General background text Pharmacogenetics – Dihydropyrimidine dehydrogenase (DPD)

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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. A person generally has two copies (**alleles**) of a gene, one on each 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 for 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.

Names of genes are given in italics. In some cases the abbreviation of the name of the gene can differ from the name of the gene product. This is the case for dihydropyrimidine dehydrogenase, where the gene is abbreviated to *DPYD* and the enzyme to DPD.

Altered metabolic capacity and clinical consequences

Fluorouracil is mainly (> 80%) converted by dihydropyrimidine dehydrogenase (DPD) to the inactive metabolite dihydrofluorouracil. DPD is mainly present in the liver, but also in most other tissues. Lower metabolic activity of DPD leads to increased intracellular concentrations of fluorodeoxyuridine monophosphate, the active metabolite of fluorouracil and its prodrug capecitabine. This leads to an increased risk of side effects such as neutropenia, thrombopenia and "hand-foot" syndrome.

Variations in the gene that encodes the DPD enzyme can result in reduced or absent enzyme activity.

The population can be divided into five phenotypes (ranked below according to decreasing activity), based on the metabolic capacity of DPD that is present and the therapy that needs to be implemented:

- Gene activity score 2 (AS 2): "normal" metabolic capacity (normal metaboliser (NM), two fully active alleles)
- Gene activity score 1.5 (AS 1.5): reduced metabolic capacity (one fully active and one reduced-activity allele)
- Gene activity score 1 (AS 1): halved metabolic capacity caused by a single gene variation (one fully active and one inactive allele)
- Phenotyping (PHENO): strongly reduced metabolic capacity (two reduced-activity alleles or one inactive and one reduced-activity allele, or more commonly two gene variations that result in a reduced-activity allele or one gene variation that results in one inactive allele and one gene variation that results in a reduced-activity allele)
- Gene activity score 0 (AS 0): absent metabolic capacity (two inactive alleles, or more commonly two gene variations resulting in an inactive allele)

There is also variation in metabolic capacity within a group with a certain gene activity score.

As the genotype only determines part of the metabolic capacity, the guidelines for dose adjustment based on the genotype are no more than a tool that can be used to achieve the desired intracellular concentration of the active metabolite. Pharmacokinetic dose adjustment (guided by plasma concentrations or AUC) may be useful for fluorouracil to optimise the dose. Although capecitabine is largely converted to fluorouracil in tissue, pharmacokinetic dose adjustment (based on the plasma concentrations or AUC of fluorouracil) can also be implemented for capecitabine.

Genotyping

The process of genotyping is used to determine the genotype. It indicates which variations of the gene for DPD are present in the tested individual. In the past, gene variations were assigned a name that consists of a star (*) and a number, an example of a possible DPD genotype is *DPYD*1/*2A*. However, this nomenclature is not available for gene variations discovered at a later stage. This is why some alleles are still referred to by nucleotide numbers followed by nucleotide changes (or by amino acid number preceded by symbol of the amino acid in the wild-type protein followed by the symbol of the amino acid in the variant).

Many variations exist for DPD; more than 30 different gene variations have been identified and described in the literature. The incidences of all variations that result in reduced DPD activity are low and whether DPD activity is affected is not fully known for each variant; a reliable genotyping test for DPD is not available. A number of these alleles are listed in Table 1, including their functionality. Genotyping usually screens for only the most common and best substantiated variant alleles. Currently, these variants are *2A, *13, 2846A>T and 1236G>A. This may result in less common variants being missed and incorrectly being designated the wild-type (also refer to the document "Uncertainties in genotyping results" on the KNMP website).

