, folic acid intake, smoking, and alcohol consumption).''</td></tr>
<tr><td>ref. 3
Du B et al.
Genetic polymorphisms of key enzymes in folate metabolism affect the efficacy of folate therapy in patients with hyperhomocysteinaemia.
Br J Nutr 2018;119:887-95.</td><td>4</td><td colspan="4">638 patients with hyperhomocysteinaemia were treated with folic acid 5 mg/day for 90 days.
Hyperhomocysteinaemia was defined as fasting total plasma homocysteine concentration ≥ 15 µM. Treatment failure was defined as hyperhomocysteinaemia still present after treatment.
Co-medication and supplements affecting folate metabolism were excluded.
ORs were adjusted for age, sex, smoking, drinking and biochemical parameters.

Genotyping:
677C>T:
- 106x 677CC
- 274x 677CT

1298A>C:
- 487x 1298AA
- 136x 1298AC

1781G>A:
- 591x 1781GG
- 47x 1781GA</td><td>Authors' conclusion:
''The MTHFR rs1801133 CT genotype, TT genotype and T allele; the MTHFR rs1801131 AC genotype, CC genotype and C allele; the MTRR rs1801394 GA genotype, GG genotype and G allele; and the</td></tr>
</table>

<table>
<tr>
<td>PubMed PMID: 29644956.

ref. 3, continuation</td>
<td>677TT: B

677CT: B

1298 CC: AA#

1298 AC: AA#

1781 GA: AA</td>
<td>- 258x 677TT - 15x 1298CC

Results:
<table>
<tr><td colspan="4">Results compared to homozygous wild type:</td></tr>
<tr><td colspan="2"></td><td>homozygous variant</td><td>heterozygous</td><td>value for homozygous wild type</td></tr>
<tr><td rowspan="3">treatment failure</td><td>677 C>T</td><td>OR_{adj} = 2.68 (95% CI: 1.59-4.54) (S)</td><td>OR_{adj} = 1.69 (95% CI: 1.00-2.85) (S)</td><td>32%</td></tr>
<tr><td>1298 A>C</td><td>OR_{adj} = 0.26 (95% CI: 0.07-0.91) (S)</td><td>OR_{adj} = 0.52 (95% CI: 0.33-0.81) (S)</td><td>52%</td></tr>
<tr><td>1781 G>A</td><td>-</td><td>NS</td><td>49%</td></tr>
<tr><td colspan="4">The authors indicate that there is a strong linkage disequilibrium between 677C>T and 1298A>C with 99.5% of alleles with a 677T-variant having a 1298A-variant. In addition, the risk for treatment failure was similarly reduced for alleles with a 677C-variant having either a 1298A-variant (OR = 0.69 (95% CI: 0.53-0.89) (S)) or a 1298C-variant (OR = 0.60 (95% CI: 0.43-0.84) (S)).</td></tr>
<tr><td colspan="4">For the MTHFR gene variants, the authors did not adjust the ORs for history of cardiovascular heart disease, history of hypertension, and history of diabetes, despite these parameters showing a significant effect on treatment failure. However, multivariate analysis showed 677C>T, history of cardiovascular heart disease and history of hypertension to be independent predictors of treatment failure with OR = 6.88 (95% CI: 3.98-16.70) (S) for the three parameters combined.</td></tr>
</table></td>
<td>MTRR rs162036 AG genotype and AG + GG genotypes were associated with the efficacy of folic acid therapy for hyperhomocysteinaemia.''</td>
</tr>
<tr>
<td>ref. 4
Huang X et al. MTHFR gene and serum folate interaction on serum homocysteine lowering: prospect for precision folic acid treatment. Arterioscler Thromb Vasc Biol 2018;38:679-85.
PubMed PMID: 29371246.</td>
<td>4</td>
<td>8218 hypertensive patients without pre-existing stroke and major cardiovascular events were treated with folic acid 0.8 mg/day and enalapril 10 mg/day for 4.5 years. The control group of 8195 patients received enalapril 10 mg/day only. This concerns the same study as Huo 2015.
Target total serum homocysteine concentration was < 10 µM.
Supplements affecting folate metabolism (B vitamins) were excluded, but other relevant co-medication was not. However, other antihypertensive drugs, lipid- or glucose-lowering drugs, and antiplatelet drugs did not differ significantly between groups stratified by genotype and treatment.
Differences in folate and total homocysteine concentrations between patients with and without folic acid treatment were adjusted for age, sex, smoking status, alcohol drinking, total homocysteine, folic acid, vitamin B12, estimated glomerular filtration rate, systolic blood pressure, systolic blood pressure, average systolic blood pressure during the treatment period and body-mass index.

