General background text Pharmacogenetics – Methylenetetrahydrofolate reductase (MTHFR)

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Definitions in pharmacogenetics
The **genotype** is the hereditary information about a specific characteristic of an individual. This information is located in the genes, in the DNA that consists of nucleotides. The piece of the DNA that carries information for one specific hereditary characteristic is called a **gene.** The DNA is divided into chromosomes, which usually occur in pairs. This means that an individual has two copies (two **alleles**) of most genes. Each allele is located on one of the chromosomes of a chromosome pair.
The **phenotype** indicates what the final manifestation (phenotypic state) of a certain genotype is. This can involve the functionality of a protein (for example the enzyme or the receptor), but also the physical manifestation of a disease. The phenotype is a result of the genotype that a person possesses, the degree of expression of the gene in question and the combination with environmental factors such as co-medication, diet and disease conditions.
Variations can exist in a population within the DNA that encodes a protein. Variations can result in alleles that encode proteins with no or reduced activity. The simplest form of variations are **"single-nucleotide polymorphisms" (SNPs)**, in which a certain part of a gene differs by only one nucleotide. If a gene variation occurs in at least 1% of the population, then this is referred to as a genetic **polymorphism. Wild-type** is the name given to the most common active allele. There can be a number of different polymorphisms for a certain allele.

Altered enzyme activity and clinical consequences
MTHFR is involved in folic acid metabolism. MTHFR converts 5,10-methylenetetrahydrofolate to 5-methyl-tetrahydrofolate. 5,10-methylenetetrahydrofolate is required for thymidine nucleotide synthesis. 5-methyl-tetrahydrofolate is required for conversion of homocysteine to methionine and therefore for protein synthesis.

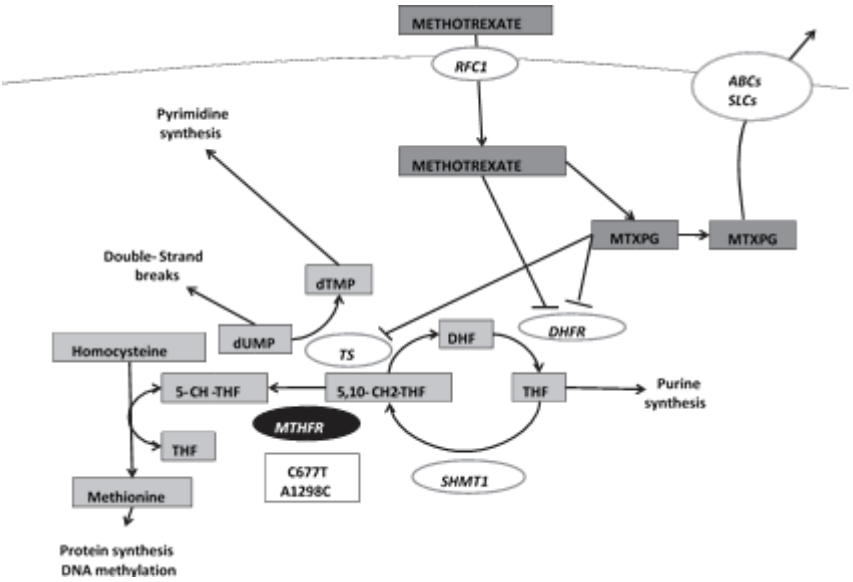

Figure 1. Folic acid metabolism and the effect of methotrexate (figure from Lopez-Lopez 2013).

MTXPG = methotrexate polyglutamate, DHFR = dihydrofolate reductase, TS = thymidylate synthetase, MTHFR = methylenetetrahydrofolate reductase.

5-CH-THF = 5-methyltetrahydrofolate, 5,10-CH2-THF = 5,10-methylenetetrahydrofolate, ABCs = ABC transporters, DHF = dihydrofolate, RFC1 = reduced folate carrier, SHMT1 = serine hydroxymethyltransferase 1, SLCs = solute carrier organic anion transporters, THF = tetrahydrofolate.

