General background text Pharmacogenetics - CYP3A4

Last updated: 24 August 2021

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 for the DNA that encodes for 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.

Most human genes consist of coding regions (**exons**) interspersed with non-coding regions (**introns**). Variations in exons usually result in variations in the protein product. Variations in introns – the non-coding regions of the gene – generally do not result in variations in the protein product. However, introns - and associated variations in these regions - can influence the level of production of the protein.

Changes in the activity of the enzyme and clinical consequences

The cytochrome P450 enzymes, which include the iso-enzyme CYP3A4, are involved in the metabolism of many medicines. CYP3A4 is involved in the metabolism of approximately half of all medicines [1,2]. CYP3A4 is responsible for a wide range of metabolising reactions, such as hydroxylation, sulfoxidation, N-dealkylation and O-dealkylation [3]. Variations in the activity of CYP3A4 can result in an increase or decrease of the metabolisation of medicines.

The activity of CYP3A4 can vary by a factor 10-100 between individuals [3]. The causes of variations in CYP3A4 activity are largely non-genetic. CYP3A4 is inhibited by grapefruit juice and by medicines such as ketoconazole, itraconazole, clarithromycin, valproic acid, ritonavir and saquinavir [3-5]. It is induced by medicines such as carbamazepine, phenytoin, efavirenz, rifampicin and hypericum [4]. In adults, the activity decreases with age [3,5], whilst the activity is higher in women than in men [6].

In addition to this, variations in the gene that encodes for CYP3A4 can result in reduced enzyme activity [7]. The population can be divided into three phenotypes, based on the metabolic capacity of CYP3A4 that is present:

- poor metaboliser (PM), severely reduced metabolic capacity (two alleles with absent or reduced activity)
- intermediate metaboliser (IM), reduced metabolic capacity (one allele with reduced or absent activity and one allele with normal activity)
- normal metaboliser (NM), "normal" metabolic capacity (two alleles with normal activity)

As the remaining activity of alleles with reduced activity has not been quantified properly and inactive alleles are not very common, the predicted phenotypes for CYP3A4 currently do not distinguish between alleles with reduced or absent activity.

Genotyping

The process of genotyping is used to determine the genotype. It indicates which alleles of the gene for CYP3A4 are present in the tested individual. Each allele has a name that consists of a star (*) and a number, an example of a possible CYP3A4 genotype is CYP3A4*1A/*22. The table at the end of this paragraph contains the alternative notations for the most important variant alleles.

There are over 40 different gene variants of CYP3A4 [1,7].

It has been determined for one gene variant (*22) that the gene variation results in reduced production and therefore reduced enzyme activity in the liver (but not in the intestines) [6]. In carriers of *22, the activity is reduced

by a factor 2.5 [6]. *22 explains 12% of the variation in CYP3A4 activity [6]. The gene variant is located in intron 6 and results in a change from a C to a T.
Other gene variants are less common than *22. One of these gene variants (*18) also has reduced activity *in vivo*, whilst three of these variants (*6, *20 and *26) have an inactivating gene variation [7]. For 6 gene variants, *in vitro* indications of reduced activity have been found [7].
Contradictory results have been found for the common variant *1B [1,6]. Five different studies found no effect of *1B on the expression of the CYP3A4 enzyme [1,2,6]. In African-Americans, *1B exhibits a strong linkage disequilibrium with gene variant CYP3A5*1, which results in CYP3A5 expression [1]. This complicates the interpretation of studies into the activity of gene variant *1B. Considering the linkage to active CYP3A5, no increased activity is attributed to CYP3A4*1B itself.
Genotyping usually screens for the presence of the most common allele variants. As a result, the reported genotype may differ from the actual genotype (also refer to the document 'Uncertainties in genotyping results' on the KNMP site).

The table below provides an overview of the alleles and the resulting enzyme activity. The previous section described which genotypes result in which phenotypes as predicted by the gene activity.

Table 1. CYP3A4 alleles and enzyme activity [1,2,7]

Source / enzyme activity / Enzyme activity	*in vivo* measure-ment	protein structure	*in vitro* measure-ment
fully functional	*1A *1B *1G		
reduced functionality	*18[a] *22		*8 *11 *12?[b] *13 *16 *17
inactive[c]		*6 *20 *26	

[a] For *18, a reduced activity has been observed *in vivo* with midazolam as substrate. However, an increase in activity was measured *in vitro* using testosterone, oestrogen or the insecticide chlorpyrifos as a substrate. The effect is therefore substrate-dependent.

[b] It was not possible to determine with certainty from the *in vitro* measurement whether the activity for *12 was reduced.

[c] As the remaining activity of alleles with reduced activity has not been quantified properly and inactive alleles are not very common, the predicted phenotypes for CYP3A4 currently do not distinguish between alleles with reduced or absent activity.
For *6 and *20, the gene variations result in a frame shift, for *26 it results in a premature stop codon. These are inactivating gene variations. For *20 and *26, this was confirmed by measurement of the *in vitro* activity.