Genotyping of the folic acid group:
- 2238x 677CC
- 4029x 677CT
- 1951x 677TT

Results:
<table>
<tr><td colspan="4">Results for folic acid treatment compared to no folic acid treatment:</td></tr>
<tr><td></td><td>677TT</td><td>677CT</td><td>677CC</td></tr>
<tr><td>increase in folate concentration (in ng/ml)</td><td>11.2 (95% CI: 10.4-12.1) (S)</td><td>11.5 (95% CI: 10.9-12.1) (S)</td><td>11.7 (95% CI: 10.9-12.6) (S)</td></tr>
<tr><td></td><td colspan="3">Both before and after treatment with folic acid, the folate concentration was slightly lower for increasing number of 677T-alleles (7.3 versus 8.6 versus 9.5 ng/ml before treatment and 23.3 versus 24.8 versus 25.7 ng/ml after treatment for 677TT versus 677CT versus 677CC) (NS).</td></tr>
</table></td>
<td>Authors' conclusion:
''Compared with CC or CT, total homocysteine in the TT group manifested a heightened L-shaped curve from low to high folate levels, but this difference in total homocysteine by genotype was eliminated when plasma folate levels reach ≈15 ng/mL or higher. Our data raised the prospect to tailor folic acid therapy according to individual MTHFR C677T genotype and folate status.''</td>
</tr>
</table>

<table>
<tr>
<td>ref. 4, continuation</td>
<td>677TT: AA#

677CT: AA</td>
<td>
<table>
<tr><td></td><td colspan="3">Folate concentration also increased in the patients receiving enalapril treatment only (increase with 4.4-4.8 ng/ml).</td></tr>
<tr><td>decrease in total homocysteine concentration (in µM)</td><td>3.3 (95% CI: 2.7-3.8) (S)</td><td>1.3 (95% CI: 1.1-1.5) (S)</td><td>1.0 (95% CI: 0.8-1.2) (S)</td></tr>
<tr><td></td><td colspan="3">The decrease in total homocysteine concentration compared to 677CC was higher for 677TT (S), but not for 677CT (NS).</td></tr>
<tr><td></td><td colspan="3">Despite the stronger decrease in total homocysteine for 677TT, the total homocysteine concentration after folic acid treatment was still numerically higher for this genotype (14.1 versus 12.4 versus 12.1 µM after treatment and 19.3 versus 13.1 versus 12.5 µM before treatment for 677TT versus 677CT versus 677CC) (NS).
The difference in total homocysteine concentration between 677TT and 677CC was significant when stratified by folate concentration, but approached zero at folate concentrations between 15 and 25 ng/ml (between 34 and 57 nM) (mean < 1.2 µM, upper limit 95% CI < 1.8 µM). The difference seemed to increase slightly at folate concentrations ≥ 25 ng/ml (mean 1.35 µM, upper limit 95% CI 2.32 µM).</td></tr>
<tr><td></td><td colspan="3">Total homocysteine was similar after treatment with enalapril only (decrease with 2.2 versus -0.6 versus -0.7 µM for 677TT versus 677CT versus 677CC) (NS).</td></tr>
</table>

<table>
<tr><td colspan="5">Percentage of patients achieving the indicated targets after treatment with folic acid (FA) and without folic acid (-) (significance of differences in percentages not determined):</td></tr>
<tr><td colspan="2"></td><td>677TT</td><td>677CT</td><td>677CC</td></tr>
<tr><td rowspan="2">folate concentration ≥ 15 ng/ml</td><td>-</td><td>24.2%</td><td>32.7%</td><td>41.3%</td></tr>
<tr><td>FA</td><td>70.1%</td><td>74.0%</td><td>76.5%</td></tr>
<tr><td rowspan="2">total homocysteine concentration < 10 µM</td><td>-</td><td>12.2%</td><td>18.4%</td><td>21.4%</td></tr>
<tr><td>FA</td><td>20.5%</td><td>27.0%</td><td>29.2%</td></tr>
<tr><td rowspan="2">total homocysteine concentration < 15 µM</td><td>-</td><td>54.6%</td><td>74.3%</td><td>76.9%</td></tr>
<tr><td>FA</td><td>73.5%</td><td>82.9%</td><td>84.8%</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 5
den Dekker HT et al.
Maternal folic acid use during pregnancy, methylenetrahydrofolate reductase gene polymorphism, and child's lung function and asthma.
Clin Exp Allergy 2018;48:175-85.
PubMed PMID: 29117460.</td>
<td>3</td>
<td>Children of 2815 mothers using folic acid supplements during pregnancy (50% start preconceptional, 34% start < 10 weeks of pregnancy and 16% start ≥ 10 weeks of pregnancy) were compared at age 10 with children from 653 non-complement users. Genotype was known for 2357 children. Frequency and dose of folic acid supplement use throughout pregnancy were not known, but 0.4-0.5 mg folic acid solely or within multivitamin supplements was recommended during pregnancy.
Current asthma was defined as ever physician-diagnosed asthma, with either wheezing or the use of inhalant medication in the past 12 months. Current asthma was present in 6% of children. Lung function parameters were measured at a median age of 9.7 years (range 8.5-12.0 years) and converted into sex-, age-, height- and ethnicity-adjusted z-scores.
Associations were tested with logistic regression models, adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational level, smoking or alcohol use during pregnancy, child’s gestational age at birth, birthweight and ethnicity.

