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Pharmacogenetics in cardiovascular diseases

Cardiovascular pharmacogenetics

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Abstract

The use of genomic markers to predict drug response and effectiveness has the potential to improve healthcare by increasing drug efficacy and minimizing adverse effects. Polymorphisms associated with inter-individual variability in drug metabolism, transport, or pharmacodynamics of major cardiovascular drugs have been identified. These include single nucleotide polymorphisms (SNP) affecting clinical outcomes in patients receiving antiplatelet agents, oral anticoagulants and statins. Based on clinical evidence supporting genetic testing in the management of cardiovascular diseases using these drug classes, this short review presents clinical guidance regarding current pharmacogenetics implementation in routine medical practice.

KEYWORDS

Pharmacogenomics; Pharmacogenetics; Cardiovascular drugs; Clinical implementation

Abbreviations

AMM: marketing authorisation (*autorisation de mise sur le marché*)

CPIC: Clinical Pharmacogenetics Implementation Consortium

CYP450: cytochromes P450

CYP2C9: cytochrome P450 family 2 subfamily C member 9

EMA: European Medicines Agency

FDA: Food and Drug Administration

INR: international normalized ratio

NHLBI: National Heart, Lung, and Blood Institute

OATP1B1: organic anion transporting polypeptide family member 1B1

RNPGx: French National Network of Pharmacogenetics (*Réseau National de Pharmacogénétique*)

rs: reference SNP cluster number

SLCO1B1: solute carrier organic anion transporter family member 1B1

SNP: single nucleotide polymorphism

TDM: Therapeutic drug monitoring

VKA: vitamin K antagonist

VKORC1: vitamin K epoxide reductase, subunit 1

Pharmacogenetics of oral vitamin K antagonists

Vitamin K antagonists (VKAs: fluindione, warfarin, acenocoumarol) are characterized by significant inter-individual variability in response that can be associated with overanticoagulation (and thus hemorrhagic risk) or resistance to treatment. Proper dosage for VKAs can be difficult to achieve due to the narrow therapeutic window and the significant inter- and intra-individual variability in response as assessed by the international normalized ratio (INR). It is estimated that the VKA dose needed to reach the target range may vary up to 20-fold from one patient to another. This variability is largely explained by the polymorphisms of two genes coding for proteins implicated respectively in the pharmacodynamics and the pharmacokinetics of VKAs (Figure 1): vitamin K epoxide reductase subunit 1 (VKORC1*)* and cytochrome P450 isoform 2C9 (CYP2C9) [1-4].

Basic concepts and main indications for pharmacogenetic testing: VKA and VKORC1 and CYP2C9 genotyping

VKORC1 genetic polymorphism

VKORC1 codes for a key enzyme of the vitamin K cycle mainly expressed in liver cell endoplasmic reticulum. The VKORC1 enzyme is the pharmacological target of VKAs whose inhibitory actions block vitamin K-dependent coagulation factors (factors II, VI, IX, and X) [Figure 1]. VKORC1 corresponds to three exons situated on chromosome 16. Dozens of genetic polymorphisms, with or without clinical expression, have been described for this gene. The polymorphism rs9934438 (c.-1639G>A) [Table 1] situated upstream from the gene in the non-coding 5' region, is associated with diminished promotor transcription activity, leading to lesser expression of the VKORC1 enzyme (allele VKORC1*2). This polymorphism is frequent and is probably responsible for the higher hemorrhagic risk in patients taking VKAs [2-4]. This allelic variant has a variable frequency depending on the ethnic background. In the Caucasian population, the frequency is about 42% (*i.e.* about 50% GA heterozygotes and 18% AA homozygotes). It is

15% in the African population and reaches 90% in the Asian population. Polymorphism rs9934438 is part of a haplotypic block, but in everyday practice it can be technically preferable to search for another single nucleotide polymorphism (SNP), rs17878363 (1173C>T), considered to be in complete linkage disequilibrium with rs9934438. Many other rare polymorphisms, generally situated in gene coding regions, are associated with structural modifications of the VKORC1 enzyme, making it less sensitive to the action of VKAs (VKA resistance). About twenty rare mutations (frequency < 1% in the Caucasian population) involving the VKORC1 gene are associated with decreased sensitivity of the target enzyme and may require significantly higher doses to reach the desired INR range [5].

