

# Clopidogrel pharmacogenetics in patients undergoing percutaneous neurointervention

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During vascular neurointervention procedures, the use of double antiaggregation therapy (mainly acetylsalicylic acid and clopidogrel) is required to minimize the risk of thromboembolic complications. Since clopidogrel is one of the most widely used agents in the perioperative management of patients undergoing neurointervention processes, knowing its mechanism of action and the factors that determine its activation and metabolism is of great importance to ensure the correct antiaggregation of these patients.

Clopidogrel is a prodrug that does not have an anti-aggregate effect by itself, but exerts its therapeutic effect once metabolized through the cytochrome P450 (CYP) enzymatic complex. Clopidogrel thiol active metabolite (R130964) specifically and irreversibly binds to the purinergic P2Y<sub>12</sub> receptor of platelets (Figure 1), inhibiting ADP-mediated platelet activation and aggregation, throughout their lifespan of 7-10 days [1].

After both oral and intravenous clopidogrel dosing, anti-aggregation activity can be observed within two hours [2]. Moreover, after administration of multiple 75 mg daily doses of clopidogrel, the steady state is reached between days 3 and 7. Once reached, the average level of platelet inhibition is between 40% and 60% [3]. In general, after treatment discontinuation, platelet aggregation and bleeding time gradually return to baseline values within 5 days [3].

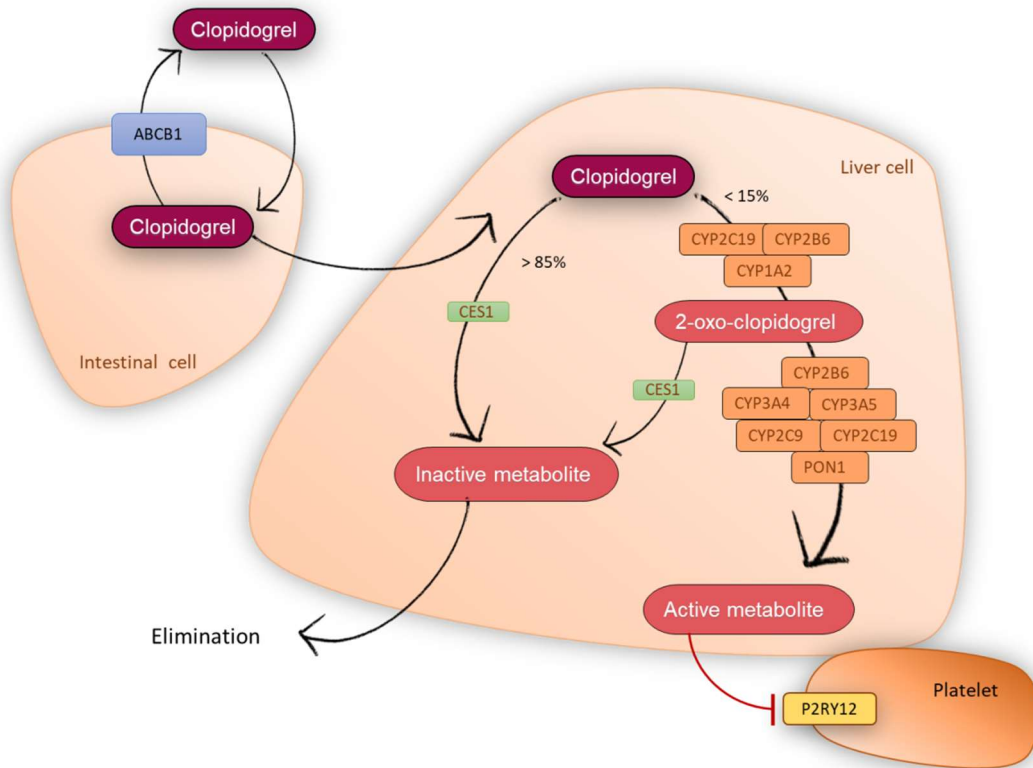
As shown in Figure 1, approximately 50% of the prodrug is absorbed, but 85% of it is hydrolyzed by the carboxylesterase 1 (CES1) to inactive forms (carboxylic acid) [4], while the remaining 15% will be accessible for transformation into the active metabolite [5]. Two consecutive oxidative processes occurred carried out by numerous CYP enzymes. First, the CYP2C19, CYP2B6 and CYP1A2 isoforms convert clopidogrel into 2-oxo-clopidogrel. Then, the isoforms CYP3A4, CYP3A5, CYP2B6, CYP2C9, CYP2C19 and the enzyme paraoxonase-1 (PON1) transform the 2-oxo-clopidogrel into its active metabolite [6,7]. However, 50% of 2-oxo-clopidogrel is metabolized by CES1 to an inactive compound, limiting the extent of active metabolite that is formed [8].

