Genetic Study of Simvastatin 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:	**2017-12-12**
Genetic laboratory referral reason:	**N.A.**		
Purpose:	**Pharmacogenetics**		
Specimen type:	**Blood**		

1. RESULTS

1.1. DOSING INFORMATION

- *1a/*15 / *1b/*5: Intermediate risk of simvastatin-associated myopathy

This patient is predicted to have intermediate SLCO1B1 function with **intermediate risk** of simvastatin-associated myopathy. As **one copy** of a decreased function allele (***5, *15, or *17**) was found **consider starting with a lower dose of simvastatin or choosing an alternate statin agent to avoid an untoward drug response.** (Adapted from [1]). Practice-based data suggests that the association between rs4149056 and muscle toxicity is stronger for simvastatin than for other drugs within the class [1]. This genetic variant causes a clear impact on simvastatin pharmacokinetics and, to a lesser degree, the pharmacokinetics of other statins, by the following order: pitavastatin, atorvastatin, pravastatin and rosuvastatin [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, gender, metabolic comorbidities and hepatic or renal dysfunction are also known to influence the risk for developing statin-induced muscle toxicity [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 has established several recommendations on the clinical interpretation and use of *SLCO1B1* genotype data for the adjustment of simvastatin dose in order to avoid adverse drug reactions [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 analysis of three genetic variants of the *SLCO1B1* gene associated with simvastatin pharmacokinetics.
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.

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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).

2.2. GENETIC PANEL

SLCO1B1 ⁝ **solute carrier organic anion transporter family, member 1B1** | ENSG00000134538

2.3. RISKS AND LIMITATIONS

The Simvastatin 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 Simvastatin 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 HGMD	Ensembl	Nucleotidic change [1]	Aminoacidic change	Observation [2]
SLCO1B1	CR043952	rs4149015	c.-910G>A	–	WT
SLCO1B1	CM043776	rs2306283	c.388A>G	p.Asn130Asp	HTZ
SLCO1B1	CM043777	rs4149056	c.521T>C	p.Val174Ala	HTZ

[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.

SLCO1B1, CR043952 / rs4149015 + SLCO1B1, CM043776 / rs2306283 + SLCO1B1, CM043777 / rs4149056

SLCO1B1 intermediate function - Intermediate risk of simvastatin-associated myopathy

The *SLCO1B1* gene encodes the SLCO1B1 protein, a liver specific transporter that mediates the transport of statins, such as simvastatin or atorvastatin, and other exogenous and/or endogenous substances into hepatocytes [6, 7]. Practice-based data suggests that the association between rs4149056 and muscle toxicity is stronger for simvastatin than for other drugs within the class [1].
The rs4149056 is a common non-synonymous coding variant that gives rise to a less functional form of the encoded protein, with reduced transport activity, leading to higher systemic statin concentrations and enhancing the risk of adverse effects [3, 8, 9, 10, 11].
The TC genotype at rs4149056, associated with intermediate function, has a prevalence of 28% among individuals of European ancestry [12]. The collective evidence indicates that statin blood concentrations are higher in people harbouring the C-allele and associate it with statin intolerance with an OR = 2.05 (95%CI = [1.02;4.09]) [8, 13]. The C-allele was identified in a genome wide association study as a dominant cause of severe statin-induced myopathy in patients taking 80 mg of simvastatin, and a meta-analysis of several studies estimates an overall OR for myopathy risk of 2.18 (95%CI = [1.39;3.43]) per C-allele [3, 13, 14]. The UK Clinical Practice Research Datalink indicates that in individuals receiving simvastatin, the OR for severe myopathy per C-allele is 4.97 (95%CI = [2.16;11.43]) [14].
The SEARCH study estimates a cumulative risk of myopathy of 3% for heterozygous carriers taking 80 mg/day simvastatin [13]. Additional evidence in other cohort shown that in patients receiving simvastatin, the C-allele (CC and TC vs TT) conferred a 3.2-fold increased risk for myopathy (OR = 3.2, 95%CI = [0.83;11.96]) [7]. In patients receiving a daily simvastatin dosage superior to 40 mg, there is an increased risk of developing myopathy (OR = 3.23, 95%CI = [1.74;5.99]) or even severe myopathy (OR = 6.28, 95%CI = [2.38;16.60]) [14].

3.3. ADDITIONAL INFORMATION

Simvastatin is one of the most commonly used statins for cholesterol reduction, for instance in the treatment of hypercholesterolemia, reducing cardiovascular morbidity and mortality [1, 11, 14]. Although generally well tolerated, some patients develop muscle-related adverse effects [14]. The most common statin-related adverse drug reaction (ADR) is skeletal muscle toxicity, including myalgia, myopathy, myositis and rabdomyolysis. Also, serum creatine kinase (CK) levels may be elevated. These ADR appear to be dose-dependent [2, 3, 14, 15, 16].
For simvastatin, there are strong evidences linking myopathy to rs4149056 in *SLCO1B1* gene, and this association has been reproduced in randomized trials and clinical practice-based cohorts. Conversely, for other statins (such as atorvastatin or pravastatin) this association has been less compelling [3, 14]. Evidences point out that *SLCO1B1*5* (rs4149056 C) allele is associated with increased risk of composite adverse events in patients with hypercholesterolemia treated with statins (atorvastatin, pravastatin or simvastatin) [3, 14].
The incidence of statin-induced myopathy has been reported to be 19% in individuals without any C alleles, 27% in heterozygous individuals and 50% in homozygous individuals for the C allele at the rs4149056 variant [3, 14]. On the basis of these observations, the Food and Drug Administration (FDA) recommends against 80 mg daily simvastatin dosage [17]. Also, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has established several recommendations on simvastatin dosage based on SLCO1B1 phenotype [1], summarized on the following table:

Dosing recommendations for simvastatin based on *SLCO1B1* phenotype			
Phenotype	**Diplotype**	**Implications for simvastatin**	**Simvastatin dosing recommendations**
Normal function	***1a/*1a; *1a/*1b; *1b/*1b**	Normal myopathy risk	Prescribe desired starting dose and adjust doses based on disease-specific guidelines.
Intermediate function	***1a/*5; *1a/*15; *1a/*17; *1b/*5; *1b/*15; *1b/*17**	Intermediate myopathy risk	Prescribe a lower dose or consider an alternative statin.
Low function	***5/*5; *5/*15; *5/*17; *15/*15; *15/*17; *17/*17**	High myopathy risk	Prescribe a lower dose or consider an alternative statin. Consider creatinine kinase surveillance.

Adapted from [1].

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