CYP2C9 genetic polymorphism

Cytochrome P450 isoform 2C9, coded for by the CYP2C9 gene on chromosome 10, is implicated in the hepatic metabolism of VKAs (warfarin, acenocoumarol and fluindione). Genetic factors lead to significant inter-individual variations in the activity level of this chromosome. In the Caucasian population, there are two main genetic variants associated with deficient CYP2C9 activity: polymorphism rs1799853 (CYP2C9*2 or c.430C>T) and rs1057910 (CYP2C9*3 or c.1075A>C). These common polymorphisms (allelic frequency ~10%) (Table 1) define slow metabolizers (genotype combining at least two mutated alleles, *i.e.* about 1% of the population) and intermediary metabolizers (heterozygous genotype for one of the alleles, *i.e.* about 18% of the population). Thus, about 20% of Caucasian individuals are carriers of at least one mutated allele associated with altered VKA metabolism [6].

Indications for VKORC1 and/or CYP2C9 genotyping

Indications for VKORC1 and/or VYP2C9 genotyping are as follows.

Search for genetic polymorphisms associated with a risk of overdosing:

- *a priori* genotyping (to predict hemorrhagic risk before initiating VKA therapy and to determine the optimal dose);

- *a posterori* genotyping (to explain overdosing observed after initiating treatment and to adapt dose).

In both situations, the test should search for polymorphisms rs9934438 or rs17878363 (VKORC1*2), rs1799853 (CYP2C9*2) and rs1057910 (CYP2C9*3).

Search for rare genetic variants associated with VKA resistance:

- *a posterori* genotyping (to explain VKA resistance in patients requiring high-dose VKA): in this situation genotyping is inappropriate; a sequencing study for gene VKORC1 should be ordered to search for rare functional mutations.

Results (drug selection, contraindications, dosage)

Genotypes associated with hemorrhagic risk with VKAs

The effects of VKORC1 and CYP2C9 polymorphisms are cumulative so that both genotypes should always be considered. In subjects with a homozygous or heterozygous c.-1639A variant of gene VKORC1, the VKORC1 enzyme is expressed less and a standard VKA dose is associated with higher risk of hemorrhage due to overexposure. Patients who are carriers of homozygous or heterozygous alleles CYP2C9*2 or *3 exhibit reduced liver metabolism of VKA and are at risk of over exposure.

When there is a risk of hemorrhage due to over exposure in patients carriers of one or more of these alleles, the VKA should be initiated at a lower dose (from 20% to 80% of standard dose) [7, 8]. This dose adaptation must also take into account the type of VKA used (warfarin, acenocoumarol or fluindione) because of the variable risk impact, the genotype (CYP2C9 and VKORC1), and other cofactors known to influence exposure to VKAs, including: comedication (statins, sulfonamides, amiodarone, azole antifungals, …); comorbidities (liver diseases, smoking, …); individual factors (age, ethnic background); and environmental factors (diet, …). Algorithms have been developed to optimize dosage as a function of these covariables [7]. Indications for warfarin doses are given by genotype (VKORC1 and CYP2C9) in Table 2 [8]. The dose reduction necessary in patients heterozygous for a mutated allele is about 20-40% of the standard dose, but can reach 90% for patients with two or more mutated alleles. Furthermore, longer drug half-life is

susceptible to modify the INR monitoring strategy. As an indication, good control of warfarin concentrations (and thus INR) is reached in 7-8 days in extensive metabolizers while it takes 11 days to reach equilibrium in slow metabolizers [9].

Genotypes associated with VKA resistance

In rarer cases, mutations of the VKORC1 gene lead to VKA resistance [10, 11]. After having ruled out potential problems related to adherence, absorption, or drug interactions, VKORC1 gene sequencing can be ordered to explain the VKA resistance in a patient requiring an abnormally high dose to achieve effective anticoagulation (assessed with the INR or clinical response) [5].

Therapeutic drug monitoring (TDM) can provide information complementary to the genetic analysis to rule out adherence or absorption problems, especially in cases of VKA resistance. For TDM, considering the variable half-life of VKAs (8-11 h for acenocoumarol, 31 h for fluindione, 35-45 h for warfarin), a 3- to 10-day delay is required to stabilize INR in the target range. Hypothrombinemia is generally observed 36-72 h after oral VKA administration. The INR should be measured at 48h and monitored regularly considering that the time necessary to achieve equilibrium (about 20 days) is basically a function of the drug's half-life and of factors modulating this half-life (genetic, pathophysiological, environmental factors), as well as the half-life of the coagulation factors.

Current level of implementation in routine practice and guidelines

- **Prescription guidelines:** In France, it is clearly stated in the marketing authorization (*autorisation de mise sur le marché* [AMM]) for the main VKAs that exposure and response to treatment are highly variable. Genetic factors and available tests are not mentioned. Nevertheless, the European Medicines Agency (EMA) recognizes the usefulness of considering the CYP2C9 and VKORC1 genotypes, together with non-genetic factors, and currently recommends the use of algorithms [7] taking into consideration these factors for determining the initial dose for VKAs. Recently, information to this effect was introduced in the warfarin labeling documents.

