Genetic Study of Clopidogrel Pharmacogenetics

PATIENT	
Name:	**N.A.**
Date of birth:	**N.A.**
Gender:	**N.A.**
Ethnicity:	**N.A.**
Consultancy referral number:	**N.A.**
Family history:	**N.A.**
Medical referral reason:	**Therapeutic adequacy**
Genetic laboratory referral reason:	**N.A.**
Purpose:	**Pharmacogenetics**
Specimen type:	**Blood**

HEALTHCARE PROVIDER	
Referring physician:	**N.A.**
Medical reference:	**N.A.**
Harvesting facility:	**N.A.**
Referring facility:	**N.A.**
Requisition date:	**N.A.**
Fulfillment date:	**2017-12-12**

1. RESULTS

1.1. DOSING INFORMATION

- *2/*2: *CYP2C19* poor metabolizer

It is recommended to consider an alternative antiplatelet therapy (if no contraindication), e.g. prasugrel or ticagrelor. Based on the genotype result, this patient is predicted to be a poor metabolizer with a significant reduction in platelet inhibition and increased residual platelet aggregation in response to clopidogrel therapy. Therefore this patient has an increased risk for adverse cardiovascular events (adapted from [1]).

1.2. GUIDELINE RECOMMENDATIONS

The results of the genetic test should be interpreted in the context of the patient's medical evaluation and racial/ethnic background. Other factors, such as age, body mass index, diabetes mellitus and the use of certain proton pump inhibitors (such as omeprazole) are also known to influence clopidogrel response [1, 2, 3].
The Clinical Pharmacogenetics Implementation Consortium (CPIC) [4] of the National Institutes of Health Pharmacogenomics Research Network develops peer-reviewed gene–drug guidelines that are published and updated periodically on `http://www.pharmgkb.org` based on new developments in the field.
CPIC established antiplatelet therapy recommendations based on the clinical interpretation and use of *CYP2C19* genotype data [5].

2. TECHNICAL INFORMATION

2.1. METHODOLOGY

1. A commercial kit was used to perform DNA extraction and purification. DNA concentration and quality were evaluated with a spectrophotometer.
2. Genotyping was performed through molecular study of three genetic variants of the *CYP2C19* gene associated with clopidogrel pharmacokinetics and pharmacodynamics.
3. Genotyping was achieved using a high-throughput DNA Microchip platform, the iPLEX® MassARRAY® system (Agena Bioscience, Inc). This array platform allows an optimal genetic analysis by combining the benefits of accurate primer extension chemistry with MALDI-TOF mass spectrometry. The different masses of each generated PCR product are then converted into genotype information.
4. In accordance with Agena Bioscience's iPLEX® chemistry flyer, the MassARRAY® system performs SNP genotyping with a high level of accuracy and reproducibility (>99% accuracy on validated assays).

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2.2. GENETIC PANEL

CYP2C19 : **Cytochrome P450, family 2, subfamily C, polypeptide 19** | ENSG00000165841

2.3. RISKS AND LIMITATIONS

The Clopidogrel PGX Kit | 2016 was built under a rigorous quality control process which may not exclude the possibility of error that might influence the test results. The reliability of the results is always guaranteed as HeartGenetics, Genetics and Biotechnology SA standard quality recommendations have been followed for the execution of this genetic test. The results presented in this report are limited to the available scientific knowledge at the time this test was developed. HeartGenetics, Genetics and Biotechnology SA guarantees the accuracy of the scientific knowledge presented in the report. It has been assumed as truthful all the above declarations about patient and medical identity, the purpose of the study, index case and nature of analysed biological products.

2.4. QUALITY ASSURANCE

The Clopidogrel PGX Kit | 2016 is a certified CE-IVD medical device developed by HeartGenetics, Genetics and Biotechnology SA. This Product has been approved, cleared, or licensed by the Portuguese Regulatory Authority INFARMED (Autoridade Nacional do Medicamento e Produtos de Saúde). HeartGenetics, Genetics and Biotechnology SA is an ISO NP 9001 and ISO 13485 certified company for Quality Management System and applies an External Quality Assessment program from UK NEQAS. The laboratory that performs this genetic test undertakes to, at all times, comply with the all applicable certifications and Law in its territory.

