The Influence of Polymorphisms in CYP2C9 and VKORC1 Genes on the Efficacy and Safety of Oral Anticoagulant Treatment

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Abstract. Inherited variants of the enzymes involved in drug metabolism, transporters, receptors may play an important role in drug response. Genotyping prior to drug administration seems to be a promising clinical approach in order to reduce the adverse effects of the drugs and to increase their efficacy. Oral anticoagulants (OAs) are drugs largely used in the prevention and treatment of thrombo-embolic diseases. Patients under treatment with OAs present a high risk for severe haemorrhage. Irreversible inhibition of VKORC1 enzyme by OA blocks regeneration of vitamin K, which leads to unfunctional pro-coagulant factors. VKORC1*2 haplotype is linked to an excessive anticoagulation risk at average doses of OAs, while other polymorphisms of the gene are linked to the resistance to OAs. Acenocoumarol is inactivated by CYP2C9 enzyme, so people carrying at least one defective allele CYP2C9*2 but particularly CYP2C9*3 are susceptible to excessive anticoagulation at average doses of acenocoumarol. In conclusion, the combined analysis of CYP2C9 and VKORC1 allows the explanation of 30-40% of the individual variability in equilibrium dose of oral anticoagulants and, consequently, in treatment response.

Keywords: oral anticoagulants, VKORC1 polymorphism, CYP2C9

Introduction

Individual variability in treatment response depends, in part, on factors which are known and easy to assess, such as age, gender, weight, renal and hepatic function, concomitant medication, disease heterogeneity, nutritional status or smoking.

In addition, inherited variants of the enzymes involved in drug metabolism (EMM), transporters, receptors and molecules of the signal transduction cascade may have a major impact on the drug response [1].

Genetic diversity contributes both to the occurrence of the disease and to the variability in treatment response. The large inter-individual and inter-ethnic variability in terms of therapeutic response may be seen as being caused, at least partially, by genetic polymorphisms, most frequently of the SNP type (Single Nucleotide Polymorphism), which are minimal changes in the genetic information existing in over 1% of the population. Such variants are viewed as normal but, still, under certain conditions, they may influence the phenotype.

Pharmacogenomics, a field developed in the latest years, approaches the way in which the variations in human genome influence the drug response and allow the adjustment of medication to the patient’s genetic constellation in the form of an individualized treatment, tailored for the patient and with increased efficacy and safety.

Recent data suggest that most of the drugs are efficient in almost 60% of clinical cases while a significant number of patients develop adverse reactions to treatment [2].

Thus, genotypic analysis prior to drug administration seems to be a promising clinical approach in order to reduce the adverse effects of the drugs and to increase their efficacy.

Oral anticoagulants

Oral anticoagulants (OAs), e.g. warfarin and
acenocoumarol, are drugs largely used in prevention and treatment of thromboembolic diseases, being among the most commonly-prescribed drugs.

They have a small therapeutic index, displaying a great inter-individual and intra-individual variability in treatment response. Patients under treatment with OAs present a risk for complications such as severe haemorrhage caused by an excessive anticoagulation or the treatment may be inefficient due to an insufficient level of coagulation [1].

OAs are used in medical practice for about 60 years but a considerable progress in understanding their mechanism of action and metabolism has been achieved in the last 10 years, especially due to pharmacogenetics. In the future, the progress of pharmacogenetics could lead to a better clinical approach of patients under treatment with OAs [3].

The Role of Polymorphisms in CYP2C9 and VKORC1 Genes during Treatment with Oral Anticoagulants

VKORC1

Identification of the coding gene for the subunit C1 of vitamin K epoxide-reductase (VKORC1) is a recent discovery, dating from 2004 [4, 5].

The gene for VKORC1 is located on chromosome 16 and it encodes a dithiol-dependant reductase which turns vitamin K epoxide into vitamin K quinone; this seems to be one of the OAs’ target-enzymes. Irreversible inhibition of VKORC1 enzyme by OA blocks regeneration of vitamin K which leads to unfunctional pro-coagulant factors.

The VKORC1 gene features several polymorphisms, most of them being grouped in four major haplotypes. Among them, VKORC1*2 haplotype seems to be the most important in terms of variability in response to oral anticoagulants and with excessive anticoagulation risk [6].

The VKORC1*2 haplotype mark is represented by the c.G-1639A polymorphism located at the level of the promoter of VKORC1 gene; its presence is associated with a lower quantity of vitamin K in its active form through perturbation of its recycling mechanism via epoxide-reductase.

Recent studies showed that the VKORC1*2 haplotype induces an excessive anticoagulation risk at average doses of acenocoumarol, leading thus to haemorrhagic events.

