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REVIEW

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

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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, p*henprocoumone 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.

Acenocoumarol 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 decompensation, diarrhoea 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 ethnical 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 phenprocoumone [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 phenprocoumone [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.

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References

1. Tomalik-Scharte D, A Lazar, U Fuhr, J Kirchheiner. The clinical role of genetic polimorphisms in drug-metabolizing enzymes. *The Pharmacogenomics Journal* **2008; 8: 4-15**

2. Ian P. Hall, Munir Pirmohamed. *Pharmacogenetics*. **1st ed. New York:Taylor & Francis Group, 2006**

3. Siguret V. Antivitamines K et pharmacogénétique: vers une meilleure compréhension de la variabilité individuelle de l'effet dose-réponse. *Patologie Biologie* **2007; 55: 295-298**

4. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortmagel K, Pelz HJ, et al. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* **2004; 427: 537-41**

5. Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. *Nature* **2004; 427:541-4**

6. Geisen C, Watzka M, Sittinger K, Steffens M, Daugela L, Seifried E, Muller CR, Wienker TF, Oldenburg J. VKORC1 haplotypes and their impact on the inter individual and inter-ethnical variability of oral anticoagulation. *Thrombosis and Haemostasis* **2005; 94: 773–9**

7. Kimmel SE, Christie J, Kealey C, Chen Z, Price M, Thorn CF, Brensinger CM, Newcomb CW, Whitehead AS. Apolipoprotein E genotype and warfarin dosing among Caucasians and African Americans. *TPJ* **2008; 8:53-60**

8. Loebstein R, Dvoskin I, Halkin H, Vecsler M, Lubetsky A, Rechavi G, Amariglio N, Cohen Y, Ken-Dror G, Almog S, Gak E. A coding VKORC1 Asp36Tyr polymorphism predisposes to warfarin resistance. *Blood* **2007; 109: 2477-2480**

9. Ansell J, Hirsh J, Poller L, Bussey H, Jacobson A, Hylek E. The pharmacology and management of the vitamin K antagonists: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* **2004; 126(3 Suppl) : 204S-233S**

10. Taube J, Halsall D, Baglin T. Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. *Blood* **2000; 96(5): 1816-9**

11. Bodin L, Verstuyft C, Tregouet DA, Robert A, Dubert L, Funck-Brentano C, Jaillon P, Beaune P, Laurent-Puig P, Becquemont L, Loriot MA. Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1) genotypes as determinants of acenocoumarol sensitivity. *Blood* **2005; 106(1): 135-140**

12. Penning-van Beest FJ, van Meegen E, Rosendaal FR, Stricker BH. Characteristics of anticoagulant therapy and comorbidity related to overanticoagulation. *Thromb Haemost* **2001; 86: 569-74**

13. Visser LE, Penning-van Beest FJ, Kasbegen AA, De Smet PA, Vulto AG, Hofman A, et al. Overanticoagulation associated with combined use of antibacterial drugs and acenocumarol or phenprocoumon anticoagulants. *Thromb Haemost* **2002; 88: 705-10**

14. Kirchheiner J, Brockmöller J. Clinical consequences of cytochrome P4502C9 polymorphisms. *Clin Pharmacol Ther* **2005; 77: 1-16**

15. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* **1999; 353: 717-9**

16. Margaglione M, Colaizzo D, D'Andrea G, Brancaccio V, Ciampa A, Grandone E, et al. Genetic modulation of oral anticoagulation with warfarin. *Thromb haemost* **2000; 84:775-8**

17. Taube J, Halsall D, Baglin T. Influence of cytochrome P450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. *Blood* **2000; 96:1816-9**

18. Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin F:, et al. Association between CYP2C9 genetic variants and anticoagulation –related

outcomes during warfarin therapy. *JAMA* **2002; 287:1690-8**

19. Kamali F, Khan TI, King BP, Frearson R, Kesteven P, Wood P, et al. Contribution of age and body size and CYP2C9 genotype to anticoagulant. **Clin** *Pharmacol Ther* **2004; 75: 204-12**

20. Siguret V, Gouin I, Golmard JL, Geoffroy S, Andreux JP, Pautas E. Polymorphismes du cytochrome P450 2C9 (CYP2C9) et posologie à l'équilibre pour des patients âgés traités par warfarine. *Rev Med Intern* **2004; 25: 271-4**

21. Visser LE, van Vliet M, van Schaik RH, Kasbergen AA, De Smet PA, Vulto AG et al. The risk of overanticoagulation in patients with cytochrome P450 CYP2C9*2 or CYP2C9*3 alleles on acenocoumarol or phenprocoumon. *Pharmacogenetics* **2004; 14: 27–33.**

22. Saraeva RB, Paskaleva ID, Doncheva E, Eap CB, Ganev VS. Pharmacogenetics of acenocoumarol: CYP2C9, CYP2C19, CYP1A2, CYP3A4, CYP3A5 and ABCB1 gene polymorphisms and dose requirements. *Journal of Clinical Pharmacy and Therapeutics* **2007; 32: 641-649**

23. Verstuyft C, Robert A, Morin S, Loriot MA, Flahault A, Beaune P, et al. Genetic and environmental risk factors for oral anticoagulant overdose. *Eur J Clin Pharmacol* **2003; 58: 739-45**

24. D'Andrea G, D'Ambrosio RL, Di Perna P, Chetta M, Santacroce R, Brancaccio V, et al. A polymorphism in VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. *Blood* **2005; 105: 645-9**

25. Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med* **2005; 352:2285-93**

26. Geisen C, Watzka M, Sittinger K, Steffens M, Daugela L, Seifried E, Muller CR, Wienker TF, Oldenburg J. VKORC1 haplotypes and their impact on the inter individual and inter-ethnical variability of oral anticoagulation. *Thrombosis and haemostasis* **2005; 94: 773-9**

27. Montes R, Ruiz de Gaona E, Martinez-Gonzales MA, Alberca I, Hermida J. The c.-1639G>A polymorphism of the VKORC1 gene is a major determinant of the response to acenocoumarol in anticoagulated patients. *British Journal of Haematology* **2006; 133: 183-7.**