The rate of clopidogrel gastrointestinal absorption can vary between individuals, thus affecting the response onset [9,10]. The levels of expression of P-glycoprotein (P-gp), encoded by ABCB1, an ATP-dependent transporter, could affect its permeability and thus alter the oral bioavailability of clopidogrel [9,11].

The effect of clopidogrel diverges broadly among patients, so that between 5 and 56% of patients are poor responders or non-responders to clopidogrel [12], and, therefore, will be at higher risk of ischemic events after stent implantation [13-15]. This different response might be due in part to genetic variability, related to clopidogrel pharmacokinetics and mechanism of action. Therefore, we are going to review the main genes associated to the response to clopidogrel. Most of these studies have been performed in patients with acute coronary syndrome.

## *CYP2C19*

Since CYP2C19 isoform is involved in the two metabolic steps and contributes to an estimated



**Figure 1:** Clopidogrel metabolic pathway.

45% of 2-oxoclopidogrel and 21% of the active metabolite [16], its genetic polymorphisms could significantly alter the response to clopidogrel treatment. Therefore, it has been the most studied enzyme of clopidogrel metabolism to date. Based on the *CYP2C19* genotype, individuals are typically categorized into different phenotypes [17]: poor metabolizers (PM), subjects carrying two non-function alleles (i.e. *CYP2C19* \*2/\*2, \*2/\*3 or \*3/\*3); intermediate metabolizers (IM), subjects who carry one non-function allele and one normal function allele, or subjects who carry one non-function allele and one increased-function allele (i.e. *CYP2C19* \*1/\*2, \*1/\*3 or \*2/\*17); normal metabolizers (NM): subjects with two normal alleles (i.e. *CYP2C19* \*1/\*1); rapid metabolizers (RM), subjects with one normal allele and one increased-function allele (i.e. *CYP2C19* \*1/\*17) and ultra-rapid metabolizers (UM), subjects with two increased-function alleles (i.e. *CYP2C19* \*17/\*17).

*CYP2C19* IM-PM phenotypes have been associated with a hyporesponse to clopidogrel, since lower levels of active metabolite will be achieved with a non-functional enzyme. Consequently, these patients showed a diminished efficacy and a higher risk of vascular adverse events, including stent thrombosis [18-21].

In contrast, the presence of the increased-function \*17 allele lead to increased platelet inhibition and hyperresponse to clopidogrel, as increased formation of active metabolite will result in increased antiplatelet efficacy [22-25]. As a consequence, these patients are at higher risk of bleeding [20,26,27].

In this regard, in 2011 and subsequently in 2013, the Clinical Pharmacogenetics Implementation Consortium (CPIC) made a series of therapeutic recommendations based on the *CYP2C19* genotype for the treatment of acute coronary syndromes with clopidogrel [28]. Briefly, NM and RM-UM patients may take standard doses of clopidogrel as indicated in the drug label. However, it is recommended that IM and PM patients receive an alternative antiplatelet therapy, such as prasugrel or ticagrelor.

The two oxidative steps necessary for the formation of the active metabolite require the activity of several cytochrome P450 enzymes, many of which are quite polymorphic. Although current evidence shows a greater contribution of *CYP2C19*, several studies have shown that other enzymes might be also relevant in the response to clopidogrel.