Genotyping of the folic acid group:
mothers: children:
- 1391x 677CC - 1101x 677CC
- 1424x 677CT+TT - 1256x 677CT+TT

Results:</td>
<td>Authors’ conclusion:
’’Preconceptional start of maternal folic acid supplement use and higher vitamin B12 concentrations at birth might adversely affect childhood lung function depending on MTHFR-C677T carriership. The clinical implications need to be evaluated.’’</td>
</tr>
</table>

ref. 5, continuation

677CT +TT: A

Results for folic acid supplementation (FA) compared to no folic acid supplementation (↓ indicates a decrease and ↑ indicates an increase):

		genotype mother		genotype child	
		CT+TT	CC	CT+TT	CC
current asthma	any FA	NS	NS	NS	NS
	start FA pre-conceptionally	NS	NS	NS	NS
	start FA ≤ 10 weeks	NS	NS	NS	NS
	start FA > 10 weeks	NS	NS	NS	NS
forced expiratory volume in 1 second	any FA	NS	↑ (S)	NS	NS
	start FA pre-conceptionally	NS	↑ (S)	NS	NS
	start FA ≤ 10 weeks	NS	↑ (S)	NS	↑ (S)
	start FA > 10 weeks	NS	↑ (S)	NS	NS
forced vital capacity	any FA	NS	↑ (S)	↑ (S)	↑ (S)
	start FA pre-conceptionally	NS	↑ (S)	↑ (S)	↑ (S)
	start FA ≤ 10 weeks	NS	↑ (S)	↑ (S)	↑ (S)
	start FA > 10 weeks	NS	↑ (S)	↑ (S)	NS
ratio (forced expiratory volume in 1 second)/(forced vital capacity)	any FA	↓ (S)	NS	NS	↓ (S)
	start FA pre-conceptionally	↓ (S)	NS	↓ (S)	↓ (S)
	start FA ≤ 10 weeks	NS	NS	NS	NS
	start FA > 10 weeks	NS	NS	NS	NS
forced expiratory flow between 25% and 75% of forced vital capacity	any FA	NS	↑ (S)	NS	NS
	start FA pre-conceptionally	↓ (S)	↑ (S)	NS	NS
	start FA ≤ 10 weeks	NS	NS	NS	NS
	start FA > 10 weeks	NS	NS	NS	NS
forced expiratory flow at 75% of forced vital capacity	any FA	NS	NS	NS	NS
	start FA pre-conceptionally	NS	NS	NS	NS
	start FA ≤ 10 weeks	NS	↑ (S)	NS	NS
	start FA > 10 weeks	NS	NS	NS	NS

For both maternal and child 677CT+TT the effect on forced expiratory flow between 25% and 75% of forced vital capacity increased with increasing duration of folic acid supplementation (S).
No other significant effects of duration of supplementation were observed.

ref. 6
Shao W et al. Association between MTHFR C677T polymorphism and methotrexate treatment outcome in rheumatoid

3

Meta-analyses of the effect of 677C>T on effectiveness and toxicity of methotrexate combined with folic acid. 7 included studies with a total of 1100 patients (569x 677T carriers and 531x 677CC) investigated effectiveness, of which 5 studies with a total of 747 patients (395x 677T carriers and 352x 677CC) reported data for 677CT and 677TT separately. 10 included studies with a total of 2007 patients (1156x 677T carriers and 851x 677CC) investigated toxicity, of which 8 studies with a total of 1657 patients (992x 677T carriers and 665x 677CC) reported data for 677CT and 677TT separately.
Meta-analyses were performed with a random-effects model in case of

Authors' conclusion:
''In addition, rheumatoid arthritis patients with the MTHFR C677T polymorphism who were supplemented with folic acid

<table>
<tr>
<td>arthritis patients: a systematic review and meta-analysis. Genet Test Mol Biomarkers 2017;21:275-85.
PubMed PMID: 28277784.

ref. 6, continuation</td>
<td>677TT: AA

677CT: AA</td>
<td>significant heterogeneity between the studies and with a fixed-effects model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent, but the data exaction method was not mentioned.
Quality of the included studies was not judged.
Publication bias and sensitivity analysis were not performed separately for the studies with folic acid supplementation, only for all studies (with or without folic acid supplementation) together.

