Genetic Study of Hereditary Thrombophilia

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:	**N.A.**
Genetic laboratory referral reason:	**N.A.**
Purpose:	**Diagnostics**
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:	**2017-12-11**

1. RESULTS

This genetic test identified 5 genetic variants (out of 14 analysed variants).

1.1. GENETIC RISK ANALYSIS

- **F2, CR961726 / rs1799963 :**
  Medium to high risk of venous and arterial thromboembolic events, isolated pulmonary thromboembolism, cardio-cerebrovascular disease, and fetal loss.
- **MTHFR, CM950819 / rs1801133 :**
  Medium risk of vascular thrombosis, coronary artery disease, cardio-cerebrovascular disease, fetal loss and intrauterine growth restriction. This risk increases in the presence of other genetic variants or risk factors associated with thrombophilia.
- **PROCR, CM052909 / rs867186 :**
  Medium risk of venous and arterial thromboembolism.
- **SERPINE1 (PAI-1), CD931038 / rs1799889 :**
  Medium risk of venous thromboembolism, coronary artery disease, fetal loss, implantation failure and pre-eclampsia in the presence of other genetic variants or risk factors associated with thrombophilia.
- **SERPINE1 (PAI-1), CR962707 / rs2227631 :**
  Medium risk of venous thromboembolism and cardiovascular disease in the presence of other genetic variants or risk factors associated with thrombophilia.

1.2. GUIDELINE RECOMMENDATIONS

General
All patients with inherited thrombophilias should undergo individualised risk assessment for appropriate clinical decision-making regarding lifestyle and pharmacologic treatment.
Hereditary Thrombophilia is a genetic disease transmitted to family relatives; therefore, it is recommended a carrier testing for this(ese) genetic variant(s) to direct members of the patient. In this situation, it is recommended a genetic consultation for the family. The risks, benefits and limitations of testing should be discussed in the context of explained inheritance and disease risk.

Clinical context
The Anticoagulation Forum [1], provides clinical guidance for thrombophilia testing in five clinical situations:
1) following provoked venous thromboembolism and 2) following unprovoked [1] venous thromboembolism; 3) in relatives of patients with thrombosis, 4) in female relatives of patients with thrombosis considering estrogen use; and 5) in female relatives of patients with thrombosis who are considering pregnancy [1].

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[1] Unprovoked means none of the following events: immobility, fracture, cancer, estrogen therapy, pregnancy, surgery within the preceding 3 months.

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 14 genetic variants of 10 genes associated with hereditary thrombophilia.
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).

2.2. GENETIC PANEL

F13A1	**Coagulation factor XIII A chain** | NG_008107.1
F2	**Coagulation factor II, thrombin** | NM_000506
F5	**Coagulation factor V** | NM_000130
FGB	**Fibrinogen beta chain** | NG_008833.1
GP1BA	**Glycoprotein Ib platelet alpha subunit** | NG_008767.2
MTHFR	**methylenetetrahydrofolate reductase (NAD(P)H)** | NM_005957
PROCR	**Protein C receptor** | NG_032899.1
PROS1	**Protein S (alpha)** | NG_009813.1
SERPINC1	**Serpin peptidase inhibitor, clade C (antithrombin), member 1** | NM_000488
SERPINE1 (PAI-1)	**Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1** | NM_000602

2.3. RISKS AND LIMITATIONS

The TromboGene 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 TromboGene 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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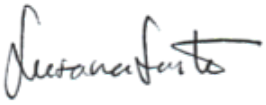

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3. APPENDIX

3.1. GENETIC INFORMATION

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

No other genetic variants from TromboGene Kit | 2016 panel have been identified, than those shown in table.

Gene	Genetic variant references HGMD	Ensembl	Nucleotidic change [1]	Aminoacidic change	Observation [2]
F2	CR961726	rs1799963	c.*+97G>A	–	Mutation in HTZ
MTHFR	CM950819	rs1801133	c.665C>T	p.Ala222Val	Polymorphism in HTZ
PROCR	CM052909	rs867186	c.655A>G	p.Ser219Gly	Polymorphism in HTZ
SERPINE1 (PAI-1)	CR962707	rs2227631	c.*-2137G>A	–	Polymorphism in HMZ
SERPINE1 (PAI-1)	CD931038	rs1799889	c.*-672delG	–	Polymorphism in 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**). This identification does not necessarily correspond to the variant designation shown in the following section.
[2] HMZ – Homozygosity; HTZ – Heterozygosity; WT – Wild type

3.2. EVIDENCES FOR MOLECULAR MARKERS

The appendix includes a detailed interpretation concerning the genetic risk that predispose to hereditary thrombophilia. All evidences are supported by PubMed scientific papers (**http://www.ncbi.nlm.nih.gov/pubmed**) and HGMD Professional database 2015.4 (**http://www.hgmd.org**), accessed on March 2016.