### **CYP2C8/CYP2C9**

A genome wide association study identified 13 polymorphisms within the CYP2C18-CYP2C19-CYP2C9-CYP2C8 cluster associated with a decreased response to clopidogrel [29]. However, there is controversy between the association of the most studied CYP2C9 alleles (\*2 and \*3) and the effect of clopidogrel. While some authors have found no significant relationship [23] others associate the presence of the \*3 allele with a higher incidence of stent thrombosis [30].

### **CYP3A4/CYP3A5**

CYP3A4 reduced activity was associated with an increased risk of stent thrombosis in patients with acute coronary syndrome treated with clopidogrel [31]. In addition, one study postulates that the role of CYP3A4/5 in clopidogrel metabolism may be more relevant than previously called [32].

### **CYP1A2**

Regarding the influence of CYP1A2 polymorphisms in the response to clopidogrel, there is no clear evidence. It has been described that the increased-function *CYP1A2*\*1F allele is not associated with an increased incidence of cardiac adverse events in patients with coronary artery disease [33]. In contrast, Cresci et al. describe an increased risk of bleeding events associated with the reduced-function *CYP1A2*\*1C allele in African-American populations with acute myocardial infarction [34].

### **CYP2B6**

The reduced-function *CYP2B6*\*5 and \*9 alleles, have not been associated with a different response to clopidogrel in patients with coronary artery disease [33,35,36].

### **PON1**

Specifically, two polymorphisms in *PON1* coding region, L55M and Q192R, have been associated with lower PON1 activity and concentration [37-39]. PON1 has been proposed as one of the crucial enzymes for clopidogrel bioactivation since it participates in the metabolism of 2-oxo-clopidogrel to its active metabolite. In patients with coronary artery disease treated with clopidogrel, Q192R Q/Q genotype was associated to an increased risk of stent thrombosis [7].

### **CES1**

Since CES1 is responsible for metabolizing 85% of clopidogrel, genetic variations that affect the expression and/or activity of CES1 may be of great relevance to clopidogrel treatment, since they will increase the amount of active metabolite that is formed. Indeed, the CES1 G143E polymorphism, with a frequency of 1%, was associated with a decreased protein functionality [40]. Lewis et al. found that, in line with the expected, carriers of the mutated allele showed increased levels of active metabolite and improved response to clopidogrel in patients with coronary heart disease [41].

### **ABCB1**

The most commonly studied variant in *ABCB1* is C3435T. Several studies have evaluated the influence of C3435T polymorphism on clopidogrel treatment. Taubert et al. described lower levels of clopidogrel and its metabolite in patients carrying

the T/T genotype, probably as a result of increased intestinal flow due to increased expression of P-gp [9]. However, this is contrary to what would be expected since the T allele has been described to reduce the expression of the protein [42-45] and, therefore, it results in increased absorption and decreased elimination. If this is the case, patients carrying the T/T genotype should achieve higher concentrations of clopidogrel and its active metabolite.

### **P2RY12**

*P2RY12* is the gene that encodes for the P2Y<sub>12</sub> G-protein-coupled receptor. It is involved in platelet aggregation and is one of the target molecules for the treatment of thromboembolisms. Multiple polymorphisms have been described in this gene, although its involvement in the functionality of the receptor requires further research. In particular, some polymorphisms have been associated with increased platelet reactivity in clopidogrel treatment [46,47]. However, these associations have not been replicated and the level of evidence is low.

## **Clopidogrel pharmacogenetics in patients undergoing neurointervention**

In spite of the vast amount of publications investigating the influence of polymorphisms on the effect of clopidogrel, most of them focus on patients with cardiological disorders, especially those with acute coronary syndrome undergoing percutaneous coronary intervention. Cerebrovascular disorders treated with neurointerventional procedures have not been investigated in depth. For this type of patients, the treatment with clopidogrel and acetylsalicylic acid is also the first therapeutic option. Moreover, extrapolation of evidence from one group of patients to others is not the appropriate approach given the specificity of the medical condition and treatment, which may influence the therapeutic outcome [48].