Results:
<table>
<tr><td colspan="3">Results compared to 677CC:</td></tr>
<tr><td></td><td>677TT</td><td>677CT</td></tr>
<tr><td rowspan="2">effectiveness</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="2">NS</td></tr>
<tr><td rowspan="2">toxicity</td><td>OR = 2.54 (95% CI: 1.36-4.75) (S)</td><td>OR = 1.66 (95% CI: 1.01-2.71) (S)</td></tr>
<tr><td colspan="2">OR = 1.57 (95% CI: 1.05-2.36) (S)</td></tr>
<tr><td colspan="3">Heterogeneity between studies was high for all 3 comparisons for toxicity.
Heterogeneity between studies was low for effectiveness and 677TC compared to 677CC, and for effectiveness and 677TT+TC compared to 677CC.
Heterogeneity between studies was absent for effectiveness and 677TT compared to 677CC.</td></tr>
<tr><td colspan="3">ORs for toxicity were numerically somewhat lower for all 25 or 18 studies together (10 or 8 with 100% folic acid supplementation, 8 or 5 with partial folic acid supplementation and 7 or 5 without folic acid supplementation) compared to only the studies with 100% folic acid supplementation, but confidence intervals strongly overlapped. So, it is not clear whether the effect of the 677T-allele on methotrexate toxicity is higher in patients receiving folic acid compared to patients not receiving folic acid.</td></tr>
</table>
</td>
<td>displayed significantly elevated risk for methotrexate toxicity.''</td>
</tr>
<tr>
<td>ref. 7
Colson NJ et al. The impact of MTHFR 677 C/T genotypes on folate status markers: a meta-analysis of folic acid intervention studies.
Eur J Nutr 2017;56:247-60.
PubMed PMID: 26497154.</td>
<td>3</td>
<td>Meta-analysis of 9 studies investigating the effect of supplementation with a minimum of 0.4 mg dietary folic acid equivalents for a period of 4 to 24 weeks. To adjust for bioequivalence between natural and synthetic folates, 1 mg dietary folic acid equivalents was defined as either 1 mg food folate or 0.6 mg folic acid with food. Studies with potential interaction of other medications/supplements with folate status, with supplementation greater than 1 mg folic acid (1.67 mg dietary folic acid equivalents) per day, with natural food folate as only folate source, with subjects with chronic illness that would affect folate status, or with pregnant women were excluded from the meta-analysis. Of the 9 included studies, 5 were randomised controlled trials and 4 were quasi-experimental trials. Based on the Rosendal score, 7 of the 9 included studies were of excellent methodological quality (Rosendal score ≥ 60%). The other 2 studies had slightly lower Rosendal scores (55% and 57%). The meta-analyses for pre-supplementation and post-supplementation fasting plasma total homocysteine, included 9 studies with a total of 841 subjects (450x 677CC and 391x 677TT) and 6 studies with a total of 326 subjects (155x 677CC and 171x 677TT), respectively. The meta-analyses for pre-supplementation and post-supplementation serum folate concentration, included 9 studies with a total of 686 subjects (371x 677CC and 315x 677TT) and 8 studies with a total of 589 subjects (301x 677CC and 288x 677TT), respectively.
One of the studies that was (partially) included in the meta-analyses is also included in our risk analysis separately (Crider 2011).
Meta-analyses were performed with the DerSimonian-Laird random-effects model, which assigns a weight to each study, inversely proportional to the within-study sampling variance, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and data exaction was standardised.
Quality of the included studies was assessed with both a modified version of Downs and Black's checklist and with the Rosendal scale.</td>
<td>Authors' conclusion:
''This meta-analysis confirms observations from observational and intervention studies that MTHFR TT genotype is associated with increased plasma homocysteine and lowered serum folate and less response to short-term supplementation.''</td>
</tr>
</table>

ref. 7, continuation	677TT: AA[#] 677TT: A	Publication bias was not assessed, but for the pre-supplementation total homocysteine concentration, the effect was similar after exclusion of the study with the largest difference between 677TT and 677CC. Results:	

Mean difference for 677TT compared to 677CC:		
total homocysteine concentration	pre-supplementation	2.8 µM (95% CI: 1.5-4.2) (S)
		The 677TT genotype group in one of the studies had mean homocysteine levels corresponding to hyperhomocysteinaemia (>15 µM), compared to none of the 677CC genotype groups.
		The difference was also significant: - for random controlled trials only (n = 308) - for females only (n = 482) - after removal of the study with the largest folic acid dose (1 mg/day) (n = 789) - after removal of the study with the largest difference (n = 618) - for Chinese populations only (n = 192)
	post-supplementation	NS
		No studies in either genotype group had mean homocysteine levels >15 µM, but the study included in the pre-supplementation meta-analysis with mean homocysteine levels >15 µM in the 677TT group was not included in the post-supplementation meta-analysis.
		A significant difference was also lacking: - for random controlled trials only (n = 247) - for females only (n = 274) - after removal of the study with the largest folic acid dose (1 mg/day) (n = 274)
serum folate concentration	pre-supplementation	-3.6 nM (95% CI: -2.1 – -5.0) (S)
		The mean serum folate concentration for all groups was above the threshold for folate deficiency (7 nM). The lowest mean concentrations were 7.3 nM and 9.1 nM in the 667TT group of two studies.
		The difference was also significant: - for random controlled trials only (n = 458) - for females only (n = 456) - after removal of the study with the largest folic acid dose (1 mg/day) (n = 634) - after removal of studies with depletion diets for a minimum of 6 weeks because of folic acid fortification of food in the countries (n = 633) - for Chinese populations only (n = 237)
	post-supplementation	- 8.7 nM (95% CI: -6.3 – -11.0) (S)
		All groups in all studies showed an increase in mean serum folate concentration with the lowest mean concentrations being 14 nM and 19 nM for the 677TT and 677CC groups for the same study.
		The difference was also significant: - for random controlled trials only (n = 357) - for females only (n = 397) - for studies with supplementation for ≥ 8 weeks (n = 319) - after removal of the study with the largest folic acid dose (1 mg/day) (n = 537) - after removal of studies with depletion

