Genetic study of Warfarin Pharmacogenetics

PATIENT		HEALTHCARE PROVIDER	
Name:	**N.A.**	Referring physician:	**N.A.**
Date of birth:	**N.A.**	Medical reference:	**N.A.**
Gender:	**N.A.**	Harvesting facility:	**N.A.**
Ethnicity:	**N.A.**	Referring facility:	**N.A.**
Consultancy referral number:	**N.A.**		
Family history:	**N.A.**	Requisition date:	**N.A.**
Medical referral reason:	**Therapeutic adequacy**	Fulfillment date:	**2017-12-11**
Genetic laboratory referral reason:	**N.A.**		
Purpose:	**Pharmacogenetics**		
Specimen type:	**Saliva**		

1. RESULTS

1.1. DOSING INFORMATION

- *VKORC1* AA - *CYP2C9* 1*/3*: High sensitivity to warfarin

Lower than standard dose requirement to achieve a stable anticoagulation INR level of 2-3.

1.2. GUIDELINE RECOMMENDATIONS

The results of the genetic test should be interpreted in the context of the patient's medical evaluation, family history and racial/ethnic background. Several drugs are known to interact with warfarin, for instance some antibiotics, cardiac drugs, and drugs active on the central nervous system. Warfarin clinical outcomes can also be influenced by the interaction with diet, in particular vegetables containing vitamin K.
The Clinical Pharmacogenetics Implementation Consortium (CPIC) [1] of the National Institutes of Health Pharmacogenomics Research Network develops gene-drug guidelines that are published and updated periodically on `http://www.pharmgkb.org` based on new developments in the field.
CPIC has established several recommendations on the clinical interpretation and use of *CYP2C9* and *VKORC1* genotype data for the adjustment of warfarin dose to achieve a therapeutic INR [2].

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 analysis of three genetic variants of the genes *VKORC1* e *CYP2C9* associated with warfarin 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

CYP2C9 ⁝ **cytochrome P450, family 2, subfamily C, polypeptide 9** | NM_000771
VKORC1 ⁝ **vitamin K epoxide reductase complex, subunit 1** | NM_024006

2.3. RISKS AND LIMITATIONS

The Warfarin 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 Warfarin 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.

TECHNICAL DIRECTION
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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 HGMD	Ensembl	Nucleotidic change [1]	Aminoacidic change	Observation [2]
CYP2C9	CM994193	rs1799853	c.430C>T	p.Arg144Cys	WT
CYP2C9	CM960481	rs1057910	c.1075A>C	p.Ile359Leu	HTZ
VKORC1	CR052440	rs9923231	c.-1639G>A	–	HMZ

[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.

CYP2C9, CM994193 / rs1799853 + CYP2C9, CM960481 / rs1057910

CYP2C9 is a member of the cytochrome P450 superfamily with a significant action in warfarin metabolism [3, 4]. *CYP2C9* polymorphisms lead to the expression of proteins with different catalytic activities, thereby impairing S-warfarin metabolism [3]. *CYP2C9**1 is considered the reference allele, associated with a normal enzymatic activity. *CYP2C9**2 and *CYP2C9**3 alleles are responsible for a reduction in enzyme activity of approximately 30-40% and 80-90%, respectively [3, 4, 5, 6]. The increased serum warfarin levels is a reflection of patients over-medication, increasing the INR above the therapeutic target level and accounting for increased bleeding incidents in some patients [4]. In this context, lower warfarin dose is required [4]. A meta-analysis study shows that both *CYP2C9**2 and *CYP2C9**3 alleles were associated with 17% and 37% reduction in daily warfarin dose, respectively, and to an increased relative risk of bleeding (OR = 1.91 95%CI = [1.16;3.17] for *CYP2C9**2 and OR = 1.77 95%CI = [1.07;2.91] for *CYP2C9**3) [7].

VKORC1, CR052440 / rs9923231

VKORC1 encodes the vitamin K-epoxide reductase, which catalyses the rate-limiting step in vitamin K recycling: the conversion of vitamin K-epoxide to vitamin K. The reduced form of vitamin K is an essential cofactor for the post-translational activation of clotting factors II, VII, IX, and X, and the anticoagulant proteins C, S and Z. Warfarin exerts its anticoagulant activity by targeting and inhibiting VKORC1 [3, 4, 8, 9].
During the initial phase of anticoagulation, genetic variants of *VKORC1* are a major determinant of variability in sensitivity to warfarin among patients [8]. The common non coding variant -1639G>A within the promoter region is significantly associated with warfarin sensitivity and reduced dose requirements, as -1639A carriers require lower initial warfarin doses than -1639G carriers [3, 4, 10]. *VKORC1* genetic variability accounts for up to 25% of the variation in warfarin dose between individuals [4, 11, 12].
Several studies demonstrate that AA genotype requires a low-dose of warfarin (OR = 4.47 p = 0.03) for an INR superior to 5 when compared with heterozygous carriers [3, 13].

3.3. ADDITIONAL INFORMATION

Warfarin is the most widely used oral anticoagulant and is among the most effective agents to prevent thromboembolic events in a variety of clinical settings [4, 8, 14, 15, 16, 17, 18]. Warfarin efficacy is highly dependent on achieving and maintaining a narrow therapeutic window, usually an international normalized ratio (INR) between 2 and 3 [14, 17]. Its use is associated with increased risk of blood clot formation when treatment is subtherapeutic (INR of less than 2) or bleeding when supratherapeutic (INR of 4 or more) [3, 8].
Clinical factors, demographic variables and variations in *CYP2C9* and *VKORC1* genes contribute significantly to the variability among patients in dose requirements for warfarin [3, 11, 15, 17, 18].
On the basis of these observations, the Food and Drug Administration (FDA) approved a labeling change for warfarin that describes the reported effects of *VKORC1* and *CYP2C9* on dose requirements [18, 19].

Warfarin dose levels according to the combined genotype of *CYP2C9* and *VKORC1*							
		***CYP2C9* Genotypes**					
		*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
VKORC1	GG	Higher	Higher	Standard	Standard	Standard	Lower
Genotype	GA	Higher	Standard	Standard	Standard	Lower	Lower
	AA	Standard	Standard	Lower	Lower	Lower	Lower

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