F2, CR961726 / rs1799963

During the coagulation process, prothrombin (factor II) is converted into thrombin (factor IIa), which in turn promotes the activation of fibrinogen (factor I) into fibrin (factor Ia) and consequently blood clot formation.
F2 G20210A mutation is associated with high levels of prothrombin, increasing the risk of venous and arterial thromboembolism [2, 3]. Most individuals (87%) with this mutation are in the highest quartile of plasma prothrombin levels, which is a risk factor for venous thromboembolism [4]. Meta-analysis studies have demonstrated that heterozygous carriers have an increased risk of developing venous thromboembolism (OR = 2.79; 95%CI = [2.25;3.46]), and that in homozygous carriers this risk is higher (OR = 6.74; 95%CI = [2.19;20.72]) [5, 6, 7, 8, 9]. It should be noted that homozygosity (20210AA) is a rare genotype.
These studies also indicate that this mutation is associated with a higher risk of venous thromboembolism in young individuals (>45 years old, OR = 3.19), isolated pulmonary thromboembolism (OR = 2.64; p<0.0001; 95%CI = [1.92;3.63]) [10], ischemic heart disease and ischemic stroke [11, 12, 13], increased risk of acute myocardial infarction in young women (>45 years old, OR = 3.84; p<0.001; 95%CI = [2.59;5.70]) [14], portal vein thrombosis (OR = 5.01; 95%CI = [3.03;8.30]) [15], and intrauterine growth restriction (OR = 4.81; 95% CI = [1.05;2.22]) [16].
Meta-analysis studies also refer that fetal loss may be associated with this mutation either in hetero- (OR = 6.8; 95%CI = [2.5;18.8]) or homozygosity (OR = 26.4; 95%CI = [1.2-559]) [9, 17, 18, 19, 20, 21, 22, 23]. It was also demonstrated that this mutation increases the risk of thromboembolism in women under therapy with oral contraceptives (OR = 7.14; 95%CI = [3.39;15.04]) [5]. It should be noted that pregnancy, puerperium and therapy with oral contraceptives constitute by themselves a predisposing risk factor for venous thromboembolism.

MTHFR, CM950819 / rs1801133

Methylenetetrahydrofolate reductase enzyme (MTHFR) converts homocysteine into methionine. The genetic variant C677T in *MTHFR* gene decreases homocysteine conversion rate, causing high levels of this aminoacid. Homocysteine accumulation is also enhanced by a vitamin B-poor diet, smoking, alcoholism and chronic therapies.
High plasma levels of homocysteine (hyper homocysteinemia) are a cause of susceptibility to thromboembolic events [24]. This genetic variant can be considered a susceptibility factor since it may increase the predisposition to vascular thrombosis [4, 25], coronary artery disease [26, 27, 28, 29], lacunar stroke [30], and peripheral arterial disease [11, 29, 31, 32]. The homozigosity for this variant may also increase the risk of acute myocardial infarction in males (OR = 3.05; p<0.001; 95%CI = [2.36;3.93]) [14].
There are also evidences of a possible linkage between this variant and either unexplained infertility in women [33]; intrauterine growth restriction (OR = 1.61; 95% CI = [0.79;3.26]) [16]; or recurrent fetal loss if in homozigosity [23, 34]. A meta-analysis study refers that this genetic variant increases venous thromboembolism risk in women under oral contraceptive therapy (OR = 2.05; 95%CI = [1.11;3.79]) [5]. It should be noted that the therapy with oral contraceptives is by itself a predisposing factor for thromboembolic events.