- Recommendations from learned societies: Several American societies, including the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the National Heart, Lung, and Blood Institute (NHLBI) recommend CYP2C9 and VKORC1 testing in the following situations (highest level of evidence: 1A): *a priori*, before initiating warfarin treatment: dose adaptation according to an algorithm taking into account the CYP2C9 genotype results and non-genetic factors; *a posteriori*, to explain an hemorrhagic event or VKA resistance [12].

- Recommendations of the French National Network of pharmacogenetics (*Réseau national de pharmacogénétique* [RNPGx]): recommendations published by the RNPGx correspond to international guidelines issuing from the CPIC and extended to VKAs available in France: fluindione (Préviscan®), acenocoumarol (Sintrom®) and warfarin (Coumadine®). The RNPGx is in favor of taking into account the VKORC1/CYP2C9 genotype and non-genetic covariables of VKA exposure: before initiating VKA treatment (testing is *advisable* to determine the optimal dose and to orient the prescription to an alternative therapeutic option such as a direct-action oral anticoagulant); after treatment initiation (testing is *advisable* to explain an hemorrhagic event or VKA resistance [13, 14].

Pharmacogenetics of clopidogrel

Clopidrogrel is a pro-drug whose metabolite inhibits platelet aggregation. Activation of clopidrogrel requires an oxidation step in the liver mainly involving CYP2C19 (Figure 2). The principal therapeutic indications for clopidrogrel are the prevention of atherothrombotic and thromboembolic events after myocardial infarction, ischemic vascular events, or lower-limb thromboangiitis obliterans and in patients with atrial fibrillation or acute coronary artery disease.

Basic concepts and indications for pharmacogenetic testing: clopidogrel and CYP2C19 genotyping

Cytochrome P450 isoform 2C19, coded for by the CYP2C19 gene on chromosome 10, is implicated in liver metabolism of clopidogrel that leads to its transformation into its active metabolite. CYP2C19 expression and/or activity are determined genetically. In the Caucasian population, the

CYP2C19*2 and *17 alleles correspond to two common genetic polymorphisms associated with a functional effect on the enzyme (Table 3).

The characteristic polymorphism of the CYP2C19*2 allele (c.681G>A or rs4244285) is located on the gene's exon 5. It leads to a splicing defect associated with a premature stop codon and absence of CYP2C19 activity, also determining slow metabolizer phenotypes (homozygous CYP2C19*2/*2 genotype). The frequency of the CYP2C19*2 allele is about 15% in the Caucasian population, which corresponds to approximately 25% of intermediary metabolizers and 2% of slow metabolizers. The CYP2C19*3 allele (rs4986893) is a nonsense SNP of the gene's exon 4. It is mainly observed in the Asian population and is associated with synthesis of an inactive truncated protein (Table 3).

In routine practice, current evidence in the literature suggests that the transformation of clopidogrel to its active metabolite, and thus therapeutic efficacy, is greatly reduced in intermediary or slow metabolizers [8, 15-16]. Consequently, these patients have a higher therapeutic risk: poor response or non-response to clopidogrel implying increased risk of recurrent cardiovascular events.

The CYP2C19*17 allele (c.805C>T or rs12248560) is located in the gene promoter and is associated with enhanced CYP2C19 expression and an ultra-rapid metabolizer phenotype. The allelic frequency reaches 25% in western populations, giving about 45% of ultra-rapid metabolizers (*1/17 and*17/*17). Studies have reported excessive reduction in platelet aggregation and higher risk of hemorrhagic events in patients carrying the CYP2C19*17 allele treated with clopidogrel [17, 18]. Certain authors have also suggested that this increased antiplatelet activity has a protective effect preventing thromboembolic cardiovascular events [18].

Indications for CYP2C19 genotyping

Indications for CYP2C19 genotyping are as follows:

- *a priori* genotyping (to predict resistance before initiating clopidogrel therapy): in this situation, the test should search for CYP2C19*2 (rs4244285) and/or potentially deleterious allele(s) specifically encountered in certain populations (e.g. CYP2C19*3 is commonly found with the *2 allele in the Asian population). At the present time, and taking into consideration available data in the literature, this indication for *a priori* testing mainly concerns patients scheduled for coronary angioplasty with stenting. In this at-risk situation for thromboembolism, the role of the CYP2C19

genotype on the efficacy of clopidogrel has been clearly demonstrated. For other indications such as the prevention of recurrent or transient ischemic vascular events, the most recent data also indicate a loss of treatment efficacy associated with the presence of allele(s) with a deleterious effect on CYP2C19 [19].