<table>
<tr><td>ref. 7, continuation</td><td></td><td>
<table>
<tr><td></td><td></td><td>diets for a minimum of 6 weeks before supplementation because of folic acid fortification of food in the countries (n = 536)
- for Chinese populations only (n = 274)</td></tr>
<tr><td colspan="3">Heterogeneity between studies was high for all 4 comparisons.</td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 8
Huo Y et al. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. JAMA 2015;313:1325-35.
PubMed PMID: 25771069.</td><td>4

677TT: AA

677CT: AA</td><td>
10348 hypertensive patients without pre-existing stroke and major cardiovascular events were treated with folic acid 0.8 mg/day and enalapril 10 mg/day for 4.5 years. The control group of 10351 patients received enalapril 10 mg/day only. Intention to treat analysis was performed, so patients violating the study protocol including patients who did not take any study drug were also included in the analysis. Follow-up visits were every 3 months.
Supplements affecting folate metabolism (B vitamins) were excluded, but other relevant co-medication was not. However, other antihypertensive drugs, lipid- or glucose-lowering drugs, and antiplatelet drugs did not differ significantly between groups stratified by genotype and treatment.
Adjusted HRs were adjusted for age, sex, systolic and diastolic blood pressure at baseline, mean systolic and diastolic blood pressure during treatment, body-mass index, study centres, baseline homocysteine, vitamin B12, creatinine, total cholesterol, triglycerides, HDL cholesterol, fasting glucose levels, smoking, and alcohol consumption.

Genotyping of the folic acid group:
- 2821x 677CC
- 5095x 677CT
- 2432x 677TT

Results:
<table>
<tr><td colspan="5">Results for folic acid treatment compared to no folic acid treatment:</td></tr>
<tr><td colspan="2"></td><td>677TT</td><td>677CT</td><td>677CC</td></tr>
<tr><td colspan="2">systolic blood pressure during treatment</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="2"></td><td colspan="3">Mean systolic blood pressure during treatment was 139.8 mm Hg.</td></tr>
<tr><td colspan="2">diastolic blood pressure during treatment</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="2"></td><td colspan="3">Mean diastolic blood pressure during treatment was 83.1 mm Hg.</td></tr>
<tr><td colspan="2">risk of first stroke</td><td>HR = 0.72 (95% CI: 0.53-0.97) (S)</td><td>NS</td><td>HR = 0.65 (95% CI: 0.48-0.89) (S)</td></tr>
<tr><td colspan="2"></td><td colspan="3">The genotype did not influence the treatment effect (NS).</td></tr>
<tr><td colspan="2"></td><td colspan="3">The incidence of first stroke was 2.7% in the folic acid group and 3.4% in the group without folic acid.</td></tr>
<tr><td rowspan="5">risk of first stroke according to baseline folate concentration quartiles</td><td>Q1</td><td>NS</td><td>trend for a decreased risk (p = 0.10) (NS)</td><td>HR_{adj} = 0.37 (95% CI: 0.19-0.73) (S); absolute reduction 2.4%</td></tr>
<tr><td>Q2</td><td>NS</td><td>NS</td><td>trend for a decreased risk (p = 0.06) (NS)</td></tr>
<tr><td>Q3</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>Q4</td><td>HR_{adj} = 0.24 (95% CI: 0.10-0.58) (S); absolute reduction 2.8%</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>The effect of folic acid was lower for Q1 compared to</td><td>Trend for a stronger effect of folic acid for Q1 compared</td><td>The effect of folic acid was stronger for Q1+Q2 com-</td></tr>
</table>
</td><td>Authors' conclusion:
''The results from the joint analyses of MTHFR genotype and baseline folate level showed that among participants with the CC or CT genotypes, the highest risk of stroke and the greatest benefit of folic acid therapy were in those with the lowest baseline folate levels. In addition, our data suggest that individuals with the TT genotype may require a higher dosage of folic acid supplementation to overcome biologically insufficient levels (as reflected in the relatively greater folate requirement with the TT genotype).''</td></tr>
</table>

<table>
<tr><td>ref. 8, continuation</td><td></td><td>
<table>
<tr><td></td><td></td><td>Q4 (S) and for Q2+Q3 compared to Q4 (S).</td><td>to Q2+Q3+Q4 (p = 0.083) (NS).</td><td>pared to Q4 (S).</td></tr>
<tr><td colspan="5">Baseline folate quartiles within each of the genotypes were 677CC: <6.6, 6.6-<9.0, 9.0-<11.5, ≥11.5 ng/ml; 677CT: <5.7, 5.7-<8.2, 8.2-<10.5, ≥10.5 ng/ml; 677TT: <4.8, 4.8-<6.5,6.5-<9.1, ≥9.1.</td></tr>
<tr><td colspan="2"></td><td colspan="3">In the group without folic acid, among subjects with the 677CC genotype, there was an inverse relationship between baseline folate level and risk of stroke (S for linear trend). A similar pattern, to a lesser degree, was observed among subjects with the 677CT genotype (S for linear trend). In contrast, subjects with the 677TT genotype had a persistently high risk of stroke across all folate quartiles (NS for linear trend).</td></tr>
<tr><td colspan="2">any adverse event other than the study outcomes</td><td colspan="3">NS</td></tr>
<tr><td colspan="2">any drug-related adverse event</td><td colspan="3">NS</td></tr>
<tr><td colspan="2">other safety outcomes, including any serious adverse events, adverse events leading to drug withdrawal, and abnormal laboratory test results with clinical significance</td><td colspan="3">NS</td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 9
Kondo A et al. C677T mutation in methylenetetrahydrofolate reductase gene and neural tube defects: should Japanese women undergo gene screening before pregnancy? Congenit Anom (Kyoto) 2014;54:30-4. PubMed PMID: 24588777.</td><td>3