Up to 9% of patients undergoing neurointervention procedures develop thromboembolic complications [49]. Dual antiplatelet therapy decreases the occurrence of vascular adverse events by up to 75-80% in patients undergoing percutaneous coronary intervention, an effect that would be similar in the cohort of patients undergoing neurointervention [49]. However, there is a subgroup of clopidogrel hyporesponders estimated at 5-30% in the cardiovascular population and up to 66% in patients undergoing neurointervention [49].

Bearing in mind that patients undergoing neurointerventional procedures represent a quite heterogeneous cohort of patients, and that there are no standardized guidelines for neurovascular conditions, it is essential to enlarge our knowledge of the effect of clopidogrel on this cohort. Following this path, several publications studied the effect of clopidogrel in relation to the different metabolizing phenotypes:

Zhu et al. conducted a study of the association between *CYP2C19* polymorphisms and the clinical efficacy of clopidogrel therapy in 241 Asian patients after ischemic stroke, who underwent carotid artery stenting. They found that patients carrying *CYP2C19*\*2 and \*3 alleles were more likely to suffer a subsequent ischemic event than those individuals with a wild-type genotype [50]. The *CYP2C19*\*17 allele was not analyzed in this study.

González et al. analyzed the influence of CYP2C19 phenotypes on the response to clopidogrel in 209 patients who underwent stent

placement in the carotid artery and who had high platelet reactivity during treatment. They found a statistically significant lower antiaggregant effect in IM/PM patients compared to UM and NM. In addition, the number of non-responders was significantly higher among the IM/PM group [35].

Ge et al. measured the effects of clopidogrel on the aggregometry of 215 patients with intracranial aneurysms treated by stent assisted coils embolization. They found that carriers of the *CYP2C19*\*2 and \*3 alleles, especially PM, had a higher risk of clopidogrel resistance, while the NM phenotype was significantly associated with the incidence of bleeding events [51]. The *CYP2C19*\*17 allele was not included in this study.

Colley et al. published a review on the involvement of the *CYP2C19*\*2 allele in neurointervention procedures, showing that this allele is associated with a hyporesponse to clopidogrel [49].

Lin et al. analyzed the response to clopidogrel in 108 patients undergoing neurointervention for intracranial aneurysms or stenosis. They found no association with the *CYP2C19*\*2 and \*3 alleles, however, they concluded that being a carrier of the *CYP2C19*\*17 allele, contrary to the expected, is related to the incidence of ischemic events, regardless of the effect of clopidogrel. However, they mention the need to confirm the influence of this allele on the clinical outcome of patients undergoing endovascular treatment [52].

All these studies suggest that the presence of no-function alleles is associated with clopidogrel resistance and the incidence of ischemic events, which is in line with studies in patients with acute coronary syndrome. However, the influence of the *CYP2C19*\*17 allele is not as clear, even contradictory to what might be expected.

Our recent research tried to confirm these results and to overcome the limitations of these studies. Our group published first in March 2019 and, second in June 2019, a retrospective observational study

in 123 and 144 patients, respectively, subjected to percutaneous neurointervention who were treated with clopidogrel [53-55]. In the first study, we aimed to evaluate the influence of *CYP2C19* phenotype on clopidogrel response. In the second, we also evaluate the influence of the main polymorphisms of the transporter ABCB1, other CYP enzymes (*CYP1A2*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, *CYP3A4* and *CYP3A5*), *PON1*, *CES1* and *P2RY12*, including 21 new cases.

Our results confirm that *CYP2C19* is the most relevant enzyme in the formation of clopidogrel active metabolite. Indeed, as expected, the *CYP2C19*\*2 allele was associated with a hyporesponse to clopidogrel in patients after percutaneous neurointervention [53]. Moreover, the *CYP2C19*\*17 allele resulted a protective factor for the incidence of ischemic events but a risk factor for the incidence of bleeding events [55]. Only two PM subjects (with genotype \*2/\*2) showed a significantly higher aggregometry value ( $268.5 \pm 26.2$  PRU) with respect to the rest of the groups ( $196.2 \pm 84.9$  PRU in IM,  $140.3 \pm 89.2$  PRU in NM,  $158.8 \pm 73.6$  PRU in RM and  $151.8 \pm 108.0$  PRU in UM;  $p=0.013$ ) (Figure 2). Moreover, regarding the type of clinical events related to the *CYP2C19* phenotype, the prevalence of ischemic events was lower in the RM-UM group (2.3%) compared to IM-PM (10.8%) and NM (15.9%),  $p=0.060$  [53-55]. On the other hand, the highest prevalence of bleeding events was in the RM-UM group (15.9%) compared to NM (7.9%) and IM-PM (2.7%),  $p=0.109$  [53-55].