- *a posteriori* genotyping (to explain recurrent thromboembolism despite treatment or hemorrhagic events occurring after starting treatment): in these two situations, the test should search for CYP2C19*2 (rs4244285) polymorphism(s) and/or, depending on the patient's ethnic background, CYP2C19*3 (rs4986893), or CYP2C9*17 (rs12248560) polymorphisms respectively.

Results (drug selection, contraindications, dosage)

Prescription of a first-line treatment with clopidogrel should take into consideration the CYP2C19 genotype as indicated in Figure 2.

- **CYP2C19*2 and *3 alleles:** in patients carrying at least one deleterious CYP2C19*2 or CYP2C19*3 allele who have a high risk of thromboembolic recurrence, an alternative antiaggregant that is not a CYP2C19 substrate (prasugrel, ticagrelor, acetylsalicylic acid, …) is preferable. Based on current knowledge, it is not recommended to increase the clopidogrel dose in patients carrying the CYP2C19*2 or *3 allele.

- **CYP2C19*17 allele**: although the presence of the CYP2C19*17 allele, whether in a heterozygous or homozygous state, has been associated with increased hemorrhagic risk in patients treated with clopidogrel, it appears to favor the drug's efficacy, reducing the incidence of recurrent thromboembolic events. Consequently, considering current knowledge, there is no guideline concerning the appropriate dosage to prescribe for a patient with the *17 allele.

Current level of implementation in routine practice and guidelines

- **Prescription guidelines**: in France, the marketing approval (AMM) warnings and precautions statement specifically mentions the risk of lower treatment efficacy associated with reduced CYP2C19 activity. Similarly, it is strongly recommended to not combine clopidogrel with agents

inhibiting and/or inducing CYP2C19 such as: omeprazole/esomeprazole, fluvoxamine, fluoxetine, moclobemide, voriconazole, fluconazole, ticlopidine, carbamazepine, and efavirenz. Nevertheless, this information, also mentioned by the EMA, has not led to specific recommendations in terms of dose adaptation or therapeutic alternatives. In the USA, in addition to providing this labeling information, the FDA has put genotype CYP2C19 on the list of Pharmacogenomic Biomarkers in Drug Labeling and recommends preferential prescription of antiplatelet agents that are not CYP2C19 substrates (prasugrel, ticagrelor, …) in patients who are slow metabolizers for CYP2C19 [20]. Interestingly, the results of a clinical trial published in 2011 have demonstrated that there is no need to increase clopidogrel dose in patients with deleterious allele variants who have had coronary angioplasty with stenting [21].

- Recommendations from learned societies: the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends CYP2C19 genotyping before initiating clopidogrel treatment in adult patients with an acute coronary syndrome who have undergone a percutaneous intervention [22]. The test should search for the main deficiency alleles (CYP2C19*2 and *3) associated with a risk of treatment resistance. The therapeutic approach proposed by the CPIC as a function of the CYP2C19 genotype is presented in Figure 3. Based on current knowledge, and considering the discordant data available in the literature, testing for the CYP2C19*17 allele, which is potentially associated with increased risk of bleeding in patients taking clopidogrel, is not recommended by the CPIC for this indication. Due to the lack of sufficient clinical evidence in the pediatric population and for other indications such as ischemic vascular events, the CPIC does not recommend CYP2C19 genotyping for these indications.

- Recommendations of the French National Network of Pharmacogenetics (RNPGx): the RNPGx is in favor of testing for the main CYP2C19 deficiency alleles before instituting clopidogrel treatment (a test is *essential* for coronary angioplasty with stenting and based on the current state of knowledge this test is *potentially useful* in the other indications). For patients carrying at least one deficiency allele, the RNPGx recommends using an alternative treatment that is not a CYP2C19 substrate (prasugrel, ticagrelor,…). If the genotype remains unknown before treatment onset, *a posteriori* testing is *advisable* to explain any recurrent thromboembolic event occurring during treatment in order to provide guidance for therapeutic optimization of the indications given in the marketing approval (testing is *advisable*) [12, 13].

Pharmacogenetics of statins

Statins inhibit 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMB-CoA reductase). This enzyme catalyzes the transformation of HMG-CoA into mevalonate, an early and limiting step of cholesterol synthesis. The statin family constitutes the main cholesterol-lowering option in the therapeutic armamentarium. The family currently includes the following compounds, listed by order of marketing approval: lovastatin (1987), simvastatin, pravastatin, fluvastatin, atorvastatin and rosuvastatin (2003). These drugs are mainly cleared by hepatobiliary excretion after capture by the solute carrier organic anion transporter family member 1B1 (SLCO1B1), also called organic anion transporting polypeptide family member 1B1 (OATP1B1), expressed on the hepatocyte membrane. Adverse effects observed in certain patients taking statins include muscle toxicity that varies from simple myalgia to potentially lethal rhabdomyolysis. This toxicity led to the removal of cerivastatin from the French market in 2003. The relationship between statin pharmacokinetics, the presence of SLCO1B1 polymorphism(s) and the occurrence of muscle toxicity has been demonstrated.