677CT: AA

677TT: AA</td><td>
Genotype frequencies in 115 mothers who had given birth to a spina bifida child were compared to genotype frequencies in 4517 population based controls. For the prevention of affected pregnancy every woman planning to conceive has to take folic acid supplements 0.4 mg daily. However, a previous study showed that the majority of pregnant Japanese women in the 1st trimester neither consumed dietary folate of 0.44 mg/day equivalent to the recommended dietary allowance nor took folic acid supplements daily (Kondo et al. 2011). Relevant co-medication was not excluded and frequency and duration of folic acid intake was not known.

Genotyping of the spina bifida mothers:
- 47x 677CC
- 56x 677CT
- 12x 677TT

Results:
<table>
<tr><td colspan="2">Odds ratios for giving birth to a spina bifida child compared to 677CC:</td></tr>
<tr><td>677CT</td><td>NS</td></tr>
<tr><td>677TT</td><td>NS</td></tr>
<tr><td colspan="2">The OR was also NS for the 677T-allele compared to the 677C-allele.</td></tr>
</table>
Note: The authors mention that similar results were published for 13 other countries. Only a Dutch study found a significantly increased risk for 677TT (Van der Put 1995). However, since mothers were recruited in 1993 and thus shortly after folic acid was proven to diminish neural
</td><td>Authors' conclusion:
''In conclusion, it is not necessary for Japanese women to undergo genetic screening C677T mutation of the MTHFR gene as a predictive marker for spina bifida prior to pregnancy, because the TT genotype is not a risk factor for having an affected infant.''</td></tr>
</table>

<table>
<tr><td>ref. 9, continuation</td><td></td><td>tube defect risk (1991 and 1992), the probability of folic acid supplementation in this study was even less likely than in Kondo 2014. Indeed, Van der Put et al. indicate that since folate administration may overcome the effects of reduced MTHFR activity, their findings provide a mechanism for the protective role of folate in the etiology of spina bifida in 677TT individuals.</td><td></td></tr>
<tr><td>ref. 10
Crider KS et al. MTHFR 677C->T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation. Am J Clin Nutr 2011;93:1365-72.
PubMed PMID: 21508090.</td><td>4

677TT: A

677CT: A</td><td>932 women without anaemia and vitamin B12 deficiency received folic acid (0.1 mg/day (in 1 or 4 doses) (n = 313), 0.4 mg/day (in 1 or 4 doses) (n = 317), 4 mg once daily (n = 167), or 4 mg once weekly (n = 135)) for 6 months and were followed up for 3 months after discontinuation of folic acid treatment.
Use of supplements and prescription drugs was excluded.
Bonferroni correction was applied to adjust for multiple testing.