2.5. TERMS AND CONDITIONS

HeartGenetics, Genetics and Biotechnology SA will not be liable whether in contract, tort, warranty, or under any statute, or any other basis of special, incidental, indirect punitive, multiple, or consequential damages in connection with in arising from this document, or improper use of the product described herein or any use of such product outside the scope of the express written licenses or permissions granted by HeartGenetics, Genetics and Biotechnology SA, to the extent allowed by law.
The results presented in Section 3.1, Genetic Information, are the responsability of the laboratory that performed the genetic test.

In the development of its work, HeartGenetics, Genetics and Biotechnology SA rigorously conforms to all the requirements set out in the legislation of the European Union institutions. It is the responsibility of partners of HeartGenetics, Genetics and Biotechnology SA to meet with the internal standards of the respective legal jurisdiction. HeartGenetics, Genetics and Biotechnology SA does not take responsibility for any eventual violations of existing regulations applicable in its partners' home countries.

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3. APPENDIX

3.1. GENETIC INFORMATION

The results, described according to HGVS nomenclature (**http:www.hgvs.org**), are presented in the following table.

Gene	Genetic variant references		Nucleotidic change [1]	Aminoacidic change	Observation [2]
	HGMD	Ensembl			
CYP2C19	CM942096	rs4986893	c.636G>A	p.Trp212Term	WT
CYP2C19	CS941458	rs4244285	c.681G>A	p.Pro227Pro	HMZ
CYP2C19	CR067132	rs12248560	c.-806C>T	–	WT

[1] The numeric identification associated with each variant is indexed to a reference sequence obtained from Ensembl database (**http://www.ensembl.org/index.html**).
[2] hmz – Homozygosity; htz – Heterozygosity; wt – Wild type

3.2. EVIDENCES FOR MOLECULAR MARKERS

This appendix includes a detailed interpretation concerning the genetic information associated with therapeutic appropriateness. All evidences are supported by scientific articles indexed in PubMed (**http://www.ncbi.nlm.nih.gov/pubmed**), PharmGKB, and HGMD Professional database 2015.4 database (**http://www.hgmd.org**), accessed in October 2016.

CYP2C19, CM942096 / rs4986893 + CYP2C19, CS941458 / rs4244285 + CYP2C19, CR067132 / rs12248560

***CYP2C19* poor metabolizer**

Clopidogrel is an inactive prodrug that requires hepatic bioactivation *via* several cythocrome P-450 enzymes, including CYP2C19.
Patients carrying two loss-of-function alleles (*CYP2C19**2 and/or *CYP2C19**3) are considered to be poor metabolizers, presenting a reduced antiplatelet effect when treated with clopidogrel [1, 6].
Meta-analysis are consistent that carriers of at least one copy of a loss-of-function allele, treated with clopidogrel, have a significantly increased risk of cardiovascular disease events [7, 8, 9], such as cardiac mortality (OR = 2.07, 95%CI = [1.22;3.52] [8]; RR = 1.28, 95%CI = [0.95;1.73] [7]), non-fatal myocardial infarction (OR = 1.69, 95%CI = [1.09;2.61] [8]; RR = 1.48, 95%CI = [1.05;2.07] [7]), stroke (OR = 5.78, 95%CI = [1.62;20.65] [8]; RR = 1.98, 95%CI = [0.77;5.09] [7]) and stent thrombosis (OR = 3.81, 95%CI = [2.27;6.40] [8]; RR = 1.75, 95%CI = [1.50;2.03] [7]) comparing with non-carriers. As expected, the combined risk of cardiovascular death, myocardial infarction and ischemic stroke is higher for carriers of two loss-of-function alleles (RR = 1.76, 95%CI = [1.24;2.50]) than for those harbouring only one allele (RR = 1.55, 95%CI = [1.11;2.17) [9]. Individuals carrying any loss-of-function allele have, however, a lower risk of bleeding when compared with carriers of either *CYP2C19**1 or *CYP2C19**17 alleles (RR = 0.84, 95%CI = [0.75;0.94]) [7].