Basic concepts and indications for pharmacogenetic testing: statins and SLCO1B1 genotyping

SLCO1B1 polymorphism

Several factors such as the activity level of the transporter OATP1B1 affect exposure to statins. OATP1B1 is coded for by the SLCO1B1 gene on chromosome 12. It is expressed on the basolateral pole of hepatocytes where it is involved in sodium-dependent capture of diverse drugs (statins, repaglinide, methotrexate) and endogenous substances such as biliary acids, estradiol, prostaglandins, thromboxane, leukotrienes or thyroxin.

Activation of the OATP1B1 transporter is genetically determined by SLCO1B1 polymorphism. The main variant of this gene is SLOC1B1*5 (rs4149056, c.521T>C) located on exon 6. SLCO1B1*5 is an antisense SNP leading to valine substitution for alanine in position 174 of the protein sequence. It is associated with significant reduction in transporter activity (Table 4). This allele has a frequency of about 16% in Caucasian and Asian populations, *i.e.* about 27% heterozygous carriers (T/C) and up to 3% homozygous carriers (CC). Interestingly, this frequency

generally does not exceed 5% in African populations. A relationship was recently demonstrated between SLOC1B1 polymorphism and the pharmacokinetics of statin muscle toxicity [23-26]. Decreased OATP1B1 transporter activity in subjects carrying the *5 allele would be associated with reduced hepatocyte capture of circulating statins and thus decreased hepatic clearance that also involves mainly CYP3A4 metabolism. This results in increased serum statin concentrations with an increased risk of muscle toxicity by overexposure [25]. In clinical practice, the effect of SLCO1B1 polymorphism on statin toxicity is directly linked to three main factors: *i*) genotype: the risk of toxicity is highest in homozygous carriers of mutated alleles, but is also considered to be high in heterozygous carriers; *ii*) dose: the risk of toxicity increases with dose in these patients; *iii*) type of statin: myotoxicity is a risk common to all statins, but the incidence appears to vary depending on the active substance (reported muscle toxicity by drug, in decreasing order of incidence: simvastatin >> pravastatin, atorvastatin, rosuvastatin > fluvastatin, lovastatin). SLCO1B1 appears to have no impact on the pharmacokinetics of fluvastatin that is not an OATP1B1 transporter substrate [24]. For instance, in carriers of the CC genotype, mean exposure is increased around +200% for simvastatin, +150% for atorvastatin, and +100% for rosuvastatin and pravastatin, in comparison with carriers of the TT genotype (homozygous wild SLCO1B1*1/*1). Thus, in subjects with SLCO1B1*5 polymorphism, whether heterozygous or homozygous, who are taking a high-dose regimen (*e.g.* simvastatin 80 mg/d) the incidence of myotoxicity is increased 3- and 17-fold respectively (23, 25].

Indications for SLCO1B1 genotyping

The current situation concerning SLCO1B1 genotyping in patients treated with statins, especially simvastatin, or who have presented muscle toxicity during the course of treatment, are presented in Figure 4. The indications for genotyping are as follows.

- *a priori* genotyping (to predict toxic risk before initiating statin treatment): in routine practice, modifications in statin pharmacokinetics associated with rs4149056 do not always lead to muscle toxicity because of the broad therapeutic index of these drugs. On the basis of current knowledge, there is no indication for general genetic screening before or after initiating statin treatment;

- *a posterori* genotyping: a SLCO1B1 test may be indicated after initiating treatment to explain muscle symptoms in a patient taking statins (myalgia and/or muscle weakness with or without elevation of creatinine phosphokinase levels).

Results (drug selection, contraindications, dosage)

Statin exposure (especially with simvastatin) and risk of myopathy are always higher in patients who are carriers of one or more SLCO1B1 alleles associated with OATP1B1 deficiency. This risk of myopathy is correlated with administered dose, the degree of the OATP1B1 activity deficit, and treatment combination with OATP1B1 and/or CYP3A4/5 isoform inhibitors (e.g. ciclosporin). The main risk factors for statin-related myotoxicity are: genetic (*e.g.* SLCO1B1, CYP3A4/5,…), pathophysiological (kidney failure, history of myopathy, diabetes mellitus, very young or very old age, female gender, hypothyroidism, …) and drug-related (dose, type of statin, potential drug interactions).