After a multivariate analysis, age, concomitant treatment with proton-pump inhibitors (PPIs) and phenotype *CYP2C19* IM-PM were predictors of worse response to clopidogrel associated with a higher value of aggregometry. Moreover, *CYP2C19* RM-UM phenotype was a protective factor, and the duration of treatment a risk factor, for the development of any ischemic event. Finally, with regard to the prediction of bleeding events, the phenotype *CYP2C19*

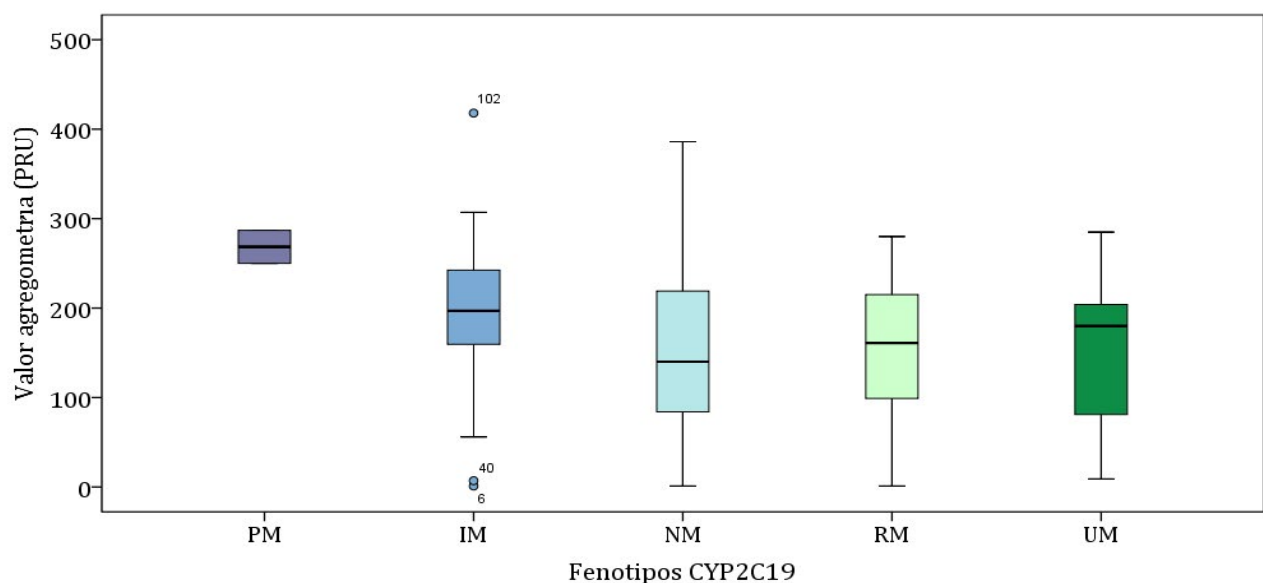


Figure 2: Aggregometry value according to *CYP2C19* phenotype.

RM-UM was the only risk factor [54].

Regarding other genes, we observed that the mutated haplotype of *ABCB1* was a predictor of better response to clopidogrel, so P-glycoprotein seems to play a relevant role in its absorption and/or distribution [54]. Furthermore, subjects carrying *CES1 G143E* polymorphism showed an increased antiaggregant effect, suggesting a higher formation of active metabolite. Finally, polymorphisms in other CYP450 enzymes, *PON1* and *P2RY12* seemed not to influence the effect of clopidogrel after a neurointervention.