The intronic polymorphism C1173T is as informative for VKORC1*2 haplotype as the c.G-1639A polymorphism is, because it is in a complete linkage imbalance with it [7].

As for the C1173T polymorphism, the frequency of allele T is about 45% in the Caucasian population, which means that half of the people belonging to the same population would be susceptible of an increased sensitivity to acenocoumarol.

If the VKORC1*2 haplotype is linked to an excessive anticoagulation risk at average doses of oral anticoagulants, there are also rare mutations of VKORC1 gene linked to the resistance against anticoagulants and to a necessary quantity of increased doses of anticoagulants.

Such a mutation is a g. G5417T transversion which leads to the substitution of an aspartate with a tyrosine in residue 36 (p.Asp36Tyr) of the VKORC1 molecule in the presence of which higher doses of warfarin are needed to obtain an anticoagulant effect [8]. It has to be mentioned that the relation between this mutation and the response to acenocoumarol is not known.

CYP2C9

OAs’ metabolism varies according to their chemical structure: derivatives from indandione (fluindione) or coumarinic derivatives (acenocoumarol, phenprocoumone or warfarin). Commonly used are coumarinic derivatives. Most of them are metabolized at the hepatocyte level through a monooxygenase, cytochrome P450 isoform 2C9 (CYP2C9), resulting in inactive products [9].

The CYP2C9 coding gene is known as a polymorphic gene and its genetic variants are associated to differences in the enzymatic activity of CYP2C9.

Studies on various ethnic groups revealed the existence of several allelic variants of the CYP2C9 gene such as CYP2C9*2, CYP2C9*3, CYP2C9*4, CYP2C9*5, CYP2C9*6 and others. Among them, the most important in regard to their frequency in the general population are CYP2C9*2 and CYP2C9*3.

The CYP2C9*2 allele is characterized by a mutation in exon 3 of the CYP2C9 gene, respectively a c.C430T transition which results in the substitution of an arginine with a cysteine in residue 144 (p.Arg144Cys) of the CYP2C9 molecule.

The CYP2C9*3 allele is characterized by a mutation in exon 7 of the CYP2C9 gene, respectively a c.A1075T transversion, which results in the substitution of an isoleucine with a leucine in residue 359 (p.Ile359Leu) of the CYP2C9 molecule.

Both of the alleles are associated to a significant decrease in the enzymatic activity of CYP2C9 – residual enzymatic activity is 12% for CYP2C9*2 and 5% for CYP2C9*3 [10].

Variants CYP2C9*2 and CYP2C9*3 are more frequently met in Caucasian population, with an allelic frequency of 10-14% (CYP2C9*2) and 8-10% (CYP2C9*3), versus 1-2% (CYP2C9*2) and 0 (CYP2C9*3) in Asian population or 0.5-1% (CYP2C9*2) and 1% (CYP2C9*3) in African population.

Aacenocoumarol is inactivated by CYP2C9 through hydroxylation, reason for which people
carrying at least one defective allele CYP2C9*2 but particularly CYP2C9*3 (allele associated to a 5% enzymatic activity of CYP2C9) are susceptible to excessive anticoagulation at average doses of acenocoumarol.

It seems that up to 14% of the variability in response and optimum dose of acenocoumarol can be explained through polymorphism CYP2C9*2 and especially through CYP2C9*3 [11].

**Individual variability in response to treatment with oral anticoagulants: environmental factors and genetic polymorphisms in VKORC1 and CYP2C9**

Difficult maneuverability of oral anticoagulants is linked to the close therapeutic margin of such drugs and to a high inter- and intra-individual variability in treatment response.

This is assessed by the International Normalized Ratio (INR) measurement, sensitive to the deficit in coagulation factors II, VII and IX – three of the coagulation factors depending on vitamin K [9].

Until recently, environmental factors had been considered the main responsible factors for inter-and intra-individual variations in oral anticoagulant treatment response. Such factors include: patient’s characteristics (age, gender, and body mass index), vitamin K alimentary intake, comorbidities (hepatic failure, severe renal insufficiency, cardiac insufficiency, thyroid diseases etc), acute intercurrent pathologies (fever, sepsis, cardiac insufficiency, diarrhea etc.) and concomitant treatments [12, 13]. In addition to demographic and environmental factors, there have been identified genetic polymorphisms which explain a part of the individual variability in response to oral anticoagulant treatment.

CYP2C9 polymorphisms were identified about 10 years ago and their variation depends on the ethnic type – Caucasian, African or Asiatic.

