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Pharmacogenetics and variability in dose response to vitamin K antagonist (VKA)

VKAs need to be used with caution. They require significant effort from the prescriber as well as the patient and are the number 1 cause of iatrogenic accidents (mainly haemorrhagic complications). This is the reason behind the narrow therapeutic scope for VKAs (INR target value 2-3 in most cases, 3-4.5 in cases of mechanical prosthesis patients with a high thrombotic risk) and the significant inter- and intra-individual variability in dose response. This notion of variability is indicated on the notice and monograph of VKA molecules: "due to significant interindividual variability, the posology of vitamin K antagonists is strictly on an individual basis"*. This variation in response is seen on a biological level (difficulty stabilising the INR, significant fluctuations, INR result lying outside of the therapeutic zone) and/or on a clinical level (minor or major haemorrhages, of which certain cases require admission to hospital and, although rare, resistance to treatment with treatment failure and thrombotic relapses). Different physiological, environmental and genetic determinants are at the root of this variability and, according to studies, represent approximately 60% of cases. It is worth noting that on the sensitivity variability to vitamin K antagonists (VKA) is not yet fully understood and is likely to have an epigenetic origin (approximately 40% of the total variability).

*Please be reminded, the mean doses of Coumadin®, Sintrom® and Previscan® are administered at steady state at doses of 5, 4 and 20 mg/day.

Pharmacodynamics and pharmaco-kinetics of vitamin K antagonists (VKA)

Vitamin K acts on the liver as a co-factor of gamma-carboxylase in the maturation of vitamin-k dependant proteins II, VII, IX, X (and protein C and protein S). Once used, vitamin K is recycled in the form of reduced vitamin K via the protein VKORC1 (vitamin-K epoxide reductase complex, VKORC1gene). Vitamin K antagonists drive vitamin-K competition as VKOR and non-reduced vitamin K can be reused for a second maturation process of vitamin-k dependant factors. This leads to a decrease in activity (see Figure 1).

Vitamin K antagonists are metabolised and inactivated in the liver, notably by enzyme CYP2C9 induced hydroxylation. Elimination is mainly renal but can also be faecal.

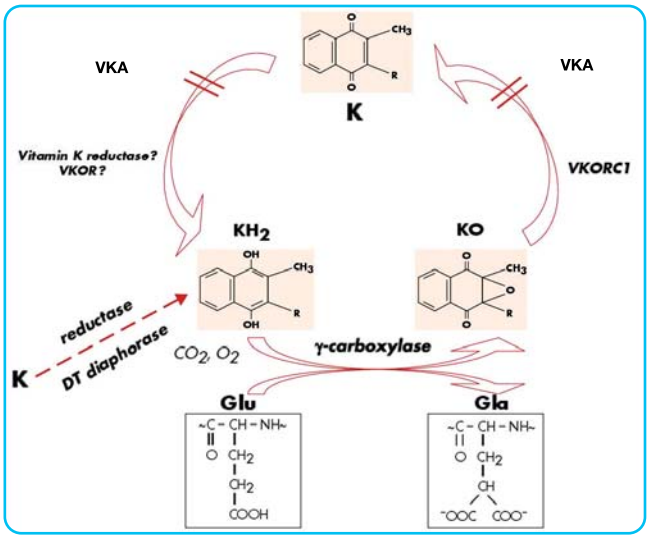

Figure 1: vitamin-K cycle

The variability in response to vitamin K antagonists ascribable to non-genetic factors (Figure 2):

- **Age:** sensitivity to VKAs and the haemorrhagic risk increase with age, particularly after 65;
- **Gender:** women tend to require lower doses than men;
- **Weight/BMI:** a decrease or increase in response to VKAs is seen in patients considered highly overweight, and as such, require dose adjustment;
- **Related disorders (comorbidities):** are described as having an influence on the VKA response, kidney failure, liver failure and acute infectious episodes (risk of overdose). In terms of tumours, the individual variation in coagulation is associated with possible interactions between VKAs and chemotherapy. This therefore requires intense INR monitoring;
- **Nutritional state:** a balanced and varied diet is required for all patients taking VKAs. It has even been shown that vitamin K

supplements can stabilise INR in patients with significant fluctuations (vitamin K supplement, the INR is often in the therapeutic zone, 28% vs.15%);

- **The taking of enzyme inducers or inhibitors (non-exhaustive list):** St. John's Wort, anti convulsive agents, azathioprine, rifampicin (decrease anticoagulant effect due to increased liver metabolism); allopurinol, amiodarone, androgens, SSRI antidepressants, fluoroquinolone antibiotics, macrolids, cephalosporins (increase the anticoagulant effect).

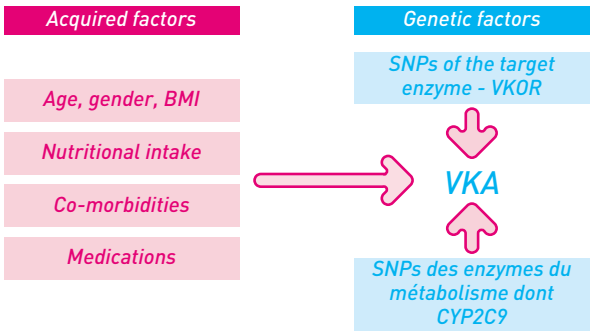

Figure 2: genetic and environmental factors that intervene in VKA dose response variability (according to Siguret *et al*).