Table 1. *DPYD* gene variations and metabolic capacity

Metabolic capacity	strong evidence for functionality (or by definition in the case of *1)	moderate evidence for functionality (*in vitro* and clinical/*ex vivo* data)	weak evidence for functionality (*in vitro* data only and/or limited clinical/*ex vivo* data)
Fully functional (wild-type; gene activity score 1)	*1 *5 = 1627A>G *9A = 85T>C	*4 = 1601G>A *6 = 2194G>A *9B = 85T>C + 2657G>A *11 = 1003G>T 62G>A 496A>G IVS10-15T>C 1896C>T	
Reduced functionality (gene activity score 0.5)	1236G>A / 1129-5923C>G (hapB3)[$] 2846A>T		
fully dysfunctional (inactive or null allele; gene activity score 0)	*2A = IVS14+1G>A *13 = 1679T>G[%]	*3 = 1897delC *7 = 295-298delTCAT *8 = 703C>T *10 = 2983G>T *12 = 1156G>T	257C>T[&] 300C>A[&] 1651G>A 1024G>A[&] 1025A>G[&] 1475C>T[&] 1774C>T[&] Duplication of exons 17+18[%]

[$] Variant 1236G>A, which does not result in a change in amino acids, is in complete linkage disequilibrium with variant 1129-5923C>G, which results in abnormal splicing of a part of the mRNA, resulting in the formation of a premature stop codon. The resulting DPD activity accounts for approximately half of the normal activity. Both variants are part of haplotype B3.

[%] For these variants, there is limited evidence from studies or from a case report that measured DPD activity, that the variants (amino acid substitution (Ile560Ser) for *13) lead to an inactive enzyme.

[&] These variants were found in a case study involving toxicity and have a very low enzyme activity *in vitro*.

Translation from genotype to phenotype

When an individual's genotype has been determined and one wants to know what the metabolic capacity for DPD is, then the genotype needs to be "translated" to the phenotype. If two gene variations are present that do not both result in an inactive allele, then this should be translated in the phenotype "phenotyping" ("PHENO"). If this is not the case, then the gene activity scores of both gene variants (both alleles) should be added together in the translation from the genotype to the phenotype (see table 2). If there are more than two gene variations, then the genotype should be translated into a "gene activity score 0" ('AS 0') if two or more of these gene variations result in an inactive allele and to "phenotyping" ("PHENO") if this is not the case. In rare cases involving two different gene variations, the variations can be located on the same allele instead of on different alleles. As it is always recommended to determine the DPD activity in the case of two or more gene variations and adjust the dose accordingly, this does not need to be taken into consideration when translating the genotyping result into the predicted phenotype.

Table 2. Relationship between genotyping result and predicted phenotype

Genotyping result	Predicted phenotype
No gene variant resulting in decreased DPD activity (*1/*1)	Gene activity score 2 (normal metaboliser)
One reduced functional gene variant (*1/1236A or *1/2846T)	Gene activity score 1.5
One fully dysfunctional gene variant (*1/*2A or *1/*13)	Gene activity score 1
Two reduced functional gene variants or one fully dysfunctional gene variant and one reduced functional gene variant (1236A/1236A, 2846T/2846T, both 1236A and 2846T, both *2A and 1236A, both *2A and 2846T, both *13 and 1236A or both *13 and 2846T)	Phenotyping
Two fully dysfunctional gene variants (*2A/*2A, *13/*13 or both *2A and *13)	Gene activity score 0

Phenotyping

The process of phenotyping is used to determine the phenotype, which means: measuring or estimating the activity of the DPD enzyme.

Possible phenotyping methods are analysing DPD enzyme activity in peripheral mononuclear blood cells [Kuilenburg, 2000] or measuring uracil concentration in plasma or urine [Ploylearmsaeng, 2006], with the first method being the most reliable. Depending on the exact method used for the determination, the average White DPD enzyme activity is 9.9 ± 2.8 nmol/hour per mg protein [Kuilenburg, 2002] or 9.6 ± 2.2 nmol/hour per mg protein [Pluim, 2015].

Methods that are less commonly used nowadays are the 2-^{13}C-uracil breath test [Mattison, 2004] or analysis of plasma uracil/dihydrouracil ratios [Ciccolini, 2006; Zhou, 2007]. The first test measures $^{13}CO_2$ formation following breakdown of 2-^{13}C-uracil mediated by DPD and other enzymes in the pyrimidine catabolic route. The second test measures conversion of the endogenous uracil substrate. The activity of the DPD enzyme can also be determined by analysing conversion of a single dose of uracil [van Staveren, 2013].