As an example, homozygous CC carriers of SLCO1B1*5/*5 5RS4149056 alleles treated with simvastatin at the dose of 80 mg/d have a 17-fold higher risk of myopathy (corresponding to a 15-20% annual incidence in this population). This risk is 3- to 5-fold higher (1-2% annual incidence) in heterozygous TC carriers (SLCO1B1*1/*5). The corresponding risk is about 0.3% in patients with the most common wild genotype TT (SLCO1B1*1/*1) receiving simvastatin 80 mg/d: the myopathy risk is multifactorial and the positive predictive value of genetic testing is thus limited.

If known prior to treatment onset, a SLCO1B1 genotype corresponding to homozygous or heterozygous presence of a C allele should be considered when assessing the patient's risk-benefit ratio before prescribing a maximal statin dose. On the basis of current knowledge, high-dose statins, as well as OATP1B1 and/or CYP3A inhibitors should be avoided in carriers of CC or TC genotypes (Figure 4) [27, 28]. The patient should also be informed about the potential toxicity of the treatment and treatment should be discontinued in the event of unexplained muscle pain.

Current level of implementation in routine practice and guidelines

- **Prescription guidelines**: data on SLCO1B1 polymorphisms were recently introduced into the warnings and precautions paragraph for certain statins such as simvastatin. The higher risk of myotoxicity associated with the presence of the rs4149056 C allele is mentioned. The FDA currently recommends avoiding doses of 80 mg/d for simvastatin because of the higher risk of myotoxicity [27]. In general, marketing approval labeling advises against combining statins with OATP1B1 and/or CYP3A4/5 inhibitors.

- **Recommendations from learned societies**: Internationally, the CPIC recommends taking into account the SLCO1B1 genotype together with the dose, the type of statin, and co-medications for assessing the risk of potential statin toxicity (Figure 4). In agreement with the FDA guidelines, the CPIC recommends: *i*) against prescribing simvastatin at 80 mg/d the first year of treatment (and more generally any maximum dose statin if there is a doubt about tolerance); *ii*) for simvastatin, to reduce the dose to 40 mg/d maximum in heterozygous patients (SLCO1B1*1/*5) and to 20 mg/d maximum in homozygous patients (SLCO1B1*5/*5). Under these conditions, in the event of treatment failure or toxicity, an alternative treatment should be envisaged; *iii*) in all situations for patients carriers of the rs4149056 allele, use of the minimal statin dose, close monitoring of creatinine phosphokinase levels, and avoiding combining the statin with OATP1B1 inhibitors (susceptible of increasing the exposure to statins) [Table 1].

- **Recommendations form the French National Network of Pharmacogenetics (RNPGx):** RNPGx is in favor of rs4149056 testing before starting treatment, or early after treatment onset (*potentially useful* test) in patients with one or more risk factors described in the preceding chapter (as a function of statin type and dose, comedications, and the pathophysiological or genetic setting). If the genotype is not known early, the RNPGx considers that a polymorphism test is *potentially useful* in the event of muscle toxicity in patients treated with statins, in order to rule out or confirm a genetic cause [12, 13].

Conclusions

Progress achieved in recent years in the field of genetics has broadened our knowledge of the molecular mechanisms implicated in the therapeutic response or the toxicity of drugs widely used for cardiovascular diseases. The level of evidence for pharmacogenetic tools available today is sufficient for clinical applications designed to predict and anticipate VKA resistance or hemorrhagic

risk, clopidogrel resistance, or statin muscle toxicity. These pharmacogenetic applications are significant in terms of both healthcare efficacy and pharmaco-economic impact, but further changes in clinical practices are still needed for widespread use. Finally, other tests whose levels of evidence are still insufficient can be expected to be developed in the future. Potentially they will concern direct-action oral anticoagulants, aspirin, beta-blockers, or converting enzyme inhibitors.

Disclosure of interest

The authors declare they have no conflict of interest concerning this article.

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Table 1 Genetic variations to search for in the event of hypersensitivity to vitamin K antagonists.

Gene*	**Name***	**polymorphism**	**rs identifier**	**allele symbol**	**frequency of the minority allele in the European population**	***in vitro* functionality**	***in vivo* functionality**
VKORC1	vitamin K epoxide reductase complex subunit 1	c.-1639G>A	rs9934438	*VKORC1*2*	39%	Significant decrease in protein expression *in vivo* and *in vitro*	
CYP2C9	cytochrome P450 family 2 subfamily C member 9	c.430C>T	rs1799853	*CYP2C9*2*	12%	Significant decrease in enzyme activity *in vivo* and *in vitro*	
		c.1075A>C	rs1057910	*CYP2C9*3*	7%		

* HNGC-approved nomenclature (see http://www.genenames.org)

Table 2 Suggested dosage for warfarin (mg/d) as a function of CYP2C9 and VKORC1 genotypes.