Genotyping:
- 162x 677CC
- 443x 677CT
- 327x 677TT

Results:
<table>
<tr><td colspan="5">Results compared to 677CC:</td></tr>
<tr><td colspan="3"></td><td>677TT</td><td>677CT</td><td>value for 677CC</td></tr>
<tr><td>plasma folate concentration</td><td colspan="2">pretreatment (0 months, all patients)</td><td>x 0.88 (S)</td><td>NS</td><td>10.3 nM</td></tr>
<tr><td rowspan="5">folic acid 0.1 mg/day</td><td>0 months</td><td>NS</td><td>NS</td><td>9.5 nM</td></tr>
<tr><td>1 month</td><td>x 0.79 (S)</td><td>NS</td><td>20.0 nM</td></tr>
<tr><td>3 months</td><td>x 0.73 (S)</td><td>NS</td><td>22.5 nM</td></tr>
<tr><td>6 months</td><td>x 0.70 (S)</td><td>NS</td><td>24.4 nM</td></tr>
<tr><td>3 months post-treatment</td><td>x 0.81 (S)</td><td>NS</td><td>15.9 nM</td></tr>
<tr><td rowspan="5">folic acid 0.4 mg/day</td><td>0 months</td><td>x 0.80 (S)</td><td>NS</td><td>11.1 nM</td></tr>
<tr><td>1 month</td><td>NS</td><td>NS</td><td>32.1 nM</td></tr>
<tr><td>3 months</td><td>NS</td><td>NS</td><td>41.6 nM</td></tr>
<tr><td>6 months</td><td>x 0.71 (S)</td><td>NS</td><td>40.8 nM</td></tr>
<tr><td>3 months post-treatment</td><td>x 0.76 (S)</td><td>NS</td><td>18.5 nM</td></tr>
<tr><td rowspan="5">folic acid 4 mg/day</td><td>0 months</td><td>x 0.71 (S)</td><td>NS</td><td>11.7 nM</td></tr>
<tr><td>1 month</td><td>x 0.63 (S)</td><td>NS</td><td>70.9 nM</td></tr>
<tr><td>3 months</td><td>NS</td><td>NS</td><td>84.3 nM</td></tr>
<tr><td>6 months</td><td>NS</td><td>NS</td><td>57.6 nM</td></tr>
<tr><td>3 months post-treatment</td><td>NS</td><td>NS</td><td>20.3 nM</td></tr>
<tr><td rowspan="5">folic acid 4 mg/week</td><td>0 months</td><td>NS</td><td>NS</td><td>10.6 nM</td></tr>
<tr><td>1 month</td><td>NS</td><td>NS</td><td>25.5 nM</td></tr>
<tr><td>3 months</td><td>NS</td><td>NS</td><td>32.8 nM</td></tr>
<tr><td>6 months</td><td>NS</td><td>NS</td><td>29.1 nM</td></tr>
<tr><td>3 months post-treatment</td><td>x 0.70 (S)</td><td>NS</td><td>18.4 nM</td></tr>
<tr><td colspan="5">In the first month and during the 6 months treatment period, the plasma folate concentration increased for all doses and genotypes (S). For all doses, the magnitude of the increase and the resulting plasma folate concentration decreased with the number of 677T-alleles (S).</td></tr>
<tr><td colspan="5">Between month 3 and month 6, there was no change in the plasma folate concentration for any dose or genotype (NS).</td></tr>
<tr><td colspan="5">At a dose of 4 mg/day, the plasma folate concentration reached a plateau after 1 month and did not change in</td></tr>
</table>
</td><td>Authors' conclusion:
''MTHFR genotype was an independent predictor of plasma and red blood cell folate and plasma homocysteine concentrations and did not have a significant interaction with folic acid dose during supplementation.''</td></tr>
</table>

ref. 10, continuation				months 2-6 for any of the genotypes (NS).			
				For all doses and genotypes, the plasma folate concentration was still elevated 3 months after the end of treatment (S). The percentage decrease in plasma folate concentration during this 3 months did not vary with the number of 677T-alleles (NS).			
				During the treatment period, there was a significant effect of the genotype on the plasma folate concentration, which did not vary with folic acid dose (S). Genotype and folic acid dose were independent predictors of plasma folate concentration.			
			red blood cell folate concentration	pretreatment (0 months, all patients)		x 0.83 (S)	NS	669 nM
				folic acid 0.1 mg/day	0 months	NS	NS	604 nM
					1 month	NS	NS	620 nM
					3 months	NS	NS	692 nM
					6 months	NS	NS	790 nM
					3 months post-treatment	NS	NS	653 nM
				folic acid 0.4 mg/day	0 months	x 0.74 (S)	NS	718 nM
					1 month	x 0.79 (S)	NS	838 nM
					3 months	x 0.80 (S)	NS	1040 nM
					6 months	x 0.79 (S)	NS	1176 nM
					3 months post-treatment	x 0.82 (S)	NS	846 nM
				folic acid 4 mg/day	0 months	x 0.70 (S)	NS	750 nM
					1 month	NS	NS	993 nM
					3 months	x 0.76 (S)	NS	1621 nM
					6 months	NS	NS	1641 nM
					3 months post-treatment	x 0.78 (S)	NS	964 nM
				folic acid 4 mg/week	0 months	NS	NS	688 nM
					1 month	NS	NS	769 nM
					3 months	NS	NS	909 nM
					6 months	NS	NS	1063 nM
					3 months post-treatment	NS	NS	750 nM
				For all doses except 0.1 mg/day, the red blood cell folate concentration decreased with the number of 677T-alleles (S).				
				At a dose of 4 mg/day, the red blood cell folate concentration reached a plateau after 3 months and did not change in months 4-6 for any of the genotypes (NS).				
				During the 6 months treatment period, the red blood cell folate concentration increased for all doses and genotypes (S).				
				In the 3 months after the end of treatment, red blood cell folate concentration rapidly declined for all genotypes and doses (S). As a result, the red blood cell folate concentration was not elevated any more 3 months after the end of treatment for all genotypes at folic acid 0.1 mg/day and 4 mg/week and for 677CC at 0.4 mg/day and 4 mg/day (NS).				
				During the treatment period, there was a significant effect of the genotype on the red blood cell folate concentration, which did not vary with folic acid dose (S). Genotype and folic acid dose were independent predictors of red blood cell folate concentration.				
				The authors indicate that an earlier study performed in				