MTHFR, CM950819 / rs1801133 + SERPINE1 (PAI-1), CD931038 / rs1799889

Methylenetetrahydrofolate reductase enzyme (MTHFR) converts homocysteine into methionine. Homocysteine accumulation is also enhanced by a vitamin B-poor diet, smoking, alcoholism and chronic therapies.

The genetic variant C677T in *MTHFR* gene decreases homocysteine conversion rate, causing high levels of this aminoacid. High plasma levels of homocysteine (hyper homocysteinemia) are a cause of susceptibility to thromboembolic events [24]. This genetic variant can be considered a susceptibility factor since it may increase the predisposition to vascular thrombosis [4, 25], coronary artery disease [26, 27, 28, 29], lacunar stroke [30] and peripheral arterial disease [11, 29, 31, 32]. There are also evidences of a possible linkage between this variant and either unexplained infertility in women [33]; intrauterine growth restriction (OR = 1.61; 95% CI = [0.79;3.26]) [16]; or recurrent fetal loss if in homozigosity [23, 34].
Plasminogen activator inhibitor type 1 (PAI-1) is an inhibitor of fibrinolysis. High concentration of PAI-1 protein may contribute to a pro-thrombotic status.
Studies show that 4G/4G and 4G/5G genotypes are associated with an increased risk for venous thromboembolism (OR = 1.62, 95%CI = [1.22;2.16]) [4], namely for deep venous thrombosis and thromboembolic events in vessels of internal organs, mainly the portal vein [35, 36]. The 4G allele may also play a role in the process of coronary artery disease [12]. This variant has been associated with an increased risk of recurrent fetal loss, with the frequency of the 4G allele being significantly higher ($P<0.001$) in cases than in control subjects [37, 38]. An association was also observed between the 4G allele and implantation failure [23, 34, 38], and pre-eclampsia (OR = 1.17, 95%CI = [1.04-1.31]) [39]. This evaluation should be performed considering a clinical context as plasma levels of PAI-1 enzyme are physiologically increased during pregnancy.

PROCR, CM052909 / rs867186
Endothelial protein C receptor (PROCR) enhances protein C activation by the thrombin-thrombomodulin complex, thus regulating thrombin formation through protein C pathway.
The results from a genome-wide association study provide evidence that this variant plays a significant role in protein C plasma levels [40], possibly by affecting the binding properties of the receptor and thereby impairing the protein C anticoagulant pathway [41]. Protein C deficiency increases the risk of arterial thromboembolism by 6.9-fold (95%CI = [2.1;22.2]) in individuals aged under 55 years [42]. An association between this variant and increased risk of venous thromboembolism was demonstrated in a meta-analysis study (OR = 1.22; 95% CI = [1.11;1.33] for every additional copy of G allele) [43].
Other studies also associate this variant with pregnancy-related venous thromboembolism (OR = 4.8; 95%CI = [2.2;10.6]) [9] and iliac deep vein thrombosis (OR = 5.5; 95%CI = [2.3;13.0]) [41]. However, this evaluation should be performed considering clinical context and familial history, as a pregnant woman has an increased risk of susceptibility to thromboembolic events.

SERPINE1 (PAI-1), CD931038 / rs1799889
Plasminogen activator inhibitor type 1 (PAI-1) is an inhibitor of fibrinolysis. High concentration of PAI-1 protein may contribute to a pro-thrombotic status.
Studies show that 4G/4G and 4G/5G genotypes are associated with an increased risk for venous thromboembolism (OR = 1.62, 95%CI = [1.22;2.16]) [4], namely for deep venous thrombosis and thromboembolic events in vessels of internal organs, mainly the portal vein [35, 36]. The 4G allele may also play a role in the process of coronary artery disease [12].
This variant has been associated with an increased risk of recurrent fetal loss, with the frequency of the 4G allele being significantly higher ($P<0.001$) in cases than in control subjects [23, 34, 37, 38]. An association was also observed between the 4G allele and implantation failure [38], and pre-eclampsia (OR = 1.17, 95%CI = [1.04;1.31]) [39].
This evaluation should be performed considering a clinical context as plasma levels of PAI-1 enzyme are physiologically increased during pregnancy.