The MTHFR polymorphisms 677C>T and 1298A>C lead to reduced activity enzyme.
The 677C>T substitution leads to replacement of alanine at position 222 in the enzyme with valine. The resulting enzyme is more thermolabile. The enzyme activity is reduced to 65% and 30% of normal activity in heterozygotes

and homozygotes respectively. The substitution is associated with increased homocysteine plasma concentrations.
The 1298A>C substitution leads to replacement of glutamic acid at position 429 in the enzyme with alanine. The effect on enzyme activity is smaller than for the 677C>T polymorphism. Homozygotes of the 1298C allele have 60% of the standard enzyme activity.
Reduced MTHFR enzyme activity leads to reduced intracellular tetrahydrofolate concentrations.

Methotrexate is converted in the body to methotrexate polyglutamate, which inhibits dihydrofolate reductase which is part of the folic acid cycle (see figure 1). Dihydrofolate reductase converts dihydrofolate to tetrahydrofolate, which is required for purine nucleotide synthesis and after conversion to 5,10-methylenetetrahydrofolate also for synthesis of thymidine nucleotides by thymidylate synthetase. Methotrexate polyglutamate also directly inhibits thymidylate synthetase. Methotrexate toxicity can be reduced by administration of the tetrahydrofolate precursors folic acid or folinic acid.
As both methotrexate and MTHFR influence folic acid metabolism, gene variants that lead to a reduced-activity MTHFR enzyme could influence the effectiveness and toxicity of methotrexate.

Genotyping
The process of genotyping is used to determine the genotype. It indicates which alleles of the gene for MTHFR are present in the tested individual. MTHFR alleles have a name that consists of the nucleotide number followed by the nucleotide change (e.g. 677T for the variant allele and 677C for the wild-type allele of the polymorphism 677C>T).

In addition to being referred to by their nucleotide change, polymorphisms can also be referred to by the amino acid change or by rs number. As all three systems appear in the literature, table 1 shows the nomenclature according to all three of these systems.

Table 1. Overview of the most important MTHFR polymorphisms

Nucleotide polymorphism according to literature	**Nucleotide polymorphism according to genome aggregation database (numbering starts at start codon)**	**rs number**	**Protein polymorphisms**
677C>T	665C>T	rs 1801133	Ala222Val
1298A>C	1286A>C	rs 1801131	Glu429Ala

The 677C>T polymorphism has a greater effect on enzyme activity than the 1298A>C polymorphism. Moreover, moderate linkage disequilibrium has been observed between the two polymorphisms. Any clinical effects of 1298A>C may therefore not be fully independent of the effects of the 677C>T polymorphism. For these two reasons, the 677C>T polymorphism is more relevant than the 1298A>C polymorphism.

Ethnic variation in prevalence of genotypes and allele frequency
Data on the prevalence of the genotypes and ethnic variation in allele frequency of the most important MTHFR polymorphism 677C>T are given below.
Approximately 8-12% of the Caucasian population are homozygous for the T allele and approximately 40% are heterozygous. This is approximately 8 and 39-42% in the Netherlands. This is equivalent to an allele frequency of 27-29% in the Netherlands.
Greater spread in allele frequencies has been found in the Asian population, ranging from a factor 3 lower to higher than in the Netherlands. The allele frequency in Africans and African Americans is clearly lower than in Dutch people.
Allele frequencies and genotypes per population are given in table 2.

Table 2. Ethnic variation in prevalence of genotypes and allele frequency

		Prevalence of phenotype (%)			**Allele frequency (%)**
population group	**area/country**	**677CC**	**677CT**	**677TT**	**677T**
White		48-52	40	8-12	19-40
	The Netherlands	50-53	39-42	8	27-29
	Europe (excluding Finland)	50	38	12	34

	Finland	70	24	6	23
Asian					9-36
	East Asia	61	30	9	29
	South Asia	50	22.5	2.5	15
African					5-9
African-American					11
African/African-American		89	9.7	1.3	11
Latin-American/American		32	42	26	50
Ashkenazi Jewish		32	47	21	46

Literature

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