		CYP2C9 Genotype					
***VKORC1* Genotype**		***1/*1**	***1/*2**	***1/*3**	***2/*2**	***2/*3**	***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

Table 3 Genetic variations to search for in a context of clopidogrel resistance or hemorrhagic risk.

Gene*	**Name***	**polymorphism**	**rs identifier**	**allele symbol**	**frequency of the minority allele in European populations**	***in vitro* functionality**	***in vivo* functionality**
CYP2C19	cytochrome P450 family 2 subfamily C member 19	c.681G>A	rs4244285	CYP2C19*2	15%	Splicing defect with appearance of a stop codon: no evidence of enzyme activity *in vivo*	
		c.636G>A	rs4986893	CYP2C19*3	<0.1% (Caucasian pop.) 6% (Asian pop.)	nonsense SNP with a premature stop codon: no evidence of enzyme activity *in vivo*	
		c. -806C>T	rs12248560	CYP2C9*17	25%	Increased gene transcription and protein expression i*n vivo* and *in vitro*	

* HNGC-approved nomenclature (see http://www.genenames.org)

Table 4 Genetic variations to search for in a context of myotoxicity in a patient taking statins.

Gene*	**Name***	**polymorphism**	**rs identifier**	**allele symbol**	**frequency of the minority allele in European populations**	***in vitro* functionality**	***in vivo* functionality**
SLCO1B1	solute carrier organic anion transporter family member 1B1	c.521T>C	rs4149056	SLCO1B1*5	16%	nonsense SNP associated with decreased enzyme activity demonstrated both *in vitro* and *in vivo*	

* HNGC-approved nomenclature (see http://www.genenames.org)

Figure 1. Schematic representation of vitamin K antagonist (VKA) mechanism of action an metabolism

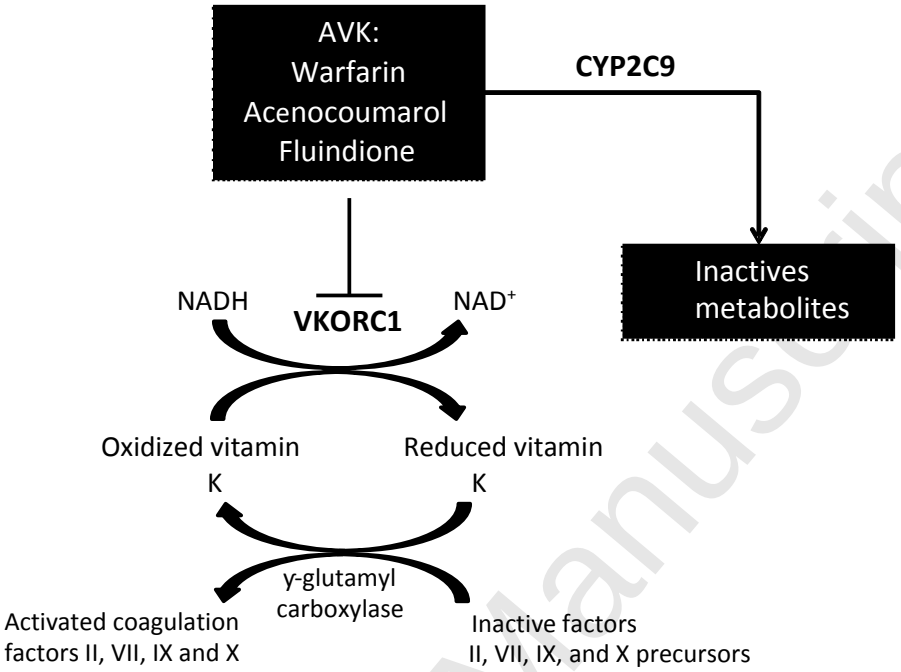

Figure 2. Schematic representation of clopidogrel mechanism of action and metabolism