Table 2. Overview of the notations used for the most important CYP3A4 alleles [6,7,11]

allele number	nucleotide change	amino acid change	rs number
*22	522-191C>T	unknown (splicing defect)	rs35599367
*20	1461_1462insA	Prof488fs (frameshift after amino acid 487)	rs67666821
*16 (*16A and *16B)	554C>G	Thr185Ser	rs12721627

Phenotyping
The process of phenotyping is used to determine the phenotype, which means: measuring or estimating the activity of the CYP3A4 enzyme. As the causes of variations in CYP3A4 activity are largely non-genetic, phenotyping of CYP3A4 does not provide any information about the presence or absence of variant alleles.

Ethnic variation in prevalence of phenotypes and allele frequency predicted according to the gene activity
So far as can be determined from the data that are currently available, there is little variation between ethnic groups in the frequency of occurrence of the CYP3A4*22 allele. The frequency for African-Americans and for Chinese individuals from the USA is in the same range as the frequency for Caucasians [8]. However, the gnomAD database lists a low frequency for Africans/Afro-Americans and found no *22 in East Asians. Considering the number of East Asian alleles for which data have been included in gnomAD, the latter corresponds to an allele frequency of less than 0.064%. There are no data for *22 in South Asians in gnomAD.
*20 appears to occur almost exclusively in Spaniards and Latin-Americans and *16 also appears to occur only in certain countries
The allele frequency is listed per population group in the table below.

Table 3. Ethnic variation in prevalence of phenotypes and allele frequency predicted according to the gene activity [1,6,8-10,12-14]

		prevalence of predicted phenotype (%)[#]			allele frequency (%)		
population group	region or sub-group	PM	IM	NM	*22	*20	*16
Caucasian					3.2-10.6		0
	The Netherlands	0.1-1.1	6.2-19.0	80-94	3.2-10.6		
	USA	0.3-0.7	9.9-15.2	84-90	5.2-8.3		
	Spain	0.2	7.5	92	3.2	0.7	0
	Finland	0.1	6.9	93	3.6	0	0
	Europe without Finland	0.2	8.5	91	4.4	0.03	0
Asian	Chinese-American	0.2	8.2	92	4.3		
	Japan	0.04-0.25	3.9-9.5	90-96			2-5
	China						0
	Southeast Asia excl. China and Japan						0
	East Asia	0.0001	0.2	100	0	0	0.1
	South Asia					0	0
African	African-American	0.2	8.2	92	4.3		0
	African/African-American	0.008	1.8	98	0.9	0.008	0
Latin-American	Mexican	0.25	9.5	96			5
	Latin-American/American, mixed ethnicity	0.07	5.1	95	2.5	0.12	0
Ashkenazi Jewish		0.8	16.4	83	9.0	0	0

[#] The prevalences of the predicted phenotypes are calculated based on the detected allele frequencies.

Literature

1. Lamba JK et al. Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. Pharmacogenetics 2002;12:121-32. PubMed PMID: 11875366.
2. García-Martín E et al. CYP3A4 variant alleles in white individuals with low CYP3A4 enzyme activity. Clin Pharmacol Ther 2002;71:196-204. PubMed PMID: 11907494.
3. van der Weide K et al. The influence of the CYP3A4*22 polymorphism on serum concentration of quetiapine in psychiatric patients. J Clin Psychopharmacol 2014;34:256-60. PubMed PMID: 24525658.
4. Flockhart DA. P450 drug interaction table [Division of Clinical Pharmacology, Indiana University], 25 januari 2012. http://medicine.iupui.edu/clinpharm/ddis/table.aspx. Accessed at 20 Januari 2016.
5. Aichhorn W et al. Influence of age, gender, body weight and valproate comedication on quetiapine plasma concentrations. Int Clin Psychopharmacol 2006;21:81-5. PubMed PMID: 16421458.
6. Wang D et al. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J 2011;11:274-86. PubMed PMID: 20386561.
7. https://www.pharmvar.org/gene/CYP3A4, accessed at 11 October 2019.
8. Thompson EE et al. CYP3A variation and the evolution of salt-sensitivity variants. Am J Hum Genet 2004;75:1059-69. PubMed PMID: 15492926.
9. Elens L et al. The new CYP3A4 intron 6 C>T polymorphism (CYP3A4*22) is associated with an increased risk of delayed graft function and worse renal function in cyclosporine-treated kidney transplant patients. Pharmacogenet Genomics 2012;22:373-80. PubMed PMID: 22388796.

10. Elens L et al. Novel CYP3A4 intron 6 single nucleotide polymorphism is associated with simvastatin-mediated cholesterol reduction in the Rotterdam Study. Pharmacogenet Genomics 2011;21:861-6. PubMed PMID: 21946898.
11. https://www.pharmgkb.org/gene/PA130/haplotype, accessed at 11 October 2019.
12. Apellániz-Ruiz M et al. Whole-exome sequencing reveals defective CYP3A4 variants predictive of paclitaxel dose-limiting neuropathy. Clin Cancer Res 2015;21:322-8. PubMed PMID: 25398452.
13. Nakajima Y et al. Impact of the haplotype CYP3A4*16B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese patients with cancer. Clin Pharmacol Ther 2006;80:179-91. PubMed PMID: 16890579.
14. genome aggregation database (gnomAD) v2.1.1, https://gnomad.broadinstitute.org.