All existing phenotyping methods have limited applicability for either accuracy or sensitivity reasons or because of the feasibility of screening large numbers of patients (costs and time commitment).

Ethnic variation in prevalence of phenotypes and allele frequency

Allele frequencies and phenotypes per population are given in table 5.

The frequency of occurrence of the various DPD alleles and the different phenotypes appears to vary significantly between nations and ethnic groups. In the Dutch population, the most common reduced-functional allele contains the gene variant 1236A and the most common fully dysfunctional allele contains the gene variant *2A.

Based on the DPD enzyme activity, approximately 3-5% of the White population have partial DPD deficiency and 0.1-0.2% have complete DPD deficiency (Tuchman 1985). This corresponds reasonably well to the prevalence calculated based on the frequencies of the gene variants *2A, 2846A>T, 1236G>A and *13 with gene activity score 1 + gene activity score 1.5 (3-12%) and gene activity score 0 + phenotyping (0.02-0.4%) respectively.

Mattison 2006 refers to studies that demonstrate increased fluorouracil toxicity (leukopaenia and anaemia) and reduced overall survival in Afro-American patients with colorectal carcinoma in comparison to White patients. Based on the DPD enzyme activity, the study accordingly demonstrated a trend towards a 2.9-fold higher prevalence of partial DPD deficiency (8%) in 149 healthy Afro-Americans compared to 109 healthy Whites (2.8%) ($p = 0.07$). The 8% Afro-Americans with partial DPD deficiency observed based on the enzyme activity is 9.5 times higher than the prevalence of gene activity score 1 + gene activity score 1.5 in Afro-Americans (0.84%) calculated based on the frequencies of the gene variants *2A, 2846A>T, 1236G>A and *13.

Determination of these four gene variants thus provides little information about the DPD enzyme activity and the associated risk of severe toxicity in Afro-Americans. Other gene variants or non-genetic factors may also play a role in Afro-Americans. The higher prevalence of DPD deficiency based on DPD enzyme activity found by Mattison 2006 in women than in men (3.1-fold higher in Afro-Americans and 1.8-fold higher in Whites) suggests that non-genetic factors also play a role.

Low frequencies of the gene variants *2A, 2846A>T, 1236G>A and *13 are also found in other non-White population groups, particularly East Asians. It is not known whether this corresponds to a higher DPD enzyme activity in these population groups or whether there are other factors, as is the case for Afro-Americans, which determine the enzyme activity in individuals from these population groups.

Table 5. Ethnic variation in prevalence of phenotypes and gene variant frequency predicted on the basis of gene activity

		prevalence predicted phenotype (%)[#]					gene variant frequency (%)			
population group	region or subgroup	GAS 0	PHENO	GAS 1	GAS 1.5	GAS 2	*2A	2846 A>T	1236 G>A	*13
White		0.001-0.023	0.02-0.36	0.2-2.8	2.6-8.8	88-97	0-1.4	1	0.3-3.7	0.1
	The Netherlands	0.005	0.14	1.35	6.1	92	0.65	0.75	2.4	0.05
	Finland	0.06	0.075	4.6	2.4	93	2.4	0.04	1.2	0.008
	Europe without Finland	0.004	0.1	1.3	5.0	94	0.6	0.5	2.1	0.06
Asian										
	Southwest Asian						0-0.75			
	East Asian	0	< 0.0001	0	0.06	100	0	0.005	0.025	0
	South Asian	0.002	0.04	0.8	3.4	96	0.4	0.05	1.7	0
African										
	Afro-American						0			
	African/ Afro-American	< 0.0001	0.002	0.14	0.7	99	0.06	0.08	0.26	0.008
Latin-American/ American, mixed ethnicity		0.0001	0.007	0.2	1.5	98	0.11	0.26	0.5	0
Ashkenazi jewish		0.003	0.01	1.0	1.5	98	0.5	0.04	0.7	0

[#] The prevalences of the predicted phenotypes are calculated from the detected gene variant frequencies. GAS = gene activity score, PHENO = phenotyping

Literature

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