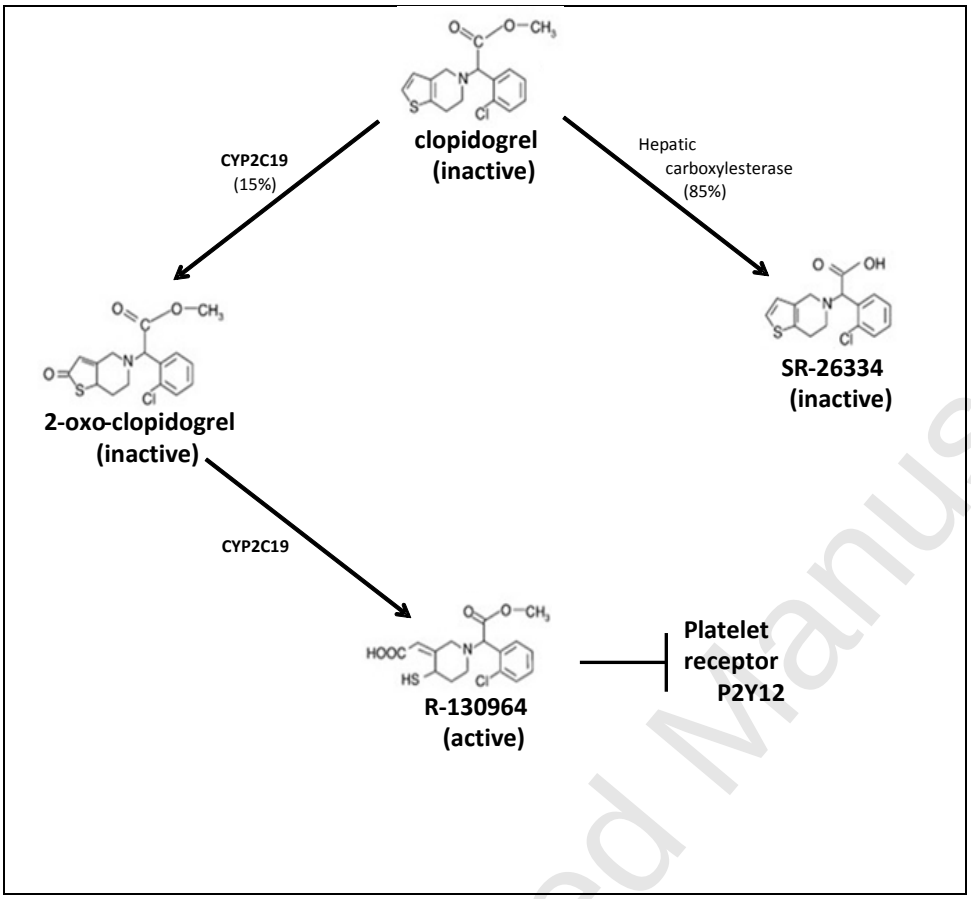

Figure 3. CYP2C19 genotyping proposed before instituting clopidogrel treatment [18].

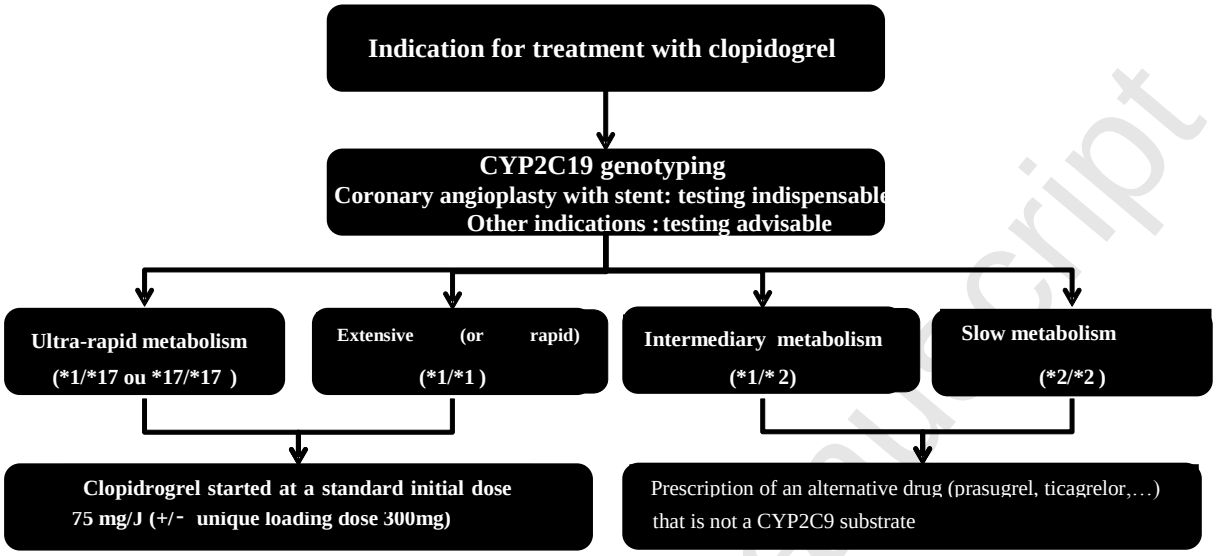

Figure 4. Indications for SLCO1B1 genotyping for simvastatin treatment. Proposed therapeutic strategy as a function of risk factors and current CPIC and FDA guidelines [23,24].