3.3. ADDITIONAL INFORMATION

The antiplatelet agent clopidogrel is extensively used worldwide, being indicated in the treatment or prevention of atherothrombotic events. It is advised when aspirin is contraindicated, in peripheral artery disease, in patients with history of coronary artery bypass surgery, and in patients with known cardiovascular disease. The dual therapy with aspirin is indicated in percutaneous angioplasty with stenting, in acute coronary syndromes without stenting, in atrial fibrillation in cases where oral anticoagulants cannot be given, and in patients with known cardiovascular disease [7, 8, 10, 11, 12, 13].
As a prodrug, clopidogrel requires biotransformation to the active metabolite Clop-AM, by cytochrome P-450 enzymes, before it exerts antiplatelet effects [2, 6, 7, 8, 9, 14]. The Clop-AM selectively inhibits the binding of adenosine diphosphate to its platelet receptor, thereby inhibiting platelet aggregation. This action is irreversible and, consequently, platelets exposed to Clop-AM are affected for the remainder of their lifespan (about 10 days) [2].
CYP2C19 is an important hepatic drug-metabolizing enzyme and is responsible for the metabolic activation of clopidogrel [6]. The metabolism of clopidogrel can be impaired by *CYP2C19* genetic polymorphisms and growing evidence reveals that the loss-of-function polymorphisms are associated with decreased exposure to Clop-AM, consequently affecting the degree of platelet inhibition [2, 8, 14].
The clinical importance of *CYP2C19* genotype on clopidogrel therapy has been extensively studied in the recent years. The *CYP2C19**2 and *CYP2C19**3 loss-of-function alleles cause an impaired antiplatelet effect in clopidogrel-treated patients, resulting in an increased risk of the recurrence of major adverse cardiovascular events [1, 6, 9, 11]. The gain-of-function allele *CYP2C19**17 is associated with increased risk of bleeding [1, 6]. The frequency of these alleles differs across populations, and among the Europeans *CYP2C19**17 is the most prevalent (21%), followed by *CYP2C19**2 (15%) and *CYP2C19**3 (0.42%) [1, 6].
In this context, the Food and Drug Administration (FDA) issued a *Boxed Warning* about patients who do not effectively metabolize clopidogrel and therefore may not receive the full benefits of the drug. It is recommended that health care professionals consider the use of other antiplatelet medications or alternative dosing strategies for clopidogrel. It was stated that *CYP2C19* genetic testing could be useful to optimize drug therapy

[2]. Also, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has established several therapeutic recommendations regarding *CYP2C19* genotype data [1], summarized on the following table:

Therapeutic recommendations for clopidogrel based on *CYP2C19* phenotype and haplotype			
Phenotype	**Haplotype**	**Implications for clopidogrel**	**Therapeutic recommendations**
Extensive metabolizer	***1/*1**	Normal platelet inhibition. Normal residual platelet aggregation.	Prescribe the label-recommended dosage.
Ultrarapid metabolizer	***1/*17; *17/*17**	Increased platelet inhibition. Decreased residual platelet aggregation.	Prescribe the label-recommended dosage.
Intermediate metabolizer	***1/*2; *1/*3; *2/*17**	Reduced platelet inhibition. Increased residual platelet aggregation. Increased risk for adverse cardiovascular events.	Consider alternative antiplatelet therapy (if no contraindication), e.g. prasugrel, ticagrelor.
Poor metabolizer	***2/*2; *2/*3; *3/*3**	Significantly reduced platelet inhibition. Increased residual platelet aggregation. Increased risk for adverse cardiovascular events.	

Adapted from [1]

NOTE: *CYP2C19**1 denotes the wildtype allele (i.e. normal function). This genetic test only studies *CYP2C19**1, *2, *3 and *17 alleles. Please note that other alleles, for instance *CYP2C19**4 and *5, also result in the absence of enzymatic activity but are rare in all ethnicities (<1%) and their effect on laboratory outcomes has not been fully documented. For this reason, the wildtype allele *1 is assumed in the absence of *2, *3 and *17.

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4. REFERENCES

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