ref. 10, continuation				the same area, showed that periconceptional supplementation with folic acid 0.4 mg/day decreased the incidence of neural tube defects by up to 85%. In this study, folic acid 0.4 mg/day increased the red blood cell folate concentrations in 677TT women to the baseline concentration of the 677CT group after 1 month and to the baseline concentration of the 677CC group after 3 months. Thus, it might be that the plasma and red blood cell folate concentrations observed in the 0.4 mg/day group approximate those necessary to reduce neural tube defect occurrence.			
			plasma homocysteine concentration	pretreatment (0 months, all patients)		x 1.7 (S)	x 1.1 (S)	6.1 µM
				folic acid 0.1 mg/day	0 months	x 1.6 (S)	NS	6.2 µM
					1 month	x 1.7 (S)	NS	6.9 µM
					3 months	x 1.6 (S)	NS	6.3 µM
					6 months	x 1.5 (S)	NS	6.2 µM
					3 months post-treatment	x 1.6 (S)	NS	6.1 µM
				folic acid 0.4 mg/day	0 months	x 1.6 (S)	NS	6.1 µM
					1 month	x 1.7 (S)	NS	5.9 µM
					3 months	x 1.6 (S)	x 1.2 (S)	6.0 µM
					6 months	x 1.4 (S)	NS	5.5 µM
					3 months post-treatment	x 1.5 (S)	NS	5.1 µM
				folic acid 4 mg/day	0 months	x 1.7 (S)	NS	6.5 µM
					1 month	x 1.5 (S)	NS	6.4 µM
					3 months	x 1.4 (S)	NS	5.2 µM
					6 months	x 1.4 (S)	NS	5.5 µM
					3 months post-treatment	x 1.5 (S)	NS	5.9 µM
				folic acid 4 mg/week	0 months	x 1.6 (S)	NS	5.7 µM
					1 month	x 1.6 (S)	NS	6.9 µM
					3 months	x 1.5 (S)	NS	5.7 µM
					6 months	x 1.5 (S)	NS	5.5 µM
					3 months post-treatment	x 1.6 (S)	NS	5.9 µM
			For all doses, the plasma homocysteine concentration increased with the number of 677T-alleles (S).					
			After the start of treatment with 0.1 mg/day and 4 mg/week, plasma homocysteine concentration temporarily increased for each of the genotypes (S). The largest increase was seen among those with the 677TT genotype (increase with 18% and 24%, respectively) (S).					
			During the 6 months treatment period, the plasma homocysteine concentration decreased for all doses and genotypes, except 677CC at 4 mg/day (S). For 677CC at 4 mg/day, there was a decrease after 3 months (S).					
			Three months after the end of treatment, plasma homocysteine concentration increased with the number of 677T-alleles for all doses, except 0.1 mg/day (S). In all dose groups, the concentration for 677TT was higher than for 677CC genotype (S). The plasma homocysteine concentration did not change in the three months after the end of treatment with 0.1 mg/day for any of the genotypes. Three months after the end of treatment, plasma homocysteine concentration was only still reduced for 677TT treated with 0.4 mg/day or 4 mg/day (S).					

ref. 10, continuation			During the treatment period, there was a significant effect of the genotype on the plasma homocysteine concentration, which did not vary with folic acid dose (S). Genotype and folic acid dose were independent predictors of plasma homocysteine concentration.				
		% of patients with high plasma homocysteine concentration (> 10.4 µM)	pretreatment	OR = 23.0 (95% CI: 9.2-57.6) (S)	NS	3.1%	
			posttreatment	OR = 17.9 (95% CI: 6.4-50.0) (S)	NS	2.5%	
				There was also an association with folic acid dose, with 0.4 mg/day and 4 mg/day resulting in a lower risk than 0.1 mg/day, whereas 4 mg/week did not.			

Risk group	-

Comments:

- Due to the large number of studies, studies and meta-analyses in which the dose of folic acid supplementation was not known were only included if they provided data on more than 1000 supplemented persons and studies and meta-analyses in which the dose of folic acid supplementation was known only if they provided data on more than 500 supplemented persons. In addition, studies with outcomes other than outcomes of established indications of folic acid or adverse events occurring during treatment for these indications (like cancer in adults or hypospadia) were excluded. An exception was made for Huo 2015 investigating stroke, because this was the primary outcome measure of this study and it is important to know whether differences in prophylaxis of folic acid deficiency or correction of homocysteine metabolism disorders translate into clinical effects. For the China Stroke Primary Prevention Trial, only two of the published articles were included: the first publications on the effect of MTHFR 677C>T on stroke and on homocysteine (Huo 2015 and Huang 2018).

Date of literature search: 8 January 2021.

	Genotype	**Code**	**Gene-drug interaction**	**Action**	**Date**
KNMP Pharmacogenetics Working Group decision	677CT	4 B	yes	no	7 June 2021
	677TT	4 B	yes	no	

Mechanism:

Folic acid is converted to tetrahydrofolate by the enzyme dihydrofolate reductase (DHFR). Tetrahydrofolate is required for the synthesis of purine nucleotides and following conversion to 5,10-methylenetetrahydrofolate, it is also required for the synthesis of thymidine nucleotides by thymidylate synthase.
The enzyme MTHFR converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. 5-methyltetrahydrofolate is required for the methylation of homocysteine to methionine and is converted to tetrahydrofolate in this process. Methionine is required for protein synthesis and DNA methylation. Reduced activity of the enzyme MTHFR results in decreased intracellular tetrahydrofolate concentrations.
Because MTHFR is involved in folate metabolism, gene variants that result in reduced MTHFR enzyme activity might influence the effect of folates like folic acid.