The most common variants in Caucasian population are CYP2C9*2 (Arg 144-Cys) and CYP2C9*3 (Ile 359-Leu) which are met in one quarter of the general population. Mutating enzymes resulted from such polymorphisms are less active than normal enzymes, leading to a decrease in the metabolism of coumarinic derivatives. Subjects carrying at least one mutating allele have an increased sensitivity to these derivatives and are named “slow metabolizers” [14].

Various studies have shown a relation between genotype and the average dose of warfarin when therapeutic balance is achieved [15-20]: in middle aged patients carrying two normal alleles (2C9*1/*1), the dose decreases by 13-22% in 2C9*1/*2 patients, by 18-40% in 2C9*2/*2 patients, by 21-49% in 2C9*1/*3 patients; by 18-73% 2C9*2/*3 patients and by 71% or over in 2C9*3/*3 patients.

Results are comparable to studies undertaken for acenocoumarol and phenprocoumon [21, 11]. Also, Saraeva et al. showed that prevalence of CYP2C9*1/*3, CYP2C9*2/*2 and CYP2C9*2/*3 had been higher in the group of patients treated with small doses of acenocoumarol versus those treated with high and average doses [22]. In a retrospective study on 185 patients treated with warfarin for a long period and with an average 13-months follow-up, Higashi et al. proved that carrying at least one mutating allele CYP2C9 is associated to (i) a significant increase in overdosage risk (hazard ratio [HR] 1.40 [CI 95%: 1.03-1.90]), (ii) an increase in the period of time to reach the equilibrium dose (HR 0.65 [CI 95%: 0.45-0.94]) and particularly (iii) an increase in major haemorrhagic risk (HR 2.39 [CI 95%: 1.18-4.86]) [18].

With regard to the overdosage risk or the haemorrhagic risk, the results are comparable to those based on acenocoumarol and phenprocoumon [21, 23].

The recent discovery of polymorphisms in the VKORC1 gene is a step forward in understanding inter-individual variability of the stabilizing dose of oral anticoagulants.

In a study on 147 patients treated with warfarin, D’Andrea et al. have identified a first polymorphism (1173C>T) as an independent factor which influences the daily average equilibrium dose: this is considerably lower in patients 1173TT (3.5 mg; p< 0.001) versus patients 1173CT (4.8 mg; p=0.002) and patients 1173CC (6.2 mg) [24].

Rieder et al. have studied the VKORC1 gene in a comprehensive manner and identified, in 186 Caucasian subjects, two types of haplotypes - A and B (including also the polymorphism found by D’Andrea et al.): A/A subjects needed a significantly lower average dose of warfarin (2.7 mg) versus A/B subjects (4.9 mg) and B/B subjects (6.2 mg) [25].

28 polymorphisms in the VKORC1 gene have been described, four of the haplotypes being responsible for the genetic variability of the VKORC1 gene. VKORC1*2 haplotype is involved in most of the variations in the response to OAs [26].

A high response to acenocoumarol was described for healthy subjects, carriers of polymorphism c.-1639G>A, a mark of VKORC1*2 haplotype [11].

Montes et al. also showed that the A allele of polymorphism c.-1639G>A in the VKORC1 gene is associated to the need of a lower dose of acenocoumarol in patients under anticoagulant treatment [27].

The VKORC1*2 haplotype seems to contribute more than CYP2C9 variants to inter-individual and inter-ethnic variability in response to acenocoumarol, contributing by up to 40% thereto. Considering
this aspect and the contribution of CYP2C9 variants by up to 14%, it would result that over 50% of the variability in response to acenocoumarol is ensured by CYP2C9 and VKORC1 variants [11].

CONCLUSIONS

As the latest studies show, the combined analysis of CYP2C9 and VKORC1 allows the explanation of 30–40% of the individual variability in equilibrium dose of oral anticoagulants and, consequently, in treatment response [3].

Although genetic testing for CYP2C9 and VKORC1 polymorphisms cannot replace the INR determination in order to adjust the dose of coumarinic anticoagulants, these procedures may help with the identification of a group of patients who need a longer period of induction for setting a stable dose and who present a potentially higher risk of major haemorrhages.

Through a fast genotyping of VKORC1 and CYP2C9 before initiating an oral anticoagulant treatment and having in view the demographic and clinical factors, the equilibrium dose of oral anticoagulant could be determined a priori. It would avoid the overdosing risk in the treatment-initiation period and, implicitly, the risk of serious haemorrhagic events. All these will contribute to an increased efficacy and safety of the oral anticoagulant treatment.

ACKNOWLEDGEMENT: This paper was supported by PNCDII grant 42-127, Trombogen

References