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In addition, as our study also analyzed the influence of the concomitant use of PPIs, we observed that its use decreases the response to clopidogrel, especially in patients carrying a *CYP2C19*\*2 allele or patients with a wild-type genotype. This is due to the inhibition of *CYP2C19* by PPIs.

Despite having found no significant association between IM-PM phenotype and the incidence of ischemic events, we believe that CPIC therapeutic recommendations in patients with acute coronary syndrome with IM-PM phenotype should also be applied in patients undergoing neurointerventional procedures (Table 1), for all the reasons mentioned above. Moreover, we suggest that therapeutic recommendations should be extended to RM-UM patients to prevent the risk of bleeding complications (Table 1).

As a conclusion from our research, we could highly recommend a routine prior genotyping of *CYP2C19* for all patients to be treated with clopidogrel, the prescription of an antiaggregant other than clopidogrel in patients carrying the *CYP2C19*\*2 or \*17 allele and to avoid the concomitant use of PPIs and clopidogrel.

According to our knowledge, no further research has been performed on this issue in the last few months. However, we would like to recommend further perspectives. First, our study should be extended with a larger sample size in order to establish significant correlations that allow the design of a treatment algorithm. It should be based on the phenotype of the patients, concomitant treatments and other factors to be taken into account, such as age and sex.

Phenotype	Genotypes	Consequences	Clopidogrel therapeutic recommendation
Rapid and Ultrarapid metabolizer (RM-UM) (~5-30% of patients)	*1/*17, *17/*17	Increased risk of bleeding	Alternative antiplatelet therapy (if no contraindication)
Normal metabolizer (NM) (~35-50% of patients)	*1/*1	Normal platelet inhibition; normal residual platelet aggregation	Clopidogrel - label recommended dosage and administration
Intermediate metabolizer (IM) (~18-45% of patients)	*1/*2, *1/*3, *2/*17	Reduced platelet inhibition, increased risk for ischemic events	Alternative antiplatelet therapy (if no contraindication)
Poor metabolizer (PM) (~2-15% of patients)	*2/*2, *2/*3, *3/*3	Reduced platelet inhibition, increased risk for ischemic events	Alternative antiplatelet therapy (if no contraindication)

**Table 1:** Therapeutic recommendations in patients receiving clopidogrel after a neurointerventional procedure according to *CYP2C19* phenotype.

Subsequently, it would be advisable to carry out a randomized clinical trial with three treatment arms: one in which patients are treated according to usual clinical practice, without available pharmacogenetic information, where decisions to change treatment or adjust doses are made according to the clinician's criteria; another treatment arm where the patient is genotyped prior to the initiation of treatment with clopidogrel, so that the adjustment of the treatment to be prescribed and the doses to be administered are guided by pharmacogenetic information (mainly CYP2C19) and the use of concomitant medication, applying the treatment algorithm. Finally, a third treatment arm where some of the alternatives to clopidogrel treatment are administered (e.g. ticagrelor). It would be useful to check whether pharmacogenetic-guided treatment, is cost-effective with respect to the administration of another antiplatelet to all patients, regardless of their genotype. Thus, it could be tested if this therapeutic individualization reduces the incidence of adverse events, improves the response to clopidogrel and avoids an over cost to the national health system. The sample size should be large in order to find subjects with minority alleles of low-frequency polymorphisms, which makes collaboration between research groups from various centres necessary.

As a final consideration, we would point out that although the implementation of pharmacogenetic results in the routine practice is difficult, it is also possible. As a result of our research, the radiologists of our hospital are more aware of the need to include genotype as an additional variable to be considered in prescribing. Therefore, we believe that multi-disciplinary teamwork is necessary to develop therapeutic guidelines that clinicians can understand and apply, so that pharmacogenetic information can be useful for the actual prescribers.

### Conflicts of Interests

F.A.S. has been consultant or investigator in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, Chemo, Cinfa, FAES, Farmalider, Ferrer, GlaxoSmithKline, Galenicum, Gilead, Italfarmaco, Janssen-Cilag, Kern, Normon, Novartis, Servier, Silverpharma, Teva and Zambon. M.S.R declares no conflicts of interest.

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