The variability in dose response to vitamin K antagonists is ascribable to genetic factors (Figure 2 and Table 2):

Many mutations have been described, but 2 represent 30 - 50% (according to studies) of the VKA variability: these are mutations in the coding genes of the vitamin K epoxide reductase complex VKORC1 and the cytochrome enzyme CYP2C9.

- **VKORC1 polymorphisms and response to VKA (25% of variability):** the VKORC1 protein is the therapeutic target of VKAs. The polymorphism 1639 G/A (rs9923231) is associated with the transcription activity of the VKORC1gene: as such, the mutated A patients (heterozygous state GA or homozygous state AA) causes a decrease in the transcriptional activity of VKORC1 and thus decreases the physiological recycling of vitamin K: this then leads to hypersensitivity and so a lower dose is required (a 25% decrease in heterozygous individuals and 50% in homozygous individuals). Symptomatic patients are rapidly put onto a treatment programme, an increase in INR, at risk of fluctuation and have an INR result › 4 times greater than "wild-type" patients.

Contrarily, rare mutations exist in the coding region of the VKORC1 gene that are responsible for VKA resistance (requiring very high doses of VKA before seeing a change in INR). These mutations are mainly point mutations (V29L, D36Y, V45A, V66M, L128R etc.).

- **CYP2C9 polymorphisms and response to VKAs (10 - 25% of variability):** the cytochrome 2C9 intervenes in the metabolism and inactivation of VKAs. The allele CYP2C9*1 represents the wild reference allele (basic normal activity of CYP2C9). The alleles *2 (Arg144Cys) and *3 (Ile359Leu) correspond to mutated alleles and a decrease in the activity of this cytochrome: the metabolism and hepatic inactivation of VKAs is therefore limited and thus leads to an accumulation of the molecule and hypersensitivity to treatment. The same can be said for the polymorphisms 1639G/A of the VKORC1 gene. Heterozygous mutated patients (*1*2 or *1*3) and homozygous/heterozygous composites (*2*2, *3*3, *2*3) require lower doses of VKA. Please note that *3 patients present with an increased hypersensitivity compared to *2 patient (decreases in dose can extend to 75% of that given to homozygous *3*3 patients versus up to 54% of *2*2 patients).

These polymorphisms are spread variably depending on ethnic origin (Table 2). Screening of the CYP2C9 alleles *2/*3 is, for example, of limited interest in Afro-Caribbean patients (the alleles CYP2C9*5, *6, *8 and *11 are of interest in this population). Whereas for VKORC1, the frequency of 1639AA homozygous patients is 15% in the Caucasian population and 80% in the Asian population. This explains the difference in the mean daily dose administered (6 mg compared with3.5 mg).

Table 2: CYP2C9 and VKORC1 allelic distribution depending on ethnic origin Johnson et al (2011).

Allele	White (n=3062)	Asian (n=1063)	Black (n=645)
CYP2C9*2	0.13	0	0.03
CYP2C9*3	0.07	0.04	0.02
Allele	**White (n=2426)**	**Asian (n=883)**	**Black (n=643)**
VKORC1: -1639G›A (rs9923231)	0.39	0.91	0.11

NB : other variants that affect VKAs have also been described (CYP4F2, CALU, GGCX).

Actions to be taken and VKA dose adjustment relative to genetic and non-genetic criteria.

The main demographic, clinical, biological and therapeutic variability factors could explain the origin of resistance or hypersensitivity to treatment. Software with algorithms (warfarindosing or pharmgkb.org) takes into account these factors and offer a personalised dose programme. The genetic parameters take into account these different algorithms are at least VKORC1 and CYP2C9;

In America, the warfarin notice includes details on the influence of these 2 mutations and different posology regimes are offered within the scope of the International warfarin pharmacogenetics consortium for CYP2C9 and VKORC1 genotypes and are validated by the FDA (Table 3). The FDA recommends in the USA to perform genetic screening for VKORC1 and CYP2C9 before initiating VKA treatment in order to establish the ideal initial dose.

In France, however, no recommendations currently exist for systematic screening of these genetic mutations before starting treatment. The question of patient advantage is subject to the pending results of prospective and pharmacoeconomic studies. In France, these pharmacogenetic tests are, for the moment, reserved for the following 3 situations:

- **Suspected VKA hypersensitivity:** Raised INR › 4 from the first days of treatment, low doses required at steady state;
- **Suspected VKA resistance:** Normal INR or slightly raised despite significantly raised doses;

- INR fluctuations and unexplained difficulty in obtaining steady state.

Table 3: daily doses of Coumadin® (mg/day) depending on the variants CYP2C9 and VKORC1 (FDA recommendations) according to Johnson et al (2011)

VKORC1: -1639›A	*CYP2C9*1/*1*	*CYP2C9*1/*2*	*CYP2C9*1/*3*	*CYP2C9*2/*2*	*CYP2C9*2/*3*	*CYP2C9*3/*3*
GG	5-7	5-7	3-4	3-4	3-4	0.5-2
GA	5-7	3-4	3-4	3-4	0.5-2	0.5-2
AA	3-4	3-4	0.5-2	0.5-2	0.5-2	0.5-2

Reproduced from updated warfarin (Coumadin) product label.

Please note

In cases of VKA hypersensitivity or resistance, it is now possible to perform a genetic study (CYP2C9 genotypes and VKORC1) to adapt each patient to a dose regime (personalised medicine). This needs to be done after having checked medication administration, other medicines being taken at the same time and all other potentially interfering factors.

Please note: the polymorphisms implicated in response sensitivity to direct oral anti-IIa and anti-Xa anticoagulants have also been discovered, notably a variant of the enzyme CYP3A4/5 implicated in rivaroxaban metabolism.

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