General background text Pharmacogenetics - VKORC1

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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 genes, which consist of DNA which in turn 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.

Most human genes consist of coding regions (**exons**) interspersed with non-coding regions (**introns**). The **promoter** is a section that precedes the gene, which is primarily responsible for regulating the activity (expression) of the gene. Variations in exons usually result in variations in the protein product. Variations in promoters or introns – the non-coding regions of the gene – generally do not result in variations in the protein product. However, promoters and introns - and associated variations in these regions - can influence the level of production of the protein.

Polymorphisms in the different positions within the gene may be in linkage disequilibrium, which means that they are always inherited together. This is called a **haplotype**: part of the genetic material that is inherited as a whole.

Changes in (the production of) the target protein and clinical consequences

Vitamin K is an essential cofactor for carboxylation of glutamic acid residues on coagulation factors II, VII, IX and X and the anticoagulation proteins C, S and Z. Vitamin K is inactive in oxidised form (vitamin K 2,3-epoxide) and active in reduced form (vitamin-K hydroquinone). The enzyme vitamin K 2,3-epoxide reductase (VKOR) regenerates inactive vitamin K to the active form. Coumarins inhibit VKOR and as such reduce clotting activity.

VKOR is an enzyme complex that consists of multiple protein chains. One of these chains is called VKOR, subunit 1 (VKORC1). VKORC1 gene variants may lead to reduced production of VKORC1 and therefore of VKOR [2] and as such may increase the effect of coumarins.

Genotyping

The process of genotyping is used to determine the genotype. It indicates which alleles of the gene for VKORC1 are present in the tested individual.

Various polymorphism have been found in the VKORC1 gene. Of these polymorphisms, two polymorphisms in linkage equilibrium (-1639G>A and 1173C>T) have been found to explain the variation in coumarin sensitivity due to differences in the VKORC1 gene [1]. -1639G>A means that nucleotide -1639 of the VKORC1 gene is guanosine (G) in het wild-type and adenosine (A) in the variant allele.

1173C>T means that nucleotide 1173 of the VKORC1 gene is cytidine (C) in het wild-type and thymidine (T) in the variant allele. Nucleotide -1639 is in the promoter of the gene, nucleotide 1173 in an intron. Variant alleles (-1639A/1173T) are associated with lower daily dosing requirements of coumarin than wild-type alleles (-1639G/1173C). As both polymorphisms are in linkage equilibrium, genotyping of only one polymorphism is required. In Caucasian populations, incidences of the variant alleles found range from 37% to 45.8% [1-5].

There is currently no standard notation of wild-type and variant VKORC1 alleles. Table 1 shows an overview of the notations used.

Different VKORC1 haplotypes (H1-H9) were initially established on the basis of polymorphisms of nucleotides -2659, -1639, 497, 698, 1173, 1542 and 3730, and hereditary classes A and B for European Caucasians [2]. Haplotypes H1 and H2 (both variant alleles either -1639A/1173T) form hereditary class A and haplotypes H7, H8 and H9 (all three wild-type alleles i.e. -1639G/1173C) form class B. Haplotypes H3 to H6 are rare in Caucasians (less than or equal to 1%).

Table 1. Overview of the notations used for the variant and wild-type VKORC1 alleles.

Hereditary class	**VKORC1 haplotypes**	**Polymorphisms***	**Short allele notation**	**Pharmacological response**
A	H1, H2	-1639A/1173T (old notation**: 3673A/6484T)	T	Higher coumarin sensitivity
B	H7, H8, H9	-1639G/1173C (old notation**: 3673G/6484C)	C	Normal coumarin sensitivity

* These polymorphisms have to date always been found in linkage equilibrium. Genotyping of one of these two polymorphisms is therefore sufficient.

** The old notation numbered nucleotides from the first nucleotide of the isolated DNA fragment containing the human VKORC1 gene. This was later replaced with the more usual numbering from the first nucleotide of the ATG start codon. In this numbering system, any preceding nucleotides - including those in the promotor region - include a minus sign.

Ethnic variation in prevalence of genotypes and allele frequency

Ethnic variation in prevalence of genotypes and haplotypes has been investigated in Americans of European, Asian and African ethnicity [2]. Significant differences have been found between these three populations.

Almost all people of European and Asian ethnicity are part of either hereditary class A or B. Hereditary class A (i.e. increased coumarin sensitivity) is found in the minority of people of European ethnicity and in the majority of people of Asian ethnicity.

Only about two thirds of people of African ethnicity are in hereditary classes A and B. There is a large third group (38%) among these people with different haplotypes. The literature does not state to what extent these 'other haplotypes' are made up of wild-type (-1639G/1173C) or variant (-1639A/1173T) alleles, but research has shown that people of African ethnicity need relatively higher daily doses than people of European ethnicity.

Table 2 shows an overview of the prevalence data.

Ethnicity	Prevalence haplotype/hereditary class (in %)			
	Class A (H1, H2)	Class B (H7, H8, H9)	Total Classes A and B	Other haplotypes
European (n=119)	37	58	96	4
Asian (n=120)	89	10	99	1
African (n=96)	14	49	62	38

Table 2. Ethnic variation in prevalence haplotypes/hereditary classes [2]

Literature

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