SERPINE1 (PAI-1), CR962707 / rs2227631
Plasminogen activator inhibitor type 1 (PAI-1) is an inhibitor of fibrinolysis. High concentration of PAI-1 protein may contribute to a pro-thrombotic status.
Studies demonstrate that this variant may be associated with thromboembolic risk [44, 45]. Individuals carrying haplotype 4G/-844A, with increased PAI-1 plasma levels and reduced levels of tissue plasminogenic activator, may have a higher risk of infarction [45]. The relevance of *PA1-1* genetic variants in cardiovascular risk assessment still needs a more meaningful clinical evidence and should be evaluated considering clinical context and familial history.

3.3. ADDITIONAL INFORMATION

The following table is merely informative and to support the interpretation of results.
The contribution of different genetic variants associated with inherited thrombophilic risk has a cumulative effect justifying the analysis of 10 genes included in the HeartGenetics panel. The following table shows the genetic variant classification (as polymorphism or mutation) and the severity prediction.

Genetic variant	Classification [1]	Severity prediction and associated risk [2]	Type of associated risk (Genetic predisposition)
F2 G20210A, CR961726 / rs1799963	DM	Severe, medium to high risk.	Thromboembolism: venous, arterial, isolated pulmonary. Cardio-cerebro-vascular disease. Fetal loss.

F5 G1691A, CM940389 / rs6025	DM	Severe, high risk.	Thromboembolism: venous, arterial, isolated pulmonary. Cardio-cerebro-vascular disease. Fetal loss.
PROCR, CM052909 / rs867186	DFP	Severe, medium risk.	Thromboembolism: venous, arterial.
PROS1, CD066393	DM	Medium risk.	Thromboembolism: venous, arterial. Cardiovascular disease. Fetal loss.
MTHFR C677T, CM950819, rs1801133	DFP	Medium risk (higher risk if combined with other risk factors or other genetic variants).	Vascular thrombosis. Coronary artery and cardio-cerebro-vascular disease. Fetal loss, and intrauterine growth restriction.
MTHFR A1298C, CM981315 / rs1801131	DFP	Low risk (higher risk if combined with other risk factors or other genetic variants).	Thrombosis. Atherosclerosis. Fetal loss.
PAI-1 4G5G, CD931038	DFP	Medium risk (higher risk if combined with other risk factors or other genetic variants).	Venous thromboembolism. Coronary artery disease Fetal loss, implantation failure and Pre-eclampsia.
PAI-1 -844AG, CR962707 / rs2227631	DP	Medium risk (if combined with other risk factors or other genetic variants).	Venous thromboembolism. Cardiovascular disease.
F13A1, CM950376	DFP	Low risk.	Venous thromboembolism. Coronary disease. Fetal loss.
FGB, CR994553 / rs1800790	DFP	Low risk (if combined with other risk factors or other genetic variants).	Venous thromboembolism. Coronary disease, stroke. Fetal loss.
F2, CM034520	DM	Severe, high risk.	Hypoprothrombinemia.
GP1BA, CM061054	DM	Severe, medium to high risk.	Thrombocytopenia.
SERPINC1, CM910058 / rs121909548	DM	Medium risk.	Venous thromboembolism. Cardiovascular disease.
SERPINC1, CM920113 / rs121909564	DM	Medium risk.	Venous thromboembolism. Cardiovascular disease.

[1] Genetic changes classification is according to reference database HGMD Professional version 2015.4 and bibliography.
DM – Disease-causing mutation;
DP – Disease-associated polymorphism;
DFP – Disease-associated polymorphisms with supporting functional evidence.

[2] Severity prediction for the genetic variant or mutation. Data supported by the software tools Polyphen (`http://genetics.bwh.harvard.edu/pph2/`), SIFT (`http://sift.jcvi.org/`) and MutPred (`http://mutpred.mutdb.org/`) that evaluate the impact of a genetic change on the structure and function of the encoded protein. This analysis is only applicable for genetic changes that involve aminoacid replacements. For some genetic variants there are still not enough evidences regarding severity.

In the context of obstetrics, thrombophilia is the leading cause of maternal thromboembolism, being associated with increased risk of recurrent fetal loss [46]. Concerning a molecular analysis in obstetrics, several evidences demonstrate that hereditary thrombophilia during pregnancy may contribute to placental insufficiency, placental abruption, pre-eclampsia and inhibition of intrauterine growth [46, 47]. In couples with a history of fetal loss, some studies argue for the importance of genetic testing for both parents [48].

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